DRUG CONJUGATE

TECHNICAL FIELD

The invention relates to antibody-drug conjugates (ADC) that are potentiated by co-administration of the ADC with a moiety comprising covalently linked saponin. The invention also relates to antibody-oligonucleotide conjugates (AOC) that are potentiated by co-administration of the AOC with a moiety comprising covalently linked saponin. The invention also relates to ADCs and AOCs which are conjugated with a saponin via a covalent linker. The invention further relates to an effector moiety such as a toxin or an antisense oligonucleotide such as for example a BNA, conjugated with a saponin via a covalent linkage. The invention also relates to a BNA covalently conjugated with a targeting moiety such as an antibody. The invention also relates to therapeutic combinations comprising a first pharmaceutical composition comprising a conjugate of a cell-targeting moiety such as an antibody and an antisense oligonucleotide such as a BNA covalently bound thereto, and comprising a second pharmaceutical composition comprising either a free saponin, or a conjugate of a cell-targeting moiety such as an antibody with a saponin covalently linked thereto. Furthermore, the invention relates to any of these conjugates or therapeutic compositions or therapeutic combinations, for use as a medicament. The invention also relates to any of these conjugates or therapeutic compositions or therapeutic combinations, for use in a method for the treatment or the prophylaxis of a cancer. Finally, the invention relates to methods for producing any of these conjugates of the invention.

BACKGROUND

Molecules with a therapeutic biological activity are in many occasions in theory suitable for application as an effective therapeutic drug for the treatment of a disease such as a cancer in human patients in need thereof. A typical example are small-molecule biologically active moieties. However, many if not all potential drug-like molecules and therapeutics currently used in the clinic suffer from at least one of a plethora of shortcomings and drawbacks. When administered to a human body, therapeutically active molecules may exert off-target effects, in addition to the biologically activity directed to an aspect underlying a to-be-treated disease or health problem. Such off-target effects are undesired and bear a risk for induction of health- or even life-threatening side effects of the administered molecule. It is the occurrence of such adverse events that cause many drug-like compounds and therapeutic moieties to fail phase III clinical trials or even phase IV clinical trials (post-market entry follow-up). Therefore, there is a strong desire to provide drug molecules such as small-molecule therapeutics, wherein the therapeutic effect of the drug molecule should, e.g., (1) be highly specific for a biological factor or biological process driving the disease, (2) be sufficiently safe, (3) be sufficiently efficacious, (4) be sufficiently directed to the diseased cell with little to no off-target activity on non-diseased cells, (5) have a sufficiently timely mode of action (e.g. the administered drug molecule should reach the targeted site in the human patient within a certain time frame and should remain at the targeted site for a certain time frame), and/or (6) have sufficiently long lasting therapeutic activity in the patient's body, amongst others. Unfortunately, to date, 'ideal' therapeutics with many or even all of the beneficial characteristics here

above outlined, are not available to the patients, despite already long-lasting and intensive research and despite the impressive progress made in several areas of the individually addressed encountered difficulties and drawbacks.

Chemotherapy is one of the most important therapeutic options for cancer treatment. However, it is often associated with a low therapeutic window because it has no specificity towards cancer cells over dividing cells in healthy tissue. The invention of monoclonal antibodies offered the possibility of exploiting their specific binding properties as a mechanism for the targeted delivery of cytotoxic agents to cancer cells, while sparing normal cells. This can be achieved by chemical conjugation of cytotoxic effectors (also known as payloads or warheads) to antibodies, to create antibody–drug conjugates (ADCs). Typically, very potent payloads such as emtansine (DM1) are used which have a limited therapeutic index (a ratio that compares toxic dose to efficacious dose) in their unconjugated forms. The conjugation of DM1 to trastuzumab (ado-trastuzumab emtansine), also known as Kadcycla, enhances the tolerable dose of DM1 at least two-fold in monkeys. In the past few decades tremendous efforts and investments have been made to develop therapeutic ADCs. However, it remains challenging to bring ADCs into the clinic, despite promising preclinical data. The first ADC approved for clinical use was gemtuzumab ozogamicin (Mylotarg, CD33 targeted, Pfizer/Wyeth) for relapsed acute myelogenous leukemia (AML) in 2000. Mylotarg was however, withdrawn from the market at the request of the Federal Drug Administration (FDA) due to a number of concerns including its safety profile. Patients treated with Mylotarg were more often found to die than patients treated with conventional chemotherapy. Mylotarg was admitted to the market again in 2017 with a lower recommended dose, a different schedule in combination with chemotherapy or on its own, and a new patient population. To date, only five ADCs have been approved for clinical use, and meanwhile clinical development of approximately fifty-five ADCs has been halted. However, interest remains high and approximately eighty ADCs are still in clinical development in nearly six-hundred clinical trials at present.

Despite the potential to use toxic payloads that are normally not tolerated by patients, a low therapeutic index (a ratio that compares toxic dose to efficacious dose) is a major problem accounting for the discontinuance of many ADCs in clinical development, which can be caused by several mechanisms such as off-target toxicity on normal cells, development of resistance against the cytotoxic agents and premature release of drugs in the circulation. A systematic review by the FDA of ADCs found that the toxicity profiles of most ADCs could be categorized according to the payload used, but not the antibody used, suggesting that toxicity is mostly determined by premature release of the payload. Of the approximately fifty-five ADCs that were discontinued, it is estimated that at least twenty-three were due to a poor therapeutic index. For example, development of a trastuzumab tesirine conjugate (ADCT-502, HER-2 targeted, ADC therapeutics) was recently discontinued due to a narrow therapeutic index, possibly due to an on-target, off-tissue effect in pulmonary tissue which expresses considerable levels of HER2. In addition, several ADCs in phase 3 trials have been discontinued due to missing primary endpoint. For example, phase 3 trials of a depatuxizumab mafodotin conjugate (ABT-414, EGFR targeted, AbbVie) tested in patients with newly diagnosed glioblastoma, and a mirvetuximab soravtansine conjugate (IMGN853, folate receptor alpha (FRα) targeted, ImmunoGen) tested in patients with platinum-resistant ovarian cancer, were recently stopped, showing no survival benefit. It is important

to note that the clinically used dose of some ADCs may not be sufficient for its full anticancer activity. For example, ado-trastuzumab emtansine has an MTD of 3.6 mg/kg in humans. In preclinical models of breast cancer, ado-trastuzumab emtansine induced tumor regression at dose levels at or above 3 mg/kg, but more potent efficacy was observed at 15 mg/kg. This suggests that at the clinically administered dose, ado-trastuzumab emtansine may not exert its maximal potential anti-tumor effect.

ADCs are mainly composed of an antibody, a cytotoxic moiety such as a payload, and a linker. Several novel strategies have been proposed and carried out in the design and development of new ADCs to overcome the existing problems, targeting each of the components of ADCs. For example, by identification and validation of adequate antigenic targets for the antibody component, by selecting antigens which have high expression levels in tumor and little or no expression in normal tissues, antigens which are present on the cell surface to be accessible to the circulating ADCs, and antigens which allows internalizing of ADCs into the cell after binding; and alternative mechanisms of activity; design and optimize linkers which enhance the solubility and the drug-to-antibody ratio (DAR) of ADCs and overcome resistance induced by proteins that can transport the chemotherapeutic agent out of the cells; enhance the DAR ratio by inclusion of more payloads, select and optimize antibodies to improve antibody homogeneity and developability. In addition to the technological development of ADCs, new clinical and translational strategies are also being deployed to maximize the therapeutic index, such as, change dosing schedules through fractionated dosing; perform biodistribution studies; include biomarkers to optimize patient selection, to capture response signals early and monitor the duration and depth of response, and to inform combination studies.

An example of ADCs with clinical potential are those ADCs such as brentuximab vedotin, inotuzumab ozogamicin, moxetumomab pasudotox, and polatuzumab vedotin, which are evaluated as a treatment option for lymphoid malignancies and multiple myeloma. Polatuzumab vedotin, binding to CD79b on (malignant) B-cells, and pinatuzumab vedotin, binding to CD22, are tested in clinical trials wherein the ADCs each were combined with co-administered rituximab, a monoclonal antibody binding to CD20 and not provided with a payload [*B. Yu and D. Liu, Antibody-drug conjugates in clinical trials for lymphoid malignancies and multiple myeloma; Journal of Hematology & Oncology (2019) 12:94*]. Combinations of monoclonal antibodies such as these examples are yet a further approach and attempt to arrive at the 'magic bullet' which combines many or even all of the aforementioned desired characteristics of ADCs.

Meanwhile in the past few decades, nucleic acid-based therapeutics are under development. Therapeutic nucleic acids can be based on deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), Anti-sense oligonucleotides (ASOs, AONs), and short interfering RNAs (siRNAs), MicroRNAs, and DNA and RNA aptamers, for approaches such as gene therapy, RNA interference (RNAi). Many of them share the same fundamental basis of action by inhibition of either DNA or RNA expression, thereby preventing expression of disease-related abnormal proteins. The largest number of clinical trials is being carried out in the field of gene therapy, with almost 2600 ongoing or completed clinical trials worldwide but with only about 4% entering phase 3. This is followed by clinical trials with ASOs. Similarly to ADCs, despite the large number of techniques being explored, therapeutic nucleic acids share two major issues during clinical development: delivery into cells and off-target effects. For instance, ASOs such as peptide

nucleic acid (PNA), phosphoramidate morpholino oligomer (PMO), locked nucleic acid (LNA) and bridged nucleic acid (BNA), are being investigated as an attractive strategy to inhibit specifically target genes and especially those genes that are difficult to target with small molecules inhibitors or neutralizing antibodies. Currently, the efficacy of different ASOs is being studied in many neurodegenerative diseases such as Huntington's disease, Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis and also in several cancer stages. The application of ASOs as potential therapeutic agents requires safe and effective methods for their delivery to the cytoplasm and/or nucleus of the target cells and tissues. Although the clinical relevance of ASOs has been demonstrated, inefficient cellular uptake, both in vitro and in vivo, limit the efficacy of ASOs and has been a barrier to therapeutic development. Cellular uptake can be < 2% of the dose resulting in too low ASO concentration at the active site for an effective and sustained outcome. This consequently requires an increase of the administered dose which induces off-target effects. Most common side-effects are activation of the complement cascade, the inhibition of the clotting cascade and toll-like receptor mediated stimulation of the immune system.

Chemotherapeutics are most commonly small molecules, however, their efficacy is hampered by the severe off-target side toxicity, as well as their poor solubility, rapid clearance and limited tumor exposure. Scaffold-small-molecule drug conjugates such as polymer-drug conjugates (PDCs) are macromolecular constructs with pharmacologically activity, which comprises one or more molecules of a small-molecule drug bound to a carrier scaffold (e.g. polyethylene glycol (PEG)).

Such conjugate principle has attracted much attention and has been under investigation for several decades. The majority of conjugates of small-molecule drugs under pre-clinical or clinical development are for oncological indications. However, up-to-date only one drug not related to cancer has been approved (Movantik, a PEG oligomer conjugate of opioid antagonist naloxone, AstraZeneca) for opioid-induced constipation in patients with chronic pain in 2014, which is a non-oncology indication. Translating application of drug-scaffold conjugates into treatment of human subjects provides little clinical success so far. For example, PK1 (N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer doxorubicin; development by Pharmacia, Pfizer) showed great anti-cancer activity in both solid tumors and leukemia in murine models, and was under clinical investigation for oncological indications. Despite that it demonstrated significant reduction of nonspecific toxicity and improved pharmacokinetics in man, improvements in anticancer efficacy turned out to be marginal in patients, and as a consequence further development of PK1 was discontinued.

The failure of scaffold-small-molecule drug conjugates is at least partially attributed to its poor accumulation at the tumor site. For example, while in murine models PK1 showed 45–250 times higher accumulation in the tumor than in healthy tissues (liver, kidney, lung, spleen, and heart), accumulation in tumor was only observed in a small subset of patients in the clinical trial.

A potential solution to the aforementioned problems is application of nanoparticle systems for drug delivery such as liposomes. Liposomes are sphere-shaped vesicles consisting of one or more phospholipid bilayers, which are spontaneously formed when phospholipids are dispersed in water. The amphiphilicity characteristics of the phospholipids provide it with the properties of self-assembly, emulsifying and wetting characteristics, and these properties can be employed in the design of new

drugs and new drug delivery systems. Drug encapsulated in a liposomal delivery system may convey several advantages over a direct administration of the drug, such as an improvement and control over pharmacokinetics and pharmacodynamics, tissue targeting property, decreased toxicity and enhanced drug activity. An example of such success is liposome-encapsulated form of a small molecule chemotherapy agent doxorubicin (Doxil: a pegylated liposome-encapsulated form of doxorubicin; Myocet: a non-pegylated liposomal doxorubicin), which have been approved for clinical use.

Therefore, a solution still needs to be found that allows for drug therapies such as anti-tumor therapies, applicable for non-systemic use when desired, wherein the drug has for example an acceptable safety profile, little off-target activity, sufficient efficacy, sufficiently low clearance rate from the patient's body, etc.

SUMMARY

For an embodiment of the present invention, it is a first goal to provide an improved biologically active compound or composition comprising such improved biologically active compound.

It is one of several objectives of embodiments of the current invention to provide a solution to the problem of non-specificity, encountered when administering small-molecule therapeutically active compounds to a human patient in need thereof. It is one of several objectives of embodiments of the current invention to provide a solution to the problem of drugs with non-optimal specificity for a biological factor or biological process driving a disease. It is one of several objectives of embodiments of the current invention to provide a solution to the problem of insufficient safety characteristics of current drugs, when administered to human patients in need thereof. It is one of several objectives of embodiments of the current invention to provide a solution to the problem of current drugs being less efficacious than desired, when administered to human patients in need thereof. It is one of several objectives of embodiments of the current invention to provide a solution to the problem of current drugs being not sufficiently directed to the diseased cell with little to no off-target activity on non-diseased cells, when administered to human patients in need thereof. It is one of several objectives of embodiments of the current invention to provide a solution to the problem that current drugs do not have a sufficiently timely mode of action (e.g. the administered drug molecule should reach the targeted site in the human patient within a certain time frame and should remain at the targeted site for a certain time frame), when administered to human patients in need thereof. It is one of several objectives of embodiments of the current invention to provide a solution to the problem that current drugs have not sufficiently long lasting therapeutic activity in the patient's body, when administered to human patients in need thereof.

At least one of the above objectives of embodiments of the invention is achieved by providing a conjugate of the invention, preferably comprising a cell-targeting moiety and at least one saponin, conjugate also being suitable for use as a medicament or suitable for implication in a pharmaceutical combination according to the invention, and suitable for use as a semi-finished product in the manufacture of an ADC or an antibody-oligonucleotide conjugate (AOC) of the invention, according to the invention. The therapeutic combination comprises for example a conjugate of the invention comprising covalently bound saponin and for example comprises a second conjugate comprising an

effector molecule, also referred to as an effector moiety or payload, wherein the conjugates comprise a different binding site for a different epitope exposed on a different cell-surface molecule of a targeted cell, wherein the different cell-surface molecules are expressed by the same target cell and exposed on the surface of the same target cell, or wherein the conjugates comprise the same binding site for the same epitope on the same cell-surface molecule of said targeted cell.

The present invention will be described with respect to particular embodiments but the invention is not limited thereto but only by the claims. The embodiments of the invention described herein can operate in combination and cooperation, unless specified otherwise.

An aspect of the invention relates to a conjugate comprising or consisting of an antibody and an antisense oligonucleotide such as an antisense BNA, covalently linked together. In Figure 1-5, the gene-silencing activity of such a conjugate is depicted (in vivo test in an animal tumor model). Reference is also made to the Examples section.

An aspect of the invention relates to a combination of a first composition comprising a conjugate comprising or consisting of an antibody and an antisense oligonucleotide such as an antisense BNA, covalently linked together, and a second composition comprising free saponin of the invention (see Table A1, Scheme I). In Figure 1-7A and in Figure 1-7C, the gene-silencing activity of such a conjugate is depicted (in vitro cell-based bioassay with human tumor cells). Reference is also made to the Examples section.

An aspect of the invention relates to a pharmaceutical combination comprising or consisting of a first composition comprising a first conjugate comprising or consisting of an antibody and an antisense oligonucleotide such as an antisense BNA, and a second composition comprising a first conjugate comprising or consisting of the same antibody and at least one saponin of the invention. In Figure 1-5, the gene-silencing activity of such a conjugate is depicted (in vivo test in an animal tumor model). In Figure 8-5, the gene-silencing activity of such a conjugate is depicted (in vitro cell-based bioassay with human tumor cells). Reference is also made to the Examples section.

An aspect of the invention relates to a pharmaceutical combination comprising or consisting of a fourth composition comprising a fourth conjugate comprising or consisting of an antibody and an antisense oligonucleotide such as an antisense BNA, and a fifth composition comprising a first conjugate comprising or consisting of a different antibody and at least one saponin of the invention. In Figure 10-6A and in Figure 10-6C, the gene-silencing activity of such a conjugate is depicted (in vitro cell-based bioassay with human tumor cells). Reference is also made to the Examples section.

An aspect of the invention relates to a conjugate comprising or consisting of an antisense oligonucleotide such as an antisense BNA, covalently linked to at least one saponin of the invention. In Figure 1-3, the gene-silencing activity of such a conjugate is depicted (in vitro cell-based bioassay with human tumor cells). Reference is also made to the Examples section.

An aspect of the invention relates to a conjugate comprising or consisting of an antisense oligonucleotide such as an antisense BNA, covalently coupled to a polymeric scaffold such as a dendron such as a G4-dendron, wherein the polymeric scaffold is covalently conjugated with one or more saponin molecules of the invention, such as four saponin molecules. In Figure 1-3, the gene-silencing activity of

such a conjugate is depicted (in vitro cell-based bioassay with human tumor cells). Reference is also made to the Examples section.

An aspect of the invention relates to a conjugate comprising or consisting of an antibody such as a monoclonal antibody with specificity for a tumor marker or tumor-cell receptor, covalently linked to at least one antisense oligonucleotide molecule such as antisense BNA, and covalently linked to at least one saponin molecule of the invention. In Figure 2-4, the gene-silencing activity of such a conjugate is depicted (in vivo test in an animal tumor model). Reference is also made to the Examples section.

An aspect of the invention relates to a conjugate comprising or consisting of an antibody such as a monoclonal antibody with specificity for a tumor marker or tumor-cell receptor, covalently linked to at least one antisense oligonucleotide molecule such as antisense BNA via a tri-functional linker such as the linker of Scheme II, and covalently linked to at least one saponin molecule of the invention via the same tri-functional linker. In Figure 1-1, the gene-silencing activity of such a conjugate is depicted (in vivo test in an animal tumor model). Reference is also made to the Examples section.

An aspect of the invention relates to a therapeutic combination consisting or comprising of a eighth composition comprising a conjugate comprising or consisting of an antibody, preferably a monoclonal antibody with specificity for a tumor marker or tumor-cell receptor, covalently linked to at least one saponin molecule of the invention, preferably via at least one linker, preferably at least one cleavable linker, cleavable under physiological acidic conditions, and further comprising a ninth composition comprising an antisense oligonucleotide such as an antisense BNA molecule. In Figure 6-2, the gene-silencing activity of such a conjugate is depicted (in vivo test in an animal tumor model). In Figure 5-2A and Figure 5-2C, the gene-silencing activity of such a conjugate is depicted (in vitro cell-based bioassay with human tumor cells). Reference is also made to the Examples section.

An aspect of the invention relates to any of the aforementioned conjugates or compositions or therapeutical combinations, for use as a medicament.

An aspect of the invention relates to any of the aforementioned conjugates or compositions or therapeutical combinations, for use in the treatment or prophylaxis of a cancer.

An aspect of the invention relates to a therapeutic molecule with chemical structure of COMPOUND I:

$$A1_m\ ((\text{-}L9_w)\ ((\text{–}\ L1_q\ \text{–}\ B1_n)_u\ ((\text{–}\ L2_r\ \text{–}\ L3_s)\ (\text{–}\ L4_v\ \text{–}\ C)_p)_t))_x$$

(compound I),

wherein

A1 is a first ligand if B1 is a first effector moiety, or A1 is the first effector moiety if B1 is the first ligand;

C is a saponin;

m = 0 or 1 if A1 is the first ligand and B1 is the first effector moiety;

m = 0 – 32 if A1 is the first effector moiety and B1 is the first ligand;

n = 0 or 1 if B1 is the first ligand and A1 is the first effector moiety, or if A1 is the first ligand and B1 is the first effector moiety;

p = any of 1-128;

L1 is at least one linker for covalently coupling two chemical groups;

L2 is at least one linker for covalently coupling two chemical groups;

L3 is at least one oligomeric or polymeric scaffold for covalently coupling two chemical groups;

L4 is at least one linker for covalently coupling two chemical groups;

L9 is a tri-functional linker for covalently coupling three chemical groups;

q = 0 or 1;

r = 0 or 1;

s = 0 or 1;

t = 0, 1 or 2 if s = 0, and t = any of 0-16 if s = 1;

u = any of 0 – 32 if A1 is the first ligand and B1 is the first effector moiety, or u = 1 if A1 is the first effector moiety and B1 is the first ligand;

v = 0 or 1;

w = 1 or 0; and

x = 1-16.

An embodiment relates to the therapeutic combination comprising the therapeutic molecule according to the invention and a second therapeutic molecule with chemical structure of COMPOUND II:

$$A2_a\ ((\text{-}L10_j)\ ((\text{–}\ L5_d\ \text{–}\ B2_b)_h\ ((\text{–}\ L6_e\ \text{–}\ L7_f)\ (\text{–}\ L8_i\ \text{–}\ C)_c)_g))_k$$

(compound II),

wherein

A2 is a second ligand if B2 is a second effector moiety, or A2 is the second effector moiety if B2 is the second ligand;

C is a saponin;

a = 0 or 1 if A2 is the second ligand and B2 is the second effector moiety, or a = 0 – 32 if A2 is the second effector moiety and B2 is the second ligand;

b = 0 or 1 if B2 is the second ligand and A2 is the second effector moiety, or if A2 is the second ligand and B2 is the second effector moiety;

c = any of 1-128;

L5 is at least one linker for covalently coupling two chemical groups;

L6 is at least one linker for covalently coupling two chemical groups;

L7 is at least one oligomeric or polymeric scaffold for covalently coupling two chemical groups;

L8 is at least one linker for covalently coupling two chemical groups;

L10 is a tri-functional linker for covalently coupling three chemical groups;

d = 0 or 1;

e = 0 or 1;

f = 0 or 1;

g = 0, 1 or 2 if f = 0, and g = any of 0-16 if f = 1;

h = any of 0 – 32 if A2 is the second ligand and B2 is the second effector moiety, or h = 1 if A2 is the second effector moiety and B2 is the second ligand;

i = 0 or 1;

j = 1 or 0 and;

k = 1-16.

An embodiment relates to the therapeutic molecule of the invention, wherein r = 0, s = 0, v = 0, s = 0, v = 0, p = 0, t = 0, ligand A1 is a monoclonal antibody or at least one binding fragment or –domain thereof according to any one of the claims 3-7, m = 1, effector moiety B1 is an effector moiety according to claim 8, preferably a BNA, either q = 0, n = 1 and u = 2-4, or q = 1, n = 2-4, u = 2-4 and linker L1 is the oligomeric or polymeric scaffold L3 according to any one of the claims 21-30.

An embodiment is the therapeutic combination of the invention, wherein the therapeutic molecule is the therapeutic molecule of the previous embodiment, and wherein the second ligand A2 is a monoclonal antibody or at least one binding fragment or –domain thereof according to the invention, a = 1, d = 0, b = 0, h = 0, e = 0, L7 is the scaffold according to the invention and f = 1, or L7 is absent and f = 0, L8 is a linker or a cleavable linker according to the invention, i = 1, c = 2-4 and g = 2-4 if f = 1 and g = 0 if f = 0, and saponin C is a saponin according to the invention, preferably the saponin C is SO1861 and/or QS-21, with the proviso that the ligand A1 and the ligand A2 are the same or are different.

An aspect of the invention relates to a therapeutic combination, wherein the therapeutic combination comprises: (a) a first pharmaceutical composition comprising the therapeutic molecule with chemical structure of COMPOUND I according to the invention, the first pharmaceutical composition optionally further comprising a pharmaceutically acceptable excipient; and (b) a second pharmaceutical composition comprising the second therapeutic molecule with chemical structure of COMPOUND II according to the invention, the second pharmaceutical composition optionally further comprising a pharmaceutically acceptable excipient.

An aspect of the invention relates to the first pharmaceutical composition of the invention for use as a medicament.

An aspect of the invention relates to a therapeutic combination for use in the treatment or prevention of cancer in a human subject, wherein the therapeutic combination comprises: (a) the first pharmaceutical composition of the invention; and (b) the second pharmaceutical composition of the invention, wherein the ligand A1 or B1 and the ligand A2 or B2 can bind to a tumor-cell epitope, preferably to a tumor-cell specific epitope, on a tumor-cell surface molecule, preferably on a tumor cell-specific surface molecule, with the proviso that the tumor-cell epitope or tumor-cell specific epitope to which the ligand A1 or B1 can bind is the same as, or is different from the tumor-cell epitope or the tumor-cell specific epitope to which the ligand A2 or B2 can bind.

An aspect of the invention relates to the first pharmaceutical composition of the invention further comprising the second therapeutic molecule of the invention.

An aspect of the invention relates to the first pharmaceutical composition of the invention further comprising the second therapeutic molecule of the invention, for use as a medicament.

An aspect of the invention relates to the first pharmaceutical composition of the invention further comprising the second therapeutic molecule of the invention, for use in the treatment or prevention of a cancer in a human subject.

An aspect of the invention relates to any of the following ADCs and AOCs, and their semi-finished conjugates, comprising a conjugate comprising covalently linked saponin of the invention and/or comprising a conjugate comprising a payload or effector moiety linked to e.g. an antibody of the invention and either optionally further comprising at least one effector molecule of the invention or optionally further comprising at least one saponin of the invention, respectively, or both:

Anti-EGFR antibody – saponin;

Anti-EGFR antibody – triterpenoid saponin and/or a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin;

Anti-EGFR antibody – SO1861;

Anti-EGFR antibody – GE1741;

Anti-EGFR antibody – SA1641;

Anti-EGFR antibody – Quil-A;

Anti-EGFR antibody – QS-21;

Anti-EGFR antibody – saponins in water soluble saponin fraction of Quillaja saponaria;

Cetuximab – saponin;

Cetuximab – triterpenoid saponin and/or a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin;

Cetuximab – SO1861;

Cetuximab – GE1741;

Cetuximab – SA1641;

Cetuximab – Quil-A;

Cetuximab – QS-21;

Cetuximab – saponins in water soluble saponin fraction of Quillaja saponaria;

Anti-HER2 antibody – saponin;

Anti-HER2 antibody – triterpenoid saponin and/or a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin;

Anti-HER2 antibody – SO1861;

Anti-HER2 antibody – GE1741;

Anti-HER2 antibody – SA1641;

Anti-HER2 antibody – Quil-A;

Anti-HER2 antibody – QS-21;

Anti-HER2 antibody – saponins in water soluble saponin fraction of Quillaja saponaria;

Trastuzumab – saponin;

Trastuzumab – triterpenoid saponin and/or a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin;

Trastuzumab – SO1861;

Trastuzumab – GE1741;

Trastuzumab – SA1641;

Trastuzumab – Quil-A;

Trastuzumab – QS-21;

Trastuzumab – saponins in water soluble saponin fraction of Quillaja saponaria;

Anti-CD71 antibody – saponin;

Anti-CD71 antibody – triterpenoid saponin and/or a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin;

Anti-CD71 antibody – SO1861;

Anti-CD71 antibody – GE1741;

Anti-CD71 antibody – SA1641;

Anti-CD71 antibody – Quil-A;

Anti-CD71 antibody – QS-21;

Anti-CD71 antibody – saponins in water soluble saponin fraction of Quillaja saponaria;

OKT-9 – saponin;

OKT-9 – triterpenoid saponin and/or a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin;

OKT-9 – SO1861;

OKT-9 – GE1741;

OKT-9 – SA1641;

OKT-9 – Quil-A;

OKT-9 – QS-21;

OKT-9 – saponins in water soluble saponin fraction of Quillaja saponaria;

Anti-EGFR antibody – oligonucleotide;

Anti-EGFR antibody – antisense oligonucleotide;

Anti-EGFR antibody – siRNA;

Anti-EGFR antibody – antisense BNA;

Anti-EGFR antibody – antisense BNA(HSP27);

Anti-EGFR antibody – proteinaceous toxin;

Anti-EGFR antibody – ribosome inactivating protein;

Anti-EGFR antibody – dianthin;

Anti-EGFR antibody – saporin;

Cetuximab – oligonucleotide;

Cetuximab – antisense oligonucleotide;

Cetuximab – siRNA;

Cetuximab – antisense BNA;

Cetuximab – antisense BNA(HSP27);

Cetuximab – proteinaceous toxin;

Cetuximab – ribosome inactivating protein;
Cetuximab – dianthin;
Cetuximab – saporin;
Anti-HER2 antibody – oligonucleotide;
Anti-HER2 antibody – antisense oligonucleotide;
Anti-HER2 antibody – siRNA;
Anti-HER2 antibody – antisense BNA;
Anti-HER2 antibody – antisense BNA(HSP27);
Anti-HER2 antibody – proteinaceous toxin;
Anti-HER2 antibody – ribosome inactivating protein;
Anti-HER2 antibody – dianthin;
Anti-HER2 antibody – saporin;
Trastuzumab – oligonucleotide;
Trastuzumab – antisense oligonucleotide;
Trastuzumab – siRNA;
Trastuzumab – antisense BNA;
Trastuzumab – antisense BNA(HSP27);
Trastuzumab – proteinaceous toxin;
Trastuzumab – ribosome inactivating protein;
Trastuzumab – dianthin;
Trastuzumab – saporin;
Anti-CD71 antibody – oligonucleotide;
Anti-CD71 antibody – antisense oligonucleotide;
Anti-CD71 antibody – siRNA;
Anti-CD71 antibody – antisense BNA;
Anti-CD71 antibody – antisense BNA(HSP27);
Anti-CD71 antibody – proteinaceous toxin;
Anti-CD71 antibody – ribosome inactivating protein;
Anti-CD71 antibody – dianthin;
Anti-CD71 antibody – saporin;
OKT-9 – oligonucleotide;
OKT-9 – antisense oligonucleotide;
OKT-9 – siRNA;
OKT-9 – antisense BNA;
OKT-9 – antisense BNA(HSP27);
OKT-9 – proteinaceous toxin;
OKT-9 – ribosome inactivating protein;
OKT-9 – dianthin;
OKT-9 – saporin;

Anti-EGFR antibody (– oligonucleotide)(– saponin), wherein the oligonucleotide is any one or more of antisense oligonucleotide, siRNA, antisense BNA, and antisense BNA(HSP27), and wherein the saponin is any one or more of a triterpenoid saponin and/or a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, SO1861, GE1741, SA1641, Quil-A, QS-21, and saponins in water soluble saponin fraction of Quillaja saponaria, wherein the anti-EGFR antibody preferably is cetuximab;

Anti-EGFR antibody (– proteinaceous toxin)(– saponin), wherein the proteinaceous toxin is any one or more of a ribosome inactivating protein, dianthin and saporin, and wherein the saponin is any one or more of a triterpenoid saponin and/or a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, SO1861, GE1741, SA1641, Quil-A, QS-21, and saponins in water soluble saponin fraction of Quillaja saponaria, wherein the anti-EGFR antibody preferably is cetuximab;

Anti-HER2 antibody (– oligonucleotide)(– saponin), wherein the oligonucleotide is any one or more of antisense oligonucleotide, siRNA, antisense BNA, and antisense BNA(HSP27), and wherein the saponin is any one or more of a triterpenoid saponin and/or a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, SO1861, GE1741, SA1641, Quil-A, QS-21, and saponins in water soluble saponin fraction of Quillaja saponaria, wherein the anti-HER2 antibody preferably is trastuzumab;

Anti-HER2 antibody (– proteinaceous toxin)(– saponin), wherein the proteinaceous toxin is any one or more of a ribosome inactivating protein, dianthin and saporin, and wherein the saponin is any one or more of a triterpenoid saponin and/or a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, SO1861, GE1741, SA1641, Quil-A, QS-21, and saponins in water soluble saponin fraction of Quillaja saponaria, wherein the anti-HER2 antibody preferably is trastuzumab;

Anti-CD71 antibody (– oligonucleotide)(– saponin), wherein the oligonucleotide is any one or more of antisense oligonucleotide, siRNA, antisense BNA, and antisense BNA(HSP27), and wherein the saponin is any one or more of a triterpenoid saponin and/or a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, SO1861, GE1741, SA1641, Quil-A, QS-21, and saponins in water soluble saponin fraction of Quillaja saponaria, wherein the anti-CD71 antibody preferably is OKT-9; and

Anti-CD71 antibody (– proteinaceous toxin)(– saponin), wherein the proteinaceous toxin is any one or more of a ribosome inactivating protein, dianthin and saporin, and wherein the saponin is any one or more of a triterpenoid saponin and/or a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, SO1861,

GE1741, SA1641, Quil-A, QS-21, and saponins in water soluble saponin fraction of Quillaja saponaria, wherein the anti-CD71 antibody preferably is OKT-9.

An embodiment is the first proteinaceous molecule of the invention, the semi-finished conjugate of the invention or the conjugate of the invention, wherein the first binding site is selected from cetuximab, trastuzumab, OKT-9, and/or wherein the effector molecule is selected from dianthin, saporin and antisense BNA(HSP27), and/or wherein the saponin is selected from SO1861, GE1741, SA1641, Quil-A, QS-21, and saponins in water soluble saponin fraction of Quillaja saponaria.

An embodiment is the conjugate according to the invention, wherein the first proteinaceous molecule is selected from cetuximab, trastuzumab, OKT-9, and/or wherein the effector molecule is selected from dianthin, saporin and antisense BNA(HSP27), and/or wherein the saponin is selected from SO1861, GE1741, SA1641, Quil-A, QS-21, and saponins in water soluble saponin fraction of Quillaja saponaria.

An aspect of the invention relates to an ADC or an AOCs or a semi-finished ADC conjugate or a semi-finished AOC conjugate comprising an antibody-saponin conjugate of the invention and comprising at least one effector molecule of the invention and/or comprising at least one saponin of the invention, of Structure C:

A (– S)b (– E)c

Structure C,

wherein A is the first binding site;

S is the saponin;

E is the effector molecule;

b = 0 – 64, preferably 0, 1, 2, 3, 4, 8, 16, 32, 64 or any whole number or fraction therein between;

c = 0 – 8, preferably 0, 1, 2, 3, 4, 6, 8 or any whole number or fraction therein between,

wherein S is coupled to A and/or E, E is coupled to A and/or S, preferably S is coupled to A and E is coupled to A.

An embodiment is the Structure C of the invention, wherein A is an anti-EGFR antibody such as cetuximab, an anti-HER2 antibody such as trastuzumab, an anti-CD71 antibody such as OKT-9, and/or wherein S is any one or more of a saponin, a triterpenoid saponin and/or a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, SO1861, GE1741, SA1641, Quil-A, QS-21, and saponins in water soluble saponin fraction of Quillaja saponaria, and/or wherein E is any one or more of an oligonucleotide, an antisense oligonucleotide, an siRNA, an antisense BNA, and an antisense BNA(HSP27), and/or any one or more of a proteinaceous toxin, a ribosome inactivating protein, dianthin and saporin.

An embodiment is the Structure C of the invention, the conjugate of the invention or the semi-finished conjugate of the invention or the first proteinaceous molecule of the invention, wherein the saponin, if present, and/or the effector molecule, if present, is covalently coupled via at least one linker, such as a cleavable linker, and/or via at least one oligomeric or polymeric scaffold, such as a linker based on N-ε-maleimidocaproic acid hydrazide (EMCH) succinimidyl 3-(2-pyridyldithio)propionate or 3-

(2-Pyridyldithio)propionic acid N-hydroxysuccinimide ester (SPDP), and 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU), and such as a scaffold based on a Dendron such as a G4-Dendron or a tri-functional linker such as the tri-functional linker of Scheme II, and/or wherein at least a lysine side chain and/or a cysteine side chain of the first binding site of the antibody, preferably a monoclonal antibody or fragments or domains thereof, is involved in the covalent bond with the saponin and/or the effector molecule and/or the linker and/or the cleavable linker and/or the scaffold, wherein preferably the saponin and/or the effector molecule is covalently linked to the first binding site of the antibody, preferably a monoclonal antibody, wherein the covalent link comprises or consists of an amide bond, a hydrazone bond, a disulphide bond.

An aspect of the invention relates to the use of any of the aforementioned conjugates of the invention or any of the the semi-finished conjugates of the invention or the antibody-saponin conjugate of the invention, as a medicament.

An aspect of the invention relates to the use of any of the conjugates of the invention or the semi-finished conjugates of the invention or the antibody-saponin conjugates of the invention, for use in the treatment or prophylaxis of a cancer or an auto-immune disease.

Figure 25 and Figure 26 show examples of ADCs of the invention with covalently coupled saponin(s) and OACs of the invention with covalently coupled saponin(s).

Of course, any and all of a, b, c, d, e, f, g, h, I, j, k, m, n, p, q, r, s, t, u, v, w and/or x have the value in accordance with each individual embodiment and aspect of the invention for any and all of the aforementioned aspects and embodiments according to the invention. In addition, (tri-functional) linkers L1, L2, L4, L5, L6, L8, L9 and/or L10, if present in a molecule or conjugate or moiety of the invention, are the (tri-functional) linkers as indicated for each and any of the aforementioned aspects and embodiments of the invention, as is readily appreciated by the skilled person. The oligomeric or polymeric scaffolds L3 and/or L7, if present in a molecule or conjugate or moiety of the invention, are the oligomeric or polymeric scaffolds as indicated for each and any of the aforementioned aspects and embodiments of the invention, as is also readily appreciated by the skilled person. Furthermore, the first ligand A1 and the first effector moiety B1, if present, and the second ligand A2 and the second effector moiety B2, if present, and the first effector moiety A1 and the first ligand B1, if present, and the second effector moiety A2 and the second ligand B2, if present, are the selected and indicated ligands and effector moieties, as disclosed for the first, second, third, fourth, fifth, and sixth series of embodiment and aspects of the invention, and all further embodiments and aspects of the invention, outlined here above. Saponin C is any one or more of the saponins referred to and listed in any of the aforementioned aspects and embodiments of the invention, in particular one or more saponins selected from Scheme I and/or Table A1.

DEFINITIONS

The term “linker” has its regular scientific meaning, and here refers to a chemical moiety or a linear stretch of amino-acid residues complexed through peptide bonds, which attaches a molecule or an atom to another molecule, e.g. to a ligand or to an effector molecule or to a scaffold. Typically, the linker comprises a chain of atoms linked by chemical bonds. Any linker molecule or linker technology known

in the art can be used in the present disclosure. Where indicated, the linker is a linker for covalently binding of molecules through a chemical group on such a molecule suitable for forming a covalent linkage or bond with the linker. The linker may be a non-cleavable linker, e.g., the linker is stable in physiological conditions. The linker may be a cleavable linker, e.g. a linker that is cleavable, in the presence of an enzyme or at a particular pH range or value, or under physiological conditions such as intracellular conditions in the endosomes such as the late endosomes and the lysosomes of mammalian cells such as human cells. Exemplary linkers that can be used in the context of the present disclosure includes, but is not limited to, N-ε-maleimidocaproic acid hydrazide (EMCH), succinimidyl 3-(2-pyridyldithio)propionate or 3-(2-Pyridyldithio)propionic acid N-hydroxysuccinimide ester (SPDP), and 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU).

The term “tri-functional linker” has its regular scientific meaning, and here refers to a linker which attaches three molecules via a chemical group on each of the three molecules. The skilled person is able to design such tri-functional linkers, based on the present disclosure and the common general knowledge. Such tri-functional linker can exhibit, for instance, a maleimido group that can be used for conjugation to targeting ligands that exhibit thiol groups to perform a thiol-ene reaction. In addition, the tri-functional linker could exhibit a dibenzocyclooctyne (DBCO) group to perform the so-called strain-promoted alkyne-azide cycloaddition (SPAAC, click chemistry) with an azido bearing saponin. Finally, the tri-functional linker could obtain a third functional group such as a trans-cyclooctene (TCO) group to perform the so-called inverse electron demand Diels–Alder (IEDDA) reaction with a tetrazine (Tz) bearing effector molecule. The skilled person will appreciate that the chemical groups of the tri-functional linker can be all three the same, or different, or the linker may comprise two of the same chemical groups for linking a molecule to the tri-functional linker. The formed bonds between the tri-functional linker can be covalent or non-covalent, and covalent bonds are preferred. The formed bonds between the tri-functional linker and the one or two or three bound molecules via respective chemical groups, can be cleavable (labile) bonds, such as cleavable under acidic conditions inside cells such as endosomes and lysosomes of mammalian cells such as human cells, or can be non-cleavable bonds. Of course, the tri-functional linker may encompass one or two chemical groups for forming covalent bonds while the further two or one chemical group(s), respectively, are/is for forming a non-covalent bond. Of course, the tri-functional linker may encompass one or two chemical groups for forming cleavable bonds while the further two or one chemical group(s), respectively, are/is for forming a non-cleavable bond.

The term “cleavable”, such as used in the term “cleavable linker” or “cleavable bond” has its regular scientific meaning, and here refers to being subject to cleavage under acidic conditions, reductive conditions, enzymatic conditions or light-induced conditions. For example, a cleavable linker may be subject to cleavage under acidic conditions, preferably said cleavable linker is subject to cleavage in vivo under acidic conditions as present in endosomes and/or lysosomes of mammalian cells, preferably human cells, preferably at pH 4.0 – 6.5, and more preferably at pH $\leq$ 5.5. As another example, a cleavable linker may be subject to cleavage by an enzyme, e.g. by cathepsin. Furthermore, an example of a covalent bond cleavable under reductive conditions is a disulphide bond.

The terms “oligomer” and “polymer” in the context of an oligomeric or polymeric scaffold has its regular scientific meaning. A polymer here refers to a substance which has a molecular structure built

up chiefly or completely from a large number of equal or similar units bonded together; an oligomer here refers to a polymer whose molecules consist of relatively few repeating units. For example, a structure comprising 5-10 or less equal or similar units, may be called an oligomeric structure, whereas a structure comprising 10-50 monomeric units or more may be called a polymeric structure, whereas a structure of 10 monomeric units may be called either oligomeric or polymeric.

The term “binding site” has its regular scientific meaning, and here refers to a region or an epitope on a molecule, e.g. a protein, DNA or RNA, to which another molecule can bind.

The term “scaffold” has its regular scientific meaning, and here refers to an oligomeric or polymeric template or a carrier or a base (base molecule or base structure), to which one or more molecules, e.g. ligand molecule, effector molecule, can be covalently bound, either directly, or via a linker, such as a cleavable linker. A scaffold may have a structurally ordered formation such as a polymer, oligomer, dendrimer, dendronized polymer, or dendronized oligomer or have an assembled polymeric structure such as a hydrogel, microgel, nanogel, stabilized polymeric micelle or liposome, but excludes structures that are composed of non-covalent assemblies of monomers such as cholesterol/phospholipid mixtures. A scaffold may comprise a polymeric or oligomeric structure, such as poly- or oligo(amines), e.g., polyethylenimine and poly(amidoamine); or structures such as polyethylene glycol, poly- or oligo(esters), such as poly(lactids), poly(lactams), polylactide-co-glycolide copolymers; or poly(dextrin), poly- or oligosaccharides, such as cyclodextrin or polydextrose; or structures such as natural and/or artificial poly- or oligoamino acids such as poly-lysine or a peptide or a protein, DNA oligo- or polymers, stabilized RNA polymers or PNA (peptide nucleic acid) polymers. Preferably, the polymeric or oligomeric structures are biocompatible, wherein biocompatible means that the polymeric or oligomeric structure does not show substantial acute or chronic toxicity in organisms and can be either excreted as it is or fully degraded to excretable and/or physiological compounds by the body’s metabolism.

The term “ligand” has its regular scientific meaning, and here refers to any molecule or molecules which may selectively bind to a target cell-surface molecule or target cell-surface receptor expressed at target cells, e.g. target cancer cells or target auto-immune cells. The ligand may bind to an epitope comprised by receptors or other antigens on the target cells. Preferably, the cell-binding ligands are antibodies.

The term "antibody" as used herein is used in the broadest sense, which may refer to an immunoglobulin (Ig) defined as a protein belonging to the class IgG, IgM, IgE, IgA, or IgD (or any subclass thereof), or a functional binding fragment or binding domain of an immunoglobulin. In the context of the present invention, a "binding fragment" or a “binding domain” of an immunoglobulin is defined as antigen-binding fragment or -domain or other derivative of a parental immunoglobulin that essentially maintains the antigen binding activity of such parental immunoglobulin. Functional fragments and functional domains are antibodies in the sense of the present invention even if their affinity to the antigen is lower than that of the parental immunoglobulin. "Functional fragments and -domains" in accordance with the invention include, but are not limited to, F(ab')2 fragments, Fab' fragments, Fab fragments, scFv, dsFv, single-domain antibody (sdAb), monovalent IgG, scFv-Fc, reduced IgG (rIgG), minibody, diabodies, triabodies, tetrabodies, Fc fusion proteins, nanobodies, variable V domains such

as VHH, Vh, and other types of antigen recognizing immunoglobulin fragments and domains. The fragments and domains may be engineered to minimize or completely remove the intermolecular disulphide interactions that occur between the CH1 and CL domains. Functional fragment and –domains offer the advantage of greater tumor penetration because of their smaller size. In addition, the functional fragment or –domain can be more evenly distributed throughout the tumor mass as compared to whole immunoglobulin.

The antibodies (immunoglobulins) of the present invention may be bi- or multifunctional. For example, a bifunctional antibody has one arm having a specificity for one receptor or antigen, while the other arm recognizes a different receptor or antigen. Alternatively, each arm of the bifunctional antibody may have specificity for a different epitope of the same receptor or antigen of the target cell.

The antibodies (immunoglobulins) of the present invention may be, but are not limited to, polyclonal antibodies, monoclonal antibodies, human antibodies, humanized antibodies, chimeric antibodies, resurfaced antibodies, anti-idiotypic antibodies, mouse antibodies, rat antibodies, rat/mouse hybrid antibodies, llama antibodies, llama heavy-chain only antibodies, heavy-chain only antibodies, and veterinary antibodies. Preferably, the antibody (immunoglobulin) of the present invention is a monoclonal antibody. The resurfaced, chimeric, humanized and fully human antibodies are also more preferred because they are less likely to cause immunogenicity in humans. The antibodies of the ADC of the present invention preferably specifically binds to an antigen expressed on the surface of a cancer cell, an autoimmune cell, a diseased cell, an aberrant cell, while leaving any healthy cell essentially unaltered (e.g. by not binding to such normal cell, or by binding to a lesser extent in number and/or affinity to such healthy cell).

Specific antibodies that can be used for the ADCs of the present invention include, but are not limited to, anti-HER2 monoclonal antibody such as trastuzumab and pertuzumab, anti-CD20 monoclonal antibody such as rituximab, ofatumumab, tositumomab and ibritumomab, anti-CA125 monoclonal antibody such as oregovomab, anti-EpCAM (17-1A) monoclonal antibody such as edrecolomab, anti-EGFR monoclonal antibody such as cetuximab, panitumumab and nimotuzumab, anti-CD30 monoclonal antibody such brentuximab, anti-CD33 monoclonal antibody such as gemtuzumab and huMy9-6, anti-vascular integrin alpha-v beta-3 monoclonal antibody such as etaracizumab, anti-CD52 monoclonal antibody such as alemtuzumab, anti-CD22 monoclonal antibody such as epratuzumab, anti-CEA monoclonal antibody such as labetuzumab, anti-CD44v6 monoclonal antibody such as bivatuzumab, anti-FAP monoclonal antibody such as sibrotuzumab, anti-CD19 monoclonal antibody such as huB4, anti-CanAg monoclonal antibody such as huC242, anti-CD56 monoclonal antibody such huN901, anti-CD38 monoclonal antibody such as daratumumab, anti-CA6 monoclonal antibody such as DS6, anti-IGF-IR monoclonal antibody such as cixutumumab and 3B7, anti-integrin monoclonal antibody such as CNTO 95, and anti-syndecan-1 monoclonal antibody such as B-B4.

Any other molecules than antibodies that bind to a cell receptor or antigen of a target cell can also be used as the cell-binding ligand for the ligand-drug conjugates of the present invention and the ligands provided with covalently bound saponin according to the invention. These ligands include, but are not limited to, proteins, polypeptides, peptides, small molecules. Examples of these non-antibody ligands are interferons (e.g. IFN-α, IFN-β, and IFN-γ), transferrins, lectins, epidermal growth factors

(EGF) and EGF-like domains, gastrin-releasing peptides (GRP), platelet-derived growth factors (PDGF), transforming growth factors (TGF), vaccinia growth factor (VGF), insulin and insulin-like growth factors (IGF, e.g. IGF-1 and IGF-2), other suitable hormones such as thyrotropin releasing hormones (TRH), melanocyte-stimulating hormones (MSH), steroid hormones (e.g. estrogen and androgen), somatostatin, lymphokines (e.g. IL-2, IL-3, IL-4, and IL-6), colony-stimulating factors (CSF, e.g. G-CSF, M-CSF and GM-CSF), bombesin, gastrin, Arg-Gly-Asp or RGD, aptamers (e.g. AS-1411, GBI-10, RNA aptamers against HIV glycoprotein), small molecules (e.g. folate, anisamide phenylboronic acid), vitamins (e.g., vitamin D), carbohydrates (e.g. hyaluronic acid, galactose).

An “effector molecule” or “effector moiety” or “payload” has its regular scientific meaning and in the context of this invention is any substance that affects the metabolism of a cell by interaction with an intracellular effector molecule target, wherein this effector molecule target is any molecule or structure inside cells excluding the lumen of compartments and vesicles of the endocytic and recycling pathway but including the membranes of these compartments and vesicles. Said structures inside cells thus include the nucleus, mitochondria, chloroplasts, endoplasmic reticulum, Golgi apparatus, other transport vesicles, the inner part of the plasma membrane and the cytosol.

The effector molecule or -moiety is a pharmaceutically active substance, such as a toxin such as a proteinaceous toxin, a drug, a polypeptide or a polynucleotide. A pharmaceutically active substance in this invention is an effector molecule or -moiety that is used to achieve a beneficial outcome in an organism, preferably a vertebrate, more preferably a mammal such as non-human subjects or a human being/subject. Benefits include diagnosis, prognosis, treatment, cure and prevention (prophylaxis) of diseases and/or symptoms and/or health problems. The pharmaceutically active substance may also lead to undesired and sometimes even harmful side effects (adverse events such as observed during clinical trials). In this case, pros and cons must be weighed to decide whether the pharmaceutically active substance is suitable in the particular case. If the effect of the pharmaceutically active substance inside a cell is predominantly beneficial for the organism as a whole, the cell is called a target cell. If the effect inside a cell is predominantly harmful for the organism as a whole, the cell is called an off-target cell. In artificial systems such as cell cultures and bioreactors, target cells and off-target cells depend on the purpose and are defined by the user. Examples of effector molecules and –moieties are a drug, a toxin, a polypeptide (such as an enzyme), a polynucleotide (including polypeptides and polynucleotides that comprise non-natural amino acids or nucleic acids), and any combination thereof.

An effector molecule or effector moiety that is a drug may include, but not limited to, anti-cancer agents, anti-inflammatory agents, and anti-infective (e.g., anti-fungal, antibacterial, anti-parasitic, anti-viral) agents. Preferably, the drug molecule of the present invention is an anti-cancer agent or an anti-auto-immune agent. Suitable anti-cancer agents include, but are not limited to, alkylating agents, antimetabolites, spindle poison plant alkaloids, cytotoxic/antitumor antibiotics, topoisomerase inhibitors, photosensitizers, and kinase inhibitors. Also included in the definition of "anti-cancer agent" are: e.g. (i) anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators; (ii) aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands; (iii) anti-androgens; (iv) protein kinase inhibitors; (v) lipid kinase inhibitors; (vi) antisense oligonucleotides, particularly those which inhibit

expression of genes in signaling pathways implicated in aberrant cell proliferation; (vii) ribozymes such as VEGF expression inhibitors and HER2 expression inhibitors; (viii) vaccines such as gene therapy vaccines; topoisomerase 1 inhibitors; (ix) anti-angiogenic agents; and pharmaceutically acceptable salts, acids, solvates and derivatives of any of the above.

An effector molecule or –moiety that is a toxin may include, but is not limited to, proteinaceous toxins (e.g. bacterial-derived toxins, and plant-derived toxins), toxins targeting tubulin filaments, toxins targeting DNA, toxins targeting RNA. Examples of proteinaceous toxins are saporin, dianthin, ricin, modeccin, abrin, volkensin, viscumin, shiga toxin, shiga-like toxin, pseudomonas exotoxin (PE, also known as exotoxin A), diphtheria toxin (DT), and cholera toxin. Examples of tubulin filaments-targeting toxins are maytansinoids (e.g. DM1 and DM4), auristatins (e.g. Monomethyl auristatin E (MMAE) and Monomethyl auristatin F (MMAF)), toxoids, tubulysins, cryptophycins, rhizoxin. Examples of DNA-targeting toxins are calicheamicins: N-Acetyl- γ-calicheamicin, CC-1065 analogs, duocarmycins, doxorubicin, methotrexate, benzodiazepines, camptothecin analogues, and anthracyclines. Examples of DNA-targeting toxins are amanitins, spliceostatins, and thailanstatins. A toxin, as used in this invention, is defined as a pharmaceutically active substance that is able to kill or inactivate a cell. Preferably, a targeted toxin is a toxin that is only, or at least predominantly, toxic for target cells but not for off-target cells. The net effect of the targeted toxin is preferably beneficial for the organism as a whole.

An effector molecule or -moiety that is a polypeptide may be, e.g., a polypeptide that recover a lost function, such as for instance enzyme replacement, gene regulating functions, or a toxin. Examples of polypeptides as effector molecules are, e.g., Cas9; toxins (e.g. saporin, dianthin, gelonin, (de)bouganin, agrostin, ricin (toxin A chain); pokeweed antiviral protein, apoptin, diphtheria toxin, pseudomonas exotoxin) metabolic enzymes (e.g. argininosuccinate lyase, argininosuccinate synthetase), enzymes of the coagulation cascade, repairing enzymes; enzymes for cell signaling; cell cycle regulation factors; gene regulating factors (transcription factors such as NF-κB or gene repressors such as methionine repressor).

An effector molecule or an effector moiety that is a polynucleotide may, e.g., be a polynucleotide that comprises coding information, such as a gene or an open reading frame encoding a protein. It may also comprise regulatory information, e.g. promotor or regulatory element binding regions, or sequences coding for micro RNAs. Such polynucleotide may comprise natural and artificial nucleic acids. Artificial nucleic acids include, e.g. peptide nucleic acid (PNA), Morpholino and locked nucleic acid (LNA), as well as glycol nucleic acid (GNA) and threose nucleic acid (TNA). Each of these is distinguished from naturally occurring DNA or RNA by changes to the backbone of the molecule. Examples of nucleotides as effector molecules are, but not limited to, e.g., DNA: single stranded DNA (e.g. DNA for adenine phosphoribosyltransferase); linear double stranded DNA (e.g. clotting factor IX gene); circular double stranded DNA (e.g. plasmids); RNA: mRNA (e.g. TAL effector molecule nucleases), tRNA, rRNA, siRNA, miRNA, antisense RNA; anti-sense oligonucleotides (ASOs, AONs e.g. PNA, PMO, LNA and BNA).

The term “proteinaceous”, used in e.g. “proteinaceous molecule” and “proteinaceous toxin”, are molecules and toxins comprising at least a string of amino acid residues that can be obtained as an

expression product from a single mRNA. Such a molecule or toxin may further comprise any post-translational modifications, a carbohydrate such as an N- or O-linked carbohydrate, disulphide bonds, phosphorylations, sulphatations, etc., as a result of any post-translational modification, and/or may further comprise any other modification such as those resulting from chemical modifications (e.g., linking of effector moieties, saponin, scaffolds, ligands, etc., either directly to e.g. an amino-acid side chain, or via at least one linker (covalently) bound to the molecule for chemically modifying the proteinaceous molecule, and chemically bound (covalently) to the proteinaceous molecule). The term "proteinaceous" also encompasses and includes assemblies of such molecules, e.g. homodimers, heterotrimers, heterohexamers or complex assemblies such as ribosomes.

The terms "specific" and "specifically", in the context of for example "specific binding" and "receptor or molecular target specifically present or expressed at the surface of a tumor cell" and the like, have their normal scientific meaning known in the art, and here refer to e.g. a binding interaction of a first molecule with a second molecule which occurs with a higher affinity relative to any putative binding of the first molecule to a further molecule different from the second molecule, or e.g. to the expression or expression to a higher extent when e.g. the number of receptors or molecular targets is considered, of a cell-surface receptor or molecular target on the surface of a first type of cell such as a tumor cell, autoimmune cell, diseased cell, aberrant cell, relative to the extent of expression of the same receptor or molecular target at a second type of cell such as a healthy cell, etc., wherein expression at the second type of cell can be fully absent or very low, relative to any extent of expression on the tumor cell, etc. Furthermore, the term "specific", for example in "specific binding", has its normal scientific meaning known in the art, and here has the meaning of indicating a molecule that can have an interaction with another molecule with higher binding affinity than background interactions between molecules. Similarly, the term "specificity" refers to an interaction, for example, between two molecules or between a cell and a molecule, which has higher binding affinity than background interactions between molecules. Binding molecules such as immunoglobulins bind via their binding site such as immunoglobulin variable regions of the immunoglobulin, to binding sites on molecules, such as epitopes, cell-surface receptors, etc., with a higher binding affinity than background interactions between molecules. In the context of the invention, background interactions are typically interactions with an affinity lower than a K_D of 10E-4 M. Similarly, "specific binding domains" are domains that preferentially bind to binding sites on molecules, such as epitopes, cell-surface receptors, etc., with a higher binding affinity than background interactions between molecules. In the context of the invention, "background interactions" are typically interactions with an affinity lower than a K_D of 10E-4 M. Preferably, specific binding domains bind with an affinity higher than a K_D of about 10E-5 M.

The term "binding" is defined as interactions between molecules that can be distinguished from background interactions.

Throughout the specification, the term "fragment" refers to an amino acid sequence which is part of a protein domain or which builds up an intact protein domain. Binding fragments according to the invention must have binding specificity for the respective target such as a cell-surface receptor, e.g. on the surface of a diseased cell such as a tumor cell.

The term “ADC” or “antibody-drug conjugate” has its regular scientific meaning known to the skilled person, and here refers to a class of biopharmaceutical drugs designed as a targeted therapy for treating e.g. cancer. Unlike chemotherapy, ADCs are intended to target and kill tumor cells while sparing healthy cells. ADCs are composed of an antibody linked to a biologically active cytotoxic (anticancer) payload or drug. ADCs combine the targeting capabilities of monoclonal antibodies with the cancer-killing ability of cytotoxic drugs. They are designed with the intention to discriminate between healthy cells and diseased tissue such as tumor cells in a tumor.

The term “Saponinum album” has its normal meaning and here refers to a mixture of saponins produced by Merck KGaA (Darmstadt, Germany) containing saponins from *Gypsophila paniculata* and *Gypsophila arostii*, containing SA1657 and mainly SA1641.

The term “Quillajasaponin” has its normal meaning and here refers to the saponin fraction of Quillaja saponaria and thus the source for all other QS saponins, mainly containing QS-18 and QS-21.

“QS-21” or “QS21” has its regular scientific meaning and here refers to a mixture of QS-21 A-apio (~63%), QS-21 A-xylo (~32%), QS-21 B-apio (~3.3%), and QS-21 B-xylo (~1.7%).

Similarly, “QS-21A” has its regular scientific meaning and here refers to a mixture of QS-21 A-apio (~65%) and QS-21 A-xylo (~35%).

Similarly, “QS-21B” has its regular scientific meaning and here refers to a mixture of QS-21 B-apio (~65%) and QS-21 B-xylo (~35%).

The term “Quil-A” refers to a commercially available semi-purified extract from Quillaja saponaria and contains variable quantities of more than 50 distinct saponins, many of which incorporate the triterpene-trisaccharide substructure Gal-(1→2)-[Xyl-(1→3)]-GlcA- at the C-3beta-OH group found in QS-7, QS-17, QS18, and QS-21. The saponins found in Quil-A are listed in van Setten (1995), Table 2 [*Dirk C. van Setten, Gerrit van de Werken, Gijsbert Zomer and Gideon F. A. Kersten, Glycosyl Compositions and Structural Characteristics of the Potential Immuno-adjuvant Active Saponins in the Quillaja saponaria Molina Extract Quil A, RAPID COMMUNICATIONS IN MASS SPECTROMETRY, VOL. 9,660-666 (1995)*]. Quil-A and also Quillajasaponin are fractions of saponins from Quillaja saponaria and both contain a large variety of different saponins with largely overlapping content. The two fractions differ in their specific composition as the two fractions are gained by different purification procedures.

The term “QS1861” and the term “QS1862” refer to QS-7 and QS-7 api. QS1861 has a molecular mass of 1861 Dalton, QS1862 has a molecular mass of 1862 Dalton. QS1862 is described in Fleck et al. (2019) in Table 1, row no. 28 [*Juliane Deise Fleck, Andresa Heemann Betti, Francini Pereira da Silva, Eduardo Artur Troian, Cristina Olivaro, Fernando Ferreira and Simone Gasparin Verza, Saponins from Quillaja saponaria and Quillaja brasiliensis: Particular Chemical Characteristics and Biological Activities, Molecules 2019, 24, 171; doi:10.3390/molecules24010171*]. The described structure is the api-variant QS1862 of QS-7. The molecular mass is 1862 Dalton as this mass is the formal mass including proton at the glucuronic acid. At neutral pH, the molecule is deprotonated. When measuring in mass spectrometry in negative ion mode, the measured mass is 1861 Dalton.

The terms first, second, third and the like in the description and in the claims, are used for distinguishing between similar elements and not necessarily for describing a sequential or chronological

order. The terms are interchangeable under appropriate circumstances. The embodiments of the invention can operate in other sequences than described or illustrated herein.

Furthermore, the various embodiments, although referred to as “preferred” or “*e.g.*” or “for example” or “in particular” are to be construed as exemplary manners in which the invention may be implemented rather than as limiting the scope of the invention.

The term “comprising”, used in the claims, should not be interpreted as being restricted to the elements or steps listed thereafter; it does not exclude other elements or steps. It needs to be interpreted as specifying the presence of the stated features, integers, steps or components as referred to, but does not preclude the presence or addition of one or more other features, integers, steps or components, or groups thereof. Thus, the scope of the expression “a pharmaceutical composition comprising A and B” should not be limited to a pharmaceutical composition consisting only of components A and B, rather with respect to the present invention, the only enumerated components of the pharmaceutical composition are A and B, and further the claim should be interpreted as including equivalents of those components. Similarly, the scope of the expression “a method comprising step A and step B” should not be limited to a method consisting only of steps A and B, rather with respect to the present invention, the only enumerated steps of the method are A and B, and further the claim should be interpreted as including equivalents of those steps.

In addition, reference to a feature by the indefinite article "a" or "an" does not exclude the possibility that more than one of the features such as for example a component, excipient, saponin, etc. are present, unless the context clearly requires that there is one and only one of the features. The indefinite article "a" or "an" thus usually means "at least one".

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1-1: *in vivo* HSP27 expression in A431 xenograph ‘nude’ mouse tumor model treated with 30mg/kg cetuximab-Cys-(SO1861-L-trifunctional linker-L-HSP27BNA), 25 mg/kg Cetuximab-(Lys-L-HSP27BNA)^4 or 25 mg/kg Cetuximab-(Cys-L-SO1861).

Figure 2-1: *in vitro* enhanced HSP27 gene silencing in EGFR expressing A431 cells by treatment with cetuximab-Cys-(SO1861-L-trifunctional linker-L-HSP27BNA), Cetuximab-(Lys-L-HSP27BNA)^4 or Cetuximab-(Cys-L-SO1861).

Figure 3-1: The legends and axes for Figures A, B, C and D are the same. **A.** cell killing activity in EGFR expressing cells (MDA-MB-468) by cetuximab, cetuxamib + 10 pM cetuximab-saporin, cetuximab-Cys-(dendron(-L-SO1861)^4)^{3,9} and cetuximab-Cys-(dendron(-L-SO1861)^4)^{3,9} + 10pM cetuximab-saporin. **B.** cell killing activity in HER2 expressing cells (SK-BR-3) by trastuzumab, trastuzumab + 50 pM trastuzumab-saporin, Trastuzumab-Cys-(dendron(-L-SO1861)^4)^4 and Trastuzumab-Cys-(dendron(-L-SO1861)^4)^4 + 50 pM trastuzumab-saporin. **C.** cell killing activity in EGFR expressing cells (HeLa) by cetuximab, cetuxamib + 10 pM cetuximab-saporin, cetuximab-Cys-(dendron(-L-SO1861)^4)^{3,9} and cetuximab-Cys-(dendron(-L-SO1861)^4)^{3,9} + 10pM cetuximab-saporin. **D.** cell killing activity in HER2 expressing cells (JIMT-1) by trastuzumab, trastuzumab + 50 pM trastuzumab-saporin, Trastuzumab-Cys-(dendron(-L-SO1861)^4)^4 and Trastuzumab-Cys-(dendron(-L-SO1861)^4)^4 + 50 pM trastuzumab-saporin.

Figure 4-1: The legends and axes for Figures A, B, C and D are the same. **A.** cell killing activity in $EGFR^{++}/CD71^{+}$ cells (MDA-MB-468) of cetuximab, cetuximab + 10 pM CD71mab-saporin , Cetuximab-Cys-$(dendron(\text{-L-SO1861})^{4})^{3,9}$, Cetuximab-Cys-$(dendron(\text{-L-SO1861})^{4})^{3,9}$ + 10 pM CD71mab-saporin , Cetuximab-Lys-$(dendron(\text{-L-SO1861})^{4})^{4,4}$ or Cetuximab-Lys-$(dendron(\text{-L-SO1861})^{4})^{4,4}$ + 10 pM CD71mab-saporin. **B.** cell killing activity in $HER2^{++}/CD71^{+}$ (SK-BR-3) cell lines of trastuzumab, trastuzumab + 10 pM CD71mab-saporin , trastuzumab-Cys-$(dendron(\text{-L-SO1861})^{4})^{4}$, trastuzumab-Cys-$(dendron(\text{-L-SO1861})^{4})^{4}$ + 10 pM CD71mab-saporin , trastuzumab-Lys-$(dendron(\text{-L-SO1861})^{4})^{4,7}$ or trastuzumab-Lys-$(dendron(\text{-L-SO1861})^{4})^{4,7}$ + 10 pM CD71mab-saporin. **C.** cell killing activity in $EGFR^{+}/CD71^{+}$ cells (CaSki) of cetuximab, cetuximab + 10 pM CD71mab-saporin , Cetuximab-Cys-$(dendron(\text{-L-SO1861})^{4})^{3,9}$, Cetuximab-Cys-$(dendron(\text{-L-SO1861})^{4})^{3,9}$ + 10 pM CD71mab-saporin , Cetuximab-Lys-$(dendron(\text{-L-SO1861})^{4})^{4,4}$ or Cetuximab-Lys-$(dendron(\text{-L-SO1861})^{4})^{4,4}$ + 10 pM CD71mab-saporin. **D.** cell killing activity in $HER2^{+/-}/CD71^{+}$ cells (JIMT-1) of trastuzumab, trastuzumab + 10 pM CD71mab-saporin , trastuzumab-Cys-$(dendron(\text{-L-SO1861})^{4})^{4}$, trastuzumab-Cys-$(dendron(\text{-L-SO1861})^{4})^{4}$ + 10 pM CD71mab-saporin , trastuzumab-Lys-$(dendron(\text{-L-SO1861})^{4})^{4,7}$ or trastuzumab-Lys-$(dendron(\text{-L-SO1861})^{4})^{4,7}$ + 10 pM CD71mab-saporin.

Figure 5-1: cell killing activity in HER2 expressing cells (SK-BR-3) of T-DM1, T-DM1 + 25.6 nM trastuzumab or T-DM1 + 5.3 nM trastuzumab-Cys-$(dendron(\text{-L-SO1861})^{4})^{4}$.

Figure 6-1: HSP27 gene silencing activity of HSP27BNA-$dendron(\text{-L-SO1861})^{4}$ compared to the HSP27BNA alone.

Figure 7-1: Schematic representation of release of SO1861 from $dendron(\text{-L-SO1861})^{4}$ under acidic conditions.

Figure 8-1: The legends and axes for Figures A and B are the same. **A.** cell killing activity in EGFR expressing A431 cells by the 'naked' dendron ($Dendron(NEM)^{4}$), $Dendron(NEM)^{4}$ + 10 pM EGFdianthin, $dendron(\text{-L-SO1861})^{4}$ or $dendron(\text{L-SO1861})^{4}$ + 10 pM EGFdianthin. **B.** cell killing activity in EGFR expressing HeLa cells by the 'naked' dendron ($Dendron(NEM)^{4}$), $Dendron(NEM)^{4}$ + 10 pM EGFdianthin, $dendron(\text{-L-SO1861})^{4}$ or $dendron(\text{L-SO1861})^{4}$ + 10 pM EGFdianthin.

Figure 9-1: The legends and axes for Figures A, B and C are the same. **A.** Effect of trastuzumab on the cell viability of a range of cancer cells. **B.** Effect of cetuximab on the cell viability of a range of cancer cells. **C.** Effect of T-DM1 on the cell viability of a range of cancer cells.

Figure 10-1: Schematic representation of the monoclonal antibody-(SO1861-scaffold-antisense BNA oligo) conjugate.

Figure 11-1: Schematic representation of the 1-target 2-component system using a monoclonal antibody bound to a toxin and the same monoclonal antibody bound to a scaffold comprising saponin.

Figure 12-1: Schematic representation of the 2-target 2-component system using a monoclonal antibody bound to a toxin and a different monoclonal antibody with a different target bound to a scaffold comprising a saponin.

Figure 13-1: Schematic representation of $Dendron(\text{-L-SO1861})^{4}$.

Figure 14-1: Schematic representation of $Dendron(\text{-L-SO1861})^{8}$.

Figure 15-1: Schematic representation of SO1861-L-trifunctional linker-L-HSP27BNA.

Figure 16-1: Reaction scheme of the synthesis of the $Dendron(SO1861)^{4}$-HSP27BNA oligo conjugate.

Figure 17-1: Model scaffold consisting of four molecular arms for saponin binding via a Schiff base (imine) and one arm for click chemistry. The polymeric structure is a pentavalent polyethylene glycol-based dendrimer of the first generation.

Figure 18-1: Cell viability of HER14 cells after treatment with a pentameric dendrimer (pentrimer), the pentrimer in the presence of SA1641, dianthin-EGF, dianthin-EFG in the presence of SA1641, the pentrimer in presence of dianthin-EGF, and the pentrimer in presence of dianthin-EGF as well as SA1641.

Figure 19-1: SO1861 structure with highlighted chemical groups for conjugation of endosomal escape enhancing saponins to a polymeric structure. Highlighted groups are aldehyde (black circle), carboxylic acid (dashed circle), alkene (dashed pentagon), and alcohol (dashed box).

Figure 20-1: **A.** Standard molecular structure of SO-1861-EMCH conjugate. Maleimide group is marked with a circle. **B.** 3D model of SO1861-EMCH conjugate. Maleimide group is marked with a circle.

Figure 21-1: Reaction scheme for the generation of poly(SO1861) using SO1861-EMCH as monomer, the APS / TMEDA system as polymerization initiator, and aminopropanethiol as radical quencher.

Figure 22-1: Schematic representation of the DNA approach. Usage of the principle of DNA-origami to generate a DNA based scaffold that is able to conjugate and release glycoside molecules. In addition, one of the DNA strands obtains a click chemistry moiety that can be used for conjugation to a targeted toxin to form a functionalized scaffold. bp: base pair.

Figure 23-1: Schematic representation of the poly(peptide-SO1861) approach. Usage of a peptide sequence that can conjugate and release glycoside molecules and which can react with itself to form a poly(peptide-SO1861) construct. The poly(peptide) chain endings can be further modified with click chemistry moieties (e.g., BCN-NHS linker) that can be used for conjugation to a toxin.

Figure 24-1: Molecular structure of G4-dendron with protected amino groups.

Figure 25-1: Schematic representation of a basic scaffold with click chemistry function to link any desired effector molecule.

Figure 26-1: Schematic representation of a functionalized scaffold with pre-bound effector molecule and click chemistry function to link any desired ligand. Optionally, a pH-sensitive linkage can be provided to release the effector molecule from the scaffold after reaching the endosomes.

Figure 1-2. *Antibody-protein toxin + unconjugated SO1861 vivo study.* BT474 tumor bearing mice treated with various concentrations of Trastuzumab-saporin (i.v.) + 1.5 mg/kg unconjugated SO1861 (subQ injection 1 hour before trastuzumab-saporin treatment).

Figure 2-2. *unconjugated saponin-mediated endosomal escape and target cell killing enhancement.* A) Cell viability analyses of HeLa cells ($EGFR^+$) treated with SO1861, SO1832, SO1862 (isomer of SO1861) or SO1904 with or without 1.5 pM EGFdianthin B) Cell viability analyses of HeLa cells ($EGFR^+$) treated with EGFdianthin and fixed concentrations of SO1861, SO1832, SO1862 (isomer of SO1861) or SO1904. C) Cell viability analyses of HeLa cells ($EGFR^+$) treated with SO1861 or GE1741 with or without 1.5 pM EGFdianthin. D) Cell viability analyses of HeLa cells ($EGFR^+$) treated with various QSmix (saponin mixture from Quillaia Saponaria) with or without 1.5 pM EGFdianthin.

Figure 3-2. *unconjugated SO1861 versus SO1861-EMCH activity. EGFR targeted antisense BNA oligo delivery and gene silencing in cancer cells, according to the invention.* A, B, C) Cell viability analyses of

A431 ($EGFR^{++}$), HeLa ($EGFR^{+}$) or A2058 ($EGFR^{-}$) cells treated with SO1861 or SO1861-EMCH with or without 1.5 pM EG dianthin. D, E) Cell viability analyses of A431 ($EGFR^{++}$) or HeLa ($EGFR^{+}$) cells treated with SO1861 or SO1861-N3 with or without 1.5 pM EGFdianthin.

Figure 4-2. *unconjugated SO1861 versus SO1861-EMCH (labile) versus SO1861-S (stable).* Cell viability analyses of HeLa cells ($EGFR^{+}$) treated with SO1861, SO1861-S (S=HATU, stable linker) and SO1861-EMCH (labile linker) with or without EGFdiantin.

Figure 5-2. *EGFR targeted antisense BNA oligonucleotide delivery and gene silencing.* HSP27 mRNA expression analyses of A431 ($EGFR^{++}$) and A2058 ($EGFR^{-}$) cells treated with cetuximab-(Cys-L-SO1861)$^{3.9}$ or cetuximab-(Cys-L-SO1861)$^{3.9}$ + 100nM HSP27BNA.

Figure 6-2. *Tumor targeted antisense BNA oligo nucleotide delivery and gene silencing in tumor bearing mice*. Mice treated with HSP27BNA + cetuximab-(Cys-L-SO1861)^{3,9} in A431 tumor bearing mice reveals efficient tumor targeted gene silencing, compared to the controls.

Figure 1-3: HSP27BNA gene silencing activity of HSP27BNA, HSP27BNA-SO1861 and HSP27BNA-dendron-(SO1861)^{4} in A431 cancer cell lines.

Figure 1-4: *Tumor targeted protein toxin delivery results in tumor volume reduction and tumor growth inhibition, in tumor bearing mice***. A.** Dose escalation (intraperitoneal, i.p.) of cetuximab-(Cys-L-SO1861)^{3,9}(Lys-S-dianthin)^{2} in A431 tumor bearing mice reveals tumor volume reduction, compared to the control. **B.** Dose escalation intraperitoneal, i.p, of cetuximab-(Cys-L-SO1861)^{3,9}(Lys-L-dianthin)^{2} in A431 tumor bearing mice reveals tumor growth reduction, compared to the controls. **C.** Dose escalation intravenous, i.v., of cetuximab-(Cys-L-SO1861)^{3,9}(Lys-L-dianthin)^{2} in A431 tumor bearing mice reveals tumor growth reduction, compared to the controls.

Figure 2-4: *Tumor targeted antisense BNA oligo nucleotide delivery and gene silencing in tumor bearing mice***.** 30 mg/kg cetuximab-(Cys-L-SO1861)^{3,9}(Lys-L-HSP27BNA)^{1,8} in A431 tumor bearing mice reveals induced efficient tumor targeted gene silencing, compared to the controls.

Figure 3-4: *Tumor targeted antisense BNA oligo nucleotide delivery and gene silencing in tumor bearing mice***.** 30 mg/kg cetuximab-Cys-(SO1861-L-trifunctional linker-L-HSP27BNA)^{3,7} in A431 tumor bearing mice reveals induced efficient tumor targeted gene silencing, compared to the controls.

Figure 4-4: *HER2 or EGFR targeted protein toxin delivery and cell killing in cancer cells, according to the invention.* **A**. Trastuzumab-(Cys-L-SO1861)^{3,8}(Lys-L-dianthin)^{1,7} or Trastuzumab-(Cys-L-SO1861)^{3,8}(Lys-S-dianthin)^{1,7} treatment and controls on SK-BR-3 cells ($HER2^{++}$) **B.** Trastuzumab-(Cys-L-SO1861)^{3,8}(Lys-L-dianthin)^{1,7} or Trastuzumab-(Cys-L-SO1861)^{3,8}(Lys-S-dianthin)^{1,7} treatment and controls on MDA-MB-468 cells ($HER2^{-}$). **C.** Cetuximab-(Cys-L-SO1861)^{3,8}(Lys-L-dianthin)^{1,7} or Cetuximab-(Cys-L-SO1861)^{3,8}(Lys-S-dianthin)^{1,7} treatment and controls on A431 cells ($EGFR^{++}$) **D.** Cetuximab-(Cys-L-SO1861)^{3,8}(Lys-L-dianthin)^{1,7} or Cetuximab-(Cys-L-SO1861)^{3,8}(Lys-S-dianthin)^{1,7} treatment and controls onA2058 cells ($EGFR^{-}$).

Figure 5-4: *EGFR targeted antisense BNA oligo delivery and gene silencing in cancer cells, according to the invention.* **A.** Cetuximab-(Cys-L-SO1861)^{3,8}(Lys-L-HSP27BNA)^{1,7} treatment and controls on A431 cells ($EGFR^{++}$) **B.** Cetuximab-(Cys-L-SO1861)^{3,8}(Lys-L-HSP27BNA)^{1,7} treatment and controls onA2058 cells ($EGFR^{-}$).

Figure 6-4: *HER2 targeted antisense BNA oligo delivery and gene silencing in cancer cells, according to the invention.* Trastuzumab-(Cys-L-SO1861)^{3,8}(Lys-L-HSP27BNA)^{3,5} treatment and controls on SK-BR-3 cells (HER2^{++}).

Figure 7-4: *EGFR targeted antisense BNA oligo delivery and gene silencing in cancer cells, according to the invention.* **A.** Cetuximab-Cys-(SO1861-L-trifunctional linker-L-HSP27BNA)^{3,7} treatment and controls on A431 cells (EGFR^{++}). **B.** Cetuximab-Cys-(SO1861-L-trifunctional linker-L-HSP27BNA)^{3,7} treatment and controls on A2058 cells (EGFR^{-}).

Figure 8-4: *Control treatments on all cell lines*. Cell viability when trastuzumab (**A**), cetuximab (**B**), T-DM1, (**C**) free toxins: saporin and dianthin (**D**) or saporin coupled to a non-cell binding IgG (**D**) are used as treatment on the indicated cell lines SK-BR-3, JIMT-1, MDA-MB-468, A431, CaSki, HeLa, A2058, BT-474.

Figure 9-4: (S)n – (L)(E) concept: *mAb-(SO1861)^{n}(protein toxin)^{n}.* Both, SO1861 at the cysteine residues (Cys) and protein toxin (ribosomal inactivating protein) at the lysine residues are conjugated to the same antibody (mAb) for delivery and internalization into the target cells. 1) mAb-(Cys-L-SO1861)^{4}(Lys-protein toxin)^{2} bind to its corresponding cell surface receptor, 2) receptor-mediated endocytosis the conjugate occurs, 3) at low endolysosomal pH and appropriate concentration, SO1861 becomes active to enable endolysosomal escape, 4) release of toxin into cytoplasm occurs and 5) toxin induces cell death.

Figure 10-4: (S)n – (L)(E) concept: *mAb-(SO1861)^{n}(antisense BNA oligo)^{n}.* Both, SO1861, at the cysteine residues (Cys) and the antisense BNA oligo nucleotide, at the lysine residues are conjugated to the same antibody (mAb) for delivery and internalization into the target cells. 1) mAb-(Cys-SO1861)^{4}(Lys-BNAoligo)^{2} bind to its corresponding cell surface receptor, 2) receptor-mediated endocytosis of both conjugates occurs, 3) at low endolysosomal pH and appropriate concentration, SO1861 becomes active to enable endolysosomal escape, 4) release of BNA oligo into cytoplasm occurs and 5) target gene silencing is induced.

Figure 11-4: (S)n – (L)(E) concept: *mAb-(SO1861-scaffold-antisense BNA oligo)^{n}.* the (SO1861-trifunctional linker-BNAoligo)^{n} is conjugated to an antibody (mAb) for delivery and internalization into the target cells. 1) mAb-(SO1861-trifunctional linker-BNAoligo)^{4} binds to its corresponding cell surface receptor, 2) receptor-mediated endocytosis of both conjugates occurs, 3) at low endolysosomal pH and appropriate concentration, SO1861 becomes active to enable endolysosomal escape, 4) release of BNA oligo into cytoplasm occurs and 5) target gene silencing is induced.

Figure 12-4: *Antibody-SO1861 conjugation procedure*. Shown is the coupling reaction of the linking of four moieties of a plant-derived saponin SO1861 to the four cysteines in the light chain of an antibody. First, the disulphide bonds in the IgG are disrupted under influence of exposure to TCEP (Tris(2-carboxyethyl)phosphine); second, the saponin SO1861 comprising a chemical linker bound to it, is added together with trifluoro acetic acid, and four saponin moieties are linked to the IgG. For producing cleavable 'ready to conjugate' saponins the aldehyde group of SO1861 was reacted with an EMCH (ε-maleimidocaproic acid hydrazide) linker. The hydrazide group of EMCH forms an acid cleavable hydrazone bond with the aldehyde of SO1861. At the same time the EMCH linker presents a maleimide group that is thiol (sulfhydryl group) reactive and thus can be conjugated to thiols of the IgG, i.e. the

ligand moiety. Herewith, an endosomal escape enhancing conjugate of the invention is provided, and/or a first binding molecule of the invention is provided.

Figure 1-5: *1T2C in vivo activity.* The 1T2C combination of 50mg/kg cetuximab-(Cys-L-SO1861)^4 + 25 mg/kg cetuximab-(-L-HSP27BNA)^4 in A431 tumor bearing mice reveals strong tumor targeted gene silencing, compared to the controls.

Figure 2-5: *1T2C in vivo activity.* The 1T2C combination of 40 mg/kg trastuzumab-(Cys-L-SO1861)^4 + 0.02/0.03 mg/kg trastuzumab-saporin in a PDX tumor mouse model (high HER2 expression) shows effective tumor growth inhibition.

Figure 3-5: *1-target 2-component.* EGFR targeted cell killing in A431 cells (EGFR^{++}) (A, C) and CaSKi cells (EGFR^{+}) (B, D) by a therapeutic combination according to the invention. A, B) Cetuximab-(Cys-L-SO1861)^{3,7} titration + fixed concentration 10 pM cetuximab-saporin and controls on A431 (A) and CaSKi (B) cells. C, D) Cetuximab-saporin titration + fixed concentration of 75nM cetuximab-(Cys-L-SO1861)^{3,7} and controls on A431 (C) and CaSKi (D) cells. Remark: For target receptor expression data of each cell line (determined by FACS analysis) see table 19.

Figure 4-5: *1-target 2-component.* EGFR targeted cell killing in HeLa cells (EGFR$^{+/-}$) (A, C) and A2058 cells (EGFR^{-}) (B, D) by a therapeutic combination according to the invention. A, B) Cetuximab-(Cys-L-SO1861)^{3,7} titration + fixed concentration 10 pM cetuximab-saporin and controls on HeLa (A) and CaSKi (B) cells. C, D) Cetuximab-saporin titration + fixed concentration of 75nM cetuximab-(Cys-L-SO1861)^{3,7} and controls on Hela (C) and A2058 (D) cells. Remark: For target receptor expression data of each cell line (determined by FACS analysis) see table 19.

Fig5-5: *1-target 2-component.* HER2 targeted cell killing in SKBR3 cells (HER2^{++}) (A, B) by a therapeutic combination according to the invention. A) Trastuzumab-(Cys-L-SO1861)^4 titration + fixed concentration 50 pM trastuzumab-saporin and controls on SKBR3 cells. B) Trastuzumab-saporin titration + fixed concentration of 2.5nM trastuzumab-(Cys-L-SO1861)^4 and controls on SKBR3 cells. Remark: For target receptor expression data of each cell line (determined by FACS analysis) see table 19.

Figure 6-5: *1-target 2-component.* HER2 targeted cell killing in JIMT-1 cells (HER2$^{+/-}$) (A, C) and MDA-MB-468 cells (HER2^{-}) (B, D) by a therapeutic combination according to the invention. A, B) Trastuzumab-(Cys-L-SO1861)^4 titration + fixed concentration of 50 pM trastuzumab-saporin and controls on JIMT-1 (A) and MDA-MB-468 (B) cells. C, D) Trastuzumab-saporin titration + fixed concentration of 2.5nM trastuzumab-(Cys-L-SO1861)^4 and controls on JIMT-1 (C) and MDA-MB-468 (D) cells. Remark: For target receptor expression data of each cell line (determined by FACS analysis) see table 19.

Figure 7-5: *Chloroquine inhibits the 1-target 2-component.* HER2 and EGFR targeted cell killing in SK-BR-3 (HER2^{++}) and A431 cells (EGFR^{++}), by a therapeutic combination according to the invention + chloroquine. A) Trastuzumab-saporin titration + fixed concentration of 5nM trastuzumab-(Cys-L-SO1861)^4 + 0.5 µM chloroquine and control on SK-BR-3 cells. B) Cetuximab-saporin titration + fixed concentration of 5 nM cetuximab-(Cys-L-SO1861)^{3,8} + 0.5 µM chloroquine and control on A431 cells. Remark: For target receptor expression data of each cell line (determined by FACS analysis) see table 19.

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Figure 8-5: *1-target 2-component.* EGFR targeted gene silencing in A431 cells ($EGFR^{++}$) and A2058 cells ($EGFR^{-}$) by a therapeutic combination according to the invention. A,B) Cetuximab-(Cys-L-SO1861)^{3,8} titration + fixed concentration of 100 nM Cetuximab-(Lys-L-HSP27BNA)^{4} and control on A431 cells (A) and A2058 cells (B). C, D) Cetuximab-(Lys-L-HSP27BNA)^{4} titration + fixed concentration of 77 nM Cetuximab-(Cys-L-SO1861)^{3,8} and control on A431 cells (C) and A2058 cells (D). Remark: For target receptor expression data of each cell line (determined by FACS analysis) see table 19.

Figure 9-5: *2-target 2-component.* A) EGFR and HER2 targeted cell killing in MDA-MB-468 cells ($EGFR^{++}$) and HeLa cells ($EGFR^{+/-}$) and HER2 targeted cell killing in SK-BR-3 cells ($HER2^{++}$) and JIMT-1 cells ($HER2^{+/-}$) by a therapeutic combination according to the invention. A) Cetuximab-Cys-(dendron(-L-SO1861)^{4})^{3,9} titration + fixed concentration 10 pM cetuximab-saporin and controls on MDA-MB-468 cells (A) and HeLa cells (B). C,D) Trastuzumab-Cys-(dendron(-L-SO1861)^{4})^{4} titration + fixed concentration 50 pM trastuzumab-saporin and controls on SK-BR-3 cells (C) and JIMT-1 cells (D). Remark: For target receptor expression data of each cell line (determined by FACS analysis) see table 19.

Figure 10-5: *1-target 2-component.* SK-BR-3 cells ($HER2^{+/-}$) can efficiently be killed with the therapeutic combination according to the invention, Tratuzumab-saporin + 2.5 nM trastuzumab-(Cys-L-SO1861)^{4}, however titration of T-DM1 + 2.5 nM trastuzumab-(Cys-L-SO1861)^{4} is not effective at such low toxin concentrations. T-DM1 is Trastuzumab-emtansine (Kadcyla®), carrying ~3.5 emtansine (DM1) toxin molecules per antibody (DAR3.5). Remark: For target receptor expression data of each cell line (determined by FACS analysis) see table 19.

Figure 11-5: *Control treatments on all cell lines.* A-D) Cell viability when trastuzumab (A), cetuximab (B), T-DM1, (C) free toxins: saporin and dianthin (D) or saporin coupled to a non-cell binding IgG (D) are treated with the indicated cell lines SK-BR-3, JIMT-1, MDA-MB-468, A431, CaSki, HeLa, A2058, BT-474. Remark: For target receptor expression data of each cell line (determined by FACS analysis) see table 19.

Figure 12-5: *1-target 2-component.* EGFR targeted cell killing in A431 cells ($EGFR^{++}$) (A) and CaSKi cells ($EGFR^{+}$) (B) and A2058 cells ($EGFR^{-}$) by a therapeutic combination according to the invention. A, B, C) Cetuximab-(Cys-L-QSmix)^{4,1} titration + fixed concentration 10 pM cetuximab-saporin or 10pM cetuximab-dianthin and controls in A431 cells (A), CaSKi cells (B) and A2058 cells (C). QSmix is a mixture of saponins from an extract Quillaja Saponaria. Remark: For target receptor expression data of each cell line (determined by FACS analysis) see table 19.

Figure 13-5: *1-target 2-component concept: mAb1-SO1861 + mAb1-protein toxin.* SO1861 and toxin (ribosomal inactivating protein) are each, independently, conjugated to an antibody (mAb1) for delivery and internalization into target cells. 1) mAb1-SO1861 and mAb1-protein toxin bind to the cell surface receptor, 2) receptor-mediated endocytosis of both conjugates occurs, 3) at low endolysosomal pH and appropriate concentration, SO1861 becomes active to enable endolysosomal escape, 4) release of toxin into cytoplasm occurs and 5) toxin induces cell death

Figure 14-5: *1-target 2-component concept: mAb1-SO1861 + mAb2-BNA oligo.* SO1861 and antisense BNA oligo nucleotide are each, independently, conjugated to an antibody (mAb1) for delivery and internalization into target cells. 1) mAb1-SO1861 and mAb1-BNAoligo bind to the cell surface receptor,

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2) receptor-mediated endocytosis of both conjugates occurs, 3) at low endolysosomal pH and appropriate concentration, SO1861 becomes active to enable endolysosomal escape, 4) release of BNA oligo into cytoplasm occurs and 5) target gene silencing.

Figure 15-5: *1-target 2-component concept: mAb1-(scaffold(-SO1861)^n)^n + mAb1-protein toxin.* Dendron(-SO1861)^n and protein toxin (ribosomal inactivating protein) are each, independently, conjugated to an antibody (mAb1) for delivery and internalization into target cells. 1) mAb1-dendron(-SO1861)^4 and mAb1-protein toxin bind to the cell surface receptor, 2) receptor-mediated endocytosis of both conjugates occurs, 3) at low endolysosomal pH and appropriate concentration, SO1861 becomes active to enable endolysosomal escape, 4) release of toxin into cytoplasm occurs and 5) toxin induces cell death.

Figure 1-6: The 2T2 component system tested in A431 tumor bearing mice model reveals tumor regression.

Figure 2-6: *The 2T2 component system tested in A431 tumor bearing mice model reveals tumor regression and eradication.*

Figure 3-6: *2-target 2-component.* EGFR/HER2 targeted cell killing in A431 cells (EGFR^{++}/HER2$^{+/-}$) (A, C) and CaSKi cells (EGFR^{++}/HER2$^{+/-}$) (B, D) by a therapeutic combination according to the invention. A, B) Cetuximab-(Cys-L-SO1861)^{3,7} titration + fixed concentration 50 pM trastuzumab-saporin and controls on A431 cells. C, D) Trastuzumab-saporin titration + fixed concentration of 75nM cetuximab-(Cys-L-SO1861)^{3,7} and controls on Caski cells. The legends and/or axes are the same for all the A,B, C or D.

Figure 4-6: *2-target 2-component.* EGFR/HER2 targeted cell killing in HeLa cells (EGFR$^{+/-}$/HER2$^{+/-}$) (A, C) and A2058 cells (EGFR^{-}/HER2$^{+/-}$) (B, D) by a therapeutic combination according to the invention. A, B) Cetuximab-(Cys-L-SO1861)^{3,7} titration + fixed concentration 50 pM trastuzumab-saporin and controls on HeLa cells. C, D) Trastuzumab-saporin titration + fixed concentration of 75nM cetuximab-(Cys-L-SO1861)^{3,7} and controls on A2058 cells. The legends and/or axes are the same for all the A,B, C or D.

Figure 5-6: *2-target 2-component.* HER2/EGFR targeted cell killing in SKBR3 cells (HER2^{++}/EGFR$^{+/-}$) (A, B) by a therapeutic combination according to the invention. A Trastuzumab-(Cys-L-SO1861)^4 titration + fixed concentration 1.5 pM EGFdianthin and controls on SKBR3 cells. B) EGFdianthin titration + fixed concentration of 2.5nM trastuzumab-(Cys-L-SO1861)^4 and controls on SKBR3 cells.

Figure 6-6: *2-target 2-component.* HER2/EGFR targeted cell killing in JIMT-1 cells (HER2$^{+/-}$EGFR$^{+/-}$) (A, C) and MDA-MB-468 cells (HER2^{-}/EGFR^{++}) (B, D) by a therapeutic combination according to the invention. A, B) Trastuzumab-(Cys-L-SO1861)^4 titration + fixed concentration 1.5 pM EGFdianthin and controls on JIMT-1 cells. C, D) EGFdianthin titration + fixed concentration of 2.5nM trastuzumab-(Cys-L-SO1861)^4 and controls on MDA-MB-468 cells. The legends and/or axes are the same for all the A,B, C or D.

Figure 7-6: *2-target 2-component.* HER2/EGFR targeted cell killing in SKBR3 cells (HER2^{++}/EGFR$^{+/-}$) (A, B) by a therapeutic combination according to the invention. A) Trastuzumab-(Cys-L-SO1861)^4 titration + fixed concentration 10 pM cetuximab-saporin and controls on SKBR3 cells. B) Cetuximab-saporin titration + fixed concentration of 2.5nM trastuzumab-(Cys-L-SO1861)^4 and controls on SKBR3 cells.

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Figure 8-6: *2-target 2-component.* HER2/EGFR targeted cell killing in JIMT-1 cells ($HER2^{+/-}EGFR^{+/-}$) (A, C) and MDA-MB-468 cells ($HER2^{-}/EGFR^{++}$) (B, D) by a therapeutic combination according to the invention. A, B) Trastuzumab-(Cys-L-SO1861)^4 titration + fixed concentration 10 pM cetuximab-saporin and controls on JIMT-1 cells. C, D) Cetuximab-saporin titration + fixed concentration of 2.5nM trastuzumab-(Cys-L-SO1861)^4 and controls on MDA-MB-468 cells. The legends and/or axes are the same for all the A,B, C or D.

Figure 9-6: *Chloroquine inhibits the 2-target 2-component.* EGFR/HER2, EGFR/CD71 or HER2/CD71 targeted cell killing in A431 cells ($EGFR^{++}/HER2^{+/-}/CD71^{+}$) (A, B), MDA-MB-468 cells ($EGFR^{++}/HER2^{-}/CD71^{+}$) (C) or SK-BR-3 ($HER2^{++}/EGFR^{+/-}/CD71^{+}$) (D) by a therapeutic combination according to the invention + chloroquine. A) Trastuzumab-dianthin or trastuzumab-saporin titration + fixed concentration of 75nM cetuximab-(Cys-L-SO1861)^{3,9} + 800 nM chloroquine and controls on A431 cells. B) CD71mab-saporin titration + fixed concentration of 10.5 nM cetuximab-(Cys-L-SO1861)^{3,9} + 500 nM chloroquine and control on A431 cells. C) CD71mab-saporin titration + fixed concentration of 10.5 nM cetuximab-(Cys-L-SO1861)^{3,9} + 500 nM chloroquine and control on MDA-MB-468 cells. D) CD71mab-saporin titration + fixed concentration of 5 nM trastuzumab-(Cys-L-SO1861)^{3,9} + 500 nM chloroquine and control on SK-BR-3 cells.

Figure 10-6: *2-target 2-component.* EGFR/HER2 targeted gene silencing in A431 cells ($EGFR^{++}/HER2^{+/-}$) (A) and A2058 cells ($EGFR^{-}/HER2^{+/-}$) (B) by a therapeutic combination according to the invention. A) Cetuximab-(Cys-L-SO1861)^{3,9} titration + fixed concentration of 100 nM trastuzumab-(Lys-L-HSP27BNA)^{4,4} and control on A431 cells (A) and A2058 cells (B) . C, D) Trastuzumab-(Lys-L-HSP27BNA)^{4,4} titration + fixed concentration of 77nM cetuximab-(Cys-L-SO1861)^{3,9} and controls on A431 cells (A) and A2058 cells (B). The legends and/or axes are the same for all the A,B, C or D.

Figure 11-6: *2-target 2-component.* A) EGFR/CD71 or HER2/CD71 targeted cell killing in MDA-MB-468 cells ($EGFR^{++}/CD71^{+}$) (A) HeLa cells ($EGFR^{+/-}$/ $CD71^{+}$), SK-BR-3 cells ($HER2^{++}/CD71^{+}$) (B) and JIMT-1 cells ($HER2^{+/-}/CD71^{+}$) by a therapeutic combination according to the invention. A) Cetuximab-Cys-(dendron(-L-SO1861)^4)^{3,9} titration + fixed concentration 10 pM CD71mab-saporin and controls on MDA-MB-468 cells. B) A) Cetuximab-Cys-(dendron(-L-SO1861)^4)^{3,9} titration + fixed concentration 10 pM CD71mab-saporin and controls on HeLa cells. C) Trastuzumab-Cys-(dendron(-L-SO1861)^4)^4 titration + fixed concentration 10 pM CD71mab-saporin and controls on SK-BR-3 cells. D) Trastuzumab-Cys-(dendron(-L-SO1861)^4)^4 titration + fixed concentration 10 pM CD71mab-saporin and controls on JIMT-1 cells.

Figure 12-6: *2-target 2-component versus T-DM1.* A431 cells ($EGFR^{++}/HER2^{+/-}$) can efficiently be killed with the therapeutic combination according to the invention, Tratuzumab-saporin + 75 nM cetuximab-(Cys-L-SO1861)^{3,9}, however titration of T-DM1 + 75 nM cetuximab-(Cys-L-SO1861)^{3,9} is not effective at such low toxin concentrations. T-DM1 is Trastuzumab-emtansine (Kadcyla®), carrying ~3.5 emtansine (DM1) toxin molecules per antibody.

Figure 13-6: *2-target 2-component.* EGFR/CD71 and EGFR/HER2 targeted cell killing in A431 cells ($EGFR^{+++}/HER2^{+/-}$) (A) and CaSKi cells ($EGFR^{++}/HER2^{+/-}$) (B) and A2058 cells ($EGFR^{-}/HER2^{+/-}$) by a therapeutic combination according to the invention. A, B,C) Cetuximab-(Cys-L-QSmix)^{4,1} titration + fixed concentration 10 pM trastuzumab-saporin or 10 pM CD71mab-saporin and controls on A431 cells (A).

CaSKi cells (B) and A2058 cells (C). QSmix is a mixture of saponins from an extract Quillaja Saponaria.

Figure 14-6: *Control treatments on all cell lines.* A-D) Cell viability when trastuzumab (A), cetuximab (B), T-DM1, (C) free toxins: saporin and dianthin (D) or saporin coupled to a non-cell binding IgG (D) are treated with the indicated cell lines SK-BR-3, JIMT-1, MDA-MB-468, A431, CaSki, HeLa, A2058, BT-474. The legends and/or axes are the same for all the A,B, C or D.

Figure 15-6: *2-target 2-component concept: mAb1-SO1861 + mAb2-protein toxin.* SO1861 and toxin (ribosomal inactivating protein) are each, separately, conjugated to an antibody (mAb) for delivery and internalization into target cells. 1) mAb1-SO1861 and mAb2-protein toxin bind to their corresponding cell surface receptor, 2) receptor-mediated endocytosis of both conjugates occurs, 3) at low endolysosomal pH and appropriate concentration, SO1861 becomes active to enable endolysosomal escape, 4) release of toxin into cytoplasm occurs and 5) toxin induces cell death.

Figure 16-6: *2-target 2-component concept: mAb1-SO1861 + mAb2-BNA oligo.* SO1861 and antisense BNA oligo nucleotide are each, separately, conjugated to an antibody (mAb) for delivery and internalization into target cells. 1) mAb1-SO1861 and mAb2-BNAoligo bind to their corresponding cell surface receptor, 2) receptor-mediated endocytosis of both conjugates occurs, 3) at low endolysosomal pH and appropriate concentration, SO1861 becomes active to enable endolysosomal escape, 4) release of BNA oligo into cytoplasm occurs and 5) target gene silencing.

Figure 1-7: EGFR targeted gene silencing in A431 cells ($EGFR^{++}$) (A) and A2058 cells ($EGFR^{-}$) (B) by a combination with unconjugated SO1861-EMCH and HSP27BNA, and SO1861-EMCH and Cetuximab-(Lys-L-HSP27BNA) titration + fixed concentration of 4000 nM SO1861-EMCH and controls on A431 cells (A) and A2058 cells (B). (A) and (B) are HSP27BNA or Cetuximab-HSP27BNA conjugate (nM) based graphs. EGFR targeted gene silencing in A431 cells ($EGFR^{++}$) (A) and A2058 cells ($EGFR^{-}$) (B) by a combination with unconjugated SO1861-EMCH with free HSP27BNA, and SO1861-EMCH and conjugate Cetuximab-(Lys-L-HSP27BNA) titration + fixed concentration of 4000 nM SO1861-EMCH and controls on A431 cells (C) and A2058 cells (D). (C) and (D) are HSP27BNA (nM) based graphs. The legend with Figure 1-7B and D also applies for Figure 1-7A and C.

DETAILED DESCRIPTION

In order for a bioactive molecule to work, the molecule must be able to engage with its target, e.g. in the blood serum, on the outside of the cell surface or inside a cell or an organelle. The active moiety of almost all protein-based targeted toxins, e.g., must enter the cytosol of the target cell to mediate its target modulatory effect. In many constellations the toxin remains ineffective since (1) the targeting moiety is poorly internalized and remains bound to the outside of the cells, (2) is recycled back to the cell surface after internalization or (3) transported to the endolysosomes where it is degraded. Although these fundamental issues are known for decades and more than 500 targeted toxins have been investigated in the past decades, the problems have not been solved yet and only one antibody-targeted protein toxin, moxetumomab pasudotox-tdfk (LUMOXITI®, AstraZeneca Pharmaceuticals LP), has been approved for relapsed or refractory hairy cell leukemia by the FDA to date.

To overcome these problems, many strategies have been described including approaches to redirect the toxins to endogenous cellular membrane transport complexes of the biosynthetic pathway

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in the endoplasmic reticulum and techniques to disrupt or weaken the membrane integrity of endosomes, i.e. the compartments of the endocytic pathway in a cell, and thus facilitating the endosomal escape. This comprises the use of lysosomotropic amines, carboxylic ionophores, calcium channel antagonists, various cell-penetrating peptides of viral, bacterial, plant, animal, human and synthetic origin, other organic molecules and light-induced techniques. Although the efficacy of the targeted toxins was typically augmented in cell culture hundred- or thousand-fold, in exceptional cases more than million-fold, the requirement to co-administer endosomal escape enhancers with other substances harbors new problems including additional side effects, loss of target specificity, difficulties to determine the therapeutic window and cell type-dependent variations.

All strategies, including physicochemical techniques, require enhancer molecules that interact more or less directly with membranes and comprise essentially small chemical molecules, secondary metabolites, peptides and proteins. A common feature of all these substances is that they are *per se* not target cell-specific and distribute with other kinetics than the targeted toxins. This is one major drawback of the current approaches.

The present invention will be described with respect to particular embodiments but the invention is not limited thereto but only by the claims. The embodiments of the invention described herein can operate in combination and cooperation, unless specified otherwise.

While the invention has been described in terms of several embodiments, it is contemplated that alternatives, modifications, permutations and equivalents thereof will become apparent to one having ordinary skill in the art upon reading the specification and upon study of the drawings and graphs. The invention is not limited in any way to the illustrated embodiments. Changes can be made without departing from the scope which is defined by the appended claims.

An aspect of the invention relates to a therapeutic molecule with chemical structure of COMPOUND I:

$$A1_m\ ((\text{-}L9_w)\ ((\text{–}\ L1_q\ \text{–}\ B1_n)_u\ ((\text{–}\ L2_r\ \text{–}\ L3_s)\ (\text{–}\ L4_v\ \text{–}\ C)_p)_t))_x$$

(compound I),

wherein

A1 is a first ligand if B1 is a first effector moiety, or A1 is the first effector moiety if B1 is the first ligand;

C is a saponin;

m = 0 or 1 if A1 is the first ligand and B1 is the first effector moiety;

m = 0 – 32 if A1 is the first effector moiety and B1 is the first ligand;

n = 0 or 1 if B1 is the first ligand and A1 is the first effector moiety, or if A1 is the first ligand and B1 is the first effector moiety;

p = any of 1-128;

L1 is at least one linker for covalently coupling two chemical groups;

L2 is at least one linker for covalently coupling two chemical groups;

L3 is at least one oligomeric or polymeric scaffold for covalently coupling two chemical groups;

L4 is at least one linker for covalently coupling two chemical groups;

L9 is a tri-functional linker for covalently coupling three chemical groups;

q = 0 or 1;

r = 0 or 1;

s = 0 or 1;

t = 0, 1 or 2 if s = 0, and t = any of 0-16 if s = 1;

u = any of 0 – 32 if A1 is the first ligand and B1 is the first effector moiety, or u = 1 if A1 is the first effector moiety and B1 is the first ligand;

v = 0 or 1;

w = 1 or 0; and

x = 1-16.

An aspect of the invention relates to a therapeutic combination comprising the therapeutic molecule according to the invention and a second therapeutic molecule with chemical structure of COMPOUND II:

$$A2_a\ ((\text{-}L10_j)\ ((\text{–}\ L5_d\ \text{–}\ B2_b)_h\ ((\text{–}\ L6_e\ \text{–}\ L7_f)\ (\text{–}\ L8_i\ \text{–}\ C)_c)_g))_k$$

(compound II),

wherein

A2 is a second ligand if B2 is a second effector moiety, or A2 is the second effector moiety if B2 is the second ligand;

C is a saponin;

a = 0 or 1 if A2 is the second ligand and B2 is the second effector moiety, or a = 0 – 32 if A2 is the second effector moiety and B2 is the second ligand;

b = 0 or 1 if B2 is the second ligand and A2 is the second effector moiety, or if A2 is the second ligand and B2 is the second effector moiety;

c = any of 1-128;

L5 is at least one linker for covalently coupling two chemical groups;

L6 is at least one linker for covalently coupling two chemical groups;

L7 is at least one oligomeric or polymeric scaffold for covalently coupling two chemical groups;

L8 is at least one linker for covalently coupling two chemical groups;

L10 is a tri-functional linker for covalently coupling three chemical groups;

d = 0 or 1;

e = 0 or 1;

f = 0 or 1;

g = 0, 1 or 2 if f = 0, and g = any of 0-16 if f = 1;

h = any of 0 – 32 if A2 is the second ligand and B2 is the second effector moiety, or h = 1 if A2 is the second effector moiety and B2 is the second ligand;

i = 0 or 1;

j = 1 or 0 and;

k = 1-16.

An embodiment is the second therapeutic molecule of the invention, wherein g = 0, 1 or 2 if f = 0 and t > 0, g = 1 or 2 if f = 0 and t = 0, g = any of 0-16 if f = 1 and t > 0, and g = any of 1-16 if f = 1 and t = 0.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the first ligand A1 or B1 and/or the second ligand A2 or B2 comprise(s) or consist(s) of an immunoglobulin, a binding domain of an immunoglobulin or a binding fragment of an immunoglobulin, such as an antibody, an IgG, a molecule comprising or consisting of a Vhh domain or Vh domain, a Fab, an scFv, an Fv, a dAb, an $F(ab)_2$, Fcab fragment, or comprise(s) or consist(s) of at least one non-proteinaceous ligand and/or at least one proteinaceous ligand, the ligand for binding to a cell-surface molecule such as EGF or a cytokine, with the proviso that the first ligand and the second ligand are the same or are different.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the first ligand A1 or B1 and/or the second ligand A2 or B2 bind(s) to a tumor-cell epitope, preferably a tumor-cell specific epitope, of a tumor-cell receptor, preferably a tumor-cell specific receptor, preferably selected from CD71, CA125, EpCAM(17-1A), CD52, CEA, CD44v6, FAP, EGF-IR, integrin, syndecan-1, vascular integrin alpha-V beta-3, HER2, EGFR, CD20, CD22, Folate receptor 1, CD146, CD56, CD19, CD138, CD27L receptor, PSMA, CanAg, integrin-alphaV, CA6, CD33, mesothelin, Cripto, CD3, CD30, CD239, CD70, CD123, CD352, DLL3, CD25, ephrinA4, MUC1, Trop2, CEACAM5, CEACAM6, HER3, CD74, PTK7, Notch3, FGF2, C4.4A, FLT3, CD38, FGFR3, CD7, PD-L1, CTLA4, CD52, PDGFRA, VEGFR1, VEGFR2, more preferably selected from CD71, EGFR and HER2, with the proviso that the first ligand and the second ligand bind to the same or to a different tumor-cell epitope, preferably a tumor-cell specific epitope, and/or wherein the tumor-cell receptor, preferably the tumor-cell specific receptor, to which the first ligand can bind is the same as, or is different from the tumor-cell receptor, preferably the tumor-cell specific receptor, to which the second ligand can bind.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the first ligand A1 or B1 and/or the second ligand A2 or B2 comprise(s) or consist(s) of cetuximab, daratumumab, gemtuzumab, trastuzumab, panitumumab, brentuximab, inotuzumab, moxetumomab, polatuzumab, obinutuzumab, OKT-9 anti-CD71 monoclonal antibody of the IgG type, pertuzumab, rituximab, ofatumumab, Herceptin, alemtuzumab, pinatuzumab, OKT-10 anti-CD38 monoclonal antibody, an antibody of Table A2 or Table A3 or Table A4, preferably cetuximab or trastuzumab or OKT-9, or at least one tumor-cell receptor binding-domain thereof and/or at least one tumor-cell receptor binding-fragment thereof which are preferably (a) tumor-cell specific receptor binding-domain(s) and/or (a) tumor-cell specific receptor binding-fragment(s), with the proviso that the first ligand is the same or different from the second ligand.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the first ligand A1 or B1 is internalized by a tumor cell after binding of the first ligand to its binding partner on the tumor cell, and wherein preferably binding of the first ligand to the

tumor cell is followed by tumor-cell receptor-mediated internalization, e.g. via endocytosis, of a complex of the first ligand and the binding partner of the first ligand on the tumor cell.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the second ligand A2 or B2 is internalized by a tumor cell after binding of the second ligand to its binding partner on the tumor cell, and wherein preferably binding of the second ligand to the tumor cell is followed by tumor-cell receptor-mediated internalization, e.g. via endocytosis, of a complex of the second ligand and the binding partner of the second ligand on the tumor cell.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the first effector moiety A1 or B1 and/or the second effector moiety A2 or B2 comprise(s) or consist(s) of at least one of any one or more of an oligonucleotide, a nucleic acid and a xeno nucleic acid, preferably selected from any one or more of a vector, a gene, a cell suicide inducing transgene, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), anti-sense oligonucleotide (ASO, AON), short interfering RNA (siRNA), microRNA (miRNA), DNA aptamer, RNA aptamer, mRNA, mini-circle DNA, peptide nucleic acid (PNA), phosphoramidate morpholino oligomer (PMO), locked nucleic acid (LNA), bridged nucleic acid (BNA), 2'-deoxy-2'-fluoroarabino nucleic acid (FANA), 2'-O-methoxyethyl-RNA (MOE), 2'-O,4'-aminoethylene bridged nucleic acid, 3'-fluoro hexitol nucleic acid (FHNA), a plasmid, glycol nucleic acid (GNA) and threose nucleic acid (TNA), or a derivative thereof, more preferably a BNA, for example a BNA for silencing HSP27 protein expression, with the proviso that the first effector moiety and the second effector moiety are the same or are different.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the first effector moiety A1 or B1 and/or the second effector moiety A2 or B2 comprise(s) or consist(s) of at least one proteinaceous molecule, preferably selected from any one or more of a peptide, a protein, an enzyme such as urease and Cre-recombinase, a proteinaceous toxin, a ribosome-inactivating protein, at least one protein toxin selected from Table A5 and/or a bacterial toxin, a plant toxin, more preferably selected from any one or more of a viral toxin such as apoptin; a bacterial toxin such as Shiga toxin, Shiga-like toxin, Pseudomonas aeruginosa exotoxin (PE) or exotoxin A of PE, full-length or truncated diphtheria toxin (DT), cholera toxin; a fungal toxin such as alpha-sarcin; a plant toxin including ribosome-inactivating proteins and the A chain of type 2 ribosome-inactivating proteins such as dianthin e.g. dianthin-30 or dianthin-32, saporin e.g. saporin-S3 or saporin-S6, bouganin or de-immunized derivative debouganin of bouganin, shiga-like toxin A, pokeweed antiviral protein, ricin, ricin A chain, modeccin, modeccin A chain, abrin, abrin A chain, volkensin, volkensin A chain, viscumin, viscumin A chain; or an animal or human toxin such as frog RNase, or granzyme B or angiogenin from humans, or any fragment or derivative thereof; preferably the protein toxin is dianthin and/or saporin, with the proviso that the first effector moiety/moieties and the second effector moiety/moieties are the same or are different.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the first effector moiety A1 or B1 and/or the second effector moiety A2 or B2 comprise(s) or consist(s) of at least one payload, preferably selected from any one or more of a toxin targeting ribosomes, a toxin targeting elongation factors, a toxin targeting tubulin, a toxin targeting DNA and a toxin targeting RNA, more preferably any one or more of emtansine, pasudotox, maytansinoid

derivative DM1, maytansinoid derivative DM4, monomethyl auristatin E (MMAE, vedotin), monomethyl auristatin F (MMAF, mafodotin), a Calicheamicin, N-Acetyl-γ-calicheamicin, a pyrrolobenzodiazepine (PBD) dimer, a benzodiazepine, a CC-1065 analogue, a duocarmycin, Doxorubicin, paclitaxel, docetaxel, cisplatin, cyclophosphamide, etoposide, docetaxel, 5-fluorouracyl (5-FU), mitoxantrone, a tubulysin, an indolinobenzodiazepine, AZ13599185, a cryptophycin, rhizoxin, methotrexate, an anthracycline, a camptothecin analogue, SN-38, DX-8951f, exatecan mesylate, truncated form of *Pseudomonas aeruginosa* exotoxin (PE38), a Duocarmycin derivative, an amanitin, α-amanitin, a spliceostatin, a thailanstatin, ozogamicin, tesirine, Amberstatin269 and soravtansine, or a derivative thereof.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the therapeutic molecule and/or the second therapeutic molecule comprise(s) or consist(s) of any one of Gemtuzumab ozogamicin, Brentuximab vedotin, Trastuzumab emtansine, Inotuzumab ozogamicin, Moxetumomab pasudotox and Polatuzumab vedotin and an antibody-drug conjugate of Table A2 and Table A3, or at least one tumor-cell specific receptor binding-domain thereof and/or at least one tumor-cell specific receptor binding-fragment thereof which are preferably (a) tumor-cell specific receptor binding-domain(s) and/or (a) tumor-cell specific receptor binding-fragment(s), with the proviso that the therapeutic molecule and the second therapeutic molecule are the same or are different.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the saponin C is a triterpenoid saponin or a bisdesmosidic triterpene saponin, belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, and/or a saponin isolated from *a Gypsophila* species and/or a *Saponaria* species and/or an *Agrostemma* species and/or a *Quillaja* species such as *Quillaja saponaria*.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the saponin C is a single specific saponin or is a mixture of two or more different saponins, such as one or more of the saponins in Table A1 or Scheme I, SO1861, SA1657, GE1741, SA1641, QS-21, QS-21A, QS-21 A-api, QS-21 A-xyl, QS-21B, QS-21 B-api, QS-21 B-xyl, QS-7-xyl, QS-7-api, QS-17-api, QS-17-xyl, QS1861, QS1862, Quillajasaponin, Saponinum album, QS-18, Quil-A, Gyp1, gypsoside A, AG1, AG2, SO1542, SO1584, SO1658, SO1674, SO1832, or any of their stereomers and/or any combinations thereof, preferably the saponin is SO1861 and/or GE1741 and/or SA1641 and/or QS-21 and/or saponin with a quillaic acid aglycon core, a Gal-(1→2)-[Xyl-(1→3)]-GlcA carbohydrate substituent at the C-3beta-OH group and a Glc-(1→3)-Xyl-(1→4)-Rha-(1→2)-[Xyl-(1→3)-4-OAc-Qui-(1→4)]-Fuc carbohydrate substituent at the C-28-OH group, and/or is 3-O-beta-D-galactopyranosyl-(1→2)-[beta-D-xylopyranosyl-(1→3)]-beta-D-glucuronopyranosyl quillaic acid 28-O-beta-D-glucopyranosyl-(1→3)-beta-D-xylopyranosyl-(1→4)- alpha-L-rhamnopyranosyl-(1→2)-[beta-D-xylopyranosyl-(1→3)-4-OAc-beta-D-quinovopyranosyl-(1→4)]-beta-D-fucopyranoside, more preferably the saponin is SO1861 and/or QS-21.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the saponin C is a bisdesmosidic saponin having a molecular mass of at least

1.500 Dalton and comprising an oleanan-type triterpene containing an aldehyde group at the C-23 position and optionally a hydroxyl group at the C-16 position, with a first branched carbohydrate side chain at the C-3 position which first branched carbohydrate side chain optionally contains glucuronic acid, wherein the saponin contains an ester group with a second branched carbohydrate side chain at the C-28 position which second branched carbohydrate chain preferably comprises at least four carbohydrate units, optionally containing at least one acetyl residue such as two acetyl residues and/or at least one deoxy carbohydrates and/or a quinovose and/or a glucose and/or 4-methoxycinnamic acid and/or optionally comprising 5-O-[5-O-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid and/or optionally comprising 5-O-[5-O-Rha-(1→2)-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid bound to a carbohydrate via an ester bond, or wherein the at least one saponin is QS-21 or any one or more of QS-21A, QS-21 A-api, QS-21 A-xyl, QS-21B, QS-21 B-api, QS-21 B-xyl, QS-7-xyl, QS-7-api, QS-17-api, QS-17-xyl, QS-18, QS1861, protonated QS1861 (QS1862), Quil-A.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the saponin C is a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23, wherein the saponin C is covalently coupled to an amino-acid residue of the first ligand A1 or B1 and/or the first effector moiety B1 or A1 and/or the second ligand A2 or B2 and/or the second effector moiety B2 or A2 via the aldehyde function in the saponin C, preferably said aldehyde function in position C-23, preferably via a linker L2, L4, L6, L8, L9 and/or L10, more preferably via a cleavable linker L2, L4, L6, L8, L9 and/or L10, wherein the amino-acid residue preferably is selected from cysteine and lysine.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the saponin C is a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23, wherein the aldehyde function in position C-23 of the at least one saponin is covalently coupled to linker N-ε-maleimidocaproic acid hydrazide, which linker is covalently coupled via a thio-ether bond to a sulfhydryl group in the first ligand A1 or B1 and/or in the first effector moiety B1 or A1 and/or in the second ligand A2 or B2 and/or in the second effector moiety B2 or A2, such as a sulfhydryl group of a cysteine.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the saponin C is a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, wherein the saponin C is covalently coupled to the amino-acid residue of the first ligand A1 or B1 and/or the first effector moiety B1 or A1 and/or the second ligand A2 or B2 and/or the second effector moiety B2 or A2 via the glucuronic acid function in the saponin C, if present, preferably via a linker L2, L4, L6, L8, L9 and/or L10, wherein the amino-acid residue preferably is selected from cysteine and lysine, more preferably the amino-acid residue is lysine.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the saponin C is a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and comprising a glucuronic acid

function in a carbohydrate substituent at the C-3beta-OH group of the saponin, wherein the glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the at least one saponin is covalently coupled to linker 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate, which linker is covalently coupled via an amide bond to an amine group in the first ligand A1 or B1 and/or in the first effector moiety B1 or A1 and/or in the second ligand A2 or B2 and/or in the second effector moiety B2 or A2, such as an amine group of a lysine or an N-terminus of the first ligand A1 or B1 and/or the first effector moiety B1 or A1 and/or the second ligand A2 or B2 and/or the second effector moiety B2 or A2.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the first ligand A1 or B1 and/or the first effector moiety B1 or A1 and/or the second ligand A2 or B2 and/or the second effector moiety B2 or A2 comprise(s) one or more than one covalently bound saponin C, preferably 2, 3, 4, 5, 6, 8, 10, 16, 32, 64, 128 or 1-100 saponins, or any number of saponins therein between, such as 7, 9, 12 saponins.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the first ligand A1 or B1 and/or the first effector moiety B1 or A1 and/or the second ligand A2 or B2 and/or the second effector moiety B2 or A2 comprise(s) one or more than one covalently bound saponin C, wherein the saponin(s) C is/are covalently bound directly to an amino-acid residue of the first ligand A1 or B1 and/or the first effector moiety B1 or A1 and/or the second ligand A2 or B2 and/or the second effector moiety B2 or A2 when r, s, v, e, f and i are 0, preferably to a cysteine and/or to a lysine, and/or is/are covalently bound via at least one linker L2, L4, L6, L8, L9 and/or L10, or via at least one cleavable linker L2, L4, L6, L8, L9 and/or L10 and/or via at least one oligomeric or polymeric scaffold L3 and/or L7, preferably 1-8 of such scaffolds or 2-4 of such scaffolds, wherein the at least one scaffold is optionally based on a dendron, wherein 1-32 saponins, preferably 2, 3, 4, 5, 6, 8, 10, 16, 32 saponins, or any number of saponins therein between, such as 7, 9, 12 saponins, are covalently bound to the at least one scaffold.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the saponin C is a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23, wherein the saponin C is covalently coupled to an amino-acid residue of the first ligand A1 or B1 and/or the first effector moiety B1 or A1 and/or the second ligand A2 or B2 and/or the second effector moiety B2 or A2 via the aldehyde function in the saponin C, preferably said aldehyde function in position C-23, preferably via a linker L2, L4, L6, L8, L9 and/or L10, more preferably via a cleavable linker L2, L4, L6, L8, L9 and/or L10, wherein the amino-acid residue preferably is selected from cysteine and lysine, and wherein the cleavable linker L2, L4, L6, L8, L9 and/or L10 is subject to cleavage under acidic conditions, reductive conditions, enzymatic conditions or light-induced conditions, and preferably the cleavable linker comprises a hydrazone bond or a hydrazide bond subject to cleavage under acidic conditions when bound to saponin, and/or comprises a bond susceptible to proteolysis, for example proteolysis by Cathepsin B, when bound to saponin, and/or the cleavable linker comprises a disulphide bond susceptible to cleavage under reductive conditions.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the saponin C is a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23, wherein the saponin C is covalently coupled to an amino-acid residue of the first ligand A1 or B1 and/or the first effector moiety B1 or A1 and/or the second ligand A2 or B2 and/or the second effector moiety B2 or A2 via the aldehyde function in the saponin C, preferably said aldehyde function in position C-23, preferably via a linker L2, L4, L6, L8, L9 and/or L10, more preferably via a cleavable linker L2, L4, L6, L8, L9 and/or L10, wherein the amino-acid residue preferably is selected from cysteine and lysine, and wherein the cleavable linker L2, L4, L6, L8, L9 and/or L10 is subject to cleavage *in vivo* under acidic conditions as present in endosomes and/or lysosomes of mammalian cells, preferably human cells, preferably at pH 4.0 – 6.5, and more preferably at pH $\leq$ 5.5.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the polymeric or oligomeric scaffold L3 and/or L7 comprises a polymeric or oligomeric structure and comprises a chemical group, the chemical group for covalently coupling of the polymeric or oligomeric scaffold L3 and/or L7 to the amino-acid residue of the first ligand and/or the first effector moiety and/or the second ligand and/or the second effector moiety.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the saponin C is a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, wherein the glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the at least one saponin is covalently coupled to linker 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate, which linker is covalently coupled via an amide bond to an amine group in the first ligand A1 or B1 and/or in the first effector moiety B1 or A1 and/or in the second ligand A2 or B2 and/or in the second effector moiety B2 or A2, such as an amine group of a lysine or an N-terminus of the first ligand A1 or B1 and/or the first effector moiety B1 or A1 and/or the second ligand A2 or B2 and/or the second effector moiety B2 or A2, and wherein the at least one saponin is covalently bound to the polymeric or oligomeric structure of the scaffold L3 and/or L7 via a cleavable linker L4 and/or L8 according to the invention.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the chemical group of the polymeric or oligomeric scaffold L3 and/or L7, for covalently coupling of the scaffold to the amino-acid residue of the first ligand and/or the first effector moiety and/or the second ligand and/or the second effector moiety, is a click chemistry group, preferably selected from a tetrazine, an azide, an alkene or an alkyne, or a cyclic derivative of these groups, more preferably the click chemistry group is an azide.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the first ligand A1 or B1 and/or the first effector moiety B1 or A1 and/or the second ligand A2 or B2 and/or the second effector moiety B2 or A2 comprise(s) one or more than one covalently bound saponin C, preferably 2, 3, 4, 5, 6, 8, 10, 16, 32, 64, 128 or 1-100 saponins, or any number of saponins therein between, such as 7, 9, 12 saponins, and wherein the at least one saponin

is covalently bound to the first ligand and/or to the first effector moiety and/or to the second ligand and/or to the second effector moiety, either directly or via at least one linker such as a bi-functional linker, for example based on N-ε-maleimidocaproic acid hydrazide and/or based on 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate, or a tri-functional linker L9 when w = 1 and/or a tri-functional linker L10 when j = 1, such as the tri-functional linker of Scheme II.

An embodiment is the therapeutic molecule of the invention and encompassing the previous embodiment, or the second therapeutic molecule of the invention encompassing the previous embodiment, wherein the tri-functional linker L9 when w = 1 and/or the tri-functional linker L10 when j = 1, comprises a second chemical group with at least one saponin covalently bound thereto, a third chemical group for covalent binding to the first and/or second ligand and a first chemical group for covalent binding to at least one first and/or second effector moiety, preferably the tri-functional linker is the trifunctional linker of Scheme II.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the at least one saponin is covalently bound to the first ligand and/or to the first effector moiety and/or to the second ligand and/or to the second effector moiety via at least one linker comprising a tri-functional linker L9 when j = 1 and/or a tri-functional linker L10 when w = 1, to which tri-functional linker both the first ligand and the at least one first effector moiety are bound and/or to which tri-functional linker both the second ligand and the at least one second effector moiety are bound, preferably the tri-functional linker is the trifunctional linker of Scheme II.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the polymeric or oligomeric structure of the scaffold L3 and/or L7 comprises a linear, branched and/or cyclic polymer, oligomer, dendrimer, dendron, dendronized polymer, dendronized oligomer, a DNA, a polypeptide, poly-lysine, a poly-ethylene glycol, or an assembly of these polymeric or oligomeric structures which assembly is preferably built up by covalent cross-linking.

An embodiment is the therapeutic molecule of the invention or the second therapeutic molecule of the invention, wherein the first ligand A1 or B1 and/or the first effector moiety B1 or A1 and/or the second ligand A2 or B2 and/or the second effector moiety B2 or A2 comprise(s) one or more than one covalently bound saponin C, preferably 2, 3, 4, 5, 6, 8, 10, 16, 32, 64, 128 or 1-100 saponins, or any number of saponins therein between, such as 7, 9, 12 saponins, and wherein the first ligand A1 or B1 is covalently bound to the first effector moiety B1 or A1, respectively, via at least one linker L1, and/or wherein the second ligand A2 or B2 is covalently bound to the second effector moiety B2 or A2, respectively, via at least one linker L5.

An embodiment is the therapeutic combination of the invention, wherein the first ligand A1 is a monoclonal antibody or at least one binding fragment or –domain thereof according to any one of the claims 3-7, m = 1, q = 0, n = 0, u = 0, r = 0, L3 is the scaffold according to the invention and s = 1, or L3 is absent and s = 0, L4 is a linker or a cleavable linker according to the invention, v = 1, p = 2-4 and t = 2-4 if s = 1 and t = 0 if s = 0, and saponin C is a saponin according to the invention, preferably the saponin C is SO1861 and/or QS-21, and effector moiety A2 is an effector moiety selected from any one or more of an oligonucleotide, a nucleic acid and a xeno nucleic acid, preferably selected from any one

or more of a vector, a gene, a cell suicide inducing transgene, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), anti-sense oligonucleotide (ASO, AON), short interfering RNA (siRNA), microRNA (miRNA), DNA aptamer, RNA aptamer, mRNA, mini-circle DNA, peptide nucleic acid (PNA), phosphoramidate morpholino oligomer (PMO), locked nucleic acid (LNA), bridged nucleic acid (BNA), 2'-deoxy-2'-fluoroarabino nucleic acid (FANA), 2'-O-methoxyethyl-RNA (MOE), 2'-O,4'-aminoethylene bridged nucleic acid, 3'-fluoro hexitol nucleic acid (FHNA), a plasmid, glycol nucleic acid (GNA) and threose nucleic acid (TNA), or a derivative thereof, more preferably a BNA, for example a BNA for silencing HSP27 protein expression, a = 1, d = 0, b = 0, h = 0, e = 0, f = 0, i = 0, c = 0 and g = 0.

An embodiment is the therapeutic molecule of the invention, wherein r = 0, s = 0, v = 0, s = 0, v = 0, p = 0, t = 0, ligand A1 is a monoclonal antibody or at least one binding fragment or –domain thereof according to any one of the invention, m = 1, effector moiety B1 is an effector moiety selected from any one or more of an oligonucleotide, a nucleic acid and a xeno nucleic acid, preferably selected from any one or more of a vector, a gene, a cell suicide inducing transgene, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), anti-sense oligonucleotide (ASO, AON), short interfering RNA (siRNA), microRNA (miRNA), DNA aptamer, RNA aptamer, mRNA, mini-circle DNA, peptide nucleic acid (PNA), phosphoramidate morpholino oligomer (PMO), locked nucleic acid (LNA), bridged nucleic acid (BNA), 2'-deoxy-2'-fluoroarabino nucleic acid (FANA), 2'-O-methoxyethyl-RNA (MOE), 2'-O,4'-aminoethylene bridged nucleic acid, 3'-fluoro hexitol nucleic acid (FHNA), a plasmid, glycol nucleic acid (GNA) and threose nucleic acid (TNA), or a derivative thereof, more preferably a BNA, for example a BNA for silencing HSP27 protein expression, either q = 0, n = 1 and u = 2-4, or q = 1, n = 2-4, u = 2-4 and linker L1 is the oligomeric or polymeric scaffold L3 according to the invention.

An embodiment is the therapeutic combination of the invention, wherein the therapeutic molecule is the therapeutic molecule of the previous embodiment, and wherein the second ligand A2 is a monoclonal antibody or at least one binding fragment or –domain thereof according to the invention, a = 1, d = 0, b = 0, h = 0, e = 0, L7 is the scaffold according to the invention and f = 1, or L7 is absent and f = 0, L8 is a linker or a cleavable linker according to the invention, i = 1, c = 2-4 and g = 2-4 if f = 1 and g = 0 if f = 0, and saponin C is a saponin according to the invention, preferably the saponin C is SO1861 and/or QS-21, with the proviso that the ligand A1 and the ligand A2 are the same or are different.

An aspect of the invention relates to a therapeutic combination, wherein the therapeutic combination comprises: (a) a first pharmaceutical composition comprising the therapeutic molecule with chemical structure of COMPOUND I according to the invention, the first pharmaceutical composition optionally further comprising a pharmaceutically acceptable excipient; and (b) a second pharmaceutical composition comprising the second therapeutic molecule with chemical structure of COMPOUND II according to the invention, the second pharmaceutical composition optionally further comprising a pharmaceutically acceptable excipient.

An aspect of the invention relates to the first pharmaceutical composition of the invention for use as a medicament.

An aspect of the invention relates to a therapeutic combination for use in the treatment or prevention of cancer in a human subject, wherein the therapeutic combination comprises: (a) the first

pharmaceutical composition of the invention; and (b) the second pharmaceutical composition of the invention, wherein the ligand A1 or B1 and the ligand A2 or B2 can bind to a tumor-cell epitope, preferably to a tumor-cell specific epitope, on a tumor-cell surface molecule, preferably on a tumor cell-specific surface molecule, with the proviso that the tumor-cell epitope or tumor-cell specific epitope to which the ligand A1 or B1 can bind is the same as, or is different from the tumor-cell epitope or the tumor-cell specific epitope to which the ligand A2 or B2 can bind.

An aspect of the invention relates to the first pharmaceutical composition of the invention, for use in the treatment or prophylaxis of cancer in a patient in need thereof, wherein the ligand A1 or B1 can bind to a tumor-cell epitope, preferably a tumor-cell specific epitope, on a tumor-cell surface molecule, preferably a tumor cell-specific surface molecule.

An embodiment is the first pharmaceutical composition for use according to the invention or the therapeutic combination for use according to the invention, wherein the second pharmaceutical composition of the invention and the first pharmaceutical composition of the invention are administered to the patient in need thereof.

An aspect of the invention relates to the first pharmaceutical composition of the invention further comprising the second therapeutic molecule of the invention.

An aspect of the invention relates to the first pharmaceutical composition of the invention further comprising the second therapeutic molecule of the invention, for use as a medicament.

An aspect of the invention relates to the first pharmaceutical composition of the invention further comprising the second therapeutic molecule of the invention, for use in the treatment or prevention of a cancer in a human subject.

An aspect of the invention within a first series of aspects and embodiments of the invention relates to a scaffold suitable for covalently binding at least one biologically active molecule to a carrier molecule, the scaffold comprising a polymeric or oligomeric structure and at least one of said biologically active molecules covalently bound to said polymeric or oligomeric structure, wherein the scaffold further comprises a first chemical group for covalently coupling of the scaffold to the carrier molecule.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the at least one biologically active molecule has a molecular mass of 3.000 Dalton or less, preferably 2.500 Dalton or less, more preferably 2.300 Dalton or less, most preferably, 2.000 Dalton or less, such as 1.700 Dalton – 1.950 Dalton.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the at least one biologically active molecule is an amphiphilic molecule.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the at least one biologically active molecule is a single specific molecule or is a mixture of different molecules, when more than one biologically active molecules are covalently bound to the polymeric or oligomeric structure comprised by the scaffold.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the at least one biologically active molecule is a glycoside, preferably a bisdesmosidic triterpene or triterpenoid saponin, more preferably a bisdesmosidic triterpene

saponin, most preferably a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the at least one biologically active molecule is a saponin that can be isolated from *a Gypsophila* species and/or a *Saponaria* species and/or an *Agrostemma* species and/or a *Quillaja* species such as *Quillaja saponaria* or is a single specific saponin or is a mixture of two or more different saponins, such as one or more of the saponins in Table A1 or Scheme I, SO1861, SA1657, GE1741, SA1641, QS-21, QS-21A, QS-21 A-api, QS-21 A-xyl, QS-21B, QS-21 B-api, QS-21 B-xyl, QS-7-xyl, QS-7-api, QS-17-api, QS-17-xyl, QS1861, QS1862, Quillajasaponin, Saponinum album, QS-18, Quil-A, Gyp1, gypsoside A, AG1, AG2, SO1542, SO1584, SO1658, SO1674, SO1832, or any of their stereomers and/or any combinations thereof, preferably the saponin is SO1861 and/or GE1741 and/or SA1641 and/or QS-21 and/or saponin with a quillaic acid aglycon core, a Gal-(1→2)-[Xyl-(1→3)]-GlcA carbohydrate substituent at the C-3beta-OH group and a Glc-(1→3)-Xyl-(1→4)-Rha-(1→2)-[Xyl-(1→3)-4-OAc-Qui-(1→4)]-Fuc carbohydrate substituent at the C-28-OH group, and/or is 3-O-beta-D-galactopyranosyl-(1→2)-[beta-D-xylopyranosyl-(1→3)]-beta-D-glucuronopyranosyl quillaic acid 28-O-beta-D-glucopyranosyl-(1→3)-beta-D-xylopyranosyl-(1→4)- alpha-L-rhamnopyranosyl-(1→2)-[beta-D-xylopyranosyl-(1→3)-4-OAc-beta-D-quinovopyranosyl-(1→4)]-beta-D-fucopyranoside, more preferably the saponin is SO1861 and/or QS-21.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the at least one biologically active molecule is a bisdesmosidic saponin having a molecular mass of at least 1.500 Dalton and comprising an oleanan-type triterpene containing an aldehyde group at the C-23 position and optionally a hydroxyl group at the C-16 position, with a first branched carbohydrate side chain at the C-3 position which first branched carbohydrate side chain optionally contains glucuronic acid, wherein the saponin contains an ester group with a second branched carbohydrate side chain at the C-28 position which second branched carbohydrate chain preferably comprises at least four carbohydrate units, optionally containing at least one acetyl residue such as two acetyl residues and/or optionally comprising deoxy carbohydrates and/or optionally comprising quinovose and/or optionally comprising glucose and/or optionally comprising 4-methoxycinnamic acid and/or optionally comprising 5-O-[5-O-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid and/or optionally comprising 5-O-[5-O-Rha-(1→2)-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid bound to a carbohydrate via an ester bond, or wherein the at least one saponin is QS-21 or any one or more of QS-21A, QS-21 A-api, QS-21 A-xyl, QS-21B, QS-21 B-api, QS-21 B-xyl, QS-7-xyl, QS-7-api, QS-17-api, QS-17-xyl, QS-18, QS1861, protonated QS1861 (QS1862), Quil-A.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the at least one biologically active molecule is covalently bound to the polymeric or oligomeric structure via a non-cleavable bond or via a cleavable bond, wherein preferably said cleavable bond is subject to cleavage under acidic conditions, reductive conditions,

enzymatic conditions or light-induced conditions, more preferably the cleavable bond is a hydrazone bond or a hydrazide bond subject to cleavage under acidic conditions, and/or is a bond susceptible to proteolysis, for example proteolysis by Cathepsin B, and/or is a bond susceptible for cleavage under reductive conditions such as a disulphide bond.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the at least one biologically active molecule is covalently bound to the polymeric or oligomeric structure via a cleavable bond, wherein said cleavable bond is subject to cleavage *in vivo* under acidic conditions as present in endosomes and/or lysosomes of mammalian cells, preferably human cells, preferably at pH 4.0 – 6.5, and more preferably at pH $\leq$ 5.5.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the at least one biologically active molecule is covalently bound to the polymeric or oligomeric structure of the scaffold via an imine bond, a hydrazone bond, a hydrazide bond, an oxime bond, a 1,3-dioxolane bond, a disulphide bond, a thio-ether bond, an amide bond, a peptide bond or an ester bond, preferably via at least one linker.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the aldehyde function in position C-23 of the at least one saponin is involved in the covalent bonding to the polymeric or oligomeric structure of the scaffold, and/or, if present, the glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the at least one saponin, is involved in the covalent bonding to the polymeric or oligomeric structure of the scaffold, either via direct binding or via at least one linker.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the aldehyde function in position C-23 of the at least one saponin is covalently coupled to linker N-ε-maleimidocaproic acid hydrazide, which linker is covalently coupled via a thio-ether bond to a sulfhydryl group in the polymeric or oligomeric structure of the scaffold, such as a sulfhydryl group of a cysteine.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the at least one saponin is covalently coupled to linker 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate, which linker is covalently coupled via an amide bond to an amine group in the polymeric or oligomeric structure of the scaffold, such as an amine group of a lysine or an N-terminus of a proteinaceous molecule.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the chemical group for covalently coupling of the scaffold to the carrier molecule is a click chemistry group.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the click chemistry group is a tetrazine, an azide, an alkene or an alkyne, or a cyclic derivative of any of these groups, preferably an azide.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the scaffold is a tri-functional linker comprising a second chemical group with at least one biologically active molecule covalently bound thereto, comprising a

third chemical group for covalent binding to a molecule and comprising the first chemical group for covalent binding to the carrier, preferably the tri-functional linker is the tri-functional linker of Scheme II and Structure B.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the at least one biologically active molecule is a defined number of glycoside molecules or a defined range of glycoside molecules, preferably 1-128 or at least 2, 3, 4, 5, 6, 8, 10, 16, 32, 64 or 128 glycoside molecules, or any number of glycoside molecules therein between, such as 7, 9, 12 glycoside molecules.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the polymeric or oligomeric structure comprises a linear, branched and/or cyclic polymer, oligomer, dendrimer, dendron, dendronized polymer, dendronized oligomer, a DNA, a polypeptide, a poly-lysine, a poly-ethylene glycol, or an assembly of these polymeric or oligomeric structures which assembly is preferably built up by covalent cross-linking.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the carrier molecule comprises or consists of any of a proteinaceous molecule, a protein, a peptide, a nucleic acid, an oligonucleotide, a lipid, a fat, a fatty acid, a nanoparticle, a carbohydrate, or any covalently bound conjugate or covalently bound complex of combinations thereof.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the carrier molecule comprises or consists of an immunoglobulin, at least one binding domain of an immunoglobulin and/or at least one binding fragment of an immunoglobulin, such as an antibody, an IgG, a molecule comprising or consisting of a Vhh domain or Vh domain, a Fab, an scFv, an Fv, a dAb, an $F(ab)_2$, Fcab fragment, or comprises or consists of at least one non-proteinaceous ligand and/or at least one proteinaceous ligand for binding to a cell-surface molecule such as EGF or a cytokine.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the carrier molecule comprises or consists of at least one binding domain and/or at least one binding fragment for binding to a cell-surface receptor such as a tumor-cell specific cell-surface receptor selected from CD71, CA125, EpCAM(17-1A), CD52, CEA, CD44v6, FAP, EGF-IR, integrin, syndecan-1, vascular integrin alpha-V beta-3, HER2, EGFR, CD20, CD22, Folate receptor 1, CD146, CD56, CD19, CD138, CD27L receptor, PSMA, CanAg, integrin-alphaV, CA6, CD33, mesothelin, Cripto, CD3, CD30, CD239, CD70, CD123, CD352, DLL3, CD25, ephrinA4, MUC1, Trop2, CEACAM5, CEACAM6, HER3, CD74, PTK7, Notch3, FGF2, C4.4A, FLT3, CD38, FGFR3, CD7, PD-L1, CTLA4, CD52, PDGFRA, VEGFR1, VEGFR2, preferably selected from CD71, EGFR, HER2.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the carrier molecule comprises or consists of any one of cetuximab, daratumumab, gemtuzumab, trastuzumab, panitumumab, brentuximab, inotuzumab, moxetumomab, polatuzumab, obinutuzumab, OKT-9 anti-CD71 monoclonal antibody of the IgG type, pertuzumab, rituximab, ofatumumab, Herceptin, alemtuzumab, pinatuzumab, OKT-10 anti-CD38

monoclonal antibody, an antibody of Table A2 or Table A3 or Table A4, preferably cetuximab or trastuzumab or OKT-9, or at least one tumor-cell receptor binding-fragment thereof and/or at least one tumor-cell receptor binding-domain thereof, such as at least one tumor-cell specific receptor binding-fragment thereof and/or at least one tumor-cell specific receptor binding-domain thereof.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the scaffold is suitable for forming a covalent bond with the carrier molecule, said covalent bond preferably involving a cysteine side-chain of the carrier molecule and/or a lysine side-chain of the carrier molecule when the carrier molecule comprises at least a cysteine and/or a lysine.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the carrier molecule comprises or consists of at least one effector molecule, or wherein the carrier further comprises at least one effector molecule, wherein the effector molecule is at least one of an active pharmaceutical substance, such as any one or more of a payload, a toxin, a drug, a polypeptide, an oligonucleotide, a nucleic acid, a xeno nucleic acid, an enzyme such as urease and Cre-recombinase, a protein toxin, a ribosome-inactivating protein.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the protein toxin comprises or consists of any one or more of a protein toxin selected from Table A5 and/or a viral toxin such as apoptin; a bacterial toxin such as Shiga toxin, Shiga-like toxin, Pseudomonas aeruginosa exotoxin (PE) or exotoxin A of PE, full-length or truncated diphtheria toxin (DT), cholera toxin; a fungal toxin such as alpha-sarcin; a plant toxin including ribosome-inactivating proteins and the A chain of type 2 ribosome-inactivating proteins such as dianthin e.g. dianthin-30 or dianthin-32, saporin e.g. saporin-S3 or saporin-S6, bouganin or de-immunized derivative debouganin of bouganin, shiga-like toxin A, pokeweed antiviral protein, ricin, ricin A chain, modeccin, modeccin A chain, abrin, abrin A chain, volkensin, volkensin A chain, viscumin, viscumin A chain; or an animal or human toxin such as frog RNase, or granzyme B or angiogenin from humans, or any fragment or derivative thereof; preferably the protein toxin is dianthin and/or saporin.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the oligonucleotide, the xeno nucleic acid or the nucleic acid comprises or consists of any one or more of a vector, a gene, a cell suicide inducing transgene, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), anti-sense oligonucleotide (ASO, AON), short interfering RNA (siRNA), microRNA (miRNA), DNA aptamer, RNA aptamer, mRNA, mini-circle DNA, peptide nucleic acid (PNA), phosphoramidate morpholino oligomer (PMO), locked nucleic acid (LNA), bridged nucleic acid (BNA), 2'-deoxy-2'-fluoroarabino nucleic acid (FANA), 2'-O-methoxyethyl-RNA (MOE), 2'-O,4'-aminoethylene bridged nucleic acid, 3'-fluoro hexitol nucleic acid (FHNA), a plasmid, glycol nucleic acid (GNA) and threose nucleic acid (TNA), or a derivative thereof, preferably a BNA, for example a BNA for silencing HSP27 protein expression.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the effector molecule comprises or consists of at least one payload, preferably selected from any one or more of a toxin targeting ribosomes, a toxin targeting elongation factors, a toxin targeting tubulin, a toxin targeting DNA and a toxin targeting RNA, more

preferably any one or more of emtansine, pasudotox, maytansinoid derivative DM1, maytansinoid derivative DM4, monomethyl auristatin E (MMAE, vedotin), monomethyl auristatin F (MMAF, mafodotin), a Calicheamicin, N-Acetyl-γ-calicheamicin, a pyrrolobenzodiazepine (PBD) dimer, a benzodiazepine, a CC-1065 analogue, a duocarmycin, Doxorubicin, paclitaxel, cisplatin, cyclophosphamide, etoposide, docetaxel, 5-fluorouracyl (5-FU), mitoxantrone, a tubulysin, an indolinobenzodiazepine, AZ13599185, a cryptophycin, rhizoxin, methotrexate, an anthracycline, a camptothecin analogue, SN-38, DX-8951f, exatecan mesylate, truncated form of *Pseudomonas aeruginosa* exotoxin (PE38), a Duocarmycin derivative, an amanitin, α-amanitin, a spliceostatin, a thailanstatin, ozogamicin, tesirine, Amberstatin269 and soravtansine, or a derivative thereof.

An embodiment within the first series of aspects and embodiments of the invention is the scaffold according to the invention, wherein the carrier molecule comprises or consists of a covalently linked combination of an effector molecule and a monoclonal antibody, preferably selected from Gemtuzumab ozogamicin, Brentuximab vedotin, Trastuzumab emtansine, Inotuzumab ozogamicin, Moxetumomab pasudotox and Polatuzumab vedotin and an antibody-drug conjugate of Table A2 and Table A3.

An aspect of the invention within the first series of aspects and embodiments of the invention relates to a method for producing a scaffold suitable for covalently binding at least one biologically active molecule to a carrier molecule, the method comprising: a) providing a polymeric or oligomeric structure comprising a first chemical group for covalently coupling of the polymeric structure or the oligomeric structure to the carrier molecule and comprising at least one of a second chemical group different from the first chemical group, wherein each second chemical group is for covalently coupling one of the at least one biologically active molecules to the oligomeric or polymeric structure; and b) covalently coupling at least one biologically active molecule to said polymeric or oligomeric structure via the second chemical group(s), wherein preferably the biologically active molecule(s) is/are any one of the biologically active molecules of the invention, more preferably SO1861 and/or GE1741 and/or SA1641 and/or QS-21, therewith providing the scaffold.

An embodiment within the first series of aspects and embodiments of the invention is the method according to the invention, the scaffold comprising at least one covalently bound biologically active molecule, the method comprising: a) providing a scaffold comprising at least one biologically active molecule covalently bound to a polymeric or oligomeric structure in said scaffold, preferably providing a scaffold according to the invention or the scaffold obtainable by the method according to the invention or the scaffold obtained with the method according to the invention; and b) covalently coupling the scaffold of a) to a carrier molecule according to the invention, therewith providing the scaffold covalently bound to a carrier molecule, the scaffold comprising at least one covalently bound biologically active molecule.

An embodiment within the first series of aspects and embodiments of the invention is the method according to the invention, wherein the scaffold is able to augment endosomal escape and/or lysosomal escape of the effector molecule according to the invention when either said effector molecule is covalently bound to the scaffold and contacted with a mammalian cell, or when said effector molecule is contacted with a mammalian cell in the presence of the scaffold.

An aspect of the invention within a second series of aspects and embodiments of the invention relates to a first proteinaceous molecule comprising a first binding site for binding to a first epitope of a first cell-surface molecule, the first proteinaceous molecule provided with at least one saponin covalently bound via at least one linker and/or via an oligomeric or polymeric scaffold to an amino-acid residue of said first proteinaceous molecule, or covalently bound directly to an amino-acid residue of said first proteinaceous molecule.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention, wherein the first binding site comprises or consists of an immunoglobulin, or at least one binding domain of an immunoglobulin and/or at least one binding fragment of an immunoglobulin, such as an antibody, an IgG, a molecule comprising or consisting of a Vhh domain or Vh domain, a Fab, an scFv, an Fv, a dAb, an $F(ab)_2$, Fcab fragment, and/or comprises or consists of at least one ligand for binding to a cell-surface molecule such as EGF or a cytokine.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention, wherein the first epitope of the first cell-surface molecule is a tumor-cell specific first epitope of a first tumor-cell surface molecule, more preferably a tumor-cell specific first epitope of a first tumor-cell surface receptor specifically present on a tumor cell.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention, wherein the at least one saponin is a triterpenoid saponin and/or a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, and/or a saponin isolated from *a Gypsophila* species and/or a *Saponaria* species and/or an *Agrostemma* species and/or a *Quillaja* species such as *Quillaja saponaria*.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention, wherein the at least one saponin is a single specific saponin or is a mixture of two or more different saponins, such as one or more of the saponins in Table A1 or Scheme I, SO1861, SA1657, GE1741, SA1641, QS-21, QS-21A, QS-21 A-api, QS-21 A-xyl, QS-21B, QS-21 B-api, QS-21 B-xyl, QS-7-xyl, QS-7-api, QS-17-api, QS-17-xyl, QS1861, QS1862, Quillajasaponin, Saponinum album, QS-18, Quil-A, Gyp1, gypsoside A, AG1, AG2, SO1542, SO1584, SO1658, SO1674, SO1832, or any of their stereomers and/or any combinations thereof, preferably the saponin is SO1861 and/or GE1741 and/or SA1641 and/or QS-21 and/or saponin with a quillaic acid aglycon core, a Gal-(1→2)-[Xyl-(1→3)]-GlcA carbohydrate substituent at the C-3beta-OH group and a Glc-(1→3)-Xyl-(1→4)-Rha-(1→2)-[Xyl-(1→3)-4-OAc-Qui-(1→4)]-Fuc carbohydrate substituent at the C-28-OH group, and/or is 3-O-beta-D-galactopyranosyl-(1→2)-[beta-D-xylopyranosyl-(1→3)]-beta-D-glucuronopyranosyl quillaic acid 28-O-beta-D-glucopyranosyl-(1→3)-beta-D-xylopyranosyl-(1→4)- alpha-L-rhamnopyranosyl-(1→2)-[beta-D-xylopyranosyl-(1→3)-4OAc-beta-D-quinovopyranosyl-(1→4)]-beta-D-fucopyranoside, more preferably the saponin is SO1861 and/or QS-21.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention, wherein the at least one saponin is a bisdesmosidic saponin having a molecular mass of at least 1.500 Dalton and comprising an oleanan-type triterpene containing an aldehyde group at the C-23 position and optionally a hydroxyl group at the C-16 position, with a first branched carbohydrate side chain at the C-3 position which first branched carbohydrate side chain optionally contains glucuronic acid, wherein the saponin contains an ester group with a second branched carbohydrate side chain at the C-28 position which second branched carbohydrate chain preferably comprises at least four carbohydrate units, optionally containing at least one acetyl residue such as two acetyl residues and/or optionally comprising deoxy carbohydrates and/or optionally comprising quinovose and/or optionally comprising glucose and/or optionally comprising 4-methoxycinnamic acid and/or optionally comprising 5-O-[5-O-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid and/or optionally comprising 5-O-[5-O-Rha-(1→2)-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid bound to a carbohydrate via an ester bond, or wherein the at least one saponin is QS-21 or any one or more of QS-21A, QS-21 A-api, QS-21 A-xyl, QS-21B, QS-21 B-api, QS-21 B-xyl, QS-7-xyl, QS-7-api, QS-17-api, QS-17-xyl, QS-18, QS1861, protonated QS1861 (QS1862), Quil-A.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention, wherein the at least one saponin is a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23, wherein the at least one saponin is covalently coupled to the amino-acid residue of the first proteinaceous molecule via an aldehyde function in the saponin, preferably said aldehyde function in position C-23, preferably via at least one linker, more preferably via at least one cleavable linker, wherein the amino-acid residue preferably is selected from cysteine and lysine.

An embodiment is the first proteinaceous molecule according to the invention, wherein the at least one saponin is a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, wherein the at least one saponin is covalently coupled to the amino-acid residue of the first proteinaceous molecule via the glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the saponin, preferably via at least one linker, wherein the amino-acid residue preferably is selected from cysteine and lysine.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention, wherein the aldehyde function in position C-23 of the at least one saponin is covalently coupled to linker N-ε-maleimidocaproic acid hydrazide, which linker is covalently coupled via a thio-ether bond to a sulfhydryl group in the first proteinaceous molecule, such as a sulfhydryl group of a cysteine.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention, wherein the glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the at least one saponin is covalently coupled to linker 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate,

which linker is covalently coupled via an amide bond to an amine group in the first proteinaceous molecule, such as an amine group of a lysine or an N-terminus of the first proteinaceous molecule.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention, wherein the first epitope of the first cell-surface molecule to which the first binding site of the first proteinaceous molecule binds is a tumor-cell specific first epitope of the tumor-cell specific receptor preferably selected from CD71, CA125, EpCAM(17-1A), CD52, CEA, CD44v6, FAP, EGF-IR, integrin, syndecan-1, vascular integrin alpha-V beta-3, HER2, EGFR, CD20, CD22, Folate receptor 1, CD146, CD56, CD19, CD138, CD27L receptor, PSMA, CanAg, integrin-alphaV, CA6, CD33, mesothelin, Cripto, CD3, CD30, CD239, CD70, CD123, CD352, DLL3, CD25, ephrinA4, MUC1, Trop2, CEACAM5, CEACAM6, HER3, CD74, PTK7, Notch3, FGF2, C4.4A, FLT3, CD38, FGFR3, CD7, PD-L1, CTLA4, CD52, PDGFRA, VEGFR1, VEGFR2, more preferably selected from CD71, EGFR, HER2.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention, wherein the tumor cell-specific first epitope, first tumor-cell surface molecule or first tumor-cell specific receptor, are a first epitope or a first molecule or a first receptor that are internalized by the tumor cell after binding of the first proteinaceous molecule of the invention to the first epitope or first molecule or first receptor, and wherein preferably the first proteinaceous molecule is subjected to tumor-cell receptor-mediated internalization, e.g. via endocytosis, or tumor-cell surface molecule mediated internalization, e.g. via endocytosis, when bound to the cell-surface molecule comprising the first epitope, the tumor-cell surface molecule or the tumor-cell specific receptor.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention, wherein the first binding site of the first proteinaceous molecule comprises or consists of any one of cetuximab, daratumumab, gemtuzumab, trastuzumab, panitumumab, brentuximab, inotuzumab, moxetumomab, polatuzumab, obinutuzumab, OKT-9 anti-CD71 monoclonal antibody of the IgG type, pertuzumab, rituximab, ofatumumab, Herceptin, alemtuzumab, pinatuzumab, OKT-10 anti-CD38 monoclonal antibody, an antibody of Table A2 or Table A3 or Table A4, preferably cetuximab or trastuzumab or OKT-9, or at least one tumor-cell receptor binding-fragment thereof and/or at least one tumor-cell receptor binding-domain thereof, preferably at least one tumor-cell specific receptor binding-fragment thereof and/or at least one tumor-cell specific receptor binding-domain thereof.

An aspect of the invention within the second series of aspects and embodiments of the invention relates to a therapeutic combination, wherein the therapeutic combination comprises: (a) a first pharmaceutical composition comprising the first proteinaceous molecule according to the invention and optionally a pharmaceutically acceptable excipient; and (b) a second pharmaceutical composition comprising a second proteinaceous molecule different from the first proteinaceous molecule, the second proteinaceous molecule comprising a second binding site for binding to a second epitope of a second cell-surface molecule different from the first cell-surface molecule, and comprising an effector moiety, the second pharmaceutical composition optionally further comprising a pharmaceutically acceptable excipient, wherein the second epitope is different from the first epitope.

An embodiment within the second series of aspects and embodiments of the invention is the therapeutic combination according to the invention, wherein the therapeutic combination comprises: (a) the first pharmaceutical composition according to the invention comprising the first proteinaceous molecule according to the invention, wherein the first epitope on the first cell-surface molecule is a tumor-cell specific first epitope on a first tumor cell-specific surface molecule, preferably a tumor-cell specific first epitope on a first cell-surface receptor specifically present at a tumor cell; and (b) the second pharmaceutical composition according to the invention, wherein the second cell-surface molecule is a second tumor cell-specific surface molecule different from the first tumor cell-specific surface molecule, preferably a second cell-surface receptor specifically present at a tumor cell different from the first cell-surface receptor specifically present at said tumor cell, and wherein the second epitope is a tumor-cell specific second epitope.

An embodiment within the second series of aspects and embodiments of the invention is the therapeutic combination according to the invention, wherein the therapeutic combination comprises: (a) the first pharmaceutical composition according to the invention comprising the first proteinaceous molecule according to the invention and comprising the first binding site for binding to the first epitope on the first cell-surface molecule, the first pharmaceutical composition optionally further comprising a pharmaceutically acceptable excipient; and (b) a third pharmaceutical composition comprising a third proteinaceous molecule, the third proteinaceous molecule comprising the first binding site for binding to the first epitope on the cell-surface molecule of (a) and an effector moiety, the third pharmaceutical composition optionally further comprising a pharmaceutically acceptable excipient, wherein the first binding site of the first proteinaceous molecule and the first binding site of the third proteinaceous molecule are the same, and wherein the first cell-surface molecule and the first epitope on the first cell-surface molecule, to which the first proteinaceous molecule can bind, and the first cell-surface molecule and the first epitope on the first cell-surface molecule, to which the third proteinaceous molecule can bind, are the same.

An embodiment within the second series of aspects and embodiments of the invention is the therapeutic combination according to the invention, wherein the therapeutic combination comprises: (a) the first pharmaceutical composition according to the invention; and (b) the third pharmaceutical composition according to the invention, wherein the first cell-surface molecule is expressed on a tumor cell surface, and preferably the first cell-surface molecule is a tumor cell-specific surface molecule, and wherein preferably the first epitope is a first tumor-cell specific epitope.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention or the therapeutic combination according to the invention, wherein the first binding site for binding to the first epitope on the first cell surface molecule is a binding site for a tumor-cell specific first epitope on a first cell-surface receptor specifically present at a tumor cell.

An embodiment within the second series of aspects and embodiments of the invention is the therapeutic combination according to the invention, wherein the second binding site of the second proteinaceous molecule and/or the first binding site of the third proteinaceous molecule comprises or consists of an immunoglobulin, at least one binding domain of an immunoglobulin and/or at least one

binding fragment of an immunoglobulin, such as an antibody, an IgG, a molecule comprising or consisting of a Vhh domain or Vh domain, a Fab, an scFv, an Fv, a dAb, an F(ab)2, Fcab fragment, and/or comprises or consists of at least one ligand for binding to a cell-surface molecule such as EGF or a cytokine.

An embodiment within the second series of aspects and embodiments of the invention is the therapeutic combination according to the invention, wherein the second binding site of the second proteinaceous molecule for binding to the second epitope is a second binding site for a tumor-cell specific second epitope on a second cell-surface receptor specifically present at the tumor cell, wherein the second binding site is different from the first binding site.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention or the therapeutic combination according to the invention, wherein said first and second proteinaceous molecules comprise the first and second binding site respectively for binding to a first and a second tumor-cell specific epitope on a first and a second tumor-cell specific receptor respectively, the receptors being different and being present at the same tumor cell, wherein the first and second binding site are different and the first and second tumor cell specific epitope are different.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention or the therapeutic combination according to the invention, wherein said first and third proteinaceous molecules comprise the same first binding site for binding to a first tumor-cell specific epitope on a first tumor-cell specific receptor.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention or the therapeutic combination according to the invention wherein the first receptor and/or the second receptor are selected from CD71, CA125, EpCAM(17-1A), CD52, CEA, CD44v6, FAP, EGF-IR, integrin, syndecan-1, vascular integrin alpha-V beta-3, HER2, EGFR, CD20, CD22, Folate receptor 1, CD146, CD56, CD19, CD138, CD27L receptor, PSMA, CanAg, integrin-alphaV, CA6, CD33, mesothelin, Cripto, CD3, CD30, CD239, CD70, CD123, CD352, DLL3, CD25, ephrinA4, MUC1, Trop2, CEACAM5, CEACAM6, HER3, CD74, PTK7, Notch3, FGF2, C4.4A, FLT3, CD38, FGFR3, CD7, PD-L1, CTLA4, CD52, PDGFRA, VEGFR1, VEGFR2, preferably selected from CD71, EGFR and HER2.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention and/or the therapeutic combination according to the invention, wherein the first and second tumor-cell specific receptors are internalized by the tumor cell after binding to the first proteinaceous molecule according to the invention and/or the second proteinaceous molecule according to the invention, and wherein preferably binding of the first proteinaceous molecule and/or the second proteinaceous molecule to the first and second tumor-cell specific receptors respectively, results in tumor-cell receptor-mediated internalization, e.g. via endocytosis, of a complex of the first proteinaceous molecule and the first tumor-cell specific receptor and of a complex of the second proteinaceous molecule and the second tumor-cell specific receptor.

An embodiment within the second series of aspects and embodiments of the invention is the therapeutic combination or the first pharmaceutical composition according the invention, wherein the

first tumor-cell receptor, preferably the first tumor-cell specific receptor, is internalized by the tumor cell after binding to the first proteinaceous molecule according to the invention and/or after binding to the third proteinaceous molecule according to the invention, and wherein preferably binding of the first proteinaceous molecule and/or the third proteinaceous molecule to the first tumor-cell receptor, such as the first tumor-cell specific receptor, is followed by tumor-cell receptor-mediated internalization, e.g. via endocytosis, of a complex of the first proteinaceous molecule and the first tumor-cell receptor and of a complex of the third proteinaceous molecule and the first tumor-cell receptor.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention, and/or therapeutic combination according to the invention, wherein the first binding site and/or the second binding site is/are or comprise(s) a monoclonal antibody or at least one cell-surface molecule binding fragment and/or –domain thereof, and preferably comprise or consist of any one of cetuximab, daratumumab, gemtuzumab, trastuzumab, panitumumab, brentuximab, inotuzumab, moxetumomab, polatuzumab, obinutuzumab, OKT-9 anti-CD71 monoclonal antibody of the IgG type, pertuzumab, rituximab, ofatumumab, Herceptin, alemtuzumab, pinatuzumab, OKT-10 anti-CD38 monoclonal antibody, and an antibody of Table A4, preferably cetuximab or trastuzumab or OKT-9, or at least one cell-surface molecule binding fragment or -domain thereof, with the proviso that the first binding site of the first proteinaceous molecule is different from the second binding site of the second proteinaceous molecule.

An embodiment within the second series of aspects and embodiments of the invention is the therapeutic combination according to the invention or the first pharmaceutical composition according to the invention, wherein the first binding site of the first proteinaceous molecule and the third proteinaceous molecule comprises a monoclonal antibody or at least one of a cell-surface molecule binding domain and/or -fragment thereof, and preferably comprise or consist of any one of cetuximab, daratumumab, gemtuzumab, trastuzumab, panitumumab, brentuximab, inotuzumab, moxetumomab, polatuzumab, obinutuzumab, OKT-9 anti-CD71 monoclonal antibody of the IgG type, pertuzumab, rituximab, ofatumumab, Herceptin, alemtuzumab, pinatuzumab, OKT-10 anti-CD38 monoclonal antibody, an antibody of Table A2 or Table A3 or Table A4, preferably cetuximab or trastuzumab or OKT-9, or at least one cell-surface molecule binding fragment and/or -domain thereof, with the proviso that the first binding site of the first proteinaceous molecule is the same as the first binding site of the third proteinaceous molecule.

An embodiment within the second series of aspects and embodiments of the invention is the therapeutic combination according to the invention, wherein the second binding site of the second proteinaceous molecule and/or the first binding site of the third proteinaceous molecule is or comprises a monoclonal antibody or at least one cell-surface molecule binding fragment or –domain thereof, and preferably comprises or consists of any one of Gemtuzumab ozogamicin, Brentuximab vedotin, Trastuzumab emtansine, Inotuzumab ozogamicin, Moxetumomab pasudotox and Polatuzumab vedotin and an antibody-drug conjugate of Table A2 and Table A3.

An embodiment within the second series of aspects and embodiments of the invention is the therapeutic combination according to the invention, wherein the effector moiety that is comprised by the second proteinaceous molecule and/or by the third proteinaceous molecule comprises or consists of

any one or more of an oligonucleotide, a nucleic acid, a xeno nucleic acid, preferably selected from any one or more of a vector, a gene, a cell suicide inducing transgene, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), anti-sense oligonucleotide (ASO, AON), short interfering RNA (siRNA), microRNA (miRNA), DNA aptamer, RNA aptamer, mRNA, mini-circle DNA, peptide nucleic acid (PNA), phosphoramidate morpholino oligomer (PMO), locked nucleic acid (LNA), bridged nucleic acid (BNA), 2'-deoxy-2'-fluoroarabino nucleic acid (FANA), 2'-O-methoxyethyl-RNA (MOE), 2'-O,4'-aminoethylene bridged nucleic acid, 3'-fluoro hexitol nucleic acid (FHNA), a plasmid, glycol nucleic acid (GNA) and threose nucleic acid (TNA), or a derivative thereof, more preferably a BNA, for example a BNA for silencing HSP27 protein expression.

An embodiment within the second series of aspects and embodiments of the invention is the therapeutic combination according to the invention, wherein the effector moiety that is comprised by the second proteinaceous molecule and/or by the third proteinaceous molecule comprises or consists of at least one proteinaceous molecule, preferably selected from any one or more of a peptide, a protein, an enzyme such as urease and Cre-recombinase, a ribosome-inactivating protein, a proteinaceous toxin, more preferably selected from any one or more of a protein toxin selected from Table A5 and/or a viral toxin such as apoptin; a bacterial toxin such as Shiga toxin, Shiga-like toxin, Pseudomonas aeruginosa exotoxin (PE) or exotoxin A of PE, full-length or truncated diphtheria toxin (DT), cholera toxin; a fungal toxin such as alpha-sarcin; a plant toxin including ribosome-inactivating proteins and the A chain of type 2 ribosome-inactivating proteins such as dianthin e.g. dianthin-30 or dianthin-32, saporin e.g. saporin-S3 or saporin-S6, bouganin or de-immunized derivative debouganin of bouganin, shiga-like toxin A, pokeweed antiviral protein, ricin, ricin A chain, modeccin, modeccin A chain, abrin, abrin A chain, volkensin, volkensin A chain, viscumin, viscumin A chain; or an animal or human toxin such as frog RNase, or granzyme B or angiogenin from humans, or any fragment or derivative thereof; preferably the protein toxin is dianthin and/or saporin.

An embodiment within the second series of aspects and embodiments of the invention is the therapeutic combination according to the invention, wherein the effector moiety comprised by the second proteinaceous molecule and/or by the third proteinaceous molecule comprises or consists of at least one payload, preferably selected from any one or more of a toxin targeting ribosomes, a toxin targeting elongation factors, a toxin targeting tubulin, a toxin targeting DNA and a toxin targeting RNA, more preferably any one or more of emtansine, pasudotox, maytansinoid derivative DM1, maytansinoid derivative DM4, monomethyl auristatin E (MMAE, vedotin), monomethyl auristatin F (MMAF, mafodotin), a Calicheamicin, N-Acetyl-γ-calicheamicin, a pyrrolobenzodiazepine (PBD) dimer, a benzodiazepine, a CC-1065 analogue, a duocarmycin, Doxorubicin, paclitaxel, docetaxel, cisplatin, cyclophosphamide, etoposide, docetaxel, 5-fluorouracyl (5-FU), mitoxantrone, a tubulysin, an indolinobenzodiazepine, AZ13599185, a cryptophycin, rhizoxin, methotrexate, an anthracycline, a camptothecin analogue, SN-38, DX-8951f, exatecan mesylate, truncated form of *Pseudomonas aeruginosa* exotoxin (PE38), a Duocarmycin derivative, an amanitin, α-amanitin, a spliceostatin, a thailanstatin, ozogamicin, tesirine, Amberstatin269 and soravtansine, or a derivative thereof.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention, wherein the first proteinaceous molecule

comprises more than one saponin, preferably 2, 3, 4, 5, 6, 8, 10, 16, 32, 64 or 1-100 saponins, or any number of saponins therein between, such as 7, 9, 12 saponins, covalently bound directly to an amino-acid residue of the first proteinaceous molecule, preferably to a cysteine and/or to a lysine, and/or covalently bound via at least one linker and/or via at least one cleavable linker and/or via at least one polymeric or oligomeric scaffold, preferably 1-8 of such scaffolds or 2-4 of such scaffolds, wherein the at least one scaffold is optionally based on a dendron, wherein 1-32 saponins such as 2, 3, 4, 5, 6, 8, 10, 16, 32 saponins, or any number of saponins therein between, such as 7, 9, 12 saponins, are covalently bound to the at least one scaffold.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention, wherein the at least one linker is a non-cleavable linker or a cleavable linker, wherein the cleavable linker is for example subject to cleavage under acidic conditions, reductive conditions, enzymatic conditions or light-induced conditions, and preferably the cleavable linker comprises a hydrazone bond or a hydrazide bond subject to cleavage under acidic conditions when bound to saponin, and/or comprises a bond susceptible to proteolysis, for example proteolysis by Cathepsin B, and/or is a bond susceptible for cleavage under reductive conditions such as a disulphide bond, when bound to saponin.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention, wherein the cleavable linker is subject to cleavage *in vivo* under acidic conditions as present in endosomes and/or lysosomes of mammalian cells, preferably human cells, preferably at pH 4.0 – 6.5, and more preferably at pH $\leq$ 5.5, when the cleavable linker is bound to a saponin.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention, wherein the oligomeric or polymeric scaffold comprises a polymeric or oligomeric structure and comprises a chemical group, the chemical group for covalently coupling of the scaffold to the amino-acid residue of said first proteinaceous molecule.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention, wherein the at least one saponin is covalently bound to the polymeric or oligomeric structure of the oligomeric or polymeric scaffold via at least one cleavable linker according to the invention.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention, wherein the chemical group of the oligomeric or polymeric scaffold, for covalently coupling of the oligomeric or polymeric scaffold to the amino-acid residue of said first proteinaceous molecule, is a click chemistry group, preferably selected from a tetrazine, an azide, an alkene or an alkyne, or a cyclic derivative of these groups, more preferably said chemical group is an azide.

An embodiment within the second series of aspects and embodiments of the invention is the first proteinaceous molecule according to the invention, wherein the polymeric or oligomeric structure of the oligomeric or polymeric scaffold comprises a linear, branched and/or cyclic polymer, oligomer, dendrimer, dendron, dendronized polymer, dendronized oligomer, a DNA, a polypeptide, poly-lysine, a

poly-ethylene glycol, or an assembly of these polymeric or oligomeric structures which assembly is preferably built up by covalent cross-linking.

An aspect of the invention within the second series of aspects and embodiments of the invention relates to a composition comprising the first proteinaceous molecule according to the invention and the second proteinaceous molecule according to the invention.

An aspect of the invention within the second series of aspects and embodiments of the invention relates to a composition comprising the first proteinaceous molecule according to the invention and the third proteinaceous molecule according to the invention.

An embodiment within the second series of aspects and embodiments of the invention is the composition according to the invention, wherein the effector moiety that is comprised by the second proteinaceous molecule or by the third proteinaceous molecule is any one of the effector moieties according to the invention, preferably a BNA.

An aspect of the invention within the second series of aspects and embodiments of the invention relates to a composition comprising the first proteinaceous molecule according to the invention and any one or more of an oligonucleotide, a nucleic acid and a xeno nucleic acid, preferably selected from at least one of a vector, a gene, a cell suicide inducing transgene, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), anti-sense oligonucleotide (ASO, AON), short interfering RNA (siRNA), microRNA (miRNA), DNA aptamer, RNA aptamer, mRNA, mini-circle DNA, peptide nucleic acid (PNA), phosphoramidate morpholino oligomer (PMO), locked nucleic acid (LNA), bridged nucleic acid (BNA), 2'-deoxy-2'-fluoroarabino nucleic acid (FANA), 2'-O-methoxyethyl-RNA (MOE), 2'-O,4'-aminoethylene bridged nucleic acid, 3'-fluoro hexitol nucleic acid (FHNA), a plasmid, glycol nucleic acid (GNA) and threose nucleic acid (TNA), or a derivative thereof, more preferably a BNA, for example a BNA for silencing HSP27 protein expression.

An aspect of the invention within the second series of aspects and embodiments of the invention relates to an antibody-drug conjugate or a ligand-drug conjugate comprising the first proteinaceous molecule according to the invention and an effector moiety.

An embodiment within the second series of aspects and embodiments of the invention is the antibody-drug conjugate or the ligand-drug conjugate according to the invention, wherein the antibody can bind to any one of CD71, CA125, EpCAM(17-1A), CD52, CEA, CD44v6, FAP, EGF-IR, integrin, syndecan-1, vascular integrin alpha-V beta-3, HER2, EGFR, CD20, CD22, Folate receptor 1, CD146, CD56, CD19, CD138, CD27L receptor, PSMA, CanAg, integrin-alphaV, CA6, CD33, mesothelin, Cripto, CD3, CD30, CD239, CD70, CD123, CD352, DLL3, CD25, ephrinA4, MUC1, Trop2, CEACAM5, CEACAM6, HER3, CD74, PTK7, Notch3, FGF2, C4.4A, FLT3, CD38, FGFR3, CD7, PD-L1, CTLA4, CD52, PDGFRA, VEGFR1, VEGFR2, preferably CD71, HER2, EGFR, and/or is or comprises any one of cetuximab, daratumumab, gemtuzumab, trastuzumab, panitumumab, brentuximab, inotuzumab, moxetumomab, polatuzumab, obinutuzumab, OKT-9 anti-CD71 monoclonal antibody of the IgG type, pertuzumab, rituximab, ofatumumab, Herceptin, alemtuzumab, pinatuzumab, OKT-10 anti-CD38 monoclonal antibody, an antibody of Table A2 or Table A3 or Table A4, preferably cetuximab or trastuzumab or OKT-9, or at least one tumor-cell receptor binding-fragment thereof and/or at least one tumor-cell receptor binding-domain thereof, and/or wherein the antibody-drug conjugate comprises any

one of Gemtuzumab ozogamicin, Brentuximab vedotin, Trastuzumab emtansine, Inotuzumab ozogamicin, Moxetumomab pasudotox and Polatuzumab vedotin and an antibody-drug conjugate of Table A2 and Table A3, or wherein the ligand-drug conjugate comprises at least one ligand for binding to a cell-surface molecule such as EGF or a cytokine.

An embodiment within the second series of aspects and embodiments of the invention is the antibody-drug conjugate or the ligand-drug conjugate according to the invention, wherein the effector moiety is any one or more of the effector moieties according to the invention.

An aspect of the invention within the second series of aspects and embodiments of the invention relates to a pharmaceutical composition comprising the composition according to the invention or the antibody-drug conjugate according to the invention or the ligand-drug conjugate according to the invention, and optionally further comprising a pharmaceutically acceptable excipient.

An aspect of the invention within the second series of aspects and embodiments of the invention relates to the therapeutic combination or the composition or the antibody-drug conjugate or the ligand-drug conjugate or the pharmaceutical composition according to the invention, for use as a medicament.

An aspect of the invention within the second series of aspects and embodiments of the invention relates to the therapeutic combination or the composition or the antibody-drug conjugate or the ligand-drug conjugate or the pharmaceutical composition according to the invention, for use in the treatment or prevention of a cancer or an autoimmune disease.

An aspect of the invention within a third series of aspects and embodiments of the invention relates to an effector moiety capable of inducing an intracellular effect when present inside a mammalian cell, the effector moiety conjugated with at least one saponin, wherein the at least one saponin is covalently bound to the effector moiety via at least one linker, or is covalently bound directly to said effector moiety.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, comprising or consisting of at least one oligonucleotide, a nucleic acid, a xeno nucleic acid, preferably selected from any one or more of a vector, a gene, a cell suicide inducing transgene, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), anti-sense oligonucleotide (ASO, AON), short interfering RNA (siRNA), microRNA (miRNA), DNA aptamer, RNA aptamer, mRNA, mini-circle DNA, peptide nucleic acid (PNA), phosphoramidate morpholino oligomer (PMO), locked nucleic acid (LNA), bridged nucleic acid (BNA), 2'-deoxy-2'-fluoroarabino nucleic acid (FANA), 2'-O-methoxyethyl-RNA (MOE), 2'-O,4'-aminoethylene bridged nucleic acid, 3'-fluoro hexitol nucleic acid (FHNA), a plasmid, glycol nucleic acid (GNA) and threose nucleic acid (TNA), or a derivative thereof, more preferably a BNA, for example a BNA for silencing HSP27 protein expression.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, comprising at least one proteinaceous molecule, the proteinaceous molecule preferably selected from any one or more of a peptide, a protein, an enzyme such as urease and Cre-recombinase, a ribosome-inactivating protein, a proteinaceous toxin such as any one or more of a protein toxin selected from Table A5 and/or a bacterial toxin or plant toxin, more preferably selected from any one or more of a viral toxin such as apoptin; a bacterial toxin such as Shiga toxin, Shiga-like toxin, Pseudomonas aeruginosa exotoxin (PE) or exotoxin A of PE, full-length or

truncated diphtheria toxin (DT), cholera toxin; a fungal toxin such as alpha-sarcin; a plant toxin including ribosome-inactivating proteins and the A chain of type 2 ribosome-inactivating proteins such as dianthin e.g. dianthin-30 or dianthin-32, saporin e.g. saporin-S3 or saporin-S6, bouganin or de-immunized derivative debouganin of bouganin, shiga-like toxin A, pokeweed antiviral protein, ricin, ricin A chain, modeccin, modeccin A chain, abrin, abrin A chain, volkensin, volkensin A chain, viscumin, viscumin A chain; or an animal or human toxin such as frog RNase, or granzyme B or angiogenin from humans, or any fragment or derivative thereof; preferably the protein toxin is dianthin and/or saporin.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, comprising at least one payload, the payload preferably selected from any one or more of a toxin targeting ribosomes, a toxin targeting elongation factors, a toxin targeting tubulin, a toxin targeting DNA and a toxin targeting RNA, more preferably any one or more of emtansine, pasudotox, maytansinoid derivative DM1, maytansinoid derivative DM4, monomethyl auristatin E (MMAE, vedotin), monomethyl auristatin F (MMAF, mafodotin), a Calicheamicin, N-Acetyl-γ-calicheamicin, a pyrrolobenzodiazepine (PBD) dimer, a benzodiazepine, a CC-1065 analogue, a duocarmycin, Doxorubicin, paclitaxel, docetaxel, cisplatin, cyclophosphamide, etoposide, docetaxel, 5-fluorouracyl (5-FU), mitoxantrone, a tubulysin, an indolinobenzodiazepine, AZ13599185, a cryptophycin, rhizoxin, methotrexate, an anthracycline, a camptothecin analogue, SN-38, DX-8951f, exatecan mesylate, truncated form of *Pseudomonas aeruginosa* exotoxin (PE38), a Duocarmycin derivative, an amanitin, α-amanitin, a spliceostatin, a thailanstatin, ozogamicin, tesirine, Amberstatin269 and soravtansine, or a derivative thereof.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the at least one saponin is a triterpenoid saponin and/or a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, and/or a saponin isolated from *a Gypsophila* species and/or a *Saponaria* species and/or an *Agrostemma* species and/or a *Quillaja* species such as *Quillaja saponaria*.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the at least one saponin is a single specific saponin or is a mixture of two or more different saponins.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the saponin is one or more of the saponins in Table A1 or Scheme I, SO1861, SA1657, GE1741, SA1641, QS-21, QS-21A, QS-21 A-api, QS-21 A-xyl, QS-21B, QS-21 B-api, QS-21 B-xyl, QS-7-xyl, QS-7-api, QS-17-api, QS-17-xyl, QS1861, QS1862, Quillajasaponin, Saponinum album, QS-18, Quil-A, Gyp1, gypsoside A, AG1, AG2, SO1542, SO1584, SO1658, SO1674, SO1832, or any of their stereomers and/or any combinations thereof, preferably the saponin is SO1861 and/or GE1741 and/or SA1641 and/or QS-21 and/or saponin with a quillaic acid aglycon core, a Gal-(1→2)-[Xyl-(1→3)]-GlcA carbohydrate substituent at the C-3beta-OH group and a Glc-(1→3)-Xyl-(1→4)-Rha-(1→2)-[Xyl-(1→3)-4-OAc-Qui-(1→4)]-Fuc carbohydrate substituent at the C-28-OH group, and/or is 3-O-beta-D-galactopyranosyl-(1→2)-[beta-D-xylopyranosyl-(1→3)]-beta-

D-glucuronopyranosyl quillaic acid 28-O-beta-D-glucopyranosyl-(1→3)-beta-D-xylopyranosyl-(1→4)- alpha-L-rhamnopyranosyl-(1→2)-[beta-D-xylopyranosyl-(1→3)-4-OAc-beta-D-quinovopyranosyl-(1→4)]-beta-D-fucopyranoside, more preferably the saponin is SO1861 and/or QS-21.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the saponin is a bisdesmosidic saponin having a molecular mass of at least 1.500 Dalton and comprising an oleanan-type triterpene containing an aldehyde group at the C-23 position and optionally a hydroxyl group at the C-16 position, with a first branched carbohydrate side chain at the C-3 position which first branched carbohydrate side chain optionally contains glucuronic acid, wherein the saponin contains an ester group with a second branched carbohydrate side chain at the C-28 position which second branched carbohydrate chain preferably comprises at least four carbohydrate units and optionally contains at least one acetyl residue such as two acetyl residues and/or optionally comprises one or more deoxy carbohydrates and/or quinovose and/or glucose and/or 4-methoxycinnamic acid and/or optionally comprising 5-O-[5-O-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid and/or optionally comprising 5-O-[5-O-Rha-(1→2)-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid bound to a carbohydrate via an ester bond, or wherein the at least one saponin is QS-21 or any one or more of QS-21A, QS-21 A-api, QS-21 A-xyl, QS-21B, QS-21 B-api, QS-21 B-xyl, QS-7-xyl, QS-7-api, QS-17-api, QS-17-xyl, QS-18, QS1861, protonated QS1861 (QS1862), Quil-A.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the at least one saponin is a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23, wherein the at least one saponin is covalently coupled to an amino-acid residue, when present, of the effector moiety via an aldehyde function in the saponin, preferably said aldehyde function in position C-23, preferably via at least one linker, more preferably via at least one cleavable linker, wherein the amino-acid residue preferably is selected from cysteine and lysine.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the at least one saponin is a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, wherein the at least one saponin is covalently coupled to an amino-acid residue, when present, of the effector moiety via the glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the saponin, preferably via at least one linker, wherein the amino-acid residue preferably is selected from cysteine and lysine.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the at least one linker comprises at least one non-cleavable linker and/or at least one cleavable linker, wherein optionally said cleavable linker is subject to cleavage under acidic, reductive, enzymatic or light-induced conditions, and preferably the cleavable linker comprises a cleavable bond selected from a hydrazone bond and a hydrazide bond subject to cleavage under acidic conditions, and/or a bond susceptible to proteolysis, for example proteolysis by

Cathepsin B, and/or a bond susceptible for cleavage under reductive conditions such as a disulphide bond.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the at least one linker comprises at least one cleavable linker which is subject to cleavage *in vivo* under acidic conditions as present in endosomes and/or in lysosomes of mammalian cells, preferably of human cells, preferably at pH 4.0 – 6.5, and more preferably at pH $\leq$ 5.5.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the at least one saponin is covalently bound to a lysine side chain, forming an amide bond, and/or to a cysteine side chain, forming a thio-ether linkage, or a disulphide bond, wherein the lysine and/or cysteine is/are comprised by the effector moiety, and wherein the saponin is bound either directly to the effector moiety, or bound via at least one linker.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the at least one saponin is covalently bound to the effector moiety via at least one linker, wherein the linker is or comprises a scaffold comprising a polymeric or oligomeric structure and a chemical group for covalently coupling of the scaffold to the effector moiety.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the at least one saponin is covalently bound to the polymeric or oligomeric structure of the scaffold via a cleavable bond.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the cleavable bond is subject to cleavage under any of acidic conditions, reductive conditions, enzymatic conditions and light-induced conditions, more preferably the cleavable bond is a hydrazone bond or a hydrazide bond subject to cleavage under acidic conditions, and/or is a bond susceptible to proteolysis, for example proteolysis by Cathepsin B, and/or is a bond susceptible for cleavage under reductive conditions such as a disulphide bond.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the cleavable bond is subject to cleavage *in vivo* under acidic conditions as present in endosomes and/or in lysosomes of mammalian cells, preferably of human cells, preferably at pH 4.0 – 6.5, and more preferably at pH $\leq$ 5.5.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the at least one saponin is covalently bound to the polymeric or oligomeric structure of the scaffold via an imine bond, a hydrazone bond, a hydrazide bond, an oxime bond, a 1,3-dioxolane bond, a disulphide bond, a thio-ether bond, an amide bond, a peptide bond and/or an ester bond, preferably via at least one linker, preferably an amide bond, a hydrazide bond, a thio-ether bond and/or a hydrazone bond.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the at least one saponin is covalently bound to the polymeric or oligomeric structure of the scaffold, involving in the covalent bond the aldehyde function in position C-23 of the at least one saponin, when present, the covalent bond being preferably an imine

bond or a hydrazone bond or an amide bond or a thio-ether bond or a disulphide bond, and/or involving in the covalent bond the glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the at least one saponin, when present, wherein preferably the covalent bond is an amide bond or a disulphide bond or a thio-ether bond.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the aldehyde function in position C-23 of the at least one saponin is covalently coupled to linker N-ε-maleimidocaproic acid hydrazide, which linker is covalently coupled via a thio-ether bond to a sulfhydryl group in the polymeric or oligomeric structure of the scaffold, such as a sulfhydryl group of a cysteine.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the at least one saponin is covalently coupled to linker 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate, which linker is covalently coupled via an amide bond to an amine group in the polymeric or oligomeric structure of the scaffold, such as an amine group of a lysine or an N-terminus of the polymeric or oligomeric structure of the scaffold.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the chemical group of the scaffold, for covalently coupling of the scaffold to the effector moiety, is a click chemistry group, preferably selected from a tetrazine, an azide, an alkene or an alkyne, or a cyclic derivative of these groups, more preferably the click chemistry group is an azide.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the polymeric or oligomeric structure of the scaffold comprises a linear, branched and/or cyclic polymer, oligomer, dendrimer, dendron, dendronized polymer, dendronized oligomer, a DNA, a polypeptide, poly-lysine, a poly-ethylene glycol, or an assembly of these polymeric or oligomeric structures which assembly is preferably built up by covalent cross-linking.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the at least one saponin is a defined number of saponins or a defined range of saponins, preferably 1-128 saponins or at least 2, 3, 4, 5, 6, 8, 10, 16, 32, 64 or 128 saponins, or any number of saponins therein between, such as 7, 9, 12 saponins.

An embodiment within the third series of aspects and embodiments of the invention is the effector moiety according to the invention, wherein the effector moiety comprises more than one saponin, preferably 2, 3, 4, 5, 6, 8, 10, 16, 32, 64 or 1-100 saponins, or any number of saponins therein between, such as 7, 9, 12 saponins, covalently bound directly to an amino-acid residue of the effector moiety, preferably to a cysteine and/or to a lysine, and/or covalently bound via at least one linker and/or via at least one cleavable linker and/or via at least one polymeric or oligomeric scaffold of any one of the claims 14-23, preferably 1-8 of such scaffolds or 2-4 of such scaffolds, wherein 1-32 saponins, preferably 2, 3, 4, 5, 6, 8, 10, 16 or 32 saponins, are covalently bound to the at least one scaffold.

An aspect of the invention within the third series of aspects and embodiments of the invention relates to an antibody-drug conjugate comprising the effector moiety according to the invention, or a ligand-drug conjugate comprising the effector moiety according to the invention.

An embodiment within the third series of aspects and embodiments of the invention is the antibody-drug conjugate or the ligand-drug conjugate according to the invention, wherein the antibody can bind to any one of CD71, CA125, EpCAM(17-1A), CD52, CEA, CD44v6, FAP, EGF-IR, integrin, syndecan-1, vascular integrin alpha-V beta-3, HER2, EGFR, CD20, CD22, Folate receptor 1, CD146, CD56, CD19, CD138, CD27L receptor, PSMA, CanAg, integrin-alphaV, CA6, CD33, mesothelin, Cripto, CD3, CD30, CD239, CD70, CD123, CD352, DLL3, CD25, ephrinA4, MUC1, Trop2, CEACAM5, CEACAM6, HER3, CD74, PTK7, Notch3, FGF2, C4.4A, FLT3, CD38, FGFR3, CD7, PD-L1, CTLA4, CD52, PDGFRA, VEGFR1, VEGFR2, preferably CD71, HER2, EGFR, and/or wherein the antibody of the antibody-drug conjugate is or comprises any one of cetuximab, daratumumab, gemtuzumab, trastuzumab, panitumumab, brentuximab, inotuzumab, moxetumomab, polatuzumab, obinutuzumab, OKT-9 anti-CD71 monoclonal antibody of the IgG type, pertuzumab, rituximab, ofatumumab, Herceptin, alemtuzumab, pinatuzumab, OKT-10 anti-CD38 monoclonal antibody, an antibody of Table A2 or Table A3 or Table A4, preferably cetuximab or trastuzumab or OKT-9, or at least one tumor-cell specific receptor binding-fragment thereof and/or at least one tumor-cell specific receptor binding-domain thereof, and/or wherein the antibody-drug conjugate comprises any one of Gemtuzumab ozogamicin, Brentuximab vedotin, Trastuzumab emtansine, Inotuzumab ozogamicin, Moxetumomab pasudotox and Polatuzumab vedotin and an antibody-drug conjugate of Table A2 and Table A3, and/or wherein the ligand-drug conjugate comprises or consists of at least one non-proteinaceous ligand and/or at least one proteinaceous ligand for binding to a cell-surface molecule such as EGF or a cytokine.

An aspect of the invention within the third series of aspects and embodiments of the invention relates to a therapeutic combination comprising: (a) the effector moiety according to the invention and optionally a pharmaceutically acceptable excipient; and (b) an antibody-drug conjugate or a ligand-drug conjugate, and optionally a pharmaceutically acceptable excipient.

An embodiment within the third series of aspects and embodiments of the invention is the therapeutic combination according to the invention, wherein the antibody-drug conjugate can bind to any one of tumor-cell receptors CD71, CA125, EpCAM(17-1A), CD52, CEA, CD44v6, FAP, EGF-IR, integrin, syndecan-1, vascular integrin alpha-V beta-3, HER2, EGFR, CD20, CD22, Folate receptor 1, CD146, CD56, CD19, CD138, CD27L receptor, PSMA, CanAg, integrin-alphaV, CA6, CD33, mesothelin, Cripto, CD3, CD30, CD239, CD70, CD123, CD352, DLL3, CD25, ephrinA4, MUC1, Trop2, CEACAM5, CEACAM6, HER3, CD74, PTK7, Notch3, FGF2, C4.4A, FLT3, CD38, FGFR3, CD7, PD-L1, CTLA4, CD52, PDGFRA, VEGFR1, VEGFR2, preferably CD71, HER2, EGFR, and/or wherein the antibody of the antibody-drug conjugate is or comprises any one of cetuximab, daratumumab, gemtuzumab, trastuzumab, panitumumab, brentuximab, inotuzumab, moxetumomab, polatuzumab, obinutuzumab, OKT-9 anti-CD71 monoclonal antibody of the IgG type, pertuzumab, rituximab, ofatumumab, Herceptin, alemtuzumab, pinatuzumab, OKT-10 anti-CD38 monoclonal antibody, an antibody of Table A2 or Table A3 or Table A4, preferably cetuximab or trastuzumab or OKT-9, or at least one tumor-cell specific receptor binding-fragment thereof and/or at least one tumor-cell specific

receptor binding-domain thereof, and/or wherein the antibody-drug conjugate comprises any one of Gemtuzumab ozogamicin, Brentuximab vedotin, Trastuzumab emtansine, Inotuzumab ozogamicin, Moxetumomab pasudotox and Polatuzumab vedotin and an antibody-drug conjugate of Table A2 and Table A3, and/or wherein the ligand-drug conjugate comprises or consists of at least one non-proteinaceous ligand and/or at least one proteinaceous ligand for binding to a cell-surface molecule such as EGF or a cytokine.

An aspect of the invention within the third series of aspects and embodiments of the invention relates to a pharmaceutical composition comprising the effector moiety according to the invention or the antibody-drug conjugate according to the invention or the ligand-drug conjugate according to the invention, and optionally a pharmaceutically acceptable excipient.

An aspect of the invention within the third series of aspects and embodiments of the invention relates to the effector moiety according to the invention or the antibody-drug conjugate according to the invention or the therapeutic combination according to the invention or the ligand-drug conjugate according to the invention or the pharmaceutical composition according to the invention, for use as a medicament.

An aspect of the invention within the third series of aspects and embodiments of the invention relates to the effector moiety according to the invention or the antibody-drug conjugate according to the invention or the ligand-drug conjugate according to the invention or the therapeutic combination according to the invention or the pharmaceutical composition according to the invention, for use in the treatment or prevention of a cancer or an autoimmune disease.

An aspect of the invention within a fourth series of aspects and embodiments of the invention relates to a conjugate comprising a cell-surface molecule targeting molecule and at least one effector moiety and further comprising at least one covalently bound saponin.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one saponin is a triterpenoid saponin and/or a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, and/or a saponin isolated from any one or more of a *Gypsophila* species and/or a *Saponaria* species and/or an *Agrostemma* species and/or a *Quillaja* species such as *Quillaja saponaria*.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one saponin is a single specific saponin or is a mixture of two or more different saponins.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one saponin has a molecular mass of 3.000 Dalton or less, preferably 2.500 Dalton or less, more preferably 2.300 Dalton or less, most preferably, 2.000 Dalton or less, such as 1.500 Dalton – 1.900 Dalton.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one saponin is one or more of the saponins in Table A1 or Scheme I, SO1861, SA1657, GE1741, SA1641, QS-21, QS-21A, QS-21 A-api, QS-21 A-xyl, QS-21B,

QS-21 B-api, QS-21 B-xyl, QS-7-xyl, QS-7-api, QS-17-api, QS-17-xyl, QS1861, QS1862, Quillajasaponin, Saponinum album, QS-18, Quil-A, Gyp1, gypsoside A, AG1, AG2, SO1542, SO1584, SO1658, SO1674, SO1832, or any of their stereomers and/or any combinations thereof, preferably the saponin is SO1861 and/or GE1741 and/or SA1641 and/or QS-21 and/or saponin with a quillaic acid aglycon core, a Gal-(1→2)-[Xyl-(1→3)]-GlcA carbohydrate substituent at the C-3beta-OH group and a Glc-(1→3)-Xyl-(1→4)-Rha-(1→2)-[Xyl-(1→3)-4-OAc-Qui-(1→4)]-Fuc carbohydrate substituent at the C-28-OH group, and/or is 3-O-beta-D-galactopyranosyl-(1→2)-[beta-D-xylopyranosyl-(1→3)]-beta-D-glucuronopyranosyl quillaic acid 28-O-beta-D-glucopyranosyl-(1→3)-beta-D-xylopyranosyl-(1→4)- alpha-L-rhamnopyranosyl-(1→2)-[beta-D-xylopyranosyl-(1→3)-4-OAc-beta-D-quinovopyranosyl-(1→4)]-beta-D-fucopyranoside, more preferably the at least one saponin is SO1861 and/or QS-21.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one saponin is a bisdesmosidic saponin having a molecular mass of at least 1.500 Dalton and comprising an oleanan-type triterpene containing an aldehyde group at the C-23 position and optionally a hydroxyl group at the C-16 position, with a first branched carbohydrate side chain at the C-3 position which first branched carbohydrate side chain optionally contains glucuronic acid, wherein the saponin contains an ester group with a second branched carbohydrate side chain at the C-28 position which second branched carbohydrate chain preferably comprises at least four carbohydrate units, optionally containing at least one acetyl residue such as two acetyl residues and/or optionally comprising deoxy carbohydrates and/or optionally comprising quinovose and/or optionally comprising glucose and/or optionally comprising 4-methoxycinnamic acid and/or optionally comprising 5-O-[5-O-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid and/or optionally comprising 5-O-[5-O-Rha-(1→2)-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid bound to a carbohydrate via an ester bond, or wherein the at least one saponin is QS-21 or any one or more of QS-21A, QS-21 A-api, QS-21 A-xyl, QS-21B, QS-21 B-api, QS-21 B-xyl, QS-7-xyl, QS-7-api, QS-17-api, QS-17-xyl, QS-18, QS1861, protonated QS1861 (QS1862), Quil-A.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the cell-surface molecule targeting molecule comprises or consists of a ligand or a proteinaceous ligand or a proteinaceous binding molecule for binding to the cell-surface molecule.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the cell-surface molecule targeting molecule comprises or consists of a non-proteinaceous ligand and/or a proteinaceous ligand for binding to a cell-surface molecule such as EGF or a cytokine, and/or comprises or consists of an immunoglobulin, at least one binding domain of an immunoglobulin and/or at least one binding fragment of an immunoglobulin, such as an antibody, an IgG, a molecule comprising or consisting of a Vhh domain or Vh domain, a Fab, an scFv, an Fv, a dAb, an $F(ab)_2$, Fcab fragment, which can bind to the cell-surface molecule.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the cell-surface molecule targeting molecule can bind to a tumor-

cell surface molecule, preferably a tumor-cell receptor such as a tumor-cell specific receptor, more preferably a receptor selected from CD71, CA125, EpCAM(17-1A), CD52, CEA, CD44v6, FAP, EGF-IR, integrin, syndecan-1, vascular integrin alpha-V beta-3, HER2, EGFR, CD20, CD22, Folate receptor 1, CD146, CD56, CD19, CD138, CD27L receptor, PSMA, CanAg, integrin-alphaV, CA6, CD33, mesothelin, Cripto, CD3, CD30, CD239, CD70, CD123, CD352, DLL3, CD25, ephrinA4, MUC1, Trop2, CEACAM5, CEACAM6, HER3, CD74, PTK7, Notch3, FGF2, C4.4A, FLT3, CD38, FGFR3, CD7, PD-L1, CTLA4, CD52, PDGFRA, VEGFR1, VEGFR2, preferably selected from CD71, HER2 and EGFR.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the tumor-cell receptor is internalized by the tumor cell after binding to the cell-surface molecule targeting molecule of the invention and the conjugate of the invention, and wherein preferably binding of the conjugate to the tumor-cell receptor is followed by tumor-cell receptor-mediated internalization, e.g. via endocytosis, of a complex of the conjugate and the tumor-cell receptor.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the cell-surface molecule targeting molecule is or comprises a monoclonal antibody or at least one cell-surface molecule binding fragment or -domain thereof, and preferably comprises or consists of any one of cetuximab, daratumumab, gemtuzumab, trastuzumab, panitumumab, brentuximab, inotuzumab, moxetumomab, polatuzumab, obinutuzumab, OKT-9 anti-CD71 monoclonal antibody of the IgG type, pertuzumab, rituximab, ofatumumab, Herceptin, alemtuzumab, pinatuzumab, OKT-10 anti-CD38 monoclonal antibody, an antibody of Table A2 or Table A3 or Table A4, preferably cetuximab or trastuzumab or OKT-9, or at least one cell-surface molecule binding fragment or -domain thereof.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one effector moiety comprises or consists of any one or more of an oligonucleotide, a nucleic acid and a xeno nucleic acid, preferably selected from any one or more of a vector, a gene, a cell suicide inducing transgene, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), anti-sense oligonucleotide (ASO, AON), short interfering RNA (siRNA), microRNA (miRNA), DNA aptamer, RNA aptamer, mRNA, mini-circle DNA, peptide nucleic acid (PNA), phosphoramidate morpholino oligomer (PMO), locked nucleic acid (LNA), bridged nucleic acid (BNA), 2'-deoxy-2'-fluoroarabino nucleic acid (FANA), 2'-O-methoxyethyl-RNA (MOE), 2'-O,4'-aminoethylene bridged nucleic acid, 3'-fluoro hexitol nucleic acid (FHNA), a plasmid, glycol nucleic acid (GNA) and threose nucleic acid (TNA), or a derivative thereof, more preferably a BNA, for example a BNA for silencing HSP27 protein expression.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one effector moiety comprises or consists of at least one proteinaceous molecule, preferably selected from any one or more of a peptide, a protein, an enzyme such as urease and Cre-recombinase, a ribosome-inactivating protein, a proteinaceous toxin selected from Table A5 and more preferably selected from any one or more of a viral toxin such as apoptin; a bacterial toxin such as Shiga toxin, Shiga-like toxin, Pseudomonas aeruginosa exotoxin (PE) or exotoxin A of PE, full-length or truncated diphtheria toxin (DT), cholera toxin; a fungal toxin such as alpha-sarcin; a plant toxin including ribosome-inactivating proteins and the A chain of type 2 ribosome-inactivating

proteins such as dianthin e.g. dianthin-30 or dianthin-32, saporin e.g. saporin-S3 or saporin-S6, bouganin or de-immunized derivative debouganin of bouganin, shiga-like toxin A, pokeweed antiviral protein, ricin, ricin A chain, modeccin, modeccin A chain, abrin, abrin A chain, volkensin, volkensin A chain, viscumin, viscumin A chain; or an animal or human toxin such as frog RNase, or granzyme B or angiogenin from humans, or any fragment or derivative thereof; preferably the protein toxin is dianthin and/or saporin.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one effector moiety comprises or consists of at least one payload, preferably selected from any one or more of a toxin targeting ribosomes, a toxin targeting elongation factors, a toxin targeting tubulin, a toxin targeting DNA and a toxin targeting RNA, more preferably any one or more of emtansine, pasudotox, maytansinoid derivative DM1, maytansinoid derivative DM4, monomethyl auristatin E (MMAE, vedotin), monomethyl auristatin F (MMAF, mafodotin), a Calicheamicin, N-Acetyl-γ-calicheamicin, a pyrrolobenzodiazepine (PBD) dimer, a benzodiazepine, a CC-1065 analogue, a duocarmycin, Doxorubicin, paclitaxel, docetaxel, cisplatin, cyclophosphamide, etoposide, docetaxel, 5-fluorouracyl (5-FU), mitoxantrone, a tubulysin, an indolinobenzodiazepine, AZ13599185, a cryptophycin, rhizoxin, methotrexate, an anthracycline, a camptothecin analogue, SN-38, DX-8951f, exatecan mesylate, truncated form of *Pseudomonas aeruginosa* exotoxin (PE38), a Duocarmycin derivative, an amanitin, α-amanitin, a spliceostatin, a thailanstatin, ozogamicin, tesirine, Amberstatin269 and soravtansine, or a derivative thereof.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one effector moiety is covalently bound to the cell-surface molecule targeting molecule, either via at least one linker or bound directly to the cell-surface molecule targeting molecule.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one effector moiety is covalently bound to the cell-surface molecule targeting molecule, thereby forming any one of antibody-drug conjugates Gemtuzumab ozogamicin, Brentuximab vedotin, Trastuzumab emtansine, Inotuzumab ozogamicin, Moxetumomab pasudotox and Polatuzumab vedotin and an antibody-drug conjugate of Table A2 and Table A3.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one saponin is covalently bound to the cell-surface molecule targeting molecule preferably an amino-acid residue of the cell-surface molecule targeting molecule, via an aldehyde function in the saponin, and/or to the at least one effector moiety preferably via an amino-acid residue in the at least one effector moiety, via an aldehyde function in the saponin, preferably an aldehyde function in position C-23 in a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the aldehyde function in the at least one saponin, preferably the aldehyde function in position C-23 of the at least one saponin, is covalently coupled to linker N-ε-maleimidocaproic acid hydrazide, which linker is covalently coupled via a thio-ether bond to a sulfhydryl

group in the cell-surface molecule targeting molecule and/or in the at least one effector moiety, such as a sulfhydryl group of a cysteine.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one saponin is a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane, with an aldehyde function in position C-23 and comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, wherein the saponin is covalently bound to an amino-acid residue of the cell-surface molecule targeting molecule and/or to the at least one effector moiety via said glucuronic acid function and preferably via an amino-acid residue in the at least one effector moiety.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the at least one saponin is covalently coupled to linker 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate, which linker is covalently coupled via an amide bond to an amine group in the cell-surface molecule targeting molecule and/or in the at least one effector moiety, such as an amine group of a lysine or an N-terminus of the cell-surface molecule targeting molecule and/or of the at least one effector moiety.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one saponin is covalently bound to the cell-surface molecule targeting molecule and/or to the at least one effector moiety either directly or via at least one linker such as a bi-functional linker, for example based on N-ε-maleimidocaproic acid hydrazide and/or based on 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate, or a tri-functional linker, such as the tri-functional linker of Scheme II and Structure B.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the tri-functional linker comprises a second chemical group with at least one saponin covalently bound thereto, a third chemical group for covalent binding to the cell-surface molecule targeting molecule and a first chemical group for covalent binding to the at least one effector moiety, preferably the tri-functional linker is the trifunctional linker of Scheme II and Structure B.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one saponin is covalently bound to the cell-surface molecule targeting molecule and to the at least one effector moiety via at least one linker comprising a tri-functional linker to which tri-functional linker both the cell-surface molecule targeting molecule and the at least one effector moiety are bound, preferably the tri-functional linker is the trifunctional linker of Scheme II and Structure B.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one linker comprises at least one cleavable linker, wherein optionally said cleavable linker is subject to cleavage under acidic, reductive, enzymatic or light-induced conditions, and preferably the cleavable linker comprises a cleavable bond selected from a hydrazone bond or a hydrazide bond subject to cleavage under acidic conditions, and/or a bond

susceptible to proteolysis, for example proteolysis by Cathepsin B, and/or a bond susceptible for cleavage under reductive conditions such as a disulphide bond.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one linker comprises at least one cleavable linker, wherein said cleavable linker is subject to cleavage *in vivo* under acidic conditions as present in endosomes and/or in lysosomes of mammalian cells, preferably of human cells, preferably at pH 4.0 – 6.5, and more preferably at pH ≤ 5.5.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one saponin is covalently bound to a lysine side chain, forming an amide bond, and/or to a cysteine side chain, forming a thio-ether linkage or a disulphide bond, wherein the lysine and/or cysteine is/are comprised by the cell-surface molecule targeting molecule and/or is/are comprised by the at least one effector moiety, and wherein the at least one saponin is either directly bound to the lysine and/or cysteine, or is bound via at least one linker optionally comprising a cleavable linker and/or a tri-functional linker such as the tri-functional linker of Scheme II and Structure B.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the linker is based on N-ε-maleimidocaproic acid hydrazide and/or based on 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate, a tri-functional linker such as the tri-functional linker of Scheme II and Structure B, a cleavable linker, and/or involves any one or more of a disulphide bond, a thio-ether bond, an amide bond, a hydrazide bond.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one saponin is covalently bound to the cell-surface molecule targeting molecule and/or to the at least one effector moiety via at least one linker, wherein the linker is or comprises a scaffold comprising a polymeric or oligomeric structure and further comprising at least one fourth chemical group for covalently coupling of the scaffold to the cell-surface molecule targeting molecule and/or to the at least one effector moiety.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one saponin is covalently bound to the polymeric or oligomeric structure of the scaffold via a cleavable bond and/or via a non-cleavable bond.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the cleavable bond is subject to cleavage under any of acidic conditions, reductive conditions, enzymatic conditions and light-induced conditions, and preferably the cleavable bond comprises a hydrazone bond or a hydrazide bond subject to cleavage under acidic conditions, and/or a bond susceptible to proteolysis, for example proteolysis by Cathepsin B, and/or a bond susceptible for cleavage under reductive conditions such as a disulphide bond.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the cleavable bond is subject to cleavage *in vivo* under acidic conditions as present in endosomes and/or in lysosomes of mammalian cells, preferably of human cells, preferably at pH 4.0 – 6.5, and more preferably at pH ≤ 5.5.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one saponin is covalently bound to the polymeric or oligomeric structure of the scaffold via any one or more of an imine bond, a hydrazone bond, a hydrazide bond, an oxime bond, a 1,3-dioxolane bond, a disulphide bond, a thio-ether bond, an amide bond, a peptide bond or an ester bond, preferably via at least one linker.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one saponin is covalently bound to the polymeric or oligomeric structure of the scaffold via any one or more of an imine bond, a hydrazone bond and a hydrazide bond, which bond is preferably cleavable according to the invention, wherein preferably the at least one saponin is covalently bound to the polymeric or oligomeric structure of the scaffold via the aldehyde function in position C-23 of the at least one saponin.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the aldehyde function in position C-23 of the at least one saponin is covalently coupled to linker N-ε-maleimidocaproic acid hydrazide, which linker is covalently coupled via a thio-ether bond to a sulfhydryl group in the polymeric or oligomeric structure of the scaffold, such as a sulfhydryl group of a cysteine.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one saponin is covalently bound to the polymeric or oligomeric structure of the scaffold via an amide bond, wherein preferably the at least one saponin is covalently bound to the polymeric or oligomeric structure of the scaffold via the glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the at least one saponin, when present.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the at least one saponin is covalently coupled to linker 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate, which linker is covalently coupled via an amide bond to an amine group in the polymeric or oligomeric structure of the scaffold, such as an amine group of a lysine or an N-terminus of the polymeric or oligomeric structure of the scaffold.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one saponin is covalently bound to the polymeric or oligomeric structure of the scaffold, involving in the covalent bond the aldehyde function in position C-23 of the at least one saponin, when present, and/or involving in the covalent bond the glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the at least one saponin, when present.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one fourth chemical group of the scaffold, for covalently coupling of the scaffold to the cell-surface molecule targeting molecule and/or to the at least one effector moiety, is a click chemistry group, preferably selected from any one or more of a tetrazine, an azide, an alkene or an alkyne, or a cyclic derivative of these groups, preferably an azide group.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the polymeric or oligomeric structure of the scaffold comprises a linear, branched and/or cyclic polymer, oligomer, dendrimer, dendron, dendronized polymer, dendronized oligomer, a DNA, a polypeptide, poly-lysine, a poly-ethylene glycol, or an assembly of these polymeric or oligomeric structures which assembly is preferably built up by covalent cross-linking.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one saponin is a defined number of saponins or a defined range of saponins, preferably 1-128 saponins or at least 2, 3, 4, 5, 6, 8, 10, 16, 32, 64 or 128 saponins, or any number of saponins therein between, such as 7, 9, 12 saponins.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the conjugate comprises more than one saponin, preferably 2, 3, 4, 5, 6, 8, 10, 16, 32, 64 or 1-100 saponins, or any number of saponins therein between, such as 7, 9, 12 saponins, covalently bound directly to an amino-acid residue of the cell-surface molecule targeting molecule and/or to the at least one effector moiety and preferably via an amino-acid residue in the at least one effector moiety, preferably to a cysteine and/or to a lysine, and/or covalently bound via at least one linker and/or via at least one cleavable linker and/or via at least one polymeric or oligomeric scaffold of the invention, preferably 1-8 of such scaffolds or 2-4 of such scaffolds, wherein 1-32 saponins, preferably 2, 3, 4, 5, 6, 8, 10, 16 or 32 saponins, or any number of saponins therein between, such as 7, 9, 12 saponins, are covalently bound to the at least one scaffold.

An embodiment within the fourth series of aspects and embodiments of the invention is the conjugate of the invention, wherein the at least one saponin is covalently bound to the cell-surface molecule targeting molecule and to the at least one effector moiety via a tri-functional linker, the tri-functional linker comprising a second chemical group with at least one saponin covalently bound thereto either directly or via a linker such as a cleavable linker and/or via the scaffold comprising a polymeric or oligomeric structure and a fourth chemical group according to the invention for covalently coupling of the scaffold to the tri-functional linker, the tri-functional linker further comprising a third chemical group for covalent binding to the cell-surface molecule targeting molecule and comprising a first chemical group for covalent binding to the at least one effector moiety, wherein the cell-surface molecule targeting molecule is bound to the third chemical group and/or the at least one effector moiety is bound to the first chemical group, preferably the trifunctional linker is the trifunctional linker of Scheme II and Structure B.

An aspect of the invention within the fourth series of aspects and embodiments of the invention relates to a pharmaceutical composition comprising the conjugate of the invention and optionally a pharmaceutically acceptable excipient and/or a pharmaceutically acceptable diluent.

An aspect of the invention within the fourth series of aspects and embodiments of the invention relates to the conjugate of the invention or the pharmaceutical composition of the invention, for use as a medicament.

An aspect of the invention within the fourth series of aspects and embodiments of the invention relates to the conjugate of the invention or the pharmaceutical composition of the invention, for use in the treatment or prevention of a cancer or an autoimmune disease.

An aspect of the invention within a fifth series of aspects and embodiments of the invention relates to a therapeutic combination for use as a medicament, wherein the therapeutic combination comprises: (a) a fourth pharmaceutical composition comprising a fourth proteinaceous molecule comprising a binding site for binding to an epitope on a cell-surface molecule and at least one saponin covalently bound to said fourth proteinaceous molecule preferably to an amino-acid residue of said fourth proteinaceous molecule, the fourth pharmaceutical composition optionally further comprising a pharmaceutically acceptable excipient; and (b) a fifth pharmaceutical composition comprising a fifth proteinaceous molecule, the fifth proteinaceous molecule comprising a binding site for binding to the epitope on the cell-surface molecule of (a) and an effector moiety, the fifth pharmaceutical composition optionally further comprising a pharmaceutically acceptable excipient, wherein the binding site of the fourth proteinaceous molecule and the binding site of the fifth proteinaceous molecule are the same, and wherein the cell-surface molecule and the epitope on the cell-surface molecule, to which the fourth proteinaceous molecule can bind, and the cell-surface molecule and the epitope on the cell-surface molecule, to which the fifth proteinaceous molecule can bind, are the same.

An aspect of the invention within the fifth series of aspects and embodiments of the invention relates to the fourth pharmaceutical composition of the invention for use as a medicament.

An aspect of the invention within the fifth series of aspects and embodiments of the invention relates to a therapeutic combination for use in the treatment or prevention of a cancer in a human subject, wherein the therapeutic combination comprises: (a) the fourth pharmaceutical composition of the invention; and (b) the fifth pharmaceutical composition of the invention, wherein the cell-surface molecule is expressed on a tumor cell surface, and preferably the cell-surface molecule is a tumor cell-specific surface molecule, and wherein preferably the epitope is a tumor-cell specific epitope.

An aspect of the invention within the fifth series of aspects and embodiments of the invention relates to the fourth pharmaceutical composition of the invention, for use in the treatment or prophylaxis of a cancer in a patient in need thereof, wherein the cell-surface molecule is expressed on a tumor cell surface, and preferably the cell-surface molecule is a tumor cell-specific surface molecule, and wherein preferably the epitope is a tumor-cell specific epitope.

An embodiment within the fifth series of aspects and embodiments of the invention is the fourth pharmaceutical composition for use according to the invention or the therapeutic combination for use of the invention, wherein the fifth pharmaceutical composition of the invention and the fourth pharmaceutical composition are administered to the patient in need thereof.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, or the fourth pharmaceutical composition for use according to the invention, wherein the binding site of the fourth proteinaceous molecule and the fifth proteinaceous molecule comprises or consists of an immunoglobulin or a binding fragment or binding domain of said immunoglobulin, such as any one or more of an antibody, an IgG, a molecule comprising or consisting of a Vhh domain or Vh domain, a Fab, an scFv, an Fv, a dAb, an F(ab)2, Fcab fragment, and/or comprises or consists of at least one ligand, the ligand for binding to a cell-surface molecule such as EGF or a cytokine.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, or the fourth pharmaceutical composition for use according to the invention, wherein the tumor-cell surface molecule is a cell-surface receptor specifically present at a tumor cell, and wherein preferably the epitope is a tumor-cell specific epitope.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, or the fourth pharmaceutical composition for use according to the invention, wherein the at least one saponin is a triterpenoid saponin and/or a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, and/or a saponin isolated from *a Gypsophila* species and/or a *Saponaria* species and/or an *Agrostemma* species and/or a *Quillaja* species such as *Quillaja saponaria*.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, or the fourth pharmaceutical composition for use according to the invention, wherein the at least one saponin is a single specific saponin or is a mixture of two or more different saponins, such as one or more of the saponins in Table A1 or Scheme I, SO1861, SA1657, GE1741, SA1641, QS-21, QS-21A, QS-21 A-api, QS-21 A-xyl, QS-21B, QS-21 B-api, QS-21 B-xyl, QS-7-xyl, QS-7-api, QS-17-api, QS-17-xyl, QS1861, QS1862, Quillajasaponin, Saponinum album, QS-18, Quil-A, Gyp1, gypsoside A, AG1, AG2, SO1542, SO1584, SO1658, SO1674, SO1832, or any of their stereomers and/or any combinations thereof, preferably the saponin is SO1861 and/or GE1741 and/or SA1641 and/or QS-21 and/or saponin with a quillaic acid aglycon core, a Gal-(1→2)-[Xyl-(1→3)]-GlcA carbohydrate substituent at the C-3beta-OH group and a Glc-(1→3)-Xyl-(1→4)-Rha-(1→2)-[Xyl-(1→3)-4-OAc-Qui-(1→4)]-Fuc carbohydrate substituent at the C-28-OH group, and/or is 3-O-beta-D-galactopyranosyl-(1→2)-[beta-D-xylopyranosyl-(1→3)]-beta-D-glucuronopyranosyl quillaic acid 28-O-beta-D-glucopyranosyl-(1→3)-beta-D-xylopyranosyl-(1→4)- alpha-L-rhamnopyranosyl-(1→2)-[beta-D-xylopyranosyl-(1→3)-4-OAc-beta-D-quinovopyranosyl-(1→4)]-beta-D-fucopyranoside, more preferably the saponin is SO1861 and/or QS-21.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, or the fourth pharmaceutical composition for use according to the invention, wherein the at least one saponin is a bisdesmosidic saponin having a molecular mass of at least 1.500 Dalton and comprising an oleanan-type triterpene containing an aldehyde group at the C-23 position and optionally a hydroxyl group at the C-16 position, with a first branched carbohydrate side chain at the C-3 position which first branched carbohydrate side chain optionally contains glucuronic acid, wherein the saponin contains an ester group with a second branched carbohydrate side chain at the C-28 position which second branched carbohydrate chain preferably comprises at least four carbohydrate units, optionally containing at least one acetyl residue such as two acetyl residues and/or optionally at least one deoxy carbohydrate and/or a quinovose and/or a glucose and/or 4-methoxycinnamic acid and/or optionally comprising 5-O-[5-O-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid and/or optionally comprising 5-O-[5-O-Rha-(1→2)-

Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid bound to a carbohydrate via an ester bond, or wherein the at least one saponin is QS-21 or any one or more of QS-21A, QS-21 A-api, QS-21 A-xyl, QS-21B, QS-21 B-api, QS-21 B-xyl, QS-7-xyl, QS-7-api, QS-17-api, QS-17-xyl, QS-18, QS1861, protonated QS-1861 (QS1862), Quil-A.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, or the fourth pharmaceutical composition for use according to the invention, wherein the at least one saponin is a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23, wherein the saponin is covalently coupled to the fourth proteinaceous molecule, preferably covalently coupled to an amino-acid residue of the fourth proteinaceous molecule, via an aldehyde function in the saponin, preferably said aldehyde function in position C-23, preferably via at least one linker, and/or via at least one cleavable linker, wherein the amino-acid residue preferably is selected from cysteine and lysine.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, or the fourth pharmaceutical composition for use according to the invention, wherein the aldehyde function in position C-23 of the at least one saponin is covalently coupled to linker N-ε-maleimidocaproic acid hydrazide, which linker is covalently coupled via a thio-ether bond to a sulfhydryl group in the fourth proteinaceous molecule, such as a sulfhydryl group of a cysteine.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, or the fourth pharmaceutical composition for use according to the invention, wherein the at least one saponin is a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, wherein the saponin is covalently coupled to an amino-acid residue of the fourth proteinaceous molecule via the glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the saponin, preferably via at least one linker, wherein the amino-acid residue preferably is selected from cysteine and lysine.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, or the fourth pharmaceutical composition for use according to the invention, wherein the glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the at least one saponin is covalently coupled to linker 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate, which linker is covalently coupled via an amide bond to an amine group in the fourth proteinaceous molecule, such as an amine group of a lysine or an N-terminus of the fourth proteinaceous molecule.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, or the fourth pharmaceutical composition for use according to the invention, wherein the epitope is an epitope on a tumor-cell receptor, preferably a tumor-cell specific epitope, and wherein the receptor is preferably a tumor-cell specific receptor, more preferably a receptor selected from CD71, CA125, EpCAM(17-1A), CD52, CEA, CD44v6, FAP, EGF-IR, integrin, syndecan-1, vascular integrin alpha-V beta-3, HER2, EGFR, CD20, CD22, Folate receptor

1, CD146, CD56, CD19, CD138, CD27L receptor, PSMA, CanAg, integrin-alphaV, CA6, CD33, mesothelin, Cripto, CD3, CD30, CD239, CD70, CD123, CD352, DLL3, CD25, ephrinA4, MUC1, Trop2, CEACAM5, CEACAM6, HER3, CD74, PTK7, Notch3, FGF2, C4.4A, FLT3, CD38, FGFR3, CD7, PD-L1, CTLA4, CD52, PDGFRA, VEGFR1, VEGFR2, most preferably selected from CD71, HER2 and EGFR.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, or the fourth pharmaceutical composition for use according to the invention, wherein the tumor-cell receptor, preferably the tumor-cell specific receptor, is internalized by the tumor cell after binding to the fourth proteinaceous molecule of the invention and/or after binding to the fifth proteinaceous molecule of the invention, and wherein preferably binding of the fourth proteinaceous molecule and/or the fifth proteinaceous molecule to the tumor-cell receptor, such as the tumor-cell specific receptor, is followed by tumor-cell receptor-mediated internalization, e.g. via endocytosis, of a complex of the fourth proteinaceous molecule and the tumor-cell receptor and of a complex of the fifth proteinaceous molecule and the tumor-cell receptor.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, or the fourth pharmaceutical composition for use according to the invention, wherein the binding site of the fourth proteinaceous molecule and the fifth proteinaceous molecule comprises a monoclonal antibody or at least one of a cell-surface molecule binding domain and/or -fragment thereof, and preferably comprise or consist of any one of cetuximab, daratumumab, gemtuzumab, trastuzumab, panitumumab, brentuximab, inotuzumab, moxetumomab, polatuzumab, obinutuzumab, OKT-9 anti-CD71 monoclonal antibody of the IgG type, pertuzumab, rituximab, ofatumumab, Herceptin, alemtuzumab, pinatuzumab, OKT-10 anti-CD38 monoclonal antibody, an antibody of Table A2 or Table A3 or Table A4, preferably cetuximab or trastuzumab or OKT-9, or at least one cell-surface molecule binding fragment and/or -domain thereof, with the proviso that the fourth binding site is the same as the fifth binding site.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the effector moiety comprised by the fifth proteinaceous molecule comprises or consists of any one or more of an oligonucleotide, a nucleic acid and a xeno nucleic acid, preferably selected from any one or more of a vector, a gene, a cell suicide inducing transgene, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), anti-sense oligonucleotide (ASO, AON), short interfering RNA (siRNA), microRNA (miRNA), DNA aptamer, RNA aptamer, mRNA, mini-circle DNA, peptide nucleic acid (PNA), phosphoramidate morpholino oligomer (PMO), locked nucleic acid (LNA), bridged nucleic acid (BNA), 2'-deoxy-2'-fluoroarabino nucleic acid (FANA), 2'-O-methoxyethyl-RNA (MOE), 2'-O,4'-aminoethylene bridged nucleic acid, 3'-fluoro hexitol nucleic acid (FHNA), a plasmid, glycol nucleic acid (GNA) and threose nucleic acid (TNA), or a derivative thereof, more preferably a BNA, for example a BNA for silencing HSP27 protein expression.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the effector moiety comprised by the fifth proteinaceous molecule comprises or consists of at least one proteinaceous molecule, preferably selected from any one or more of a peptide, a protein, an enzyme such as urease and Cre-recombinase,

a proteinaceous toxin, a ribosome-inactivating protein, a protein toxin selected from Table A5 and/or a bacterial toxin, a plant toxin, more preferably selected from any one or more of a viral toxin such as apoptin; a bacterial toxin such as Shiga toxin, Shiga-like toxin, Pseudomonas aeruginosa exotoxin (PE) or exotoxin A of PE, full-length or truncated diphtheria toxin (DT), cholera toxin; a fungal toxin such as alpha-sarcin; a plant toxin including ribosome-inactivating proteins and the A chain of type 2 ribosome-inactivating proteins such as dianthin e.g. dianthin-30 or dianthin-32, saporin e.g. saporin-S3 or saporin-S6, bouganin or de-immunized derivative debouganin of bouganin, shiga-like toxin A, pokeweed antiviral protein, ricin, ricin A chain, modeccin, modeccin A chain, abrin, abrin A chain, volkensin, volkensin A chain, viscumin, viscumin A chain; or an animal or human toxin such as frog RNase, or granzyme B or angiogenin from humans, or any fragment or derivative thereof; preferably the protein toxin is dianthin and/or saporin.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the effector moiety comprised by the fifth proteinaceous molecule comprises or consists of at least one payload, preferably selected from any one or more of a toxin targeting ribosomes, a toxin targeting elongation factors, a toxin targeting tubulin, a toxin targeting DNA and a toxin targeting RNA, more preferably any one or more of emtansine, pasudotox, maytansinoid derivative DM1, maytansinoid derivative DM4, monomethyl auristatin E (MMAE, vedotin), monomethyl auristatin F (MMAF, mafodotin), a Calicheamicin, N-Acetyl-γ-calicheamicin, a pyrrolobenzodiazepine (PBD) dimer, a benzodiazepine, a CC-1065 analogue, a duocarmycin, Doxorubicin, paclitaxel, docetaxel, cisplatin, cyclophosphamide, etoposide, docetaxel, 5-fluorouracyl (5-FU), mitoxantrone, a tubulysin, an indolinobenzodiazepine, AZ13599185, a cryptophycin, rhizoxin, methotrexate, an anthracycline, a camptothecin analogue, SN-38, DX-8951f, exatecan mesylate, truncated form of *Pseudomonas aeruginosa* exotoxin (PE38), a Duocarmycin derivative, an amanitin, α-amanitin, a spliceostatin, a thailanstatin, ozogamicin, tesirine, Amberstatin269 and soravtansine, or a derivative thereof.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the fifth proteinaceous molecule comprises or consists of any one of Gemtuzumab ozogamicin, Brentuximab vedotin, Trastuzumab emtansine, Inotuzumab ozogamicin, Moxetumomab pasudotox and Polatuzumab vedotin and an antibody-drug conjugate of Table A2 and Table A3, or at least one tumor-cell receptor binding-domain thereof and/or at least one tumor-cell receptor binding-fragment thereof, wherein said domain(s) or fragment(s) comprise(s) the effector moiety and are preferably (a) tumor-cell specific receptor binding-domain(s) and/or (a) tumor-cell specific receptor binding-fragment(s).

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the fourth proteinaceous molecule comprises more than one covalently bound saponin, preferably 2, 3, 4, 5, 6, 8, 10, 16, 32, 64, 128 or 1-100 saponins, or any number of saponins therein between, such as 7, 9, 12 saponins.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the at least one saponin is covalently bound directly to an amino-acid residue of the fourth proteinaceous molecule, preferably to a cysteine and/or

to a lysine, and/or is covalently bound via at least one linker and/or via at least one cleavable linker and/or via at least one oligomeric or polymeric scaffold, preferably 1-8 of such scaffolds or 2-4 of such scaffolds, wherein the at least one scaffold is optionally based on a dendron, wherein 1-32 saponins, such as 2, 3, 4, 5, 6, 8, 10, 16, 32 saponins, or any number of saponins therein between, such as 7, 9, 12 saponins, are covalently bound to the at least one scaffold.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the cleavable linker is subject to cleavage under acidic conditions, reductive conditions, enzymatic conditions or light-induced conditions, and preferably the cleavable linker comprises a cleavable bond selected from a hydrazone bond and a hydrazide bond subject to cleavage under acidic conditions, and/or a bond susceptible to proteolysis, for example proteolysis by Cathepsin B, and/or a bond susceptible for cleavage under reductive conditions such as a disulphide bond.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the cleavable linker is subject to cleavage *in vivo* under acidic conditions as present in endosomes and/or lysosomes of mammalian cells, preferably human cells, preferably at pH 4.0 – 6.5, and more preferably at pH $\leq$ 5.5.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the oligomeric or polymeric scaffold comprises a polymeric or oligomeric structure and comprises at least one chemical group, the at least one chemical group for covalently coupling of the scaffold to the amino-acid residue of said fourth proteinaceous molecule.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the at least one saponin is covalently bound to the polymeric or oligomeric structure of the scaffold via a cleavable linker according to the invention.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the at least one saponin is covalently bound to the polymeric or oligomeric structure of the scaffold via any one or more of an imine bond, a hydrazone bond, a hydrazide bond, an oxime bond, a 1,3-dioxolane bond, a disulphide bond, a thio-ether bond, an amide bond, a peptide bond or an ester bond, preferably via at least one linker.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the at least one saponin comprises an aldehyde function in position C-23 and optionally a glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the at least one saponin, which aldehyde function is involved in the covalent bonding to the polymeric or oligomeric structure of the scaffold, and/or, if present, the glucuronic acid function is involved in the covalent bonding to the polymeric or oligomeric structure of the scaffold, either via direct binding or via at least one linker.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the aldehyde function in position C-23 of the at least one saponin is covalently coupled to linker N-ε-maleimidocaproic acid hydrazide, which linker is

covalently coupled via a thio-ether bond to a sulfhydryl group in the polymeric or oligomeric structure of the scaffold, such as a sulfhydryl group of a cysteine.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the at least one saponin is covalently coupled to linker 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate, which linker is covalently coupled via an amide bond to an amine group in the polymeric or oligomeric structure of the scaffold, such as an amine group of a lysine or an N-terminus of a proteinaceous molecule.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the at least one chemical group of the scaffold, for covalently coupling of the oligomeric or polymeric scaffold to the amino-acid residue of said fourth proteinaceous molecule, is a click chemistry group, preferably selected from a tetrazine, an azide, an alkene or an alkyne, or a cyclic derivative of these groups, more preferably the click chemistry group is an azide.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the polymeric or oligomeric structure of the scaffold comprises a linear, branched and/or cyclic polymer, oligomer, dendrimer, dendron, dendronized polymer, dendronized oligomer, a DNA, a polypeptide, poly-lysine, a poly-ethylene glycol, or an assembly of these polymeric or oligomeric structures which assembly is preferably built up by covalent cross-linking.

An embodiment within the fifth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the fourth pharmaceutical composition and the fifth pharmaceutical composition are administered to the patient in need thereof.

An aspect of the invention within the fifth series of aspects and embodiments of the invention relates to the fourth pharmaceutical composition of the invention, further comprising the fifth proteinaceous molecule of the invention.

An aspect of the invention within the fifth series of aspects and embodiments of the invention relates to the fourth pharmaceutical composition of the invention, further comprising the fifth proteinaceous molecule of the invention, for use as a medicament.

An aspect of the invention within the fifth series of aspects and embodiments of the invention relates to the fourth pharmaceutical composition of the invention, further comprising the fifth proteinaceous molecule of the invention, for use in the treatment or prophylaxis of cancer in a patient in need thereof.

An aspect of the invention within a sixth series of aspects and embodiments of the invention relates to a therapeutic combination for use as a medicament, wherein the therapeutic combination comprises: (a) a sixth pharmaceutical composition comprising a sixth proteinaceous molecule comprising a sixth binding site for binding to a sixth cell-surface molecule and at least one saponin covalently bound to said sixth proteinaceous molecule preferably covalently bound to an amino-acid residue of said sixth proteinaceous molecule, the sixth pharmaceutical composition optionally further

comprising a pharmaceutically acceptable excipient; and (b) a seventh pharmaceutical composition comprising a seventh proteinaceous molecule preferably different from the sixth proteinaceous molecule, the seventh proteinaceous molecule comprising a seventh binding site for binding to a seventh cell-surface molecule different from the sixth cell-surface molecule and an effector moiety, the seventh pharmaceutical composition optionally further comprising a pharmaceutically acceptable excipient.

An aspect of the invention within the sixth series of aspects and embodiments of the invention relates to the sixth pharmaceutical composition of the invention for use as a medicament.

An aspect of the invention within the sixth series of aspects and embodiments of the invention relates to a therapeutic combination for use in the treatment or prevention of cancer in a human subject, wherein the therapeutic combination comprises: (a) the sixth pharmaceutical composition of the invention, wherein the sixth cell-surface molecule is a sixth tumor-cell surface molecule, preferably a sixth tumor cell-specific surface molecule; and (b) the seventh pharmaceutical composition of the invention, wherein the seventh cell-surface molecule is a seventh tumor-cell surface molecule different from the sixth tumor-cell surface molecule, preferably the seventh cell-surface molecule is a seventh tumor cell-specific surface molecule different from the sixth tumor cell-specific surface molecule.

An aspect of the invention within the sixth series of aspects and embodiments of the invention relates to the sixth pharmaceutical composition of the invention, for use in the treatment or prophylaxis of cancer in a patient in need thereof, wherein the sixth cell-surface molecule is a sixth tumor-cell surface molecule, preferably a sixth tumor cell-specific surface molecule.

An embodiment within the sixth series of aspects and embodiments of the invention is the sixth pharmaceutical composition for use according to the invention or the therapeutic combination of the invention, wherein the seventh pharmaceutical composition of the invention and the sixth pharmaceutical composition are administered to the patient in need thereof, and wherein the seventh tumor-cell surface molecule is different from the sixth tumor-cell surface molecule, preferably the seventh tumor cell-specific surface molecule is different from the sixth tumor cell-specific surface molecule.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention or the sixth pharmaceutical composition for use according to the invention, wherein the sixth binding site of the sixth proteinaceous molecule comprises or consists of an immunoglobulin or at least one binding fragment or –domain of said immunoglobulin for binding to the sixth cell-surface molecule, such as any one or more of an antibody, an IgG, a molecule comprising or consisting of a Vhh domain or Vh domain, a Fab, an scFv, an Fv, a dAb, an F(ab)2, Fcab fragment, and/or comprises or consists of at least one ligand, preferably at least one ligand for binding to the sixth cell-surface molecule such as EGF or a cytokine.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention or the sixth pharmaceutical composition for use according to the invention, wherein the sixth binding site for binding to the sixth tumor-cell surface molecule, preferably a tumor cell-specific surface molecule, is a sixth binding site for a sixth cell-surface receptor present at a tumor cell, preferably specifically present at a tumor cell.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention or the sixth pharmaceutical composition for use according to the invention, wherein the at least one saponin is a triterpenoid saponin or a bisdesmosidic triterpene saponin, belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, and/or a saponin isolated from a *Gypsophila* species and/or a *Saponaria* species and/or an *Agrostemma* species and/or a *Quillaja* species such as *Quillaja saponaria*.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention or the sixth pharmaceutical composition for use according to the invention, wherein the at least one saponin is a single specific saponin or is a mixture of two or more different saponins, such as one or more of the saponins in Table A1 or Scheme I, SO1861, SA1657, GE1741, SA1641, QS-21, QS-21A, QS-21 A-api, QS-21 A-xyl, QS-21B, QS-21 B-api, QS-21 B-xyl, QS-7-xyl, QS-7-api, QS-17-api, QS-17-xyl, QS1861, QS1862, Quillajasaponin, Saponinum album, QS-18, Quil-A, Gyp1, gypsoside A, AG1, AG2, SO1542, SO1584, SO1658, SO1674, SO1832, or any of their stereomers and/or any combinations thereof, preferably the saponin is SO1861 and/or GE1741 and/or SA1641 and/or QS-21 and/or saponin with a quillaic acid aglycon core, a Gal-(1→2)-[Xyl-(1→3)]-GlcA carbohydrate substituent at the C-3beta-OH group and a Glc-(1→3)-Xyl-(1→4)-Rha-(1→2)-[Xyl-(1→3)-4-OAc-Qui-(1→4)]-Fuc carbohydrate substituent at the C-28-OH group, and/or is 3-O-beta-D-galactopyranosyl-(1→2)-[beta-D-xylopyranosyl-(1→3)]-beta-D-glucuronopyranosyl quillaic acid 28-O-beta-D-glucopyranosyl-(1→3)-beta-D-xylopyranosyl-(1→4)- alpha-L-rhamnopyranosyl-(1→2)-[beta-D-xylopyranosyl-(1→3)-4-OAc-beta-D-quinovopyranosyl-(1→4)]-beta-D-fucopyranoside, more preferably the saponin is SO1861 and/or QS-21.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention or the sixth pharmaceutical composition for use according to the invention, wherein the at least one saponin is a bisdesmosidic saponin having a molecular mass of at least 1.500 Dalton and comprising an oleanan-type triterpene containing an aldehyde group at the C-23 position and optionally a hydroxyl group at the C-16 position, with a first branched carbohydrate side chain at the C-3 position which first branched carbohydrate side chain optionally contains glucuronic acid, wherein the saponin contains an ester group with a second branched carbohydrate side chain at the C-28 position which second branched carbohydrate chain preferably comprises at least four carbohydrate units, optionally containing at least one acetyl residue such as two acetyl residues and/or at least one deoxy carbohydrates and/or a quinovose and/or a glucose and/or 4-methoxycinnamic acid and/or optionally comprising 5-O-[5-O-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid and/or optionally comprising 5-O-[5-O-Rha-(1→2)-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid bound to a carbohydrate via an ester bond, or wherein the at least one saponin is QS-21 or any one or more of QS-21A, QS-21 A-api, QS-21 A-xyl, QS-21B, QS-21 B-api, QS-21 B-xyl, QS-7-xyl, QS-7-api, QS-17-api, QS-17-xyl, QS-18, QS1861, protonated QS1861 (QS1862), Quil-A.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention or the sixth pharmaceutical composition for use according to the invention, wherein the at least one saponin is a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 , wherein the saponin is covalently coupled the sixth proteinaceous molecule, preferably covalently coupled to an amino-acid residue of the sixth proteinaceous molecule, via an aldehyde function in the saponin, preferably said aldehyde function in position C-23, preferably via at least one linker, and/or via at least one cleavable linker, wherein the amino-acid residue preferably is selected from cysteine and lysine.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention or the sixth pharmaceutical composition for use according to the invention, wherein the aldehyde function in position C-23 of the at least one saponin is covalently coupled to linker N-ε-maleimidocaproic acid hydrazide, which linker is covalently coupled via a thio-ether bond to a sulfhydryl group in the sixth proteinaceous molecule, such as a sulfhydryl group of a cysteine.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention or the sixth pharmaceutical composition for use according to the invention, wherein the at least one saponin is a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, wherein the saponin is covalently coupled to an amino-acid residue of the sixth proteinaceous molecule via the glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the saponin, preferably via at least one linker, wherein the amino-acid residue preferably is selected from cysteine and lysine.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention or the sixth pharmaceutical composition for use according to the invention, wherein the glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the at least one saponin is covalently coupled to linker 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate, which linker is covalently coupled via an amide bond to an amine group in the sixth proteinaceous molecule, such as an amine group of a lysine or an N-terminus of the sixth proteinaceous molecule.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention or the sixth pharmaceutical composition for use according to the invention, wherein the seventh binding site of the seventh proteinaceous molecule comprises or consists of an immunoglobulin, at least one binding domain of said immunoglobulin and/or at least one binding fragment of said immunoglobulin for binding to the seventh cell-surface molecule, such as an antibody, an IgG, a molecule comprising or consisting of a Vhh domain or Vh domain, a Fab, an scFv, an Fv, a dAb, an F(ab)2, Fcab fragment, and/or comprises or consists of at least one ligand, preferably a ligand for binding to the seventh cell-surface molecule such as EGF or a cytokine.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention or the sixth pharmaceutical composition for use

according to the invention, wherein the seventh binding site for binding to the seventh tumor-cell surface molecule, preferably the seventh tumor cell-specific surface molecule, is a seventh binding site for a seventh cell-surface receptor present at a tumor cell, preferably specifically present at a tumor cell.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention or the sixth pharmaceutical composition for use according to the invention, wherein the sixth binding site and the seventh binding site are binding sites for binding to a sixth and seventh tumor-cell receptor respectively, preferably for binding to a sixth and seventh tumor-cell specific receptor respectively, preferably present at the same tumor cell, and wherein the sixth and seventh tumor-cell receptor are preferably tumor-cell specific receptors, and/or are selected from CD71, CA125, EpCAM(17-1A), CD52, CEA, CD44v6, FAP, EGF-IR, integrin, syndecan-1, vascular integrin alpha-V beta-3, HER2, EGFR, CD20, CD22, Folate receptor 1, CD146, CD56, CD19, CD138, CD27L receptor, PSMA, CanAg, integrin-alphaV, CA6, CD33, mesothelin, Cripto, CD3, CD30, CD239, CD70, CD123, CD352, DLL3, CD25, ephrinA4, MUC1, Trop2, CEACAM5, CEACAM6, HER3, CD74, PTK7, Notch3, FGF2, C4.4A, FLT3, CD38, FGFR3, CD7, PD-L1, CTLA4, CD52, PDGFRA, VEGFR1, VEGFR2, more preferably selected from HER2, CD71 and EGFR, with the proviso that the sixth binding site is different from the seventh binding site and with the proviso that the sixth and seventh tumor-cell specific receptor, preferably the sixth and seventh tumor-cell specific receptor, are different receptors.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention or the sixth pharmaceutical composition for use according to the invention, wherein the sixth binding site and the seventh binding site comprise or consist of cetuximab, daratumumab, gemtuzumab, trastuzumab, panitumumab, brentuximab, inotuzumab, moxetumomab, polatuzumab, obinutuzumab, OKT-9 anti-CD71 monoclonal antibody of the IgG type, pertuzumab, rituximab, ofatumumab, Herceptin, alemtuzumab, pinatuzumab, OKT-10 anti-CD38 monoclonal antibody, an antibody of Table A2 or Table A3 or Table A4, preferably cetuximab or trastuzumab or OKT-9, or at least one tumor-cell receptor binding-domain thereof and/or at least one tumor-cell receptor binding-fragment thereof, with the proviso that the sixth binding site is different from the seventh binding site.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention or the sixth pharmaceutical composition for use according to the invention, wherein the sixth tumor-cell receptor is internalized by the tumor cell after binding to the sixth proteinaceous molecule of the invention, and wherein preferably binding of the sixth proteinaceous molecule to the sixth tumor-cell receptor is followed by tumor-cell receptor-mediated internalization, e.g. via endocytosis, of a complex of the sixth proteinaceous molecule and the sixth tumor-cell receptor, wherein the sixth tumor-cell receptor is preferably a sixth tumor-cell specific receptor.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the seventh tumor-cell receptor is internalized by the tumor cell after binding to the seventh proteinaceous molecule of the invention, and wherein preferably binding of the seventh proteinaceous molecule to the seventh tumor-cell receptor is followed

by tumor-cell receptor-mediated internalization, e.g. via endocytosis, of a complex of the seventh proteinaceous molecule and the seventh tumor-cell receptor, wherein the seventh tumor-cell receptor is preferably a seventh tumor-cell specific receptor.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention or the sixth pharmaceutical composition for use according to the invention, wherein the effector moiety comprised by the seventh proteinaceous molecule comprises or consists of at least one of an oligonucleotide, a nucleic acid and a xeno nucleic acid, preferably selected from any one or more of a vector, a gene, a cell suicide inducing transgene, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), anti-sense oligonucleotide (ASO, AON), short interfering RNA (siRNA), microRNA (miRNA), DNA aptamer, RNA aptamer, mRNA, mini-circle DNA, peptide nucleic acid (PNA), phosphoramidate morpholino oligomer (PMO), locked nucleic acid (LNA), bridged nucleic acid (BNA), 2'-deoxy-2'-fluoroarabino nucleic acid (FANA), 2'-O-methoxyethyl-RNA (MOE), 2'-O,4'-aminoethylene bridged nucleic acid, 3'-fluoro hexitol nucleic acid (FHNA), a plasmid, glycol nucleic acid (GNA) and threose nucleic acid (TNA), or a derivative thereof, more preferably a BNA, for example a BNA for silencing HSP27 protein expression.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention or the sixth pharmaceutical composition for use according to the invention, wherein the effector moiety comprised by the seventh proteinaceous molecule comprises or consists of at least one proteinaceous molecule, preferably selected from any one or more of a peptide, a protein, an enzyme such as urease and Cre-recombinase, a proteinaceous toxin, a ribosome-inactivating protein, a protein toxin selected from Table A5 and/or a bacterial toxin, a plant toxin, more preferably selected from any one or more of a viral toxin such as apoptin; a bacterial toxin such as Shiga toxin, Shiga-like toxin, Pseudomonas aeruginosa exotoxin (PE) or exotoxin A of PE, full-length or truncated diphtheria toxin (DT), cholera toxin; a fungal toxin such as alpha-sarcin; a plant toxin including ribosome-inactivating proteins and the A chain of type 2 ribosome-inactivating proteins such as dianthin e.g. dianthin-30 or dianthin-32, saporin e.g. saporin-S3 or saporin-S6, bouganin or de-immunized derivative debouganin of bouganin, shiga-like toxin A, pokeweed antiviral protein, ricin, ricin A chain, modeccin, modeccin A chain, abrin, abrin A chain, volkensin, volkensin A chain, viscumin, viscumin A chain; or an animal or human toxin such as frog RNase, or granzyme B or angiogenin from humans, or any fragment or derivative thereof; preferably the protein toxin is dianthin and/or saporin.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention or the sixth pharmaceutical composition for use according to the invention, wherein the effector moiety comprised by the seventh proteinaceous molecule comprises or consists of at least one payload, preferably selected from any one or more of a toxin targeting ribosomes, a toxin targeting elongation factors, a toxin targeting tubulin, a toxin targeting DNA and a toxin targeting RNA, more preferably any one or more of emtansine, pasudotox, maytansinoid derivative DM1, maytansinoid derivative DM4, monomethyl auristatin E (MMAE, vedotin), monomethyl auristatin F (MMAF, mafodotin), a Calicheamicin, N-Acetyl-γ-calicheamicin, a pyrrolobenzodiazepine (PBD) dimer, a benzodiazepine, a CC-1065 analogue, a duocarmycin, Doxorubicin, paclitaxel, docetaxel, cisplatin, cyclophosphamide, etoposide, docetaxel, 5-fluorouracyl (5-

FU), mitoxantrone, a tubulysin, an indolinobenzodiazepine, AZ13599185, a cryptophycin, rhizoxin, methotrexate, an anthracycline, a camptothecin analogue, SN-38, DX-8951f, exatecan mesylate, truncated form of *Pseudomonas aeruginosa* exotoxin (PE38), a Duocarmycin derivative, an amanitin, α-amanitin, a spliceostatin, a thailanstatin, ozogamicin, tesirine, Amberstatin269 and soravtansine, or a derivative thereof.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the seventh proteinaceous molecule comprises or consists of any one of Gemtuzumab ozogamicin, Brentuximab vedotin, Trastuzumab emtansine, Inotuzumab ozogamicin, Moxetumomab pasudotox and Polatuzumab vedotin and an antibody-drug conjugate of Table A2 and Table A3, or at least one tumor-cell receptor binding-domain thereof and/or at least one tumor-cell receptor binding-fragment thereof, wherein said domain(s) or fragment(s) comprise(s) the effector moiety and are preferably (a) tumor-cell specific receptor binding-domain(s) and/or (a) tumor-cell specific receptor binding-fragment(s).

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention or the sixth proteinaceous molecule for use according to the invention, wherein the sixth proteinaceous molecule comprises more than one covalently bound saponin, preferably 2, 3, 4, 5, 6, 8, 10, 16, 32, 64, 128 or 1-100 saponins, or any number of saponins therein between, such as 7, 9, 12 saponins.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention or the sixth proteinaceous molecule for use according to the invention, wherein the more than one covalently bound saponin are covalently bound directly to an amino-acid residue of the sixth proteinaceous molecule, preferably to a cysteine and/or to a lysine, and/or are covalently bound via at least one linker and/or via at least one cleavable linker and/or via at least one oligomeric or polymeric scaffold, preferably 1-8 of such scaffolds or 2-4 of such scaffolds, wherein the at least one scaffold is optionally based on a dendron, wherein 1-32 saponins, preferably 2, 3, 4, 5, 6, 8, 10, 16, 32 saponins, or any number of saponins therein between, such as 7, 9, 12 saponins, are covalently bound to the at least one scaffold.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the cleavable linker is subject to cleavage under acidic conditions, reductive conditions, enzymatic conditions or light-induced conditions, and preferably the cleavable linker comprises a cleavable bond selected from a hydrazone bond and a hydrazide bond subject to cleavage under acidic conditions, and/or a bond susceptible to proteolysis, for example proteolysis by Cathepsin B, and/or a bond susceptible for cleavage under reductive conditions such as a disulphide bond.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the cleavable linker is subject to cleavage *in vivo* under acidic conditions as present in endosomes and/or lysosomes of mammalian cells, preferably human cells, preferably at pH 4.0 – 6.5, and more preferably at pH $\leq$ 5.5.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the oligomeric or polymeric scaffold comprises

a polymeric or oligomeric structure and comprises at least one chemical group, the at least one chemical group for covalently coupling of the scaffold to the amino-acid residue of said sixth proteinaceous molecule.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the at least one saponin is covalently bound to the polymeric or oligomeric structure of the scaffold via a cleavable linker according to the invention.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the at least one saponin is covalently bound to the polymeric or oligomeric structure of the scaffold via any one or more of an imine bond, a hydrazone bond, a hydrazide bond, an oxime bond, a 1,3-dioxolane bond, a disulphide bond, a thio-ether bond, an amide bond, a peptide bond or an ester bond, preferably via at least one linker.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the at least one saponin comprises an aldehyde function in position C-23 and optionally a glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the at least one saponin, which aldehyde function is involved in the covalent bonding to the polymeric or oligomeric structure of the scaffold, and/or, if present, the glucuronic acid function is involved in the covalent bonding to the polymeric or oligomeric structure of the scaffold, either via direct binding or via at least one linker.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the aldehyde function in position C-23 of the at least one saponin is covalently coupled to linker N-ε-maleimidocaproic acid hydrazide, which linker is covalently coupled via a thio-ether bond to a sulfhydryl group in the polymeric or oligomeric structure of the scaffold, such as a sulfhydryl group of a cysteine.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the at least one saponin is covalently coupled to linker 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate, which linker is covalently coupled via an amide bond to an amine group in the polymeric or oligomeric structure of the scaffold, such as an amine group of a lysine or an N-terminus of a proteinaceous molecule.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the chemical group of the polymeric or oligomeric scaffold, for covalently coupling of the scaffold to the amino-acid residue of the sixth proteinaceous molecule, is a click chemistry group, preferably selected from a tetrazine, an azide, an alkene or an alkyne, or a cyclic derivative of these groups, more preferably the click chemistry group is an azide.

An embodiment within the sixth series of aspects and embodiments of the invention is the therapeutic combination for use of the invention, wherein the polymeric or oligomeric structure of the scaffold comprises a linear, branched and/or cyclic polymer, oligomer, dendrimer, dendron, dendronized polymer, dendronized oligomer, a DNA, a polypeptide, poly-lysine, a poly-ethylene glycol, or an

assembly of these polymeric or oligomeric structures which assembly is preferably built up by covalent cross-linking.

An aspect of the invention within the sixth series of aspects and embodiments of the invention relates to the therapeutic combination for use of the invention, wherein the sixth pharmaceutical composition and the seventh pharmaceutical composition are administered to the patient in need thereof.

An aspect of the invention within the sixth series of aspects and embodiments of the invention relates to the sixth pharmaceutical composition of the invention, further comprising the seventh proteinaceous molecule of the invention.

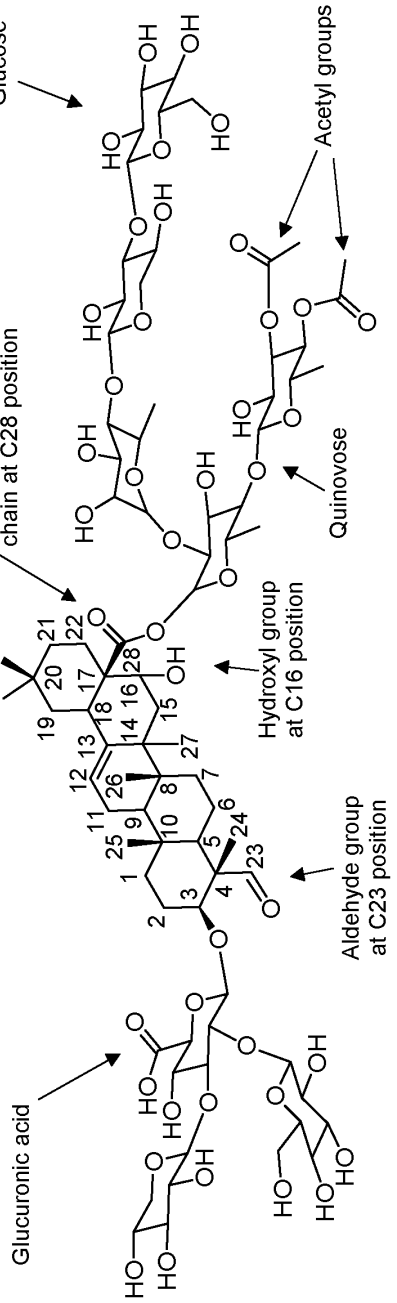
An aspect of the invention within the sixth series of aspects and embodiments of the invention relates to the sixth pharmaceutical composition of the invention, further comprising the seventh proteinaceous molecule of the invention, for use as a medicament.

An aspect of the invention within the sixth series of aspects and embodiments of the invention relates to the sixth pharmaceutical composition of the invention, further comprising the seventh proteinaceous molecule of the invention, for use in the treatment or prophylaxis of cancer in a patient in need thereof.

Of course, any and all of a, b, c, d, e, f, g, h, l, j, k, m, n, p, q, r, s, t, u, v, w and/or x have the value in accordance with each individual embodiment and aspect of the invention for any and all of the aforementioned aspects and embodiments according to the invention. In addition, (tri-functional) linkers L1, L2, L4, L5, L6, L8, L9 and/or L10, if present in a molecule or conjugate or moiety of the invention, are the (tri-functional) linkers as indicated for each and any of the aforementioned aspects and embodiments of the invention, as is readily appreciated by the skilled person. The oligomeric or polymeric scaffolds L3 and/or L7, if present in a molecule or conjugate or moiety of the invention, are the oligomeric or polymeric scaffolds as indicated for each and any of the aforementioned aspects and embodiments of the invention, as is also readily appreciated by the skilled person. Furthermore, the first ligand A1 and the first effector moiety B1, if present, and the second ligand A2 and the second effector moiety B2, if present, and the first effector moiety A1 and the first ligand B1, if present, and the second effector moiety A2 and the second ligand B2, if present, are the selected and indicated ligands and effector moieties, as disclosed for the first, second, third, fourth, fifth, and sixth series of embodiment and aspects of the invention, and all further embodiments and aspects of the invention, outlined here above. Saponin C is any one or more of the saponins referred to and listed in any of the aforementioned aspects and embodiments of the invention, in particular one or more saponins selected from Scheme I and/or Table A1.

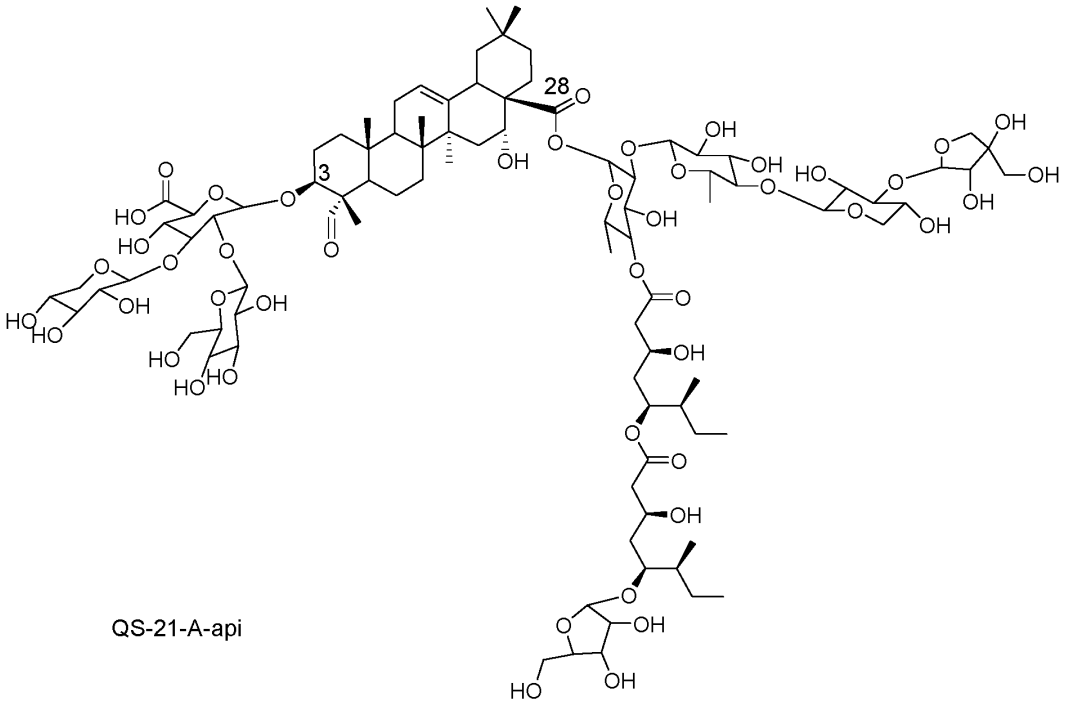
An embodiment is the first, fourth or sixth proteinaceous molecule of the invention comprising a saponin comprising one or several or all of the indicated structural features of the saponin of Structure A in Scheme I, the saponin of structure A referred to as a saponin with an ‘ideal’ structure when endosomal escape enhancing activity towards an effector moiety present in the endosome of a cell contacted with first proteinaceous molecule, and/or a saponin selected from any one or more of the further saponins in Scheme I:

SCHEME I

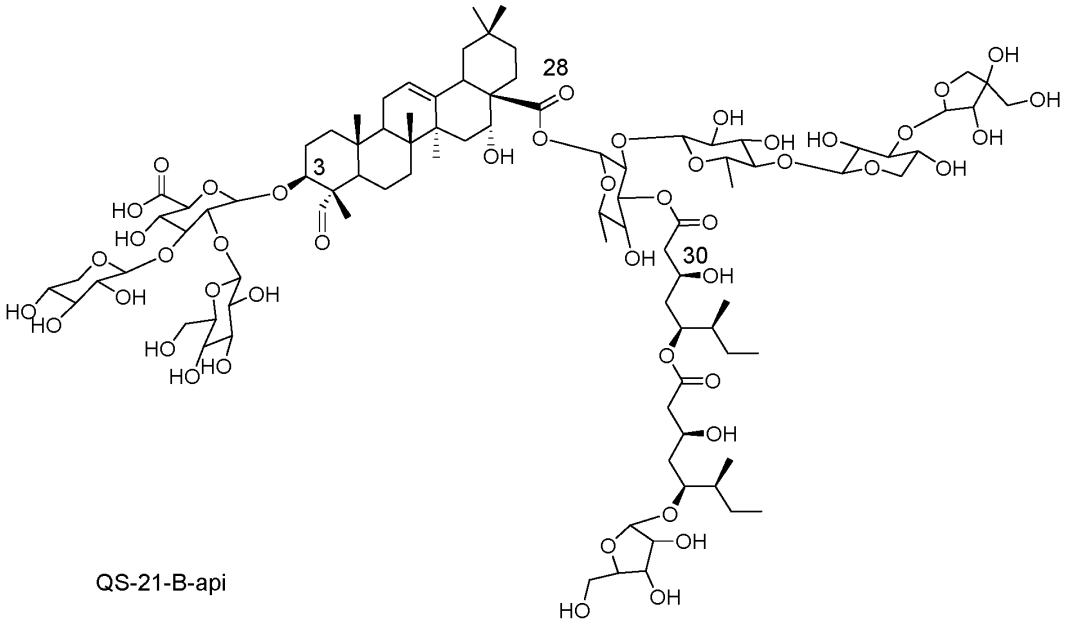


STRUCTURE A

SCHEME I (continued)

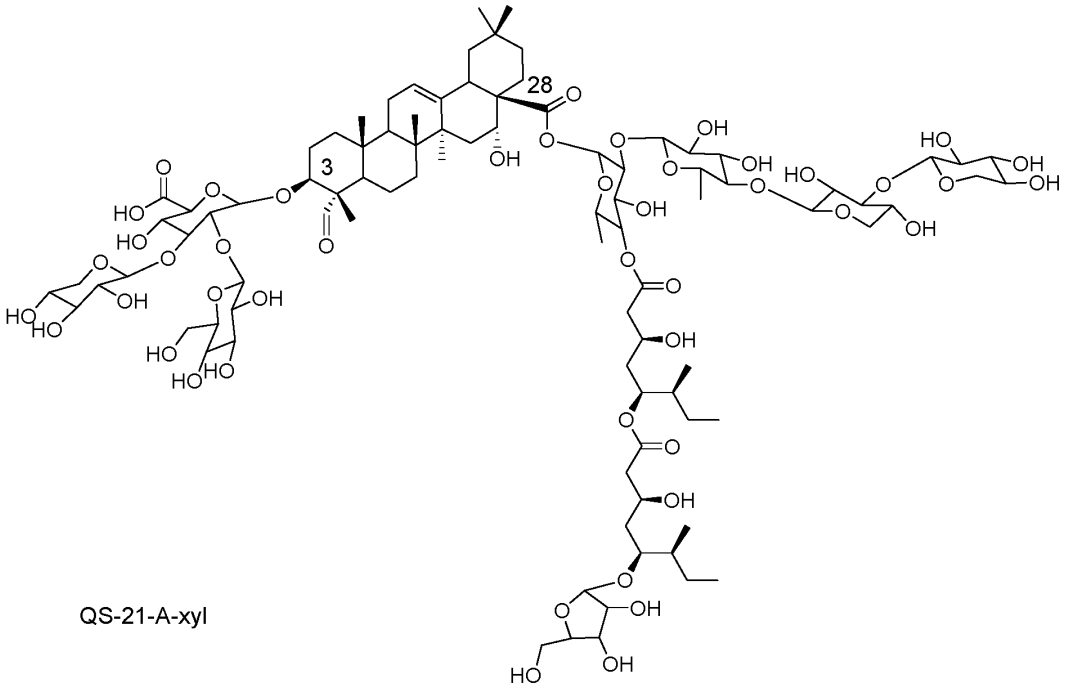


QS-21-A-api

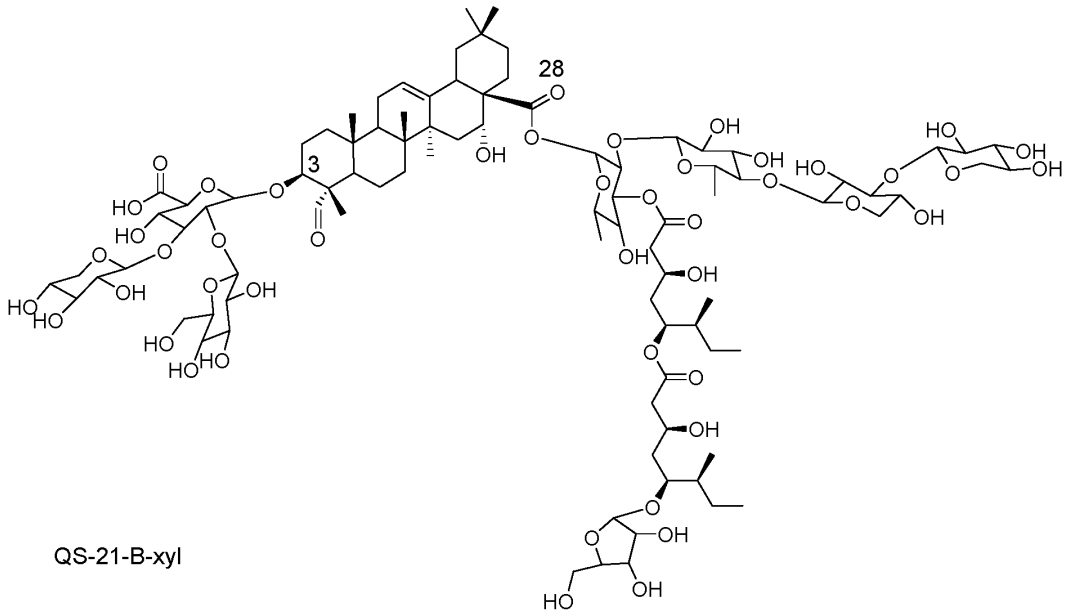


QS-21-B-api

SCHEME I (continued)

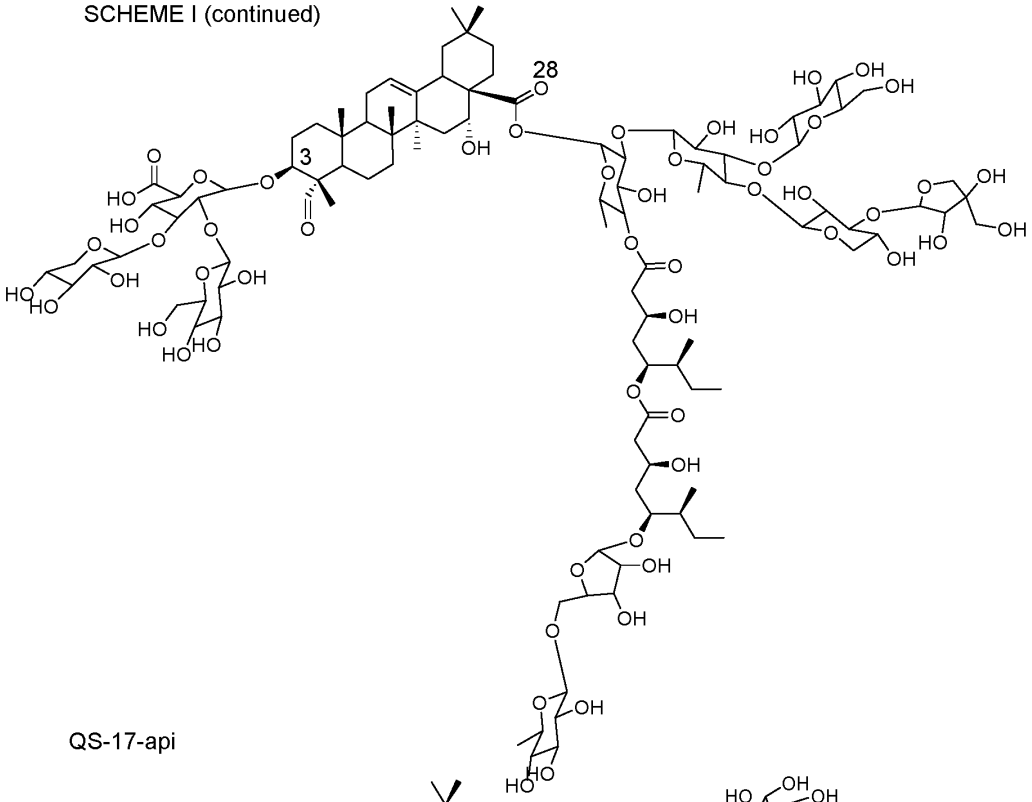


QS-21-A-xyl

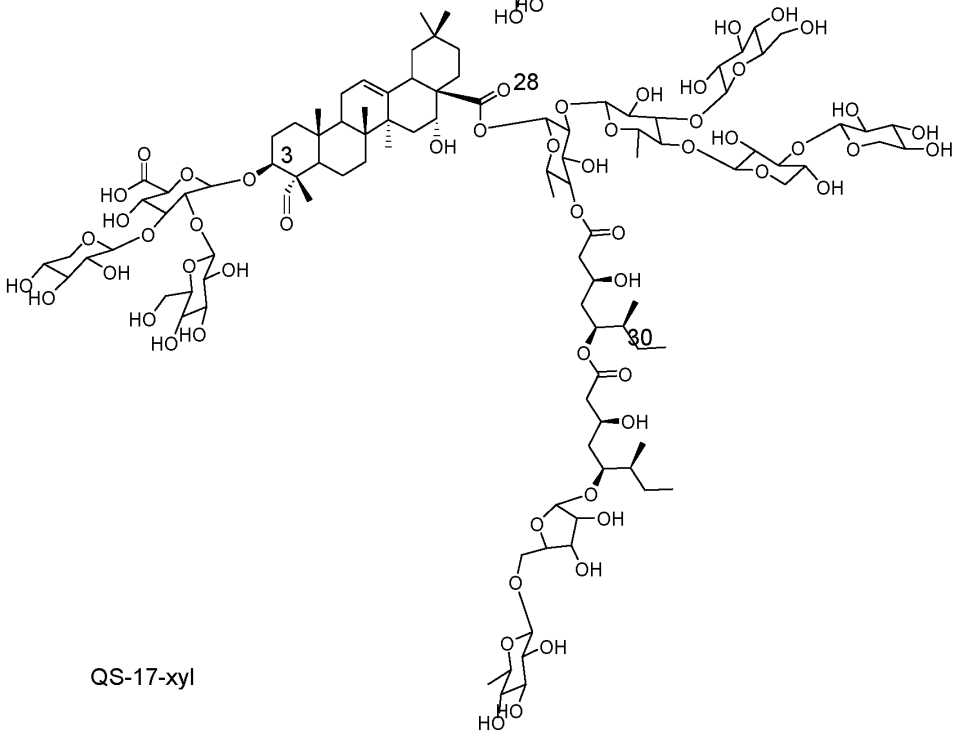


QS-21-B-xyl

SCHEME I (continued)

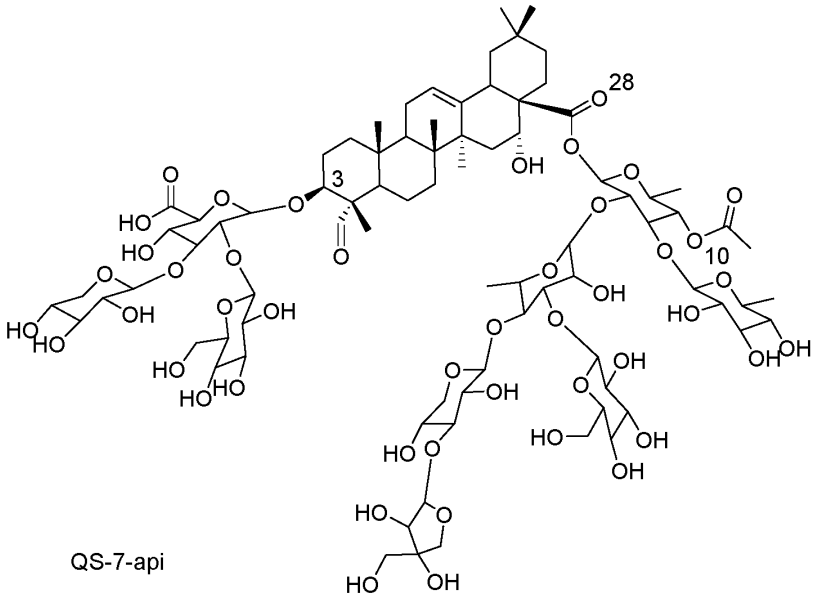


QS-17-api

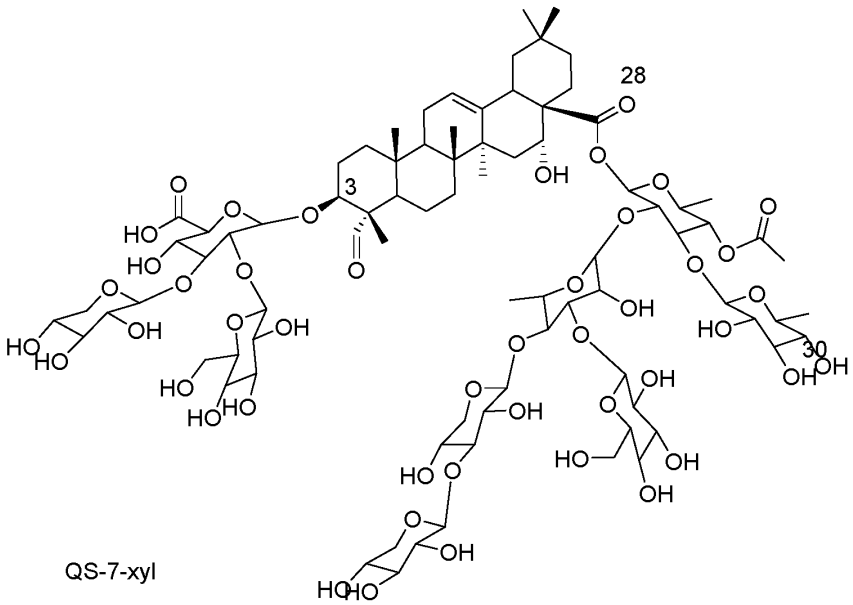


QS-17-xyl

SCHEME I (continued)



QS-7-api

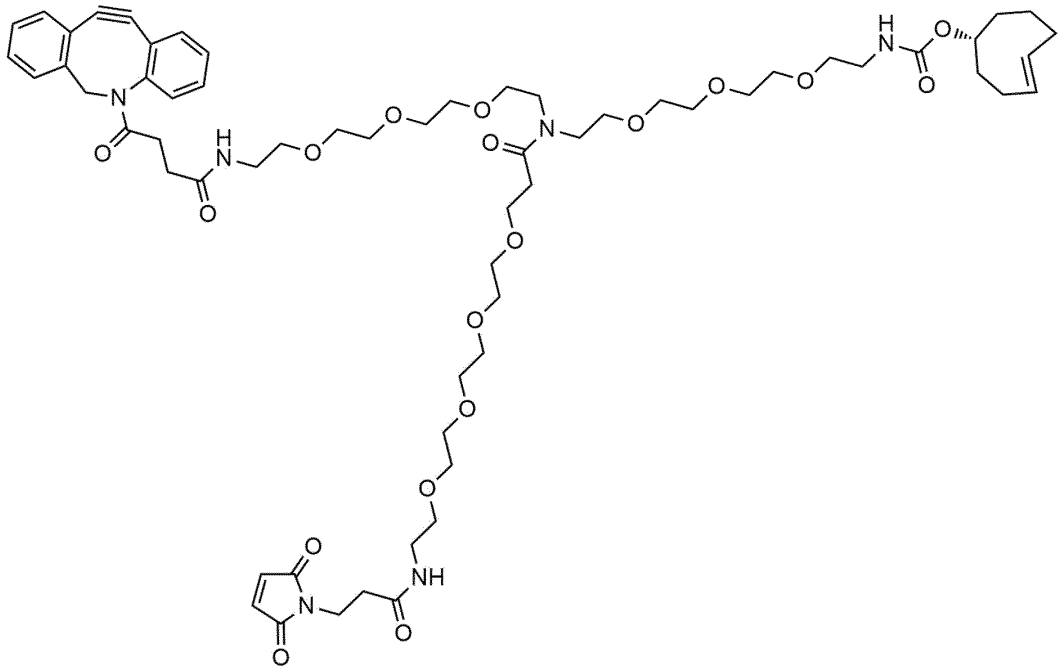


QS-7-xyl

According to the invention, a glycoside, such as a saponin C according to the invention, bound to the first, fourth and/or sixth proteinaceous molecule of the invention, which has the 'ideal' structure for the purpose of enhancing endosomal escape of an effector molecule bound to the second or third or fifth or seventh proteinaceous molecule of the invention is a bisdesmosidic saponin according to Structure A of Scheme I, having a molecular mass of at least 1.500 Dalton and comprising an oleanan-type triterpene containing an aldehyde group at the C-23 position and optionally a hydroxyl group at the C-16 position, with a first branched carbohydrate side chain at the C-3 position which first branched carbohydrate side chain optionally contains glucuronic acid, wherein the saponin contains an ester group with a second branched carbohydrate side chain at the C-28 position which second branched carbohydrate chain preferably comprises at least four carbohydrate units, optionally containing at least one acetyl residue such as two acetyl residues and/or optionally comprising deoxy carbohydrates and/or optionally comprising quinovose and/or optionally comprising glucose and/or optionally comprising 4-methoxycinnamic acid and/or optionally comprising 5-O-[5-O-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid and/or optionally comprising 5-O-[5-O-Rha-(1→2)-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid bound to a carbohydrate via an ester bond.

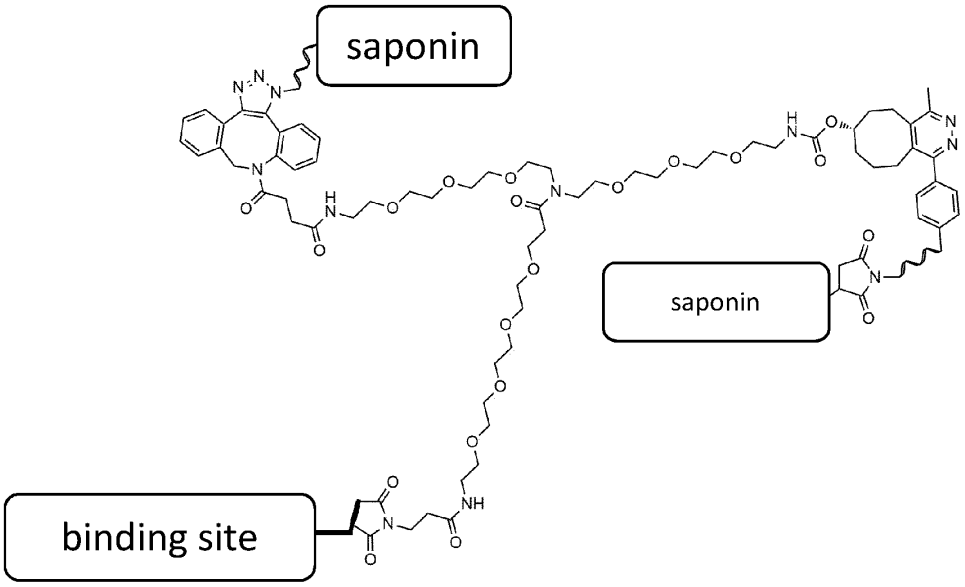
SO1861 is different from the "*ideal structure*" displayed in Scheme I, Structure A, only in having only one acetyl residue at the quinovose and having an additional xylose. The "ideal structure" of a saponin for enhancing endosomal escape of an effector molecule or effector moiety or a payload, is a saponin which preferably has the Structure A of Scheme I, and saponins which display the endosomal escape enhancing activity have one or more of the structural features displayed in Structure A of Scheme I. Without wishing to be bound by any theory, the inventors belief that the Structure A of Scheme I represents an "ideal saponin" (and not a minimum requirement saponin) for endosomal escape enhancing activity, which means that not all of the structures (chemical groups) can or must be present in each saponin with at least sufficient endosomal escape enhancing activity to promote accumulation of the effector moiety in the cytosol, and which means that some saponins might have other structure elements such as acyl chains, and/or for yet other saponins that display endosomal escape enhancing activity, the sugars can be different than the sugars displayed in Scheme I. For example, the QS-21 saponin and some of the saponins in the water soluble fraction of Quillaja saponaria (Quillaja saponins; Quil-A) differ in the carbohydrate modification at C-28 when the ideal structure of Structure A in Scheme I is considered: presence of an acyl chain in QS-21 for example. In the water soluble fraction of Quillaja saponaria, saponins such as QS-7, QS1862, are similar to the ideal Structure A, and are similar to SO1861.

An embodiment is the first, fourth or sixth proteinaceous molecule of the invention, comprising the oligomeric tri-functional linker as the scaffold core structure, according to Scheme II:



SCHEME II,

wherein the saponins are covalently bound to the tri-functional linker scaffold L9 and/or L10 via labile, cleavable hydrazone linkers (acid sensitive) and/or via a maleimide comprising bond, whereas the binding of the scaffold to the binding site such as an antibody is established via labile, cleavable hydrazone linkers (acid sensitive) and/or via a maleimide comprising bond with cysteines in the binding site such as 1, 2, 3 or 4 cysteines, therewith forming Structure B:



Structure B,

such that 1-4 scaffolds are covalently bound to a single e.g. antibody such as a monoclonal antibody. According to the invention, one of the two displayed saponins in Structure B can also be replaced by a covalently coupled effector moiety, effector molecule, payload, or both saponins are absence with two covalently coupled effector moieties, effector molecules, payloads coupled to the tri-functional linker. The binding site is for example an antibody or a binding fragment or binding domain thereof.

The inventors established that the therapeutic window of an antibody drug conjugate, such as the second and third and fifth and seventh proteinaceous molecules in the second or third or fifth or seventh pharmaceutical composition of the invention, respectively, increases when administered to a tumor-bearing mammal (mouse) to whom also the first, fourth or sixth pharmaceutical composition is administered. The first, fourth or sixth proteinaceous protein has at least one glycoside such as a saponin C bound thereto, preferably covalently, more preferably via a cleavable linker. The saponin C augments the therapeutic efficacy of the effector moiety A1, B1, A2 or B2, bound to the second and third and fifth and seventh proteinaceous molecule, likely by enhancing the endosomal escape of the effector moiety into the cytosol where the activity of the effector moiety is desired. This way, already at a lower dose than the conventional dose of the ADC, i.e. the second or third or fifth or seventh proteinaceous molecule, therapeutic effect is established under influence of the presence of the first, fourth or sixth proteinaceous molecule comprising the saponin C near, at and/or inside the targeted cell. The targeted cell is for example a diseased cell such as a tumor cell or an auto-immune cell or a B-cell disease related B-cell, etc. The effector moiety A1, B1, A2 or B2 is for example a toxin as part of an ADC or an oligonucleotide such as a BNA as part of an AOC according to the invention.

By targeting (two) different cell-surface molecules with the first and second, or fourth and fifth, or sixth and seventh proteinaceous molecule, the delivery of the saponin C and the effector molecule A1, B1, A2 or B2 at and inside the cytosol of the very same targeted cell, exposing (both different) cell-surface molecules on the cell surface, is improved and more specific, compared to exposure of such cells to only the second, third, fifth or seventh proteinaceous molecule such as an ADC or an AOC, without the presence of the cell-targeted saponin C (first, fourth or sixth proteinaceous molecule). An aberrant cell selected for separate targeting by the binding site of the first, fourth or sixth proteinaceous molecule and by the binding site of the second, third, fifth or seventh proteinaceous molecule, wherein the binding sites are different and wherein the epitope to which the first and second proteinacous molecules bind are different and are located in/on a different kind and type of cell-surface molecule such as two different receptors, ideally bears the first epitope and the second epitope on the first cell-surface molecule and the second cell-surface molecule respectively, to a high extent (i.e. relativelty higher expression of the two distinct and different cell-surface molecules on the targeted cell such as for example a tumor cell or an auto-immune cell, than the expression on a non-targeted cell such as for example a healthy cell) and/or expose the first and second cell-surface molecules specifically, when (neighboring) healthy cells in a patient are considered. Preferably, both cell-surface molecules targeted by the first and second binding sites are relatively highly and/or specifically expressed on the targeted (diseased, tumor) cell compared to healthy cells, which are not intended to be targeted with the molecules and conjugates of the invention. An embodiment is the pharmaceutical combination, wherein at least one of the first (fourth, sixth) and second (fifth, seventh) binding site and thus at least one of the first (fourth, sixth) and second (fifth, seventh) cell-surface molecule such as a first (fourth, sixth) and second (fifth, seventh) tumor-cell receptor, is expressed specifically or to a relatively higher extent when compared to expression of the first (fourth, sixth) cell-surface molecule and/or the second (fifth, seventh) cell-surface molecule on the surface of a healthy (neighbouring) cell. Thus, the first (fourth, sixth) epitope or the second (fifth, seventh) epitope, preferably the first (fourth, sixth) epitope and the second (fifth, seventh) epitope, on the targeted cell-surface molecule is/are ideally unique to the targeted diseased cells, and is/are at least specifically present and exposed at the surface of the targeted cells. Binding of the first (fourth, sixth) and second (fifth, seventh) proteinaceous molecules to their respective first (fourth, sixth) and second (fifth, seventh) epitope on a targeted cell is followed by endocytosis of the complexes of the first (fourth, sixth) proteinaceous molecule and the first (fourth, sixth) target cell-surface molecule and the second (fifth, seventh) proteinaceous molecule and the second (fifth, seventh) target cell-surface molecule. Since the first and second proteinaceous molecules have to enter the same target cell through binding interaction with two different cell-surface molecules both expressed to a sufficient extent or uniquely on the targeted cell when compared to healthy cells that should not be targeted, accumulation of a therapeutically active amount of first and second proteinaceous molecules inside the target cells is only possible and occurring if expression levels of the two distinct targeted cell-surface molecules is both above a certain minimal expression threshold. At the same time, the fact that the effector moiety bound to the second (fifth, seventh) proteinaceous molecule is only capable of exerting its intracellular (e.g. cytotoxic or gene silencing) activity in the presence of the first (fourth, sixth) proteinaceous molecule bearing the covalently bound saponin, when both the first and second proteinaceous

molecules were capable to enter the target cell in sufficient amounts by binding to sufficiently exposed and expressed first and second cell-surface molecules, also provides a safeguard against negative and undesired side effects of the effector moiety towards e.g. healthy cells and healthy tissue not meant to be targeted and affected by the effector moiety, when expression of at least on of the first and second cell-surface molecules is sufficiently low at the healthy cells and preferably when expression of both the first and second targeted cell-surface molecules is sufficiently low at the healthy cells. That is to say, sufficiently low expression or even absence of exposed first and second cell-surface molecules with regard to the first and second cell-surface molecules, and at least either the first cell-surface molecule or the second cell-surface molecule, bound by the first and second binding site of the first and second proteinaceous molecules respectively, does ideally not allow entrance into (non-targeted) healthy cells of both the first and second proteinaceous molecules to amounts that would in concert result in endosomal escape of the effector moiety under influence of the saponin bound to the first proteinaceous molecule. Since the ADC or the AOC can be used at lower dose compared to when the first proteinaceous molecule was not added to the therapeutic regimen, ADC or AOC entrance in healthy cells to low extent already bears a lower risk for occurrence of unwanted side effects when for example the targeting and killing of target diseased cells such as tumor cells and auto-immune cells is considered.

Synchronization is the missing link between a successful delivery strategy for mice and its application in humans. Indeed, the inventors established in a series of in vivo mouse tumor models that separately administering to the mice a dose of free saponin and a dose of ADC (second or third or fifth or seventh proteinaceous molecule according to the invention) did not result in any desired anti-tumor activity such as delayed tumor growth, tumor regression, diminished and slower tumor growth, compared to control animals not treated with the ADC and free saponin. The free saponin was administered using various routes of administration and using various time points of administering the free saponin compared to the moment of administering the ADC (administering free saponin before, during and after administering the ADC). The ADC tested in in vivo tumor models was cetuximab-dianthin (with free SO1861), or trastuzumab-saporin (with free SO1861). Varying the dose of free saponin did not provide for an efficacious anti-tumor activity. The ADCs referred to were administered at a dose that in itself did not inflict any beneficial anti-tumor effect on the tumor-bearing animals. Surprisingly, the inventors now established that beneficial anti-tumor activity in various in vitro mammalian cell-based bioassays and/or in various in vivo animal tumor models can be achieved by treating the animals with conjugates according to the invention, optionally comprising a scaffold according to the invention, i.e. combinations of first and second or first and third or fourth and fifth or sixth and seventh proteinaceous molecules of the invention. The scaffold for example being a tri-functional linker with a covalently bound saponin (e.g. SO1861, QS-21) via a cleavable or non-cleavable linkage, and/or with a covalently bound effector moiety (e.g. dianthin, silencing BNA (HSP27) via a non-cleavable bond or a cleavable bond, and/or with a covalently bound monoclonal antibody such as cetuximab, trastuzumab, OKT-9, or the scaffold being a dendron, such as a dendron to which for example four moieties can bind such as four saponin molecules, or a dendron for binding for example two saponins and two effector molecules, the dendron comprising a chemical group for (covalent) coupling to a ligand or an antibody or fragment or domain thereof. Reference is made to the Examples

section, exemplifying various of these scaffolds according to the invention, showing in vivo and/or in vitro anti-tumor cell activity when cell toxicity exerted by e.g. a proteinaceous toxin is considered or when gene silencing in the tumor cell is considered.

Without wishing to be bound by any theory, in view of the failures observed when treatment of tumor-bearing animals with an ADC together with free saponin is considered, it is preferred to synchronize the presence of both, the at least one saponin, and the effector moiety, preferably a toxin or an (antisense) oligonucleotide, in compartments or vesicles of the endocytic pathway of the target cell, e.g. a tumor cell or an auto-immune cell. With ADC (and/or AOC) and free saponin, synchronizing the presence of the molecules in the late endosomes, in order to obtain the synergistic effects *in vivo* was not beneficially obtainable according to attempts of the inventors. In one aspect, the invention preferably solves at least the following problem with respect to combining the effector moiety comprised by the second, third, fifth or seventh proteinaceous molecule and the saponins comprised by the first, fourth or sixth proteinaceous molecule: without wishing to be bound by any theory the only reasonable chemical group within, e.g., the saponins that can be used for (covalent), in particular single and cleavable, retainable coupling is required for the endosomal escape activity. Known restrictions are most likely the reason why saponins have not been used in combination with pharmaceutically active substances in clinical investigations other than the application of saponins in vaccination regimes wherein the use of an immune-potentiating adjuvant substance was implied, although the striking endosomal escape enhancer effect of, e.g., saponins listed in Table A1 and Scheme I is known for more than 10 years. For example providing a first, fourth or sixth proteinaceous molecule of the invention with a covalently conjugated scaffold solves these difficulties, at least in part. Surprisingly, the saponins previously applied for their immune-potentiating activity in the vaccination context involving saponins as adjuvant component, are now also suitably for (covalent) coupling to the first, fourth or sixth proteinaceous molecule of the invention, for anti-tumor activity in vitro and in vivo.

An effector moiety useful in the present invention preferably relies on late endosomal escape for exerting its effect. Some effectors, such as, e.g., a pseudomonas exotoxin, are rerouted to other organelles prior to the “late endosomal stage” and, thus, would normally not benefit from coupling to the second proteinaceous molecule according to the present invention. However, such toxin may be adapted for use with the present invention, e.g., by deleting the signal peptide responsible rerouting. In particular toxins that are highly toxic and would require only one molecule to escape the endosomes to kill a cell maybe modified to be less potent. It is preferred to use a toxin that kills a cell if at least 2, more preferably at least 5, more preferably at least 10, more preferably at least 20, more preferably at least 50, most preferably at least 100 toxin molecules escape the endosome. It is further preferred that a second proteinaceous molecule of the invention comprises a covalently conjugated functionalized scaffold, i.e. a scaffold comprising covalently bound effector moietie(s) for targeting the scaffold comprising the bound effector moietie(s) at a target cell such as a tumor cell or an auto-immune cellFurther, in order to reduce off-target toxicity, cell membrane non-permeable small molecule toxins are preferred effector molecules over cell membrane permeable toxins.

The term “ligand” as used in this invention has its ordinary meaning and preferably means a molecule or structure that is able to bind another molecule or structure on the cell surface of a target

cell, wherein said molecule or structure on the cell surface can be endocytosed and is preferably absent or less prominent on off-target cells. Preferably, said molecule or structure on the cell surface is constitutively endocytosed. More preferably a ligand in this invention induces endocytosis of said molecule or structure on the cell surface of target cells after binding to said molecule or structure. This is for instance the case for Epidermal Growth Factor Receptor (EGFR), present on the surface of a variety of cancer cells. Examples of molecules or structures on the cell surface of target cells that are constitutively endocytosed, are for instance Claudin-1 or major histocompatibility complex class II glycoproteins. A ligand can, e.g., be an antibody, a growth factor or a cytokine. Combining in a carrier molecule a toxin with a ligand is one possibility to create a targeted toxin. A toxin that is only toxic in a target cell because it interferes with processes that occur in target cells only can also be seen as a targeted toxin (as in off-target cells it cannot exert its toxic action, e.g. apoptin). Preferably, a targeted toxin is a toxin that is combined with a ligand or e.g. a monoclonal antibody in order to be active in target cells and not in off-target cells (as it is only bound to and endocytosed by target cells). In a functionalized scaffold comprising a carrier molecule comprising a ligand and an effector moiety (i.e. a second or third proteinaceous molecule), the ligand or the monoclonal antibody guides the effector moiety and scaffold to the target cells. After internalization, the at least one glycoside, preferably a saponin comprised by the conjugate of the first proteinaceous molecule and the saponin, mediates the endosomal escape of the effector moiety. The saponin is typically a saponin listed in Table A1 and Scheme I, and preferably the saponin is SO1861 and/or QS-21, and/or SA1641 and/or GE1741.

The inventors established that immunoglobulins, domains thereof, ligands, etc., are particularly suitable for application as the (first) binding site of the first, fourth or sixth proteinaceous molecule (and the same binding site of the third proteinaceous molecule) comprising the (first) binding site. Similarly, the inventors established that immunoglobulins, domains thereof, ligands, etc., are particularly suitable for application as the second, fifth or seventh binding site of the second, fifth or seventh proteinaceous molecule comprising the second, fifth or seventh binding site. For example, antibodies and binding domains of antibodies are suitable for targeting an epitope on the exposed surface of a selected cell-surface molecule, resulting in targeting the first and third and fourth and sixth (and separately the second, fifth and seventh) proteinaceous molecule to target cells expressing the cell-surface molecule targeted by the first and third and fourth and sixth proteinaceous molecule and/or target also cells expressing the second or fifth or seventh cell-surface molecule targeted by the second or fifth or seventh proteinaceous molecule, these cells also expressing the first and third and fourth and sixth cell-surface molecule (which is the same cell-surface molecule), and having said cell-surface molecules on their cell surface. Similarly, ligands such as EGF, targeting the EGFR on target cells, are suitable for application as the binding site in the first and third and fourth and sixth proteinaceous molecules, or as the second or fifth or seventh binding site in the second or fifth or seventh proteinaceous molecule with the proviso that the second, fifth and seventh binding site is different from the first and third and fourth and sixth binding site which first and third binding site are the same. Preferred are binding sites for the first and third, fourth, sixth epitope or for the second, fifth, seventh epitope, which are specific for the binding of the first and third, fourth, sixth proteinaceous molecules to the first, fourth, sixth cell-surface molecule and/or for the binding of the second, fifth, seventh proteinaceous molecule to the second, fifth, seventh

cell-surface molecule, the first (third, fourth, sixth) and second (fifth, seventh) cell-surface molecules exposed on the very same target cell. Binding sites based on antibodies or domains or binding fragments thereof for example provide for such desired specificity for a selected first, second, third, fourth, fifth, sixth, seventh epitope on a selected first or second or third (same as the first), fourth, fifth, sixth, seventh cell-surface molecule of a selected cell for targeting such as a diseased cell, a tumor cell, an auto-immune cell, etc. Therefore, first, second and third and fourth, fifth, sixth and seventh binding sites based on antibodies or binding molecules (fragments, domains) are preferred for the first and second and third and fourth and fifth and sixth ad seventh proteinaceous molecules.

By targeting the same cell-surface molecule with the first and third, and fourth and fifth proteinaceous molecule, the delivery of the saponin C and the effector moiety A1, B1, A2 or B2 at and inside the cytosol of the very same targeted cell is improved and more specific. An aberrant cell selected for targeting by the binding site of the first and third, or the fourth and fifth proteinaceous molecule ideally bears the cell-surface molecule to a high extent and/or specifically, when (neighboring) healthy cells in a patient are considered. Thus, the epitope on the targeted cell-surface molecule is ideally unique to the targeted diseases cells, and is at least specifically present and exposed at the surface of the targeted cells. Binding of the first and third or fourth and fifth proteinaceous molecules is followed by endocytosis of the complexes of the first proteinaceous molecule and the target cell-surface molecule and the third proteinaceous molecule and the target cell-surface molecule, or of the fourth proteinaceous molecule and the target cell-surface molecule and the fifth proteinaceous molecule and the target cell-surface molecule. Since the first and third, or fourth and fifth, proteinaceous molecules have to enter the same target cell through binding interaction with the very same cell-surface molecules, accumulation of a therapeutically active amount of first and third, or fourth and fifth, proteinaceous molecules inside the target cells is only possible and occurring if expression levels of the targeted cell-surface molecule is above a certain minimal expression threshold. At the same time, the fact that the effector moiety bound to the third and fifth proteinaceous molecule is only capable of exerting its intracellular (e.g. cytotoxic or gene silencing) activity in the presence of the first or fourth proteinaceous molecule bearing the covalently bound saponin, when both the first and third, or both the fourth and fifth, proteinaceous molecules were capable to enter the target cell in sufficient amounts by binding to sufficiently exposed and expressed cell-surface molecule, also provides a safeguard against negative and undesired side effects of the effector moiety towards e.g. healthy cells and healthy tissue not meant to be targeted and affected by the effector moiety, when expression of the targeted cell-surface molecule is sufficiently low at the healthy cells. That is to say, low expression of the cell-surface molecule bound by the binding site of the first and third, or fourth and fifth, proteinaceous molecules, does not allow entrance of both the first and third, or both the fourth and fifth, proteinaceous molecules to amounts that would in concert result in endosomal escape of the effector moiety under influence of the saponin bound to the first and fourth proteinaceous molecule. Since the ADC or AOC can be used at lower dose compared to when the first or fourth proteinaceous molecule was not added to the therapeutic regimen, ADC or AOC entrance in healthy cells to low extent already bears a lower risk for occurrence of unwanted side effects when for example the targeting and killing of target diseased cells such as tumor cells and auto-immune cells is considered.

Throughout the description and claims (the whole application), the terms 'first' and 'third' have the same meaning when the first and third epitope, the first and third binding site, the first and third cell-surface molecule are considered. That is to say, for the first and third proteinaceous molecules, the targeted epitope is the same, the binding site is the same, the targeted cell-surface molecule such as a tumor-cell (specific) receptor is the same. The same for the terms 'fourth' and 'fifth'.

Tables A2, A3 and A4 list preferred examples of the first, third, fourth and sixth cell-surface molecule comprising the first (third, fourth and sixth) epitope for the first (third, fourth and sixth) binding site of the first (third, fourth and sixth) proteinaceous molecule. In addition, Tables A2, A3 and A4 also list preferred examples of the second, fifth and seventh cell-surface molecule comprising the second, fifth and seventh epitope for the second, fifth and seventh binding site of the second, fifth, seventh proteinaceous molecule. When the first, third, fourth, sixth and/or second, fifth, seventh cell-surface molecule is specifically expressed on the target cell, preferably both the first, third, fourth and sixth, and the second, fifth, seventh cell-surface molecules, and when the first and second epitopes on the first and second cell-surface molecules respectively, to which the first binding site and/or the second binding site can bind respectively, is specifically present in the first and/or second cell-surface molecule, specific targeting of the first, third and/or second proteinaceous molecule to the same desired target cell such as a tumor cell exposing the first and second tumor-cell surface molecules, is facilitated, whereas other cells such as healthy cells, which do not express the first and/or second cell-surface molecule or do express the first and/or second cell-surface molecule to a lower extent, preferably which which do not express the first and second cell-surface molecule or do express the first and second cell-surface molecule to a lower extent compared to expression of the cell-surface molecule(s) on the targeted (aberrant) cell, are not targeted by the first, third and second proteinaceous molecule or are targeted to a lower extent.

A pharmaceutically active substance in this invention is an effector moiety that is used to achieve a beneficial outcome in an organism, preferably a vertebrate, more preferably a human being such as a cancer patient or an auto-immune patient. Benefit includes diagnosis, prognosis, treatment, cure and/or prevention of diseases and/or symptoms. The pharmaceutically active substance may also lead to undesired harmful side effects. In this case, pros and cons must be weighed to decide whether the pharmaceutically active substance is suitable in the particular case. If the effect of the pharmaceutically active substance inside a cell is predominantly beneficial for the whole organism, the cell is called a target cell. If the effect inside a cell is predominantly harmful for the whole organism, the cell is called an off-target cell. In artificial systems such as cell cultures and bioreactors, target cells and off-target cells depend on the purpose and are defined by the user.

An effector moiety that is a polypeptide may be, e.g., a polypeptide that recover a lost function, such as for instance enzyme replacement, gene regulating functions, or a toxin.

An embodiment is the first, fourth or sixth proteinaceous molecule of the invention, wherein the first, fourth or sixth proteinaceous molecule comprises more than one saponin C, preferably 2, 3, 4, 5, 6, 8, 10, 16, 32, 64 or 1-100 saponins, or any number of saponins therein between, such as 7, 9, 12 saponins, covalently bound directly to an amino-acid residue of the first, fourth or sixth proteinaceous molecule, preferably to a cysteine and/or to a lysine, and/or covalently bound via at least one linker

and/or via at least one cleavable linker and/or via at least one polymeric or oligomeric scaffold L3, L7, preferably 1-8 of such scaffolds or 2-4 of such scaffolds, wherein the at least one scaffold is optionally based on a dendron, wherein 1-32 saponins such as 2, 3, 4, 5, 6, 8, 10, 16, 32 saponins, or any number of saponins therein between, such as 7, 9, 12 saponins, are covalently bound to the at least one scaffold.

Table A1 and Scheme I and the above embodiments summarize a series of saponins C that have been identified for their endosomal escape enhancing activity when contacted to mammalian cells, in particular human tumor cells, in free form together with a second molecule (e.g. an effector moiety or effector molecule, payload, such as a toxin, an oligonucleotide). Indeed, in cell-based bioassays using human tumor cells it was established for the saponins tabulated in Table A1 and those in Scheme I and in the various embodiments of the invention described herein, that under influence of these saponins, when bound to the first, fourth or sixth proteinaceous molecule, a second molecule (effector moiety) such as a nucleic acid and/or a toxin such as a protein toxin (e.g. one or more of the protein toxins listed in Table A5), bound to the second or third or fifth or seventh proteinaceous molecule, is delivered into the cytosol with increased efficiency and/or efficacy, presumably through intracellular release from the (late) endosomes and lysosomes. That is to say, endosomal and/or lysosomal escape of such second molecules (effector moieties bound to a second or to a third or to a fifth or to a seventh proteinaceous molecule of the invention), e.g. nucleic acids and/or toxins, is less efficient in the absence of the saponin.

Surprisingly, the inventors now demonstrate that a water-soluble saponin fraction from Quillaja saponaria, comprising QS-21 and its family members QS-21A, QS-21 A-api, QS-21 A-xyl, QS-21B, QS-21 B-api, QS-21 B-xyl, QS-7-xyl, QS-7-api, QS-17-api, QS-17-xyl, QS1861, QS1862, QS-18 and Quil-A, also exhibits the ability to potentiate a biological effect in vitro of e.g. a nucleic acid bound to a monoclonal antibody or a protein toxin bound to a monoclonal antibody (examples of a second and/or third and/or fifth and/or seventh proteinaceous molecule of the invention comprising covalently bound oligonucleotide or payload such as a (protein) toxin), when administered to tumor cells of a mammalian species (human) in the form of a covalent conjugate comprising a monoclonal antibody (first, fourth, sixth proteinaceous molecule of the invention), together with the second and/or third and/or fifth and/or seventh proteinaceous molecule comprising the effector moiety (the aforementioned second and/or third and/or fifth and/or seventh proteinaceous molecule) and the at least one glycoside such as the QS-21 and its family member saponins encompassed by such QS-21 preparation (e.g. water soluble fraction of Quillaja saponaria), comprised by the first, fourth or sixth proteinaceous molecule as a covalent conjugate, wherein the effector molecule and the glycoside, e.g. saponin fraction of Quillaja saponaria, QS-21, SO1861, SA1641, GE1741, are covalently bound to for example the proteinaceous molecules directly or via a linker or via a polymeric or oligomeric scaffold, either directly or via at least one linker. Without wishing to be bound by any theory, the observed stimulation or potentiation of for example antisense BNA mediated reduction of tumor-cell HSP27 expression (HSP27 gene silencing) in the presence of saponins derived from Quillaja saponaria in vitro may (also) relate to activation of the inflammasome in the tumor cell by the saponins, for example resulting in tumor cell pyroptosis. The inventors established that second and third and fifth and seventh proteinaceous molecules conjugated to for example antisense BNA or dianthin or saporin, exerted any anti-tumor cell activity in vitro at all or improved anti-tumor cell activity when contacted with cells in bio-based cell assays, when in the

presence of the first or fourth or sixth proteinaceous molecule of the invention, comprising the saponin, and targeted to the same (tumor) cells as the cell surface molecule targeted by the second and/or third and/or fifth and/or seventh proteinaceous molecule, whereas in the absence of the first, fourth, sixth proteinaceous molecule and thus in the absence of saponin, no such activity towards the tumor cell was observed.

QS-21, and also the water-soluble saponins fraction comprising QS-21 from Quillaja saponaria is already for a long time known and previously intensively applied for its immune-potentiating abilities, e.g. as an adjuvant in e.g. sub-unit vaccines. For example, QS-21 is applied in two phase III clinical trials with human patients, who were vaccinated with a sub-unit vaccine mixed with an adjuvant comprising QS-21 (Glaxo-Smith-Kline, MAGRIT trial, DERMA study), wherein the sub-unit was MAGE-A3 protein, which is specifically expressed and presented by tumor cells. The anti-tumor vaccinations, potentiated with QS-21, aimed for extension of disease-free survival of the cancer patients (melanoma; non-small cell lung cancer). In addition, QS-21 has been tested as an adjuvant in clinical trials for developing anti-cancer vaccine treatment, for vaccines for HIV-1 infection, in development of a vaccine against hepatitis B, and for anti-malaria vaccine development using QS-21 comprising adjuvants AS01 and AS02 of Glaxo-Smith-Kline. Previous studies revealed an immune response elicited against MAGE-A3 peptides presented at the cancer cell surface, under influence of the QS-21 saponin comprising adjuvant (AS15; GSK). To the surprise of the inventors, the saponin fraction of Quillaja saponaria, and thus likely QS-21 (as part of the water soluble saponin fraction of Quillaja saponaria) potentiates the anti-tumor cell activity of e.g. a payload such as a protein toxin (dianthin), bound to the second, fifth, seventh proteinaceous molecule (e.g. the ligand EGF).

The inventors show that a tumor-cell targeting monoclonal antibody provided with covalently coupled antisense BNA such as BNA(HSP27), and contacted with the tumor cells together with a first or fourth proteinaceous molecule of the invention with covalently coupled saponin (e.g. SO1861, QS-21), both the BNA and the saponin coupled to the respective antibody (e.g. cetuximab) of the first and third or fourth and fifth proteinaceous molecule via a cleavable bond is capable of silencing HSP27 in vivo in tumors, compared to control and compared to AOC (third and fifth proteinaceous molecule) only, without presence of first or fourth proteinaceous molecule with coupled saponin. Co-administering an ADC or an antibody-oligonucleotide conjugate (AOC), such as an antibody-BNA conjugate, with a first or fourth proteinaceous molecule with a saponin thus endows the ADC or AOC with anti-tumor cell activity not seen with only the ADC or only the AOC at the same dose. Noteworthy, the AOC (the second or third or fifth proteinaceous molecule) and the monoclonal antibody with covalently coupled saponin (first or fourth proteinaceous molecule) increase HSP27 expression in tumor cells, when administered to tumor-bearing mice separately in separate groups of mice, compared to a control group (vehicle administered, only). Only co-administration of the AOC comprising the effector moiety of the invention (second or third or fifth proteinaceous molecule) and the first or fourth proteinaceous molecule with covalently coupled saponin, displays reduced HSP27 expression when compared to controls. The antisense BNA (HSP27) was BNA with oligo nucleic acid sequence 5'-GGCacagccagtgGCG-3' according to Zhang et al. (2011) [*Y Zhang, Z Qu, S Kim, V Shi, B Liao1, P Kraft, R Bandaru, Y Wu, LM Greenberger and ID Horak, Down-modulation of cancer targets using locked nucleic acid (LNA)-based*

antisense oligonucleotides without transfection, Gene Therapy (2011) 18, 326–333]. Noteworthy, to the best of the knowledge of the inventors, BNA is designed for application as a free nucleic acid. The inventors are now the first to demonstrate that the antisense BNA can be covalently coupled through a (non-)cleavable linker with a ligand or an antibody, in a way that gene-silencing activity is retained in vitro and more importantly in vivo in the tumor cells of a tumor-bearing animal. This approach of providing BNA-based AOCs opens new ways to administer targeted BNA to human (cancer) patients in need thereof.

The inventors disclose here that covalently coupling saponins such as saponins in the water-soluble fraction of Quillaja saponaria, QS-21, SA1641, SO1861, Table A1, Scheme I, to a first or fourth or sixth proteinaceous molecule, such as via a tri-functional linker, e.g. the tri-functional linker of Scheme II and Structure B, or via an oligomeric or polymeric structure of a scaffold comprising covalently bound saponins, results in improved cell toxicity exerted by the effector moiety such as a toxin, comprised by the second and/or third and/or fifth and/or seventh proteinaceous molecule, under influence of the covalently coupled saponin in the first, fourth or sixth proteinaceous molecule.

According to the invention, typically the saponin is a saponin listed in Table A1, Scheme I. It has been proven beneficial for the activity of the saponin, e.g. the endosomal escape enhancing activity inside cells when the entry into the cell and the accumulation inside the cytosol of an effector moiety covalently coupled to the second or third or fifth or seventh proteinaceous molecule, is considered, when the saponin is covalently coupled to the first or fourth or sixth proteinaceous molecule involving a hydrazone bond, and/or a hydrazide bond, and/or a disulphide bond. Such bond types readily cleave under the acidic conditions inside (late) endosomes and lysosomes of mammalian cells, e.g. human cells, and/or under the reductive conditions. Alternatively, the inventors also demonstrate that covalent coupling of saponin to the first, fourth or sixth proteinaceous molecule via a bond that is not readily cleavable under the physiological conditions inside cells, e.g. (late) endosomes, lysosomes, cytosol, is also beneficial to the potentiating activity of the saponin on the biological effect of e.g. an effector moiety such as a nucleic acid (e.g. BNA silencing HSP27) and a proteinaceous toxin such as saporin. Throughout the application, including the claims, the term ‘cleavable linker’, ‘cleavable bond’, etc., is also referred to as ‘labile linker’ (‘L’) and ‘labile bond’, for example in the context of cleavage of such a bond or linker in the (late) endosome and/or lysosome when a conjugate of the invention, e.g. a first, fourth or sixth proteinaceous molecule optionally comprising a scaffold with saponins coupled to the first, fourth or sixth proteinaceous molecule through a linker and/or via the scaffold via hydrazone bonds or disulphide bonds, is referred to. For example, Figure 1-1, Figure 6-2, Figure 2-4 and Figure 1-5 show the in vivo HSP27 gene silencing in human tumors in mice. The tumor-bearing mice were for example treated with a first proteinaceous molecule consisting of monoclonal antibody with saponin bound thereto via a labile linker (hydrazone bond) according to the invention, whereas the third proteinaceous molecule comprised bound antisense BNA for silencing the HSP27 gene in the tumor cells, covalently coupled to the monoclonal antibody (same type as the first monoclonal antibody) via a a disulphide bond. That is to say, without wishing to be bound by any theory, the hydrazone bond and the disulphide bond are cleaved in the (late) endosomes and/or lysosomes of the targeted tumor cells that express the epitope on the targeted cell-surface molecule, here the EGFR, at the cell surface, once the therapeutic

combination of the invention is internalized by e.g. endocytosis. Cleavage of the bonds likely contributes to the endosomal escape enhancing activity of the saponin when the entry of the BNA from the endosome and/or lysosome into the cytosol is considered, although such cleavage is not a necessity for observing the gene silencing effect of the combination of the cetuximab-SO1861 conjugate and the cetuximab-BNA conjugate of the invention.

The skilled person will appreciate that a tri-functional linker is a scaffold of the invention suitable for covalently coupling one, two or three saponin moieties. For the tri-functional linker covalent coupling of one or two saponin moieties is preferred. The second and/or third binding site is for example suitable for covalent coupling a proteinaceous ligand such as the first, fourth or sixth proteinaceous molecule. Typical proteinaceous ligands are EGF for targeting (tumor) cells expressing EGFR at the cell surface, and cytokines for targeting tumor cells or autoimmune cells. Moreover, the second or third binding site of the tri-functional linker is suitable for covalent coupling of an immunoglobulin such as a monoclonal antibody, i.e. the first, fourth or sixth proteinaceous molecule for binding to a cell surface molecule such as a tumor cell surface molecule, preferably a tumor-cell specific molecule, more preferably a tumor cell receptor that is specifically (over-)expressed at the surface of the tumor cell. Similarly, the immunoglobulin, or any fragment(s) and/or domain(s) thereof which encompass the binding specificity of the immunoglobulin, is suitable for binding to a cell surface molecule such as a receptor, expressed at the surface of an autoimmune cell. Thus, in an embodiment, the first, fourth or sixth proteinaceous molecule comprises the tri-functional linker, said linker comprises or consists of a covalently bound saponin, e.g. QS-21, SO1861, and the covalently bound binding site such as a cell targeting moiety such as a ligand or an antibody for (specific) binding to a tumor cell, an auto-immune cell, a diseased cell, an aberrant cell, a non-healthy cell, a B-cell disease.

A first, fourth or sixth proteinaceous molecule according to the invention thus comprises at least one saponin. With “at least one” in this context is meant that the first , fourth or sixth proteinaceous molecule comprises one saponin molecule but may also comprise a couple (e.g. two, three or four) of saponins or a multitude (e.g. 10, 20 or 100) of saponins. Depending on the application, the first, fourth or sixth proteinaceous molecule may comprise a covalently bound scaffold with covalently bound saponins, wherein the scaffold may be designed such that it comprises a defined number of saponins. Preferably, a first, fourth or sixth proteinaceous molecule according to the invention comprises a defined number or range of saponins, rather than a random number. This is especially advantageous for drug development in relation to marketing authorization. A defined number in this respect means that a first, fourth or sixth proteinaceous molecule preferably comprises a previously defined number of saponins. This is, e.g., achieved by designing a scaffold comprising a polymeric structure with a certain number of possible moieties for the saponin(s) to attach. Under ideal circumstances, all of these moieties are coupled to a saponin and the scaffold than comprises the prior defined number of saponins. It is envisaged to offer a standard set of scaffolds, comprising, e.g., two, four, eight, sixteen, thirty-two, sixty-four, etc., saponins so that the optimal number can be easily tested by the user according to his needs. An embodiment is the first, fourth or sixth proteinaceous molecule of the invention comprising the scaffold of the invention, wherein the saponin is present in a defined range as, e.g., under non-ideal circumstances, not all moieties present in a polymeric structure bind a saponin. Such ranges may for

instance be 2 – 4 saponin molecules per scaffold, 3 – 6 saponin molecules per scaffold, 4 – 8 saponin molecules per scaffold, 6 – 8 saponin molecules per scaffold, 6 – 12 saponin molecules per scaffold and so on. In such case, a first proteinaceous molecule comprising a scaffold according to the invention thus comprises 2, 3 or 4 saponins if the range is defined as 2 – 4.

The scaffold is fundamentally independent of the type of saponin covalently bound to the scaffold, the scaffold subsequently (in sequential order) covalently coupled to the first, fourth or sixth proteinaceous molecule. Thus, first, fourth or sixth proteinaceous molecule comprising the scaffold is the basis product for a new platform technology. Since the at least one covalently bound saponin mediates intracellular delivery of the effector moiety bound to the second, third, fifth or seventh proteinaceous molecule, the scaffold technology according to the invention is the first system known that mediates controlled intracellular effector moiety delivery by saponins. The scaffold provides an optimized and functionally active unit that can be linked to the saponin(s) and to the binding site comprised by the first, fourth or sixth proteinaceous molecule, e.g. a ligand, an antibody, etc., at a single and defined position.

An embodiment is the first, fourth or sixth proteinaceous molecule comprising a scaffold according to the invention, wherein the number of monomers of the polymeric or oligomeric structure is an exactly defined number or range. Preferably, the polymeric or oligomeric structure comprises structures such as poly(amines), e.g., polyethylenimine and poly(amidoamine), or structures such as polyethylene glycol, poly(esters), such as poly(lactides), poly(lactams), polylactide-co-glycolide copolymers, poly(dextrin), or a peptide or a protein, or structures such as natural and/or artificial polyamino acids, e.g. poly-lysine, DNA polymers, stabilized RNA polymers or PNA (peptide nucleic acid) polymers, either appearing as linear, branched or cyclic polymer, oligomer, dendrimer, dendron, dendronized polymer, dendronized oligomer or assemblies of these structures, either sheer or mixed. Preferably, the polymeric or oligomeric structures are biocompatible, wherein biocompatible means that the polymeric or oligomeric structure does not show substantial acute or chronic toxicity in organisms and can be either excreted as it is or fully degraded to excretable and/or physiological compounds by the body's metabolism. Assemblies can be built up by covalent cross-linking or non-covalent bonds and/or attraction. They can therefore also form nanogels, microgels, or hydrogels, or they can be attached to carriers such as inorganic nanoparticles, colloids, liposomes, micelles or particle-like structures comprising cholesterol and/or phospholipids. Said polymeric or oligomeric structures preferably bear an exactly defined number or range of coupling moieties for the coupling of glycoside molecules (and/or effector molecules and/or carrier molecules such as a ligand, monoclonal antibody or a fragment thereof). Preferably at least 50%, more preferably at least 75%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%, more preferably at least 99%, most preferably 100% of the exactly defined number or range of coupling moieties in the polymeric or oligomeric structure is occupied by a glycoside molecule in a scaffold according to the invention.

Preferably, a dendron is a branched, clearly defined tree-like polymer with a single chemically addressable group at the origin of the tree, called the focal point. A dendrimer is a connection of two or more dendrons at their focal point. A dendronized polymer is a connection of the focal point of one or

more dendrons to a polymer. In a preferred embodiment, a scaffold according to the invention is provided, wherein the polymeric or oligomeric structure comprises a linear, branched or cyclic polymer, oligomer, dendrimer, dendron, dendronized polymer, dendronized oligomer or assemblies of these structures, either sheer or mixed, wherein assemblies can be built up by covalent cross-linking or non-covalent attraction and can form nanogels, microgels, or hydrogels, and wherein, preferably, the polymer is a derivative of a poly(amine), e.g., polyethylenimine and poly(amidoamine), and structures such as polyethylene glycol, poly(esters), such as poly(lactids), poly(lactams), polylactide-co-glycolide copolymers, and poly(dextrin), and structures such as natural and/or artificial polyamino acids such as poly-lysine, or a peptide or a protein or DNA polymers, stabilized RNA polymers or PNA (peptide nucleic acid) polymers. Preferably, the polymeric or oligomeric structures are biocompatible.

An embodiment is the therapeutic combination of the invention or the therapeutic combination for use according to the invention, wherein the first, fourth or sixth proteinaceous molecule comprises more than one covalently bound saponin, preferably 2, 3, 4, 5, 6, 8, 10, 16, 32, 64, 128 or 1-100 saponins, or any number of saponins therein between, such as 7, 9, 12 saponins.

An embodiment is the first, fourth or sixth proteinaceous molecule of the invention, wherein the at least one saponin is covalently bound to the polymeric or oligomeric structure of the oligomeric or polymeric scaffold via at least one cleavable linker according to the invention.

An embodiment is the first, fourth or sixth proteinaceous molecule of the invention, wherein the chemical group of the oligomeric or polymeric scaffold, for covalently coupling of the oligomeric or polymeric scaffold to the amino-acid residue of said first, fourth or sixth proteinaceous molecule, is a click chemistry group, preferably selected from a tetrazine, an azide, an alkene or an alkyne, or a cyclic derivative of these groups, more preferably said chemical group is an azide.

An embodiment is the first, fourth or sixth proteinaceous molecule of the invention, wherein the polymeric or oligomeric structure of the oligomeric or polymeric scaffold comprises a linear, branched and/or cyclic polymer, oligomer, dendrimer, dendron, dendronized polymer, dendronized oligomer, a DNA, a polypeptide, poly-lysine, a poly-ethylene glycol, or an assembly of these polymeric or oligomeric structures which assembly is preferably built up by covalent cross-linking.

The inventors established that covalent coupling, preferably via cleavable bonds or linkers, of the saponin to the first, fourth or sixth proteinaceous molecule, according to any of the embodiments here above, provides efficient and cell-targeted potentiation of the activity of an effector moiety bound to the second and to the third and to the fifth and to the seventh proteinaceous molecule, wherein the first and third and fourth and sixth proteinaceous molecules comprise the same first, fourth or sixth binding site and wherein the first (fourth, sixth) and second (fifth, seventh) proteinaceous molecules comprise a first (third, fourth, sixth) and second (fifth, seventh) binding site which are different. Coupling saponin to a cysteine side chain or a lysine side chain of the first, fourth or sixth proteinaceous molecule such as a monoclonal antibody, directly or via a linker, proved to be a beneficial way of specific and efficient delivery of effector-moiety potentiating activity inside the target cell, when also the effector moiety is delivered in the same target cell by using the second and/or third and/or fifth and/or seventh proteinaceous molecule comprising the same first binding site as the first proteinaceous molecule when

the third or fifth proteinaceous molecule is considered and comprising different first and second, seventh binding sites respectively when the first and second, seventh proteinaceous molecules are considered.

To explain the invention in more detail, the process of cellular uptake of substances (although the inventors do not wish to be bound by any theory) and the used terminology in this invention is described. The uptake of extracellular substances into a cell by vesicle budding is called endocytosis. Said vesicle budding can be characterized by (1) receptor-dependent ligand uptake mediated by the cytosolic protein clathrin, (2) lipid-raft uptake mediated by the cholesterol-binding protein caveolin, (3) unspecific fluid uptake (pinocytosis), or (4) unspecific particle uptake (phagocytosis). All types of endocytosis run into the following cellular processes of vesicle transport and substance sorting called the endocytic pathways. The endocytic pathways are complex and not fully understood. Without wishing to be bound by any theory, organelles may be formed *de novo* and mature into the next organelle along the endocytic pathway. It is however, now hypothesized that the endocytic pathways involve stable compartments that are connected by vesicular traffic. A compartment is a complex, multifunctional membrane organelle that is specialized for a particular set of essential functions for the cell. Vesicles are considered to be transient organelles, simpler in composition, and are defined as membrane-enclosed containers that form *de novo* by budding from a preexisting compartment. In contrast to compartments, vesicles can undergo maturation, which is a physiologically irreversible series of biochemical changes. Early endosomes and late endosomes represent stable compartments in the endocytic pathway while primary endocytic vesicles, phagosomes, multivesicular bodies (also called endosome carrier vesicles), secretory granules, and even lysosomes represent vesicles. The endocytic vesicle, which arises at the plasma membrane, most prominently from clathrin-coated pits, first fuses with the early endosome, which is a major sorting compartment of approximately pH 6.5. A large part of the cargo and membranes internalized are recycled back to the plasma membrane through recycling vesicles (recycling pathway). Components that should be degraded are transported to the acidic late endosome (pH lower than 6) via multivesicular bodies. Lysosomes are vesicles that can store mature lysosomal enzymes and deliver them to a late endosomal compartment when needed. The resulting organelle is called the hybrid organelle or endolysosome. Lysosomes bud off the hybrid organelle in a process referred to as lysosome reformation. Late endosomes, lysosomes, and hybrid organelles are extremely dynamic organelles, and distinction between them is often difficult. Degradation of an endocytosed molecule occurs inside an endolysosome or lysosome. Endosomal escape is the active or passive release of a substance from the inner lumen of any kind of compartment or vesicle from the endocytic pathway, preferably from clathrin-mediated endocytosis, or recycling pathway into the cytosol. Endosomal escape thus includes but is not limited to release from endosomes, endolysosomes or lysosomes, including their intermediate and hybrid organelles.

Unless specifically indicated otherwise and in particular when relating to the endosomal escape mechanism of the glycoside molecule such as the saponin of the invention, whenever the word “endosome” or “endosomal escape” is used herein, it also includes the endolysosome and lysosome, and escape from the endolysosome and lysosome, respectively. After entering the cytosol, said substance might move to other cell units such as the nucleus.

In formal terms, a glycoside is any molecule in which a sugar group is bound through its anomeric carbon to another group via a glycosidic bond. Glycoside molecules, such as saponins, in the context of the invention are such molecules that are further able to enhance the effect of an effector moiety, without wishing to be bound by any theory, in particular by facilitating the endosomal escape of the effector moiety. Without wishing to be bound by any theory, the glycoside molecules (saponins, such as those listed in Table A1) interact with the membranes of compartments and vesicles of the endocytic and recycling pathway and make them leaky for said effector moieties resulting in augmented endosomal escape. With the term "the scaffold is able to augment endosomal escape of the effector moiety" is meant that the at least one saponin (glycoside molecule), which is coupled to the polymeric or oligomeric structure of the scaffold, is able to enhance endosomal escape of an effector moiety when both molecules are within an endosome, e.g. a late endosome, optionally and preferably after the at least one glycoside such as a saponin is released from the first, fourth, sixth proteinaceous molecule such as from a linker or polymeric or oligomeric structure comprised by said first, fourth, sixth proteinaceous molecule, e.g., by cleavage of a cleavable bond between the at least one glycoside (saponin) and the the first, fourth, sixth proteinaceous molecule (for example via a polymeric or oligomeric structure of a scaffold and/or via a linker). Even though a bond between the at least one glycoside such as a saponin according to the invention and the first, fourth, sixth proteinaceous molecule, optionally via a linker or a scaffold, may be a "stable bond", that does not mean that such bond cannot be cleaved in the endosomes by, e.g., enzymes. For instance, the glycoside or saponin, optionally together with a linker or a part of the oligomeric or polymeric structure of a scaffold, may be cleaved off from the remaining linker fragment or oligomeric or polymeric structure. It could, for instance be that a protease cuts a (proteinaceous) linker or proteinaceous polymeric structure, e.g., albumin, thereby releasing the at least one glycoside, saponin. It is, however, preferred that the glycoside molecule (preferably saponin) is released in an active form, preferably in the original form that it had before it was (prepared to be) coupled to the first, fourth, sixth proteinaceous molecule optionally via a linker and/or an oligomeric or polymeric scaffold; thus the glycoside (saponin) has its natural structure after such cleavage or the glycoside (saponin) has (part of) a chemical group or linker bound thereto, after such cleavage, while glycoside biological activity (saponin biological activity), e.g. endosomal/lysosomal escape enhancing activity towards an effector moiety present in the same endosome or lysosome, is maintained or restored upon said cleavage of the bond between the glycoside (saponin) and the carrier molecule, i.e. the first, fourth, sixth proteinaceous molecule optionally comprising a linker and/or a scaffold of the invention. With regard to the present invention the term "stable" with respect to bonds between e.g. saponins and amino-acid residues of the first, fourth, sixth proteinaceous molecule, a linker, a polymeric or oligomeric structures (of the scaffold), ligands, (monoclonal) immunoglobulins or binding domains or –fragments thereof, and/or effectors (effector moieties, effector molecules), is meant that the bond is not readily broken or at least not designed to be readily broken by, e.g., pH differences, salt concentrations, or UV-light, reductive conditions. With regard to the present invention the term "cleavable" with respect to bonds between e.g. saponins and the first, fourth, sixth proteinaceous molecule, linkers, amino-acid residues, polymeric or oligomeric structures of the scaffold, ligands, antibodies and/or effectors, is meant that the bond is designed to be readily broken

by, e.g., pH differences, salt concentrations, under reductive conditions, and the like. The skilled person is well aware of such cleavable bonds and how to prepare them.

Before the present invention one of the major hurdles of introducing ADCs and AOCs on the market was the small therapeutic window: a therapeutically effective dose of an ADC or an AOC is accompanied with (unacceptable) side effects, hampering development and implication in treatment of patients with the ADCs. By the application of the first, fourth or sixth proteinaceous molecule of the invention it has now become possible to guide one or multiple glycoside molecules (saponin) to a (target) cell, together with the ADC carrying a payload or together with a (monoclonal) antibody conjugated with an oligonucleotide such as a BNA according to the invention (i.e. a particular second or third or fifth or seventh proteinaceous molecule of the invention). In particular, it was previously not possible to specifically guide an effector moiety of a second or third or fifth or seventh proteinaceous molecule and a (predefined, controllable) particular number or range of glycoside molecules (saponins) per effector moiety at the same time to the cytosol of cells, such as via the endocytic pathway of a cell.

A solution provided for by the invention comprises the covalent binding of at least one saponin to the first or fourth or sixth proteinaceous molecule. A further solution provided for by the invention comprises (first) polymerizing the glycoside molecules (saponins) using an oligomeric or polymeric scaffold, and providing the first or fourth or sixth proteinaceous molecule with a cluster of covalently bound saponins, enabling re-monomerization of the one or more saponins at the intracellular site where the mode of action of the saponin is desired, e.g. after endocytosis. ”Polymerizes” in this context means the reversible and/or irreversible multiple conjugation of saponin molecules to the first, fourth, sixth proteinaceous molecule, either via linker, or directly or via a polymeric or oligomeric structure to form a scaffold or the reversible and/or irreversible multiple conjugation of (modified) saponins thereby forming a polymeric or oligomeric structure to form a scaffold. “Re-monomerization” in this context means the cleavage of the saponins from the first, fourth, sixth proteinaceous molecule, from the linker linking the saponin(s) to the first, fourth, sixth proteinaceous molecule or from the scaffold, for example after endocytosis, and regaining the (native) chemical state of the unbound saponins, which unbound saponins may or may not comprise additional chemical groups such as a chemical group for linking the saponin to a linker, an amino-acid residue of the first proteinaceous molecule or to the scaffold, and/or a (chemical) linker bound to a chemical group of the saponin such as an aldehyde group or carboxylic acid group. Due to the complex chemistry of the saponins for example the ´polymerization´ of saponins at a scaffold or other linking linker and their ´re-monomerization´ at a desired location such as intracellularly e.g. after endocytosis, was a challenging task. In particular, the chemical reactions used for providing the linkers and the scaffold comprising covalently linked glycosides for covalent binding to the first, fourth, sixth proteinaceous molecule, e.g. triterpenoid saponins (polymerization of the glycosides), normally occur in water-free organic solvents, but saponins and for example biocompatible polymers applied as a scaffold for bearing bound saponins, are water-soluble molecules. The chemical properties of the unmodified saponin further prohibited polymerization by itself and, one other possible solution, to bind multiple saponins (directly) to the effector molecule was estimated not to be very promising, as an effector molecule (drug, toxin, polypeptide or polynucleotide) does typically not provide sufficient binding sites and because the coupling product would become quite heterogeneous and/or

coupling biologically active molecules such as a saponin and e.g. a peptide, a toxin, a nucleic acid together bears the risk for influencing and hampering the activity of one or even both molecules bound together in such saponin-comprising conjugate. Further, there was a considerable risk that the effector moiety comprised by the second or third or fifth or seventh proteinaceous molecule loses its function after coupling of a saponin to the e.g. ADC or antibody-oligonucleotide conjugate (AOC). Embodiments of the present invention solves at least one of these drawbacks.

An aspect of the invention relates to a composition comprising the first or fourth or sixth proteinaceous molecule of the invention and the second, third, fifth or seventh proteinaceous molecule of the invention.

An aspect of the invention relates to a composition comprising the first, fourth or sixth proteinaceous molecule of the invention and the third or fifth proteinaceous molecule of the invention.

An embodiment is the composition comprising the first, fourth or sixth proteinaceous molecule of the invention and the second, or seventh proteinaceous molecule of the invention, or is the composition comprising the first, fourth or sixth proteinaceous molecule of the invention and the third or fifth proteinaceous molecule of the invention, wherein the effector moiety that is comprised by the second, fifth or seventh proteinaceous molecule or by the third proteinaceous molecule is any one of the effector moieties according to the invention, preferably a BNA.

An aspect of the invention relates to a composition comprising the first, fourth or sixth proteinaceous molecule of the invention and any one or more of an oligonucleotide, a nucleic acid and a xeno nucleic acid, preferably selected from at least one of a vector, a gene, a cell suicide inducing transgene, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), anti-sense oligonucleotide (ASO, AON), short interfering RNA (siRNA), microRNA (miRNA), DNA aptamer, RNA aptamer, mRNA, mini-circle DNA, peptide nucleic acid (PNA), phosphoramidate morpholino oligomer (PMO), locked nucleic acid (LNA), bridged nucleic acid (BNA), 2'-deoxy-2'-fluoroarabino nucleic acid (FANA), 2'-O-methoxyethyl-RNA (MOE), 2'-O,4'-aminoethylene bridged nucleic acid, 3'-fluoro hexitol nucleic acid (FHNA), a plasmid, glycol nucleic acid (GNA) and threose nucleic acid (TNA), or a derivative thereof, more preferably a BNA, for example a BNA for silencing HSP27 protein expression (antisense BNA(HSP27)).

An effector molecule, or effector moiety, in the context of this invention is any substance that affects the metabolism of a cell by interaction with an intracellular effector molecule target, wherein this effector molecule target is any molecule or structure inside cells excluding the lumen of compartments and vesicles of the endocytic and recycling pathway but including the membranes of these compartments and vesicles. Said structures inside cells thus include the nucleus, mitochondria, chloroplasts, endoplasmic reticulum, Golgi apparatus, other transport vesicles, the inner part of the plasma membrane and the cytosol. Cytosolic delivery of an effector moiety in the context of the invention preferably means that the effector moiety is able to escape the endosome (and/or lysosome), which, as defined previously, also includes escaping the endolysosome and the lysosome, and is preferably able to reach the effector moiety target as described herein. The invention also encompasses a new type of molecule, referred to as scaffold that serves to bring both an effector moiety and at least one glycoside molecule such as a saponin of the invention in an endosome at the same time in a pre-defined ratio,

when the effector moiety is comprised by the second or third or fifth or seventh proteinaceous molecule of the invention and the saponin is comprised by the first, fourth or sixth proteinaceous molecule. Within the context of the present invention, the polymeric or oligomeric structure of the scaffold is a structurally ordered formation such as a polymer, oligomer, dendrimer, dendronized polymer, or dendronized oligomer or it is an assembled polymeric structure such as a hydrogel, microgel, nanogel, stabilized polymeric micelle or liposome, but excludes structures that are composed of non-covalent assemblies of monomers such as cholesterol/phospholipid mixtures. The terms “polymer, oligomer, dendrimer, dendronized polymer, or dendronized oligomer“ have their ordinary meaning. In particular a polymer is a substance which has a molecular structure built up chiefly or completely from a large number of equal or similar units bonded together and an oligomer is a polymer whose molecules consist of relatively few repeating units. There is no consensus about one specific cut-off for “many” and “a few” as used in the above definition of polymer and oligomer, respectively. However, as the scaffold may comprise a polymeric or an oligomeric structure, or both, the full range of numbers of similar units bonded together applies to such structure, i.e. from 2 monomeric units to 100 monomeric units, 1000 monomeric units, and more. A structure comprising 5 or less, for instance maybe called an oligomeric structure, whereas a structure comprising 50 monomeric units maybe called a polymeric structure. A structure of 10 monomeric units maybe called either oligomeric or polymeric. A scaffold as defined herein, further comprises at least one glycoside molecule such as a saponin of the invention. A scaffold preferably includes a polymeric or oligomeric structure such as poly- or oligo(amines), e.g., polyethylenimine and poly(amidoamine), and biocompatible structures such as polyethylene glycol, poly- or oligo(esters), such as poly(lactids), poly(lactams), polylactide-co-glycolide copolymers, and poly(dextrin), poly- or oligosaccharides, such as cyclodextrin or polydextrose, and poly- or oligoamino acids, such as poly-lysine or a peptide or a protein, or DNA oligo- or polymers. An assembled polymeric structure as defined herein comprises at least one scaffold and, optionally, other individual polymeric or oligomeric structures. Other individual polymeric or oligomeric structures of said assembly may be (a) scaffolds (thus comprising at least one glycoside molecule such as a saponin of the invention), (b) functionalized scaffolds (thus comprising at least one glycoside molecule such as a saponin, and a ligand, antibody, etc. as the first proteinaceous molecule, (c) polymeric or oligomeric structures without a glycoside molecule such as a saponin of the invention (See Table A1 for example), without a ligand, antibody, etc., as the first proteinaceous molecule. A functionalized assembled polymeric structure is an assembled polymeric structure that contains (a) at least one functionalized scaffold or (b) at least one scaffold and at least one polymeric structure comprising at least one ligand, antibody, etc. as the first proteinaceous molecule. Polymeric or oligomeric structures within an assembled polymeric structure that do not comprise any of the above mentioned molecules (i.e. no glycosides such as saponins, no first proteinaceous molecule such as ligands, antibodies) are in particular added as structural components of the assembled structures, which help to build up or to stabilize the assembled structure (“glue-like“).Without wishing to be bound by any theory, the acidic environment seems to be a prerequisite for the synergistic action between glycoside (saponin) and effector moiety.

Whether or not a first, fourth or sixth proteinaceous molecule comprising saponins, either or not further comprising one or more (cleavable) linkers and/or optionally a scaffold, is able to disturb the

acidic environment and inhibit the endosomal escape function of the at least one glycoside (saponin) can be easily determined with an assay known in the art. The inhibition is described as “fold amount increases of glycoside necessary to induced 50% cell killing”. It is preferred that the scaffold does not lead to an increase that is at least the increase in glycoside molecules (saponins) necessary to obtain 50% cell killing observed when using Chloroquine as a positive control. Alternatively, and preferably, the first, fourth, sixth proteinaceous molecule comprising saponins, either or not further comprising one or more (cleavable) linkers and/or optionally a scaffold does not lead to an at least 4-fold increase of glycoside molecules to induce 50% cell killing, more preferably does not lead to an at least 2-fold increase. The fold increase is to be measured in assay, wherein Chloroquine, as a positive control, induces a 2-fold increase in glycoside amount, preferably saponin amount wherein the saponin is any one or more of the saponins of the invention (see Table A1, Scheme I, previous embodiments) to observe 50% cell killing.

With the term “improving or enhancing an effect of an effector moiety” is meant that the glycoside molecule, preferably a saponin of the invention, increases the functional efficacy of that effector moiety (e.g. the therapeutic index of a toxin or a drug or an oligonucleotide such as a BNA; the metabolic efficacy of a modifier in biotechnological processes; the transfection efficacy of genes in cell culture research experiments), preferably by enabling or improving its target engagement. Acceleration, prolongation, or enhancement of antigen-specific immune responses are preferably not included. Therapeutic efficacy includes but is not limited to a stronger therapeutic effect, preferably with lower dosing and/or with less side effects. “Improving an effect of an effector moiety” can also mean that an effector moiety, which could not be used because of lack of effect (and was e.g. not known as being an effector moiety), becomes effective when used in combination with the present invention. Any other effect, which is beneficial or desired and can be attributed to the combination of effector moiety and the second or third proteinaceous molecule, as provided by the invention is considered to be “an improved effect”. In an embodiment, the scaffold comprising bound saponin(s) and comprised by the first, fourth or sixth proteinaceous molecule enhances an effect of the effector moiety comprised by the second, third, fifth or seventh proteinaceous molecule which effect is intended and/or desired. In case of a first, fourth or sixth proteinaceous molecule comprising saponin bound to a proteinaceous scaffold, the proteinaceous polymeric structure of the scaffold as such may have, for instance, an effect on colloid osmotic pressure in the blood stream. If such effect is not the intended or desired effect of such a functionalized scaffold comprised by the first, fourth or sixth proteinaceous molecule, the proteinaceous structure of the scaffold is not an effector moiety as defined in the invention. Or, for instance in case of a DNA- or RNA-based scaffold carrying bound saponins and comprised by the first proteinaceous molecule, parts of that DNA or RNA may have an (unintended) function, e.g., by interfering with expression. If such interference is not the intended or desired effect of the ultimate functionalized scaffold, the DNA- or RNA polymeric structure of the scaffold is not the effector moiety as defined in the invention.

A number of preferred features can be formulated for endosomal escape enhancers comprised by the first, fourth or sixth proteinaceous molecule, i.e. a glycoside or saponin, preferably a saponin according to the invention: (1) they are preferably not toxic and do not invoke an immune response, (2)

they preferably do not mediate the cytosolic uptake of the effector moiety into off-target cells, (3) their presence at the site of action is preferably synchronized with the presence of the effector moiety, (4) they are preferably biodegradable or excretable, and (5) they preferably do not substantially interfere with biological processes of the organism unrelated to the biological activity of the effector molecule with which the endosomal escape enhancer is combined with, e.g. interact with hormones. Examples of glycoside molecules such as saponins of the invention that fulfill the before mentioned criteria, at least to some extent, are bisdesmosidic triterpenes, preferably bisdesmosidic triterpene saponins, such as SO1861, SA1641, QS-21, GE1741, and the saponins in Table A1, Scheme I.

An aspect of the invention relates to an antibody-drug conjugate or an antibody-oligonucleotide conjugate or a ligand-drug conjugate comprising the first, fourth or sixth proteinaceous molecule of the invention and an effector moiety.

As said before, the at least one saponin that is comprised by the first, fourth or sixth proteinaceous molecule according to the invention increases the efficacy of at least current and new effector moieties as defined in this invention. Potential side-effects will be decreased due to lowering of dosing of the effector moiety comprised by the second or third or fifth or seventh proteinaceous molecule, without lowering the efficacy. Therefore, the invention provides a first, fourth or sixth proteinaceous molecule according to the invention for use in medicine or for use as a medicament. Thus, an aspect of the invention relates to a first, fourth or sixth proteinaceous molecule according to the invention, the first, fourth or sixth proteinaceous molecule comprising at least a saponin, for use as a medicament. Also provided is the use of a first, fourth or sixth proteinaceous molecule according to the invention for manufacturing a medicament. Especially cancer medicines, and in particular the classical chemotherapy medicaments, are notorious for their side effects. Because of targeting and synchronization in time and place of both the pharmaceutically active substance comprised by the second or third or fifth or seventh proteinaceous molecule and the saponin comprised by the first, fourth or sixht proteinaceous molecule, since the first and third, andor the fourth and fifth, proteinaceous molecule bear the same binding site for the same epitope on the same cell-surface molecule, or since the first and second, or sixth and seventh, proteinaceous molecule bear different binding sites for different first and second, or sixth and seventh, epitopes on the first and second, or on the sixth and seventh, cell-surface molecules respectively, a therapeutic combination according to the invention is especially valuable for use as a medicament, in particular for use in a method of treating cancer. The invention thus provides a therapeutic combination according to the invention or a first, fourth or sixth proteinaceous molecule of the invention for use in a method of treating cancer. The invention also provides a therapeutic combination according to the invention or a first, fourth or sixth proteinaceous molecule of the invention for use in a method of treating acquired or hereditary disorders, in particular monogenic deficiency disorders. The therapeutic combination thus comprises the first and second proteinaceous molecule and/or comprises the first and third proteinaceous molecule, and/or the fourth and fifth proteinaceous molecule, and/or the sixth and seventh proteinaceous molecule. Thus, an aspect of the invention relates to a therapeutic combination according to the invention, wherein the second or third or fifth or seventh proteinaceous molecule comprises a covalently bound effector moiety, for use in a method for the treatment of a cancer or an auto-immune disease.

A further application of the first, second and third, fourth, fifth, sixth, seventh proteinaceous molecules of the invention in medicine is the substitution of intracellular enzymes in target cells that produce these enzymes in insufficient amount or insufficient functionality. The resulting disease might be hereditary or acquired. In most cases, only symptomatic treatment is possible and for a number of rare diseases, insufficient treatment options lead to a shortened life span of concerned patients. An example for such a disease is phenylketonuria, which is an inborn error of metabolism that results in decreased metabolism of the amino acid phenylalanine. The disease is characterized by mutations in the gene for the hepatic enzyme phenylalanine hydroxylase. Phenylketonuria is not curable to date. The incidence is approximately 1:10,000 with the highest known incidence in Turkey with 1:2,600. A second or third or fifth or seventh proteinaceous molecule, preferably an antibody, with bound phenylalanine hydroxylase or with a bound polynucleotide that encodes phenylalanine hydroxylase can be used to target liver cells by use of a suitable specific antibody, and to substitute the defect enzyme in hepatocytes. This is one example of use of the therapeutic combination of the invention comprising a first, fourth or sixth proteinaceous molecule with a saponin bound thereto and a second or third or fifth or seventh proteinaceous molecule with the enzyme or the oligonucleotide bound thereto according to the invention for substitution or gene therapy. In a preferred embodiment, a therapeutic combination according to the invention for use in a method of gene therapy or substitution therapy is provided.

The present invention also provides a method of treating cancer, the method comprising administering a medicament comprising a therapeutic combination according to the invention to a patient in need thereof, preferably administering an effective dose of said medicament to a patient in need thereof, preferably a human cancer patient.

Considerations concerning forms suitable for administration are known in the art and include toxic effects, solubility, route of administration, and maintaining activity. For example, pharmacological compositions injected into the bloodstream should be soluble.

Suitable dosage forms, in part depend upon the use or the route of entry, for example transdermal or by injection. Such dosage forms should allow the compound to reach a target cell whether the target cell is present in a multicellular host. Other factors are known in the art, and include considerations such as toxicity and dosage form which retard the compound or composition from exerting its effect.

An embodiment is the combination of an endosomal escape enhancing conjugate according to the invention, comprising the first, fourth or sixth proteinaceous molecule comprising at least one covalently bound saponin, and a binding moiety, wherein the binding moiety comprises at least one effector moiety, the binding moiety being the second or third or fifth or seventh proteinaceous molecule comprising the bound effector moiety, wherein the endosomal escape enhancing conjugate and the binding moiety are, independently from one another, able to specifically bind to a target cell-specific surface molecule or structure, thereby inducing receptor-mediated endocytosis of a complex of the endosomal escape enhancing conjugate and the target cell-specific surface molecule, and of the complex of the binding moiety and the target cell-specific surface molecule, wherein the endosomal escape enhancing conjugate and the binding moiety can bind to the same target cell-specific surface molecule via their same binding site, or wherein the endosomal escape enhancing conjugate and the

binding moiety can bind to the different target cell-specific surface molecules via their different binding sites. An embodiment is the combination according to the invention, wherein the endosomal escape enhancing conjugate is able to compete with the binding moiety for binding to the target cell-specific surface molecule or structure. An embodiment is the combination according to the invention, wherein the endosomal escape enhancing conjugate and the binding moiety are, independently from one another, able to specifically bind to the same epitope, or to a different epitope. An embodiment is the combination for use in a method for the treatment of an aberrancy such as a cancer according to the invention, wherein said endosomal escape enhancing conjugate and said binding moiety are to be administered concomitant or sequentially, preferably concomitant.

An aspect of the invention relates to a kit comprising a first container containing an endosomal escape enhancing conjugate according to the invention (i.e. the first, fourth or sixth proteinaceous molecule) and a second container containing a binding moiety according to the invention (i.e. the second and/or third and/or fifth and/or seventh proteinaceous molecule), the kit further comprising instructions for using the binding molecules (i.e. the therapeutic combination comprising the first and second or the first and third or the fourth and fifth or the sixth and seventh pharmaceutical compositions).

It is part of the invention that the therapeutic combination, the first pharmaceutical composition, the first proteinaceous molecule, the second or third pharmaceutical composition or the second or third proteinaceous molecule of the invention is further combined with a covalent conjugate (complex) of a binding molecule or a binding moiety and a saponin, or is further combined with a pharmaceutical compound, an antibody, etc., therewith providing a composition comprising three or more enhancers, pharmaceutically active ingredients, etc., e.g. a conjugate of the invention (e.g. a first proteinaceous molecule and/or a second or third proteinaceous molecule) combined with a binding moiety complexed with an effector molecule, further combined with a pharmaceutical, which is either or not linked to a saponin, and which is either or not coupled to a ligand such as a targeting immunoglobulin, a domain or a fragment thereof. Furthermore, an embodiment is the therapeutic combination, the first pharmaceutical composition, the first proteinaceous molecule, the second or third pharmaceutical composition or the secondor third proteinaceous molecule of the invention, wherein the second or third proteinaceous molecule is provided with two or more effector moieties such as a toxin or immunotoxin, wherein the two or more effector moieties are the same or different.

TABLE A1. Saponins displaying (late) endosomal/lysosomal escape enhancing activity, and saponins comprising a structure reminiscent to such saponins displaying (late) endosomal/lysosomal escape enhancing activity

Saponin Name	**Aglycon core**	**Carbohydrate substituent at the C-3beta-OH group**	**Carbohydrate substituent at the C-28-OH group**
NP-005236	2alpha-Hydroxyoleanolic acid	GlcA-	Glc/Gal-

AMA-1	16alpha-Hydroxyoleanolic acid	Glc-	Rha-(1→2)-[Xyl-(1→4)]-Rha-
AMR	16alpha-Hydroxyoleanolic acid	Glc-	Rha-(1→2)-[Ara-(1→3)-Xyl-(1→4)]-Rha-
alpha-Hederin	Hederagenin (23-Hydroxyoleanolic acid)	Rha-(1→2)-Ara-	-
NP-012672	16alpha,23-Dihydroxyoleanolic acid	Ara/Xyl-(1→4)-Rha/Fuc-(1→2)-Glc/Gal-(1→2)-Rha/Fuc-(1→2)-GlcA-	Ara/Xyl-
NP-017777	Gypsogenin	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Xyl-(1→4)-Rha-(1→2)-[R-(→4)]-Fuc- (R = 4E-Methoxycinnamic acid)
NP-017778	Gypsogenin	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Xyl-(1→4)-Rha-(1→2)-[R-(→4)]-Fuc- (R = 4Z-Methoxycinnamic acid)
NP-017774	Gypsogenin	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Xyl-(1→4)-[Gal-(1→3)]-Rha-(1→2)-4-OAc-Fuc-
NP-018110[c], NP-017772[d]	Gypsogenin	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Xyl-(1→4)-[Glc-(1→3)]-Rha-(1→2)-3,4-di-OAc-Fuc-
NP-018109	Gypsogenin	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Xyl-(1→4)-[Glc-(1→3)]-Rha-(1→2)-[R-(→4)]-3-OAc-Fuc- (R = 4E-Methoxycinnamic acid)
NP-017888	Gypsogenin	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Glc-(1→3)-Xyl-(1→4)-[Glc-(1→3)]-Rha-(1→2)-4-OAc-Fuc-
NP-017889	Gypsogenin	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Glc-(1→3)-Xyl-(1→4)-Rha-(1→2)-4-OAc-Fuc-
NP-018108	Gypsogenin	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Ara/Xyl-(1→3)-Ara/Xyl-(1→4)-Rha/Fuc-(1→2)-[4-OAc-Rha/Fuc-(1→4)]-Rha/Fuc-
SA1641[a], AE X55[b]	Gypsogenin	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Xyl-(1→3)-Xyl-(1→4)-Rha-(1→2)-[Qui-(1→4)]-Fuc-
NP-017674	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Api-(1→3)-Xyl-(1→4)-[Glc-(1→3)]-Rha-(1→2)-Fuc-
NP-017810	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Xyl-(1→4)-[Gal-(1→3)]-Rha-(1→2)-Fuc-
AG1	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Xyl-(1→4)-[Glc-(1→3)]-Rha-(1→2)-Fuc-
NP-003881	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Ara/Xyl-(1→4)-Rha/Fuc-(1→4)-[Glc/Gal-(1→2)]-Fuc-
NP-017676	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Api-(1→3)-Xyl-(1→4)-[Glc-(1→3)]-Rha-(1→2)-[R-(→4)]-Fuc- (R = 5-O-[5-O-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid)
NP-017677	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Api-(1→3)-Xyl-(1→4)-Rha-(1→2)-[R-(→4)]-Fuc- (R = 5-O-[5-O-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid)
NP-017706	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Api-(1→3)-Xyl-(1→4)-Rha-(1→2)-[Rha-(1→3)]-4-OAc-Fuc-

NP-017705	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Api-(1→3)-Xyl-(1→4)-[Glc-(1→3)]-Rha-(1→2)-[Rha-(1→3)]-4-OAc-Fuc-
NP-017773	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	6-OAc-Glc-(1→3)-Xyl-(1→4)-Rha-(1→2)-[3-OAc-Rha-(1→3)]-Fuc-
NP-017775	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Glc-(1→3)-Xyl-(1→4)-Rha-(1→2)-[3-OAc--Rha-(1→3)]-Fuc-
SA1657	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Xyl-(1→3)-Xyl-(1→4)-Rha-(1→2)-[Qui-(1→4)]-Fuc-
AG2	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Glc-(1→3)-[Xyl-(1→4)]-Rha-(1→2)-[Qui-(1→4)]-Fuc-
SO1861	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Glc-(1→3)-Xyl-(1→4)-Rha-(1→2)-[Xyl-(1→3)-4-OAc-Qui-(1→4)]-Fuc-
GE1741	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Xyl-(1→3)-Xyl-(1→4)-Rha-(1→2)-[3,4-di-OAc-Qui-(1→4)]-Fuc-
SO1542	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Glc-(1→3)-[Xyl-(1→4)]-Rha-(1→2)-Fuc-
SO1584	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	6-OAc-Glc-(1→3)-[Xyl-(1→4)]-Rha-(1→2)-Fuc-
SO1658	Gypsogenin	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Glc-(1→3)-[Xyl-(1→3)-Xyl-(1→4)]-Rha-(1→2)-Fuc-
SO1674	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Glc-(1→3)-[Xyl-(1→3)-Xyl-(1→4)]-Rha-(1→2)-Fuc-
SO1832	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Xyl-(1→3)-Xyl-(1→4)-Rha-(1→2)-[Xyl-(1→3)-4-OAc-Qui-(1→4)]-Fuc-
QS-7 (also referred to as QS1861)	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Api/Xyl-(1→3)-Xyl-(1→4)-[Glc-(1→3)]-Rha-(1→2)-[Rha-(1→3)]-4OAc-Fuc-
QS-7 api (also referred to as QS1862)	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Api-(1→3)-Xyl-(1→4)-[Glc-(1→3)]-Rha-(1→2)-[Rha-(1→3)]-4OAc-Fuc-
QS-17	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Api/Xyl-(1→3)-Xyl-(1→4)-[Glc-(1→3)]-Rha-(1→2)-[R-(→4)]-Fuc- (R = 5-O-[5-O-Rha-(1→2)-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid)
QS-18	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Api/Xyl-(1→3)-Xyl-(1→4)-[Glc-(1→3)]-Rha-(1→2)-[R-(→4)]-Fuc- (R = 5-O-[5-O-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid)
QS-21 A-apio	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Api-(1→3)-Xyl-(1→4)-Rha-(1→2)-[R-(→4)]-Fuc- (R = 5-O-[5-O-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid)
QS-21 A-xylo	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Xyl-(1→3)-Xyl-(1→4)-Rha-(1→2)-[R-(→4)]-Fuc- (R = 5-O-[5-O-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid)

QS-21 B-apio	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Api-(1→3)-Xyl-(1→4)-Rha-(1→2)-[R-(→3)]-Fuc- (R = 5-O-[5-O-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid)
QS-21 B-xylo	Quillaic acid	Gal-(1→2)-[Xyl-(1→3)]-GlcA-	Xyl-(1→3)-Xyl-(1→4)-Rha-(1→2)-[R-(→3)]-Fuc- (R = 5-O-[5-O-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid)
beta-Aescin (described: Aescin Ia)	Protoaescigenin-21(2-methylbut-2-enoate)-22-acetat	Glc-(1→2)-[Glc-(1→4)]-GlcA-	-
Teaseed saponin I	23-Oxo-barringtogenol C - 21,22-bis(2-methylbut-2-enoate)	Glc-(1→2)-Ara-(1→3)-[Gal-(1→2)]-GlcA-	-
Teaseedsaponin J	23-Oxo-barringtogenol C - 21,22-bis(2-methylbut-2-enoate)	Xyl-(1→2)-Ara-(1→3)-[Gal-(1→2)]-GlcA-	-
Assamsaponin F	23-Oxo-barringtogenol C - 21(2-methylbut-2-enoate)-16,22-diacetat	Glc-(1→2)-Ara-(1→3)-[Gal-(1→2)]-GlcA-	-
Digitonin	Digitogenin	Glc-(1→3)-Gal-(1→2)-[Xyl-(1→3)]-Glc-(1→4)-Gal-	-
Primula acid 1	3,16,28-Trihydroxyoleanan-12-en	Rha-(1→2)-Gal-(1→3)-[Glc-(1→2)]-GlcA-	-
AS64R	Gypsogenic acid	-	Glc-(1→3)-[Glc-(1→6)]-Gal-
		Carbohydrate substituent at the C-23-OH group	
AS6.2	Gypsogenic acid	Gal-	Glc-(1→3)-[Glc-(1→6)]-Gal-
a, b: Different names refer to different isolates of the same structure c, d: Different names refer to different isolates of the same structure			

TABLE A2 - ADCs which were previously investigated in the human clinical setting, and subsequently retracted from further clinical investigation

Drug Name	Indication	Target	Last Development Stage
Monoclonal Antibody Conjugate to	Oncology	Cells Expressing Epidermal Growth Factor Receptor (Proto Oncogene c ErbB 1 or Receptor Tyrosine Protein Kinase erbB 1 or HER1 or ERBB1 or EGFR or EC 2.7.10.1)	Discovery

Drug Name	Indication	Target	Last Development Stage
Target EGFR for Oncology			
Affilutin	Multiple Myeloma (Kahler Disease)		Discovery
IMGN-779	Myelodys-plastic Syndrome	Cells Expressing Myeloid Cell Surface Antigen CD33 (Sialic Acid Binding Ig Like Lectin 3 or gp67 or CD33)	IND/CTA Filed
Neuradiab	Non-Hodgkin Lymphoma	Cells Expressing Tenascin (Cytotactin or GMEM or GP 150-225 or Glioma Associated Extracellular Matrix Antigen or Hexabrachion or JI or Myotendinous Antigen or Neuronectin or Tenascin C or TNC)	Phase I
IMGN-779	Refractory Acute Myeloid Leukemia; Relapsed Acute Myeloid Leukemia	Cells Expressing Myeloid Cell Surface Antigen CD33 (Sialic Acid Binding Ig Like Lectin 3 or gp67 or CD33)	Phase I
AGS-67E	Acute Myelocytic Leukemia (AML, Acute Myeloblas-tic Leukemia)	Cells Expressing Leukocyte Antigen CD37 (Tetraspanin 26 or CD37)	Phase I
AGS-67E	Hairy Cell Leukemia; Non-Hodgkin Lymphoma; Refractory Chronic Lymphocy-tic Leukemia (CLL); Relapsed Chronic Lymphocy-tic Leukemia (CLL); T-Cell Leukemia	Cells Expressing Leukocyte Antigen CD37 (Tetraspanin 26 or CD37)	Phase I
ASG-15ME	Metastatic Transitional (Urothelial) Tract Cancer	Cells Expressing SLIT And NTRK Like Protein 6 (SLITRK6)	Phase I
vandortuzumab vedotin	Metastatic Hormone Refractory (Castration Resistant, Androgen-Indepen-dent) Prostate Cancer	Cells Expressing Metalloreductase STEAP1 (Six Transmembrane Epithelial Antigen Of The Prostate 1 or STEAP1 or EC 1.16.1.)	Phase I
CDX-014	Ovarian Cancer	Cells Expressing Hepatitis A Virus Cellular Receptor 1 (Kidney Injury Molecule 1 or T Cell Immunoglobulin And Mucin Domain Containing Protein 1 or T-Cell Immunoglobulin Mucin Receptor 1 or T Cell Membrane Protein 1 or CD365 or HAVCR1)	Phase I
AGS-16M18	Liver Cancer; Renal Cell Carcinoma		Phase I
vorsetuzumab mafodotin	Non-Hodgkin Lymphoma; Renal Cell Carcinoma	Cells Expressing CD70 Antigen (CD27 Ligand or Tumor Necrosis Factor Ligand Superfamily Member 7 or CD70)	Phase I
denintuzumab mafodotin	Acute Lymphocy-tic Leukemia (ALL, Acute Lympho-blastic Leukemia); B-Cell Non-Hodgkin Lymphoma; Burkitt Lymphoma; Lympho-blastic Lymphoma; Mantle Cell Lymphoma	Cells Expressing B Lymphocyte Antigen CD19 (B Lymphocyte Surface Antigen B4 or Differentiation Antigen CD19 or T Cell Surface Antigen Leu 12 or CD19)	Phase I

Drug Name	Indication	Target	Last Development Stage
SGN-CD70A	Diffuse Large B-Cell Lymphoma; Follicular Lymphoma; Mantle Cell Lymphoma; Metastatic Renal Cell Carcinoma; Non-Hodgkin Lymphoma	Cells Expressing CD70 Antigen (CD27 Ligand or Tumor Necrosis Factor Ligand Superfamily Member 7 or CD70)	Phase I
RG-7636	Metastatic Melanoma	Endothelin B Receptor (Endothelin Receptor Non Selective Type or EDNRB)	Phase I
SC-006	Metastatic Colorectal Cancer		Phase I
MM-310	Breast Cancer; Endome-trial Cancer; Esophageal Cancer; Gastric Cancer; Gastroeso-phageal (GE) Junction Carcino-mas; Head And Neck Cancer Squamous Cell Carcinoma; Non-Small Cell Lung Cancer; Ovarian Cancer; Pancreatic Ductal Adenocar-cinoma; Prostate Cancer; Small-Cell Lung Cancer; Soft Tissue Sarcoma; Solid Tumor; Transitional Cell Carcinoma (Urothelial Cell Carcinoma)	Ephrin Type A Receptor 2 (Epithelial Cell Kinase or Tyrosine Protein Kinase Receptor ECK or EPHA2 or EC 2.7.10.1)	Phase I
PF-06647263	Metastatic Breast Cancer; Ovarian Cancer	Cells Expressing Ephrin A4 (EPH Related Receptor Tyrosine Kinase Ligand 4 or EFNA4)	Phase I
PF-06263507	Solid Tumor	Cells Expressing Trophoblast Glycoprotein (M6P1 or 5T4 Oncofetal Antigen or 5T4 Oncofetal Trophoblast Glycoprotein or Wnt Activated Inhibitory Factor 1 or TPBG)	Phase I
PF-06650808	Metastatic Breast Cancer; Non-Small Cell Lung Cancer; Ovarian Cancer	Cells Expressing Neurogenic Locus Notch Homolog Protein 3 (NOTCH3)	Phase I
XMT-1522	Breast Cancer; Gastric Cancer; Non-Small Cell Lung Cancer	Receptor Tyrosine Protein Kinase ERBB 2 (Metastatic Lymph Node Gene 19 Protein or Proto Oncogene Neu or Proto Oncogene C ErbB 2 or Tyrosine Kinase Type Cell Surface Receptor HER2 or p185erbB2 or HER2 or CD340 or ERBB2 or EC 2.7.10.1); Tubulin	Phase I
AMG-595	Anaplastic Astrocyto-ma; Recurrent Glioblasto-ma Multiforme (GBM)	Cells Expressing Epidermal Growth Factor Receptor (Proto Oncogene c ErbB 1 or Receptor Tyrosine Protein Kinase erbB 1 or HER1 or ERBB1 or EGFR or EC 2.7.10.1)	Phase I
pinatuzumab vedotin	Chronic Lymphocytic Leukemia (CLL)	Cells Expressing B Cell Receptor CD22 (B Lymphocyte Cell Adhesion Molecule or Sialic Acid Binding Ig Like Lectin 2 or T Cell Surface Antigen Leu 14 or CD22)	Phase I
cantuzumab ravtansine	Colorectal Cancer; Non-Small Cell Lung Cancer; Pancreatic Cancer; Solid Tumor		Phase I

Drug Name	Indication	Target	Last Development Stage
AVE-9633	Acute Myelocytic Leukemia (AML, Acute Myeloblas-tic Leukemia)	Cells Expressing Myeloid Cell Surface Antigen CD33 (Sialic Acid Binding Ig Like Lectin 3 or gp67 or CD33)	Phase I
BIWI-1[(1)]	Breast Cancer; Carcino-mas; Esophageal Cancer; Head And Neck Cancer Squamous Cell Carcinoma	Cells Expressing CD44 Antigen (CDw44 or Epican or Extracellular Matrix Receptor III or GP90 Lymphocyte Homing/Adhesion Receptor or HUTCH I or Heparan Sulfate Proteoglycan or Hermes Antigen or Hyaluronate Receptor or Phagocytic Glycoprotein 1 or CD44)	Phase I
RG-7882	Epithelial Ovarian Cancer; Fallopian Tube Cancer; Pancreatic Cancer; Peritoneal Cancer	Cells Expressing Mucin 16 (Ovarian Cancer Related Tumor Marker CA125 or Ovarian Carcinoma Antigen CA125 or MUC16)	Phase I
ASG-5ME	Adenocar-cinoma; Hormone Refractory (Castration Resistant, Androgen-Indepen-dent) Prostate Cancer; Metastatic Adenocar-cinoma of The Pancreas	Cells Expressing Choline Transporter Like Protein 4 (Solute Carrier Family 44 Member 4 or SLC44A4)	Phase I
DCDS-0780A	B-Cell Non-Hodgkin Lymphoma		Phase I
SC-004	Endome-trial Cancer; Epithelial Ovarian Cancer; Fallopian Tube Cancer; Peritoneal Cancer		Phase I
RG-7600	Ovarian Cancer; Pancreatic Ductal Adenocar-cinoma		Phase I
sofituzumab vedotin	Epithelial Ovarian Cancer; Fallopian Tube Cancer; Ovarian Cancer; Pancreatic Cancer; Peritoneal Cancer	Cells Expressing Mucin 16 (Ovarian Cancer Related Tumor Marker CA125 or Ovarian Carcinoma Antigen CA125 or MUC16)	Phase I
IMGN-289	Breast Cancer; Esophageal Cancer; Gastric Cancer; Head And Neck Cancer Squamous Cell Carcinoma; Non-Small Cell Lung Cancer; Solid Tumor	Cells Expressing Epidermal Growth Factor Receptor (Proto Oncogene c ErbB 1 or Receptor Tyrosine Protein Kinase erbB 1 or HER1 or ERBB1 or EGFR or EC 2.7.10.1)	Phase I
SAR-428926	Breast Cancer; Colorectal Cancer; Gastric Cancer; Non-Small Cell Lung Cancer; Ovarian Cancer; Prostate Cancer; Solid Tumor	Cells Expressing Lysosome Associated Membrane Glycoprotein 1 (CD107 Antigen Like Family Member A or CD107a or LAMP1)	Phase I
SGNCD-19B	B-Cell Non-Hodgkin Lymphoma; Diffuse Large B-Cell Lymphoma; Follicular Lymphoma	Cells Expressing B Lymphocyte Antigen CD19 (B Lymphocyte Surface Antigen B4 or Differentiation Antigen CD19 or T Cell Surface Antigen Leu 12 or CD19)	Phase I
SGNCD-123A	Refractory Acute Myeloid Leukemia; Relapsed Acute Myeloid Leukemia	Cells Expressing Interleukin 3 Receptor Subunit Alpha (CD123 or IL3RA)	Phase I

Drug Name	Indication	Target	Last Development Stage
SGNCD-352A	Refractory Multiple Myeloma; Relapsed Multiple Myeloma	Cells Expressing SLAM Family Member 6 (Activating NK Receptor or NK T B Antigen or CD352 or SLAMF6)	Phase I
RG-7841	Breast Cancer; Non-Small Cell Lung Cancer; Solid Tumor	Cells Expressing Lymphocyte Antigen 6E (Retinoic Acid Induced Gene E Protein or Stem Cell Antigen 2 or Thymic Shared Antigen 1 or LY6E)	Phase I
IMGN-388	Solid Tumor	Cells Expressing Integrin Alpha V (Vitronectin Receptor Subunit Alpha or CD51 or ITGAV)	Phase I
lorvotuzumab mertansine	Refractory Multiple Myeloma; Relapsed Multiple Myeloma	Cells Expressing Neural Cell Adhesion Molecule 1 (Antigen Recognized By Monoclonal Antibody 5.1H11 or CD56 or NCAM1)	Phase I
lorvotuzumab mertansine	Neuroendo-crine Carcinoma; Neuroendo-crine Tumors; Non-Small Cell Lung Cancer; Ovarian Cancer; Skin Cancer	Cells Expressing Neural Cell Adhesion Molecule 1 (Antigen Recognized By Monoclonal Antibody 5.1H11 or CD56 or NCAM1)	Phase I
BAY-794620	Lung Cancer; Solid Tumor	Cells Expressing Carbonic Anhydrase 9 (Carbonate Dehydratase IX or pMW1 or Membrane Antigen MN or P54/58N or Renal Cell Carcinoma Associated Antigen G250 or CA9 or EC 4.2.1.1)	Phase I
RG-7598	Refractory Multiple Myeloma; Relapsed Multiple Myeloma		Phase I
Oncolysin B	B-Cell Leukemia; Lymphoma	Cells Expressing B Lymphocyte Antigen CD19 (B Lymphocyte Surface Antigen B4 or Differentiation Antigen CD19 or T Cell Surface Antigen Leu 12 or CD19)	Phase I
ADCT-502[1]	Bladder Cancer; Breast Cancer; Esophageal Cancer; Gastric Cancer; Non-Small Cell Lung Cancer	Cells Expressing Receptor Tyrosine Protein Kinase ERBB 2 (Metastatic Lymph Node Gene 19 Protein or Proto Oncogene Neu or Proto Oncogene C ErbB 2 or Tyrosine Kinase Type Cell Surface Receptor HER2 or p185erbB2 or HER2 or CD340 or ERBB2 or EC 2.7.10.1)	Phase I
AMG-172	Renal Cell Carcinoma	Cells Expressing CD70 Antigen (CD27 Ligand or Tumor Necrosis Factor Ligand Superfamily Member 7 or CD70)	Phase I
ImmuRAIT-LL2	B-Cell Non-Hodgkin Lymphoma	Cells Expressing B Cell Receptor CD22 (B Lymphocyte Cell Adhesion Molecule or Sialic Acid Binding Ig Like Lectin 2 or T Cell Surface Antigen Leu 14 or CD22)	Phase I/II
indusatumab vedotin	Adenocar-cinoma Of The Gastroe-sophageal Junction; Gastric Cancer	Cells Expressing Heat Stable Enterotoxin Receptor (Guanylyl Cyclase C or or	Phase I/II

Drug Name	Indication	Target	Last Development Stage
		Intestinal Guanylate Cyclase or GUCY2C or EC 4.6.1.2)	
clivatuzumab tetraxetan	Pancreatic Cancer	Cells Expressing Mucin 1 (Breast Carcinoma Associated Antigen DF3 or Episialin or H23AG or Krebs Von Den Lungen 6 or PEMT or Peanut Reactive Urinary Mucin or Polymorphic Epithelial Mucin or Tumor Associated Epithelial Membrane Antigen or Tumor Associated Mucin or CD227 or MUC1)	Phase I/II
depatuxizumab mafodotin[2]	Recurrent Malignant Glioma	Epidermal Growth Factor Receptor (Proto Oncogene c ErbB 1 or Receptor Tyrosine Protein Kinase erbB 1 or HER1 or ERBB1 or EGFR or EC 2.7.10.1)	Phase I/II
CDX-014	Metastatic Renal Cell Carcinoma; Papillary Renal Cell Carcinoma	Cells Expressing Hepatitis A Virus Cellular Receptor 1 (Kidney Injury Molecule 1 or T Cell Immunoglobulin And Mucin Domain Containing Protein 1 or T-Cell Immunoglobulin Mucin Receptor 1 or T Cell Membrane Protein 1 or CD365 or HAVCR1)	Phase I/II
vadastuximab talirine[1]	Refractory Acute Myeloid Leukemia; Relapsed Acute Myeloid Leukemia	Cells Expressing Myeloid Cell Surface Antigen CD33 (Sialic Acid Binding Ig Like Lectin 3 or gp67 or CD33)	Phase I/II
vadastuximab talirine	Myelodys-plastic Syndrome	Cells Expressing Myeloid Cell Surface Antigen CD33 (Sialic Acid Binding Ig Like Lectin 3 or gp67 or CD33)	Phase I/II
MLN-2704	Metastatic Hormone Refractory (Castration Resistant, Androgen-Indepen-dent) Prostate Cancer	Cells Expressing Glutamate Carboxypeptidase 2 (Folate Hydrolase 1 or Prostate Specific Membrane Antigen or PSMA or Pteroylpoly Gamma Glutamate Carboxypeptidase or Cell Growth Inhibiting Gene 27 Protein or FOLH1 or EC 3.4.17.21)	Phase I/II
Oncolysin B	AIDS - Related Lymphoma	Cells Expressing B Lymphocyte Antigen CD19 (B Lymphocyte Surface Antigen B4 or Differentiation Antigen CD19 or T Cell Surface Antigen Leu 12 or CD19)	Phase I/II
coltuximab ravtansine	Diffuse Large B-Cell Lymphoma	Cells Expressing B Lymphocyte Antigen CD19 (B Lymphocyte Surface Antigen B4 or Differentiation Antigen CD19 or T Cell Surface Antigen Leu 12 or CD19)	Phase II
coltuximab ravtansine	Acute Lymphocy-tic Leukemia (ALL, Acute Lympho-blastic Leukemia)	Cells Expressing B Lymphocyte Antigen CD19 (B Lymphocyte Surface Antigen B4	Phase II

Drug Name	Indication	Target	Last Development Stage
		or Differentiation Antigen CD19 or T Cell Surface Antigen Leu 12 or CD19)	
coltuximab ravtansine	Diffuse Large B-Cell Lymphoma	Cells Expressing B Lymphocyte Antigen CD19 (B Lymphocyte Surface Antigen B4 or Differentiation Antigen CD19 or T Cell Surface Antigen Leu 12 or CD19)	Phase II
indusatumab vedotin[2]	Adenocar-cinoma Of The Gastroe-sophageal Junction; Gastric Cancer; Metastatic Adenocar-cinoma of The Pancreas	Cells Expressing Heat Stable Enterotoxin Receptor (Guanylyl Cyclase C or or Intestinal Guanylate Cyclase or GUCY2C or EC 4.6.1.2)	Phase II
depatuxizumab mafodotin	Squamous Non-Small Cell Lung Cancer	Epidermal Growth Factor Receptor (Proto Oncogene c ErbB 1 or Receptor Tyrosine Protein Kinase erbB 1 or HER1 or ERBB1 or EGFR or EC 2.7.10.1)	Phase II
depatuxizumab mafodotin[2]	Anaplastic Astrocyto-ma; Anaplastic Oligoastro-cytoma; Gliosar-coma; High-Grade Glioma; Oligoden-droglioma; Pediatric Diffuse Intrinsic Pontine Glioma; Recurrent Glioblasto-ma Multiforme (GBM)	Epidermal Growth Factor Receptor (Proto Oncogene c ErbB 1 or Receptor Tyrosine Protein Kinase erbB 1 or HER1 or ERBB1 or EGFR or EC 2.7.10.1)	Phase II
lifastuzumab vedotin	Non-Small Cell Lung Cancer	Sodium Dependent Phosphate Transport Protein 2B (Sodium Phosphate Transport Protein 2B or NaPi3b or Sodium/Phosphate Cotransporter 2B or NaPi 2b or Solute Carrier Family 34 Member 2 or SLC34A2)	Phase II
lifastuzumab vedotin	Ovarian Cancer	Sodium Dependent Phosphate Transport Protein 2B (Sodium Phosphate Transport Protein 2B or NaPi3b or Sodium/Phosphate Cotransporter 2B or NaPi 2b or Solute Carrier Family 34 Member 2 or SLC34A2)	Phase II
Bismab-A	Acute Myelocytic Leukemia (AML, Acute Myeloblas-tic Leukemia)	Cells Expressing Myeloid Cell Surface Antigen CD33 (Sialic Acid Binding Ig Like Lectin 3 or gp67 or CD33)	Phase II
denintuzumab mafodotin	Diffuse Large B-Cell Lymphoma; Follicular Lymphoma	Cells Expressing B Lymphocyte Antigen CD19 (B Lymphocyte Surface Antigen B4 or Differentiation Antigen CD19 or T Cell Surface Antigen Leu 12 or CD19)	Phase II
Avicidin[1]	Colorectal Cancer; Prostate Cancer	Cells Expressing Epithelial Cell Adhesion Molecule (Adenocarcinoma Associated Antigen or Cell Surface Glycoprotein Trop 1 or Epithelial Cell Surface Antigen or Epithelial Glycoprotein 314 or KS 1/4 Antigen or KSA or Tumor Associated Calcium Signal Transducer 1 or CD326 or EPCAM)	Phase II

Drug Name	Indication	Target	Last Development Stage
pinatuzumab vedotin	Diffuse Large B-Cell Lymphoma; Follicular Lymphoma	Cells Expressing B Cell Receptor CD22 (B Lymphocyte Cell Adhesion Molecule or Sialic Acid Binding Ig Like Lectin 2 or T Cell Surface Antigen Leu 14 or CD22)	Phase II
SGN-15	Metastatic Breast Cancer; Non-Small Cell Lung Cancer; Ovarian Cancer; Prostate Cancer	Cells Expressing Lewis Y Antigen (CD174)	Phase II
cantuzumab ravtansine	Gastric Cancer; Gastroe-sophageal (GE) Junction Carcino-mas		Phase II
ASP-6183	Ovarian Cancer		Phase II
SAR-566658	Metastatic Breast Cancer	Cells Expressing Sialoglycotope CA6 Antigen	Phase II
Oncolysin S	Small-Cell Lung Cancer	Cells Expressing Neural Cell Adhesion Molecule 1 (Antigen Recognized By Monoclonal Antibody 5.1H11 or CD56 or NCAM1)	Phase II
lorvotuzumab mertansine	Small-Cell Lung Cancer	Cells Expressing Neural Cell Adhesion Molecule 1 (Antigen Recognized By Monoclonal Antibody 5.1H11 or CD56 or NCAM1)	Phase II
glembatumumab vedotin	Metastatic Melanoma; Metastatic Uveal Melanoma; Osteosar-coma; Squamous Non-Small Cell Lung Cancer	Cells Expressing Transmembrane Glycoprotein NMB (Transmembrane Glycoprotein HGFIN or GPNMB)	Phase II
MM-302	Metastatic Breast Cancer	Cells Expressing Receptor Tyrosine Protein Kinase ERBB 2 (Metastatic Lymph Node Gene 19 Protein or Proto Oncogene Neu or Proto Oncogene C ErbB 2 or Tyrosine Kinase Type Cell Surface Receptor HER2 or p185erbB2 or HER2 or CD340 or ERBB2 or EC 2.7.10.1)	Phase II/III
Neuradiab	Brain Cancer; Glioblasto-ma Multiforme (GBM)	Cells Expressing Tenascin (Cytotactin or GMEM or GP 150-225 or Glioma Associated Extracellular Matrix Antigen or Hexabrachion or JI or Myotendinous Antigen or Neuronectin or Tenascin C or TNC)	Phase III
clivatuzumab tetraxetan	Metastatic Adenocar-cinoma of The Pancreas	Cells Expressing Mucin 1 (Breast Carcinoma Associated Antigen DF3 or Episialin or H23AG or Krebs Von Den Lungen 6 or PEMT or Peanut Reactive Urinary Mucin or Polymorphic Epithelial Mucin or Tumor Associated Epithelial Membrane Antigen or Tumor Associated Mucin or CD227 or MUC1)	Phase III

Drug Name	Indication	Target	Last Development Stage
depatuxizumab mafodotin[2]	Glioblasto-ma Multiforme (GBM)	Epidermal Growth Factor Receptor (Proto Oncogene c ErbB 1 or Receptor Tyrosine Protein Kinase erbB 1 or HER1 or ERBB1 or EGFR or EC 2.7.10.1)	Phase III
vadastuximab talirine[1]	Acute Myelocytic Leukemia (AML, Acute Myeloblas-tic Leukemia)	Cells Expressing Myeloid Cell Surface Antigen CD33 (Sialic Acid Binding Ig Like Lectin 3 or gp67 or CD33)	Phase III
glembatumumab vedotin[2]	Metastatic Breast Cancer	Cells Expressing Transmembrane Glycoprotein NMB (Transmembrane Glycoprotein HGFIN or GPNMB)	Phase III
Oncolysin B	B-Cell Leukemia; Lymphoma	Cells Expressing B Lymphocyte Antigen CD19 (B Lymphocyte Surface Antigen B4 or Differentiation Antigen CD19 or T Cell Surface Antigen Leu 12 or CD19)	Phase III
ImmuRAIT-LL2	B-Cell Leukemia	Cells Expressing B Cell Receptor CD22 (B Lymphocyte Cell Adhesion Molecule or Sialic Acid Binding Ig Like Lectin 2 or T Cell Surface Antigen Leu 14 or CD22)	Preclinical
indusatumab vedotin	Metastatic Colorectal Cancer	Cells Expressing Heat Stable Enterotoxin Receptor (Guanylyl Cyclase C or or Intestinal Guanylate Cyclase or GUCY2C or EC 4.6.1.2)	Preclinical
ASG-15ME	Lung Cancer	Cells Expressing SLIT And NTRK Like Protein 6 (SLITRK6)	Preclinical
HTI-1511	Bile Duct Cancer (Cholangiocarcinoma) ; Breast Cancer; Colorectal Cancer; Non-Small Cell Lung Cancer	Cells Expressing Epidermal Growth Factor Receptor (Proto Oncogene c ErbB 1 or Receptor Tyrosine Protein Kinase erbB 1 or HER1 or ERBB1 or EGFR or EC 2.7.10.1)	Preclinical
ZW-33	Gastric Cancer; Metastatic Breast Cancer	Cells Expressing Receptor Tyrosine Protein Kinase ERBB 2 (Metastatic Lymph Node Gene 19 Protein or Proto Oncogene Neu or Proto Oncogene C ErbB 2 or Tyrosine Kinase Type Cell Surface Receptor HER2 or p185erbB2 or HER2 or CD340 or ERBB2 or EC 2.7.10.1)	Preclinical
ZW-33	Ovarian Cancer	Cells Expressing Receptor Tyrosine Protein Kinase ERBB 2 (Metastatic Lymph Node Gene 19 Protein or Proto Oncogene Neu or Proto Oncogene C ErbB 2 or Tyrosine Kinase Type Cell Surface Receptor HER2 or p185erbB2 or HER2 or CD340 or ERBB2 or EC 2.7.10.1)	Preclinical
SGNCD-352A	Non-Hodgkin Lymphoma	Cells Expressing SLAM Family Member 6 (Activating NK Receptor or NK T B Antigen or CD352 or SLAMF6)	Preclinical

Drug Name	Indication	Target	Last Development Stage
HuMax-CD74-ADC	Oncology	Cells Expressing HLA Class II Histocompatibility Antigen Gamma Chain (HLA DR Antigens Associated Invariant Chain or Ia Antigen Associated Invariant Chain or p33 or CD74)	Preclinical
sacituzumab govitecan	Pancreatic Ductal Adenocar-cinoma	Cells Expressing Tumor Associated Calcium Signal Transducer 2 (Cell Surface Glycoprotein Trop 2 or Membrane Component Chromosome 1 Surface Marker 1 or Pancreatic Carcinoma Marker Protein GA733-1 or TACSTD2)	
sacituzumab govitecan	Adenocar-cinoma; Cervical Cancer; Colorectal Cancer; Endome-trial Cancer; Epithelial Ovarian Cancer; Esophageal Cancer; Follicular Thyroid Cancer; Gastric Cancer; Glioblasto-ma Multiforme (GBM); Head And Neck Cancer Squamous Cell Carcinoma; Hepato-cellular Carcinoma; Kidney Cancer (Renal Cell Cancer); Metastatic Hormone Refractory (Castration Resistant, Androgen-Indepen-dent) Prostate Cancer; Metastatic Transitional (Urothelial) Tract Cancer; Transitional Cell Cancer (Urothelial Cell Cancer)	Cells Expressing Tumor Associated Calcium Signal Transducer 2 (Cell Surface Glycoprotein Trop 2 or Membrane Component Chromosome 1 Surface Marker 1 or Pancreatic Carcinoma Marker Protein GA733-1 or TACSTD2)	
sacituzumab govitecan	Hepato-cellular Carcinoma	Cells Expressing Tumor Associated Calcium Signal Transducer 2 (Cell Surface Glycoprotein Trop 2 or Membrane Component Chromosome 1 Surface Marker 1 or Pancreatic Carcinoma Marker Protein GA733-1 or TACSTD2)	
sacituzumab govitecan	Metastatic Breast Cancer; Transitional Cell Cancer (Urothelial Cell Cancer)	Cells Expressing Tumor Associated Calcium Signal Transducer 2 (Cell Surface Glycoprotein Trop 2 or Membrane Component Chromosome 1 Surface Marker 1 or Pancreatic Carcinoma Marker Protein GA733-1 or TACSTD2)	
sacituzumab govitecan	Non-Small Cell Lung Cancer; Small-Cell Lung Cancer	Cells Expressing Tumor Associated Calcium Signal Transducer 2 (Cell Surface Glycoprotein Trop 2 or Membrane Component Chromosome 1 Surface Marker 1 or Pancreatic Carcinoma Marker Protein GA733-1 or TACSTD2)	

Drug Name	Indication	Target	Last Development Stage
sacituzumab govitecan	Metastatic Breast Cancer	Cells Expressing Tumor Associated Calcium Signal Transducer 2 (Cell Surface Glycoprotein Trop 2 or Membrane Component Chromosome 1 Surface Marker 1 or Pancreatic Carcinoma Marker Protein GA733-1 or TACSTD2)	
(1) Discontinued due to adverse events (2) Discontinued due to lack of efficacy			

TABLE A3 – ADCs that reached phase III clinical development

Drug Name	Indication	Development Stage	Last Development Stage	Reason for Discontinuation
trastuzumab emtansine	Gastric Cancer	Marketed	Phase II/III	Unspecified
MM-302	Metastatic Breast Cancer	Discontinued	Phase II/III	Business/Strategic Decision
trastuzumab emtansine	Metastatic Breast Cancer	Marketed	Phase III	Unspecified
trastuzumab emtansine	Gastric Cancer	Marketed	Phase III	Unspecified
ibritumomab tiuxetan	Diffuse Large B-Cell Lymphoma	Marketed	Phase III	
inotuzumab ozogamicin	Follicular Lymphoma	Marketed	Phase III	
inotuzumab ozogamicin	Diffuse Large B-Cell Lymphoma; Non-Hodgkin Lymphoma	Marketed	Phase III	Lack of Efficacy
rovalpituzumab tesirine	Small-Cell Lung Cancer	Phase III	Phase III	
rovalpituzumab tesirine	Small-Cell Lung Cancer	Phase III	Phase III	
Neuradiab	Brain Cancer; Glioblastoma Multiforme (GBM)	Inactive	Phase III	Unspecified
clivatuzumab tetraxetan	Metastatic Adenocarcinoma of The Pancreas	Inactive	Phase III	Unspecified
depatuxizumab mafodotin	Glioblastoma Multiforme (GBM)	Inactive	Phase III	Lack of Efficacy
vadastuximab talirine	Acute Myelocytic Leukemia (AML, Acute Myeloblastic Leukemia)	Discontinued	Phase III	Adverse Events

glembatumumab vedotin	Metastatic Breast Cancer	Discontinued	Phase III	Lack of Efficacy
Oncolysin B	B-Cell Leukemia; Lymphoma	Discontinued	Phase III	Business/Strategic Decision

TABLE A4. Tumor-specific cell-surface receptor targets which can be targeted by any of the antibody-comprising conjugates of the invention, immunoglobulins according to the invention, and antibodies that can be used for the ADCs and the antibodies provided with a saponin, and the ADCs provided with a saponin, of the present invention (not presented as a limitation; further immunoglobulins are equally suitable for the invention)

Target cell-surface receptor	**Example monoclonal antibodies**
HER2	anti-HER2 monoclonal antibody such as trastuzumab and pertuzumab
CD20	anti-CD20 monoclonal antibody such as rituximab, ofatumumab, tositumomab and ibritumomab
CA125	anti-CA125 monoclonal antibody such as oregovomab
EpCAM (17-1A)	anti-EpCAM (17-1A) monoclonal antibody such as edrecolomab
EGFR	anti-EGFR monoclonal antibody such as cetuximab, panitumumab and nimotuzumab
CD30	anti-CD30 monoclonal antibody such brentuximab
CD33	anti-CD33 monoclonal antibody such as gemtuzumab and huMy9-6
vascular integrin alpha-v beta-3	anti-vascular integrin alpha-v beta-3 monoclonal antibody such as etaracizumab
CD52	anti-CD52 monoclonal antibody such as alemtuzumab
CD22	anti-CD22 monoclonal antibody such as epratuzumab
CEA	anti-CEA monoclonal antibody such as labetuzumab
CD44v6	anti-CD44v6 monoclonal antibody such as bivatuzumab
FAP	anti- FAP monoclonal antibody such as sibrotuzumab
CD19	anti-CD19 monoclonal antibody such as huB4
CanAg	anti-CanAg monoclonal antibody such as huC242
CD56	anti-CD56 monoclonal antibody such huN901
CD38	anti-CD38 monoclonal antibody such as daratumumab
CA6	anti-CA6 monoclonal antibody such as DS6
IGF-IR	anti-IGF-IR monoclonal antibody such as cixutumumab and 3B7
integrin	anti-integrin monoclonal antibody such as CNTO 95
syndecan-1	anti-syndecan-1 monoclonal antibody such as B-B4

Table A5: RIPs from plants*

Plant Family	**Plant Species**	**Proteins**	**Classification**
Adoxaceae	Sambucus ebulus L.	Ebulitin α, Ebulitin β, Ebulitin γ	RIP 1

		Ebulin f, Ebulin l, Ebulin r1, Ebulin r2, SEA	RIP 2
		SEAII, SELfd, SELId, SELIm	lectin
	Sambucus nigra L.	α-Nigritin, β-Nigritin, γ-Nigritin, Nigritin f1, Nigritin f2	RIP 1
		basic Nigrin b, Nigrin b = SNA-V, Nigrin f = SNA-Vf, Nigrin I1, Nigrin I2, Nigrin s, SNA-I, SNA-I', SNA-If, SNAflu-I, SNLRP1, SNLRP2	RIP 2
		SNA-Id, SNA-Im, SNA-II, SNA-III, SNA-IV = SNA-IVf, SNA-IVI, SNApol-I, SNApol-II, TrSNA-I, TrSNA-If	lectin
	Sambucus racemosa L.	basic racemosin b, SRA	RIP 2
		SRLbm = SRAbm	lectin
	Sambucus sieboldiana (Miq.) Blume ex Graebn.	SSA = SSA-b-1, Sieboldin-b = SSA-b-2	RIP 2
		SSA-b-3, SSA-b-4	lectin
Aizoaceae	Mesembryanthe-mum crystallinum L.	RIP1	RIP 1
Amaranthaceae	Amaranthus caudatus L.	Amaranthin = ACA	lectin
	Amaranthus cruentus L.	ACL	lectin
	Amaranthus hypochondriacus L. [Syn.: Amaranthus leucocarpus S. Watson]	A. leucocarpus lectin	lectin
	Amaranthus mangostanus L.	Amaramangin	RIP 1
	Amaranthus tricolor L.	AAP-27	RIP 1
	Amaranthus viridis L.	Amaranthin	RIP 1
	Beta vulgaris L.	Beetin-27 = BE27, Beetin-29 = BE29, Betavulgin	RIP 1
	Celosia argentea L. [Syn.: Celosia cristata L.]	CCP-25, CCP-27	RIP 1
	Chenopodium album L.	CAP30	RIP 1
	Spinacia oleracea L.	SoRIP1 = BP31	RIP 1
		SoRIP2	RIP 1 candidate
Araliaceae	Aralia elata (Miq.) Seem.	Aralin	RIP 2
	Panax ginseng C.A.Mey	Panaxagin	peculiar RIP 1 candidate/RNase
	Panax quinquefolius L.	Quinqueginsin	peculiar RIP 1 candidate/RNase
Asparagaceae	Asparagus officinalis L.	Asparin 1, Asparin 2	RIP 1
	Drimia maritima (L.) Stearn [Syn.: Charybdis maritima (L.) Speta]	Charybdin	RIP 1
	Muscari armeniacum Leichtlin ex Baker	Musarmin 1, Musarmin 2, Musarmin 3, Musarmin 4	RIP 1
	Polygonatum multiflorum (L.) All.	PMRIPm, PMRIPt	RIP 2
	Yucca gloriosa var. tristis Carrière [Syn.: Yucca recurvifolia Salisb.]	Yucca leaf protein = YLP	RIP 1
Basellaceae	Basella rubra L.	Basella RIP 2a, Basella RIP 2b, Basella RIP 3	RIP 1
Caryophyllaceae	Agrostemma githago L.	Agrostin 2, Agrostin 5, Agrostin 6, Agrostin	RIP 1

	Dianthus barbatus L.	Dianthin 29	RIP 1
	Dianthus caryophyllus L.	Dianthin 30, Dianthin 32	RIP 1
	Dianthus chinensis L. [Syn.: Dianthus sinensis Link]	D. sinensis RIP	RIP 1
	Gypsophila elegans M.Bieb.	Gypsophilin	RIP 1
	Silene chalcedonica (L.) E.H.L.Krause [Syn.: Lychnis chalcedonica L.]	Lychnin	RIP 1
	Silene glaucifolia Lag. [Syn.: Petrocoptis glaucifolia (Lag.) Boiss.]	Petroglaucin 1, Petroglaucin 2	RIP 1
	Silene laxipruinosa Mayol & Rosselló [Syn.: Petrocoptis grandiflora Rothm.]	Petrograndin	RIP 1
	Saponaria ocymoides L.	Ocymoidin	RIP 1
	Saponaria officinalis L.	Saporin-L1 = SO-L1, Saporin-L2 = SO-L2, Saporin-L3 = SO-L3, Saporin-I = SO-I = SO-4, Saporin-R1 = SO-R1, Saporin-R2 = SO-R2, Saporin-R3 = SO-R3, SO3a, SO3b, Saporin-S5 = Saporin 5 = SO-S5, Saporin-S6 = Saporin 6 = SO-6 = SO-S6, Saporin-S8 = SO-S8, Saporin-S9 = Saporin 9 = SO-S9, SAP-C, SAP-S	RIP 1
	Myosoton aquaticum (L.) Moench [Syn.: Stellaria aquatica (L.) Scop.]	Stellarin	RIP 1
	Stellaria media (L.) Vill.	RIP Q3	RIP 1
	Vaccaria hispanica (Mill.) Rauschert [Syn.: Vaccaria pyramidata Medik.]	Pyramidatin	RIP 1
Cucurbitaceae	Benincasa hispida (Thunb.) Cogn.	Hispin	RIP 1
		α-benincasin, β-benincasin	sRIP 1
	Bryonia cretica subsp. dioica (Jacq.) Tutin. [Syn.: Bryonia dioica L.]	Bryodin 1 = BD1, Bryodin 2, Bryodin-L, Bryodin-R	RIP 1
		BDA	lectin/ RIP 2 like
	Citrullus colocynthis (L.) Schrad.	Colocin 1, Colocin 2	RIP 1
	Cucurbita foetidissima Kunth	Foetidissimin	peculiar RIP 2
		Foetidissimin II	RIP 2
	Cucumis ficifolius A.Rich. [Syn.: Cucumis figarei Delile ex Naudin]	Cucumis figarei RIP = CF-RIP	RIP 1 candidate
	Cucurbita maxima Duchesne	Cucurmoschin	sRIP 1 candidate
	Cucurbita moschata Duchesne [Syn.: Cucurbita moschata (Duchesne ex Lam.) Duchesne ex Poir.]	Cucurmosin, Cucurmosin 2, C. moschata RIP, Moschatin, PRIP 1, PRIP 2	RIP 1
		α-moschin, β-moschin	sRIP 1 candidate
	Cucurbita pepo L.	Pepocin	RIP 1
	Cucurbita pepo var. texana (Scheele) D.S.Decker [Syn.:	Texanin	RIP 1

	Cucurbita texana (Scheele) A. Gray]		
	Gynostemma pentaphyllum (Thunb.) Makino	Gynostemmin	RIP 1
	Lagenaria siceraria (Molina) Standl.	Lagenin	RIP 1 candidate
	Luffa acutangula (L.) Roxb.	Luffaculin-1, Luffaculin-2	RIP 1
		Luffangulin	sRIP 1
		Luffa acutangula fruit lectin	lectin
	Luffa cylindrica (L.) M.Roem [Syn.: Luffa aegyptiaca Mill.]	Luffin, Luffin-a, Luffin-b, α-luffin, β-luffin, LRIP	RIP 1
		Luffacylin, Luffin P1	sRIP 1
		Luffin-S, LuffinS(1), LuffinS(2) = luffin S2, LuffinS(3)	sRIP 1 candidate
	Marah oreganus (Torr. & A. Gray) Howell	MOR-I, MOR-II	RIP 1
	Momordica balsamina L.	Balsamin, MbRIP-1, Momordin II	RIP 1
	Momordica charantia L.	MAP 30, α-momorcharin = α-MC = α-MMC, β-momorcharin = β-MC = β-MMC, δ-momorcharin = δ-MMC, Momordin, Momordin = Momordica charantia inhibitor, Momordin II, Momordin-a, Momordin-b	RIP 1
		γ-momorcharin = γ-MMC, Charantin	sRIP 1
		RIP 1 candidate	RIP 1 candidate
		MCL = M. charantia lectin, anti-H Lectin, Momordica agglutinin, Momordin, protein fraction 1, protein fraction 2	lectin
		MCL = Momordica charantia seed lectin = Momordica charantia lectin, MCL1	RIP 2
	Momordica cochinchinensis Spreng.	Cochinin B, Momorcochin, Momorcochin-S	RIP 1
	Siraitia grosvenorii (Swingle) C.Jeffrey ex A.M.Lu & Zhi Y.Zhang [Syn.: Momordica grosvenorii Swingle]	Momorgrosvin	RIP 1
	Sechium edule (Jacq.) Sw.	Sechiumin	RIP 1
		Sechium edule fruit lectin	lectin
	Trichosanthes anguina L.	Trichoanguin	RIP 1
		SGSL	lectin/ RIP 2 like
	Trichosanthes cordata Roxb.	TCA-I, TCA-II	lectin
	Trichosanthes cucumerina L.	TCSL	lectin/ RIP 2 candidate
	Trichosanthes cucumeroides (Ser.) Maxim.	β-trichosanthin = β-TCS	RIP 1
	Trichosanthes kirilowii Maxim.	α-kirilowin, β-kirilowin, TAP 29, TK-35, Trichobitacin, Trichokirin, Trichomislin = TCM, Trichosanthin = Trichosanthes antiviral protein = TAP = TCS = α-trichosanthin = α-TCS = GLQ223, Trichosanthin, β-trichosanthin = β-TCS, γ-trichosanthin = γ-TCS	RIP 1

		Trichokirin S1, S-Trichokirin, Trichosanthrip	sRIP 1
		TKL-1 = Trichosanthes kirilowii lectin-1	lectin/ RIP 2 candidate
		TK-I, TK-II, TK-III, Trichosanthes kirilowii lectin	lectin
	Trichosanthes kirilowii Maximovicz var. japonica (Miquel) Kitamura	Karasurin-A, Karasurin-B, Karasurin-C	RIP 1
	Trichosanthes lepiniate	Trichomaglin	RIP 1
	Trichosanthes dioica Roxb.	TDSL	lectin/ RIP 2 candidate
	Trichosanthes sp. Bac Kan 8-98	Trichobakin	RIP 1
Cupressaceae	Thuja occidentalis L.	Arborvitae RIP	RIP candidate
Euphorbiaceae	Croton tiglium L.	Crotin I	RIP 1 candidate
		Crotin 2	RIP 1
	Euphorbia characias L.	E. characias lectin	lectin
	Suregada multiflora (A.Juss.) Baill. [Syn.: Gelonium multiflorum A.Juss.]	Gelonin = GAP 31	RIP 1
	Hura Crepitans L.	Hura crepitans RIP, Hura crepitans RIP-5	RIP 1
		Hura crepitans latex lectin	RIP 2
		Crepitin, Hurin, Hura crepitans seed lectin	lectin
	Jatropha curcas L.	Curcin, Curcin 2, Curcin-L, Jc-SCRIP	RIP 1
	Manihot palmata Müll. Arg.	Mapalmin	RIP 1
	Manihot esculenta Crantz. [Syn.: Manihot utilissima Pohl]	Manutin 1, Manutin 2	RIP 1
	Ricinus communis L.	Ricin = crystalline Ricin = Ricin D, Ricin E, RCA = Ricinus communis agglutinin = RCAI = RCA120 = R. communis hemagglutinin = RCB-PHA I, RCAII = RCA60 = RCB-PHA II	RIP 2
	Ricinus communis, USA	Ricin 1, Ricin 2, Ricin 3	RIP 2
	Ricinus communis, India	Ricin I, Ricin II, Ricin III	RIP 2
	Ricinus sanguienus, France	$Ricin_{11}$, $Ricin_{12}$, $Ricin_2$	RIP 2
Fabaceae	Abrus precatorius L.	Abrin, Abrin-a = Abrin C = Abrin-III, Abrin-b, Abrin-c = Abrin A = Abrin-I, Abrin-d, Abrin-II, APA = Abrus precatorius agglutinin = Abrus lectin = AAG, APA-I, APA-II	RIP 2
	Abrus pulchellus Thwaites	Pulchellin, Pulchellin PI, Pulchellin PII, Pulchellin PIII	RIP 2
	Pisum sativum subsp. sativum L. [Syn.: Pisum sativum var. arvense (L.) Poir.]	α-pisavin, β-pisavin	RIP 1
	Pisum sativum var. macrocarpon	Sativin	RIP 1 candidate
Iridaceae		IrisRIP = IRIP, IrisRIP.A1, IrisRIP.A2, IrisRIP.A3	RIP 1

	Iris hollandica var. Professor Blaauw	IRA, IRAb, IRAr	RIP 2
Lamiaceae	Clerodendrum aculeatum (L.) Schltdl.	CA-SRI	RIP 1 candidate
	Clerodendrum inerme (L.) Gaertn.	CIP-29	RIP 1
		CIP-34	RIP 1 candidate
	Leonurus japonicus Houtt.	Leonurin	RIP candidate
Lauraceae	Cinnamomum bodinieri H. Lév.	Bodinierin	RIP 2
	Cinnamomum camphora (L.) J.Presl	Camphorin	RIP 1
		Cinnamomin, Cinnamomin 1, Cinnamomin 2, Cinnamomin 3	RIP 2
		Cinphorin	sRIP 2
	Cinnamomum parthenoxylon (Jack) Meisn. [Syn.: Cinnamomum porrectum (Roxb.) Kosterm.]	Porrectin	RIP 2
Malvaceae	Abelmoschus esculentus (L.) Moench	Abelesculin	RIP 1
Nyctaginaceae	Boerhaavia diffusa L.	Boerhaavia inhibitor	RIP 1 candidate
	Bougainvillea spectabilis Willd.	BAP I, Bouganin = Bougainvillea RIP I	RIP 1
	Bougainvillea × buttiana cv. Enid Lancester	BBP-24, BBP-28	RIP 1
	Bougainvillea × buttiana cv. Mahara	BBAP1	RIP 1
	Mirabilis expansa (Ruiz & Pav.) Standl.	ME1, ME2	RIP 1
	Mirabilis jalapa L.	MAP, MAP-2, MAP-3, MAP-4, MAP-S	RIP 1
Olacaceae	Malania oleifera Chun & S. K. Lee	Malanin	lectin/ RIP 2 candidate
	Ximenia americana L.	Riproximin = Rpx, Rpx-I, Rpx-II	RIP 2
Passifloraceae	Adenia digitata (Harv.) Engl.	Modeccin = Modeccin 4B, Modeccin 6B	RIP 2
	Adenia ellenbeckii Harms	A. ellenbeckii lectin	RIP 2 candidate
	Adenia fruticosa Burtt Davy	A. fruticosa lectin	lectin
	Adenia glauca Schinz	A. glauca lectin	RIP 2 candidate
	Adenia goetzei Harms (unresolved name)	A. goetzei lectin	RIP 2
	Adenia keramanthus Harms	A. keramanthus lectin	RIP 2 candidate
	Adenia lanceolata Engl.	Lanceolin	RIP 2
	Adenia racemosa W. J. de Wilde	A. racemosa lectin	lectin
	Adenia spinosa Burtt Davy	A. spinosa lectin	RIP 2 candidate
	Adenia stenodactyla Harms	Stenodactylin	RIP 2
	Adenia venenata Forssk.	A. venenata lectin	RIP 2 candidate
	Adenia volkensii Harms	Volkensin	RIP 2

Phytolaccaceae	Phytolacca americana L.	α-PAP, PAP = Phytolacca americana protein = pokeweed antiviral protein, PAP-I, PAP-II, PAP-III, PAP-C, PAP-H, PAP-R, PAP-S, PAP-S1, PAP-S2	RIP 1
	Phytolacca dioica L.	Diocin 1, Diocin 2, PD-L1, PD-L2, PD-L3, PD-L4, PD-S1, PD-S2, PD-S3	RIP 1
	Phytolacca dodecandra L'Hér.	Dodecandrin, Dodecandrin C	RIP 1
	Phytolacca heterotepala H. Walter	Heterotepalin 4, Heterotepalin 5b	RIP 1
	Phytolacca insularis Nakai	Insularin = PIP = Phytolacca insularis antiviral protein, PIP2 = P. insularis antiviral protein 2	RIP 1
Poaceae	Hordeum vulgare L.	Barley toxin = Barley translation inhibitor = Barley Protein Synthesis Inhibitor = BPSI = RIP 30, Barley toxin I = Barley translation inhibitor I, Barley toxin II = Barley translation inhibitor II = Barley Protein Synthesis Inhibitor II = BPSI II, Barley toxin III = Barley translation inhibitor III, JIP60	RIP 1
	Oryza sativa L.	Oryza sativa RIP	RIP 1
	Secale cereale L.	RPSI	RIP 1
	Triticum aestivum L.	Tritin, Tritin 1, Tritin 2, Tritin 3, Tritin-S, Tritin-L	RIP 1
	Zea mays L.	b-32 = maize RIP = maize proRIP1, Maize proRIP2	RIP 3/ peculiar RIP 1
Ranunculaceae	Eranthis hyemalis (L.) Salisb.	EHL	RIP 2
Santalaceae	Phoradendron californicum Nutt.	PCL	RIP 2
	Viscum album L. (Himalayan mistletoe)	HmRip, HmRip 1, HmRip 2, HmRip 3, HmRip 4	RIP 2
	Viscum album L. (European mistletoe)	ML-I = Mistletoe lectin I = Viscumin = Eu-ML = EML-1 = VAA-I, ML-II = Mistletoe lectin II = VAA-II, ML-III = Mistletoe lectin III = VAA-III	RIP 2
	Viscum articulatum Burm. f.	Articulatin-D	RIP 2
	Viscum coloratum (Kom.) Nakai [Syn.: Viscum album subsp. coloratum Kom.]	KML, KML-C, KML-IIL, KML-IIU, VCA	RIP 2
Solanaceae	Nicotiana tabacum L.	CIP31	RIP-like protein
		TRIP	RIP 1 candidate
Thymelaeaceae	Phaleria macrocarpa (Scheff.) Boerl.	P. macrocarpa RIP	RIP candidate

* Schrot J, Weng A, Melzig MF, et al. Ribosome-inactivating and related proteins. Toxins (Basel). 2015 May 8;7(5):1556-615.

An aspect of the invention relates to a conjugate comprising or consisting of an antibody and an antisense oligonucleotide such as an antisense BNA, covalently linked together. In Figure 1-5, the gene-silencing activity of such a conjugate is depicted (in vivo test in an animal tumor model). Reference is also made to the Examples section.

An aspect of the invention relates to a combination of a first composition comprising a conjugate comprising or consisting of an antibody and an antisense oligonucleotide such as an antisense BNA,

covalently linked together, and a second composition comprising free saponin of the invention (see Table A1, Scheme I). In Figure 1-7A and in Figure 1-7C, the gene-silencing activity of such a conjugate is depicted (in vitro cell-based bioassay with human tumor cells). Reference is also made to the Examples section.

An aspect of the invention relates to a pharmaceutical combination comprising or consisting of a first composition comprising a first conjugate comprising or consisting of an antibody and an antisense oligonucleotide such as an antisense BNA, and a second composition comprising a first conjugate comprising or consisting of the same antibody and at least one saponin of the invention. In Figure 1-5, the gene-silencing activity of such a conjugate is depicted (in vivo test in an animal tumor model). In Figure 8-5, the gene-silencing activity of such a conjugate is depicted (in vitro cell-based bioassay with human tumor cells). Reference is also made to the Examples section.

An aspect of the invention relates to a pharmaceutical combination comprising or consisting of a fourth composition comprising a fourth conjugate comprising or consisting of an antibody and an antisense oligonucleotide such as an antisense BNA, and a fifth composition comprising a first conjugate comprising or consisting of a different antibody and at least one saponin of the invention. In Figure 10-6A and in Figure 10-6C, the gene-silencing activity of such a conjugate is depicted (in vitro cell-based bioassay with human tumor cells). Reference is also made to the Examples section.

An aspect of the invention relates to a conjugate comprising or consisting of an antisense oligonucleotide such as an antisense BNA, covalently linked to at least one saponin of the invention. In Figure 1-3, the gene-silencing activity of such a conjugate is depicted (in vitro cell-based bioassay with human tumor cells). Reference is also made to the Examples section.

An aspect of the invention relates to a conjugate comprising or consisting of an antisense oligonucleotide such as an antisense BNA, covalently coupled to a polymeric scaffold such as a dendron such as a G4-dendron, wherein the polymeric scaffold is covalently conjugated with one or more saponin molecules of the invention, such as four saponin molecules. In Figure 1-3, the gene-silencing activity of such a conjugate is depicted (in vitro cell-based bioassay with human tumor cells). Reference is also made to the Examples section.

An aspect of the invention relates to a conjugate comprising or consisting of an antibody such as a monoclonal antibody with specificity for a tumor marker or tumor-cell receptor, covalently linked to at least one antisense oligonucleotide molecule such as antisense BNA, and covalently linked to at least one saponin molecule of the invention. In Figure 2-4, the gene-silencing activity of such a conjugate is depicted (in vivo test in an animal tumor model). Reference is also made to the Examples section.

An aspect of the invention relates to a conjugate comprising or consisting of an antibody such as a monoclonal antibody with specificity for a tumor marker or tumor-cell receptor, covalently linked to at least one antisense oligonucleotide molecule such as antisense BNA via a tri-functional linker such as the linker of Scheme II, and covalently linked to at least one saponin molecule of the invention via the same tri-functional linker. In Figure 1-1, the gene-silencing activity of such a conjugate is depicted (in vivo test in an animal tumor model). Reference is also made to the Examples section.

An aspect of the invention relates to a therapeutic combination consisting or comprising of a eighth composition comprising a conjugate comprising or consisting of an antibody, preferably a

monoclonal antibody with specificity for a tumor marker or tumor-cell receptor, covalently linked to at least one saponin molecule of the invention, preferably via at least one linker, preferably at least one cleavable linker, cleavable under physiological acidic conditions, and further comprising a ninth composition comprising an antisense oligonucleotide such as an antisense BNA molecule. In Figure 6-2, the gene-silencing activity of such a conjugate is depicted (in vivo test in an animal tumor model). In Figure 5-2A and Figure 5-2C, the gene-silencing activity of such a conjugate is depicted (in vitro cell-based bioassay with human tumor cells). Reference is also made to the Examples section.

An aspect of the invention relates to any of the aforementioned conjugates or compositions or therapeutical combinations, for use as a medicament.

An aspect of the invention relates to any of the aforementioned conjugates or compositions or therapeutical combinations, for use in the treatment or prophylaxis of a cancer.

Of course, as said before, any and all of a, b, c, d, e, f, g, h, l, j, k, m, n, p, q, r, s, t, u, v, w and/or x have the value in accordance with each individual embodiment and aspect of the invention for any and all of the aforementioned aspects and embodiments according to the invention. In addition, (tri-functional) linkers L1, L2, L4, L5, L6, L8, L9 and/or L10, if present in a molecule or conjugate or moiety of the invention, are the (tri-functional) linkers as indicated for each and any of the aforementioned aspects and embodiments of the invention, as is readily appreciated by the skilled person. The oligomeric or polymeric scaffolds L3 and/or L7, if present in a molecule or conjugate or moiety of the invention, are the oligomeric or polymeric scaffolds as indicated for each and any of the aforementioned aspects and embodiments of the invention, as is also readily appreciated by the skilled person. Furthermore, the first ligand A1 and the first effector moiety B1, if present, and the second ligand A2 and the second effector moiety B2, if present, and the first effector moiety A1 and the first ligand B1, if present, and the second effector moiety A2 and the second ligand B2, if present, are the selected and indicated ligands and effector moieties, as disclosed for the first, second, third, fourth, fifth, and sixth series of embodiment and aspects of the invention, and all further embodiments and aspects of the invention, outlined here above. Saponin C is any one or more of the saponins referred to and listed in any of the aforementioned aspects and embodiments of the invention, in particular one or more saponins selected from Scheme I and/or Table A1.

The invention is further illustrated by the following examples, which should not be interpreted as limiting the present invention in any way.

EXAMPLES

EXAMPLE A – TREATING A MAMMALIAN TUMOR-BEARING ANIMAL WITH A CONJUGATE OF THE INVENTION IN COMBINATION WITH AN ADC RESULTS IN SURVIVAL AND TUMOR REGRESSION

Female Balb/c nude mice were injected subcutaneously with a suspension of human A431 tumor cells. Under the skin of the mice, a human epidermal carcinoma developed in the xenograft animal tumor

model. After injection of the tumor cells, the xenograft tumor was allowed to develop to a size of approximately 170-180 mm^3. The A431 tumor cells have the following characteristics: high EGFR expressors, medium CD71 expressors, low HER2 expressors.

In Table A, the results of the treatment of control mice and tumor-bearing mice are presented. Tumor-bearing mice were treated with the indicated antibodies directed to either human Her2/neu, human EGFR, or human CD71, which are cell-surface receptors on the xenograft tumor. Cetuximab was covalently conjugated with saponin SO1861. The SO1861 was first provided with the linker EMCH (N-ε-maleimidocaproic acid hydrazide), which EMCH is a maleimide-and-hydrazide crosslinker for covalently conjugating sulfhydryls (reduced cysteines of the antibody)) to carbonyls (aldehyde or ketones; here the carbonyl of the aldehyde at position C-23 of the saponin). The saponin-EMCH was covalently coupled to reduced cysteines of the Cetuximab, forming a covalent thio-ether bond between the EMCH and the cysteine side chain. The ADCs trastuzumab-saporin (covalent conjugate) and anti-CD71 mAb (OKT-9, IgG) – saporin (covalent conjugate) were tested for their tumor-attacking efficacy in the mice, measured as tumor volume in time after start of the treatment with the ADCs. The dose of the ADCs was sub-optimal in the tumor model. That is to say, from previous experiments, it was established at which sub-optimal dose of the ADCs no tumor-regression or arrest of tumor growth would be observable.

TABLE A: RESULTS OF TREATING A MAMMALIAN TUMOR-BEARING ANIMAL WITH A CONJUGATE OF THE INVENTION IN COMBINATION WITH AN ADC RESULTS IN SURVIVAL AND TUMOR REGRESSION

Treatment group	**Patient / healthy animal**	**treatment**	**tumor size (volume in mm^3 or '+' for growth, '-' for regression, and 'stable' for growth nor regression)**
1	xenograft	vehicle	2000 mm^3 (death/euthanasia)
2	xenograft	Trastuzumab-saporin	2000 mm^3 (death/euthanasia)
3	xenograft	Anti-CD71 mAb OKT-9 – saporin (covalent conjugate)	2000 mm^3 (death/euthanasia)
4	xenograft	Cetuximab-SO1861 (covalent conjugate)	2000 mm^3 (death/euthanasia)
5	xenograft	Cetuximab	> 170 mm^3, but < 2000 mm^3 (death/euthanasia)
6	**xenograft**	**Trastuzumab-saporin** (covalent conjugate) **+**	**Tumor regression from 180 mm^3 at the start of treatment back to 80 mm^3 (survival)**

		Cetuximab-SO1861 (covalent conjugate)	
7	**xenograft**	**Anti-CD71 mAb OKT-9 - saporin** (covalent conjugate) **+ Cetuximab-SO1861** (covalent conjugate)	**Tumor regression from 180 mm^3 at the start of treatment back to 40 mm^3 (survival)**

These results demonstrate that the combination therapy of an ADC at a dose which is ineffective when treatment of tumor-bearing mice with the ADC alone is considered (tumor growths, death of the mice is not prevented (euthanasia)), with a conjugate of the invention consisting of a tumor-cell specific receptor targeting antibody covalently bound to a saponin, i.e. SO1861, the covalent conjugate administered to the mice suffering from cancer, at a non-effective dose when administered alone (tumor growths, death of the mice is not prevented (euthanasia)), provides an efficient and efficacious treatment regimen, expressed as tumors in regression and prolonged survival of the treated animals (beyond the duration of the experiment). The sub-optimal dose of ADC combined with a covalently bound saponin-comprising conjugate of the invention which has no anti-tumor activity when administered alone, thus provide for an effective treatment option for cancer patients, wherein a relative low dose of the ADC is efficacious. A lower dose of ADC bears the promise of less risk for adverse events, or even no side effects at all. In addition, the stimulatory effect of the saponin-bearing conjugate of the invention when the efficacy of the ADC is considered, shows that ADCs which previously have proven to lack efficacy when tumor patient treatment is concerned, may gain renewed attention and value, since ADC efficacy is improved in combination therapy setting, as the current example demonstrated. Reference is made to Table A2 and Table A3, summarizing ADCs which were previously investigated in the human clinical setting, but then were for some ADCs retracted from further clinical investigation. Especially the ADCs for which clinical development was terminated due to observed lack of efficacy and/or due to occurrence of unacceptable adverse event are ADCs which may gain renewed value for cancer patients when combined with a covalently bound saponin-comprising conjugate of the invention, such as the cetuximab-saponin tested.

EXAMPLE B – **saponins mixture of Quillaja saponaria comprising QS-21, with endosomal/lysosomal escape enhancing activity**

Scheme I displays the common molecular structure of a series of QS-21 saponins (in part adapted from: Conrado Pedebos, Laércio Pol-Fachin, Ramon Pons, Cilaine V. Teixeira Hugo Verli, Atomic Model and Micelle Dynamics of QS-21 Saponin, Molecules 2014, 19, 3744-3760). A mixture of water-soluble saponins obtained from Quillaja saponaria (Sigma-Aldrich, product No. S4521; Roth, Item No. 6857; InvivoGen, product 'Quil-A') may be applied in the endosomal/lysosomal escape enhancing conjugate, composition, combination of the invention, based on endosomal/lysosomal escape enhancing properties of at least one individual saponin present in the mixture, e.g. QS-21, or based on a combination of two or more of the saponins comprised by the mixture, such as QS-21 and QS-7.

The inventors demonstrated that the mixture of saponins from Quillaja saponaria at 2,5 microgram/ml dose was capable of enhancing endosomal escape of dianthin, as tested with mammalian tumor cells in a cell-based bioassay. The effector moiety exposed to the cells was dianthin covalently coupled to the ligand EGF: EGF-dianthin. Cells tested were tumor cell lines HeLa for free saponins, and A431, MDA-MB-468, CaSki and A2058 for testing the saponins when covalently coupled to cetuximab.

Example 1

A trifunctional linker scaffold was designed and produced with specific chemical end groups (DBCO, TCO) for conjugation (labile, (L) conjugation) with on one arm an SO1861 molecule and on the other arm an antisense HSP27BNA oligo nucleotide (targeting and inducing degradation of the onco-target *hsp27* mRNA in cancer cells) to produce SO1861-L-trifunctional linker-L-HSP27BNA (Figure 16-1). SO1861-L-trifunctional linker-L-HSP27BNA was conjugated with its the third arm (maleimide) to the cysteine residues (Cys) anti-EGFR antibody, cetuximab (cetuximab-Cys-(SO1861-L-trifunctional linker-L-HSP27BNA)^4).

This scaffold comprising conjugate was tested in a A431 xenograph 'nude' mouse tumor model for EGFR-mediated tumor targeted gene silencing activity. Dosings started at day 12 when tumors reached ~170mm^3 in size and tumor samples were collected at 72h after the first dosing and analysed for HSP27 gene expression compared to cellular control mRNA expression (reference genes). This revealed that 1 dosing of 25mg/kg cetuximab-Cys-(SO1861-L-trifunctional linker-L-HSP27BNA)^{3,7} resulted in a 40% reduction in HSP27 gene expression in the tumors compared to single dosing of cetuximab-(Cys-L-SO1861)^{3,8} or cetuximab-(Lys-L-HSP27BNA)^4 mono therapies (Figure 1-1). Compared to the vehicle control tumors a reduction of 25% gene silencing was observed. This shows and enables that conjugated SO1861 efficiently can induce targeted delivery of therapeutic oligo nucleotides in tumors, *in vivo*.

To further strengthen this, cetuximab-Cys-(SO1861-L-trifunctional linker-L-HSP27BNA DAR4)^4 was tested for enhanced HSP27 gene silencing in EGFR expressing (A431) , *in vitro* as illustrated in Figure 2-1. Cetuximab-Cys-(SO1861-L-trifunctional linker-L-HSP27BNA)^{3,7} efficiently induces HSP27 gene silencing in A431 cells (IC50=…) compared to Cetuximab-(Lys-L-HSP27BNA)^4 or Cetuximab-(Cys-L-SO1861)^{3,8} alone (Figure 2-1).

Example 2

1 target 2-components system is the combination treatment of mAb1-(dendron(SO1861)^n)^n and mAb1-effector as illustrated in Figure 11-1 and whereas the 2 target 2-component system is the combination of mAb1-(dendron(SO1861)^n)^n + mAb2-effector as illustrated in Figure 12-1.

Dendron(-L-SO1861)^4 was conjugated to the anti-EGFR antibody, cetuximab via cysteine residues (Cys) conjugation with a DAR3,9, cetuximab-Cys-(dendron(-L-SO1861)^4)^{3,9} and tested for enhanced cell killing activity in combination with an anti-EGFR antibody-protein toxin conjugate (cetuximab-saporin) in EGFR expressing cells (MDA-MB-468). Cetuximab-Cys-(dendron(-L-SO1861)^4)^{3,9} + 10 pM cetuximab-saporin efficiently induces toxin-mediated cell killing in high EGFR expressing cells (IC50=…), whereas this was not induced by Cetuximab-Cys-(dendron(-L-SO1861)^4)^{3,9} or cetuximab (equivalent) + 10pM

cetuximab-saporin or cetuximab (Figure 3-1A). Similar experiments in cells that express low levels of EGFR (HeLa) revealed no activity of Cetuximab-Cys-(dendron(-L-SO1861)^4)^{3,9} (Figure 3-1C) indicating that in the absence of sufficient EGFR receptor expression, effective intracellular SO1861 concentrations are not reached (threshold) to induce endosomal protein toxin escape and toxin-mediated cell killing.

Next, dendron(-L-SO1861)^4 was conjugated to the anti-HER2 antibody, trastuzumab via cysteine conjugation (Cys) with a DAR4, trastuzumab-Cys-(dendron(-L-SO1861)^4)^4 and tested for enhanced cell killing activity in combination with an anti-HER2 antibody-protein toxin conjugate (trastuzumab-saporin) in HER2 expressing cells (SK-BR-3). trastuzumab-Cys-(dendron(-L-SO1861)^4)^4 + 50 pM trastuzumab-saporin efficiently induce toxin-mediated cell killing (IC50 =…), whereas this was not induced by trastuzumab-Cys-(dendron(-L-SO1861)^4)^4 or trastuzumab (equivalent) + 50nM trastuzumab-saporin or trastuzumab (Figure 3-1B). Similar experiments in cells that express low levels of HER2 (JIMT-1) revealed no activity of Trastuzumab-Cys-(dendron(-L-SO1861)^4)^4 (Figure 3-1D) indicating that in the absence of sufficient HER2 receptor expression, effective intracellular SO1861 concentrations are not reached (threshold) to induce endosomal protein toxin escape and toxin-mediated cell killing.

Next, Cetuximab-Cys-(dendron(-L-SO1861)^4)^{3,9} or Cetuximab-Lys-(dendron(-L-SO1861)^4)^{4,4} (Lys=dendron(-L-SO1861)^4 conjugated to lysines of antibody) was tested in combination with 10 pM CD71mab-saporin in a 2 target 2 components system in EGFR^{++}/CD71$^+$ cells (MDA-MB-468). This showed for both conjugates a strong enhancement of the cell killing activity (IC50=.. IC50=.. resp.), whereas this was not induced by Cetuximab-Cys-(dendron(-L-SO1861)^4)^{3,9} or Cetuximab-Lys-(dendron(-L-SO1861)^4)^{4,4} or cetuximab (equivalent) + 10 pM CD71mab-saporin or cetuximab (Figure 4-1A). Similar experiments in cells that express lower levels of EGFR (CaSKi, EGFR$^+$/CD71$^+$) revealed reduced activity for both cetuximab-Cys-(dendron(-L-SO1861)^4)^{3,9} or cetuximab-Lys-(dendron(-L-SO1861)^4)^{4,4} (Figure 4-1C) compared to the activity in high expressors (Figure 4-1A) indicating that in cells with lower EGFR receptor expression levels, the effective intracellular SO1861 concentrations is lower resulting in reduced toxin-mediated cell killing activity.

Same experiment was performed with trastuzumab-Cys-(dendron(-L-SO1861)^4)^4 or trastuzumab-Lys-(dendron(-L-SO1861)^4)^{4,7} in combination with CD71mab-saporin on HER2^{++}/CD71$^+$ (SK-BR-3) cell lines revealing strong cell killing activity compared to the controls (Figure 4-1B). When trastuzumab-Cys-(dendron(-L-SO1861)^4)^4 or trastuzumab-Lys-(dendron(-L-SO1861)^4)^{4,7} was tested on HER2$^{+/-}$/CD71$^+$ (JIMT-1) in combination with 10 pM CD71mab-saporin no cell killing activity could be observed indicating that in the absence of sufficient HER2 receptor expression, effective intracellular SO1861 concentrations are not reached (threshold) to induce endosomal protein toxin escape and toxin-mediated cell killing.

Next, trastuzumab-Cys-(dendron(-L-SO1861)^4)^4 + trastuzumab-emtansine (T-DM1, antibody-small molecule toxin conjugate) was tested for enhanced cell killing activity in HER2 expressing cells (SK-BR-3). No enhanced cell killing was observed with this combination, compared to T-DM1 alone or T-DM1 + equivalent trastuzumab, since the endosomal membrane forms no barrier for small molecules to reach the cytoplasm. (Figure 5-1).

Example 3

Materials and methods

dendron(SO1861)$_4$-BNA oligo synthesis (Figure 17-1)

HSP27BNA oligo disulfide (1.1 mg, 0.187 µmol) was dissolved in 20 mM NH_4HCO_3 with 1.0 mM TCEP (500 µL) and the mixture was shaken for 1 min and left standing at room temperature. After 1 hour the reaction mixture was filtered by using a centrifugal filter with a molecular weight cut-off of 3000 Da (14000 × g for 30 min). The residue solution was diluted with 20 mM NH_4HCO_3 with 1.0 mM TCEP (500 µL) and the resulting mixture was filtered again under the same conditions described above. The residue solution was diluted with 20 mM NH_4HCO_3/acetonitrile (3:1, v/v, 1.0 mL) and the resulting mixture was added to dendron(SO1861)4-maleimide1 (3.54 mg, 0.375 µmol) (Figure 17-1) . The reaction mixture was shaken for 1 min and left standing at room temperature. After 10 min the reaction mixture was subjected to preparative LC-MS.[4A] Fractions corresponding to the product were immediately pooled together, frozen and lyophilized overnight to give the title compound (1.25 mg, 85%) as a white fluffy solid. Purity based on LC-MS 94%

LRMS (m/z): 1896 $[M-8]^{8-}$, 2167 $[M-7]^{7-}$

LC-MS r.t. (min): 3.77[6B]

results

HSP27BNA oligo, (antisense BNA oligo targeting the mRNA transcript of the cancer target, heat shock protein 27 (HSP27BNA)) was conjugated to a dendron(-L-SO1861)^4 (HSP27BNA-dendron(-L-SO1861)^4, Figure 17-1) and co-administrated to A431 cancer cells. As readout, gene silencing of HSP27 mRNA in A431 cells was determined. This revealed that HSP27BNA-dendron(-L-SO1861)^4 treatment resulted in an improvement of HSP27 gene silencing activity compared to the HSP27BNA alone (Figure 6-1).

Example 4

Methods

SO1861 releasing assay

To dendron(SO1861)$_4$-Cbz (0.05 mg) (Figure 7-1) was added 50 µL of solution containing water/acetonitrile/TFA (1.00 mL/1.00 mL/4 drops). The reaction mixture was shaken for 1 min and left standing at room temperature. The SO1861 release was followed over time by using UPLC-MS.[4]

Results

The release efficiency of the SO1861 molecules from the dendron(-L-SO1861)^4 under acid conditions has been determined (Figure 7-1).

Next, dendron(-L-SO1861)^4 was tested for enhanced delivery of a targeted toxin, EGFdianthin on EGFR expressing cells (A431 and HeLa). This shows that dendron(L-SO1861)^4 + 10 pM EGFdianthin can induce enhanced toxin-mediated cell killing (IC50 ….nM), whereas the 'naked' dendron (Dendron(NEM)^4) or dendron(-L-SO1861)^4 or Dendron(NEM)^4 + 10 pM EGFdianthin is not showing enhanced cell killing at these concentrations (Figure 8-1A, 8-1B).

Example 5

Materials and methods

In our current work, we investigated a model scaffold consisting of four molecular arms for saponin binding via a Schiff base (imine) and one arm for click chemistry. The polymeric structure (Figure 19-1) is a pentavalent polyethylene glycol-based dendrimer of the first generation (i.e. number of repeated branching cycles) that was purchased from Iris Biotech GmbH (Marktredwitz, Germany). The saponin (in this example SA1641) was purified from a saponin composite raw extract from Gypsophila species called Saponinum album obtained from Merck (Darmstadt, Germany). The powdered raw extract (2.5 g) was hydrolyzed in water (100 mL) with sodium hydroxide (0.2 g). The solution was stirred for 20 h at 40 °C and then supplemented with glacial acetic acid until pH 5.0 was reached. To remove tannins, the solution was shaken in a separatory funnel with 30 mL butanol. The aqueous phase was recaptured and butanol extraction repeated two times. The butanol phases were supplemented with anhydrous sodium sulfate, filtered and pooled. Butanol was evaporated and the remaining saponin powder resolved in 20% methanol to a final concentration of 30 mg/mL. After short sonication, different saponins were separated by high performance liquid chromatography (HPLC). Tubes (excluding column) were rinsed with warm water (40 °C) at a flow of 1.5 mL/min and then including Eurospher RP-C18-column (5 µm, 250 × 8 mm) with isopropanol (100%). Saponins were applied to the column and eluted with a methanol gradient (20% methanol to 70% methanol within 30 min at 1.5 mL/min in water supplemented with 0.01% trifluoroacetic acid followed by 70% methanol for further 60 min) (Sama et al, 2018). Aliquots of the fractions were analyzed for their SA1641 content by electrospray ionization mass spectrometry (ESI-MS). Fractions containing pure SA1641 were pooled and methanol evaporated. The aqueous solution was frozen as a thin film in a rotating round-bottom flask by use of dry ice. After storage for 16 h at –80 °C, the sample was lyophilized. To produce the scaffold as defined in the invention, the polymeric structure (0.2 mM) and SA1641 (3.2 mM) were solved in water (approx. pH 8) and equal volumes mixed and shaken for 24 h at 26 °C. Then sodium cyanoborohydride ($NaCNBH_3$; 0,1 M) was added in 4-fold molar excess referred to SA1641 and the sample incubated for further 24 h. The structure was then verified by ultra performance liquid chromatography (UPLC)/ESI-MS. The samples were applied to a RP-C4-column and eluted with a methanol gradient (25% methanol to 80% methanol within 15 min in water supplemented with 0.01% trifluoroacetic acid followed by 80% methanol for further 10 min). The fractions were analyzed by use of LockSpray™ that is an ion source designed specifically for exact mass measurement with electrospray ionization using LC-time-of-flight (LC-TOF) mass spectrometers from Waters Corporation.

Example 6

Materials and methods

As an example for a pharmaceutical active substance, we used the targeted toxin dianthin-Epidermal Growth Factor (dianthin-EGF). The plasmid His-dianthin-EGF-pET11d (Weng et al, 2009) (100 ng) was added to 20 µL Escherichia coli Rosetta™ 2 (DE3) pLysS Competent Cells (Novagen, San Diego, CA, USA). Cells were transformed by a heat-shock (30 min on ice, 90 s at 42 °C and 1 min on ice). Thereafter, 300 µL lysogeny broth (LB) was added and the suspension incubated for 1 h at 37 °C while

shaking at 200 rpm. A preheated lysogeny broth agar plate with 50 µg/mL ampicillin was inoculated with 100 µl bacteria suspension and the plate incubated overnight at 37 °C. Lysogeny broth (3 mL) with 50 µg/mL ampicillin was inoculated with a colony from the plate and the bacteria were incubated for 8 h at 37 °C and 200 rpm. The suspension (50 µL) was added to 500 mL of lysogeny broth with 50 µg/mL ampicillin and incubated overnight at 37 °C and 200 rpm. Subsequently, the volume was scaled-up to 2.0 L and bacteria grew under the same conditions until an optical density at wavelength 600 nm of 0.9 was reached. Thereafter, protein expression was induced by the addition of isopropyl β-D-1-thiogalactopyranoside (IPTG) at a final concentration of 1 mM. Protein expression lasted for 3 h at 37 °C and 200 rpm. Finally, the bacterial suspension was centrifuged at 5,000 × *g* and 4 °C for 5 min, resuspended in 20 mL PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.47 mM KH_2PO_4) and stored at –20 °C until use. For purification, bacterial suspensions were thawed and lysed by sonication. Lysates were centrifuged (15,800 × *g*, 4 °C, 30 min) and imidazole added to a final concentration of 20 mM. The supernatant was incubated with 2 mL of Ni-nitrilotriacetic acid agarose under continuous shaking for 30 min at 4 °C in the presence of 20 mM imidazole. Subsequently, the material was poured into a 20-mL-column and washed three times with 10 mL wash buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole) and dianthin-EGF eluted by 10-mL-portions of increasing concentrations of imidazole (31, 65, 125 and 250 mM) in wash buffer. Eluate fractions (2 mL) were dialyzed overnight at 4 °C against 2.0 L PBS. Desalted dianthin-EGF was concentrated by an Amicon® Ultra-15 (10 kDa) and the protein concentration quantified.

To introduce a suitable click chemistry group into dianthin-EGF, alkyne-PEG_5-N-hydroxysuccinimidyl ester in 8-fold molar excess referred to dianthin-EGF was solved in dimethyl sulfoxide and added to 9 volumes of dianthin-EGF (1 mg in 0.2 M NaH_2PO_4/Na_2HPO_4, pH 8). After incubation at room temperature for 4 h, non-bound alkyne was separated by use of a PD10 column (GE-Healthcare, Freiburg, Germany). Click chemistry with the polymeric structure was conducted by copper(I)-catalyzed alkyne-azide cycloaddition. Alkyne-dianthin-EGF (0.02 mM), dendrimer (0.05 mM), $CuSO_4$ (0.1 mM), tris(3-hydroxypropyltriazolylmethyl)amine (0.5 mM) and sodium ascorbate (5 mM) were incubated under gentle agitation for 1 h at room temperature in 0.1 M NaH_2PO_4/Na_2HPO_4, pH 8. Low molecular mass substances were then separated using a PD10 column.

To test the efficacy of the invention, we conducted a viability assay with HER14 cells. These cells are fibroblasts stably transfected with the human epidermal growth factor receptor and therefore target cells for the targeted toxin dianthin-EGF. HER14 cells (2,000 cells/100 µL/well) were seeded into wells of 96-well-cell culture plates and incubated for 24 h in DMEM medium supplemented with 10% fetal calf serum and 1% penicillin/streptomycin at 37 °C, 5% CO_2 and 98% humidity. The different test substances (see results and Figure 21-1) were then added in triplicates in a volume of 25 µL and supplemented with further 25 µL of medium. After an incubation of 72 h, 30 µL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (0.5 mg/mL in water) was added per well and incubated for 2 h. Thereafter, the medium was carefully removed and replaced by an aqueous solution containing 10% (v/v) isopropanol, 5% (w/v) sodium dodecyl sulfate and 400 mM HCl, and incubated for 5 min. Solubilized formazan was photometrically quantitated at 570 nM in a microplate reader (Spectra MAX 340 PC,

Molecular Devices, Sunnyvale, CA, USA). Untreated cells were normalized to 1 and all samples referred to the untreated control. Significance was determined by unpaired two-sample t-tests.

Results

The polymeric structure, in the example a pentameric dendrimer (pentrimer), does not have any cytotoxic effect on the target cells, neither in absence nor in presence of SA1641 (Figure 21-1, column 2 and 3). In the absence of the scaffold, the targeted toxin (dianthin-EGF) shows half maximal toxicity at a concentration of 0.1 nM (column 4). In the presence of SA1641 the same concentration results in death of all cells indicating the general ability of SA1641 to act as an enhancer of the endosomal escape (column 5). The presence of the polymeric structure does not affect the toxicity of dianthin-EGF neither in the presence nor in the absence of SA1641 (columns 6 and 7), indicating that the scaffold does not affect the toxicity of dianthin-EGF. To couple the model polymeric structure via click chemistry to the example pharmaceutically active substance of dianthin-EGF, the substance had to be coupled with an alkyne group before. . A manufacturer of a pharmaceutically active substance can introduce the click position during synthesis directly into the substance at a position of his choice where the activity of the substance remains unaffected. There was no additional loss of activity when clicking the alkyne-modified pharmaceutically active substance to the polymeric structure indicating that the polymeric structure itself was not toxic.

Example 7

Considering available chemical groups for conjugation reactions to the SO1861 molecule, four chemical groups have been identified. The alcohols and diols of the sugar residues, the aldehyde group on the triterpenoid backbone, the carboxylic acid on one of the sugar residues (glucuronic acid), and the alkene group on the triterpenoid backbone as highlighted in Figure 19-1.

In view of the pros and cons of each identified chemical group (Table 1), the aldehyde and alcohol groups are best suitable for reversible conjugation reactions, while the alkene and the carboxylic acid (glucuronic acid) are the groups best suitable for irreversible / stable conjugation reactions. The aldehyde group within the molecule structure of SO1861, however, is the most suitable for reversible conjugation reactions over the alcohols. On the one hand, because there is only one aldehyde present in the structure that allows chemoselective reactions. On the other hand, because the aldehyde can perform reversible conjugation reactions with a variety of chemical groups such as amines, hydrazides, and hydroxylamines forming acid-cleavable moieties like imines, hydrazones, and oximes. This factor enables a freedom of choice over the chemical group for the desired reversible conjugation reaction. Contrary, the alcohols are good candidates for reversible conjugation reaction via the formation of acetals and ketals as well, but lack in chemoselectivity since they are present in a large quantity on the glycosidic structure.

For the formation of an irreversible and stable bond the carboxylic acid is the most suitable since it can form amides and esters with the common tools used in peptide chemistry (e.g. reaction with amines via carbodiimide mediated amide formation).

Table 1. Functional groups that are available for saponin conjugation reactions

Functional Group	Pros	Cons
Alcohol (Diols)	- Suitable for reversible acetal/ketal formation - Suitable for ester formations with activated carboxylic acids	- Acetal/ketal formation without chemoselectivity - Ester formation without chemoselectivity
Aldehyde	- Suitable for chemoselective reversible hydrazone formation with hydrazides - Suitable for chemoselective reversible imine formation with amines - Suitable for chemoselective reversible oxime formation with hydroxylamines	- Not suitable for acetal formation in the presence of unprotected saponin sugar diols
Alkene	- Suitable for chemoselective irreversible radical reactions	- Not suitable for reversible conjugation reactions - Not suitable for reactions involving a hydrogenation step
Carboxylic acid	- Suitable for chemoselective amide / ester formation with amines and alcohols after activation	- Not suitable for reversible conjugation reactions under mild conditions

Regarding an ideal EMCH spacer length for conjugation to a polymeric structure, computer simulation (PerkinElmer, ChemBio3D, Ver. 13.0.0.3015) shows that the maleimide group on SO1861-EMCH is located at the periphery of the molecule and thus should be accessible for thiol bearing polymeric structures (Figure 27-1).

As a polymeric structure, a G4-dendron (PFd-G4-Azide-NH-BOC, Polymer Factory) with 16 functional amino end groups and an azido group at the focal point was utilized for the conjugation to SO1861 (Figure 24-1). The advantage of using a dendron over a dendrimer is the focal point that the dendron structure is exhibiting.

Another approach for the development of a SO1861 scaffold among the discussed polymer, and protein approach is the poly(SO1861) approach. The idea of this approach is to generate a polymer that consists of SO1861 molecules only, with pH sensitive cleavable bonds that release the SO1861. In addition, the poly(SO1861) should be able to perform conjugation reactions to toxins and biopolymers. The main goal with this approach is to keep it as simple and cost effective as possible. Since a protocol for the generation of acid cleavable SO1861 has been developed already (SO1861-EMCH approach) it would be interesting to see if it is possible to polymerize the SO1861-EMCH through simple addition of

a polymerization initiator without further modifying the SO1861 or identifying other conjugation sites on the SO1861 molecule. In the past, several papers have discussed the polymerization of maleimide groups by using radical initiators which attack the double bond of the maleimide group and thus initiate a radical polymerization along the double bonds of the maleimides. Since SO1861-EMCH reveals a maleimide group in its structure this group could potentially be explored for radical polymerization reactions to yield a poly(SO1861) with acid cleavable function. If the polymerization reaction has a reasonable reaction time the generated SO1861 polymers could be quenched with a radical quencher that not only quenches the reaction but also generates a functional group for toxin or biopolymer conjugation. Here, the system of ammonium persulfate (APS) and tetramethylethylenediamine (TMEDA) is indicated in an exemplary way as radical generator and aminopropanethiol serves as a model radical quencher. Using aminopropanethiol as a quencher exemplary, the generated amine group could be specifically further modified to a click-able group or being used to directly conjugate the poly(SO1861) to a toxin.

Another approach for the development of a SO1861 scaffold is the DNA approach. The idea of this approach is to utilize the concept of the so-called DNA-origami (Kolb et al, 2004; Bird et al, 1988). DNA-origami as the polymeric or assembled polymeric structure to conjugate saponins to it, can offer several inherent advantages including stability, scalability, and precise control of the final size and shape of the resulting DNA-saponin scaffold. Since these DNA nanocarriers are comprised of natural DNA, they are biocompatible and do not show toxicity to living cells, and can ease the release of cargo from internal cellular compartments. The multivalency of such a structure can further allow fine-tuning targeting capabilities and high capacity for a variety of payloads such as fluorophores and toxins. Thus, in this approach DNA strands are identified that offer chemical functional groups on the 3' and 5' endings respectively, and that are able to hybridize only in certain wanted areas of the sequence that allow a control over the final shape of the construct. The chemical groups should be utilized to couple saponins, for instance though a thiol-ene reaction between the already developed SO1861-EMCH and a thiol group on one of the 3' and 5' DNA strands. The complementary DNA strand can offer a click function group that can be used for coupling to a targeted toxin. The concept is illustrated in Figure 23-1.

A similar approach is imaginable by using a specific peptide sequence instead of DNA strands that is able to bind and release saponins and that can be polymerized forming a large poly(peptide)-like structure. In this approach, a peptide sequence has been identified and purchased that has a length fitting the calculated size of a SO1861-EMCH molecule, that offers a cysteine residue in the middle of the sequence, and that obtains an amine group at both the N-terminus and C-terminus. The cysteine residue can be utilized to conjugate SO1861-EMCH via a thiol-ene reaction of the maleimide group of SO1861-EMCH and the thiol group of the cysteine residue. The two amine groups can be utilized to polymerize the peptide-SO1861 conjugate with a suitable crosslinker.

Example 8

SO1861-BNA oligo conjugation

HSP27 BNA oligo disulfide (1.10 mg, 0.187 µmol) was dissolved in 20 mM NH_4HCO_3 with 1.0 mM TCEP (500 µL) and the mixture was shaken for 1 min and left standing at room temperature. After 1

hour the reaction mixture was filtered by using a centrifugal filter with a molecular weight cut-off of 3000 Da (14000 × g for 30 min). The residue solution was diluted with 20 mM NH_4HCO_3 with 1.0 mM TCEP (500 µL) and the resulting mixture was filtered again under the same conditions described above. The residue solution was diluted with 20 mM NH_4HCO_3/acetonitrile (3:1, v/v, 1.00 mL) and the resulting mixture was added to SO1861-EMCH (3.54 mg, 0.375 µmol). The reaction mixture was shaken for 1 min and left standing at room temperature. After 10 min the reaction mixture was subjected to preparative LC-MS.[4A] Fractions corresponding to the product were immediately pooled together, frozen and lyophilized overnight to give the title compound (1.25 mg, 85%) as a white fluffy solid. Purity based on LC-MS 100%.

LRMS (m/z): 1561 $[M-5]^{5-}$, 1951 $[M-4]^{4-}$

LC-MS r.t. (min): 2.46[6B]

dendron(SO1861)^4-BNA oligo conjugation

HSP27 BNA oligo disulfide (1.1 mg, 0.187 µmol) was dissolved in 20 mM NH_4HCO_3 with 1.0 mM TCEP (500 µL) and the mixture was shaken for 1 min and left standing at room temperature. After 1 hour the reaction mixture was filtered by using a centrifugal filter with a molecular weight cut-off of 3000 Da (14000 × g for 30 min). The residue solution was diluted with 20 mM NH_4HCO_3 with 1.0 mM TCEP (500 µL) and the resulting mixture was filtered again under the same conditions described above. The residue solution was diluted with 20 mM NH_4HCO_3/acetonitrile (3:1, v/v, 1.0 mL) and the resulting mixture was added to dendron(SO1861)4-maleimide1 (3.54 mg, 0.375 µmol). The reaction mixture was shaken for 1 min and left standing at room temperature. After 10 min the reaction mixture was subjected to preparative LC-MS.[4A] Fractions corresponding to the product were immediately pooled together, frozen and lyophilized overnight to give the title compound (1.25 mg, 85%) as a white fluffy solid. Purity based on LC-MS 94%

LRMS (m/z): 1896 $[M-8]^{8-}$, 2167 $[M-7]^{7-}$

LC-MS r.t. (min): 3.77[6B]

Cell culture

Cells were seeded in DMEM (PAN-Biotech GmbH) supplemented with 10% fetal bovine serum (PAN-Biotech GmbH) and 1% penicillin/streptomycin (PAN-Biotech GmbH), in a 96 well plate at 5,000 c/w in 100 µL/well and incubated overnight at 37 °C and 5% CO_2. The next day samples were prepared in DMEM and cells were treated.

Gene silencing

RNA isolation and Qpcr analysis was performed according to standard procedures and protocols.

HSP27 primers: F: R:

HSP27BNA oligo

HSP27BNA(-thiol) oligos (sequence 5'-GGCacagccagtgGCG-3') (Zhang et al., 2011) were ordered at Bio-synthesis Inc. (Lewisville, Texas)

Results

BNAoligo, antisense BNA oligo targeting the mRNA transcript of the cancer target (upregulated in cancer cells), heat shock protein 27 (HSP27BNA) was conjugated to SO1861-EMCH (HSP27BNA-L-SO1861) or dendron(-L-SO1861)^4 (HSP27BNA-dendron(-L-SO1861)^4) and co-administrated to an A431 cancer

cell line, according to the invention. As readout, gene silencing of HSP27 mRNA in A431 cells was determined. This revealed that HSP27BNA-L-SO1861 treatment resulted in an improvement of HSP27 gene silencing activity compared to the HSP27BNA alone, whereas the activity of HSP27BNA-dendron(-L-SO1861)^4 (4 SO1861 molecules/BNA) is even stronger (3-fold) compared to the gene silencing activity of HSP27BNA alone (Figure 1-3). This shows that conjugation of 1 or more SO1861 molecules improves the gene silencing activity of the therapeutic BNA oligo nucleotide due to the enhancement of SO1861-mediated endosomal escape and cytoplasmic delivery of the antisense BNA.

Example 9

SO1861 was conjugated (labile) via cysteine residues (Cys) and dianthin (protein toxin) was conjugated (stable) via lysine residues (Lys) to cetuximab (monoclonal antibody recognizing and binding human EGFR), resulting in the production of: Cetuximab-(Cys-L-SO1861)^{3,9}(Lys-S-dianthin)^2. The conjugate was tested in a A431 (EGFR^{++}) xenograph mouse tumor model for EGFR tumor targeted cell killing as illustrated in Figure 9-4. Dosings started at day 12 when tumors reached ~150mm^3 in size and tumor volume was determined after every dosing. Mice (n=3) were treated (intraperitoneal; i.p.; dose escalation) at day 12: 0.5 mg/kg; day15: 1 mg/kg and day24: 1.5 mg/kg with cetuximab-(Cys-L-SO1861)^{3,9}(Lys-S-dianthin)^2 or cetuximab-(Lys-S-dianthin)^{1,6}. At day 26, compared to the control group, tumor volume reduction could be observed in the tumor bearing mice treated with cetuximab-(Cys-L-SO1861)^{3,9}(Lys-S-dianthin)^2 (Figure 1-4A). This shows that labile conjugation of SO1861 to an antibody-protein toxin (stable) conjugate can enhance the targeted therapeutic efficacy of the tumor targeted antibody-protein toxin, thereby inducing a more effective tumor targeted therapy.

Next, SO1861 was conjugated (labile) via cysteine residues (Cys) and dianthin (protein toxin) was conjugated (labile) via lysine residues (Lys) to cetuximab (monoclonal antibody recognizing and binding human EGFR), resulting in the production of: Cetuximab-(Cys-L-SO1861)^{3,9}(Lys-L-dianthin)^2. The conjugate was tested in a A431 (EGFR^{++}) xenograph mouse tumor model for EGFR tumor targeted cell killing as illustrated in Figure 9-4. Dosings started at day 12 when tumors reached ~150mm^3 in size and tumor volume was determined after every dosing. Mice (n=3) were treated (intraperitoneal; i.p.; dose escalation) at day 12: 0.5 mg/kg; day15: 1 mg/kg, day24: 1.5 mg/kg with cetuximab-(Cys-L-SO1861)^{3,9}(Lys-L-dianthin)^2 or cetuximab-(Lys-L-dianthin)^{1,6}. This revealed that after 35 days compared to the control, tumor bearing mice treated with cetuximab-(Cys-L-SO1861)^{3,9}(Lys-L-dianthin)^2 showed tumor growth inhibition (Figure 1-4B). When mice (n=3; were treated (intravenous, i.v.; dose escalation) day 12: 0.5 mg/kg; day15: 1 mg/kg, day18: 2 mg/kg, day24: 2.5 mg/kg with the cetuximab-(Cys-L-SO1861)^{3,9}(Lys-L-dianthin)^2 according to the invention also tumor growth inhibition could be observed compared to the control (data represents 1 mice, since 2 mice died during the treatments). This shows that labile conjugation of SO1861 to an antibody-protein toxin (labile) conjugate can enhance the targeted therapeutic efficacy of the tumor targeted antibody-protein toxin, thereby inducing a more effective tumor targeted therapy.

Next, SO1861-EMCH was conjugated via cysteine residues (Cys) to cetuximab (monoclonal antibody recognizing and binding human EGFR), with a DAR 3,9 and the antisense HSP27BNA oligo nucleotide (targeting and inducing degradation of the onco-target *hsp27* mRNA (gene silencing) in cancer cells)

via a labile (L) linker to the lysine residues (Lys) of the antibody, with a DAR 1,8 resulting in the production of cetuximab-(Cys-L-SO1861)^{3,9}(Lys-L-HSP27BNA)^{1,8}. Cetuximab-(Cys-L-SO1861)^{3,9}(Lys-L-HSP27BNA)^{1,8} was tested in a A431 xenograph 'nude' mouse tumor model for EGFR-mediated tumor targeted HSP27 gene silencing, according to the invention as illustrated in Figure 10-4. Dosing started at day 12 when tumors reached ~150mm^3 in size and HSP27 mRNA expression was determined. For this, tumor samples were collected at 72h after the first dosing and analysed for HSP27 gene expression levels compared to cellular control mRNA expression levels (reference genes). Tumor bearing mice (n=3) treated (intraperitoneal; i.p.) with 30 mg/kg cetuximab-(Cys-L-SO1861)^{3,9}(Lys-L-HSP27BNA)^{1,8} showed after 1 dosing 40% reduction in HSP27 mRNA expression in the tumors compared to single dosing of cetuximab-(Cys-L-SO1861)^{3,8} or cetuximab-(Lys-L-HSP27BNA)^{1,5} (Figure 2-4). Compared to the tumor of the vehicle control a reduction of 25% HSP27 gene expression was observed. This shows and enables that conjugation of SO1861 and HSP27BNA to the same targeting antibody, according to the invention, efficiently induces SO1861-mediated enhanced cytoplasmic delivery of a therapeutic antisense oligo nucleotide in solid tumors of tumor bearing mice, inducing tumor targeted gene silencing. In another example, a trifunctional linker scaffold was designed and produced with 3 specific chemical end groups for conjugation with SO1861 on one arm and the HSP27BNA on the other arm to produce SO1861-L-trifunctional linker-L-HSP27BNA. Next, SO1861-L-trifunctional linker-L-HSP27BNA was conjugated with its third arm to cysteine residues (Cys) of the anti-EGFR antibody, cetuximab (cetuximab-Cys-(SO1861-L-trifunctional linker-L-HSP27BNA)^{3,7}) and tested in a A431 xenograph 'nude' mouse tumor model for EGFR-mediated tumor targeted gene silencing activity, according to the invention as illustrated in Figure 11-4. Dosings started at day 12 when tumors reached ~150mm^3 in size and HSP27 mRNA expression was determined. For this, tumor samples were collected at 72h after the first dosing and analysed for HSP27 gene expression levels compared to cellular control mRNA expression levels (reference genes). This revealed that 1 dosing of 30 mg/kg cetuximab-Cys-(SO1861-L-trifunctional linker-L-HSP27BNA)^{3,7} resulted in a 40% reduction in HSP27 gene expression in the tumors compared to single dosing of 25 mg/kg cetuximab-(Cys-L-SO1861)^{3,8} or 25 mg/kg cetuximab-(Lys-L-HSP27BNA)^4 mono therapies (Figure 3-4). Compared to the vehicle control tumors, a reduction of 25% HSP27 gene expression was observed in tumor bearing mice treated with 1 dosing of cetuximab-Cys-(SO1861-L-trifunctional linker-L-HSP27BNA)^{3,7}. This shows and enables that cetuximab-Cys-(SO1861-L-trifunctional linker-L-HSP27BNA)^{3,7} efficiently induces SO1861-mediated enhanced cytoplasmic delivery of a therapeutic antisense oligo nucleotide in a solid tumor of tumor bearing mice, inducing targeted gene silencing, *in vivo*.

Example 10

In another example according to the invention, SO1861 (labile) and the protein toxin, dianthin (labile or stable) were conjugated to the HER2 targeting antibody, trastuzumab. Trastuzumab-(Cys-L-SO1861)^{3,8}(Lys-L-dianthin)^{1,7} or trastuzumab-(Cys-L-SO1861)^{3,8}(Lys-S-dianthin)^{1,7}, were produced and tested for enhanced cell killing in SK-BR-3 (HER2^{++}) and MDA-MB-468 (HER2$^-$) cells as illustrated in Figure 9-4. Both, trastuzumab-(Cys-L-SO1861)^{3,8}(Lys-L-dianthin)^{1,7} (IC50= 0,8 nM) and trastuzumab-(Cys-L-SO1861)^{3,8}(Lys-S-dianthin)^{1,7} (IC50= 0,8 nM) efficiently induces cell killing of SK-BR-3 cells

(HER2^{++}) (Figure 4-4A). This was not observed in SK-BR-3 cells treated with trastuzumab, trastuzumab-(Lys-L-dianthin)^{1,7}, trastuzumab-(Lys-S-dianthin)^{1,7} or trastuzumab-(L-SO1861)^{3,8} alone (Figure 4-4A). In MDA-MB-468 cells (HER2^{-}) no cell killing activity can be observed for any of the conjugates, according to the invention (Figure 4-4B). This shows that conjugation of SO1861 to an HER targeting antibody-protein toxin conjugate, efficiently induces SO1861-mediated enhanced cytoplasmic delivery of the protein toxin in the target cell resulting in target cell death.

In another example according to the invention, SO1861 (labile) and the protein toxin, dianthin (labile or stable) were conjugated to the EGFR targeting antibody, cetuximab. Cetuximab-(Cys-L-SO1861)^{3,9}(Lys-L-dianthin)^{2} or cetuximab-(Cys-L-SO1861)^{3,9}(Lys-S-dianthin)^{2}, was tested for enhanced cell killing in A431 cells (EGFR^{++}) and A2058 cells (EGFR^{-}) as illustrated in Figure 9-4. Both, cetuximab-(Cys-L-SO1861)^{3,9}(Lys-L-dianthin)^{2} (IC50= 0,3 nM) and cetuximab-(Cys-L-SO1861)^{3,8}(Lys-S-dianthin)^{1,7} (IC50= 0,3 nM) showed enhanced cell killing in A431 cells (EGFR^{++}) compared to cetuximab-(Lys-L-dianthin)^{1,6} (IC50= 2pM), cetuximab-(Lys-S-dianthin)^{1,6} (IC5= 2pM) alone (Figure 4-4C). In A2058 cells (EGFR^{-}) the combination according to the invention did not show any cell killing activity (IC50> 200nM; Figure 4-4D). This shows that conjugation of SO1861 to an EGFR targeting antibody-protein toxin conjugate, efficiently enhances SO1861-mediated cytoplasmic delivery of the protein toxin in the target cell resulting in enhanced target cell death.

Example 11

In another example according to the invention, SO1861 (labile) and the HSP27BNA oligo (labile) were conjugated to the EGFR targeting antibody, cetuximab. Cetuximab-(Cys-L-SO1861)^{3,8}(Lys-L-HSP27BNA)^{3,8} was tested for enhanced HSP27 gene silencing in A431 cells (EGFR^{++}) and A2058 (EGFR^{-}) cells, according to the invention as illustrated in Figure 10-4. Cetuximab-(Cys-L-SO1861)^{3,8}(Lys-L-HSP27BNA)^{3,8} efficiently induces HSP27 gene silencing in A431 cells (IC50= 3nM) compared to cetuximab, cetuximab-(Lys-L-HSP27BNA)^{3,9} or cetuximab-(Cys-L-SO1861)^{3,8} alone (Figure 5-4A). In A2058 cells (EGFR^{-}) no gene silencing activity can be observed with cetuximab-(Cys-L-SO1861)^{3,8}(Lys-L-HSP27BNA)^{3,8} (IC50> 100nM; Figure 5-4B). This shows and enables that conjugation of SO1861 and HSP27BNA to the same targeting antibody, according to the invention, efficiently induces SO1861-mediated enhanced cytoplasmic delivery of a therapeutic antisense oligo nucleotide in the target cells, inducing targeted gene silencing.

In another example according to the invention, SO1861 (labile) and the HSP27BNA oligo (labile) were conjugated to the HER2 targeting antibody, trastuzumab. Trastuzumab-(Cys-L-SO1861)^{3,8}(Lys-L-HSP27BNA)^{3,5} was tested for enhanced HSP27 gene silencing in SK-BR-3 cells (HER2^{++}) cells, according to the invention as illustrated in Figure 10-4. Trastuzumab-(Cys-L-SO1861)^{3,8}(Lys-L-HSP27BNA)^{3,5} efficiently induces HSP27 gene silencing in SK-BR-3 cells (IC50= 9 nM) compared to trastuzumab-(Lys-L-HSP27BNA)^{4,4} alone (Figure 6-4). This shows and enables that conjugation of SO1861 and HSP27BNA to an HER2 targeting antibody, according to the invention, efficiently induces SO1861-mediated enhanced cytoplasmic delivery of a therapeutic antisense oligo nucleotide in the target cells, inducing targeted gene silencing.

In another example, cetuximab-Cys-(SO1861-L-trifunctional linker-L-HSP27BNA)^{3,7} was tested for enhanced HSP27 gene silencing in A431 (EGFR^{++}) and A2058 (EGFR^{-}) cells according to the invention as illustrated in Figure 11-4. Cetuximab-Cys-(SO1861-L-trifunctional linker-L-HSP27BNA)^{3,7} efficiently induces HSP27 gene silencing in A431 cells (IC50= 2nM) compared to Cetuximab-(Lys-L-HSP27BNA)^{4} or Cetuximab-(Cys-L-SO1861)^{3,7} alone (Figure 7-4A). In A2058 cells (EGFR^{-}) gene silencing activity was only observed at high (> 80nM) concentrations of Cetuximab-Cys-(SO1861-L-trifunctional linker-L-HSP27BNA)^{3,7} (IC50=100nM; Figure 7-4B). This shows and enables that in high EGFR expressing cells cetuximab-Cys-(SO1861-L-trifunctional linker-L-HSP27BNA)^{3,7} efficiently induces SO1861-mediated enhanced cytoplasmic delivery of a therapeutic antisense oligo nucleotide in the target cells, inducing targeted gene silencing.

Example 12

Figure 8-4A-D displays the relative cell viability when trastuzumab (Figure 8-4A), cetuximab (Figure 8-4B) or T-DM1 (Figure 8-4C), unconjugated protein toxins, saporin, dianthin and saporin conjugated to a (non-cell binding) IgG antibody (Figure 8-4D) are administered to various cancer cell lines SK-BR-3, JIMT-1, MDA-MB-468, A431, CaSki, HeLa, A2058.

Trastuzumab and cetuximab do not or hardly influence cell viability when exposed to most of the cell lines, with some effect on cell growth inhibition via blocking the function of the HER2 growth factor receptor when trastuzumab is exposed to SK-BR-3 cells at relatively high dose and with some effect on cell growth inhibition via blocking the function of the EGFR growth factor receptor when cetuximab is exposed to MDA-MB-468 cells at relatively high dose.

TDM-1, or ado-trastuzumab emtansine, is a targeted therapy approved by the U.S. Food and Drug Administration to treat: HER2-positive metastatic breast cancer that has previously been treated with Herceptin (chemical name: trastuzumab) and taxane chemotherapy; early-stage HER2-positive breast cancer after surgery if residual disease was found after neoadjuvant (before surgery) treatment with Herceptin and taxane chemotherapy. The TDM-1 is a combination of Herceptin (Trastuzumab) and the chemotherapy medicine emtansine. Figure 8-4C shows that the TDM-1 results in decreased cell viability for all cell lines tested at >1000 pM concentrations

The free toxins saporin and dianthin and the toxin saporin coupled to a control IgG with no affinity for any of the cell surface molecules on the cell lines tested, do not or hardly have any influence on cell viability over a wide range of concentrations toxin tested, up to 100.000 pM (Figure 8-4D).

Example 13 (Example 1 invention 5)

The 1 target 2-components system (1T2C) is the combination treatment of mAb1-protein toxin and mAb1-SO1861, as illustrated in Figure 13-5. SO1861-EMCH was conjugated via cysteine residues (Cys) and HSP27BNA oligo was conjugated via lysine residues to cetuximab (monoclonal antibody recognizing and binding human EGFR), both with a DAR 4 resulting in the production of 2 conjugates: cetuximab-(Cys-L-SO1861)^{4} and cetuximab-(Lys-L-HSP27BNA)^{4}. The combination of cetuximab-(Cys-L-SO1861)^{4} (intraperitoneal administration, (i.p.)) and cetuximab-(Lys-L-HSP27BNA)^{4} (intravenous administration, (i.v.)) was tested in a A431 xenograph 'mouse tumor model for EGFR tumor targeted

gene silencing activity. Dosings started at day 12 when tumors reached ~150mm^3 in size and tumor samples were collected at 72h after the first dosing and analysed for HSP27 gene expression compared to control gene mRNA expression levels (reference genes). This revealed that 1 dosing of 50 mg/kg cetuximab-(Cys-L-SO1861)^4 + 25 mg/kg cetuximab-(Lys-L-HSP27BNA)^4 resulted in a 50% reduction in HSP27 gene expression in the A431 tumors compared to single dosing of cetuximab-(Cys-L-SO1861)^4 or cetuximab-(Lys-L-HSP27BNA)^4 mono therapies (Figure 1-5). Compared to the vehicle control tumors, a reduction of 40% HSP27 gene silencing was observed. This shows and enables that the combination of cetuximab-conjugated SO1861 + cetuximab-conjugated HSP27BNA oligo, according to the 1T2C invention, induces efficient targeted delivery of a therapeutic antisense oligo nucleotide in the cytoplasm of solid tumor cells, thereby inducing tumor targeted gene silencing, *in vivo*.

Next, SO1861-EMCH was conjugated via cysteine residues (Cys) to trastuzumab (monoclonal antibody recognizing and binding human HER2), with a DAR 4 resulting in the production of trastuzumab-(Cys-L-SO1861)^4. The combination of trastuzumab-(Cys-L-SO1861)^4 and trastuzumab-saporin (trastuzumab protein toxin conjugate) was tested in a mouse tumor model (patient derived xenograph tumor model, PDX) with high HER2 expression levels and resistant for trastuzumab mono therapy. The combination, according to the 1T2C invention of 40 mg/kg trastuzumab-(Cys-L-SO1861)^4 (intraperitoneal administration, (i.p.)) + 0.03 (Day1, 8)/ 0.02 (Day 15, 22, 30, 36,43) mg/kg trastuzumab-saporin (intravenous administration, (i.v.)) revealed strong tumor growth inhibition compared to the vehicle control and the 40 mg/kg trastuzumab-(Cys-L-SO1861)^4 or 0.03/0.02 mg/kg trastuzumab-saporin mono therapies (Figure 2-5). Besides, in tumor bearing mice that were treated with a lower dosing combination (40 mg/kg trastuzumab-(Cys-L-SO1861)^4 + 0.01 mg/kg trastuzumab-saporin) no tumor growth inhibiting activity was observed (Figure 2-5). This shows and enables that the 1T2C combination of trastuzumab conjugated SO1861 + trastuzumab conjugated protein toxin induces efficient targeted delivery of a therapeutic protein toxin in the cytoplasm of solid tumor cells, thereby inducing tumor cell death and tumor growth inhibition, *in vivo*.

Example 14

The 1 target 2-components system (1T2C) is the combination treatment of mAb1-SO1861 and mAb1-protein toxin (Figure 13-5)

SO1861-EMCH was conjugated via cysteine residues (Cys) to cetuximab (monoclonal antibody recognizing and binding human EGFR), with a DAR 3,7 (cetuximab-(Cys-L-SO1861)^{3,7}). Cetuximab-(Cys-L-SO1861)^{3,7} was titrated on a fixed concentration of 10 pM cetuximab-saporin (cetuximab, conjugated to the protein toxin, saporin) and targeted protein toxin mediated cell killing on EGFR expressing cells (A431, EGFR^{++}; CaSKi, EGFR^{+}) was determined. This revealed strong cell killing at low concentrations of cetuximab-(Cys-L-SO1861)^{3,7} (A431: IC50= 0,6 nM and Caski IC50= 1 nM; Figure 5A, 3-5B) whereas cetuximab, cetuximab-(Cys-L-SO1861)^{3,7} or cetuximab + 10 pM cetuximab-saporin could not induce any cell killing activity in EGFR expressing cells. This shows that cetuximab conjugated SO1861 efficiently enhances endosomal escape of the cetuximab conjugated protein toxin (at non-effective concentrations), thereby inducing cell killing of EGFR expressing cells. The cell killing activity in A431 is more effective compared to CaSki correlating with EGFR expression levels in these cell lines.

EGFR receptor binding competition between both conjugates within the 1T2C is also observed when cetuximab-(Cys-L-SO1861)^{3,7} concentrations increase, cell killing activity declines due to outcompeting receptor binding and internalization of cetuximab-saporin (Figure 3-5A, 3-5B).

Next, cetuximab-saporin was titrated on a fixed concentration of 75 nM cetuximab-(Cys-L-SO1861)^{3,7} and targeted protein toxin mediated cell killing on EGFR expressing cells was determined. This revealed that 75nM cetuximab-(Cys-L-SO1861)^{3,7} in combination with low concentrations cetuximab-saporin induced already efficient cell killing in EGFR expressing cells (A431: IC50= 0.4 pM; and CaSKi: (IC50= 2 pM; Figure 3-5C and 3-5D), whereas cetuximab-saporin alone or cetuximab-saporin + 75 nM cetuximab showed cell killing only at high concentrations cetuximab-saporin (IC50= 40 pM, IC50= 1000 pM, resp.) in both cell lines (Figure 3-5C, 3-5D). All this shows that relatively low concentrations of cetuximab-saporin can be effective and induce cell killing only in combination with low cetuximab-SO1861 concentrations in high EGFR expressing cells. The receptor competition between both conjugates within the 1T2C system is also observed in the cetuximab-toxin titration treatments when the cell killing activity of cetuximab-saporin with and without 75 nM cetuximab was compared (Figure 3-5C, 3-5D).

Next, cetuximab-(Cys-L-SO1861)^{3,7} was titrated on a fixed concentration of 10 pM cetuximab-saporin and targeted protein toxin mediated cell killing on low EGFR expressing cells or cells without EGFR expression (HeLa, EGFR$^{+/-}$; A2058, EGFR^{-}) was determined. Cells with low (HeLa) or no (A2058) EGFR expression were not sensitive at all for any combination of cetuximab-(Cys-L-SO1861)^{3,7} + 10 pM cetuximab-saporin (HeLa: IC50> 1000 nM; A2058: IC50> 1000 nM; Figure 4-5A, 4-5B). This shows that in the absence of sufficient EGFR receptor expression, effective intracellular delivered SO1861 concentrations are not optimal (threshold) to induce endosomal protein toxin escape and toxin-mediated cell killing. Next, cetuximab-saporin was titrated on a fixed concentration of 75 nM cetuximab-(Cys-L-SO1861)^{3,7} and targeted protein toxin mediated cell killing on low (HeLa) or no (A2058) EGFR expressing cells was determined. Low EGFR expressing cells (HeLa) showed cell killing only at high cetuximab-saporin concentrations in combination with 75 nM cetuximab-(Cys-L-SO1861)^{3,7} (HeLa: IC50= 60 pM), Figure 4-5C), whereas A2058 cells (EGFR^{-}) are not sensitive at any of the tested concentrations (A2058: IC50> 10.000 pM; Figure 4-5D). All this shows that cells with low or no EGFR receptor expression are not susceptible for the combination of cetuximab-(Cys-L-SO1861)^{3,7} + cetuximab-saporin, due to a lack of sufficient EGFR receptor that facilitates the antibody-mediated delivery of sufficient SO1861 within the endolysosomal compartments, to facilitate the escape of the protein toxin.

Next, SO1861-EMCH was conjugated via cysteine residues (Cys) to trastuzumab (monoclonal antibody recognizing and binding human HER2, with a DAR 4, (trastuzumab-(Cys-L-SO1861)^{4}). Trastuzumab-(Cys-L-SO1861)^{4} was titrated on a fixed concentration of 50 pM trastuzumab-saporin (trastuzumab, conjugated to the protein toxin, saporin) and targeted protein toxin mediated cell killing on HER2 expressing cells (SK-BR-3, HER2^{++}) was determined. This revealed strong cell killing at low concentrations of trastuzumab-(Cys-L-SO1861)^{4} (SK-BR-3: IC50= 0,8 nM; Figure 5-5A) whereas equivalent concentrations trastuzumab, trastuzumab-(Cys-L-SO1861)^{4} or trastuzumab + 50 pM trastuzumab-saporin could not induce any cell killing activity in HER2 expressing cells. This shows that

trastuzumab conjugated SO1861 efficiently enhances endosomal escape of the trastuzumab conjugated protein toxin (at non-effective concentrations), thereby inducing cell killing of HER2 expressing cells. The receptor competition between both conjugates within the 1T2C is also observed when trastuzumab-(Cys-L-SO1861)^4 concentrations increase, cell killing activity declines due to outcompeting receptor binding and internalization of trastuzumab-saporin (Figure 5-5A).

Next, trastuzumab-saporin was titrated on a fixed concentration of 2,5 nM trastuzumab-(Cys-L-SO1861)^4, according to the invention and targeted protein toxin mediated cell killing on HER2 expressing cells was determined. This revealed that 2,5 nM trastuzumab-(Cys-L-SO1861)^4 in combination with low concentrations trastuzumab-saporin induced already efficient cell killing in HER2 expressing cells (SK-BR-3: IC50= 2 pM; Figure 5-5B), whereas trastuzumab-saporin alone or trastuzumab-saporin + 2,5 nM trastuzumab showed cell killing only at high concentrations trastuzumab-saporin (Figure 5-5B). All this shows that relatively low concentrations of trastuzumab-saporin can be effective and induce cell killing only in combination with low trastuzumab-(Cys-L-SO1861)^4 concentrations in high HER2 expressing cells.

Next, trastuzumab-(Cys-L-SO1861)^4 was titrated on a fixed concentration of 50 pM trastuzumab-saporin, according to the invention and targeted protein toxin mediated cell killing on low HER2 expressing cells (A431 HER2$^{+/-}$) or cells without HER2 expression (JIMT-1: HER2$^+$; MDA-MB-468: HER2$^-$) was determined. Cells with low or no HER2 expression were not sensitive at all for any combination of trastuzumab-(Cys-L-SO1861)^4 + 50 pM trastuzumab-saporin (JIMT-1: IC50> 1000 nM; MDA-MB-468: IC50> 1000 nM; Figure 6-5A, 6-5B). This shows that in the absence of sufficient HER2 receptor expression, effective intracellular delivered SO1861 concentrations are not optimal (threshold) to induce endosomal protein toxin escape and toxin-mediated cell killing. Next, trastuzumab-saporin was titrated on a fixed concentration of 2,5 nM trastuzumab-(Cys-L-SO1861)^4 and targeted protein toxin mediated cell killing on low or no HER2 expressing cells was determined. Low HER2 expressing cells (JIMT-1) showed cell killing only at high trastuzumab-saporin concentrations in combination with 2,5 nM trastuzumab-(Cys-L-SO1861)^4 (JIMT-1: IC50> 10.000 pM; Figure 6-5C), whereas MDA-MB-468 cells (HER2$^-$) are not sensitive at any of the tested concentrations (MDA-MB-468: IC50> 10.000 pM; Figure 6-5D).

All this shows that cells with low or no HER2 receptor expression are not susceptible for the combination of trastuzumab-(Cys-L-SO1861)^{3,7} + trastuzumab-saporin, due to a lack of sufficient HER2 receptor that facilitates the antibody-mediated delivery of sufficient SO1861 within the endolysosomal compartments, to facilitate the escape of the protein toxin.

Example 15

In order to show that the activity of the 1T2C system is driven by the acidification of the endolysosomal compartments, the 1T2C system , according to the invention was tested in combination with an endosomal acidification inhibitor, chloroquine. Trastuzumab-saporin was titrated in combination with 5 nM trastuzumab-(Cys-L-SO1861)^4 in combination with or without chloroquine. Trastuzumab-saporin + 5 nM trastuzumab-(Cys-L-SO1861)^4 showed a strong cell killing activity in high HER2 expressing cells (SK-BR-3, HER2^{++}; IC50= 0.2 pM;), however, trastuzumab-saporin + 5 nM trastuzumab-(Cys-L-

SO1861)^4 + 0.5 µM chloroquine resulted in strong inhibition of the 1T2C cell killing activity in SK-BR-3 (HER2^{++}) cells (IC50= 40pM). This shows that activity of the antibody conjugated SO1861 is reduced/blocked when acidification of endoslysomes is prohibited (Figure 7-5A). Same results were derived with the 1T2C combination, according to the invention of cetuximab-saporin + 5 nM cetuximab-(Cys-L-SO1861)^{3,8} (IC50= 1 pM) compared with cetuximab-saporin + 5 nM cetuximab-(Cys-L-SO1861)^{3,8} + 0.5 µM chloroquine (IC50= 200 pM) in EGFR expressing cells (A431, EGFR^{++}; Figure 7-5B).

Example 16

The 1 target 2-components system (1T2C) can also be the combination treatment of mAb1-SO1861 and mAb1-antisense BNA oligo nucleotide as illustrated in Figure 14-5. For this we used an antisense BNA oligonucleotide against the mRNA of a cancer specific target gene (upregulated in cancer cells), heat shock protein 27 (HSP27). Upon release into the cytoplasm the antisense BNA recognizes and binds the mRNA encoding for HSP27, targeting the mRNA for destruction thereby depleting the HSP27 mRNA expression within the cancer cell. HSP27BNA was conjugated to cetuximab with a DAR4 (Cetuximab-(Lys-L-HSP27BNA)^4) and tested in combination with cetuximab-(Cys-L-SO1861)^{3,8} for enhanced HSP27 gene silencing activity in EGFR expressing cells (A431, EGFR^{++}) and non-expressing cells (A2058, EGFR$^-$), according to the invention (Figure 14-5). Cetuximab-(Cys-L-SO1861)^{3,8} + 100 nM cetuximab-(Lys-L-HSP27BNA)^4 showed strong HSP27 gene silencing in EGFR expressing cells (A431: IC50= nM, Figure 8-5A), whereas cetuximab-(Cys-L-SO1861)^{3,8} alone did not show any gene silencing activity. In A2058 cells (EGFR$^-$) no gene silencing activity was observed in the 1T2C combination (Figure 8-5B). Next, cetuximab-(Lys-L-HSP27BNA)^4 + 76.9 nM Cetuximab-(Cys-L-SO1861)^{3,8} show strong HSP27 gene silencing activity in EGFR expressing cells (A431: IC50= 4 nM, Figure 8-5C), whereas cetuximab-(Lys-L-HSP27BNA)^4 or cetuximab-(Cys-L-SO1861)^{3,8} or the combination of Cetuximab-(Lys-L-HSP27BNA)^4 + 77 nM cetuximab did not reveal any significant gene silencing activity (IC50> 100nM). When the experiment was performed in EGFR non-expressing cells (A2058) no gene silencing activity was observed in the 1T2C combination (IC50>100 nM; Figure 8-5D). All this shows that the 1T2C system efficiently delivers an antisense BNA oligo to the cytoplasm of high EGFR expressing cells, thereby inducing mRNA degradation of the BNA target mRNA resulting in target gene silencing.

Example 17

The 1 target 2-components system (1T2C) can also be the combination treatment of mAb1-(scaffold(-SO1861)^n)^n and mAb1-protein toxin as illustrated in Figure 15-5. Dendron(-L-SO1861)^4 was conjugated to cetuximab via cysteine residues (Cys) conjugation with a DAR3,9 and cetuximab-Cys-(dendron(-L-SO1861)^4)^{3,9} was tested for enhanced cell killing activity in combination with an anti-EGFR antibody-protein toxin conjugate (cetuximab-saporin) in EGFR expressing cells (MDA-MB-468). Cetuximab-Cys-(dendron(-L-SO1861)^4)^{3,9} + 10 pM cetuximab-saporin efficiently induces toxin-mediated cell killing in high EGFR expressing cells (IC50= 0.4 nM; Figure 9-5A), whereas this was not induced by cetuximab-Cys-(dendron(-L-SO1861)^4)^{3,9} or cetuximab + 10 pM cetuximab-saporin or cetuximab (Figure 9-5A). This shows that according to the 1T2C invention, cetuximab conjugated dendron(-L-SO1861)^4 efficiently

enhances endosomal escape of the cetuximab conjugated protein toxin (at non-effective concentrations), thereby inducing cell killing of high HER2 expressing cells.Similar 1T2C experiments were performed in cells that express low levels of EGFR (HeLa, $EGFR^{+/-}$) and this revealed no cell killing activity when the 1T2C combination, according ot the invention was used (IC50> 100pM; Figure 9-5B) indicating that in the absence of sufficient EGFR receptor expression, effective intracellular SO1861 concentrations are not optimal (threshold) to induce cytoplasmic delivery of the protein toxin that results in toxin-mediated cell killing.

Next, dendron(-L-SO1861)^4 was conjugated to the anti-HER2 antibody, trastuzumab via cysteine conjugation (Cys) with a DAR4, trastuzumab-Cys-(dendron(-L-SO1861)^4)^4 and tested for enhanced cell killing activity in combination with an anti-HER2 antibody-protein toxin conjugate (trastuzumab-saporin) in HER2 expressing cells (SK-BR-3, $HER2^{++}$). Trastuzumab-Cys-(dendron(-L-SO1861)^4)^4 + 50 pM trastuzumab-saporin efficiently induces toxin-mediated cell killing (IC50= 2 nM, Figure 9-5C), whereas this was not induced by trastuzumab-Cys-(dendron(-L-SO1861)^4)^4 or trastuzumab + 50nM trastuzumab-saporin or trastuzumab (Figure 9-5C). This shows that trastuzumab conjugated dendron(-L-SO1861)^4 efficiently enhances endosomal escape of the trastuzumab conjugated protein toxin (at non-effective concentrations), thereby inducing cell killing of high HER2 expressing cells. Similar experiments in cells that express low levels of HER2 (JIMT-1, $HER2^{+/-}$) revealed no activity of Trastuzumab-Cys-(dendron(-L-SO1861)^4)^4 + 50 pM trastuzumab-saporin (IC50> 200 nM; Figure 9-5D) indicating that in the absence of sufficient HER2 receptor expression, effective intracellular SO1861 concentrations are not optimal (threshold) to induce endosomal protein toxin escape and toxin-mediated cell killing.

Example 18

The clinical approved ADC, trastuzuzmab-emtansine (T-DM1) is a conjugate of the anti-Her2 antibody, trastuzumab and the small molecule toxin emtansine (DAR3.5). T-DM1 was titrated in combination with trastuzumab-(Cys-L-SO1861)^4 and compared with the antibody protein toxin conjugate, trastuzumab-saporin + Trastuzumab-(Cys-L-SO1861)^4, according to the invention. Whereas trastuzumab-saporin + 2.5 nM trastuzumab-(Cys-L-SO1861)^4 showed enhanced activity compared to Trastuzumab-saporin + 2.5 nM trastuzumab or trastuzumab-saporin alone (IC50= 2 pM, Figure 10-5), T-DM1 + 25.6 nM trastuzumab-(Cys-L-SO1861)^4 showed no enhanced cell killing activity (IC50> 100 pM; Figure 10-5). This shows that the 1T2C system, according to the invention, cannot enhance the delivery of an antibody small molecule conjugate, since small molecules can already passively cross (endolysosomal) membranes.

Example 19

Figure 11-5A-D displays the relative cell viability when trastuzumab (Figure 11-5A), cetuximab (Figure 11-5B) or T-DM1 (Figure 11-5C), unconjugated protein toxins, saporin, dianthin and saporin conjugated to a (non-cell binding) IgG antibody (Figure 11-5D) are administrated to various cancer cell lines SK-BR-3, JIMT-1, MDA-MB-468, A431, CaSki, HeLa, A2058.

Trastuzumab and cetuximab do not or hardly influence cell viability when exposed to most of the cell lines, with some effect on cell growth inhibition via blocking the function of the HER2 growth

factor receptor when trastuzumab is exposed to SK-BR-3 cells at relatively high dose and with some effect on cell growth inhibition via blocking the function of the EGFR growth factor receptor when cetuximab is exposed to MDA-MB-468 cells at relatively high dose.

TDM-1, or ado-trastuzumab emtansine, is a targeted therapy approved by the U.S. Food and Drug Administration to treat: HER2-positive metastatic breast cancer that has previously been treated with Herceptin (chemical name: trastuzumab) and taxane chemotherapy; early-stage HER2-positive breast cancer after surgery if residual disease was found after neoadjuvant (before surgery) treatment with Herceptin and taxane chemotherapy. The TDM-1 is a combination of Herceptin (Trastuzumab) and the chemotherapy medicine emtansine. Figure 11-5C shows that the TDM-1 results in decreased cell viability for all cell lines tested at >1000 pM concentrations

The free toxins saporin and dianthin and the toxin saporin coupled to a control IgG with no affinity for any of the cell surface molecules on the cell lines tested, do not or hardly have any influence on cell viability over a wide range of concentrations toxin tested, up to 100.000 pM (Figure 11-5D).

Example 20

The 1 target 2-components system (1T2C) can also be the combination treatment of mAb1-QSmix (mixture of saponins from Quillaja Saponaria) and mAb1-protein toxin.

QSmix-EMCH was conjugated via cysteine residues (Cys) to cetuximab (monoclonal antibody recognizing and binding human EGFR), with a DAR 4.1 (cetuximab-(Cys-L-QSmix)$^{4.1}$). Cetuximab-(Cys-L-QSmix)$^{4.1}$ was titrated on a fixed concentration of 10 pM cetuximab-saporin or 10pM cetuximab-dianthin and targeted protein toxin mediated cell killing on A431 (EGFR^{++}), CaSKi (EGFR^{+}) and A2058 (EGFR^{-}) cells was determined. This revealed strong cell killing at low concentrations of cetuximab-(Cys-L-QSmix)$^{4.1}$ + 10 pM cetuximab-saporin or 10pM cetuximab-dianthin in A431 (EGFR^{++}) and CaSKi (EGFR^{+}) cells (A431: IC50= 3 nM, Figure 12-5A; CaSKi: IC50= 1nM, Figure 12-5B) whereas all control treatments could not induce any cell killing in EGFR expressing cells. In cells that do not express EGFR (A2058; EGFR^{-}) no HSP27 gene silencing is observed with the combination, according to the invention (IC50> 1000 nM; Figure 12-5C). This shows that cetuximab conjugated QS21mix efficiently enhances endosomal escape of the cetuximab conjugated protein toxin (at non-effective concentrations), thereby inducing cell killing only in EGFR expressing cells.

Example 21 *2 target 2-component system (in vivo)*

The 2 target 2-components system (2T2C) is the combination treatment of mAb1-SO1861 and mAb2-protein toxin, (Figure 15-6; 16-6; 17-6). SO1861-EMCH was conjugated via cysteine residues (Cys) to cetuximab (monoclonal antibody recognizing and binding human EGFR), with a DAR 4 resulting in the production of: cetuximab-(Cys-L-SO1861)^{4}. The combination of cetuximab-(Cys-L-SO1861)^{4} and trastuzumab-saporin or CD71mab-saporin was tested in a A431 (EGFR^{++}/HER2$^{+/-}$/CD71^{+}) xenograph 'nude' mouse tumor model for EGFR tumor targeted cell killing as illustrated in Figure 1-6 and Figure 2-6. Dose escalation was performed to determine the therapeutic efficacy *(Day 9: 0.3 mg/kg trastuzumab-saporin or 0.1 mg/kg CD71mab-saporin + 5 mg/kg cetuximab-(Cys-L-SO1861)^{4}; Day 14, 18: 0.1 mg/kg trastuzumab-saporin or 0.05 mg/kg CD71mab-saporin + 5 mg/kg cetuximab-(Cys-L-SO1861)^{4}; Day 21:*

0.05 mg/kg trastuzumab-saporin or 0.05 mg/kg CD71mab-saporin + 15 mg/kg cetuximab-(Cys-L-SO1861)^4; Day 28: 0.02 mg/kg trastuzumab-saporin or 0.02 mg/kg CD71mab-saporin + 15 mg/kg cetuximab-(Cys-L-SO1861)^4 trastuzumab-saporin/cetuximab-SO1861. Controls were on the same dosing scheme respectively, only cetuximab (i.v.) was given 25 mg/kg every treatment day). At day 32 (dashed line), 35 and 39 we started the combination, according to the 2T2C invention of 25 mg/kg cetuximab-(Cys-L-SO1861)^4 (intraperitoneal injection (i.p.) + 0.02 mg/kg trastuzumab-saporin or 0.02 CD71mab-saporin (intravenous administration, (i.v.)) and this revealed strong tumor regression for both 2T2C combination groups compared to the vehicle control, 25 mg/kg cetuximab-(Cys-L-SO1861)^4 or 0.02 mg/kg trastuzumab-saporin/CD71mab-saporin mono therapies (Figure 1-6, 2-6). The 2T2C system even outcompetes cetuximab, the clinically used monoclonal antibody against EGFR. Next we performed the same experiment but then we started with 25 mg/kg cetuximab-(Cys-L-SO1861)^4 (intraperitoneal injection (i.p.) + 0.03 mg/kg trastuzumab-saporin or 0.03 CD71mab-saporin (intravenous administration, (i.v.)) treatment with a dosing at day 9 and 14 and thereafter 1 dosing per week. The 2T2C system according to the invention showed tumor regression in all mice and even in 1 mice in both 2T2C groups, complete tumor eradication (tumor volume= 0 mm^3) (Figure 2-6). Also here the controls showed a strong increased in tumor volume whereas the positive control for this A431 mice model, cetuximab showed only tumor growth inhibition, but no regression (Figure 2-6). This shows and enables the 2T2C system approach, according to the invention, of cetuximab conjugated SO1861 + trastuzumab conjugated protein toxin or CD71mab conjugated protein toxin inducing highly efficient targeted delivery of a therapeutic protein toxin in the cytoplasm of solid tumors of tumor bearing mice, *in vivo*, thereby inducing even full tumor eradication in some mice and strong tumor regression in others even in large size tumors (2000 mm^3).

Example 22 *2 target 2-component system (in vitro)*

Results

The 2 target 2-components system (2T2C) is the combination treatment of mAb1-SO1861 and mAb2-protein toxin, (see also Figure 1-6, 2-6, 15-6, 16-6, 17-6). SO1861-EMCH was conjugated via cysteine residues (Cys) to cetuximab (monoclonal antibody recognizing and binding human EGFR), with a DAR 3,7 (cetuximab-(Cys-L-SO1861)^{3,7}). Cetuximab-(Cys-L-SO1861)^{3,7} was titrated on a fixed concentration of 50 pM trastuzumab-saporin (trastuzumab, conjugated to the protein toxin, saporin) and targeted protein toxin mediated cell killing on EGFR/HER2 expressing cells (A431, EGFR^{++}/HER2$^{+/-}$; CaSKi, EGFR^{+}/HER2$^{+/-}$) was determined as illustrated in Figure 3-6. This revealed strong cell killing at low concentrations of cetuximab-(Cys-L-SO1861)^{3,7} (A431: IC50= 3 nM and CaSKi IC50= 10 nM; Figure 3-6A, 3-6B) whereas equivalent concentrations cetuximab, cetuximab-(Cys-L-SO1861)^{3,7} or cetuximab + 50 pM trastuzumab -saporin could not induce any cell killing activity in EGFR/HER2 expressing cells. This shows that relatively low concentrations of cetuximab-SO1861 conjugate efficiently enhances endosomal escape of the trastuzumab conjugated protein toxin (at non-effective concentrations), thereby inducing efficient cell killing of high EGFR/low HER2 expressing cells.

Next, trastuzumab-saporin was titrated on a fixed concentration of 75 nM cetuximab-(Cys-L-SO1861)^{3,7} and targeted protein toxin mediated cell killing on EGFR/HER2 expressing cells was determined. This revealed that 75 nM cetuximab-(Cys-L-SO1861)^{3,7} in combination with low concentrations trastuzumab-saporin induced already efficient cell killing in EGFR/HER2 expressing cells (A431: IC50= 5 pM; and CaSKi: IC50= 1 pM; Figure 3-6C and 3-6D), whereas trastuzumab-saporin alone or trastuzumab-saporin + 75 nM cetuximab did not show significant cell killing activity (IC50> 10.000 pM) in both cell lines (Figure 3-6C, 3-6D). All this shows that relatively low concentrations of trastuzumab-saporin can be effective and induce cell killing in combination with low cetuximab-SO1861 conjugate concentrations in high EGFR/low HER2 expressing cells.

Next, cetuximab-(Cys-L-SO1861)^{3,7} was titrated on a fixed concentration of 50 pM trastuzumab-saporin and targeted protein toxin-mediated cell killing on HeLa (EGFR$^{+/-}$/HER2$^{+/-}$) or A2058 (EGFR^{-}/HER2$^{+/-}$) was determined as illustrated in Figure 4-6, 15-6 – 17-6. Both HeLa (EGFR$^{+/-}$/HER2$^{+/-}$) and A2058 (EGFR^{-}/HER2$^{+/-}$) cells do not show cell killing at low concentrations of cetuximab-(Cys-L-SO1861)^{3,7} + 50 pM trastuzumab-saporin (HeLa: IC50= 400 nM; A2058: IC50> 400 nM; Figure 4-6A, 4-6B). This shows that in the absence of sufficient receptor expression, effective intracellular delivered SO1861 concentrations are not reached (threshold) to induce endosomal escape and cytoplasmic delivery of the protein toxin. Next, trastuzumab-saporin was titrated on a fixed concentration of 75 nM cetuximab-(Cys-L-SO1861)^{3,7} and targeted protein toxin mediated cell killing on HeLa (EGFR$^{+/-}$/HER2$^{+/-}$) or A2058 (EGFR^{-}/HER2$^{+/-}$) was determined. Both HeLa (EGFR$^{+/-}$/HER2$^{+/-}$) and A2058 (EGFR^{-}/HER2$^{+/-}$) cells showed no cell killing activity (HeLa: IC50> 10.000 pM; A2058: IC50> 10.000 pM; Figure 4-6C, 4-6D). All this shows that cells with low or no EGFR receptor expression are not susceptible for the combination of cetuximab-(Cys-L-SO1861)^{3,7} + trastuzumab-saporin, due to a lack of sufficient EGFR receptor that facilitates the antibody-mediated delivery of sufficient SO1861 (threshold) to ensure endosomal escape of the toxin within the cytoplasm of the cell.

Next, SO1861-EMCH was conjugated via cysteine residues (Cys) to trastuzumab (monoclonal antibody recognizing and binding human HER2), with a DAR 4 (trastuzumab-(Cys-L-SO1861)^{4}). Trastuzumab-(Cys-L-SO1861)^{4} was titrated on a fixed concentration of 1.5 pM EGFdianthin (EGFR targeted ligand toxin fusion protein) and targeted protein toxin mediated cell killing on HER2/EGFR expressing cells (SK-BR-3: HER2^{++}/EGFR$^{+/-}$) was determined. This revealed strong cell killing at low concentrations of trastuzumab-(Cys-L-SO1861)^{4} + 1.5 pM EGFdianthin (SK-BR-3: IC50= 1 nM; Figure 5-6A) whereas equivalent concentrations trastuzumab, trastuzumab-(Cys-L-SO1861)^{4} or trastuzumab + 1.5 pM EGFdianthin could not induce any cell killing activity in HER2^{++}/EGFR$^{+/-}$ expressing cells. This shows that trastuzumab conjugated SO1861 efficiently enhances endosomal escape of the EGF fusion protein toxin (at non-effective concentrations), thereby inducing cell killing of high HER2/low EGFR expressing cells.

Next, EGFdianthin was titrated on a fixed concentration of 2.5 nM trastuzumab-(Cys-L-SO1861)^{4} and targeted protein toxin mediated cell killing on SK-BR-3 (HER2^{++}/EGFR$^{+/-}$) expressing cells was determined. This revealed that 2.5 nM trastuzumab-(Cys-L-SO1861)^{4} in combination with low concentrations EGFdianthin induced already efficient cell killing in HER2/EGFR expressing cells (SK-BR-3: IC50= 1 pM) (Figure 5-6B), whereas EGFdianthin alone or EGFdianthin + 2.5 nM trastuzumab

showed no cell killing activity (IC50>10.000 pM) (Figure 5-6B). All this shows that relatively low concentrations of EGFdianthin can be effective and induce cell killing only in combination with low trastuzumab-(Cys-L-SO1861)^4 concentrations in high HER2/low EGFR expressing cells.

Next, trastuzumab-(Cys-L-SO1861)^4 was titrated on a fixed concentration of 1.5 pM EGFdianthin and targeted protein toxin mediated cell killing on JIMT-1 (HER2$^{+/-}$/EGFR$^{+/-}$) or MDA-MB-468: HER2$^-$/EGFR^{++}) was determined. Both cell lines were not sensitive for any combination of trastuzumab-(Cys-L-SO1861)^4 + 1.5 pM EGFdianthin (JIMT-1: IC50> 1000 nM; MDA-MB-468: IC50> 1000 nM; Figure 6-6A, 6-6B). This shows that in the absence of sufficient HER2 receptor expression, effective intracellular delivered SO1861 concentrations are not reached (threshold) to induce endosomal escape and cytoplasmic delivery of the protein toxin.

Next, EGFdianthin was titrated on a fixed concentration of 2.5 nM trastuzumab-(Cys-L-SO1861)^4 and targeted protein toxin mediated cell killing on JIMT-1 (HER2$^{+/-}$/EGFR$^{+/-}$) or MDA-MB-468 (HER2$^-$/EGFR^{++}) was determined. Both cell lines showed cell killing at high EGFdianthin concentrations with or without 2,5 nM trastuzumab-(Cys-L-SO1861)^4 (JIMT-1: IC50= 10.000 pM; MDA-MB-468: IC50= 200 pM Figure 6-6C, 6-6D).

All this shows that cells with low or no HER2 receptor expression are not susceptible for the combination of trastuzumab-(Cys-L-SO1861)^{3,7} + 1.5 pM EGFdianthin, due to a lack of sufficient HER2 receptor that facilitates the antibody-mediated delivery of sufficient SO1861 (threshold) to ensure endosomal escape of the toxin within the cytoplasm of the cell.

Next, SO1861-EMCH was conjugated via cysteine residues (Cys) to trastuzumab (monoclonal antibody recognizing and binding human HER2), with a DAR 4, (trastuzumab-(Cys-L-SO1861)^4). Trastuzumab-(Cys-L-SO1861)^4 was titrated on a fixed concentration of 5 pM cetuximab-saporin (EGFR targeting antibody-protein toxin conjugate) and targeted protein toxin mediated cell killing on HER2/EGFR expressing cells (SK-BR-3: HER2^{++}/EGFR$^{+/-}$) was determined as illustrated in Figure 15-6 – 17-6. This revealed strong cell killing at low concentrations of trastuzumab-(Cys-L-SO1861)^4 + 5 pM cetuximab-saporin (SK-BR-3: IC50= 1 nM; Figure 7-6A) whereas equivalent concentrations trastuzumab, trastuzumab-(Cys-L-SO1861)^4 or trastuzumab + 5 pM cetuximab-saporin could not induce any cell killing activity in HER2^{++}/EGFR$^{+/-}$ expressing cells. This shows that trastuzumab conjugated SO1861 efficiently enhances endosomal escape of the cetuximab conjugated protein toxin (at non-effective concentrations), thereby inducing cell killing of HER2^{++}/EGFR$^{+/-}$ expressing cells.

Next, cetuximab-saporin was titrated on a fixed concentration of 2.5 nM trastuzumab-(Cys-L-SO1861)^4 and 75 nM trastuzumab-(Cys-L-SO1861)^4 and targeted protein toxin mediated cell killing on HER2/EGFR expressing cells (SK-BR-3: HER2^{++}/EGFR$^{+/-}$) was determined. This revealed that 2.5 nM trastuzumab-(Cys-L-SO1861)^4 in combination with low concentrations cetuximab-saporin induced already efficient cell killing in SK-BR-3 cells (SK-BR-3: IC50= 1 pM; Figure 7-6B), whereas cetuximab-saporin alone or cetuximab-saporin + 2.5 nM trastuzumab showed cell killing only at high concentrations trastuzumab-saporin (SK-BR-3: IC50> 4000 pM; Figure 7-6B). All this shows that relatively low concentrations of cetuximab-saporin can be effective and induce cell killing only in combination with low trastuzumab-(Cys-L-SO1861)^4 concentrations in HER2^{++}/EGFR$^{+/-}$ expressing cells.

Next, trastuzumab-(Cys-L-SO1861)^4 was titrated on a fixed concentration of 5 pM cetuximab-saporin and targeted protein toxin mediated cell killing on JIMT-1 (HER2$^{+/-}$/EGFR$^{+/-}$) and MDA-MB-468 (HER2$^-$/EGFR^{++}) cells was determined. Both cell lines were not sensitive for the combination of trastuzumab-(Cys-L-SO1861)^4 + 5 pM cetuximab-saporin (JIMT-1: IC50> 1000 nM; MDA-MB-468: IC50> 1000 nM; Figure 8-6A, 8-6B). This shows that in the absence of sufficient HER2 receptor expression, effective intracellular delivered SO1861 concentrations are not reached (threshold) to induce endosomal escape and cytoplasmic delivery of the protein toxin.

Next, cetuximab-saporin was titrated on a fixed concentration of 2.5 nM trastuzumab-(Cys-L-SO1861)^4 and targeted protein toxin mediated cell killing on JIMT-1 (HER2$^{+/-}$/EGFR$^{+/-}$) and MDA-MB-468 (HER2$^-$/EGFR^{++}) cells was determined. Both cell lines showed cell killing at similar cetuximab-saporin concentrations with or without 2.5 nM trastuzumab-(Cys-L-SO1861)^4 (JIMT-1: IC50= 80pM; MDA-MB-468: IC50= 100 pM; Figure 8-6C, 8-6D).

All this shows that cells with low or no HER2 receptor expression are not susceptible for the combination of trastuzumab-(Cys-L-SO1861)^4 + cetuximab-saporin, due to a lack of sufficient HER2 receptor that facilitates the antibody-mediated delivery of sufficient SO1861 (threshold) to ensure endosomal escape of the toxin within the cytoplasm of the cell.

Example 23

In order to show that the activity of the conjugated SO1861 is driven by the acidification of the endosomal compartments, the 2T2 components system, according to the invention was tested in combination with an endosomal acidification inhibitor, chloroquine. Trastuzumab-saporin + 77 nM cetuximab-(Cys-L-SO1861)^{3,9} or trastuzumab-dianthin + 77 nM cetuximab-(Cys-L-SO1861)^{3,9} showed strong cell killing activity in A431 (EGFR^{++}/HER2$^{+/-}$) cells, whereas this 2T2C activity, according to the invention, was inhibited when 800 nM chloroquine was co-administrated to both combinations (Figure 9-6A). Same results were observed when CD71mab-saporin + 10.5 nM cetuximab-(Cys-L-SO1861)^{3,9} + 500 nM chloroquine was tested in A431 (EGFR^{++}/CD71$^+$) and MDA-MB-468 (EGFR^{++}/CD71$^+$) cells (Figure 9-6B, 9C) or when CD71mab-saporin + 5 nM trastuzumab-(Cys-L-SO1861)^4 + 500 nM chloroquine was tested in SK-BR-3 (HER2^{++}/CD71$^+$) cells (Figure 9-6D). This shows that the intracellular activity of conjugated SO1861 within the 2T2C system can be inhibited when acidification of endosomes is blocked.

Example 24

The 2 target 2-components system (2T2C) is also the combination treatment of mAb1-SO1861 and mAb2-antisense BNA oligo nucleotide, (Figure 16-6). Therefore, the 2T2C system was also tested in combination with an antisense BNA oligonucleotide against the mRNA of a cancer specific target gene, heat shock protein 27 (HSP27). Upon release into the cytoplasm the antisense BNA recognizes and binds the mRNA encoding for HSP27, targeting the mRNA for destruction thereby depleting the HSP27 expression within the cancer cell. HSP27BNA was conjugated to trastuzumab with a DAR4.4 (trastuzumab-(Lys-L-HSP27BNA)^{4,4}) and tested in combination with Cetuximab-(Cys-L-SO1861)^{3,9} for enhanced HSP27 gene silencing activity in A431 (EGFR^{++}/HER2$^{+/-}$) cells and A2058 (EGFR$^-$/HER2$^{+/-}$)

cells as illustrated in Figure 16-6. Cetuximab-(Cys-L-SO1861)^{3,9} was titrated on a fixed concentration of 100 nM Trastuzumab-(Lys-L-HSP27BNA)^{4,4} and targeted HSP27BNA-mediated gene silencing activity was determined. Cetuximab-(Cys-L-SO1861)^{3,9} + 100 nM Trastuzumab-(Lys-L-HSP27BNA)^{4,4} show strong gene silencing activity in A431 cells (EGFR^{++}/HER2$^{+/-}$) (A431: IC50= 1 nM; Figure 10-6A), compared to Cetuximab-(Cys-L-SO1861)^{3,9} alone. In A2058 cells (EGFR^{-}/HER2$^{+/-}$), the combination according to the invention showed no HSP27 gene silencing (A2058: IC50> 100nM; Figure 10-6B). This shows that cetuximab conjugated SO1861 efficiently enhances endosomal escape of the trastuzumab conjugated BNA oligo nucleotide (at non-effective concentrations), thereby inducing target gene silencing in EGFR^{++}/HER2$^{+/-}$ expressing cells.

Next, Trastuzumab-(Lys-L-HSP27BNA)^{4,4} was titrated on a fixed concentration of Cetuximab-(Cys-L-SO1861)^{3,9} and targeted HSP27BNA-mediated gene silencing activity was determined in A431 (EGFR^{++}/HER2$^{+/-}$) cells and A2058 (EGFR^{-}/HER2$^{+/-}$) cells as illustrated in Figure 16-6. Trastuzumab-(Lys-L-HSP27BNA)^{4,4} + 77 nM Cetuximab-(Cys-L-SO1861)^{3,9} show strong gene silencing activity in A431 cells (EGFR^{++}/HER2$^{+/-}$) (A431: IC50= 1 nM; Figure 10-6C), whereas trastuzumab-(Lys-L-HSP27BNA)^{4,4} alone or Cetuximab-(Cys-L-SO1861)^{3,9} alone or trastuzumab-(Lys-L-HSP27BNA)^{4,4} + 77 nM cetuximab did not reveal any significant gene silencing activity (IC50>100nM). A2058 (EGFR^{-}/HER2$^{+/-}$) cells did not show any gene silencing activity in the combination according to the invention (A2058: IC50> 100nM; Figure 10-6D). All this shows that relatively low concentrations of trastuzumab-HSP27BNA can be effective and induce cell killing only in combination with low concentrations of cetuximab-(-L-SO1861) concentrations in HER2^{++}/EGFR$^{+/-}$ expressing cells.

Example 25

The 2 target 2-components system (2T2C) can also be the combination treatment of mAb1-(dendron(-SO1861)^{n})^{n} and mAb2-protein toxin. Dendron(-L-SO1861)^{4} was conjugated to the anti-EGFR antibody, cetuximab via cysteine residues (Cys) with a DAR3,9, (cetuximab-Cys-(dendron(-L-SO1861)^{4})^{3,9}) and tested for enhanced cell killing activity in combination with an anti-CD71 antibody protein toxin conjugate (CD71mab-saporin) in MDA-MB-468 (EGFR^{++}/CD71^{+}) expressing cells as illustrated in Figure 17-6. Cetuximab-Cys-(dendron(-L-SO1861)^{4})^{3,9} + 10 pM CD71mab-saporin efficiently induces toxin-mediated cell killing in MDA-MB-468 (EGFR^{++}/CD71^{+}) expressing cells (IC50= 0.4 nM, Figure 11-6A), whereas this could not be induced by Cetuximab-Cys-(dendron(-L-SO1861)^{4})^{3,9}) or cetuximab + 10 pM CD71mab-saporin or cetuximab (Figure 11-6A). This shows that cetuximab conjugated dendron(-L-SO1861)^{4} efficiently enhances endosomal escape of the CD71mab-protein toxin (at non-effective concentrations), thereby inducing cell killing of EGFR^{++}/CD71^{+} expressing cells. Similar experiments were performed in HeLa cells (HER2$^{+/-}$/CD71^{+}) cells and this revealed no activity of cetuximab-Cys-(dendron(-L-SO1861)^{4})^{3,9}) + 10 pM CD71mab-saporin (IC50> 100 nM Figure 11-6B) indicating that in the absence of sufficient EGFR receptor expression, effective intracellular SO1861 concentrations are not reached (threshold) to induce endosomal escape and cytoplasmic delivery of the protein toxin.

Next, dendron(-L-SO1861)^{4} was conjugated to the anti-HER2 antibody, trastuzumab via cysteine conjugation (Cys) with a DAR4, trastuzumab-Cys-(dendron(-L-SO1861)^{4})^{4} and tested for enhanced cell killing activity in combination with an anti-CD71 antibody protein toxin conjugate (CD71mab-saporin) in

SK-BR-3 cells ($HER2^{++}/CD71^{+}$) expressing cells. Trastuzumab-Cys-(dendron(-L-SO1861)^4)^4 + 10 pM CD71mab-saporin efficiently induces toxin-mediated cell killing in SK-BR3 cells (IC50= 3 nM, Figure 11-6C), whereas this was not induced by trastuzumab-Cys-(dendron(-L-SO1861)^4)^4 or trastuzumab (equivalent) + 10 pM CD71mab-saporin or trastuzumab (Figure 11-6C). This shows that trastuzumab conjugated dendron(-L-SO1861)^4, according to the invention efficiently enhances endosomal escape of the CD71mab-protein toxin (at non-effective concentrations), thereby inducing cell killing of $HER2^{++}/CD71^{+}$ expressing cells. Similar experiments were performed in JIMT-1 cells ($HER2^{+/-}/CD71^{+}$) and this revealed no activity of trastuzumab-Cys-(dendron(-L-SO1861)^4)^4 + 10 pM CD71mab-saporin (IC50> 100 nM Figure 11-6C) indicating that in the absence of sufficient HER2 receptor expression, effective intracellular SO1861 concentrations are not reached (threshold) to induce endosomal escape and cytoplasmic delivery of the protein toxin.

Example 26

The clinical approved ADC, trastuzumab-emtansine (T-DM1) is a conjugate of the anti-Her2 antibody, trastuzumab and the small molecule toxin emtansine (DAR3-4). T-DM1 was tested within the 2T2C system, according to the invention in combination with cetuximab-(Cys-L-SO1861)^4. T-DM1 + 77 nM cetuximab-(Cys-L-SO1861)^{3,9} showed no enhanced cell killing activity compared to T-DM1 alone or T-DM1 + 77 nM cetuximab (IC50= 80.000 pM, Figure 12-6), whereas trastuzumab-saporin + 75 nM cetuximab-(Cys-L-SO1861)^{3,7}, according to the invention showed enhanced cell killing activity compared to trastuzumab-saporin + 75 nM cetuximab or trastuzumab-saporin alone (IC50=3 pM, Figure 12-6). All this shows that the 2T2C system does not enhance the delivery of antibody conjugated small molecules, that are already able to passively cross cellular (endosomal) membranes.

Example 1-2

Various concentrations of trastuzumab-saporin (HER2 targeted protein-toxin conjugate; intravenous) were tested in combination with 1.5 mg/kg SO1861 (1 hour before antibody-toxin injection ;subcutaneous) for enhanced efficacy in a BT474 (HER2++) xenograph mouse model. Dosing started at day 13 when tumors reached ~150mm^3 in size and tumor volume was determined after every treatment. Although tumor growth inhibition was observed in the mice treated with 1 mg/kg and 0.3 mg/kg trastuzumab-saporin, there was no enhanced tumor growth inhibition observed in the mice treated with the combination of trastuzumab-saporin + SO1861. This shows that unconjugated SO1861 is not able to enhance antibody-protein toxins within the current settings and mouse model.

Example 2-2

Materials:

QSmix (1): S4521 (Sigma Aldrich); QSmix (2) : 6857.1 (Carl Roth) QSmix (3): Quil-A® Adjuvant: vac-quil (InvivoGen/Brenntag).

Previously, the efficacy of various saponins (SO1861, SO1642) were co administrated as 'free' unconjughated molecules to cells in combination with a ligand toxin fusion (e.g. EGFdianthin) or an antibody-protein toxin conjugate, resulting in enhanced cell killing activity of target expressing cells.

Here, three different saponin molecules (SO1861, SO1862 (isomer of SO1861), SO1832 and SO1904) isolated from a root extract of *Saponaria officinalis* were titrated in the presence and absence of a non-effective fixed concentration of 1.5 pM EGFdianthin on HeLa ($EGFR^+$) cells. This revealed a strong enhancement of cell killing activity for all tested saponin variants (IC50= 300 nM; Figure 2-2A) compared to the treatments without EGFdianthin. Next, EGFdianthin was titrated with a fixed concentration of saponin (~1000nM) and this revealed strong targeted cell killing enhancement at low pM concentrations of EGFdianthin (IC50= 0.4 pM; Figure 2-2B), observed for all used saponins SO1861, SO1862 (isomer of SO1861), SO1832 and SO1904. EGF-dianthin alone could only induce cell killing at very high concnetrations (IC50=10.000 pM). This shows that these specific types of saponins, all have the intrinsic capacity to efficiently induce endosomal escape with only a very low amount of targeted toxin available.

To extend this test, saponins from other sources were analyzed. A saponin purified from a root extract of *Gypsophila elegans* M.Bieb. (GE1741) was titrated on HeLa cells in the presence and absence of 1.5 pM EGFdianthin and compared with purified SO1861. GE1741 also enhances the EGFdianthin induced HeLa cell killing, but shows slightly less efficacy compared to SO1861. (GE1741 IC50= 800 nM; Figure 2-2C) and also displays a higher general toxicity (IC50= 5.000 nM in absence of EGFdianthin; Figure 2-2C). A similar test in which different partially purified mixtures of *Quillaja saponaria* saponins (QSmix 1-3) were co-administrated with 1.5 pM EGFdianthin on HeLa cells and this revealed for 2 out of 3 (QSmix 1 and QSmix 3) similar activity as SO1861 (IC50 QSmix/QSmix3=300nM; Figure 2-2D). QSmix (2) is less efficient in enhancing 1.5 pM EGFdianthin induced cell killing (IC50= 2000 nM; Figure 2-2D), however, no general toxicity is observed. This shows that also in QS extracts, specific type of saponins are available that efficiently induce endosomal escape of the targeting ligand toxin EGFdianthin.

Example 3-2

In order to conjugate SO1861 molecules to antibodies, according to the invention, labile/acid sensitive linkers (-EMCH or -N3), was conjugated to SO1861 via the aldehyde group, producing SO1861-EMCH or SO1861-N3. To verify the activity of SO1861-EMCH the molecule was titrated in the presence and absence of a fixed non-effective (1.5 pM) EGFdianthin concentration on EGFR expressing (A431, HeLa) and non-expressing cells (A2058). In all three cell lines SO1861 alone showed a strong cell viability reduction, whereas SO1861-EMCH as single compound showed no toxicity up to 25.000 nM (Figure 3-2A-C). When SO1861-EMCH was combined with 1.5 pM EGFdianthin a strong target specific cell viability reduction is observed in the $EGFR^+$ A431 and HeLa cells (IC50= 3.000 nM; Figure 3-2A,B), while the $EGFR^-$ A2058 cells are not affected at all (Figure 3-2C). Similar results were obtained for SO1861-N3. SO1861-N3 co-administrated with 1.5 pM EGFdianthin also shows efficient cell killing on A431 and HeLa cells (IC50= 3.000 nM), but without EGFdianthin a general toxicity is observed at above 10.000 nM (Figure 3-2D, 3-2E).

For the stable conjugation of SO1861 to antibodies, according to the invention, a stable linker (HATU) was conjugated to SO1861 via the carboxylic acid group of SO1861 producing, SO1861-(S). To determine the activity different concentrations of SO1861-(S) were co-administrated with 1.5 pM EGFdianthin and tested for cell killing activity in EGFR expressing HeLa cells. SO1861-(S) showed a

similar activity as SO1861, indicating that conjugation to the carboxylic acid does not affect the endosomal escape enhancing potency of the molecule as is observed with SO1861-EMCH (Figure 4-2).

Example 4-2

Labile SO1861 was conjugated via cysteine residues (Cys) to the anti-EGFR antibody cetuximab (monoclonal antibody recognizing and binding human EGFR), with DAR3.9 (cetuximab-(Cys-L-SO1861)$^{3.9}$) and tested for its enhanced delivery of antisense BNA oligo nucleotides resulting in enhanced target gene silencing. In this study we used an antisense BNA oligonucleotide against the mRNA of a cancer specific target gene, heat shock protein 27 (HSP27). Within the cytoplasm of the cell HSP27BNA bind the mRNA encoding for HSP27, target the mRNA for destruction, thereby reducing the HSP27 expression within the cancer cell. Cetuximab-(Cys-L-SO1861)$^{3.9}$ was titrated on fixed concentration of 100 nM HSP27BNA on EGFR^{++} (A431) and EGFR^{-} (A2058) cells. The combination according ot the invention showed efficient HSP27 silencing on A431 (IC50= 2 nM; Figure 5-2A), while no silencing was observed for cetuximab-(Cys-L-SO1861)$^{3.9}$ alone. Cetuximab-(Cys-L-SO1861)$^{3.9}$ + 100nM HSP27BNA showed no gene silencing activity in EGFR^{-} cells (A2058) (Figure 5-2B). This shows that low concentrations of antibody-conjugated SO1861 efficiently can enhance cytoplasmic delivery and endolysosomal escape of an antisense BNA oligo nucleotide, thereby inducing efficient gene silencing in target expressing cells.

Next the HSP27BNA was titrated on EGFR^{++} (A431) and EGFR^{-} (A2058) cells combined with fixed concentration of cetuximab-(Cys-L-SO1861)$^{3.9}$. This shows that HSP27BNA in combination with 28.6 nM cetuximab-(Cys-L-SO1861)$^{3.9}$ or 77 nM cetuximab-(Cys-L-SO1861)$^{3.9}$ very efficiently enhances HSP27 gene silencing in A431 cells (IC50= 10 nM; Figure 5-2C). HSP27BNA alone or combined with a fixed equivalent of 77 nM cetuximab are less efficient (IC50= 1.000 nM; Figure 5-2C). The combination treatment of HSP27BNA + 77 nM cetuximab-(Cys-L-SO1861)$^{3.9}$ was also tested on EGFR- cells (A2058) and this revealed no HSP27 gene silencing enhancement (IC50=1.000 nM; Figure 5-2D). This shows that cells with low or no EGFR receptor expression are not susceptible for the combination of cetuximab-(Cys-L-SO1861)^{3,9} + HSP27BNA, while cetuximab targeted SO1861 can enhance HSP27 gene silencing efficiently at low concentrations of non-targeted HS27BNA in high EGFR cells.

Next, SO1861-EMCH was conjugated via cysteine residues (Cys) to cetuximab (monoclonal antibody recognizing and binding human EGFR), with a DAR 3,8. The combination according to the invention, cetuximab-(Cys-L-SO1861)^{3,8} + HSP27BNA (antisense HSP27BNA oligo nucleotide targeting and inducing degradation of the onco-target *hsp27* mRNA (gene silencing) in cancer cells) was tested in a A431 xenograph 'nude' mouse tumor model for EGFR-mediated tumor targeted HSP27 gene silencing. Dosing started at day 12 when tumors reached ~150mm^3 in size and HSP27 mRNA expression was determined. For this, tumor samples were collected at 72h after the first dosing and analysed for HSP27 gene expression levels compared to cellular control mRNA expression levels (reference genes). Tumor bearing mice (n=3) were treated (intraperitoneal; i.p.) at day 12: 25 mg/kg cetuximab-(Cys-L-SO1861)^{3,8} + 25 mg HSP27BNA and at day 15: 25 mg/kg cetuximab-(Cys-L-SO1861)^{3,8} + 10 mg HSP27BNA and this revealed a 25% reduction in HSP27 mRNA expression in the tumors compared to vehicle control or

single dosing of 25 mg/kg HSP27BNA (Figure 6-2). This shows and enables that conjugation of SO1861 to a targeting antibody, according to the invention, efficiently induces SO1861-mediated enhanced cytoplasmic delivery of a therapeutic antisense oligo nucleotide in solid tumors of tumor bearing mice, inducing tumor targeted gene silencing, *in vivo*.

EXAMPLE 1-7 – targeted antisense oligonucleotide coupled to an antibody

Cetuximab-(Lys-L-HSP27BNA) + saponin SO1861

An antisense BNA oligonucleotide against the mRNA of a cancer specific target gene, heat shock protein 27 (HSP27), will upon release into the cytoplasm recognize and bind the mRNA encoding for HSP27, target the mRNA for destruction and thereby lower the HSP27 expression within the cancer cell. Non-targeted HSP27BNA was titrated on $EGFR^{++}$ (A431) and $EGFR^{-}$ (A2058) cells to test for HSP27 gene silencing in combination with saponins. Data revealed efficient HS27 silencing on both A431 A2058 cells when HSP27BNA was combined with 4000 nM SO1861-ECMH (IC50= 10 nM; Figure 1-7A, 1-7B), while no silencing was observed for single HSP27BNA treatment (IC50≥ 1.000 nM; Figure 1-7A, 1-7B).

Next, HSP27BNA was conjugated to the lysines of cetuximab with DAR1.5 and DAR3.9, resulting in cetuximab-(Lys-L-HSP27BNA)$^{1.5}$ and cetuximab-(Lys-L-HSP27BNA)$^{3.9}$. The conjugated cetuximab-HSP27BNA samples were again titrated on $EGFR^{++}$ (A431) and $EGFR^{-}$ (A2058) cells to test for targeted HSP27 gene silencing in combination with saponins. These conjugates show very efficient HSP27 gene silencing in A431 ($EGFR^{++}$) cells in the presence of 4000 nM SO1861-EMCH (DAR1.5 IC50= 0.05 nM and DAR3.9 IC50= 0.3 nM; Figure 1-7A), while the silencing of the targeted HSP27BNA samples is comparable to the non-targeted HSP27BNA in the absence of SO1861-EMCH. The silencing in A2058 ($EGFR^{-}$) cells is not improved compared to non-targeted HSP27BNA in general. Both in the presence and absence of SO1861 similar HSP27BNA (conjugate) amounts are required to induce silencing (Figure 1-7B). This shows that cells with high EGFR receptor expression very efficient targeted HSP27 gene silencing can be achieved using targeted HSP27BNA in combination with SO1861.

An antisense BNA oligonucleotide against the mRNA of a cancer specific target gene, heat shock protein 27 (HSP27), will upon release into the cytoplasm recognize and bind the mRNA encoding for HSP27, target the mRNA for destruction and thereby lower the HSP27 expression within the cancer cell. Non-targeted HSP27BNA was titrated on $EGFR^{++}$ (A431) and $EGFR^{-}$ (A2058) cells to test for HSP27 gene silencing in combination with saponins. Data revealed efficient HS27 silencing on both A431 A2058 cells when HSP27BNA was combined with 4000 nM SO1861-ECMH (IC50= 10 nM; Figure 1-7C, 1-7D), while no silencing was observed for single HSP27BNA treatment (IC50≥ 1.000 nM; Figure 1-7C, 1-7D).

Next, HSP27BNA was conjugated to the lysines of cetuximab with DAR1.5 and DAR3.9, resulting in cetuximab-(Lys-L-HSP27BNA)$^{1.5}$ and cetuximab-(Lys-L-HSP27BNA)$^{3.9}$. The conjugated cetuximab-HSP27BNA samples were again titrated on $EGFR^{++}$ (A431) and $EGFR^{-}$ (A2058) cells to test for targeted HSP27 gene silencing in combination with saponins. These conjugates show very efficient HSP27 gene silencing in A431 ($EGFR^{++}$) cells in the presence of 4000 nM SO1861-EMCH, requiring less HSP27BNA oligo (IC50= 0.04 nM; Figure 1-7C), while the silencing of the targeted HSP27BNA samples is comparable to the non-targeted HSP27BNA in the absence of SO1861-EMCH. The silencing

in A2058 ($EGFR^-$) cells is not improved compared to non-targeted HSP27BNA in general. It even seems that about 10x more HSP27BNA oligo is required in the presence of SO1861, while no significant silencing is observed without SO1861 (Figure 1-7D). This shows that cells with high EGFR receptor expression very efficient targeted HSP27 gene silencing can be achieved using targeted HSP27BNA in combination with SO1861.

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CLAIMS

1. Therapeutic molecule with chemical structure of COMPOUND I:

$$A1_m ((-L9_w) ((– L1_q – B1_n)_u ((– L2_r – L3_s) (– L4_v – C)_p)_t))_x$$

(compound I),

wherein

A1 is a first ligand if B1 is a first effector moiety, or A1 is the first effector moiety if B1 is the first ligand;
C is a saponin;
m = 0 or 1 if A1 is the first ligand and B1 is the first effector moiety;
m = 0 – 32 if A1 is the first effector moiety and B1 is the first ligand;
n = 0 or 1 if B1 is the first ligand and A1 is the first effector moiety, or if A1 is the first ligand and B1 is the first effector moiety;
p = any of 1-128;
L1 is at least one linker for covalently coupling two chemical groups;
L2 is at least one linker for covalently coupling two chemical groups;
L3 is at least one oligomeric or polymeric scaffold for covalently coupling two chemical groups;
L4 is at least one linker for covalently coupling two chemical groups;
L9 is a tri-functional linker for covalently coupling three chemical groups;
q = 0 or 1;
r = 0 or 1;
s = 0 or 1;
t = 0, 1 or 2 if s = 0, and t = any of 0-16 if s = 1;
u = any of 0 – 32 if A1 is the first ligand and B1 is the first effector moiety, or u = 1 if A1 is the first effector moiety and B1 is the first ligand;
v = 0 or 1;
w = 1 or 0; and
x = 1-16.

2. Therapeutic combination comprising the therapeutic molecule according to claim 1 and a second therapeutic molecule with chemical structure of COMPOUND II:

$$A2_a ((-L10_j) ((– L5_d – B2_b)_h ((– L6_e – L7_f) (– L8_i – C)_c)_g))_k$$

(compound II),

wherein

A2 is a second ligand if B2 is a second effector moiety, or A2 is the second effector moiety if B2 is the second ligand;
C is a saponin;
a = 0 or 1 if A2 is the second ligand and B2 is the second effector moiety, or a = 0 – 32 if A2 is the second effector moiety and B2 is the second ligand;
b = 0 or 1 if B2 is the second ligand and A2 is the second effector moiety, or if A2 is the second ligand and B2 is the second effector moiety;
c = any of 1-128;
L5 is at least one linker for covalently coupling two chemical groups;
L6 is at least one linker for covalently coupling two chemical groups;
L7 is at least one oligomeric or polymeric scaffold for covalently coupling two chemical groups;
L8 is at least one linker for covalently coupling two chemical groups;
L10 is a tri-functional linker for covalently coupling three chemical groups;
d = 0 or 1;
e = 0 or 1;
f = 0 or 1;
g = 0, 1 or 2 if f = 0, and g = any of 0-16 if f = 1;
h = any of 0 – 32 if A2 is the second ligand and B2 is the second effector moiety, or h = 1 if A2 is the second effector moiety and B2 is the second ligand;
i = 0 or 1;
j = 1 or 0 and;
k = 1-16.

3. Second therapeutic molecule of claim 2, wherein g = 0, 1 or 2 if f = 0 and t > 0, g = 1 or 2 if f = 0 and t = 0, g = any of 0-16 if f = 1 and t > 0, and g = any of 1-16 if f = 1 and t = 0.

4. Therapeutic molecule of any of the claims 1-3 or the second therapeutic molecule of claim 2 or 3, wherein the first ligand A1 or B1 and/or the second ligand A2 or B2 comprise(s) or consist(s) of an immunoglobulin, a binding domain of an immunoglobulin or a binding fragment of an immunoglobulin, such as an antibody, an IgG, a molecule comprising or consisting of a Vhh domain or Vh domain, a Fab, an scFv, an Fv, a dAb, an $F(ab)_2$, Fcab fragment, or comprise(s) or consist(s) of at least one non-proteinaceous ligand and/or at least one proteinaceous ligand, the ligand for binding to a cell-surface molecule such as EGF or a cytokine, with the proviso that the first ligand and the second ligand are the same or are different.

5. Therapeutic molecule of any one of the claims 1-4 or the second therapeutic molecule of any of the claims 2-4, wherein the first ligand A1 or B1 and/or the second ligand A2 or B2 bind(s) to a tumor-cell epitope, preferably a tumor-cell specific epitope, of a tumor-cell receptor, preferably a tumor-cell specific receptor, preferably selected from CD71, CA125, EpCAM(17-1A), CD52, CEA, CD44v6, FAP, EGF-IR,

integrin, syndecan-1, vascular integrin alpha-V beta-3, HER2, EGFR, CD20, CD22, Folate receptor 1, CD146, CD56, CD19, CD138, CD27L receptor, PSMA, CanAg, integrin-alphaV, CA6, CD33, mesothelin, Cripto, CD3, CD30, CD239, CD70, CD123, CD352, DLL3, CD25, ephrinA4, MUC1, Trop2, CEACAM5, CEACAM6, HER3, CD74, PTK7, Notch3, FGF2, C4.4A, FLT3, CD38, FGFR3, CD7, PD-L1, CTLA4, CD52, PDGFRA, VEGFR1, VEGFR2, more preferably selected from CD71, EGFR and HER2, with the proviso that the first ligand and the second ligand bind to the same or to a different tumor-cell epitope, preferably a tumor-cell specific epitope, and/or wherein the tumor-cell receptor, preferably the tumor-cell specific receptor, to which the first ligand can bind is the same as, or is different from the tumor-cell receptor, preferably the tumor-cell specific receptor, to which the second ligand can bind.

6. Therapeutic molecule of any one of the claims 1-5 or the second therapeutic molecule of any one of the claims 2-5, wherein the first ligand A1 or B1 and/or the second ligand A2 or B2 comprise(s) or consist(s) of cetuximab, daratumumab, gemtuzumab, trastuzumab, panitumumab, brentuximab, inotuzumab, moxetumomab, polatuzumab, obinutuzumab, OKT-9 anti-CD71 monoclonal antibody of the IgG type, pertuzumab, rituximab, ofatumumab, Herceptin, alemtuzumab, pinatuzumab, OKT-10 anti-CD38 monoclonal antibody, an antibody of Table A2 or Table A3 or Table A4, preferably cetuximab or trastuzumab or OKT-9, or at least one tumor-cell receptor binding-domain thereof and/or at least one tumor-cell receptor binding-fragment thereof which are preferably (a) tumor-cell specific receptor binding-domain(s) and/or (a) tumor-cell specific receptor binding-fragment(s), with the proviso that the first ligand is the same or different from the second ligand.

7. Therapeutic molecule of any one of the claims 1-6 or the second therapeutic molecule of any one of the claims 2-6, wherein the first ligand A1 or B1 is internalized by a tumor cell after binding of the first ligand to its binding partner on the tumor cell, and wherein preferably binding of the first ligand to the tumor cell is followed by tumor-cell receptor-mediated internalization, e.g. via endocytosis, of a complex of the first ligand and the binding partner of the first ligand on the tumor cell.

8. Therapeutic molecule of any one of the claims 1-7 or the second therapeutic molecule of any one of the claims 2-7, wherein the second ligand A2 or B2 is internalized by a tumor cell after binding of the second ligand to its binding partner on the tumor cell, and wherein preferably binding of the second ligand to the tumor cell is followed by tumor-cell receptor-mediated internalization, e.g. via endocytosis, of a complex of the second ligand and the binding partner of the second ligand on the tumor cell.

9. Therapeutic molecule of any one of the claims 1-8 or the second therapeutic molecule of any one of the claims 2-8, wherein the first effector moiety A1 or B1 and/or the second effector moiety A2 or B2 comprise(s) or consist(s) of at least one of any one or more of an oligonucleotide, a nucleic acid and a xeno nucleic acid, preferably selected from any one or more of a vector, a gene, a cell suicide inducing transgene, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), anti-sense oligonucleotide (ASO, AON), short interfering RNA (siRNA), microRNA (miRNA), DNA aptamer, RNA aptamer, mRNA, mini-

circle DNA, peptide nucleic acid (PNA), phosphoramidate morpholino oligomer (PMO), locked nucleic acid (LNA), bridged nucleic acid (BNA), 2'-deoxy-2'-fluoroarabino nucleic acid (FANA), 2'-O-methoxyethyl-RNA (MOE), 2'-O,4'-aminoethylene bridged nucleic acid, 3'-fluoro hexitol nucleic acid (FHNA), a plasmid, glycol nucleic acid (GNA) and threose nucleic acid (TNA), or a derivative thereof, more preferably a BNA, for example a BNA for silencing HSP27 protein expression, with the proviso that the first effector moiety and the second effector moiety are the same or are different.

10. Therapeutic molecule of any one of the claims 1-9 or the second therapeutic molecule of any one of the claims 2-9, wherein the first effector moiety A1 or B1 and/or the second effector moiety A2 or B2 comprise(s) or consist(s) of at least one proteinaceous molecule, preferably selected from any one or more of a peptide, a protein, an enzyme such as urease and Cre-recombinase, a proteinaceous toxin, a ribosome-inactivating protein, at least one protein toxin selected from Table A5 and/or a bacterial toxin, a plant toxin, more preferably selected from any one or more of a viral toxin such as apoptin; a bacterial toxin such as Shiga toxin, Shiga-like toxin, Pseudomonas aeruginosa exotoxin (PE) or exotoxin A of PE, full-length or truncated diphtheria toxin (DT), cholera toxin; a fungal toxin such as alpha-sarcin; a plant toxin including ribosome-inactivating proteins and the A chain of type 2 ribosome-inactivating proteins such as dianthin e.g. dianthin-30 or dianthin-32, saporin e.g. saporin-S3 or saporin-S6, bouganin or de-immunized derivative debouganin of bouganin, shiga-like toxin A, pokeweed antiviral protein, ricin, ricin A chain, modeccin, modeccin A chain, abrin, abrin A chain, volkensin, volkensin A chain, viscumin, viscumin A chain; or an animal or human toxin such as frog RNase, or granzyme B or angiogenin from humans, or any fragment or derivative thereof; preferably the protein toxin is dianthin and/or saporin, with the proviso that the first effector moiety/moieties and the second effector moiety/moieties are the same or are different.

11. Therapeutic molecule of any one of the claims 1-10 or the second therapeutic molecule of any one of the claims 2-10, wherein the first effector moiety A1 or B1 and/or the second effector moiety A2 or B2 comprise(s) or consist(s) of at least one payload, preferably selected from any one or more of a toxin targeting ribosomes, a toxin targeting elongation factors, a toxin targeting tubulin, a toxin targeting DNA and a toxin targeting RNA, more preferably any one or more of emtansine, pasudotox, maytansinoid derivative DM1, maytansinoid derivative DM4, monomethyl auristatin E (MMAE, vedotin), monomethyl auristatin F (MMAF, mafodotin), a Calicheamicin, N-Acetyl-γ-calicheamicin, a pyrrolobenzodiazepine (PBD) dimer, a benzodiazepine, a CC-1065 analogue, a duocarmycin, Doxorubicin, paclitaxel, docetaxel, cisplatin, cyclophosphamide, etoposide, docetaxel, 5-fluorouracyl (5-FU), mitoxantrone, a tubulysin, an indolinobenzodiazepine, AZ13599185, a cryptophycin, rhizoxin, methotrexate, an anthracycline, a camptothecin analogue, SN-38, DX-8951f, exatecan mesylate, truncated form of *Pseudomonas aeruginosa* exotoxin (PE38), a Duocarmycin derivative, an amanitin, α-amanitin, a spliceostatin, a thailanstatin, ozogamicin, tesirine, Amberstatin269 and soravtansine, or a derivative thereof.

12. Therapeutic molecule of any one of the claims 1-11 or the second therapeutic molecule of any one of the claims 2-11, wherein the therapeutic molecule and/or the second therapeutic molecule comprise(s) or consist(s) of any one of Gemtuzumab ozogamicin, Brentuximab vedotin, Trastuzumab emtansine, Inotuzumab ozogamicin, Moxetumomab pasudotox and Polatuzumab vedotin and an antibody-drug conjugate of Table A2 and Table A3, or at least one tumor-cell specific receptor binding-domain thereof and/or at least one tumor-cell specific receptor binding-fragment thereof which are preferably (a) tumor-cell specific receptor binding-domain(s) and/or (a) tumor-cell specific receptor binding-fragment(s), with the proviso that the therapeutic molecule and the second therapeutic molecule are the same or are different.

13. Therapeutic molecule of any one of the claims 1-12 or the second therapeutic molecule of any one of the claims 2-12, wherein the saponin C is a triterpenoid saponin or a bisdesmosidic triterpene saponin, belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and optionally comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, and/or a saponin isolated from *a Gypsophila* species and/or a *Saponaria* species and/or an *Agrostemma* species and/or a *Quillaja* species such as *Quillaja saponaria*.

14. Therapeutic molecule of any one of the claims 1-13 or the second therapeutic molecule of any one of the claims 2-13, wherein the saponin C is a single specific saponin or is a mixture of two or more different saponins, such as one or more of the saponins in Table A1 or Scheme I, SO1861, SA1657, GE1741, SA1641, QS-21, QS-21A, QS-21 A-api, QS-21 A-xyl, QS-21B, QS-21 B-api, QS-21 B-xyl, QS-7-xyl, QS-7-api, QS-17-api, QS-17-xyl, QS1861, QS1862, Quillajasaponin, Saponinum album, QS-18, Quil-A, Gyp1, gypsoside A, AG1, AG2, SO1542, SO1584, SO1658, SO1674, SO1832, or any of their stereomers and/or any combinations thereof, preferably the saponin is SO1861 and/or GE1741 and/or SA1641 and/or QS-21 and/or saponin with a quillaic acid aglycon core, a Gal-(1→2)-[Xyl-(1→3)]-GlcA carbohydrate substituent at the C-3beta-OH group and a Glc-(1→3)-Xyl-(1→4)-Rha-(1→2)-[Xyl-(1→3)-4-OAc-Qui-(1→4)]-Fuc carbohydrate substituent at the C-28-OH group, and/or is 3-O-beta-D-galactopyranosyl-(1→2)-[beta-D-xylopyranosyl-(1→3)]-beta-D-glucuronopyranosyl quillaic acid 28-O-beta-D-glucopyranosyl-(1→3)-beta-D-xylopyranosyl-(1→4)- alpha-L-rhamnopyranosyl-(1→2)-[beta-D-xylopyranosyl-(1→3)-4-OAc-beta-D-quinovopyranosyl-(1→4)]-beta-D-fucopyranoside, more preferably the saponin is SO1861 and/or QS-21.

15. Therapeutic molecule of any one of the claims 1-14 or the second therapeutic molecule of any one of the claims 2-14, wherein the saponin C is a bisdesmosidic saponin having a molecular mass of at least 1.500 Dalton and comprising an oleanan-type triterpene containing an aldehyde group at the C-23 position and optionally a hydroxyl group at the C-16 position, with a first branched carbohydrate side chain at the C-3 position which first branched carbohydrate side chain optionally contains glucuronic acid, wherein the saponin contains an ester group with a second branched carbohydrate side chain at the C-28 position which second branched carbohydrate chain preferably comprises at least four carbohydrate units, optionally containing at least one acetyl residue such as two acetyl residues and/or

at least one deoxy carbohydrates and/or a quinovose and/or a glucose and/or 4-methoxycinnamic acid and/or optionally comprising 5-O-[5-O-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid and/or optionally comprising 5-O-[5-O-Rha-(1→2)-Ara/Api-3,5-dihydroxy-6-methyl-octanoyl]-3,5-dihydroxy-6-methyl-octanoic acid bound to a carbohydrate via an ester bond, or wherein the at least one saponin is QS-21 or any one or more of QS-21A, QS-21 A-api, QS-21 A-xyl, QS-21B, QS-21 B-api, QS-21 B-xyl, QS-7-xyl, QS-7-api, QS-17-api, QS-17-xyl, QS-18, QS1861, protonated QS1861 (QS1862), Quil-A.

16. Therapeutic molecule of any one of the claims 1-15 or the second therapeutic molecule of any one of the claims 2-15, wherein the saponin C is a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23, wherein the saponin C is covalently coupled to an amino-acid residue of the first ligand A1 or B1 and/or the first effector moiety B1 or A1 and/or the second ligand A2 or B2 and/or the second effector moiety B2 or A2 via the aldehyde function in the saponin C, preferably said aldehyde function in position C-23, preferably via a linker L2, L4, L6, L8, L9 and/or L10, more preferably via a cleavable linker L2, L4, L6, L8, L9 and/or L10, wherein the amino-acid residue preferably is selected from cysteine and lysine.

17. Therapeutic molecule of any one of the claims 1-16 or the second therapeutic molecule of any one of the claims 2-16, wherein the saponin C is a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23, wherein the aldehyde function in position C-23 of the at least one saponin is covalently coupled to linker N-ε-maleimidocaproic acid hydrazide, which linker is covalently coupled via a thio-ether bond to a sulfhydryl group in the first ligand A1 or B1 and/or in the first effector moiety B1 or A1 and/or in the second ligand A2 or B2 and/or in the second effector moiety B2 or A2, such as a sulfhydryl group of a cysteine.

18. Therapeutic molecule of any one of the claims 1-17 or the second therapeutic molecule of any one of the claims 2-17, wherein the saponin C is a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, wherein the saponin C is covalently coupled to the amino-acid residue of the first ligand A1 or B1 and/or the first effector moiety B1 or A1 and/or the second ligand A2 or B2 and/or the second effector moiety B2 or A2 via the glucuronic acid function in the saponin C, if present, preferably via a linker L2, L4, L6, L8, L9 and/or L10, wherein the amino-acid residue preferably is selected from cysteine and lysine, more preferably the amino-acid residue is lysine.

19. Therapeutic molecule of any one of the claims 1-18 or the second therapeutic molecule of any one of the claims 2-18, wherein the saponin C is a bisdesmosidic triterpene saponin belonging to the type of a 12,13-dehydrooleanane with an aldehyde function in position C-23 and comprising a glucuronic acid function in a carbohydrate substituent at the C-3beta-OH group of the saponin, wherein the glucuronic acid function in the carbohydrate substituent at the C-3beta-OH group of the at least one

saponin is covalently coupled to linker 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate, which linker is covalently coupled via an amide bond to an amine group in the first ligand A1 or B1 and/or in the first effector moiety B1 or A1 and/or in the second ligand A2 or B2 and/or in the second effector moiety B2 or A2, such as an amine group of a lysine or an N-terminus of the first ligand A1 or B1 and/or the first effector moiety B1 or A1 and/or the second ligand A2 or B2 and/or the second effector moiety B2 or A2.

20. Therapeutic molecule of any one of the claims 1-19 or the second therapeutic molecule of any one of the claims 2-19, wherein the first ligand A1 or B1 and/or the first effector moiety B1 or A1 and/or the second ligand A2 or B2 and/or the second effector moiety B2 or A2 comprise(s) one or more than one covalently bound saponin C, preferably 2, 3, 4, 5, 6, 8, 10, 16, 32, 64, 128 or 1-100 saponins, or any number of saponins therein between, such as 7, 9, 12 saponins.

21. Therapeutic molecule of any one of the claims 1-20 or the second therapeutic molecule of any one of the claims 2-20, wherein the first ligand A1 or B1 and/or the first effector moiety B1 or A1 and/or the second ligand A2 or B2 and/or the second effector moiety B2 or A2 comprise(s) one or more than one covalently bound saponin C, wherein the saponin(s) C is/are covalently bound directly to an amino-acid residue of the first ligand A1 or B1 and/or the first effector moiety B1 or A1 and/or the second ligand A2 or B2 and/or the second effector moiety B2 or A2 when r, s, v, e, f and i are 0, preferably to a cysteine and/or to a lysine, and/or is/are covalently bound via at least one linker L2, L4, L6, L8, L9 and/or L10, or via at least one cleavable linker L2, L4, L6, L8, L9 and/or L10 and/or via at least one oligomeric or polymeric scaffold L3 and/or L7, preferably 1-8 of such scaffolds or 2-4 of such scaffolds, wherein the at least one scaffold is optionally based on a dendron, wherein 1-32 saponins, preferably 2, 3, 4, 5, 6, 8, 10, 16, 32 saponins, or any number of saponins therein between, such as 7, 9, 12 saponins, are covalently bound to the at least one scaffold.

22. Therapeutic molecule of claim 16 or the second therapeutic molecule of claim 16, wherein the cleavable linker L2, L4, L6, L8, L9 and/or L10 is subject to cleavage under acidic conditions, reductive conditions, enzymatic conditions or light-induced conditions, and preferably the cleavable linker comprises a hydrazone bond or a hydrazide bond subject to cleavage under acidic conditions when bound to saponin, and/or comprises a bond susceptible to proteolysis, for example proteolysis by Cathepsin B, when bound to saponin, and/or the cleavable linker comprises a disulphide bond susceptible to cleavage under reductive conditions.

23. Therapeutic molecule of any one of the claims 16-22 or the second therapeutic molecule of any one of the claims 16-22, wherein the cleavable linker L2, L4, L6, L8, L9 and/or L10 is subject to cleavage *in vivo* under acidic conditions as present in endosomes and/or lysosomes of mammalian cells, preferably human cells, preferably at pH 4.0 – 6.5, and more preferably at pH $\leq$ 5.5.

24. Therapeutic molecule of any one of the claims 1-23 or the second therapeutic molecule of any one of the claims 2-23, wherein the polymeric or oligomeric scaffold L3 and/or L7 comprises a polymeric or oligomeric structure and comprises a chemical group, the chemical group for covalently coupling of the polymeric or oligomeric scaffold L3 and/or L7 to the amino-acid residue of the first ligand and/or the first effector moiety and/or the second ligand and/or the second effector moiety.

25. Therapeutic molecule of any one of the claims 1-24 or the second therapeutic molecule of any one of the claims 2-24, wherein the at least one saponin is covalently bound to the polymeric or oligomeric structure of the scaffold L3 and/or L7 via a cleavable linker L4 and/or L8 according to any one of the claims 19-23.

26. Therapeutic molecule of any one of the claims 20-25 or the second therapeutic molecule of any one of the claims 20-25, wherein the chemical group of the polymeric or oligomeric scaffold L3 and/or L7, for covalently coupling of the scaffold to the amino-acid residue of the first ligand and/or the first effector moiety and/or the second ligand and/or the second effector moiety, is a click chemistry group, preferably selected from a tetrazine, an azide, an alkene or an alkyne, or a cyclic derivative of these groups, more preferably the click chemistry group is an azide.

27. Therapeutic molecule of any one of the claims 20-26 or the second therapeutic molecule of any one of the claims 20-26, wherein the at least one saponin is covalently bound to the first ligand and/or to the first effector moiety and/or to the second ligand and/or to the second effector moiety, either directly or via at least one linker such as a bi-functional linker, for example based on N-ε-maleimidocaproic acid hydrazide and/or based on 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate, or a tri-functional linker L9 when w = 1 and/or a tri-functional linker L10 when j = 1, such as the tri-functional linker of Scheme II.

28. Therapeutic molecule of claim 27 or the second therapeutic molecule of claim 27, wherein the tri-functional linker L9 when w = 1 and/or the tri-functional linker L10 when j = 1, comprises a second chemical group with at least one saponin covalently bound thereto, a third chemical group for covalent binding to the first and/or second ligand and a first chemical group for covalent binding to at least one first and/or second effector moiety, preferably the tri-functional linker is the trifunctional linker of Scheme II.

29. Therapeutic molecule of any one of the claims 1-28 or the second therapeutic molecule of any one of the claims 2-28, wherein the at least one saponin is covalently bound to the first ligand and/or to the first effector moiety and/or to the second ligand and/or to the second effector moiety via at least one linker comprising a tri-functional linker L9 when j = 1 and/or a tri-functional linker L10 when w = 1, to which tri-functional linker both the first ligand and the at least one first effector moiety are bound and/or to which tri-functional linker both the second ligand and the at least one second effector moiety are bound, preferably the tri-functional linker is the trifunctional linker of Scheme II.

30. Therapeutic molecule of any one of the claims 20-29 or the second therapeutic molecule of any one of the claims 20-29, wherein the polymeric or oligomeric structure of the scaffold L3 and/or L7 comprises a linear, branched and/or cyclic polymer, oligomer, dendrimer, dendron, dendronized polymer, dendronized oligomer, a DNA, a polypeptide, poly-lysine, a poly-ethylene glycol, or an assembly of these polymeric or oligomeric structures which assembly is preferably built up by covalent cross-linking.

31. Therapeutic molecule of any one of the claims 20-30 or the second therapeutic molecule of any one of the claims 20-30, wherein the first ligand A1 or B1 is covalently bound to the first effector moiety B1 or A1, respectively, via at least one linker L1, and/or wherein the second ligand A2 or B2 is covalently bound to the second effector moiety B2 or A2, respectively, via at least one linker L5.

32. Therapeutic combination of any one of the claims 2-31, wherein the first ligand A1 is a monoclonal antibody or at least one binding fragment or –domain thereof according to any one of the claims 3-7, m = 1, q = 0, n = 0, u = 0, r = 0, L3 is the scaffold according to any one of the claims 20-29 and s = 1, or L3 is absent and s = 0, L4 is a linker or a cleavable linker according to any one of the claims 15-28, v = 1, p = 2-4 and t = 2-4 if s = 1 and t = 0 if s = 0, and saponin C is a saponin according to any one of the claims 12-18, preferably the saponin C is SO1861 and/or QS-21, and effector moiety A2 is an effector moiety according to claim 8, preferably a BNA, a = 1, d = 0, b = 0, h = 0, e = 0, f = 0, i = 0, c = 0 and g = 0.

33. Therapeutic molecule of claim 1, wherein r = 0, s = 0, v = 0, s = 0, v = 0, p = 0, t = 0, ligand A1 is a monoclonal antibody or at least one binding fragment or –domain thereof according to any one of the claims 3-7, m = 1, effector moiety B1 is an effector moiety according to claim 8, preferably a BNA, either q = 0, n = 1 and u = 2-4, or q = 1, n = 2-4, u = 2-4 and linker L1 is the oligomeric or polymeric scaffold L3 according to any one of the claims 21-30.

34. Therapeutic combination of any one of the claims 2-31, wherein the therapeutic molecule is the therapeutic molecule of claim 33, and wherein the second ligand A2 is a monoclonal antibody or at least one binding fragment or –domain thereof according to any one of the claims 3-7, a = 1, d = 0, b = 0, h = 0, e = 0, L7 is the scaffold according to any one of the claims 20-29 and f = 1, or L7 is absent and f = 0, L8 is a linker or a cleavable linker according to any one of the claims 15-24, i = 1, c = 2-4 and g = 2-4 if f = 1 and g = 0 if f = 0, and saponin C is a saponin according to any one of the claims 12-18, preferably the saponin C is SO1861 and/or QS-21, with the proviso that the ligand A1 and the ligand A2 are the same or are different.

35. Therapeutic combination, wherein the therapeutic combination comprises:

(a) a first pharmaceutical composition comprising the therapeutic molecule with chemical structure of COMPOUND I according to any one of the claims 1-34, the first pharmaceutical composition optionally further comprising a pharmaceutically acceptable excipient; and

(b) a second pharmaceutical composition comprising the second therapeutic molecule with chemical structure of COMPOUND II according to any one of the claims 2-34, the second pharmaceutical composition optionally further comprising a pharmaceutically acceptable excipient.

36. The first pharmaceutical composition of claim 35 for use as a medicament.

37. Therapeutic combination for use in the treatment or prevention of cancer in a human subject, wherein the therapeutic combination comprises:

(a) the first pharmaceutical composition of claim 35; and

(b) the second pharmaceutical composition of claim 35,

wherein the ligand A1 or B1 and the ligand A2 or B2 can bind to a tumor-cell epitope, preferably to a tumor-cell specific epitope, on a tumor-cell surface molecule, preferably on a tumor cell-specific surface molecule, with the proviso that the tumor-cell epitope or tumor-cell specific epitope to which the ligand A1 or B1 can bind is the same as, or is different from the tumor-cell epitope or the tumor-cell specific epitope to which the ligand A2 or B2 can bind.

38. The first pharmaceutical composition of any one of the claims 35-37, for use in the treatment or prophylaxis of cancer in a patient in need thereof, wherein the ligand A1 or B1 can bind to a tumor-cell epitope, preferably a tumor-cell specific epitope, on a tumor-cell surface molecule, preferably a tumor cell-specific surface molecule.

39. The first pharmaceutical composition for use according to claim 36 or 38 or the therapeutic combination for use of claim 35 or 37, wherein the second pharmaceutical composition of claim 35 or 37 and the first pharmaceutical composition of any one of the claims 35-38 are administered to the patient in need thereof.

40. The first pharmaceutical composition of claim 35 further comprising the second therapeutic molecule of any one of the claims 2-35.

41. The first pharmaceutical composition of claim 40, for use as a medicament.

42. The first pharmaceutical composition of claim 40, for use in the treatment or prevention of a cancer in a human subject.