Use of oligonucleotides for the treatment of tumors

The invention relates to the use of oligonucleotides for the specific therapy of tumor diseases at various stages directly during surgical intervention.

Short single- or double-stranded oligonucleotides referred to as oligonucleotides may be synthesized by a chemical process established in the prior art (Herdewijn, 2005; Surhone et al., 2010). Currently, DNA oligonucleotides up to a length of about 200 bases or base pairs may be synthesized efficiently, wherein, for many technical reasons, DNA may be synthesized more easily and in longer fragments compared to RNA. Technically, longer syntheses are possible, enzymatic or *in vivo* preparations, however, are simpler and more efficient. It is expected though that in the future oligonucleotides of increasing length will also be synthesized. As of now, machines are used in the synthesis process, which perform automatically the repetitive steps of synthesis.

One advantage of chemically synthesized oligonucleotides lies in their modifiability. Such modifications may comprise, for example, non-natural nucleotide building blocks such as inosine or natural modifications such as cytosine methylation. Furthermore, nucleotides may be modified at the base residue, the sugar residue, or the phosphate residue, as present in embodiments of the current invention. Modifications include, for example, substitutions with alkyl, alkoxy, amino, deaza, halogen, hydroxyl, thiol groups or combinations thereof. Nucleotides may also be replaced with analogs of higher stability, for example, a ribonucleotide may be replaced with a deoxyribonucleotide or the 2' OH group of its sugar moiety may be replaced with 2'amino groups, 2'O-methyl groups, 2' methoxyethyl groups, or a 2'-O, 4'-C methylene bridge. Examples of a purine or pyrimidine analog of nucleotides include xanthine, hypoxanthine, azapurine, methylnthioadenine, 7-deaza-adenosine, and O- or N-modified nucleotides. The phosphate residue of the nucleotide may be modified by replacing one or more oxygen atoms of the phosphate group with nitrogen or with sulfur (phosphorothioates). Further modifications include Locked Nucleic Acids LNA (W09914226 A2), Unlocked Nucleic Acids UNA, 2'OMe-methoxy and 2'F fluoro modifications. The 3' and 5' ends of a single strand also allow the addition of further molecular residues such as simple or glycosylated peptides and proteins as well as lipids, chitosan and other chitosan derivatives, polymers or dyes. Modifications of this type may be linked to the oligonucleotides directly or via spacers, for example one or more glycine residues. These modifications are developed with a variety of objectives, for example for influencing the stability of the oligonucleotides or the melting temperature of a double strand, for changing the affinity to other molecules or surfaces or the biological availability or activity *in vivo*. Modifications are also used, for example, for improving the function of the oligonucleotide, their stability or their transfer properties, or for controlling their localization or targeting. In the following, the term oligonucleotides includes all of these modifications.

Many types of oligonucleotides occur naturally in humans and animals or generally in living beings or viruses or are derived there from. They have very different functions such as regulation of protein expression, interference with said expression or communication between cells. They may be present in various forms, such as double strand, double strand with single-strand overhangs, hairpin, cyclically or as single strand. Examples of naturally occurring oligonucleotides are microRNA (miRNA) or long noncoding RNA (lnRNA); examples of naturally derived or artificial oligonucleotides are short interfering RNA (siRNA,) short activating RNA (saRNA), CRISPR-Cas oligonucleotides or antisense-DNA, some of which are suitable to serve as therapeutically active substances. Substances approved for first applications are antisense DNA (Mipomersen™, Kastle Theraputics, Inc., Chicago, II., USA) used in the

treatment of homozygous, hereditary hypercholesterolemia and siRNA (Patisaran ™, Alnylam, Inc., Cambridge, MA., USA) for the treatment of polyneuropathy in hereditary, transthyretin mediated amyloidosis. Both drugs are injected systemically.

The term “tumor” in the narrower sense is understood to refer to a benign or malignant neoplasm (neoplasia) of body tissues resulting from a dysregulation of cell growth (DocCheck, https://flexikon.doccheck.com/de/Tumor). Colloquially, malignant tumors are referred to as cancer. To this day, there is no cure for many forms of malignant, especially solid tumors. For example, among tumors with a very short survival time after diagnosis, are those of the pancreas, of the adrenal glands, of the mesothelium, the brain and the lungs, although great efforts have been undertaken in recent decades in order to improve or develop diagnoses, individual therapies and treatment methods for these tumors. Examples for these efforts include the surgical removal of the tumor, drug treatment or radiation, and various combinations thereof. Newer or experimental treatment methods include specific gene or cell therapies, including, in particular, chimeric antigen receptor-bearing T cells (CAR-T).

Despite better diagnostic technologies and despite enormous efforts concerning therapy and the early detection of tumors, improvements achieved so far concerning treatment and survival are very limited. For many of these tumors, patients are cured only in exceptional cases. Many therapies, however, at least slow disease progression and extend life expectancy of the patient. Depending on the type of tumor, the mean lifetime gained is between years and only a few months. There is a considerable need for new therapies, particularly for those tumors, in the case of which patients under treatment so far have gained hardly any additional life expectancy.

Many solid tumors are treated early and in a first step using surgery. A complete removal of the primary tumor is not always possible, however. Even if there appears to be a complete removal of the tumor mass appears, is very likely that the tumor bed or the edges of the surgical cavity still harbor tumor cells, from which a recurrence may arise. Even when using sensitive pathological procedures, these tumor cells may not be detected. Metastases occurring at a short distance from the primary tumor are also likely (colloquially direct seeding metastases). In some types of tumors, a complete removal is almost impossible, for example in advanced glioblastomas. Glioblastomas exhibit infiltrating growth and then often affect indispensable brain regions. In some types of tumors, even the entire affected organ is surgically removed, such as the adrenal gland in adrenocortical carcinoma. In this case, drug substitution of organ function is necessary and also possible. Even with radical resection, recurrences are common and almost always fatal in this tumor. Despite these limitations, surgical removal of the tumor often is the essential component of an efficient tumor treatment for which there is no substitute. Tumor resection often is associated with an adjuvant or neoadjuvant therapy. Drug treatment and radiation are then used in a neoadjuvant manner, i.e., before the surgery, if the tumor mass of the primary tumor and the risk of surgery both are to be reduced, as for example in the case of large tumors or tumors that are difficult to reach.

When applied as an adjuvant, i.e., after the operation, drug treatment and radiation therapy are intended to prevent the occurrence of recurrences and to act on distant metastases which are not accessible to surgery or whose existence is only suspected. Postoperative radiation is mainly used in order to prevent a recurrence or to treat known local metastases, since as of now it is applied almost only locally. Drugs are applied systemically in most cases, especially if metastases are suspected but escape detection.

In above-mentioned applications, drugs either may be chemotherapeutics exerting a cytotoxic effect predominantly on proliferating cells, or targeted therapeutics such as antibodies or antibodies loaded with cytotoxins for attacking tumor cells via tumor-specific receptors. Since with regard to metastases, the receptor composition often is not known, targeted therapeutics are rarely used as adjuvants.

For tumors not amenable to surgery, only radiation and drug therapies are available. In order to reduce the undesirable effects of drug treatment, drugs are now being applied locally. This is often accomplished via injection directly into the tumor or into surrounding tissue if an assumed spread of tumor cells is to be avoided during direct injection.

Less often, drugs are administered locally during surgery. It is assumed that by using this administration route, targeting of distant metastases is not accomplished any better in comparison to adjuvant systemic applications. In the case of glioblastoma, which often is only partially resectable, such treatment strategies are pursued more often, aiming at attacking the non-resectable tumor mass.

To this end, chemotherapeutical drugs are used, which are inserted into the surgical cavity after surgery. Regarding glioblastoma, local administration is also considered, since many drugs do not cross the blood-brain barrier - even though the blood-brain barrier may not be completely intact in a glioblastoma. Moreover, polymeric carriers are also used, with the intention to enable easier application and longer availability by slow release of the medication stored within the depot, in addition to enabling significantly higher drug dosages without the occurrence of toxic systemic effects.

Gliadel™, which was approved in the USA in 1999, is an example of a biodegradable, disc-shaped carrier having a diameter of 14 mm and a thickness of 1 mm, wherein the carrier is provided with 7.7 mg of the cytostatic carmustine (also: bis-chloroethyl-nitroso-urea, abbreviated BCNU); up to eight specimens are inserted into the patient's surgical cavity by the surgeon towards the end of the operation after removal of the tumor tissue. In clinical studies, an extension of the median lifespan by approximately 2 months could be demonstrated for both, patients diagnosed for the first time and patients with recurrences (Hart et al. 2008); the significance of the studies is questioned, however (Chowdhary et al 2015). The wafers may be detected up to 232 days after surgery and are therefore present longer than the assumed proliferation cycle of the tumor cells.

Gliadel™ carriers, however, are large and brittle, therefore limiting contact between carrier and surgical cavity. Instructions of application, however, do not provide for the carriers to be broken up into smaller fragments since there is at issue an increased risk of surgery. A whole series of other carrier principles have therefore been developed, which have reached different stages of clinical development, including microbeads (Paclimer™, Guildford Pharmaceuticals) with a diameter of 53 µm, which have been provided with paclitaxel (taxol) for intraperitoneal administration in recurrent ovarian cancer. They also were used in a modified form for the treatment of glioma (Li et al 2003).

Another form of nanoparticles has been developed able to expand at acidic pH (<5). Supposedly, these nanoparticles release their substances in the acidic environment of the endosome. These expanding particles were also provided with paclitaxel for use in the treatment of lung cancer (Griset et al., 2009), mesothelioma (Schulz et al., 2011) and peritoneal carcinomatosis (Colson et al., 2011). The particles were administered intravenously or intraperitoneally after resection of the primary tumor in mouse models. Apparently, these particles have not yet been tested clinically.

Chitosan-based hydrogels: Chitosan is obtained by deacetylating chitin, the material from which insect and crustacean shells are made essentially. Due to its excellent biodegradability, chitosan has found its way into biomedical applications for some time, for example as wound healing material (Kim et al.,

2008). Paclitaxel-loaded, chitosan-based hydrogels have been suggested for treating wound healing edges after tumor resection. Therefore, the combination was injected *in vivo* a few days after tumor inoculation in order to simulate this situation (Ruel-Gariäpy et al., 2004). Campthotecin was used in another study, but without aiming at an application during resection (Berrada et al., 2005).

In the case of radio frequency or thermal ablation, micro rods provided with doxorubicin were injected as adjuvants. In the case of ablation, recurrences are most frequently found at the edges of the treatment zone and around blood vessels. In animal experiments, rods were injected after ablation of the liver (Qian et al., 2003) and into a pre-punctured xenograft liver tumor (Weinberg et al., 2007). The formation of a fibrotic capsule around the rod was reduced by a simultaneous administration of dexomethasone, which was complexed with hydroxypropyl ß-cydodextrin (Blanco et al., 2006). In another experiment, 5-FU was applied to the rods (Haaga et al., 2005).

Further possible carriers are flexible films which adapt better to the surface of wound edges than rigid carriers, so that substances may diffuse into the tissue from a larger area. Poly(glycerol monostearate co-epsilon-caprolactone) films with paclitaxel were implanted after resection in a lung cancer model (Liu et al., 2010); in a similar model, films contained hydroxycamptothecin (Wolinski et al. -2, 2010) and once again paclitaxel in a sarcoma-model (Liu et al., 2012).

The often limited success of these strategies led to further refinements of local administration, for example, using liposomes for applying cytostatics (Yang et al., 2016), or coupling targeting molecules to nanoparticles or inducing controlled release of drugs via various external stimuli such as magnetic fields or light (Rosenblum et al., 2018).

A novel, intraoperative form of application is called hyperthermic intraperitoneal chemotherapy (HIPEC) (Glehen et al., 2008), aiming at achieving high local concentrations of cytostatics in the peritoneum and absorption of the substance in the upper cell layers in order to reduce systemic toxicity. The additional hyperthermia is believed to increase the therapeutic potential of the applied cytostatics by improving tissue penetration. However, hyperthermia also produces its own direct cytotoxic effect (Ceelen et al., 2010).

For the application of HIPEC, several drainage lines are placed in different areas of the abdomen at the end of surgery. A roller pump system with a heat exchanger is used and the temperature curve is checked. Target temperature is 42-43^{o}C, and perfusion time is between 30 and 120 minutes, depending on the protocol used. So far, mitomycin C is the most frequently used cytostatic drug. The drugs oxaliplatin and irinotecan used in standard systemic therapy are being used increasingly in HIPEC (Piso et al., 2011).

Pressure aerosol chemotherapy (Pressurized IntraPeritoneal Aerosol Chemotherapy, PIPAC) is another form of application. The application of an aerosol under pressure results in a particularly effective drug distribution in body cavities such as the abdomen or the chest. During PIPAC, drugs such as cisplatin, doxorubicin, oxaliplatin, paclitaxel are used. Local administration directly at the tumor site means that otherwise existing pharmacological limitations of intraperitoneal chemotherapy, are eliminated, such as poor distribution within the body cavity and low diffusion into the tissue. The local dose of the cytostatic may be reduced by a factor of 10 without losing efficacy against tumor tissue. Thus, dose-dependent local toxicity of intraperitoneal chemotherapy is better controlled, and organ toxicity as well as systemic side effects of therapy are significantly reduced.

In PIPAC, a 12 mmHg CO2 capnoperitoneum, i.e., an overpressure is applied, and two balloon trocars are inserted through the abdominal wall. Then, a micropump is inserted into the abdomen and

connected to a high-pressure contrast medium injector. In a typical protocol, doxorubicin (1.5 mg/m^2 body surface area (BSA) in 50 ml 0.9% NaCl solution) and cisplatin (7.5 mg/m^2BSA in 150 ml 0.9% NaCl solution) are applied successively via micropump and injector as a pressure chemotherapy aerosol. Injection parameters are set at a flow rate of 30 ml/min and a maximum inlet pressure of 200 psi. The micropump generates a polydisperse aerosol with a droplet size between 6 to 11 pm. The small size ensures that the dispersed droplets remain within the gas for an extended period of time, for example over 30 minutes. The therapeutic capnoperitoneum is kept at a temperature of 37°C for 30 minutes. The chemotherapy aerosol is then excreted into the air disposal system via a closed line. Finally, the trocars are pulled (Reymond et al., 2014).

An adjuvant gene therapy strategy, Sitimagene ceradenovec (Cerepro), has been published (EP 1 135 513 B2). After tumor resection, an adenovirus is injected into the tissue adjacent to the surgical cavity, wherein the virus carries a functional thymidine kinase gene, for example of the herpes-simplex virus. In transfected cells, the gene causes thymidine kinase expression. When ganciclovir is applied after surgery and taken up by transfected cells, it is phosphorylated preferably in these cells, thereby forming an active substance able to interfere with gene synthesis. By using this procedure, the effect of ganciclovir is confined locally to the area around the surgical cavity. Clinical test results, however, did not result in an approval of this therapy; in the negative recommendation of the Committee for Medicinal Products for Human Use (CHMP), based on the evaluation by the Committee for Advanced Therapies (CAT) of the European Medicines Agency (EMA), a low observed effectiveness and a significant risk of serious side effects was argued (EMA website for withdrawn applications for approval: Cerepro). Later on, this approach was developed further by additionally administering the cytostatic temezolomide, which is part of the standard therapy for glioblastomas, due to an observed synergistic effect (EP 2 665 489 B1).

Furthermore, gene therapy is proposed for mesothelioma and ovarian cancer (WO 2015/002861 A1), wherein an adenovirus loaded with a gene for human interferon alpha 2b is used. Application as an adjuvant is not intended explicitly.

Recently, attempts have been made to use antibodies labeled with dyes, in particular, fluorescent dyes, during tumor resection in order to visualize tumor-positive resection margins during surgery. In a breast cancer model, the dye IRDye800CW was coupled to antibodies bevacizumab, cetuximab, panitumumab, trastuzumab and tocilizumab and their local distribution was investigated (Korb et al., 2014). In another study, a monoclonal antibody against PD-1 was labeled with the same dye and injected intraperitoneally after surgery as an adjuvant without dye (Du et al., 2017). Moreover, it has been proposed to use photoimmunotherapy, i.e., antibody-based photodynamic therapy, intraoperatively during tumor resection in the tumor bed in order to visualize and fight simultaneously remaining tumor cells (de Boer, 2016).

In other studies, however, antibodies were also used as adjuvants in tumor treatment by applying multiple systemic injections after resection of the tumor. For treatment of colon cancer, for example, adjuvant chemotherapy after surgery has become state of the art and is often used in medical practice. 5-FU and oxaliplatin are used. In other studies, the angiogenesis-inhibiting monoclonal antibody Avastin™ (bevacizumab) was systemically administered postoperatively instead. This antibody binds to the vascular endothelial growth factor (VEGF), thereby preventing angiogenesis signaling. After 36 months, there was, however, no improvement in the risk of recurrence in the adjuvant antibody arm of the study. In another study, cetuximab, an EGFR-binding antibody used in breast cancer therapy, was added to the standard for adjuvant chemotherapy. As a result, the relapse risk for the antibody

arm hardly changed; on the contrary, the risk of recurrence increased for some patient groups (De Gramont et al., 2011; Oyan, 2012). The antibodies are believed to accelerate resistance to chemotherapy. From preclinical studies is also known that their use as an adjuvant promotes the formation of metastases. Furthermore, it cannot be excluded that new pro-survival paths are stimulated, functioning as enhancers of resistance. It also appears that by combining therapies, different mechanisms of action may interfere with each other (Huang et al., 2017)

Immune check point-targeted monoclonal antibodies (ICT mAbs), for example PD-1 antibodies, were administered intratumorally in first clinical tests. Compared to a systemic use, resistance to ICT mAbs appears to be reduced (Maraballe et al., 2017).

Several oligonucleotides have been developed already for the treatment of tumors. Usually, they are directed against specific cellular targets, for example, cell cycle proteins such as kinesin spindle protein (KSP), polo-like kinase 1 (PLK), protein kinase N3 (PKN3), ribonucleotide reductase (RRM2), or tenasion-2. Also included is a prodrug from WO 2010/102615, wherein a conjugated protease substrate inhibits the effectiveness of the siRNA until the substrate is cleaved by a protease.

In the case of oligonucleotides, however, current developments focus primarily on the formulation and the delivery as nanoparticles or gel (Kim et al., 2012), both of which are intended for systemic or intraperitoneal application by injection. siRNA may also be applied, for example, "naked" in saline, or complexed with polycations, cationic lipid/lipid transfection reagents or cationic peptides, as components of defined molecular conjugates (e.g., cholesterol-modified siRNA, TAT-DRBD/siRNA complexes), or as components of liposomes. For applications in regenerative medicine, the use of a matrix made of porous polyester urethane was tested as a subcutaneous implant, releasing siRNA-binding nanoparticles from a diblock copolymer (Nelson et al., 2013), thereby requiring two carrier systems, which means additional complexity. Simultaneous inclusion of cells and siRNA in alginate or collagen has also been described (Krebs et al., 2009) and has been proposed for applications in which the loaded alginate or collagen, present as a hydrogel, may be injected.

Local applications of oligonucleotides have been and are being developed as intratumoral injections for a variety of therapeutic approaches against tumors. Han et al. used chitosan particles formulated with siRNA for reducing *in vivo* expression of transglutaminase in breast cancer and melanoma (Han et al., 2011). Hydrogels with a gelling temperature of 40°C in combination with gold-containing nanoshells were injected intratumorally to induce a local effect of siRNA by using optical radiation (Strang et al., 2014). Polyethyleneimide-conjugated organophosphazene also exhibits a thermal reaction and was injected intratumorally with siRNA against VEGF and Cyclin B1 (Kim et al., 2012). These approaches were not used perioperatively or as an adjuvant; the focus was solely on achieving a high local concentration and thus a high efficiency against the primary tumor, and on reducing the speed of diffusion of oligonucleotides, compared to small molecules, from the site of action. The same also applies to the study on ductal pancreatic adenocarcinoma (PDA), in which expression of a mutated form of the Kirsten rat sarcoma virus (KRAS) gene was to be reduced (Khvalevsky et al., 2013). In this approach, a polylactide-co-glycolide (PLGA) matrix is used as well. Matrix elements were even sutured to the tumor in order to optimize efficiency against the primary tumor due to extensive contact. Immunostimulatory RNA (CPG 1826, Coley Pharmaceuticals) was administered intratumorally as adjuvant in animal experiments; here, mice were treated with antibodies against OX40, CTLA4, GITR and FR4, but not operated. An improved effect is reported by the authors, this approach was not pursued clinically, however (Houot et al., 2009).

Plasmids and oligonucleotides also have been locally applied already in order to promote wound healing, e.g., plasmids for platelet-derived growth factor (pPDGF) or vascular-endothelial growth factor (pVEGF) in poly (lactic-co-glycolic acid) PLGA nanoparticles (Tokatiian et al., 2014) or prolyl hydroxylase domain 2 (PHD2)-siRNA in acellular dermal matrix as an implant (Vandegrift et al., 2015), or p53 (Nguyen et al., 2010). In these applications, however, the focus is on wound healing after an injury without any connection to tumors. A potential risk of these approaches lies in the fact that the target genes addressed for wound healing may constitute an increased risk for tumor formation. This circumstance is preventing the use of these local approaches in tumor treatments.

According to the state-of-the-art, cytostatics in particular are used as adjuvants after tumor resection. In fact, locally applied cytostatics are expected to exhibit many advantages with respect to their systemic application:

- the substances, which often are hydrophobic, reach the intended site of action within the vicinity of the resected tumor mass much more directly
- the otherwise rapid breakdown of small molecule cytostatics is counteracted by their application via carriers from which the molecules are eluted over a longer period of time. As a result, more cell cycles of the proliferating cells may be covered by the medication.
- adverse effects outside the tumor, which are dose-limiting for many cytostatics, are limited to the area around the surgical cavity and are significantly lower.

The reasons for the limited success of such adjuvant systems so far are not known. To date, Gliadel™, a combination of macroscopic carrier and carmustine as a cytostatic, is the only system approved in the United States and Europe. The approval extends to the adjuvant use in glioblastoma.

The advantages of local, adjuvant application expected for small molecules are not expected for macromolecules. Macromolecules are usually readily soluble and are excreted more slowly than small molecules. In addition, the achievable concentration gain is lower with the local application of macromolecules than with small molecules. The diffusion paths of macromolecules are also shorter compared to those of the small molecules, i.e., they do not penetrate far into the tumor bed and its surroundings. The depth of penetration becomes particularly small if a fibrotic capsule is formed around the carrier during the healing process of the surgical cavity.

In tumor resection, macromolecules are therefore used for other purposes. An elaborate approach was followed with Sitimagene ceradenovec, which approach involves the conversion of a prodrug injected systemically after surgery, which in turn is a small molecule. However, the conversion only occurs locally in cells transfected previously during surgery with a gene coding for herpes simplex thymidine kinase, which exhibits a length of several hundred nucleotides. The gene is packaged in a replication incompetent adenovirus for infecting cells. The gene is expressed after transfection of the cells and causes the expression of herpes simplex thymidine kinase, which in turn converts the Ganciclovir™ prodrug into the active form. By using the small molecule Gangciclovir™, a high local concentration may be achieved.

Antibodies are used in surgical procedures, however, primarily due to their possible use as fluorescence-labeled markers during surgery. Such antibodies are often used, which otherwise serve as systemic tumor therapeutics. Their ability to bind to tumor-specific receptors enables them to serve as tumor cell specific markers. After excitation of fluorescence, the tumor cells thus marked light up and provide the surgeon with information about the spread of the tumor. Antibodies may be injected

locally into the tumor for therapy, i.e., without tumor resection. As adjuvants in the course of tumor resection they are only applied systemically.

For adjuvant, local tumor therapy, there are primarily small molecules available that have so far shown only limited therapeutic success. There is therefore a need for improved adjuvant therapy in order to improve the chances of a cure after tumor resection.

Object of the invention

The object of the invention therefore is the provision of a simple of an adjuvant therapy, to be applied locally within a surgical cavity, by which therapy those tumor cells are destroyed, which remain after at least partial tumor resection and which emerge after resection within the tumor bed and within its vicinity or by which therapy seeding metastases in the vicinity or surroundings of the primary tumor are destroyed.

Solution according to the invention

The object of the invention is achieved surprisingly by the use of tumor-effective oligonucleotides which are applied directly to the tumor bed after resection of the tumor. The oligonucleotides are preferably applied together with resorbable, gel-like or elastic carriers or solid carriers such as gauze material or particles. Also, if the surgical cavity is too complex for other forms of application, a liquid formulation may be preferred in order to reach all potential locations, where tumor cells may be located. In turn, the liquid form may comprise particulate or gel-like particulate carriers. When using a liquid formulation, the volume to be applied is preferably chosen such that it fills the surgical cavity by more than a half, or, in particular, completely. If drainage is required after surgery, the liquid application form may also be applied as a perfusion several times or continuously for a longer period of time. Furthermore, an aerosolic application form may also be preferred, in which the formulated or non-formulated oligonucleotides are aerosolized from a liquid solution and introduced into the surgical cavity.

The effect is surprising because oligonucleotides are expected to be less suitable for this application compared to other classes of molecules, in particular to small molecules, because of their charge, size, lability, their low rate of uptake into cells and due of their rapid degradation. Formulated oligonucleotides are sometimes significantly larger and diffuse even more slowly, rendering them even less suitable for this use according to expectations.

Small molecules, on the other hand, are expected to reach not only the top of a cell layer but also deeper layers of the adjacent tissue after local application. This deep-reaching effect is preferred because tumor cells are also expected there.

A simple form of therapy in the sense of the invention is characterized by using at least one type of oligonucleotide as an active ingredient component, which achieves individually an antitumor effect already. This simple form of therapy is preferred in order to maintain a low risk of undesirable effects.

Preferred embodiments

A treatment using only one application is preferred, since access to the surgical cavity is limited in time, taking into account the rapid development of laparoscopic surgical techniques used for tumor surgery, and, ideally, currently does not exceed more than 30 minutes. Each subsequent opening of the surgical cavity would also increase the risk of disseminating tumor cells.

According to the invention, pleiotropic oligonucleotides are used preferably in order to act on as many tumor cells as possible, irrespective of the phase of the cell cycle in which the cell is currently located, in particular in the case of a single application. According to the invention, pleiotropic oligonucleotides are those oligonucleotides which affect simultaneously in a cell at least two targets, for example two mRNAs with the same target sequence, coding for different proteins. Examples of pleiotropic oligonucleotides are prodrugs according to WO 2010/102615, wherein preferred embodiments are effective in tumor cells against several physiological targets in parallel (WO 2012/098234).

According to the invention, biodegradable carrier materials are preferred exhibiting low immunogenicity, which are characterized by hydrogel-like or elastic properties, and from which oligonucleotides elute by diffusion or during *in vivo* degradation or by changes in environmental parameters such as pH. Carriers comprising medically proven materials such as collagen, atelocollagen, gelatin, fibrin, chitosan or hyaluronic acid, synthetic or recombinant variants thereof and their synthetic modifications are used with particular preference.

It is particularly preferred if the use of oligonucleotides according to the invention is in carriers which are temporarily connected, such that large amounts of oligonucleotide are available locally and over an extended period of time. According to the invention, “temporarily connected” means that the carrier may be firm, elastic or even deformable, but constitutes a unit over several hours or days. Biodegradable carriers also are considered temporarily connected, wherein the carriers become smaller as a result of breakdown processes within the body and which disintegrate into smaller units after hours or days, which are dismantled from a carrier like a gauze, or which undergo a transformation into a liquid phase.

It is particular preferred if the use of the oligonucleotides according to the invention is in large amounts per application. The total amount of oligonucleotides used depends on the surgical cavity and the structure of the surrounding tissue. For the connected carriers, this relationship is described as loading density. It is preferred to use oligonucleotides in carriers with loading densities of more than 3 micrograms of oligonucleotide per milliliter of carrier volume, particularly preferably more than 12, 50, 250, 1000 or 5000 micrograms per milliliter, respectively.

It may also be advantageous to use carriers from which the oligonucleotides elute over an extended period of time, in particular over several days. Carriers that are absorbed by the body are particularly advantageous. Carriers that enable simple laparoscopic handling, such as flexible foils or rods, are particularly advantageous. Oligonucleotides and smaller particulate carriers may be applied in nets, such as nets made of gauze material, or in a hydrogel. It is preferred to use carriers which have an initial elution rate of more than 1 microgram/square centimeter and day, in particular more than 2, 5, 10, 25 or 100 microgram/square centimeter and day, respectively.

According to the invention, the oligonucleotides are used locally after the tumor resection in order to prevent a recurrence or metastases. Accordingly, they are applied during surgery to the ensuing surgical margins after the complete or partial removal of the primary tumor and the surrounding tissue. This also includes surrounding connective and fatty tissues. For mechanical application of the oligonucleotides, gel-like formulations are advantageous, which may be distributed or sprayed onto the tissue with a brush or similar tool.

A suitable carrier material for the use of the oligonucleotides is collagen, which may be provided with gel-like or elastic properties. Collagen is absorbed by the body and exhibits low immunogenicity.

The use of oligonucleotides in liquid formulation according to the invention is advantageous if the oligonucleotides may be distributed in the surgical cavity using a syringe. This form of application is advantageous with respect to complex forms of surgical cavities because the formulation distributes itself within the surgical cavity and may flow out of its openings, just as likely as seeding metastases. “Complex” in the sense of the invention means that the surgical cavity is open to cavities such as the peritoneum, enabling the spread of tumor cells to remote edges of the peritoneum.

A particularly suitable distribution is ensured by the use of liquid volumes that are of similar size, particularly preferably of the same size or even 1, 2, 5 times as large as the volume of the surgical cavity alone or including cavities connected thereto. For example, after removal of an adrenal gland, a surgical cavity of approximately 25 ml is created, which is connected, however, to the much larger retroperitoneal space. Large volumes of liquid enable carrying out perfusions, such that tumor-effective oligonucleotides are continuously supplied to the surgical cavity and high local concentrations of oligonucleotides may be achieved everywhere in the cavity.

The use of oligonucleotides according to the invention as aerosol is also preferred. For this purpose, an overpressure is created in the surgical cavity or in the cavities adjacent thereto. The aerosol is introduced into the resulting cavity by means of a trocar, tube or similar tool or is even generated with an inserted aerosol generator. The aerosol is distributed almost uniformly in the cavity, such that even remote areas of the cavity may be reached. The cavity is maintained open for more than two hours, and particularly more than an hour or half an hour. The use of the oligonucleotides according to the invention in aerosol form may also be repeated several times if access to the cavity is continuous or may be re-established quickly and easily.

It is preferred to use oligonucleotides in aerosols amounting to more than 1 microgram/square centimeter of cavity volume, and particularly preferably of more than 2.5, 10, 25, 100 micrograms.

The use of the oligonucleotides according to the invention includes combinations with methods and therapies known from the prior art. This includes, for example, the simultaneous application of drugs such as cytostatics and oligonucleotides, and also, for example, a radiotherapy carried out in parallel.

Embodiment: adrenocortical carcinoma

Carcinomas of the adrenal cortex are rare in occurrence and as of today are usually treated by laparoscopic adrenalectomy, i.e., the minimally invasive surgical removal of the complete adrenal gland. Prognosis for patients thus treated nevertheless is poor, particularly in the case of late-identified primary tumors, recurrences or metastases are very likely to occur and usually lead to the patient's death within a few months. Laparoscopic adrenalectomy is conducted using either abdominal/transperitoneal or retroperitoneal access. The renal fascia is pierced and a surgical cavity is created, which is pressurized at 20 - 30 mm Hg positive pressure for stabilization. The positive pressure also reduces or prevents bleeding into the surgical cavity following any injury to blood vessels. Subsequently, further incisions are used to mobilize surrounding organs, to sever arteries and veins supplying the adrenal gland, and to expose the adrenal gland. Ideally, the adrenal gland as a whole is dissected together with the surrounding adipose tissue, transferred to a retrieval pouch, which in turn is pulled out of the patient via one of the trocars. A lymphadenectomy may be performed in addition. The surgical cavity is rinsed with distilled water and antibiotics and, in simple cases, closed without drainage. This surgical procedure carefully avoids damaging the tumor capsule as well as leaving any tumor tissue in the patient. Nevertheless, local recurrences of unclear origin commonly occur. Tumor cells may have been transported from the adrenal gland into the environment prior to surgery. This

includes seeding metastases occurring in body cavities on other organs or parts of organs caused by caudal migration of detached tumor cells by gravity. These processes are facilitated by the fact that the renal fascia surrounding the kidney opens medially and caudally into the retroperitoneal space.

According to the invention, oligonucleotides are used locally after tumor resection in order to prevent recurrences or metastases and to fight remaining tumor cells or metastases. To this end, for example, the oligonucleotides are applied during surgery after removal of the adrenal gland to the developing surgical margins and the surrounding tissue. This also includes surrounding connective and fatty tissue, especially the renal fascia. Particularly suitable for this purpose are gel-like formulations which may be applied laparoscopically to the wound edges using an instrument such as a brush. In the surgical cavity of an adrenocortical carcinoma, wound margins develop with a surface area of about 50 square centimeters. Gels are applied with a layer thickness of 0.2 to 1 mm, which corresponds to a gel volume of 1 to 5 ml. This gel volume preferably contains 70 micrograms of oligonucleotide and particularly preferably more than 250 or 1000 micrograms, or 5, 25 or 100 milligrams. The gel is degraded within a few weeks and the oligonucleotides are continuously released.
In a further exemplary embodiment, elastic carriers made of collagen, for example, are used, which are introduced into the surgical cavity. As highly elastic carriers, they are introduced into the surgical cavity via the aperture of a trocar as a capsule. After the encapsulation has been removed, the carrier takes on the size and shape corresponding to the resected organ of about 4 * 3 * 2 cm, having a volume or partial volume of less than 25 ml. The carrier harbors oligonucleotides in amounts of preferably more than 70 micrograms, and particularly preferably more than 250 or 1000 micrograms, or 5, 25 or 100 milligrams. The carrier is degraded within a few weeks and the oligonucleotides are continuously released.

The degradation rate of the gel and the elastic carrier may be controlled by manufacturing parameters, in the case of collagen, for example, by the degree of cross-linking of the collagen. *In vivo* half-lives of more than 1 week are preferred, half-lives of 2 weeks, 4 weeks and 3 months are particularly preferred.

In a further embodiment according to the invention, a liquid formulation for the oligonucleotides is used. Here, the oligonucleotides, for example, are distributed in the surgical cavity by means of a syringe. An advantage of this form of application is that the formulation distributes itself within the surgical cavity and may flow out of its openings, just as likely as seeding metastases. In adrenocortical carcinoma with the aforementioned expansion of the renal fascia, liquid application forms reach its extensions much better than solid application forms. The injected volume in this example is 20 ml, which comprises preferably more than 70 micrograms, and particularly preferred more than 250 or 1000 micrograms, or 5, 25 or 100 milligrams of oligonucleotide. The oligonucleotides may be packed in particles or liposomes such that they are released slowly only when the carriers are degraded or absorbed by the cells. The rate of degradation of the particles may be influenced by manufacturing parameters such as the degree of crosslinking. *In vivo* half-lives of more than 1 week are preferred, half-lives of 2 weeks, 4 weeks and 3 months are particularly preferred.

It may also be advantageous to use carriers exhibiting simpler geometries, from which the oligonucleotides elute over an extended period, in particular over several days. Carriers which may be absorbed by the body are particularly advantageous. Carriers enabling simple laparoscopic handling, such as flexible foils or rods, are particularly advantageous. Smaller particulate carriers may be applied in nets, such as gauze nets, or in a hydrogel.

In the case of adrenocortical carcinoma, the use of the oligonucleotides in an aerosol may be preferred, in particular in amounts of 70 micrograms, and particularly preferred in the amount of more than 250 or 1000 micrograms, or 5, 25 or 100 milligrams of oligonucleotide.

The combination of the use of the oligonucleotides as an adjuvant with adjuvant therapies such as mitotane and the combination with radiotherapy may be preferred.

Embodiment: ovarian cancer

Currently, ovarian cancer is the sixth most common malignant disease in women (Guideline Ovarian Cancer 2013). The surgical removal of an ovary constitutes an essential part of treatment. In the case of ovarian cancer, the result of the diagnosis strongly influences the extent of tissue removal. In addition to the ovary itself, the fallopian tube and lymph nodes, and, in later stages, also other organs may be affected, in particular those extending into or adjacent to the peritoneum. Tumor resection is carried out as far as possible, but complete removal often is not possible in these cases because the surrounding tissue is already affected. In contrast to adrenocortical carcinoma, laparoscopic surgery is not recommended.

Adjuvant chemotherapy is recommended in most cases; an exception to this recommendation relates to cases of very early stages at the time of surgery. Systemic application of carboplatin, a low molecular weight cytostatic, is recommended, which often is associated with significant side effects such as changes in blood count, dysfunction of the liver and nerves, as well as impaired cardiovascular function. In cases of higher staging levels, systemic therapy using paclitaxel and bevacizumab, a monoclonal antibody against VEGF, is also recommended. Relapses nevertheless occur frequently.

According to the invention, the oligonucleotides are used locally after tumor resection in order to prevent recurrences or metastases and to fight remaining tumor cells or metastases. To this end, they are applied, for example, during surgery after removal of the ovary and other tissue to the resulting surgical margins and the surrounding tissue. This also includes surrounding connective and fatty tissue, especially parts of the peritoneum.

The size of an ovary in an adult is about 3.5 * 2 * 1 cm, its volume amounts to about 3 - 6 ml. Similar to adrenocortical carcinoma, various formulations are suitable, including a gel-like formulation. Despite the smaller organ volume compared to the adrenal cortex, volumes of 1 - 5 ml are used preferably since also tissue surrounding the ovary should be coated to a greater extent. The gel volume preferably contains 70 micrograms and particularly preferred more than 250 or 1000 micrograms, or 5, 25 or 100 milligrams. The gel is degraded within a few weeks providing for the continuously release of the oligonucleotides. The oligonucleotides may be packed in particles or liposomes such that they are released slowly only in the case when carriers are degraded or absorbed by the cells. The rate of degradation of the particles may be influenced by manufacturing parameters such as the degree of crosslinking. *In vivo* half-lives of more than 1 week are preferred, half-lives of 2 weeks, 4 weeks and 3 months are particularly preferred.

In a further exemplary embodiment, elastic carriers made of collagen, for example, are used, which are introduced into the surgical cavity and which completely or partially expand to the volume of an ovary. A carrier harbors oligonucleotides in amounts of preferably more than 70 micrograms, and particularly preferred of more than 250 or 1000 micrograms, or 5, 25 or 100 milligrams. The carrier is degraded by the body within a few weeks, allowing for a continuous release of the oligonucleotides.

The degradation rate of the gel and the elastic carrier may be controlled by manufacturing parameters, in the case of collagen, for example, by the degree of cross-linking of the collagen. *In vivo* half-lives of more than 1 week are preferred, half-lives of 2 weeks, 4 weeks and 3 months are particularly preferred.

In cases of ovarian cancer, a liquid formulation of the oligonucleotides may also be advantageous, since the peritoneum surrounding the ovaries is characterized by a large extension. The injected volume for ovarian cancer in this example is 20 ml, comprising preferably more than 70 micrograms, and particularly preferred more than 250 or 1000 micrograms, or 5, 25 or 100 milligrams of oligonucleotide. The oligonucleotides may be packed in particles or liposomes such that they are released slowly only when the carriers are degraded or absorbed by the cells. The rate of degradation of the particles may be influenced by manufacturing parameters such as the degree of crosslinking. *In vivo* half-lives of more than 1 week are preferred, half-lives of 2 weeks, 4 weeks and 3 months are particularly preferred.

It may also be advantageous to use carriers exhibiting simpler geometries, from which the oligonucleotides elute over an extended period, in particular over several days. Carriers which may be absorbed by the body, such as flexible foils or rods, are particularly advantageous. Smaller particulate carriers may be applied in nets, such as gauze nets, or in a hydrogel.

In the case of ovarian cancer, the use of the oligonucleotides in an aerosol may be preferred, in particular in amounts of 70 micrograms, and particularly preferred in the amount of more than 250 or 1000 micrograms, or 5, 25 or 100 milligrams of oligonucleotide.

The combination of the use of the oligonucleotides as an adjuvant with adjuvant cytostatics such as carboplatin, cisplatin, paclitaxel and bevacizumab, and the combination with radiation therapy may be preferred.

Exemplary embodiment: mesothelioma

Malignant diffuse mesothelioma is a tumor originating from the mesothelial or submesothelial cells of the pleura, peritoneum or pericardium. The prognosis for patients with malignant pleural mesothelioma is poor with median survival times of 4 to 12 months. A curative treatment is currently not available.

The majority (> 80%) of mesotheliomas originate from the pleura. Malignant mesotheliomas are comparatively rare. Most commonly they signal a previous exposure to asbestos (Neumann et al., 2013). It is also expected that cases of mesothelioma, e.g., after the attack on the World Trade Center in New York on September 11, 2001, will increase significantly in numbers during the next 15 years among those exposed at that time (Povtak, 2016). Depending on the subtype of mesothelioma, in more than 50% of pleural mesotheliomas tumor cells are released into the associated pleural effusion.

There is no standard therapy for treating mesothelioma. Recommended therapy concepts range from standalone symptomatic treatment to aggressive multimodal treatment comprising surgery, chemotherapy and radiation. Currently, two surgical strategies exist, namely, pleurectomy/ ecortication or extrapleural pleuropneumectomy, in order to achieve as complete a macroscopic tumor removal as possible. These resections are not performed laparoscopically. Due to the diffuse growth of the mesothelioma, however, a complete tumor removal generally is not possible. Residual parts of the tumor remain, which often are detectable only by microscope, in the case of which adjuvant radiotherapy and chemotherapy are recommended (Rice, 2011). According to the prior art, a combination of intraperitoneal carboplatin and pemetrexed is recommended. Regarding patient survival, successes were noted by the adjuvant use of check point inhibitors (Scherpereel et al 2017).

In contrast to carcinomas of the adrenal cortex and ovary, the size of the surgical cavity differs widely; after resection of a lung in pleural mesothelioma, it may amount to a size of several liters with an area of several hundred square centimeters. In the case of these volumes, a gel may also be used advantageously and applied with tools such as brushes; concentrations of more than 15 or 70 micrograms per milliliter of gel are preferred, particularly preferred of more than 250 or 1000 micrograms, or 5, 25 or 100 milligrams per milliliter of gel.

When preserving the affected lung, a liquid formulation is suitable, preferably at concentrations of more than 15 or 70 micrograms per milliliter and particularly preferred at more than 250 or 1000 micrograms, or 5, 25 or 100 milligrams per milliliter.

In the case of large-volume resections, elastic carriers having a total volume corresponding to that of the resected organs are no longer useful. Instead, flat carriers such as membranes, foils or gauze are used preferably, harboring oligonucleotides directly, packaged in particles such as liposomes, conjugated or formulated as a gel. Loading densities of 10 micrograms per square centimeter are preferred, particularly preferred are densities of 50 or 200 micrograms, or 1, 5 or 20 milligrams of oligonucleotide per square centimeter. It is preferred to apply oligonucleotides to implants, such as those used after resection of the diaphragm, with the same oligonucleotide density.

Also in the case of mesothelioma, the use of supports with a simpler geometry may be preferred, in particular if the oligonucleotides elute from these supports over a longer period, in particular over several days. Carriers that are absorbed by the body, such as flexible foils or rods, are particularly advantageous. Smaller particulate carriers can be applied in nets, such as gauze nets, or in a hydrogel.

In mesothelioma, the use of the oligonucleotides in an aerosol may be preferred, in particular in amounts of 70 micrograms, and particularly preferred in the amount of more than 250 or 1000 micrograms, or 5, 25 or 100 milligrams of oligonucleotide.

The combination of the use of the oligonucleotides as an adjuvant with adjuvant therapies such as carboplatin and pemetrexed, and the combination with radiotherapy may be preferred.

Embodiment: glioblastoma

Glioblastomas belong to the diffusely infiltrating, highly malignant gliomas and are the most common brain neoplasms with a share of 16%. According to the WHO classification, they are classified as grade IV tumors associated with a poor prognosis. Since glioblastomas exhibited a markedly infiltrative growth, a cure by surgical resection of the tumor is not possible. It is a goal to reduce the tumor mass surgically as complete as possible. Adjuvant therapy, combining radiotherapy with chemotherapy, is therefore recommended according to the European Organization for Research and Treatment of Cancer and National Cancer Institute of Canada Clinical Trials Group (EORTC-NCIC) protocol (Javamanne et al., 2018). It commences the earliest at four weeks after the operation, however, when the surgical wound healing process has progressed. The current treatment standard is temozolomide (Davis, 2016), an oral alkylating chemotherapy drug exhibiting genotoxicity and teratogenicity. An intraoperative deposition of Gliadel ™ wafers as adjuvant as described above constitutes a treatment variant not part of the standard of treatment, but which also is carried out together with temozolomide treatment. In contrast, the simultaneous use of temozolomide and bevavizumab is not recommended (Holdhoff et al., 2011). A newer experimental option of treatment includes DCVax, a procedure in which monocytes are removed from the patient and differentiated extracorporeally into dendritic cells carrying tumor antigens, and which are subsequently transferred back to the patient., however,

In glioblastoma, size and shape of the surgical cavity differ individually. The size of the surgical cavity, however, also amounts to a few milliliters, making it comparable to that of adrenocortical and ovarian carcinoma. The shape is different, however, and as individual as the size. In addition, partial resections are performed almost exclusively in the case of glioblastoma.

For the use according to the invention in the case of glioblastoma, gel-like formulations, which are applied to the wound edges with an instrument such as a brush, are also particularly suitable. The gel volume of 1 to 5 milliliters is preferred to comprise 70 micrograms, particularly preferred to comprise more than 250 or 1000 micrograms, or 5, 25 or 100 milligrams. The gel is degraded within a few weeks allowing for continuous release of the oligonucleotides. The oligonucleotides may be packed in particles or liposomes such that they are released slowly only when the carriers are degraded or absorbed by the cells. The rate of degradation of the particles may be influenced by manufacturing parameters such as the degree of crosslinking. *In vivo* half-lives of more than 1 week are preferred, half-lives of 2 weeks, 4 weeks, 3 months or the whole year are particularly preferred.

In a further exemplary embodiment, elastic carriers made of collagen, for example, are used, which are introduced into the surgical cavity and which completely or partially expand to the volume of the surgical cavity. The one or more carrier harbors oligonucleotides in amounts of preferably more than 70 micrograms, particularly preferred of more than 250 or 1000 micrograms, or 5, 25 or 100 milligrams. The one or more carriers is degraded by the body within a few weeks, allowing for a continuous release of the oligonucleotides.

The degradation rate of a gel or of an elastic carrier may be controlled by manufacturing parameters, in the case of collagen, for example, by the degree of cross-linking of the collagen. *In vivo* half-lives of more than 1 week are preferred, half-lives of 2 weeks, 4 weeks and 3 months are particularly preferred.

In cases of glioblastoma, a liquid formulation of the oligonucleotides may also be advantageous, since it is not known whether the glioblastoma also spreads in a manner other than infiltrating. In the case of glioblastoma, the injected volume, for example, amounts to 20 ml, comprising preferably more than 70 micrograms, particularly preferred more than 250 or 1000 micrograms, or 5, 25 or 100 milligrams of oligonucleotide. The oligonucleotides may be packed in particles or liposomes such that they are released slowly only when the carriers are degraded or absorbed by the cells. The rate of degradation of the particles may be influenced by manufacturing parameters such as the degree of crosslinking. *In vivo* half-lives of more than 1 week are preferred, half-lives of 2 weeks, 4 weeks and 3 months are particularly preferred.

In the case of glioblastoma, the use of the oligonucleotides in an aerosol may be preferred, in particular in amounts of 70 micrograms, and particularly preferred in the amount of more than 250 or 1000 micrograms, or 5, 25 or 100 milligrams of oligonucleotide.

It may also be advantageous to use carriers exhibiting simpler geometries, from which the oligonucleotides elute over an extended period, in particular over several days. Carriers which may be absorbed by the body, such as flexible foils or rods, are particularly advantageous. Smaller particulate carriers may be applied in nets, such as gauze nets, or in a hydrogel.Another example are wafers as used in Gliadel.

The combination of the use of the oligonucleotides as an adjuvant with adjuvant cytostatics such as temozolomide, with cellular therapies such as DCVax, and the combination with radiation may be preferred.

Embodiment: Peritoneal carcinosis

Peritoneal carcinosis refers to the infestation of the peritoneum with multiple malignant tumor cells. The cause of peritoneal cancer is usually not a tumor of the peritoneum itself, but a malignant tumor of another abdominal organ. Most commonly, this is an advanced metastatic tumor of the gastrointestinal tract, of the pancreas or ovaries, as described above. In some cases, and identification of the primary tumor is not possible (https://flexikon.doccheck.com/de/Peritonealkarzinose).

With 15-20 cases per 100,000 people, peritoneal carcinosis occurs rarely, but with an increasing tendency; in Germany there are about 35,000 new cases per year (Glockzin et al., 2007). The life time prognosis in peritoneal cancer is on average about 6 months after diagnosis.

Unless advanced, peritoneal cancer is being treated increasingly using multimodal therapy combining surgical cytoreduction with an intraoperative, hyperthermal intraperitoneal chemotherapy (Piso et al., 2011). PIPAC, as described above, also is used.

In contrast to the adrenocortical and ovarian carcinomas, the size of the surgical cavity differs greatly due to the varying number of affected organs. The use of a gel is nevertheless advantageous for these volumes and wherein the gel is applied with tools such as brushes; concentrations of more than 15 or 70 micrograms per milliliter of gel are preferred, particularly preferred are concentrations of more than 250 or 1000 micrograms, or 5, 25 or 100 milligrams per milliliter of gel.

In the case of large-volume resections, elastic carriers having a total volume corresponding to that of the resected organs are no longer useful. Instead, flat carriers such as membranes, foils or gauze are used preferably, harboring oligonucleotides directly, packaged in particles such as liposomes, conjugated or formulated as a gel. Loading densities of 10 micrograms per square centimeter are preferred, particularly preferred are densities of 50 or 200 micrograms, or 1, 5 or 20 milligrams of oligonucleotide per square centimeter. It is preferred to apply oligonucleotides to implants, such as those used after resection of the diaphragm, with the same oligonucleotide density.

Also in the case of peritoneal carcinosis the use of supports with a simpler geometry may be preferred, in particular if the oligonucleotides elute from these supports over a longer period, in particular over several days. Carriers that are absorbed by the body, such as flexible foils or rods, are particularly advantageous. Smaller particulate carriers can be applied in nets, such as gauze nets, or in a hydrogel.

In peritoneal carcinosis, the use of the oligonucleotides in an aerosol may be preferred, in particular in amounts of 70 micrograms, and particularly preferred in the amount of more than 250 or 1000 micrograms, or 5, 25 or 100 milligrams of oligonucleotide.

The combination of the use of the oligonucleotides as an adjuvant with adjuvant therapies such as carboplatin and pemetrexed, and the combination with radiotherapy may be preferred.

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Claims

1. Use of a tumor-effective oligonucleotide in a tumor patient after complete or partial resection or ablation of a solid tumor of this patient, characterized in that the oligonucleotide is applied in a body cavity created during the resection or ablation in order to fight tumor cells remaining in the tumor bed and within its vicinity after surgery or ablation or to fight metastases, and in order to counteract recurrences or the formation of new metastases in this area.

2. Use of an oligonucleotide according to claim 1, characterized in that the oligonucleotide used has a pleiotropic effect in at least one type of tumor cell.

3. Use of an oligonucleotide according to anyone of claims 1 or 2, characterized in that it is applied in a gel, in a liquid or an elastic material.

4. Use of an oligonucleotide according to any one of claims 1, 2 or 3 as a load of a carrier in the form of gel, liquid or an elastic material, characterized in that the carrier comprises oligonucleotides preferably at a concentration of 15 or 70 micrograms per milliliter, and particularly preferred at a concentration of more than 250 or 1000 micrograms, or 5, 25 or 100 milligrams per milliliter.

5. Use of an oligonucleotide according to claim 4, characterized in that the carrier consists of resorbable elastic or gel-like material and at least partially reproduces the shape of the resected organ.

6. Use of an oligonucleotide according to any one of the claims 1, 2 or 3 as a load of a flat carrier such as membranes, foils or gauze, wherein the oligonucleotides are carried directly, packaged in particles such as liposomes, conjugated or formulated as a gel, characterized in that that the loading densities are at least 10 micrograms per square centimeter, particularly preferred 50 or 200 micrograms, or 1, 5 or 20 milligrams of oligonucleotide per square centimeter.

7. Use of an oligonucleotide according to any one of claims 4 to 6, characterized in that the carrier contains materials with low immunogenicity, in particular collagen, atelocollagen, gelatin, chitosan or hyaluronic acid.

8. Use of an oligonucleotide according to claim 6 or 7, characterized in that the carrier additionally has markers for specific detection, in particular absorptive or fluorescent dyes.

9. Use of an oligonucleotide according to any one of claims 4 to 8, characterized in that the crosslinking or other stabilization of the carrier is stopped such that the carrier material is degraded in the patient's body at a preferred rate, in particular with an *in vivo* half-life of more than a week, and particularly preferred with a half-life of more than 2 weeks, 4 weeks and 3 months.

10. Use of an oligonucleotide according to any one of claims 4 to 9, characterized in that in addition to the oligonucleotides, other tumor-effective molecules, in particular cytostatics, are applied to the carrier in therapeutically relevant dosages.

11. Use of an oligonucleotide according to any one of claims 4 to 10, characterized in that carriers are combined with each another, in particular gel or gauze in combination with liquid formulation, such as to reach optimally tumor cells which are located at a distance from the wound edge.

12. Use of an oligonucleotide according to any one of claims 4 to 11, characterized in that the diagnostic elements are integrated in the carrier, in particular sensors for heart rate, LDL or HDL concentrations.

13. Use of the oligonucleotides according to any one of claims 1 to 12, characterized in that standard treatments, such as operations, aftercare and adjuvant chemotherapy, are carried out in parallel with or almost simultaneously with the method according to the invention.

14. Use of oligonucleotides according to any one of claims 1 to 13 for the adjuvant therapy of tumors, in particular of adrenocortical carcinoma, ovarian cancer, mesothelioma and glioblastoma.