# Antibodies and molecular conjugates thereof targeting uPAR

## Technical field

The present invention relates to antibodies and molecular conjugates targeting the receptor uPAR, in particular antibody-drug conjugates (ADCs) of said antibodies and their use in delivery of active agents to cells and tissues expressing uPAR. The invention further relates to the use of said ADCs in the treatment of diseases involving uPAR expressing cells, such as certain cancers.

## Background

Urokinase-type plasminogen activator receptor (uPAR), also known as CD87 is a glycolipid-anchored membrane protein part of the plasminogen activation system, which is a well-established tumor target, due to its aberrant overexpression, pathophysiological role, and negative prognostic value in virtually all human aggressive cancers. The attractive expression profile of this receptor, namely, the simultaneous presence on both neoplastic cells and associated tumor microenvironment, coupled with the almost undetectable baseline levels in normal tissues, and an efficient endocytic ability, have fostered a gradual shift in the corresponding therapeutic landscape from function inhibition to cytotoxic approaches to selectively eradicate the uPAR-expressing cells utilizing a targeted cytotoxic insult (Metrangolo et al., 2021).

One of the explored therapeutic strategies involves the use of antibody-drug conjugates (ADCs), an emerging and actively growing class of anti-cancer therapeutics that have reached clinical and regulatory milestones, with +10 FDA-approved ADCs currently marketed for oncologic indications, and over hundreds under advanced clinical and preclinical evaluation. ADCs are designed as prodrugs consisting of a preferably tumor-selective monoclonal antibody (mAb) chemically linked to a cytotoxic moiety, which is selectively delivered into the target cells, upon receptor binding and subsequent internalization, leading to tumor-selective kill.
This approach confers a more favorable therapeutic profile to the cytotoxic agent and advantageously limits off-target toxicity to healthy tissues, a typical drawback of standard chemo- and radiotherapies (Dumontet C et al., 2023).
Two published examples of anti-uPAR ADCs exist (Lourenço et al., 2023; Harel et al., 2019), both in relation to the treatment of triple-negative breast cancer.

Treatment methods currently exist for most cancer types. However, in most cases with unsatisfactory efficiency or with detrimental side effects due to the lack of specificity of the treatment. Thus, there is a need for more potent treatments with increased specificity, especially against very aggressive types of cancer such as those characterized by overexpression of the uPAR receptor.

**Summary**

The present invention relates to novel antibodies or antigen-binding fragments thereof directed against the uPAR receptor, as well as antibody-drug conjugates (ADCs) based thereon. The ADCs discloses within the present invention demonstrate a high-affinity, cross-species reactivity and applicability as a therapeutic agent for various aggressive uPAR-positive malignancies with unmet medical needs, particularly pancreatic ductal adenocarcinoma (PDAC), which stands outs as the cancer indication with the highest receptor levels, both in cancer and stromal compartments, and largest expression separation between neoplastic and normal tissues. Notwithstanding, the consistent evidence supporting uPAR's pro-invasive role in advancing PDAC, no uPAR-targeted ADCs are currently approved or being pre- or clinically investigated for this oncological indication (Kumar et al., 2022).

One aspect of the present invention is thus an antibody or antigen-binding fragment thereof which binds to the Urokinase-type plasminogen activator receptor (uPAR), wherein said antibody is selected from the group consisting of:

a. an antibody or antigen-binding fragment thereof comprising
    i. an immunoglobulin light chain comprising or consisting of the amino acid sequence of SEQ ID NO: 5 or a sequence having at least 90% sequence identity thereto, and
    ii. an immunoglobulin heavy chain comprising or consisting of the amino acid sequence of SEQ ID NO: 10 or a sequence having at least 90% sequence identity thereto,
b. an antibody or antigen-binding fragment thereof comprising
    i. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 6 or a sequence having at least 90% sequence identity thereto, and

ii. an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 11 or a sequence having at least 90% sequence identity thereto,

c. an antibody or antigen-binding fragment thereof comprising

i. an immunoglobulin light chain variable region comprising as complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 35, as CDR2 an amino acid sequence of SEQ ID NO: 36, and as CDR3 and amino acid sequence of SEQ ID NO: 37, and

ii. an immunoglobulin heavy chain variable region comprising as complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 38, as CDR2 an amino acid sequence of SEQ ID NO: 39, and as CDR3 and amino acid sequence of SEQ ID NO: 40,

d. an antibody or antigen-binding fragment thereof comprising

i. an immunoglobulin light chain variable region comprising as complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 7, as CDR2 an amino acid sequence of SEQ ID NO: 8, and as CDR3 and amino acid sequence of SEQ ID NO: 9, and

ii. an immunoglobulin heavy chain variable region comprising as complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 12, as CDR2 an amino acid sequence of SEQ ID NO: 13, and as CDR3 and amino acid sequence of SEQ ID NO: 14,

e. a humanized version of the antibody or antigen-binding fragment thereof of any one of a, b, c or d.

f. a chimeric version of the antibody or antigen-binding fragment thereof of any one of a, b, c or d,

g. an antibody or antigen-binding fragment thereof comprising

i. an immunoglobulin light chain variable region comprising or consisting of an amino acid sequence selected from SEQ ID NOs: 53-57 or a sequence having at least 90% sequence identity thereto, and

ii. an immunoglobulin heavy chain variable region comprising or consisting of an amino acid sequence selected from SEQ ID NOs: 47-51 or a sequence having at least 90% sequence identity thereto.

Another aspect of the present invention is an antibody or antigen-binding fragment thereof which binds to the Urokinase-type plasminogen activator receptor (uPAR), wherein said antibody is selected from the group consisting of:

a. an antibody or antigen-binding fragment thereof comprising
   i. an immunoglobulin light chain comprising or consisting of the amino acid sequence of SEQ ID NO: 15 or a sequence having at least 90% sequence identity thereto, and
   ii. an immunoglobulin heavy chain comprising or consisting of the amino acid sequence of SEQ ID NO: 20 or a sequence having at least 90% sequence identity thereto,
b. an antibody or antigen-binding fragment thereof comprising
   iii. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 16 or a sequence having at least 90% sequence identity thereto, and
   iv. an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 21 or a sequence having at least 90% sequence identity thereto,
c. an antibody or antigen-binding fragment thereof comprising
   v. an immunoglobulin light chain variable region comprising as complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 17, as CDR2 an amino acid sequence of SEQ ID NO: 18, and as CDR3 and amino acid sequence of SEQ ID NO: 19, and
   vi. an immunoglobulin heavy chain variable region comprising as complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 22, as CDR2 an amino acid sequence of SEQ ID NO: 23, and as CDR3 and amino acid sequence of SEQ ID NO: 24,
d. a humanized version of the antibody or antigen-binding fragment thereof of any one of a, b, or c,

e. a chimeric version of the antibody or antigen-binding fragment thereof of any one of a, b, or c.

Yet another aspect of the present invention is an antibody or antigen-binding fragment thereof which binds to the Urokinase-type plasminogen activator receptor (uPAR), wherein said antibody is selected from the group consisting of:

a. an antibody or antigen-binding fragment thereof comprising
   vii. an immunoglobulin light chain comprising or consisting of the amino acid sequence of SEQ ID NO: 25 or a sequence having at least 90% sequence identity thereto, and
   viii. an immunoglobulin heavy chain comprising or consisting of the amino acid sequence of SEQ ID NO: 30 or a sequence having at least 90% sequence identity thereto,
b. an antibody or antigen-binding fragment thereof comprising
   ix. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 26 or a sequence having at least 90% sequence identity thereto, and
   x. an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 31 or a sequence having at least 90% sequence identity thereto,
c. an antibody or antigen-binding fragment thereof comprising
   xi. an immunoglobulin light chain variable region comprising as complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 27, as CDR2 an amino acid sequence of SEQ ID NO: 28, and as CDR3 and amino acid sequence of SEQ ID NO: 29, and
   xii. an immunoglobulin heavy chain variable region comprising as complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 32, as CDR2 an amino acid sequence of SEQ ID NO: 33, and as CDR3 and amino acid sequence of SEQ ID NO: 34,
d. a humanized version of the antibody or antigen-binding fragment thereof of any one of a, b, or c,
e. a chimeric version of the antibody or antigen-binding fragment thereof of any one of a, b, or c.

Further aspects of the present invention includes also antibody-drug conjugates comprising the antibodies or antigen-binding fragments thereof defined herein and further comprising an active agent, and optionally a linker which links the antibody or antigen-binding fragment thereof to the active agent.

Even further aspects of the present invention includes also methods for treatment of a disease characterised by cells expressing and/or overexpressing uPAR, said method comprising administering to a subject the antibody as defined herein above, the ADCs as defined herein above, or a pharmaceutical composition comprising the antibody or the ADCs as defined herein above.

**Description of Drawings**

**Figure 1:** The topographic landscape of mAbs binding epitopes on uPAR-receptor. Surface representation of uPAR in its semi-open conformation (PDB code: 1YWH). The epitopes for FL1 (domain D2), FL2 (domain D1), and FL3 (domain D3) are highlighted.

**Figure 2:** Internalization profile of a-uPAR mAbs in uPAR-positive U937 leukemic cells. Histograms showing internalized fractions of Iodine-125 ($^{125}$I)-labelled a-uPAR mAbs in the reference uPAR-positive leukemic cell line, U937, as quantified by radioactive-based internalization assay (see Examples), after 4h of incubation at 37° C. The cross-reactive FL1 mAb, exhibiting the highest internalization degree, was selected as the lead candidate for ADC development. NC: negative control.

**Figure 3:** Schematical representation of FL1 linked to DBCO-PEG4-VC-PAB-DMEA-PNU-159682.
FL1-based PNU ADCs harbor two linker-payload moieties coupled at the conserved N-Glycans sites of the Fc fragment of the mAb via Click chemistry, as described in the Examples section. FL1 linked to DBCO-PEG4-VC-PAB-DMEA-PNU-159682 is also referred to as FL1-PNU ADC herein.

**Figure 4:** FL1-PNU-ADC exhibits robust and selective *in vitro* cytotoxicity in uPAR-expressing PDAC cells.
Dose-response curves showing *in vitro* antitumor activity of FL1-PNU ADC, in the target panel of cells, as compared with unmodified Ab, NC (Negative Control) aTNP-

PNU ADC, and free payload. Cells were incubated with serial dilutions of test compounds and cell viability was assessed by a colorimetric assay (MTS assay), after 76 (unmodified Ab and free toxin) and 120 h (ADCs), respectively, and expressed as a relative percentage to untreated control cells. While the free toxin was indiscriminately potent across the entire cell panel, regardless of the concentration tested, and uPAR status, the unconjugated FL1 did not exert any antiproliferative activity. Conversely, robust cell killing was elicited by the derived FL1-PNU conjugate, with a clear concentration-dependent profile in all receptor-positive cells, and average picomolar EC50 values. Conversely, the uPAR null KPC cells remained largely unaffected, as the ones treated with the negative control ADC, aTNP-PNU, which confirmed the on-target antitumor effect by FL1-PNU. In both cases, non-specific cell killing was only observed at the highest concentrations, although still leaving a notably broad therapeutic window for FL1-PNU selective activity.

**Figure 5:** *In vitro* cytotoxicity in additional relevant uPAR-positive oncological indications.
Besides PDAC cells, the efficacy of FL1-PNU ADC was tested in other relevant uPAR-positive cancer cell types, respectively, the leukemic cell line U937, the glioblastoma cells U87, the triple-negative breast cancer cells MDA-MB-231, and REN-luc, a mesothelioma cell line. Cells were incubated with serial dilutions of FL1-PNU ADC and negative control aTNP-FL1 ADC and cell viability was assessed by a colorimetric assay (MTS assay) after 120 h, and expressed as a relative percentage to untreated control cells. Treatment with FL1-PNU ADC yielded a potent and dose-dependent killing response in all examined cells, with a large window of specificity, as shown by the unresponsiveness to the non-targeted control ADC based on a-TNP.

**Figure 6:** *In vivo* efficacy of FL1-PNU-ADC in xenograft and orthotopic PDAC models.
Balb-c nude mice were subcutaneously engrafted with human PDAC AsPC1 cells. When the tumors reached 50-120 $mm^3$, mice were randomized into three groups of 11-12, and subsequently administered with three cycles of a weekly intravenous (i.v.) dose of 0.75 mg/kg of ADC products, respectively FL1-PNU and the non-binding control ADC, aTNP-PNU, or PBS vehicle control. Animals were followed for a total of 62 days, with tumor volume and body weight measured three times per week (Fig. 6A). Compared to the PBS and negative control (NC) ADC arms, sacrificed due to excessive tumor burden, tumors shrunk in the FL1-PNU-treated group, upon the

second dosing, with complete eradication attained in all but one mouse after treatment cessation. In five of the animals, small palpable tumors reoccurred around day 48 but subsequently halted to a stable disease until the end of the observation period. A full cure was achieved in 6 out of 12 mice, with no sign of relapse over the entire follow-up. Accordingly, the antitumor effect of FL1-PNU translated to a highly significant long-term survival benefit, compared to both control groups, with 9 mice surviving until the endpoint (Fig. 6B). None of the groups experienced weight loss (Fig. 6C).

A Schematic design and timeline of treatment in the orthotopic PDAC model is presented in Fig. 6D. Female C567BL/6 mice (n = 8-10) were orthotopically injected into the head of the pancreas with luciferase-expressing KPC2 cancer cells. Tumor growth was monitored by IVIS imaging. Treatment was started on day 14 from tumor implantation following mice randomization and using the same weekly schedule as in A), for a total of two doses (0.75 mg/kg ADC).

Fig. 6E shows pictures of harvested tumors for each treatment group at the endpoint (Day 28).

Fig. 6F shows mean of tumor weights (in grams) at the endpoint (Day 28) for each treatment group.

Fig 6G shows mouse body weight measured twice a week and expressed relative to initial body weight. FL1-PNU ADC was able to induce a profound antitumor effect reflected by a 3- and 2.2-fold reduction of final tumor weights compared to the vehicle- and isotype-control ADC-treated groups, respectively, with no weight loss observed over the treatment period. *$p \leq 0.05$, ***, $p \leq 0.001$, ns: non-significant (by one-way ANOVA, GraphPad Prism 9). Both studies demonstrate a highly potent and selective efficacy of anti-uPAR FL1-PNU ADC across various models of PDAC, accompanied by an overall favourable tolerability profile.

**Figure 7:** FL1-PNU reshapes the suppressive TME and promotes immunomodulation. Immunophenotyping was performed to investigate the ADC impact on the immune microenvironment, including main myeloid and lymphoid lineage markers. A significant increase of both T- and B-lymphocytes was verified in the FL1-PNU ADC-treated samples, which was almost absent in the PBS ones. On the myeloid ($CD11b^+$) cell compartment, this population which constituted nearly 90% of immune cells in the control samples, was almost halved in the FL1-PNU treated group. A similar reduction was observed in the percentage of $Ly6G^+/Ly6C^{lo/in}$, corresponding to neutrophils/polymorphonuclear myeloid-derived suppressor cells (PMN-MDSC), which

dominated the myeloid compartment in the vehicle-treated samples. For the Ly6C$^{hi}$ cells identifying monocytes or macrophages with the classical inflammatory phenotype and lacking in the vehicle-treated tumors, were significantly expanded in the FL1-PNU-ADC-treated ones. *, $p \leq 0.05$; **, $P \leq 0.01$; ***, $p \leq 0.001$ (by one-way ANOVA, GraphPad Prism 9).

**Figure 8**: Comparison of in vitro efficacy of FL1-PNU, 2G10-PNU and ATN658-PNU conjugates. To compare the efficacy of ADCs comprising FL1 with 2G10 and ATN658, the antibodies were conjugated with the same linker payload and coupling method as described in the Examples. The conjugates' efficacy was assessed on the uPAR-positive TNBC cells MDA-MB-231 after four days of treatment, as described in Figure 4. FL1-PNU appears more potent than 2G10-PNU and ATN658-PNU, confirming FL1 as a superior anti-uPAR mAb candidate in an ADC format.

**Figure 9**: Comparison of in vitro efficacy of FL1-PNU, FL2-PNU, FL3-PNU, and 2G10-PNU conjugates. To compare the efficacy of ADCs comprising FL1, FL2, and FL3 mAbs with 2G10, the antibodies were conjugated with the same linker payload and coupling method as described in the Examples. The conjugates' efficacy was assessed on the uPAR-positive TNBC cells MDA-MB-231 after three days of treatment, as described in Figure 4. FL1-PNU appears more potent than all other tested conjugates including 2G10-PNU, which confirms FL1 as a superior anti-uPAR mAb candidate in an ADC format. FL2-PNU appears more potent than 2G10 and FL3-PNU, respectively.

**Figure 10**: Comparison of in vitro efficacy of FL1-PNU and the humanized candidates HC1-LC4-PNU and HC5-LC3-PNU. To compare the efficacy of an ADC comprising FL1 with ADCs comprising the humanized mAb candidates comprising VH1 (SEQ ID NO: 47) and VL4 (SEQ ID NO: 56), referred to as HC1-LC4, and VH5 (SEQ ID NO: 51) and VL3 (SEQ ID NO: 55), referred to as HC5-LC3, the antibodies were conjugated with the same linker payload and coupling method as described in the Examples for PNU ADCs. The conjugates' efficacy was assessed on the uPAR-positive TNBC cells MDA-MB-231 after three days of treatment, as described in Figure 4. Hyhel-PNU was included as a non-targeting negative control mAb. HC1-LC4-PNU and HC5-LC3-PNU display similar potency and appear more potent than the parent FL1-PNU based on murine FL1.

**Figure 11**: In vitro efficacy of DAR 4 FL1-MMAE. Cells were incubated with serial dilutions of ADCs (0.2 – 1.2 x $10^{-5}$ µM) and cell viability was assessed after 120 h as described in Figure 4. FL1-MMAE induces uPAR-specific dose responses with EC50 values of 3.7 and 0.36 nM for MDA-MB-231 and HT-1080, respectively.

**Figure 12**: Analysis of uPAR-expression in cultured fibroblasts and M2-polarized BMDM and normal mouse skin. (A) Flow cytometry analysis of surface uPAR-levels in human 1BR3.G, murine NIH3T3 fibroblast cells, and M2-differentiated BMDM. Bone marrow precursor cells were isolated from the pelvis, femur, and tibia of FVB/N mice (8-10 weeks). The collected cells were then differentiated to M2-like macrophages in 96-well plates (5 x $10^4$ cells in 100 uL per well) in media (DMEM, 10% FBS, 1% P/S) containing 20 ng/mL mM-CSF (Biotechne) for four days, followed by an additional two days of stimulation with 20 ng/mL mM-CSF and 20 ng/mL mIL-4 (Biotechne). For the flow cytometry analysis, cells were incubated with A647-labeled-FL1 at a pre-determined saturating concentration on ice in the dark for 1 hour. After staining, samples were stained wth 1 µg/ml with 7-AAD (Invitrogen, # 1890506) for cell death exclusion and finally acquired on a BD LSR Fortessa 20X (BD Biosciences). The detected uPAR-positive signal on the surface is expressed as median fluorescence intensity, MFI. (B) Immunohistochemical staining of uPAR in normal mouse skin. Mouse skin from naïve C57BL/6 mice was immunostained for muPAR using a rabbit anti-smuPAR pAb P47. Weak uPAR staining is detected in sparse quiescent fibroblasts in the skin (black arrows). Scale bar, 100 µm.

**Figure 13**: FL1-PNU targets uPAR-positive immune and stromal cells and induces bystander killing of uPAR-negative cancer cells. (A) Cell viability profiles of M2-polarized BMDM, human and murine fibroblast cell lines, 1BR3.G and NIH3T3, as assessed by MTS assay following a three-day exposure to FL1-PNU and aTNP-PNU ADCs (n = 3, ± SD). (B) Scheme of established transwell co-culture systems. $uPAR^{KO}$ KPC2 cancer cells were seeded in 6-well transwell plates, while uPAR-positive fibroblasts (1BR3.G or NIHT3T) were seeded in the upper transwell permeable inserts with a membrane pore size of 0.4 µm. FL1-PNU and aTNP-PNU ADCs were added at 4 nM [EC80 for 1BR3.G and NIH3T3, (C)] to the upper chamber, and viable cells in both compartments were quantified after 72 hours of incubation. The percentage of cell viability relative to aTNP-PNU-treated cells (referred to as control) is represented (D) (n = 2).

**Detailed description**

The inventors herein provide a series of alternative anti-uPAR antibodies and antibody-drug conjugates thereof, which display improved ligand affinity and efficacy compared to prior art anti-uPAR antibodies as well as improved internalization and *in vivo* efficacy compared to other anti-uPAR antibodies.

Anti-uPAR antibodies

The anti-uPAR antibody of the present disclosure may be of any immunoglobulin class including IgG, IgM, IgD, IgE, IgA, and any subclass thereof. IgG subclasses are also well known to those in the art and include but are not limited to human IgGl, IgG2, IgG3 and IgG4. In one embodiment the anti-uPAR antibody is an IgG monoclonal antibody. In one embodiment the anti-uPAR antibody is IgG1κ. In one embodiment the anti-uPAR antibody is an antigen-binding fragment.

In one embodiment of the present disclosure, the FL1 antibody described herein comprises a light chain variable region of amino acids comprising SEQ ID NO: 6, which is the variable region of SEQ ID NO: 5, and a heavy chain variable region of amino acids comprising SEQ ID NO:11, which is the variable region of SEQ ID NO: 10.

In one embodiment of the present disclosure, the FL1 antibody described herein may comprise a light chain of amino acids comprising or consisting of SEQ ID NO: 5, and a heavy chain of amino acids comprising or consisting of SEQ ID NO:10.

In one embodiment of the present disclosure, the anti-uPAR antibody as defined herein comprises:

a. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 6; and/or
b. an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 11.

In one embodiment of the present disclosure, the antibody which binds to uPAR as defined herein comprises:

a. an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 5; and/or
b. an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 10.

In one embodiment of the present disclosure, the antibody which binds to uPAR as defined herein comprises:

a. an immunoglobulin light chain consisting of the amino acid sequence of SEQ ID NO: 5; and
b. an immunoglobulin heavy chain consisting of the amino acid sequence of SEQ ID NO: 10).

The antibody or antigen-binding fragment thereof directed against uPAR may in some embodiments be characterized by a number of complementarity determining regions (CDRs) comprised in the variable regions of both heavy chain and light chain of the antibody.

Complementarity Determining Regions (CDRs) were predicted according to the IMGT numbering scheme as determined by Lefranc et al. (2005) using the online-available antibody region-specific alignment (AbRSA) tool (see references). Alternatively, CDRs were predicted according to the definition scheme of Kabat et al. as specified in the references Kabat et al. (1983), Kabat et al. (1991) and Wu and Kabat (2008) using a computerized Kabat-numbering programme as published by Dunbar and Deane (2016).

Thus in one embodiment of the present disclosure, the antibody described herein as FL1 which binds uPAR may comprise an immunoglobulin light chain variable region comprising a CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 7, 8 and 9, respectively, and further comprising an immunoglobulin heavy chain variable region comprising a CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs: 12, 13 and 14, respectively.

In one embodiment of the present disclosure, the antibody which binds to uPAR as defined herein comprises:

a. an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 15; and/or
b. an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 20.

In one embodiment of the present disclosure, the antibody which binds to uPAR as defined herein comprises:

a. an immunoglobulin light chain consisting of the amino acid sequence of SEQ ID NO: 15; and
b. an immunoglobulin heavy chain consisting of the amino acid sequence of SEQ ID NO: 20.

In one embodiment of the present disclosure, the antibody which binds uPAR may be further characterized by comprising an immunoglobulin light chain variable region comprising a CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs 17, 18 and 19, respectively, and further comprising an immunoglobulin heavy chain variable region comprising a CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs 22, 23 and 24, respectively.

In one embodiment of the present disclosure, the antibody which binds to uPAR as defined herein comprises:

a. an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 25; and/or
b. an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 30.

In one embodiment of the present disclosure, the antibody which binds to uPAR as defined herein comprises:

a. an immunoglobulin light chain consisting of the amino acid sequence of SEQ ID NO: 25; and
b. an immunoglobulin heavy chain consisting of the amino acid sequence of SEQ ID NO: 30.

In one embodiment of the present disclosure, the antibody which binds uPAR may be further characterized by comprising an immunoglobulin light chain variable region

comprising a CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs 27, 28 and 29, respectively, and further comprising an immunoglobulin heavy chain variable region comprising a CDR1, CDR2, and CDR3 comprising the amino acid sequences of SEQ ID NOs 32, 33 and 34, respectively.

In one embodiment of the present disclosure, the antibody which binds to uPAR as defined herein comprises:

a. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 53 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 47 or a sequence having at least 90% sequence identity thereto,
b. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 53 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 48 or a sequence having at least 90% sequence identity thereto,
c. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 53 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 49 or a sequence having at least 90% sequence identity thereto,
d. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 53 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 50 or a sequence having at least 90% sequence identity thereto,
e. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 53 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid

sequence of SEQ ID NO: 51 or a sequence having at least 90% sequence identity thereto,

f. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 54 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 47 or a sequence having at least 90% sequence identity thereto,

g. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 54 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 48 or a sequence having at least 90% sequence identity thereto,

h. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 54 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 49 or a sequence having at least 90% sequence identity thereto,

i. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 54 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 50 or a sequence having at least 90% sequence identity thereto,

j. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 54 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 51 or a sequence having at least 90% sequence identity thereto,

k. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 55 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy

chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 47 or a sequence having at least 90% sequence identity thereto,

l. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 55 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 48 or a sequence having at least 90% sequence identity thereto,

m. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 55 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 49 or a sequence having at least 90% sequence identity thereto,

n. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 55 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 50 or a sequence having at least 90% sequence identity thereto,

o. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 55 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 51 or a sequence having at least 90% sequence identity thereto,

p. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 56 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 47 or a sequence having at least 90% sequence identity thereto,

q. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 56 or a sequence having at

least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 48 or a sequence having at least 90% sequence identity thereto,

r. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 56 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 49 or a sequence having at least 90% sequence identity thereto,

s. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 56 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 50 or a sequence having at least 90% sequence identity thereto,

t. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 56 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 51 or a sequence having at least 90% sequence identity thereto,

u. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 57 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 47 or a sequence having at least 90% sequence identity thereto,

v. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 57 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 48 or a sequence having at least 90% sequence identity thereto,

w. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 57 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 49 or a sequence having at least 90% sequence identity thereto,

x. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 57 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 50 or a sequence having at least 90% sequence identity thereto, or

y. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 57 or a sequence having at least 90% sequence identity thereto, and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 51 or a sequence having at least 90% sequence identity thereto.

In one embodiment of the present disclosure, the antibody which binds to uPAR comprises an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 56 and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 47.

In one embodiment of the present disclosure, the antibody which binds to uPAR comprises an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 55 and an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 51.

In one embodiment of the present disclosure, the antibody which binds to uPAR comprises a light chain (LC) IgK isotype constant domain. In one embodiment of the present disclosure, the antibody which binds to uPAR comprises a light chain (LC) constant domain comprising or consisting of an amino acid sequence selected from SEQ ID NO: 58 or SEQ ID NO: 59. In one embodiment of the present disclosure, the

antibody which binds to uPAR comprises a light chain (LC) constant domain comprising or consisting of an amino acid sequence of SEQ ID NO: 59.

In one embodiment of the present disclosure, the antibody which binds to uPAR comprises a heavy chain (HC) IgG1 constant domain. In one embodiment of the present disclosure, the antibody which binds to uPAR comprises a heavy chain (HC) constant domain comprising or consisting of the amino acid sequence of SEQ ID NO: 52.

Surface plasmon resonance (SPR) studies were performed to determine the real-time binding kinetics between the specified immobilized antibodies and soluble recombinant $uPAR^{WT}$. The FL1, FL2 and FL3 binding epitopes on uPAR were determined by surface plasmon resonance using single-point alanine-scanning mutagenesis and are shown in Figure 1. Another anti-uPAR mAb denoted 2G10 is also included in Table 1 for reference and comparison. The * denotes the affinity reported (Harel et al. 2019) for the interaction between immobilized uPAR and the Fab fragment of 2G10 in solution.

As can be seen directly from Table 1 below, FL1, FL2 and FL3 all demonstrate superior (i.e. lower) $K_D$ values compared to 2G10 against human uPAR (denoted huPAR in Table 1). No $K_D$ value is given for FL3 and 2G10 against murine uPAR (muPAR) because of lack of cross-species reactivity.

**Table 1 – properties of selected monoclonal anti-uPAR antibodies.**

| mAb | uPA inhibition | Epitope | Subtype | Cross-reactivity | Affinity $K_D$ (nM) |
|---|---|---|---|---|---|
| FL1 | no | D2 | $IgG_1$, κ | Yes (human and mouse) | 0.24 (huPAR)<br>2.32 (muPAR) |
| FL2 | yes | D1 | $IgG_1$, $\kappa^6$ | Yes (human and mouse) | 0.54 (huPAR)<br>5.2 (muPAR) |
| FL3 | no | D3 | $IgG_1$, κ | No (only human) | 0.02 |
| 2G10 | yes | Not known | $IgG_1$ | No (only human) | 10* |

FL2 and FL3, exhibiting similarly high affinity, particularly FL3, and cross-reactivity, in the case of FL2, and able to promote target-mediated internalization upon binding of unique uPAR epitopes (Fig. 2), are deemed valuable mAbs candidates for usage as targeting vehicles in uPAR-targeted therapeutics, including, but not limited to ADCs.

The data presented herein suggests that anti-uPAR antibodies targeting domains D1-D3 of uPAR provide a surprisingly strong interaction between antibody and receptor. Furthermore, it is demonstrated that in particular anti-uPAR antibodies targeting D2 of uPAR are internalized to a particularly large extent, thereby making these highly suitable and efficient for use in ADCs.

The anti-uPAR antibody of the present disclosure may in one embodiment be a humanised antibody or a murine antibody, which binds specifically to the uPAR receptor, more so, the antibody disclosed herein binds specifically at least one of domains D1 (SEQ ID NO: 2), D2 (SEQ ID NO: 3) or D3 (SEQ ID NO: 4) of the uPAR receptor.

In one embodiment, the antibody described herein as FL1 binds domain D2 (SEQ ID NO: 3) of the uPAR receptor. More specifically, the antibody described herein as FL1 may in one embodiment be characterized by binding to the conformational epitope on domain D2, said conformational epitope comprising or consisting of amino acid residues 8 (S), 10 (D), 12 (S), 15 (R), and 17 (R) of uPAR domain D2 (SEQ ID NO: 3). The same epitope may alternatively be defined as amino acid residues $Ser^{122}$, $Asp^{124}$, $Ser^{126}$, $Arg^{129}$, $Arg^{131}$ of full-length human uPAR (SEQ ID NO: 1). One embodiment of the present disclosure is thus an antibody or antigen-binding fragment thereof directed against uPAR which binds to the conformational epitope $Ser^{122}$, $Asp^{124}$, $Ser^{126}$, $Arg^{129}$, and $Arg^{131}$ of full-length human uPAR (SEQ ID NO: 1), alternatively which binds to the conformational epitope comprising or consisting of amino acid residues 8 (S), 10 (D), 12 (S), 15 (R), and 17 (R) of uPAR domain D2 (SEQ ID NO: 3). A further embodiment of the present disclosure is an isolated antibody or antigen-binding fragment thereof which binds to the conformational epitope comprising or consisting of amino acid residues number 8 (S), 10 (D), 12 (S), 15 (R), and 17 (R) of uPAR domain D2 (SEQ ID NO: 3).

In one embodiment, the antibody described herein as FL2 binds to domain D1 (SEQ ID NO: 2) of the uPAR receptor. More specifically, the antibody described herein as FL2 may in one embodiment be characterized by binding to the conformational epitope $Leu^{19}$, $Asp^{22}$ of uPAR domain D1 (SEQ ID NO: 2).

In one embodiment, the antibody described herein as FL3 binds to domain D3 (SEQ ID NO: 4) of the uPAR receptor. More specifically, the antibody described herein as FL3 may in one embodiment be characterized by binding to the linear epitope $Asp^{84}$, $Leu^{85}$ of uPAR domain D3 (SEQ ID NO: 4).

The data presented herein suggests that anti-uPAR antibodies binding an epitope located in Domain 2 of uPAR are internalized to a particularly large extent, which can also be seen in Fig. 2. uPAR-dependent uptake and lysosomal trafficking in murine uPAR-positive KPC cells were additionally evaluated by confocal microscopy using fluorescently labelled A647-FL1 (A647 refers to Alexa Fluor™-647 (Thermo Fisher Scientific)) to verify lack of uptake of FL1 in uPAR knock-out control cells (unpublished results).

It is well accepted and understood within the field that the CDRs are largely responsible for antigen recognition of the antibodies disclosed herein. Therefore, in a preferred embodiment, any sequence variation is located outside the CDRs. All variant antibodies and antigen binding fragments disclosed herein retain the capability to bind to uPAR (SEQ ID NO: 1) efficiently. Residues 1 to 22 of SEQ ID NO: 1 takes the form of a signal peptide.

Methods of generating antibodies are well known in the art. For example, antibodies may be generated via any one of several methods which employ induction of *in vivo* production of antibody molecules, screening of immunoglobulin libraries, or generation of monoclonal antibody molecules by cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the Epstein-Barr virus (EBV)-hybridoma technique.

Likewise, antibody fragments can be obtained using methods well known in the art. For example, antibody fragments according to the present invention can be prepared by proteolytic hydrolysis of the antibody with various enzymes or by expression in *E. coli* or mammalian cells (*e.g.* chinese hamster ovary cell culture or other protein expression systems) of DNA encoding the fragment. Alternatively, antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods.

By “antibody” we include substantially intact antibody molecules, murine antibodies,

chimeric antibodies, humanised antibodies, human antibodies, single chain antibodies, bispecific antibodies, antibody heavy chains, antibody light chains, homodimers and heterodimers of antibody heavy and/or light chains, and antigen-binding fragments and derivatives of the same.

By "antigen-binding fragment" we mean a functional fragment of an antibody that is capable of binding to uPAR.

In one embodiment, the anti-uPAR antibody according to the present disclosure is selected from a mouse antibody, a chimeric antibody, a human antibody, a humanised antibody, a humanised antigen-binding fragment, a Fab fragment, a Fab' fragment, a F(ab')2 fragment, an Fv fragment, a single chain antibody (SCA) such as an scFv, the variable portion of the heavy and/or light chains thereof, or a Fab miniantibody, where these fragments or modified antibodies may be derived from mouse, chimeric, human or humanized antibodies.

In one embodiment the anti-uPAR antibody is a humanised or fully human monoclonal antibody or antigen-binding fragment thereof.

In one embodiment, the anti-uPAR antibody of the present disclosure is a recombinant antibody.

The advantages of using antibody fragments, rather than whole antibodies, are several-fold. The smaller size of the fragments may lead to improved pharmacological properties, such as better tissue penetration. Moreover, antigen-binding fragments can be expressed in and secreted from *E. coli* or other non-mammalian host cells, thus allowing the facile production of large amounts of said fragments.

Fab is the fragment which contains a monovalent antigen-binding fragment of an antibody molecule which can be produced by digestion of whole antibody with the enzyme papain, or other specific means of proteolysis to yield a light chain and a portion of the heavy chain.

F(ab')2 is the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin, or other specific means of proteolysis to yield a bivalent antigen-

binding fragment without subsequent reduction; F(ab')2 is a dimer of two Fab fragments held together by two disulfide bonds.

Fv is a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain, expressed as two chains.

Single chain antibody (SCA) is a genetically engineered molecule containing the variable region of the light chain and the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused, single chain molecule, including an scFv.

Polypeptides, polynucleotides, vectors and host cells

One embodiment of the present disclosure is an isolated polynucleotide encoding any of the polypeptides disclosed herein, i.e. an isolated polynucleotide which encodes the amino acid sequence of any one of SEQ ID NOs: 5, 6, 10 and 11.

In one embodiment, the polynucleotide comprises SEQ ID NO: 41 and/or SEQ ID NO: 42, encoding SEQ ID NO: 5 and SEQ ID NO: 10, respectively.

In one embodiment this disclosure provides an isolated polynucleotide comprising SEQ ID NO: 41, optionally wherein the polynucleotide further comprises SEQ ID NO: 42.

One embodiment of the present disclosure is an isolated polynucleotide encoding any of the polypeptides disclosed herein, i.e. an isolated polynucleotide which encodes the amino acid sequence of any one of SEQ ID NOs: 15, 16, 20, and 21.

In one embodiment, the polynucleotide comprises SEQ ID NO: 43 and/or SEQ ID NO: 44, encoding SEQ ID NO: 15 and SEQ ID NO: 20, respectively.

In one embodiment this disclosure provides an isolated polynucleotide comprising SEQ ID NO: 43, optionally wherein the polynucleotide further comprises SEQ ID NO: 44.

One embodiment of the present disclosure is an isolated polynucleotide encoding any of the polypeptides disclosed herein, i.e. an isolated polynucleotide which encodes the amino acid sequence of any one of SEQ ID NOs: 25, 26, 30 and 31.

In one embodiment, the polynucleotide comprises SEQ ID NO: 45 and/or SEQ ID NO: 46, encoding SEQ ID NO: 25 and SEQ ID NO: 30, respectively.

In one embodiment this disclosure provides an isolated polynucleotide comprising SEQ ID NO: 45, optionally wherein the polynucleotide further comprises SEQ ID NO: 46.

In one embodiment the polypeptide is an isolated polypeptide.

One embodiment of the present disclosure is an isolated polynucleotide encoding any of the polypeptides disclosed herein, i.e. an isolated polynucleotide which encodes an antibody or antigen-binding fragment thereof comprising

i. an immunoglobulin light chain variable region comprising or consisting of an amino acid sequence selected from SEQ ID NOs: 53-57 or a sequence having at least 90% sequence identity thereto, and
ii. an immunoglobulin heavy chain variable region comprising or consisting of an amino acid sequence selected from SEQ ID NOs: 47-51 or a sequence having at least 90% sequence identity thereto.

One embodiment of the present disclosure is a vector, such as an expression vector, comprising the polynucleotide as defined herein.

In one embodiment of the present disclosure, the vector is a mammalian expression vector.

In one embodiment of the present disclosure, the vector is a plasmid vector, such as a plasmid vector selected from pD2610-v13 (ATUM), pSV and the pCMV series of plasmid vectors.

In one embodiment of the present disclosure, the vector is a viral vector, such as a viral vector selected from the group consisting of adenoviral vectors, lentiviral vectors, adeno-associated viral vectors, herpesviral vectors, vaccinia viral vectors, poxviral vectors, baculoviral vectors and oncolytic viral vectors.

A further embodiment of the present disclosure is a host cell comprising the polynucleotide and/or the vector as defined herein.

In one embodiment of the present disclosure, the host cell comprising the polynucleotide and/or the vector as described herein is selected from the group consisting of CHO (Chinese hamster ovary) cells, COS (CV-1 (simian) in Origin, and carrying the SV40 genetic material) cells, HEK (Human embryonic kidney) cells and HeLa (Henrietta Lacks) cells.

In one embodiment the host cell is a recombinant host cell.

Antibody-drug conjugates (ADCs) comprising anti-uPAR humanised antibodies

Antibody-drug conjugates are highly potent biopharmaceutical drugs designed as a targeted therapy with very high specificity, in particular for the treatment of cancer which can otherwise be challenging to target.

The present inventors have with data demonstrated that anti-uPAR ADCs are a potent tool in treatment of cancers characterized by overexpression of uPAR. As such, the presented data shows a significantly greater reduction in overall cell viability after anti-uPAR ADC treatment compared to treatment with ADCs based on non-targeting aTNP.

One particularly preferred embodiment of the present disclosure is an antibody-drug conjugate (ADC) comprising:

a. an antibody or antigen-binding fragment thereof as defined herein,
b. an active agent, and
c. optionally a linker which links a) to b).

The antibody as defined herein may individually be selected from the antibody described herein as FL1, FL2 and/or FL3 or an antigen-binding fragment thereof.

In one embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

a. an antibody or antigen-binding fragment thereof, comprising:
    i) an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 6; and/or

ii) an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 11

b. an active agent, and

c. optionally a linker which links a) to b).

In one embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

a. an antibody or antigen-binding fragment thereof, comprising:

i) an immunoglobulin light chain comprising or consisting of the amino acid sequence of SEQ ID NO: 5; and/or

ii) an immunoglobulin heavy chain comprising or consisting of the amino acid sequence of SEQ ID NO: 10

b. an active agent, and

c. optionally a linker which links a) to b).

In one embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

a. an antibody or antigen-binding fragment thereof, comprising:

i) an immunoglobulin light chain consisting of the amino acid sequence of SEQ ID NO: 5; and

ii) an immunoglobulin heavy chain consisting of the amino acid sequence of SEQ ID NO: 10

b. an active agent, and

c. optionally a linker which links a) to b).

In another embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

a. an antibody or antigen-binding fragment thereof, comprising:

i) an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 16; and/or

ii) an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 21

b. an active agent, and

c. optionally a linker which links a) to b).

In one embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

a. an antibody or antigen-binding fragment thereof, comprising:

i) an immunoglobulin light chain comprising or consisting of the amino acid sequence of SEQ ID NO: 15; and/or

ii) an immunoglobulin heavy chain comprising or consisting of the amino acid sequence of SEQ ID NO: 20

b. an active agent, and

c. optionally a linker which links a) to b).

In one embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

a. an antibody or antigen-binding fragment thereof, comprising:

i) an immunoglobulin light chain consisting of the amino acid sequence of SEQ ID NO: 15; and

ii) an immunoglobulin heavy chain consisting of the amino acid sequence of SEQ ID NO: 20

b. an active agent, and

c. optionally a linker which links a) to b).

In yet another embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

a. an antibody or antigen-binding fragment thereof, comprising:

i) an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 26; and/or

ii) an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 31

b. an active agent, and

c. optionally a linker which links a) to b).

In one embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

a. an antibody or antigen-binding fragment thereof, comprising:
   i) an immunoglobulin light chain comprising or consisting of the amino acid sequence of SEQ ID NO: 25; and/or
   ii) an immunoglobulin heavy chain comprising or consisting of the amino acid sequence of SEQ ID NO: 30
b. an active agent, and
c. optionally a linker which links a) to b).

In one embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

a. an antibody or antigen-binding fragment thereof, comprising:
   i) an immunoglobulin light chain consisting of the amino acid sequence of SEQ ID NO: 25; and
   ii) an immunoglobulin heavy chain consisting of the amino acid sequence of SEQ ID NO: 30
b. an active agent, and
c. optionally a linker which links a) to b).

In one embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

a. an antibody or antigen-binding fragment thereof, comprising:
   i) an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 56; and/or
   ii) an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 47
b. an active agent, and
c. optionally a linker which links a) to b).

In one embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

a. an antibody or antigen-binding fragment thereof, comprising:
   i) an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 55; and/or
   ii) an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 51
b. an active agent, and
c. optionally a linker which links a) to b).

Active agent

The ADCs of the present disclosure comprise an active agent, e.g. a drug, which can be delivered intracellularly to cells expressing uPAR. The active agent may e.g. be a therapeutic agent, a radioisotope or a detectable label. In a preferred embodiment the active agent is a therapeutic agent.

In one embodiment, the active agent may be or comprise a radioisotope. The radioisotope may serve as a radiation emitter either for treatment of affected tissues or for diagnostic purposes. In one embodiment, the radioisotope may consist of or comprise $^{60}$Co, $^{89}$Sr, $^{90}$Y, $^{99m}$Tc, $^{131}$I, $^{137}$Cs, $^{153}$Sm, or $^{223}$Rd. In one embodiment of the present disclosure, the radioisotope may be in combination with a chelator such as DOTA or EDTA or others which are well known in the art.

In one embodiment the active agent is a therapeutic agent. Classes of therapeutic agents include DNA crosslinking agents, DNA alkylating agents, DNA strand scission agents, anthracyclines, antimetabolites, anti-microtubule/anti-mitotic agents, histone deacetylase inhibitors, kinase inhibitors, metabolism inhibitors, peptide antibiotics, immune checkpoint inhibitors, platinum-based antineoplastics, topoisomerase inhibitors, DNA or RNA polymerase inhibitors, nucleotide based agents, and cytotoxic antibiotics.

In a preferred embodiment the active agent is a cytotoxic agent allowing for efficient killing of the cells expressing uPAR.

In one embodiment the active agent is a chemotherapeutic agent.

In one embodiment, the active agent is a DNA-crosslinking agent, such as a DNA crosslinking agent selected from cisplatin or a derivative of cisplatin such as carboplatin or oxaliplatin, mitomycin C (MMC), pyrrolobenzodiazepine (PBD), and dimeric pyrrolobenzodiazepine derivatives such as SGD-1882 or a derivative of any of these.

In one embodiment of the present disclosure, the active agent is a DNA alkylating agent, such as a DNA alkylating agent selected from nitrogen mustards such as tris(2-chloroethyl)amine, pyridinobenzodiazepines or a pyridinobenzodiazepine derivative, indolinobenzodiazepine dimers, and Duocarmycin SA or a derivative of any of these.

In one embodiment, the active agent is a DNA strand scission agent, such as a DNA strand scission agent selected from calicheamicin and hamiltrone or a derivative of any of these.

In one embodiment the active agent is an anthracycline, such as an anthracycline selected from Daunorubicin, doxorubicin, epirubicin, idarubicin, and PNU-159682 or a derivative of any of these.

In one embodiment the active agent is PNU-159682 or a derivative thereof.

In one embodiment the active agent is an antimetabolite, such as an antimetabolite selected from folic acid antagonists such as methotrexate, purine antimetabolites such as 6-mercaptopurine or 6-thioguanine or fludarabine phosphate or pentostatin or cladribine, and pyrimidine antimetabolites such as 5-fluorouracil or 5-fluorodeoxyuridine or cytarabine or gemcitabine, or a derivative of any of these.

In one embodiment the active agent is an anti-mitotic agent, such as selected from the group consisting of derivatives of auristatin or dolastatin such as monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF) and more, a taxane such as Paclitaxel or Docetaxel and more, a vinca alkaloid such as Vinblastine, Vincristine, Vindesine or Vinorelbine and more, a mayatansinoid, Colchicine, and Podophyllotoxin or a derivative of any of these.

In one embodiment, the active agent is monomethyl auristatin E (MMAE) or a derivative thereof.

In one embodiment, the active agent is a histone deacetylase inhibitor, such as a histone deacetylase inhibitor selected from trichostatin A, vorinostat, belinostat, panabiostat, givinostat, resminostat, abexinostat, quisinostat, rocilinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entinostat, tacedinaline, 4SC202, mocetinostat, romidepsin, nicotinamide, sirtinol, cambinol, and EX-527 or a derivative of any of these.

In one embodiment, the active agent is a kinase inhibitor, such as a kinase inhibitor selected from genistein, lavendustin C, PP1-AG1872, PP2-AG1879, SU6656, CGP77675, PD166285, imatinib, erlotinib, gefitinib, lavendustin A, cetuximab, UCS15A, herbimycin A, and radicicol or a derivative of any of these.

In one embodiment, the active agent is a metabolism inhibitor, such as an NAMPT inhibitor. Examples of NAMPT inhibitors include APO866, GMX-1777, GMX-1778 ATG-019, and OT-82 or a derivative of any of these.

In one embodiment, the active agent is an immune checkpoint inhibitor, such as a PD-1 inhibitor or a PD-L1 inhibitor. Examples of PD-1 inhibitors include Pembrolizumab, Nivolumab, Cemiplimab, JTX-4014, Spartalizumab, Camrelizumab, Sintilimab, Tislelizumab, Toripalimab, Dostarlimab, AMP-224 and AMP-514. Examples of PD-L1 inhibitors include Atezolizumab, Avelumab, Durvalumab, KN035, CK-301, AUNP12, CA-170 and BMS-986189 or a derivative of any of these.

In one embodiment, the active agent is a platinum-based antineoplastic, such as a platinum-based antineoplastic selected from lipoplatin, cisplatin, carboplatin, oxaliplatin, nedaplatin, picoplatin, phenanthriplatin, satraplatin, and triplatin tetranitrate or a derivative of any of these.

In one embodiment, the active agent is a topoisomerase inhibitor, such as a topoisomerase inhibitor selected from camptothecin or derivatives thereof such as

topotecan, belotecan, lurtotecan, irinotecan, SN-38, exatecan, and Dxd or a derivative of any of these.

In one embodiment, the active agent is a DNA- or RNA-polymerase inhibitor, such as a polymerase inhibitor selected from amanitin or alpha-amanitin or derivatives thereof, actinomycin D, and aphidicolin or a derivative of any of these.

In one embodiment, the active agent is a nucleotide-based agent, such as an RNA- or DNA-oligonucleotide, such as an siRNA or a miRNA.

There may be one or more units of drug per antibody molecule. The ratio between the number of drug molecules per antibody is denoted the drug-to-antibody ratio (DAR). In one embodiment, the DAR is between 1 and 10, such as between 2 and 8, for example between 2 and 6, such as 2 or 4, such as 2.

Linker

A stable link between the antibody and the active agent is an important aspect of ADC technology. Linkers may e.g. be based on chemical motifs including disulfides, hydrazones or peptides (cleavable), or thioethers (noncleavable), and control the distribution and delivery of the cytotoxic agent to the target cell. Cleavable and noncleavable types of linkers have been proven to be safe in preclinical and clinical trials.

The type of linker, cleavable or non-cleavable, lends specific properties to the delivered drug. For example, cleavable linkers can e.g. be cleaved by enzymes in the target cell, leading to efficient intracellular release of the active agent, for example a cytotoxic agent. In contrast, an ADC containing a non-cleavable linker has no mechanism for drug release, and must rely on mechanisms such as degradation of the targeting antibody, for drug release. Furthermore, as is appreciated by those skilled in the art, the linker composition may influence critical factors such as solubility and pharmacokinetic properties of the ADC as a whole.

In a preferred embodiment of the present disclosure, the ADC targeting uPAR as disclosed herein comprises a linker that links the antibody to the active agent.

In one embodiment of the present disclosure, the linker may be cleavable or non-cleavable.

Cleavable groups include a disulfide bond, an amide bond, a substituted amide bond in the form of a peptide bond, a thioamide, bond, an ester bond, a thioester bond, a vicinal diol bond, or a hemiacetal. These, or other cleavable bonds, may include enzymatically-cleavable bonds, such as peptide bonds (cleaved by peptidases), phosphate bonds (cleaved by phosphatases), nucleic acid bonds (cleaved by endonucleases), and sugar bonds (cleaved by glycosidases).

In a further embodiment of the present disclosure, the linker is a cleavable linker allowing for intracellular release of the active agent inside the target cells.

In a further embodiment the linker is a peptide linker. The choice of peptide sequence is critical to the success of the conjugate. In some embodiments the linker is stable to serum proteases, yet is cleaved by lysosomal enzymes in the target cell.

In a further embodiment the linker is an enzyme-cleavable peptide-containing linker, such as a cathepsin cleavable peptide-containing linker. Cathepsin can be one of several cathepsin types, being one of a group of lysosomal proteases.

In a further embodiment of the present disclosure, the linker comprises or consists of a dipeptide, such as valine-citrulline (VC) or valine-alanine (VA).

In one embodiment the linker comprises or consists of a dipeptide, such as valine-citrulline (VC) or valine-alanine (VA), which may be further connected through an amide linkage to other structural elements. Valine-citrulline-based linkers, in which the citrulline carboxyl function is modified to a substituted amide, can be cleaved by lysosomal cathepsins, whereas valine-alanine-based linkers, in which the alanine carboxyl function is modified to a substituted amide, can be cleaved by other lysosomal proteases, including other cathepsins.

In a further embodiment of the present disclosure, the antibody-drug conjugate as defined herein further comprises a spacer, such as a spacer comprising p-

aminobenzoic acid, p-aminobenzyl (PAB), p-aminobenzylcarbamate (PABC), p-aminobenzoyloxycabonyl, or polyethylenglycol (PEG).

In one embodiment of the present disclosure, the antibody-drug conjugate as defined herein comprises p-aminobenzylcarbamate (PABC).

In one embodiment of the present disclosure, the antibody-drug conjugate as defined herein comprises p-aminobenzyl (PAB).

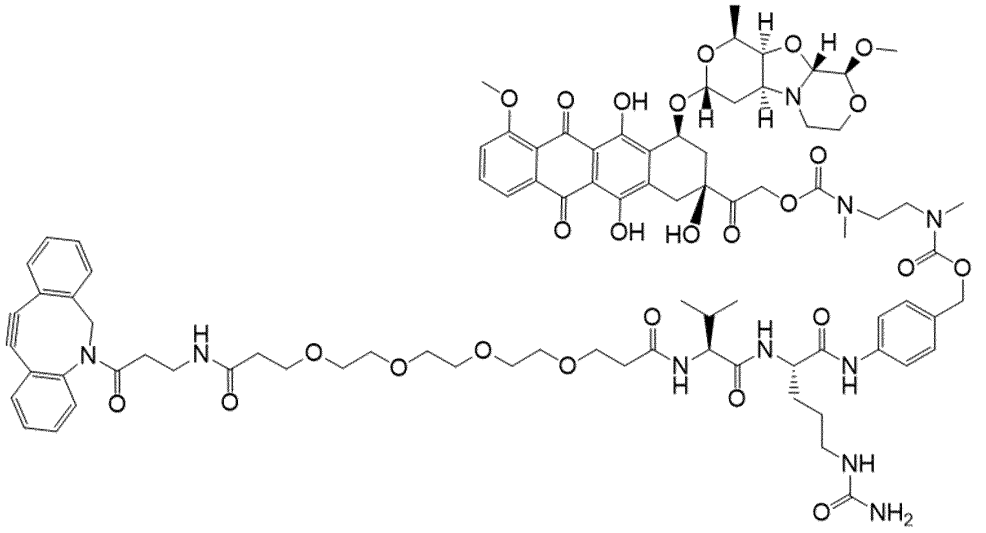
In one embodiment of the present disclosure, the antibody-drug conjugate as defined herein comprises one or more polyethylenglycols (PEGs), such as one PEG, two PEGs, three PEGs, four PEGs or five PEGs. In one embodiment of the present disclosure, the antibody-drug conjugate as defined herein comprises a PEG spacer, such as PEG, PEG2, PEG3, PEG4 or PEG5.

In a further embodiment of the present disclosure, the antibody-drug conjugate as defined herein further comprises an attachment group, such as an attachment group comprising or consisting of maleimide and caproic acid (MC), N-hydroxysuccinimide, reactive attachment groups directed to modified or unmodified protein-bound carbohydrate, peptide sequences that are required for enzymatic reactions, azides or alkynes or being derived from these by reaction with the antibody or a chemically or enzymatically generated derivative thereof.

In one embodiment of the present disclosure, the ADC of the present disclosure further comprises an attachment entity. The attachment entity may for example connect the antibody and the cleavable linker, where the attachment entity is the reaction product between an antibody functionalized with a moiety having a specific reactivity and another moiety having a matching reactivity functionalized onto the cleavable linker. Such matching chemical reactivity is well-known in the field of chemistry and may in one embodiment take the form of alkyne/azide click chemistry, such as SPAAC or CuAAC type reactivity. In another embodiment, this reactive attachment group comprises or consists of maleimide and caproic acid (MC), where maleimide reacts preferably with cysteine thiols during coupling. In other embodiments, the attachment group comprises or consists of N-hydroxysuccinimide, reactive attachment groups directed to modified or unmodified protein-bound carbohydrate, peptide sequences

that are required for enzymatic reactions, azides or alkynes or being derived from these by reaction with the antibody or a chemically or enzymatically generated derivative thereof.

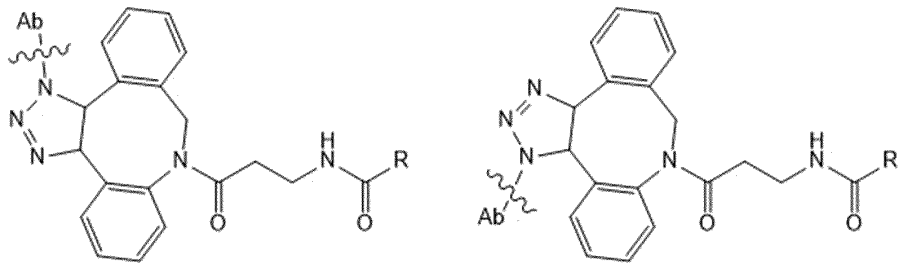
In one embodiment of the present disclosure, the ADC comprises an antibody targeting uPAR such as any of FL1, FL2 and/or FL3 defined herein including humanized variants thereof, and a linker-drug complex. In one embodiment the linker-drug complex comprises or consists of the cytotoxic agent PNU-159682, one or more of a spacer (such as PEG3, PEG4, PAB and DMEA), a cathepsin-cleavable linker (such as Valine-citrulline, VC) and an attachment group (such as DBCO). In one embodiment of the present disclosure, the drug-linker may comprise or consist of DBCO-PEG4-VC-PAB-DMEA-PNU-159682 with the formula



In another embodiment, the cytotoxic drug is an anti-mitotic cytotoxin, such as monomethyl auristatin E (MMAE) and the drug-linker part of an ADC within the scope of the present invention may comprise or consist of DBCO-PEG3-VC-PAB-MMAE. In one embodiment of the present disclosure, the ADC comprises an antibody targeting uPAR as defined herein, and the linker-drug complex DBCO-(PEG)n-VC-PAB-DMEA-PNU159682 or DBCO-(PEG)n-VC-PAB-MMAE, wherein n is representative of the length of the polyethyleneglycol spacer and may take any value from 1 to 6, preferably 3 or 4, such as in DBCO-PEG4-VC-PAB-DMEA-PNU159682 or DBCO-PEG3-VC-PAB-MMAE. In one embodiment, the linker-drug complex comprised in the ADC as

described herein may also be identified by CAS Registry number 2259318-56-2 or 2754384-60-4. These types of linker-drug complexes feature a spacer (p-aminobenzyl), a cathepsin-cleavable linker (Valine-citrulline (VC) dipeptide) and an attachment group comprising dibenzocyclooctyne-amine (DBCO), also sometimes referred to as azadibenzocyclooctyne (ADIBO) which undergoes SPAAC-type reaction with azide-functionalized antibodies or fragments thereof to create a stable conjugation. DBCO as used herein may refer to CAS: 1255942-06-3, whereas DBCO-PEG4 together may refer to CAS 1537170-85-6. In one embodiment, SPAAC-type reaction results in one or more regioisomers, all of which are within the scope of the present disclosure.

As used herein, the term "regioisomer" or "regioisomers" refers to the positional isomers, which is a category of structural isomers, wherein the position or the substituent changes position on the parent structure. Herein the term regioisomer without departing from the chemical structure of azide-functionalized DBCO-PEG4-VC-PAB-DMEA-PNU159682 inherently includes all regioisomers either as a pure regioisomer or mixture of two or more regioisomers thereof. The regioisomers identified herein preferably refers to the following positional and/or structural isomers (I) and (II) of the DBCO-moiety:




(I) (II)

wherein Ab represents the anti-uPAR antibody or antigen-binding fragment thereof, and R represents a linking moiety connected to a cytotoxic drug, preferably PEG4-VC-PAB-DMEA-PNU159682 or PEG3-VC-PAB-MMAE.

In one embodiment, the ADC of the present disclosure targeting uPAR comprises or consists of:

a. the antibody as defined herein, comprising:

i) an immunoglobulin light chain consisting of the amino acid sequence of SEQ ID NO: 5; and

ii) an immunoglobulin heavy chain consisting of the amino acid sequence of SEQ ID NO: 10.

b. a VC linker,

c. a DBCO or ADIBO attachment group,

d. a PAB or a PABC spacer, and

e. PNU159682 as active agent,

wherein the ADC may optionally further comprise a PEG such as PEG4.

In one embodiment, the ADC of the present disclosure targeting uPAR comprises or consists of:

a. the antibody as defined herein, comprising:

i) an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 6; and/or

ii) an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 11,

b. a VC linker,

c. a DBCO or ADIBO attachment group,

d. a PAB or a PABC spacer, and

e. PNU159682 as active agent,

wherein the ADC may optionally further comprise a PEG such as PEG4.

In one embodiment, the ADC of the present disclosure targeting uPAR comprises or consists of:

a. the antibody as defined herein, comprising:

i) an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 56; and/or

ii) an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 47,

b. a VC linker,

c. a DBCO or ADIBO attachment group,

d. a PAB or a PABC spacer, and

e. PNU159682 as active agent,

wherein the ADC may optionally further comprise a PEG such as PEG4.

In one embodiment, the ADC of the present disclosure targeting uPAR comprises or consists of:

a. the antibody as defined herein, comprising:

i) an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 55; and/or

ii) an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 51,

b. a VC linker,

c. a DBCO or ADIBO attachment group,

d. a PAB or a PABC spacer, and

e. PNU159682 as active agent,

wherein the ADC may optionally further comprise a PEG such as PEG4.

In one embodiment, the ADC of the present disclosure may further comprise a DMEA spacer and/or group, in particular in those embodiments wherein the cytotoxic agent is PNU-159682.

Therapeutic use

The ADCs directed against uPAR as described herein are useful for the delivery of active agents, such as therapeutic or cytotoxic agents to cells expressing uPAR and similar proteins and thus for the treatment of a range of diseases and disorders characterized by either expression or overexpression of said proteins.

Thus, one embodiment of the present disclosure is the antibody or the antibody-drug conjugate directed against uPAR as defined herein for use as a medicament.

Another embodiment of the present disclosure is a method for treatment of a disease characterised by cells in a subject expressing and/or overexpressing uPAR, said method comprising administering to the subject the antibody or the antibody-drug conjugate as defined herein.

With its optimal tumor-selective expression profile, and simultaneous presence in the stromal compartments of most aggressive cancers, the urokinase receptor represents

an attractive and versatile target for cytotoxic-based targeted approaches, like antibody-drug conjugates.

In one embodiment of the present disclosure, the method is the method as defined herein, wherein the disease characterized by cells expressing and/or overexpressing uPAR is cancer.

In one embodiment of the present disclosure, the method is the method as defined herein, wherein the cancer is selected from pancreatic adenocarcinoma (PAAD) such as pancreatic ductal adenocarcinoma (PDAC), bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), glioblastoma, glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSCC), leukemia, acute myeloid leukemia (LAML), ovarian serous cystadenocarcinoma (OV), rectum adenocarcinoma (READ), sarcoma (SARC), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD).

In one embodiment of the present disclosure, the cancer is selected from pancreatic cancer, breast cancer, colon cancer, gastric cancer, esophageal cancer, hepatocellular cancer, prostate cancer, bladder cancer, mesothelioma, fibrosarcoma, and squamous cell carcinomas.

In one embodiment of the present disclosure, the cancer is breast cancer such as selected from triple-negative breast cancer (TNBC), basal-like breast cancer (BLBC), HER-2 breast cancer, Luminal A type breast cancer and Luminal B type breast cancer.

In one embodiment of the present disclosure, the cancer is preferably pancreatic cancer such as pancreatic ductal adenocarcinoma.

In one embodiment of the present disclosure, the cancer is mesothelioma such as selected from pleural mesothelioma, peritoneal mesothelioma, and pericardial mesothelioma.

In one embodiment of the present disclosure, the cancer is leukaemia, such as acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), chronic lymphocytic leukaemia (CLL), and chronic myeloid leukaemia (CML), or subtypes of these.

In one embodiment of the present disclosure, the method is the method as defined herein, wherein the cancer is metastatic cancer

In one embodiment of the present disclosure, the method is the method as defined herein, wherein the cancer is a solid tumour.

In one embodiment of the present disclosure, the method is the method as defined herein, wherein the cancer is glioblastoma.

One embodiment of the present disclosure is a pharmaceutical composition comprising an effective amount of the antibody or the antibody-drug conjugate as defined herein, and a pharmaceutically acceptable buffer, diluent, carrier, adjuvant or excipient.

A 'therapeutically effective amount', or 'effective amount', or 'therapeutically effective', as used herein, refers to that amount that provides a therapeutic effect for a given condition and administration regimen. This is a predetermined quantity of active material calculated to produce a desired therapeutic effect in association with the required additive and diluent, *i.e.* a carrier or administration vehicle. Further, it is intended to mean an amount sufficient to reduce, and most preferably prevent, a clinically significant deficit in the activity, function, and response of the host. Alternatively, a therapeutically effective amount is sufficient to cause an improvement in a clinically significant condition in a host. As is appreciated by those skilled in the art, the amount of a compound may vary depending on its specific activity. Suitable dosage amounts may contain a predetermined quantity of active composition calculated to produce the desired therapeutic effect in association with the required diluent.

The ADCs of the present disclosure may be formulated into any type of pharmaceutical composition known in the art to be suitable for the delivery thereof.

The pharmaceutical compositions may be prepared in a manner known in the art that is sufficiently storage stable and suitable for administration to humans and/or animals. For

example, the pharmaceutical compositions may be lyophilized, *e.g.* through freeze drying, spray drying, spray cooling, or through the use of particle formation from supercritical particle formation.

By “pharmaceutically acceptable" we mean a non-toxic material that does not decrease the effectiveness of the ADC. Such pharmaceutically acceptable buffers, carriers or excipients are well-known in the art (see Remington's Pharmaceutical Sciences, 18th edition, A.R Gennaro, Ed., Mack Publishing Company (1990) and handbook of Pharmaceutical Excipients, 3rd edition, A. Kibbe, Ed., Pharmaceutical Press (2000), the disclosures of which are incorporated herein by reference).

The term "buffer" is intended to mean an aqueous solution containing an acid-base mixture with the purpose of stabilizing pH. Pharmaceutically acceptable buffers are well known in the art.

The term "diluent" is intended to mean an aqueous or non-aqueous solution with the purpose of diluting the agent in the pharmaceutical preparation.

The term "adjuvant" is intended to mean any compound added to the formulation to increase the biological effect of the agent of the invention. The adjuvant may be one or more of zinc, copper or silver salts with different anions, for example, but not limited to fluoride, chloride, bromide, iodide, thiocyanate, sulfite, hydroxide, phosphate, carbonate, lactate, glycolate, citrate, borate, tartrate, and acetates of different acyl composition. The adjuvant may also be cationic polymers such as cationic cellulose ethers, cationic cellulose esters, deacetylated hyaluronic acid, chitosan, cationic dendrimers, cationic synthetic polymers such as poly(vinyl imidazole), and cationic polypeptides such as polyhistidine, polylysine, polyarginine, and peptides containing these amino acids.

The excipient may be one or more of carbohydrates, polymers, lipids and minerals. Examples of carbohydrates include lactose, glucose, sucrose, mannitol, and cyclodextrines, which are added to the composition, e.g., for facilitating lyophilisation. Examples of polymers are starch, cellulose ethers, cellulose carboxymethylcellulose, hydroxypropylmethyl cellulose, hydroxyethyl cellulose, ethylhydroxyethyl cellulose, alginates, carageenans, hyaluronic acid and derivatives thereof, polyacrylic acid, polysulphonate, polyethylenglycol/polyethylene oxide, polyethyleneoxide/polypropylene

oxide copolymers, polyvinylalcohol/polyvinylacetate of different degree of hydrolysis, and polyvinylpyrrolidone, all of different molecular weight, which are added to the composition, e.g., for viscosity control, for achieving bioadhesion, or for protecting the lipid from chemical and proteolytic degradation. Examples of lipids are fatty acids, phospholipids, mono-, di-, and triglycerides, ceramides, sphingolipids and glycolipids, all of different acyl chain length and saturation, egg lecithin, soy lecithin, hydrogenated egg and soy lecithin, which are added to the composition for reasons similar to those for polymers. Examples of minerals are talc, magnesium oxide, zinc oxide and titanium oxide, which are added to the composition to obtain benefits such as reduction of liquid accumulation or advantageous pigment properties.

The ADCs of the present disclosure or pharmaceutical compositions comprising the ADCs may be administered via any suitable route known to those skilled in the art. Thus, possible routes of administration include parenteral (intravenous, subcutaneous, and intramuscular), topical, ocular, nasal, pulmonar, buccal, oral, vaginal and rectal. Also, administration from implants is possible.

In one preferred embodiment, the pharmaceutical compositions are administered parenterally, for example, intravenously, intracerebroventricularly, intraarticularly, intra-arterially, intraperitoneally, intrathecally, intraventricularly, intrasternally, intracranially, intramuscularly or subcutaneously, or they may be administered by infusion techniques. They are conveniently used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered if necessary.

In one embodiment of the present disclosure, the method is the method as defined herein, wherein the antibody-drug conjugate is administered parenterally, for example, intravenously, intracerebroventricularly, intraarticularly, intra-arterially, intraperitoneally, intrathecally, intraventricularly, intrasternally, intracranially, intramuscularly or subcutaneously, or by infusion techniques.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending

agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

In one embodiment of the present disclosure, the method is the method as defined herein, wherein the antibody-drug conjugate or the antibody is administered intravenously.

In one embodiment of the present disclosure, the method is the method as defined herein, wherein the antibody-drug conjugate or the antibody is administered subcutaneously.

In one embodiment of the present disclosure, the method is the method as defined herein, wherein the antibody-drug conjugate or the antibody is administered in combination with one or more further agents, such as one or more further therapeutic agents.

In one embodiment of the present disclosure, the ADC or the antibody of the present disclosure is administered in conjunction with additional reagents and/or therapeutics that may increase the functional efficiency of the ADC, such as established or novel drugs that increase lysosomal membrane permeability, thereby facilitating molecular entry from the lysosome interior to the cytoplasm, or drugs that increase the permeability of the blood-brain barrier.

In one embodiment of the present disclosure, the ADCs or the antibodies described herein may be administered in combination with a range of anti-cancer agents, such as antimetabolites, alkylating agents, anthracyclines and other cytotoxic antibiotics, vinca alkyloids, anti-microtubule/anti-mitotic agents, histone deacetylase inhibitors, kinase inhibitors, peptide antibiotics, immune checkpoint inhibitors, platinum-based antineoplastics, etoposide, taxanes, topoisomerase inhibitors, antiproliferative immunosuppressants, corticosteroids, sex hormones and hormone antagonists, cytotoxic antibiotics and other therapeutic agents.

The data presented herein demonstrates that FL1-PNU-ADC provides significant immunomodulation in the tumor microenvironment post-treatment including also in the stromal compartments. This is the first report of a uPAR-targeted ADC endowed with this complementary mechanism.

In one embodiment of the present disclosure, the ADCs or the anitbodies described herein induce an immunomodulation of the tumour associated cells, such as the stromal compartment of pancreatic cancers as part of the anti-cancer treatment. The observed immunomodulatory effect by FL1-PNU ADC allows for combination with other immunotherapies and may in a preferred embodiment be combined with immune checkpoint inhibitors for a more efficacious ADC treatment.

Thus, in one embodiment of the present disclosure, the method is the method as defined herein, wherein the cell expressing uPAR displays uPAR overexpression.

In one embodiment of the present disclosure, the method is the method as defined herein, wherein the cell expressing uPAR is a tumour cell.

In one embodiment of the present disclosure, the method is the method as defined herein, wherein the cell expressing uPAR is a tumour associated cell.

Tumour associated cells include, but are not limited to, activated fibroblasts, myofibroblasts, neovasculature and infiltrating cells of the macrophage-monocyte lineage or other leukocytic cell types, as well as cells of the stromal tissue surrounding the tumour which is of particular importance in cancers such as pancreatic cancer.

In one embodiment of the present disclosure, the method is the method as defined herein, wherein the antibody or the antibody-drug conjugate induces cell death and/or inhibits the growth and/or proliferation of the uPAR expressing cell.

In one embodiment of the present disclosure, the method is the method as defined herein, wherein the antibody or the antibody-drug conjugate induces liberation of free cytotoxin from the uPAR expressing cells, leading to cell death and/or inhibition of the

growth and/or proliferation of neighbouring cancer cells. This might be referred to as "bystander effect" within the present disclosure.

In one embodiment of the present disclosure, the method is the method as defined herein, wherein the treatment is ameliorative or curative.

A further embodiment of the present disclosure is a method for inhibiting tumour progression in a subject, comprising administering to the subject the antibody or the antibody-drug conjugate or the pharmaceutical composition as defined herein to said subject.

A further embodiment of the present disclosure is a method for inhibiting, lowering or eliminating metastatic capacity of a tumour in a subject, comprising administering to the subject the antibody or the antibody-drug conjugate or the pharmaceutical composition as defined herein to said subject.

An even further embodiment of the present disclosure is a kit comprising the antibody or the antibody-drug conjugate or the pharmaceutical compositions as defined herein, optionally further comprising means for administering said antibody-drug conjugate or pharmaceutical composition to a subject and/or instructions for use.

In one embodiment, the present disclosure relates to an antibody-drug conjugate as described herein or a pharmaceutical composition as described herein for use in the manufacture of a medicament for treatment of a disease characterised by cells expressing uPAR, such as cancer.

Without wishing to be bound by theory, it is believed that specific antibody binding to the D2 and/or D1 domain of uPAR enables species cross-reactivity as evidenced with the herein tested antibody candidates FL1 and FL2.

**Examples**

**Antibodies**

All anti-uPAR mAbs, and the isotype-matched negative control mAb directed against trinitrophenol, aTNP, were produced in-house by standard hybridoma technology.

Alternatively, the recombinant HyHEL-10 Mouse IgG1 Kappa Isotype Control Antibody (Sydlabs, #PA007154.m1) was employed as non-targeting negative control mAb. FL1 and FL2 display cross-reactivity towards human and murine uPAR and were obtained by immunizing uPAR-deficient mice with recombinant purified soluble human and mouse uPAR, respectively.

The antibody 2G10 was produced in-house by state-of-the-art recombinant technology starting from the antibody sequences disclosed in WO 2019/113248 A1 (UNIV. CALIFORNIA), while recombinant mouse ATN-658 was purchased by Creative Biolabs (#:PABL-355).

The anti-muPAR pAb, P47, has been previously described in H. Solberg et al 2001.

All interactions studied, such as measured by surface plasmon resonance, were carried out on a Biacore 3000 (Biacore, Sweden) using 10 mM HEPES, 150 mM NaCl, 3mM EDTA, and 0.005% (v/v) surfactant P-20 at pH 7.4 as running buffer. Purified anti-uPAR mAbs (1µg/ml) were covalently immobilized on a carboxymethylated dextran matrix (CM5 sensor chip) using *N*-hydroxysuccinimide / *N*-ethyl-*N'*-[3-(diethylamino)propyl]carbodiimide, yielding levels of 400-1000 RU-immobilized mAbs. To establish the kinetics for the mAb-uPAR interactions, serial 2-fold dilutions of purified human uPAR (0.4 - 200 nM in running buffer) were analyzed at 20 °C with 50 l/min flow rates. To certify reproducibility of the analyses, 50 nM uPAR was re-tested at the end of each experiment. After each run, the sensor chip was regenerated by two consecutive injections of 0.1 M acetic acid in 0.5 M NaCl. The kinetic rate constants, $k_{on}$ and $k_{off}$, were subsequently derived from these real-time interaction analyses by fitting the association and dissociation phases to a bimolecular interaction model using the BIAevaluation 4.1 software (Biacore).

To identify the functional epitopes for for the mAbs FL1, FL2 and FL3 of the present disclosure, their binding kinetics toward a large number of purified uPAR mutants carrying defined single-site alanine substitutions covering the entire sequence of the mature protein (except for cysteine residues) were analyzed.
The uPAR mutants were tested at 10, 30 and 90 nM. The kinetic constants for the interaction between intact soluble uPAR (residues 23–305 of SEQ ID NO: 1) and the immobilized mAbs (approx. 7 fmol/mm$^2$) were measured at 20 °C at a flow rate of 50

µl/min as specified above. Functional hotspot residues in epitopes for these mAbs are defined as the 1–4 residues, where alanine substitutions have the greatest impact on the dissociation rate constants ($k_{off}$) as measured by surface plasmon resonance (see above).

**Humanized antibodies**

Production of humanized FL1 variants was conducted by Fusion antibodies. Using a combined IMGT/Kabat CDR numbering system, the CDRs were identified. Five human framework sequences derived from mature Human IgGs were used as "acceptor" frameworks for the FL1 LCDR and HCDR sequences. Briefly, databases of Human IgG sequences were searched for comparison to the murine VH domain using BLAST search algorithms, and the top candidates were selected from the top 200 BLAST results based on a combination of framework homology, maintaining key framework residues, and canonical loop structure. As a result, the humanized sequences are expected to be non-immunogenic and retain the canonical structure of the CDR loops. Each of the VH and VL domains was then synthesized in-frame with human IgG1 constant domain sequence (SEQ ID NO: 52) and IgK isotype constant domain sequence (SEQ ID NO: 59), respectively. The entire heavy and light chain sequences were codon optimized and the DNA sequence was verified by DNA sequencing analysis. A total of 25 humanized variants and 1 chimeric antibody (having the murine variable domains and the human Ig constant domains), used as a positive control, were expressed using a CHO-based transient expression system (see Table 2 below). The produced antibodies were then purified from cell culture supernatants using standard chromatography technique. The purified antibodies were then characterized for yield, binding affinity (towards muPAR and huPAR), purity, monodispersity, and endotoxin levels, and ranked accordingly. Ten humanized variants were finally selected as top candidates for further ADC development. Pilot ADC testing was performed on MDA-MB-231 cells and results of two representative candidates, HC1-LC4-PNU comprising VH1 (SEQ ID NO: 47) and VL4 (SEQ ID NO: 56) and HC5-LC3-PNU comprising VH5 (SEQ ID NO: 51) and VL3 (SEQ ID NO: 55), are shown in Fig. 10.

**Table 2: Heavy chain variable region (VH) and light chain variable region (VL) of humanized FL1 antibodies.** VH1 = SEQ ID NO: 47, VH2 = SEQ ID NO: 48, VH3 = SEQ ID NO: 49, VH4 = SEQ ID NO: 50, VH5 = SEQ ID NO: 51, VL1 = SEQ ID NO: 53, VL2 = SEQ ID NO: 54, VL3 = SEQ ID NO: 55, VL4 = SEQ ID NO: 56 and VL5 = SEQ ID NO: 57.

| Chimeric Antibody | HC0:LC0 | | | | |
|---|---|---|---|---|---|
| Humanized Variants | VH1:VL1 | VH1:VL2 | VH1:VL3 | VH1:VL4 | VH1:VL5 |
| | VH2:VL1 | VH2:VL2 | VH2:VL3 | VH2:VL4 | VH2:VL5 |
| | VH3:VL1 | VH3:VL2 | VH3:VL3 | VH3:VL4 | VH3:VL5 |
| | VH4:VL1 | VH4:VL2 | VH4:VL3 | VH4:VL4 | VH4:VL5 |
| | VH5:VL1 | VH5:VL2 | VH5:VL3 | VH5:VL4 | VH5:VL5 |

**Cell lines**

All cancer cell lines in Table 3 were purchased from the American Type Culture Collection (ATCC). The murine PDAC cell lines, KPC1 and KPC2, and relative knock-out clones for the uPAR gene, PLAUR (designated in the text as $uPAR^{KO}$) were courtesy of Prof. Matthew J. Flick. The parental KPC cells were individually generated from primary tumors of KPC (*Kras*$^{G12D/+}$, *p53*$^{R172H/+}$, *Elas*$^{CreER/+}$) mice, and the relative $uPAR^{KO}$ clones derived using a CRISPR RNA-guided FokI nuclease strategy, as detailed by Yang Y., et al., 2019. The fibroblast cell lines used for the bystander effect are the murine NIH3T3 (ATCC) and human 1BR3.6 (European Collection of Authenticated Cell Cultures, ECACC) cells. Cell lines were maintained in a 37°C, 5% $CO_2$ incubator in supplier-recommended media supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin; additionally, 2,5% horse serum was included in MIA PaCa-2 media formulation.

**Table 3 – Panel of tested uPAR-positive cells**

| Cancer cell type | Origin | Name |
|---|---|---|
| PDAC | Human | AsPC-1 |
| | | BxPC-3 |
| | | MIA PaCa-2 |
| | Murine | KPC1 |
| | | KPC1 $uPAR^{KO}$ |
| | | KPC2 |
| | | KPC2 $uPAR^{KO}$ |
| Leukemia | Human | U937 |
| Glioblastoma | | U87 |
| Triple-Negative Breast Cancer | | MDA-MB-231 |
| Mesothelioma | | REN-Luc |
| Fibrosarcoma | | HT-1080 |

The M2-polarized macrophages were derived from bone-marrow-derived macrophages, as described in Fig. 12 and detailed in (H. J. Jürgensen et al 2019).

**Fluorescence and radioactive labeling**

Alexa Fluor-647 (A647)-labeled FL1 was generated using a Mouse IgG1 Labeling Kit (Thermo Scientific™, #A20186), in accordance with the manufacturer´s instructions. Briefly, 1 mg/ml of mAb in sodium bicarbonate buffer, pH = 8.3, was incubated with a 5-fold molar excess of A647 reactive dye (at 10 mg/ml in DMSO) for 1 h at room temperature in the dark, under gentle agitation, and then purified by gel filtration on kit´s provided PD-10 desalting columns. The A647-conjugate was characterized for the degree of labeling (DOL) by absorbance measurements, following the vendor protocol.

$^{125}$I-labeling of the FL1 mAb was carried out as illustrated in Engelholm et al., (2003), followed by reducing SDS-page analysis and visualization on a Phosphor Imager (Fuji Fla 3000).

**Radioactive-based assessment of anti-uPAR mAbs uptake in uPAR-positive leukemic cells U937**

Cells, seeded in triplicates in 24-well plates ($10^5$/well), were incubated with $^{125}$I-labeled a-uPAR mAbs for 4 h at 37 °C, to allow endocytosis. $^{125}$I-Collagen was included as non-binding control (NC). After incubation, the excess dye was removed by three washes with ice-cold 1X PBS, followed by a short treatment with 0.25% trypsin-EDTA supplemented with 50 µg/mL proteinase K to remove surface-bound mAbs. Cells were collected by centrifugation and, after resuspension in FACS buffer, the radioactivity of the pellets, corresponding to the internalized fractions, was measured in a gamma counter (Nucleocounter3000), and expressed as counts per minute (CPM).

**Confocal microscopy**

Microscopy-based qualitative assessment of A647-labeled-FL1 uptake was performed in adherent KPC cells seeded in triplicates at 5000 cells/well in 24-well plates, with adherent cells plated on coverslips. After 4 h incubation at 37 °C, unbound antibodies were removed by three washes with ice-cold 1X PBS. Surface staining was performed on live cells following 15 min incubation at RT with A488-WGA (Invitrogen, #W11261) at 1 µg/ml in 1X PBS, followed by three additional washing steps. Cells were then fixed in 4 % PFA for 15 min at RT, mounted using ProLong™ Gold Antifade Mountant with DAPI as nuclear counterstain (Thermo Scientific™, #P36941), and left to dry overnight. Image acquisition was performed on a Zeiss LSM 800 or LSM900 confocal microscope using a 40X oil objective.

To investigate the lysosomal trafficking of the antibody, cells were pre-incubated with 100 µM of E64d protease inhibitor (Sigma-Aldrich, #330005) for 1 h at 37 °C prior to the assay.

**Histology and Immunohistochemistry**

Tissue sections were deparaffinized (using tissue clear (Sakura finetek #1466) and decreasing ethanol concentrations), rinsed in tap water, and subjected to uPAR staining as follows: After deparaffinization, tissue sections were subjected to antigen retrieval by treatment with 5 µg/mL proteinase K at 37 °C for 15 min. After washing, samples were incubated in 1% $H_2O_2$ for 15 min for blocking of endogenous peroxidases, followed by subsequent washing in tap water and 0.5% TBS-T. Afterwards, slides were incubated with primary antibody (rabbit anti-smuPAR pAb P47 at 0.5 µg/ml) at 1:1000, all diluted in antibody diluent (Dako, #S3022:) overnight at 4 °C. Finally, detection was performed with the following secondary antibody; Envision-HRP anti-rabbit (#4003, Dako). Incubation was carried out for 45 min at RT before adding the chromogenic DAB substrate (Origene, # C09-12) for an additional 5 min. Sections were finally rinsed and counterstained with Mayer's hematoxylin for 30 s, followed by dehydration, drying, and mounting, as formerly detailed. All stained sections were scanned by NanoZoomer-XR Digital slide scanner C12000-01(Hamamatsu) and analyzed with NDP.view2 Plus software.

**Generation and characterization of site-specific DAR2 and DAR4 glycan-linked ADC**

Site-specific glycan-linked PNU-159682-ADCs were generated by copper-free click chemistry via GlyCLICK™ conjugation with the commercially available click reactive linker-toxin DBCO-PEG4-VC-PAB-DMEA-PNU-159682 (Levena) (Fig. 3). This approach follows the strain promoted alkyne-azide cycloaddition known as SPAAC to those skilled in the art. The GlyCLICK Azide Activation kit (Genovis) was used to produce azide-modified mAbs, as stated by the manufacturer protocol. Briefly, antibodies, buffer exchanged in 1X TBS, were deglycosylated by immobilized GlycINATOR© to the inner N-Acetylglucosamine (GlcNac) moieties of the IgG Fc N-glycans and then azide-activated at the exposed GlcNAc using GalT (Y289L) and UDP-GalNAz. Following overnight incubation at 30°C, unbound UDP-GalNAz was removed by purification on 2 ml Desalting Spin Columns (40K MWCO; Thermo Scientific™, #87768). Successful azide modification was confirmed by Coomassie staining on reducing SDS-PAGE gels,

prior to conjugation. The linker-payload (dissolved in a mixture of 80% propylene glycol (PG) and 20% N,N-dimethylformamide (DMF), was added at a 15-times molar excess of the Az-modified mAbs in TBS, in a 3:1 ratio, and the reaction incubated overnight at 25°C, under gentle agitation. Whereafter, SDS-PAGE analysis was conducted, and conjugates were subsequently purified via Zeba Spin Desalting Columns or left to incubate for an additional 24 h, in case of incomplete coupling. The concentration, purity, and drug-to-antibody ratio (DAR) of ADC products were further examined via UV-vis spectroscopy and MALDI-TOF. Successful conjugation, and expected DAR of 2, were confirmed in ADC constructs based on FL1, FL2, FL3, HC1-LC4, HC5-LC3, 2G10, ATN658 or aTNP.

Likewise, the generation of glycan-linked DAR 4 MMAE-ADCs was performed via a similar site-specific glyconjugation method using the SiteClick™ Antibody Azido Modification Kit (Invitrogen, ##S20026) per manufacturer´s instructions. Briefly, terminal galactose residues on the N-linked sugars in the Fc region of the antibodies (in TBS 1X) were removed by β-Galactosidase cleavage, followed by 1,4-galactosyltransferase (Gal-T)-catalyzed azide-activation of the terminal GlcNAc residues using UDP-GalNAz. Following overnight incubation at 30°C, unbound UDP-GalNAz was removed by purification on 2 ml Desalting Spin Columns (40K MWCO; Thermo Scientific™, #87768). Successful azide modification was confirmed by Coomassie staining by reducing SDS-PAGE analysis. Finally, the Azide-modified antibodies were conjugated with 15-times molar excess of linker-payload DBCO-(PEG)3-VC-PAB-MMAE (MedChemExpress, # HY-111012, CAS: 2754384-60-4) (dissolved in a mixture of 80% propylene glycol (PG) and 20% N,N-dimethylformamide (DMF), in a 3:1 ratio, and the reaction incubated overnight at 25°C, under gentle agitation. Conjugation was confirmed via SDS-page analysis and conjugates were purified using Zeba Spin Desalting Columns. DAR determination via UV-vis analysis confirmed the expected DAR 4 for both FL1- or Hyhel-based-ADCs.

***In vitro* cytotoxicity assay**

Cells plated in triplicates at 3 x $10^4$ cells/ml per well in 96-well plates were dosed with eight 4-fold serial dilutions of ADC conjugates (FL1-PNU, FL2-PNU, FL3-PNU, HC1-LC4-PNU, HC5-LC3-PNU, 2G10-PNU, ATN658-PNU, FL1-MMAE and NC-PNU and MMAE-NC ADCs), and, where indicated, unconjugated FL1 (Naked FL1), and free toxin

(PNU) in 100 µl of complete growth media, with control wells receiving PBS-supplemented media alone. Three- or five-days post-incubation at 37°C 5% $CO_2$, cell viability was evaluated following 1h incubation with 15 µl of CellTiter 96® Aqueous one-solution cell-proliferation assay reagent (MTS, Promega). Plate absorbance was then measured at 490 nm and 650 nm (for background subtraction) using a Spectra Max Plus plate reader (Molecular Devices). Cell viability was calculated as a percentage of untreated control samples. It can be derived from Figures 4, 5 (FL1-PNU) and 11 (FL1-MMAE) that FL1-based ADCs have a superior and uPAR-dependent cytotoxic effect on cell viability (except uPAR knock-out cells) compared to the non-targeting NC-PNU ADC. This effect is observed for all tested uPAR-expressing cell-lines. Furthermore, the FL1 based ADC FL1-PNU appears more potent than 2G10-PNU and ATN658-PNU (Figure 8) and the humanized candidates HC1-LC4-PNU and HC5-LC3-PNU appear more potent than the parent FL1-PNU based on murine FL1 (Figure 11).

***In vivo* efficacy of FL1-PNU in PDAC models**

The efficacy of FL1-PNU was tested in xenograft and allograft mouse models of PDAC, taking advantage of FL1-PNU cross-reactivity. Associated data is presented in Fig. 6. AsPC-1 cell line-derived xenografts were engrafted subcutaneously into the upper right flanks of 7–10 weeks-old female BALB/cAnNRj-Foxn1 nu/nu mice (JANVIER LABS) at 2.5 x $10^6$/100 µl sterile PBS per mouse. When tumors reached 80-150 $mm^3$, animals were block-randomized into treatment groups of 12 according to mean tumor size and body weight, and then administered with a weekly tail vein injection of 0.75 mg/ml of test articles or PBS vehicle control, over three consecutive weeks. Tumor volume ($mm^3$ = length × $width^2$/2) and body weight were recorded two to three times weekly, and mice were closely monitored for any signs of distress or abnormal behaviour. Euthanasia was performed at predetermined endpoint criteria (tumor size exceeding 1000 $mm^3$ or 12 mm in diameter; tumor ulcers over 8 mm (in length), ~ 15% weight loss or abnormal behaviours). Syngeneic graft studies were performed using 8–weeks-old wild-type C57BL/6 mice (Jackson Laboratories) orthotopically injected into the pancreas with 5 x $10^4$ Luc-KPC2 cells in 30 µl sterile PBS, following an established surgical procedure. Two weeks after recovery, tumor growth was qualitatively monitored using the IVIS Spectrum Imaging System (PerkinElmer), following i.p. injection of D-luciferin (PerkinElmer, #122799). Mice with no detectable luciferase activity were excluded from the study (2/30). The remaining animals (28/30) were randomized into the three treatment arms (n = 8-10) with equal average luciferase activity, dosed twice as

described above, and finally sacrificed at day 28 from the tumor implant. The treatment efficacy was evaluated by comparing tumor weight at termination.

**Immunophenotyping**

At the time of harvest, mice were euthanized, and tumors were excised. Spleens from tumor-free mice were collected as well and used as a control for proper staining. Briefly, the dissected tumors were gently rinsed with ice-cold 1X PBS, and any attached healthy tissues were removed using a sterile scalpel. Tumors were then finely minced with a razor blade into the wells of a 6-multi-well plate on ice and enzymatically digested in 2-3 ml of digestion buffer consisting of RPMI 1640 medium supplemented with 5 mM of CaCl2, 1.5 mg/ml Collagenase D (11088858001, Roche) and 100 ug/ml DNase I. The samples were then incubated for 35 min at 37°C, with gentle shaking every 10 min, to aid digestion. Spleens were physically ground into a sterile Petri dish and kept on ice until the tumor digestion was complete. The dissociated single-cell suspensions were then rinsed with ice-cold media to neutralize enzymatic activity, filtered through a 70 µm cell strainer, and collected by centrifugation. Lysis of red cells was then performed by resuspending cell pellets in 5 ml of ACK lysing buffer (Gibco™, #A1049201) per manufacturer's instructions. After a washing step in ice-cold 1X PBS, cells were counted and $2*10^6$ cells/100 µl were aliquoted to the wells of a 96-well polypropylene round bottom plate (Corning® 96, #3879), and collected by centrifugation for subsequent staining. The live-dead stain was first performed by resuspending cell pellets in 100 µl of diluted Zombie Aqua™ (1:400) in 1X PBS, followed by a 20 min incubation, in the dark, at room temperature. A mixture of live and heat-killed splenocytes was included as a live-dead single-stained cell control (1:800). Immunostaining was then carried out using a general panel for lymphoid and myeloid cell discrimination. The following anti-mouse antibodies/dies (purchase from BD Biosciences unless otherwise stated) were used: CD45-BUV395 (1:800), TCR β-Pacific blue (BioLegend, 1:500), CD19-PE-Cy7 (1:800), CD11b-PerCP-eFluor 710 (Invitrogen; 1:1600), Ly6C-PE (1:800), Ly6G-APC-H7 (1:400). Samples were stained using 100 µl of freshly prepared Ab master mixes in FACS staining buffer (SB, 1X PBS, 1% BSA), including True-Stain Monocyte Blocker™ (BioLegend, #426102) for Fc blocking, and incubated, at 4°C for 20 min, protected from light. Single-stained controls were prepared using UltraComp eBeads (Invitrogen, #01-2222-42). After surface staining and two washing steps with SB, samples and relative eBeads were fixed in paraformaldehyde and stored at 4°C in the dark, until detection. Samples were acquired on the Becton Dickinson LSRFortessa using FACSDiva 8.0.1 software located

in the UNC Flow Cytometry Core Facility. A minimum of $10^6$ and $10^4$ events/tube were collected for cell samples and beads, respectively. The FlowJo™ Software v10.8.1 was used for data analysis.

**FL-1-PNU targets uPAR-positive immune and stromal cells and elicits bystander cytotoxicity *in vitro***

Transwell Co-culture Assay

The ADC bystander activity was assessed by a co-culture of uPAR-positive fibroblasts, human 1BR3.G, and murine NIH3T3 cells, respectively, and $uPAR^{KO}$ KPC2 cancer cells in dual-chamber transwell-clear dishes. A total of $7.5 \times 10^4$ $uPAR^{KO}$ KPC2 were plated in the bottom chamber of the 6-well cell culture plates, while $1.5 \times 10^5$ fibroblasts were seeded in the upper transwell-inserts (Corning, #3450), with microscopically transparent polyester membranes with a pore size 0.4 µm, designed for drug transport studies. At 24 h after seeding, FL1-PNU or aTNP-PNU ADCs (at 4 nM) were added to the upper inserts. Following 72 h of incubation, cells in both chambers were detached and viability was measured using a NucleoCounter NC-3000 (ChemoMetec) after staining for live/dead cells with Solution-13 AO-DAPI stain (ChemoMetec, # 910-3013).

Given the established negative impact of the dysplastic TME in PDAC drug resistance, simultaneous targeting of cancer, stromal, and immunosuppressive components may enhance the therapeutic response. Therefore, we investigated FL1-PNU´s activity on M2-differentiated bone marrow-derived macrophages (M2-BMDM), as well as human and murine fibroblast cell lines, namely 1BR3.G and NIH3T3. These cells served as *in vitro* models of tumor-associated macrophages and activated cancer-associated fibroblasts (CAFs), which dominate the PDAC TME and are known to upregulate uPAR, compared to normal quiescent cells (Fig. 12).

Bone marrow precursor cells were isolated from the pelvis, femur, and tibia of FVB/N mice (8-10 weeks). The collected cells were then differentiated to M2-like macrophages in 96-well plates ($5 \times 10^4$ cells in 100 uL per well) in media (DMEM, 10% FBS, 1% P/S) containing 20 ng/mL mM-CSF (Biotechne) for four days, followed by an additional two days of stimulation with 20 ng/mL mM-CSF and 20 ng/mL mIL-4 (Biotechne). For the flow cytometry analysis, cells were incubated with A647-labeled-FL1 at a pre-determined saturating concentration on ice in the dark for 1 hour. After staining,

samples were stained wth 1 µg/ml with 7-AAD (Invitrogen, # 1890506) for cell death exclusion and finally acquired on a BD LSR Fortessa 20X (BD Biosciences).

Both M2-BMDM and fibroblasts exhibited a dose- and uPAR-expression-dependent response to FL1-PNU, compared to aTNP-PNU, following a three-day exposure to the ADCs (Fig. 13A).

We next assessed whether this uPAR-mediated stromal/immune targeting by FL1-PNU could also promote the killing of uPAR-negative cancer cells by bystander cytotoxicity. For this purpose, we employed dual-chamber Transwell dishes with uPAR-positive fibroblasts seeded in the upper permeable inserts and uPAR$^{KO}$ KPC2 cancer cells in the bottom wells (Fig. 13B). In this system, any potential bystander factors can diffuse through the porous membrane to the bottom compartment. Based on the monoculture results, we selected a concentration of 4 nM FL1-PNU to achieve minimal targeting of the uPAR-null cells and maximum cytotoxicity of the uPAR-positive fibroblasts (EC80) (Fig. 13C). Both mono- and transwell co-cultures were treated with the FL1- and aTNP-PNU ADCs and the cell viability was assessed after three days of incubation. As expected, in the monoculture setting, direct FL1-PNU treatment markedly impacted the viability of the uPAR-positive fibroblasts, but not of the uPAR$^{KO}$ KPC2 cells, when compared to the aTNP-PNU ADC. However, in the co-culture systems, we observed a striking reduction in both uPAR-positive and negative cell populations following exposure of the uPAR-positive fibroblasts to FL1-PNU (Fig. 13D). These results indicated that free PNU diffused in the media from the FL1-PNU-targeted uPAR-positive fibroblasts, resulting in the killing of the neighboring uPAR$^{KO}$ cells, which are sensitive to PNU itself (data not shown) but not to the uPAR-ADC.

Combined, these data indicate that FL1-PNU can effectively target stromal and immune cells in a uPAR-dependent fashion. This leads to free drug release and bystander killing of cytotoxin-sensitive uPAR-negative tumor cells.

**Results and conclusions**

Internalization data in uPAR-expressing U937 cells indicate a significant internalization of all tested mAbs FL1, FL2 and FL3 supporting their potential as ADC candidates. FL1 emerged as the best internalized and was therefore selected as the lead candidate.

Importantly, no uptake was observed in cells devoid of the receptor, namely KPC knock-out cells, thus confirming a uPAR-mediated internalization process (unpublished results).

We then evaluated the *in vitro* and *in vivo* efficacy of FL1-PNU-based ADC, comprising the highly potent anthracycline derivative, PNU159682, in relevant preclinical models of PDAC . We also assessed the in vitro efficacy of FL1 coupled to a payload with a different mode of cytotoxicity, namely the anti-mitotic cytotoxin, MMAE.

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In the *in vitro* setting, various human and murine cells with distinct uPAR levels were investigated and proven to be highly sensitive to the FL1-ADC's antitumor activity, in a uPAR- and concentration-dependent manner, with EC50 values in the low nanomolar/picomolar range, and a broad window of selective efficacy (Figures 4, 5 and 11). Indeed, both uPAR-null cells and cells exposed to the non-targeted ADC, aTNP-PNU were unresponsive, further confirming a specific cytotoxic activity by the worked FL1-based a-uPAR ADCs. Cytotoxicity was proven to derive from the selective delivery of the cytotoxic moiety, as no antitumor effect was elicited by FL1 when unconjugated.

Likewise, potent *in vitro* cytotoxicity was also observed for FL1-ADCs based on humanized variants of FL1. As evidenced in Figure 10, these humanized-FL1-ADCs appear more potent than the parent murine-FL1-based ADC

In addition to PDAC cells, FL1-PNU also showed strong activity against in vitro models of uPAR-positive tumor-associated macrophages and CAFs (Fig. 12 and Fig. 13). Direct targeting of these cells may help to alleviate the fibrotic and immunosuppressive barriers and impair the tumor-stroma cross-talks driving PDAC progression and metastasis. Furthermore, the ability of uPAR-positive cells to mediate a bystander effect after targeting by FL1-PNU, as exemplified by our transwell co-culture experiments, may further amplify the antitumor response by facilitating intratumoral drug distribution within tumors with heterogeneous uPAR expression, like PDAC.

FL1 efficacy was further validated *in vivo* in distinct mouse models of PDAC, to better recapitulate the biological complexities of the human disease. The simplest setup involved a xenograft immunocompromised model based on one of the sensitive human cell lines of the investigated panel, AsPC-1, and three-treatment arms consisting of both

uPAR-targeted and non-specific ADC, and a PBS vehicle control, under an optimized treatment schedule to ensure tolerability. Compared to both control arms, terminated due to excessive tumor burden, FL1-PNU was able to rapidly induce considerable tumor shrinkage in all but one of the treated mice, with a complete cure achieved in half of the animals after treatment cessation (Fig. 6A). Tumor relapse was observed in 5 of the mice, with small masses reoccurring later in the study, but halted to a stable disease stable until the end of the follow-up. Importantly, the FL1-PNU-induced antitumor effect resulted in significantly longer overall survival (Fig. 6B), with 9 out of 12 mice still alive at the study endpoint, and no significant weight loss associated with the treatment (Fig. 6C).

To validate these promising findings in a more biologically relevant model of PDAC, and immunologically preserved context, the study was repeated in a well-recognized syngeneic orthotopic model, established from KPC cells in immune-competent hosts (Fig. 6D). With only two given doses, FL1-PNU exerted a remarkable uPAR-dependent antitumor effect, confirmed by the significantly reduced tumor volumes of the treated mice, as opposed to both control groups (Figs. 6E and 6F). Notably, mice did not experience any weight loss or clinically evident signs of toxicity, indicating that FL1-PNU is well tolerated under the experimented treatment setup (Fig. 6G). This was further confirmed by histological evaluation of major animal organs, like the liver or kidney, where no gross morphological abnormalities were detected.

Importantly, immune profiling of the syngeneic orthotopic tumors revealed a significant infiltration of T lymphocytes and inflammatory cells following treatment with FL1-PNU (Figure 8). In striking contrast, the PBS group almost lacked these immune cell components, in line with the immunologically “cold” nature of the PDAC tumor microenvironment (TME). In addition, the myeloid cells ($CD11b^+$) and, among them, neutrophils, and myeloid derived suppressor cells, which belong to and dominate the suppressive arsenal of the tumor stroma, as seen in the vehicle samples, were almost halved in the FL1-PNU-treated ones. Combined these data suggest that FL1-PNU tumorigenic activity triggers a broad immunomodulation, engaging both adaptive and innate immune players, which may, in turn, boost the ADC effect. In parallel, targeting of the myeloid-cell-dominated suppressive milieu, may relax the immunological tolerance and facilitate the activated antitumor immune responses. As uPAR is highly expressed on these cells, the observed reduction may realistically derive from their direct targeting

by the ADC, which suggest a potential uPAR-specific stromal targeting effect by the presently worked ADC.

The potency of FL1-PNU was compared to the potency of ADCs comprising two known antibodies, 2G10 and ATN658, conjugated with the same linker payload. It can be derived from Figure 8 that FL1-PNU appears more potent than 2G10-PNU and ATN658-PNU, confirming FL1 as a superior anti-uPAR mAb candidate in an ADC format.

Likewise, the *in vitro* efficacy of ADCs comprising FL1, FL2, and FL3 mAbs was compared to the efficacy of an ADC comprising a known antibody, 2G10. The antibodies were conjugated with the same linker payload. It can be derived from Figure 9 that FL1-PNU appears more potent than all other tested conjugates including 2G10-PNU, which confirms FL1 as a superior anti-uPAR mAb candidate in an ADC format. FL2-PNU appears more potent than 2G10 and FL3-PNU, respectively.

Based on the presented results, particularly the anti-proliferative impact of FL1-based ADCs on various *in vitro* and *in vivo* models, including PDAC models, we consider that a conjugate comprising FL1 and a payload, such as PNU159682 or MMAE, such as the FL1-DBCO-PEG4-VC-PAB-DMEA-PNU-159682 or FL1-DBCO-(PEG)3-VC-PAB-MMAE formats worked in the present example are particularly suitable for the treatment of uPAR expressing cancers, in particular PDAC. ADCs comprising humanized versions of FL1 are also considered particularly suitable for the treatment of uPAR expressing cancers.

Furthermore, given the proven similarly robust and selective *in vitro* efficacy of the FL1-PNU conjugate in uPAR-positive U937, U87, MDA-MB-231, and REN-Luc cells, and FL1-MMAE ADC on uPAR-positive HT-1080 cells, we evaluate the therapeutic value of this ADC for use in these other aggressive treatment indications, respectively, leukemia, glioblastoma, triple-negative breast cancer, fibrosarcoma, and mesothelioma, and make solid ground for its exploitation in all existing uPAR-positive oncological indications, for instance as Pan-Cancer therapy.

**Sequence overview**

| SEQ ID NO | Sequence | Comment |
|---|---|---|
| 1 | MGHPPLLPLLLLLHTCVPASWGLRCM QCKTNGDCRVEECALGQDLCRTTIVR LWEEGEELELVEKSCTHSEKTNRTLS YRTGLKITSLTEVVCGLDLCNQGNSG RAVTYSRSRYLECISCGSSDMSCERG RHQSLQCRSPEEQCLDVVTHWIQEG EEGRPKDDRHLRGCGYLPGCPGSNG FHNNDTFHFLKCCNTTKCNEGPILELE NLPQNGRQCYSCKGNSTHGCSSEET FLIDCRGPMNQCLVATGTHEPKNQSY MVRGCATASMCQHAHLGDAFSMNHI DVSCCTKSGCNHPDLDVQYRSGAAP QPGPAHLSLTITLLMTARLWGGTLLW T | Human uPAR aa sequence (UniProtKB/Swiss-Prot: Q03405.1), aa1-aa22 is signal peptide |
| 2 | LRCMQCKTNGDCRVEECALGQDLCR TTIVRLWEEGEELELVEKSCTHSEKTN RTLSYRTGLKITSLTEVVCGLDLCNQG NSGRAVTYSRSRY | uPAR domain 1 (D1), aa 23-114 |
| 3 | LECISCGSSDMSCERGRHQSLQCRS PEEQCLDVVTHWIQEGEEGRPKDDR HLRGCGYLPGCPGSNGFHNNDTFHF LKCCNTTKCNEGPILELENLPQNG | uPAR domain 2 (D2), aa 115-213 |
| 4 | RQCYSCKGNSTHGCSSEETFLIDCRG PMNQCLVATGTHEPKNQSYMVRGCA TASMCQHAHLGDAFSMNHIDVSCCT KSGCNHPDLDVQYRSG | uPAR domain 3 (D3), aa 214-305 |
| 5 | DIVMTQTPLSLPVSLGDQASISCRSSQ SVVSNNGKTYLEWYLQKPGQSPKLLI YKVSNRFSGVPDRFSGSGSGTDFTLK ISRVEAEDLGVYYCFQGSHVPWTFG GGTKLEVKRADAAPTVSIFPPSSEQLT SGGASVVCFLNNFYPKDINVKWKIDG SERQNGVLNSWTDQDSKDSTYSMSS TLTLTKDEYERHNSYTCEATHKTSTS PIVKSFNRNEC | FL1 light chain |
| 6 | DIVMTQTPLSLPVSLGDQASISCRSSQ SVVSNNGKTYLEWYLQKPGQSPKLLI YKVSNRFSGVPDRFSGSGSGTDFTLK | FL1 light chain variable region |

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|---|---|---|
| | ISRVEAEDLGVYYCFQGSHVPWTFG GGTKLEVK | |
| 7 | QSVVSNNGKTY | FL1 IMGT-predicted LC CDR1 |
| 8 | KVS | FL1 IMGT-predicted LC CDR2 |
| 9 | FQGSHVPWT | FL1 IMGT-predicted LC CDR3 |
| 10 | EVQLQQSGSVLVRPGTSVNLSCKAS GYTFTSYWMSWVKQRPGQGLEWIG EIFPKSGRINSNENFRGKATLTVDTSS STAYINLSSLTSEDSAVYYCSPIYSGD YGFADWGQGTLVTVSAAKTTPPSVY PLAPGSAAQTNSMVTLGCLVKGYFPE PVTVTWNSGSLSSGVHTFPAVLQSDL YTLSSSVTVPSSTWPSETVTCNVAHP ASSTKVDKKIVPRDC | FL1 heavy chain |
| 11 | EVQLQQSGSVLVRPGTSVNLSCKAS GYTFTSYWMSWVKQRPGQGLEWIG EIFPKSGRINSNENFRGKATLTVDTSS STAYINLSSLTSEDSAVYYCSPIYSGD YGFADWGQGTLVTVSA | FL1 heavy chain variable region |
| 12 | GYTFTSYW | FL1 IMGT-predicted HC CDR1 |
| 13 | IFPKSGRI | FL1 IMGT-predicted HC CDR2 |
| 14 | SPIYSGDYGFAD | FL1 IMGT-predicted HC CDR3 |
| 15 | DIVMTQTPASLAVSLGQSVTISCRASE SLEYYGNKLMQWYQQKPGQPPRLLI YGASNVESGVPDRFSGSGSGTDFSL NIHPVEEDDIAMYFCQQSRKVPSTFG GGTKLEIKRADAAPTVSIFPPSSEQLT SGGASVVCFLNNFYPKDINVKWKIDG SERQNGVLNSWTDQDSKDSTYSMSS TLTLTKDEYERHNSYTCEATHKTSTS PIVKSFNRNEC | Fab FL2 light chain |
| 16 | DIVMTQTPASLAVSLGQSVTISCRASE SLEYYGNKLMQWYQQKPGQPPRLLI YGASNVESGVPDRFSGSGSGTDFSL | FL2 light chain variable region |

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|---|---|---|
| | NIHPVEEDDIAMYFCQQSRKVPSTFG GGTKLEIK | |
| 17 | ESLEYYGNKL | FL2 IMGT-predicted LC CDR1 |
| 18 | GAS | FL2 IMGT-predicted LC CDR2 |
| 19 | QQSRKVPST | FL2 IMGT-predicted LC CDR3 |
| 20 | EVKLVESGGGLVKPGGSLKLSCAASG FTFSRHAMSWVRQTPEKRLEWVAYIS GGGDYIHYVDTVQGRFTISRDNARNT LYLQMSSLKSEDTAMYFCTSLIYYHD RTYAYFAYWGQGTLVTVSAAKTTPPS VYPLAPGSAAQTNSMVTLGCLVKGYF PEPVTVTWNSGSLSSGVHTFPAVLQS DLYTLSSSVTVPSSTWPSETVTCNVA HPASSTKVDKKIVPRDC | Fab FL2 heavy chain |
| 21 | EVKLVESGGGLVKPGGSLKLSCAASG FTFSRHAMSWVRQTPEKRLEWVAYIS GGGDYIHYVDTVQGRFTISRDNARNT LYLQMSSLKSEDTAMYFCTSLIYYHD RTYAYFAYWGQGTLVTVSA | FL2 heavy chain variable region |
| 22 | GFTFSRHA | FL2 IMGT-predicted HC CDR1 |
| 23 | ISGGGDYI | FL2 IMGT-predicted HC CDR2 |
| 24 | TSLIYYHDRTYAYFAY | FL2 IMGT-predicted HC CDR3 |
| 25 | DILMTQSPASLSASVGETVTITCRASE NIYSYLAWYQQKQGKSPQLLVYHAQS LTEGVPSRFSGSGSGSQFSLKINSLQ PEDFGTYYCQHHYATPRTFGGGTRL ELKRADAAPTVSIFPPSSEQLTSGGAS VVCFLNNFYPKDINVKWKIDGSERQN GVLNSWTDQDSKDSTYSMSSTLTLTK DEYERHNSYTCEATHKTSTSPIVKSF NRNEC | Fab FL3 light chain |
| 26 | DILMTQSPASLSASVGETVTITCRASE NIYSYLAWYQQKQGKSPQLLVYHAQS | FL3 light chain variable region |

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|---|---|---|
| | LTEGVPSRFSGSGSGSQFSLKINSLQ PEDFGTYYCQHHYATPRTFGGGTRL ELK | |
| 27 | ENIYSY | FL3 IMGT-predicted LC CDR1 |
| 28 | HAQ | FL3 IMGT-predicted LC CDR2 |
| 29 | QHHYATPRT | FL3 IMGT-predicted LC CDR3 |
| 30 | QVQLQESGPGLVAPSQRLSINCTVSG FSLIRYDISWIRQPPGKGLEWLGVIWT GGGTNYNSAFMSRLTISKDNSKSQVF LKVNSLQTDDTAIYYCVRGGALFAYW GQGTLVTVSEAKTTPPSVYPLAPGSA AQTNSMVTLGCLVKGYFPEPVTVTW NSGSLSSGVHTFPAVLQSDLYTLSSS VTVPSSTWPSETVTCNVAHPASSTKV DKKIVPRDC | Fab FL3 heavy chain |
| 31 | QVQLQESGPGLVAPSQRLSINCTVSG FSLIRYDISWIRQPPGKGLEWLGVIWT GGGTNYNSAFMSRLTISKDNSKSQVF LKVNSLQTDDTAIYYCVRGGALFAYW GQGTLVTVSE | FL3 heavy chain variable region |
| 32 | GFSLIRYD | FL3 IMGT-predicted HC CDR1 |
| 33 | IWTGGGT | FL3 IMGT-predicted HC CDR2 |
| 34 | VRGGALFAY | FL3 IMGT-predicted HC CDR3 |
| 35 | RSSQSVVSNNGKTYLE | FL1 KABAT-predicted LC CDR1 |
| 36 | KVSNRFS | FL1 KABAT-predicted LC CDR2 |
| 37 | FQGSHVPWT | FL1 KABAT-predicted LC CDR3 |
| 38 | SYWMS | FL1 KABAT-predicted HC CDR1 |
| 39 | EIFPKSGRINSNENFRG | FL1 KABAT-predicted HC CDR2 |
| 40 | YSGDYGFAD | FL1 KABAT-predicted HC CDR3 |

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| 41 | GACATTGTGATGACCCAAACTCCAC<br>TCTCCCTGCCTGTCAGTCTTGGAGA<br>TCAAGCCTCCATCTCTTGCAGATCC<br>AGTCAGAGCGTTGTATCTAATAATG<br>GAAAAACCTATTTAGAATGGTACCT<br>GCAGAAACCAGGCCAGTCTCCAAAG<br>CTCCTGATCTACAAAGTTTCCAACC<br>GATTTTCTGGGGTCCCAGACAGGTT<br>CAGTGGCAGTGGATCAGGGACAGA<br>TTTCACACTCAAGATCAGCAGAGTG<br>GAGGCTGAGGATCTGGGAGTTTATT<br>ACTGCTTTCAAGGTTCACATGTTCC<br>GTGGACGTTCGGTGGAGGCACCAA<br>ACTGGAAGTCAAACGGGCTGATGCT<br>GCACCAACTGTATCCATCTTCCCAC<br>CATCCAGTGAGCAGTTAACATCTGG<br>AGGTGCCTCAGTCGTGTGCTTCTTG<br>AACAACTTCTACCCCAAAGACATCA<br>ATGTCAAGTGGAAGATTGATGGCAG<br>TGAACGACAAAATGGCGTCCTGAAC<br>AGTTGGACTGATCAGGACAGCAAAG<br>ACAGCACCTACAGCATGAGCAGCAC<br>CCTCACGTTGACCAAGGACGAGTAT<br>GAACGACATAACAGCTATACCTGTG<br>AGGCCACTCACAAGACATCAACTTC<br>ACCCATTGTCAAGAGCTTCAACAGG<br>AATGAGTGT | Nucleotide encoding FL1 light chain |
| 42 | GAGGTTCAGCTGCAGCAGTCTGGG<br>TCTGTGCTGGTGAGGCCTGGAACTT<br>CAGTGAACCTGTCCTGCAAGGCTTC<br>TGGCTACACTTTCACCAGCTACTGG<br>ATGAGCTGGGTGAAGCAGAGGCCT<br>GGACAAGGCCTTGAGTGGATTGGA<br>GAAATTTTTCCTAAAAGTGGTAGAAT<br>TAACTCCAATGAGAACTTCAGGGGC<br>AAGGCCACACTGACTGTTGACACAT<br>CCTCCAGCACAGCCTACATTAATCT<br>CAGCAGCCTGACCTCTGAGGACTCT<br>GCGGTCTATTACTGTTCACCTATCTA<br>CTCTGGTGACTACGGATTTGCTGAC<br>TGGGGCCAAGGGACTCTGGTCACT<br>GTCTCTGCAGCCAAAACGACACCCC<br>CATCTGTCTATCCACTGGCCCCTGG<br>ATCTGCTGCCCAAACTAACTCCATG<br>GTGACCCTGGGATGCCTGGTCAAG | Nucleotide encoding FL1 heavy chain |

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|---|---|---|
| | GGCTATTTCCCTGAGCCAGTGACAG<br>TGACCTGGAACTCTGGATCCCTGTC<br>CAGCGGTGTGCACACCTTCCCAGCT<br>GTCCTGCAGTCTGACCTCTACACTC<br>TGAGCAGCTCAGTGACTGTCCCCTC<br>CAGCACCTGGCCCAGCGAGACCGT<br>CACCTGCAACGTTGCCCACCCGGC<br>CAGCAGCACCAAGGTGGACAAGAA<br>AATTGTGCCCAGGGATTGT | |
| 43 | GACATTGTGATGACCCAAACTCCAG<br>CTTCTTTGGCTGTGTCTCTAGGGCA<br>GAGTGTCACCATCTCCTGCAGAGCC<br>AGTGAAAGTCTTGAGTATTATGGCA<br>ATAAGTTAATGCAGTGGTACCAACA<br>GAAACCGGGGACAGCCACCCCGACT<br>CCTCATCTATGGTGCATCCAACGTA<br>GAATCTGGGGTCCCTGACAGGTTTA<br>GTGGCAGTGGGTCTGGGACAGACT<br>TCAGCCTCAACATCCATCCTGTGGA<br>GGAGGATGATATTGCAATGTATTTCT<br>GTCAGCAAAGTAGGAAGGTTCCTTC<br>GACGTTCGGTGGAGGCACCAAGCT<br>GGAAATCAAACGGGCTGATGCTGCA<br>CCAACTGTATCCATCTTCCCACCAT<br>CCAGTGAGCAGTTAACATCTGGAGG<br>TGCCTCAGTCGTGTGCTTCTTGAAC<br>AACTTCTACCCCAAAGACATCAATGT<br>CAAGTGGAAGATTGATGGCAGTGAA<br>CGACAAAATGGCGTCCTGAACAGTT<br>GGACTGATCAGGACAGCAAAGACA<br>GCACCTACAGCATGAGCAGCACCCT<br>CACGTTGACCAAGGACGAGTATGAA<br>CGACATAACAGCTATACCTGTGAGG<br>CCACTCACAAGACATCAACTTCACC<br>CATCGTCAAGAGCTTCAACAGGAAT<br>GAGTGT | Nucleotide encoding FL2 light chain |
| 44 | GAAGTGAAGCTGGTGGAGTCTGGG<br>GGAGGCTTAGTGAAGCCTGGAGGG<br>TCCCTGAAACTCTCCTGTGCAGCCT<br>CTGGATTCACTTTCAGTAGGCATGC<br>CATGTCTTGGGTTCGCCAGACTCCA<br>GAGAAGAGGCTGGAGTGGGTCGCA<br>TACATTAGTGGTGGTGGTGATTATAT<br>CCACTATGTCGACACTGTGCAGGGC | Nucleotide encoding FL2 heavy chain |

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| | CGATTCACCATCTCCAGAGACAATG<br>CCAGGAACACCCTCTACTTGCAAAT<br>GAGCAGTCTGAAGTCTGAGGACACA<br>GCCATGTATTTCTGTACAAGTCTGAT<br>TTATTACCACGATAGAACCTACGCC<br>TACTTTGCTTACTGGGGCCAAGGGA<br>CTCTGGTCACTGTCTCTGCAGCCAA<br>AACGACACCCCCATCTGTCTATCCA<br>CTGGCCCCTGGATCTGCTGCCCAAA<br>CTAACTCCATGGTGACCCTGGGATG<br>CCTGGTCAAGGGCTATTTCCCTGAG<br>CCAGTGACAGTGACCTGGAACTCTG<br>GATCCCTGTCCAGCGGTGTGCACAC<br>CTTCCCAGCTGTCCTGCAGTCTGAC<br>CTCTACACTCTGAGCAGCTCAGTGA<br>CTGTCCCCTCCAGCACCTGGCCCA<br>GCGAGACCGTCACCTGCAACGTTG<br>CCCACCCGGCCAGCAGCACCAAGG<br>TGGACAAGAAAATTGTGCCCAGGGA<br>TTGT | |
| 45 | GACATTCTGATGACCCAGTCTCCAG<br>CCTCCCTATCTGCATCTGTGGGAGA<br>AACTGTCACCATCACATGTCGAGCA<br>AGTGAGAATATTTACAGTTATTTAGC<br>ATGGTATCAGCAGAAACAGGGAAAA<br>TCTCCTCAACTCCTGGTCTATCATG<br>CACAATCCTTAACAGAAGGTGTGCC<br>ATCAAGGTTCAGTGGCAGTGGATCA<br>GGCTCACAGTTTTCTCTGAAGATCA<br>ACAGCCTGCAGCCTGAAGATTTTGG<br>GACTTATTACTGTCAACATCATTATG<br>CTACTCCTCGGACGTTCGGTGGAG<br>GCACCAGGCTGGAACTCAAACGGG<br>CTGATGCTGCACCAACTGTATCCAT<br>CTTCCCACCATCCAGTGAGCAGTTA<br>ACATCTGGAGGTGCCTCAGTCGTGT<br>GCTTCTTGAACAACTTCTACCCCAAA<br>GACATCAATGTCAAGTGGAAGATTG<br>ATGGCAGTGAACGACAAAATGGCGT<br>CCTGAACAGTTGGACTGATCAGGAC<br>AGCAAAGACAGCACCTACAGCATGA<br>GCAGCACCCTCACGTTGACCAAGGA<br>CGAGTATGAACGACATAACAGCTAT<br>ACCTGTGAGGCCACTCACAAGACA | Nucleotide encoding FL3 light chain |

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| | TCAACTTCACCCATTGTCAAGAGCTT CAACAGGAATGAGTGT | |
| 46 | CAGGTACAGCTGCAGGAGTCAGGA CCTGGCCTGGTGGCGCCCTCACAG AGACTGTCCATTAACTGCACTGTCT CTGGGTTCTCATTAATCAGATATGAT ATAAGCTGGATTCGCCAGCCACCAG GAAAGGGTCTGGAGTGGCTTGGAG TAATATGGACTGGTGGAGGCACAAA TTATAATTCAGCTTTCATGTCCAGAC TGACCATCAGCAAGGACAACTCCAA GAGCCAAGTTTTCTTAAAAGTGAAC AGTCTGCAAACTGATGACACAGCCA TATATTACTGTGTAAGAGGGGGCGC TCTGTTTGCTTACTGGGGCCAAGGG ACTCTGGTCACTGTCTCTGAAGCCA AAACGACACCCCCATCTGTCTATCC ACTGGCCCCTGGATCTGCTGCCCAA ACTAACTCCATGGTGACCCTGGGAT GCCTGGTCAAGGGCTATTTCCCTGA GCCAGTGACAGTGACCTGGAACTCT GGATCCCTGTCCAGCGGTGTGCAC ACCTTCCCAGCTGTCCTGCAGTCTG ACCTCTACACTCTGAGCAGCTCAGT GACTGTCCCCTCCAGCACCTGGCC CAGCGAGACCGTCACCTGCAACGTT GCCCACCCGGCCAGCAGCACCAAG GTGGACAAGAAAATTGTGCCCAGGG ATTGT | Nucleotide encoding FL3 heavy chain |
| 47 | QVQLQQSGAEVKKPGSSVKVSCKAS GYTFTSYWMSWVKQRPGHGLEWMG EIFPKSGRINSNENFRGRVTITADTST STAYMELSSLRSEDTAVYYCSPIYSG DYGFADWGQGTLVTVSS | FL1 humanized heavy chain variable region 1 (VH1) |
| 48 | QVQLVQSGAEVKQPGASVKVSCKAS GYTFTSYWMSWVKQRPGQGLEWLG EIFPKSGRINSNENFRGRVTLTTDTSIS TAYMELSSLRSDDSAVYYCSPIYSGD YGFADWGQGTLVTVSS | FL1 humanized heavy chain variable region 2 (VH2) |
| 49 | EVQLLESGGGLVRPGGSLRLSCAAS GYTFTSYWMSWVKQRPGKGLEWVS EIFPKSGRINSNENFRGRFTISVDNSK | FL1 humanized heavy chain variable region 3 (VH3) |

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| | NTLYLQMNSLRAEDSAVYYCSPIYSG DYGFADWGQGTLVTVSA | |
| 50 | QVQLVQSGSEVKQPGASVKVSCKAS GYTFTSYWMSWVKQRPGQGLEWMG EIFPKSGRINSNENFRGRFVFSMDTS ASTAYLQISSLKAEDSAMYYCSPIYSG DYGFADWGQGTLVTVSA | FL1 humanized heavy chain variable region 4 (VH4) |
| 51 | QVQLVQSGAEVKKPGASVKVSCKAS GYTFTSYWMSWVKQRPGQGLEWIG EIFPKSGRINSNENFRGRVTLTVDTST STVYMELSSLRSEDSAVYYCSPIYSG DYGFADWGQGTLVTVSA | FL1 humanized heavy chain variable region 5 (VH5) |
| 52 | ASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCD KTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK | FL1 humanized HC constant domain (IgG1) |
| 53 | DIVMTQSPLSLSVTPGQPASISCRSSQ SVVSNNGKTYLEWYLQKPGQSPQLLI YKVSNRFSGVPDRFSGSGSGTDFTLK ISRVEAEDVGVYYCFQGSHVPWTFG QGTKVEIK | FL1 humanized light chain variable region 1 (VL1) |
| 54 | DVVMTQSPLSLPVTLGQPASISCRSS QSVVSNNGKTYLEWFLQRPGQSPRR LLYKVSNRFSGVPDRFSGSGSGTDFT LKISRVEAEDVGVYYCFQGSHVPWTF GQGTKVEIK | FL1 humanized light chain variable region 2 (VL2) |
| 55 | DIVMTQTPLSLPVTPGQPASISCKSSQ SVVSNNGKTYLEWYLQKPGQPPQLLI YKVSNRFSGVPDRFSGSGSGTDFTLK ISRVEAEDVGVYYCFQGSHVPWTFG GGTKVEIK | FL1 humanized light chain variable region 3 (VL3) |
| 56 | DIVMTQSPDSLAVSLGERATINCRSS QSVVSNNGKTYLEWYQQKPGQPPKL LIYKVSNRFSGVPDRFSGSGSGTDFT LTISSLQAEDVAVYYCFQGSHVPWTF GGGTKVEIK | FL1 humanized light chain variable region 4 (VL4) |

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| 57 | DIVMTQTPLSLSVTPGQPASISCKSSQSVVSNNGKTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPWTFGGGTKLEVK | FL1 humanized light chain variable region 5 (VL5) |
| 58 | TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC | FL1 humanized LC constant domain variant 1 (IgK isotype) |
| 59 | RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC | FL1 humanized LC constant domain variant 2 (IgK isotype) |

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**Claims**

1. An antibody or antigen-binding fragment thereof which binds to the Urokinase-type plasminogen activator receptor (uPAR), wherein said antibody is selected from the group consisting of:
    a. an antibody or antigen-binding fragment thereof comprising
        i. an immunoglobulin light chain comprising or consisting of the amino acid sequence of SEQ ID NO: 5 or a sequence having at least 90% sequence identity thereto, and
        ii. an immunoglobulin heavy chain comprising or consisting of the amino acid sequence of SEQ ID NO: 10 or a sequence having at least 90% sequence identity thereto,
    b. an antibody or antigen-binding fragment thereof comprising
        i. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 6 or a sequence having at least 90% sequence identity thereto, and
        ii. an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 11 or a sequence having at least 90% sequence identity thereto,
    c. an antibody or antigen-binding fragment thereof comprising
        i. an immunoglobulin light chain variable region comprising as complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 35, as CDR2 an amino acid sequence of SEQ ID NO: 36, and as CDR3 and amino acid sequence of SEQ ID NO: 37, and
        ii. an immunoglobulin heavy chain variable region comprising as complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 38, as CDR2 an amino acid sequence of SEQ ID NO: 39, and as CDR3 and amino acid sequence of SEQ ID NO: 40,
    d. an antibody or antigen-binding fragment thereof comprising
        i. an immunoglobulin light chain variable region comprising as complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 7, as CDR2 an amino acid sequence of SEQ ID NO: 8, and as CDR3 and amino acid sequence of SEQ ID NO: 9, and

ii. an immunoglobulin heavy chain variable region comprising as complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 12, as CDR2 an amino acid sequence of SEQ ID NO: 13, and as CDR3 and amino acid sequence of SEQ ID NO: 14,

e. a humanized version of the antibody or antigen-binding fragment thereof of any one of a, b, c or d.,

f. a chimeric version of the antibody or antigen-binding fragment thereof of any one of a, b, c or d,

g. an antibody or antigen-binding fragment thereof comprising

i. an immunoglobulin light chain variable region comprising or consisting of an amino acid sequence selected from SEQ ID NOs: 53-57 or a sequence having at least 90% sequence identity thereto, and

ii. an immunoglobulin heavy chain variable region comprising or consisting of an amino acid sequence selected from SEQ ID NOs: 47-51 or a sequence having at least 90% sequence identity thereto.

2. An antibody or antigen-binding fragment thereof which binds to the Urokinase-type plasminogen activator receptor (uPAR), wherein said antibody is selected from the group consisting of:

a. an antibody or antigen-binding fragment thereof comprising

i. an immunoglobulin light chain comprising or consisting of the amino acid sequence of SEQ ID NO: 15 or a sequence having at least 90% sequence identity thereto, and

ii. an immunoglobulin heavy chain comprising or consisting of the amino acid sequence of SEQ ID NO: 20 or a sequence having at least 90% sequence identity thereto,

b. an antibody or antigen-binding fragment thereof comprising

i. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 16 or a sequence having at least 90% sequence identity thereto, and

ii. an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 21 or a sequence having at least 90% sequence identity thereto,

c. an antibody or antigen-binding fragment thereof comprising

i. an immunoglobulin light chain variable region comprising as complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 17, as CDR2 an amino acid sequence of SEQ ID NO: 18, and as CDR3 and amino acid sequence of SEQ ID NO: 19, and

ii. an immunoglobulin heavy chain variable region comprising as complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 22, as CDR2 an amino acid sequence of SEQ ID NO: 23, and as CDR3 and amino acid sequence of SEQ ID NO: 24,

d. a humanized version of the antibody or antigen-binding fragment thereof of any one of a, b, or c,

e. a chimeric version of the antibody or antigen-binding fragment thereof of any one of a, b, or c.

3. An antibody or antigen-binding fragment thereof which binds to the Urokinase-type plasminogen activator receptor (uPAR), wherein said antibody is selected from the group consisting of:

a. an antibody or antigen-binding fragment thereof comprising

i. an immunoglobulin light chain comprising or consisting of the amino acid sequence of SEQ ID NO: 25 or a sequence having at least 90% sequence identity thereto, and

ii. an immunoglobulin heavy chain comprising or consisting of the amino acid sequence of SEQ ID NO: 30 or a sequence having at least 90% sequence identity thereto,

b. an antibody or antigen-binding fragment thereof comprising

i. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 26 or a sequence having at least 90% sequence identity thereto, and

ii. an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 31 or a sequence having at least 90% sequence identity thereto,

c. an antibody or antigen-binding fragment thereof comprising

i. an immunoglobulin light chain variable region comprising as complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 27, as CDR2 an amino acid sequence of SEQ ID NO: 28, and as CDR3 and amino acid sequence of SEQ ID NO: 29, and

ii. an immunoglobulin heavy chain variable region comprising as complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 32, as CDR2 an amino acid sequence of SEQ ID NO: 33, and as CDR3 and amino acid sequence of SEQ ID NO: 34,

d. a humanized version of the antibody or antigen-binding fragment thereof of any one of a, b, or c,

e. a chimeric version of the antibody or antigen-binding fragment thereof of any one of a, b, or c.

4. The antibody according to any of the preceding claims, wherein said antibody is:

a. an antibody or antigen-binding fragment thereof comprising

i. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 56 or a sequence having at least 90% sequence identity thereto, and

ii. an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 47 or a sequence having at least 90% sequence identity thereto, or

b. an antibody or antigen-binding fragment thereof comprising

i. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 55 or a sequence having at least 90% sequence identity thereto, and

ii. an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 51 or a sequence having at least 90% sequence identity thereto.

5. The antibody according to any of the preceding claims, wherein any sequence variance is outside the complementarity-determining regions.

6. The antibody according to any of the preceding claims, wherein the antibody is selected from a mouse antibody, a chimeric antibody, a human antibody, a humanised antibody, a humanised antigen-binding fragment, a Fab fragment, a Fab' fragment, an F(ab')2 fragment, an Fv, a single chain antibody (SCA) such as an scFv, the variable portion of the heavy and/or light chains thereof, and a Fab miniantibody.

7. The antibody according to any of the preceding claims, wherein the antibody is a monoclonal antibody

8. The antibody according to any of the preceding claims, wherein the antibody is a humanised or fully human monoclonal antibody or antigen-binding fragment thereof.

9. The antibody according to any of the preceding claims, wherein the antibody is a recombinant antibody.

10. The antibody according to any one of the preceding claims, wherein said antibody is an immunoglobulin class antibody such as IgG, IgM, IgD, IgE, IgA, and any subclass thereof, such as IgG1, IgG2, IgG3 or IgG4.

11. The antibody according to any one of the preceding claims, wherein said antibody comprise a light chain (LC) constant domain comprising or consisting of an amino acid sequence selected from SEQ ID NOs: 58-59 or a sequence having at least 90% sequence identity thereto

12. The antibody according to any one of the preceding claims, wherein said antibody comprise a heavy chain (HC) constant domain comprising or consisting of the amino acid sequence of SEQ ID NO: 52 or a sequence having at least 90% sequence identity thereto.

13. The antibody according to claim 1, wherein the antibody does not bind SEQ ID NO: 2 and/or does not bind SEQ ID NO: 4.

14. The antibody according to claim 1, wherein the antibody binds to a conformational epitope comprising or consisting of amino acid residues number 8 (S), 10 (D), 12 (S), 15 (R), and 17 (R) of uPAR domain D2 (SEQ ID NO: 3).

15. An antibody-drug conjugate (ADC) comprising:
a. the antibody as defined in any one of the preceding claims,
b. an active agent, and
c. optionally a linker which links a) to b).

16. The antibody-drug conjugate according to claim 15, wherein the active agent is selected from a therapeutic agent, a cytotoxic agent, a radioisotope, and a detectable label.

17. The antibody-drug conjugate according to any one of claims 15 to 16, wherein the active agent is a cytotoxic agent.

18. The antibody-drug conjugate according to any one of claims 15 to 17, wherein the active agent is a therapeutic agent, such as a therapeutic agent selected from the group consisting of anti-microtubule/anti-mitotic agents, DNA crosslinking agents, DNA alkylating agents, DNA strand scission agents, anthracyclines, antimetabolites, histone deacetylase inhibitors, kinase inhibitors, metabolism inhibitors, peptide antibiotics, immune checkpoint inhibitors, platinum-based antineoplastics, topoisomerase inhibitors, DNA or RNA polymerase inhibitors, nucleotide based agents, and cytotoxic antibiotics.

19. The antibody-drug conjugate according to any one of claims 15 to 18, wherein the active agent is an anti-mitotic agent, such as selected from the group consisting of derivatives of auristatin or dolastatin such as monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF) and more, a taxane such as Paclitaxel or Docetaxel and more, a vinca alkaloid such as Vinblastine, Vincristine, Vindesine or Vinorelbine and more, a mayatansinoid, Colchicine, and Podophyllotoxin.

20. The antibody-drug conjugate according to claim 19, wherein the active agent is monomethyl auristatin E (MMAE).

21. The antibody-drug conjugate according to any one of claims 15 to 18, wherein the active agent is a DNA-crosslinking agent, such as a DNA crosslinking agent selected from cisplatin or a derivative of cisplatin such as carboplatin or oxaliplatin, mitomycin C (MMC), pyrrolobenzodiazepine, and dimeric pyrrolobenzodiazepine derivatives such as SGD-1882.

22. The antibody-drug conjugate according to any one of claims 15 to 18, wherein the active agent is a DNA alkylating agent, such as a DNA alkylating agent selected from nitrogen mustards such as tris(2-chloroethyl)amine, pyridinobenzodiazepines or a pyridinobenzodiazepine derivative, indolinobenzodiazepine dimers, and Duocarmycin SA.

23. The antibody-drug conjugate according to any one of claims 15 to 18, wherein the active agent is a DNA strand scission agent, such as a DNA strand scission agent selected from calicheamicin and hamiltrone.

24. The antibody-drug conjugate according to any one of claims 15 to 18, wherein the active agent is an anthracycline, such as an anthracycline selected from Daunorubicin, doxorubicin, epirubicin, idarubicin, and PNU-159682.

25. The antibody-drug conjugate according to claim 24, wherein the active agent is PNU-159682.

26. The antibody-drug conjugate according to any one of claims 15 to 18, wherein the active agent is an antimetabolite, such as an antimetabolite selected from folic acid antagonists such as methotrexate, purine antimetabolites such as 6-mercaptopurine or 6-thioguanine or fludarabine phosphate or pentostatin or cladribine, and pyrimidine antimetabolites such as 5-fluorouracil or 5-fluorodeoxyuridine or cytarabine or gemcitabine.

27. The antibody-drug conjugate according to any one of claims 15 to 18, wherein the active agent is a histone deacetylase inhibitor, such as a histone deacetylase inhibitor selected from trichostatin A, vorinostat, belinostat, panabiostat, givinostat, resminostat, abexinostat, quisinostat, rocilinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entinostat, tacedinaline, 4SC202, mocetinostat, romidepsin, nicotinamide, sirtinol, cambinol, and EX-527.

28. The antibody-drug conjugate according to any one of claims 15 to 18, wherein the active agent is a kinase inhibitor, such as a kinase inhibitor selected from genistein, lavendustin C, PP1-AG1872, PP2-AG1879, SU6656, CGP77675, PD166285, imatinib, erlotinib, gefitinib, lavendustin A, cetuximab, UCS15A, herbimycin A, and radicicol.

29. The antibody-drug conjugate according to any one of claims 15 to 18, wherein the active agent is a metabolism inhibitor, such as an NAMPT inhibitor selected from APO866, GMX-1777, GMX-1778 ATG-019, and OT-82.

30. The antibody-drug conjugate according to any one of claims 15 to 18, wherein the active agent is an immune checkpoint inhibitor, such as a PD-1 inhibitor selected from Pembrolizumab, Nivolumab, Cemiplimab, JTX-4014, Spartalizumab, Camrelizumab, Sintilimab, Tislelizumab, Toripalimab, Dostarlimab, AMP-224 and AMP-514; or a PD-L1 inhibitor selected from Atezolizumab, Avelumab, Durvalumab, KN035, CK-301, AUNP12, CA-170 and BMS-986189.

31. The antibody-drug conjugate according to any one of claims 15 to 18, wherein the active agent is a platinum-based antineoplastic, such as a platinum-based antineoplastic selected from lipoplatin, cisplatin, carboplatin, oxaliplatin, nedaplatin, picoplatin, phenanthriplatin, satraplatin, and triplatin tetranitrate.

32. The antibody-drug conjugate according to any one of claims 15 to 18, wherein the active agent is a topoisomerase inhibitor, such as a topoisomerase inhibitor selected from camptothecin or derivatives thereof such as topotecan, belotecan, lurtotecan, irinotecan, SN-38, exatecan, and Dxd.

33. The antibody-drug conjugate according to any one of claims 15 to 18, wherein the active agent is a DNA- or RNA-polymerase inhibitor, such as a polymerase inhibitor selected from amanitin or alpha-amanitin or derivatives thereof, actinomycin D, and aphidicolin.

34. The antibody-drug conjugate according to any one of claims 15 to 33, wherein the active agent comprises a radioisotope selected from $^{60}Co$, $^{89}Sr$, $^{90}Y$, $^{99m}Tc$, $^{131}I$, $^{137}Cs$, $^{153}Sm$, and $^{223}Rd$.

35. The antibody-drug conjugate according to any one of claims 15 to 34, wherein the drug-to-antibody ratio (DAR) is between 1 and 10, such as between 2 and 8, for example between 2 and 6, such as 2 or 4, such as 2.

36. The antibody-drug conjugate according to any one of claims 15 to 34, wherein the drug-to-antibody ratio (DAR) is from 2 to 6, such as 2 or 4, such as 2.

37. The antibody-drug conjugate according to any one of claims 15 to 36, wherein the antibody-drug conjugate comprises a linker selected from a cleavable and a non-cleavable linker, optionally wherein the linker is a peptide linker.

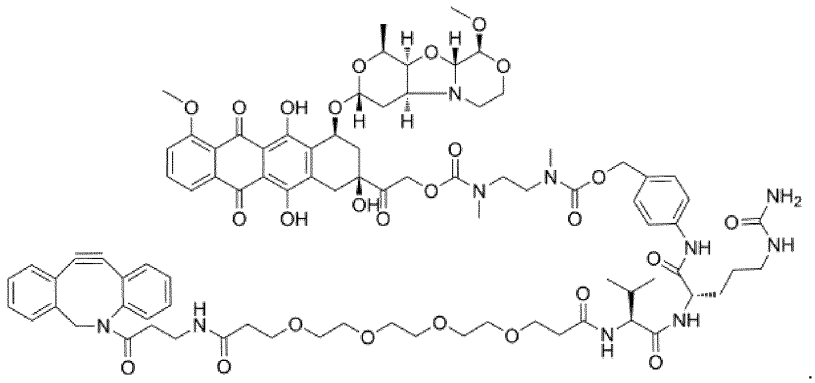
38. The antibody-drug conjugate according to any one of claims 15 to 37, wherein the linker comprises or consists of a dipeptide, such as valine-citrulline (VC) or valine-alanine (VA).

39. The antibody-drug conjugate according to any one of claims 15 to 38, wherein the antibody-drug conjugate further comprises a spacer, such as a spacer comprising p-aminobenzoic acid, p-aminobenzyl (PAB), p-aminobenzylcarbamate (PABC), p-aminobenzoyloxycabonyl, or polyethylenglycol (PEG).

40. The antibody-drug conjugate according to any one of claims 15 to 39, wherein the antibody-drug conjugate further comprises an attachment group, such as a an attachment group comprising or consisting of a dibenzocyclooctyne including amine-functionalized dibenzocyclooctynes, optionally modified with a PEG such

as PEG3 or PEG4, maleimide, N-hydroxysuccinimide, reactive attachment groups directed to modified or unmodified protein-bound carbohydrate, peptide sequences that are required for enzymatic reactions, azides or alkynes or being derived from these by reaction with the antibody or a chemically or enzymatically generated derivative thereof.

41. The antibody-drug conjugate according to any one of claims 15 to 40, wherein the antibody-drug conjugate prior to antibody conjugation comprises the following molecular structure:



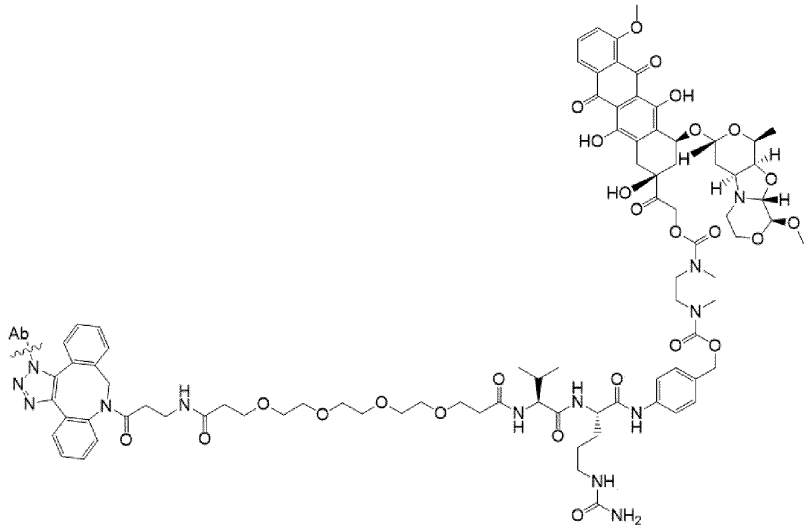
42. The antibody-drug conjugate according to any one of claims 15 to 18 and 34 to 41, wherein the antibody-drug conjugate comprises or consists of:
a. the antibody as defined in any one of claims 1 to 14,
b. a VC linker,
c. a dibenzocyclooctyne-amine (DBCO) attachment group, optionally N-functionalized with PEG4,
d. a PAB or a PABC spacer, and
e. PNU-159682 as active agent.

43. The antibody-drug conjugate according to any one of claims 15 to 18 and 34 to 42, wherein the antibody-drug-conjugate comprises CAS identifier 2259318-56-2.

44. The antibody-drug conjugate according to any one of claims 15 to 18 and 34 to 43, wherein the antibody-drug conjugate comprises the antibody as defined in claim 1 and DBCO-PEG4-VC-PAB-DMAE-PNU159682.

45. The antibody-drug conjugate according to any one of claims 15 to 18 and 34 to 44, wherein the antibody-drug conjugate comprises the antibody as defined in claim 1 and ADIBO-PEG4-VC-PAB-DMAE-PNU159682 linked via a triazole moiety.

46. The antibody-drug conjugate according to any one of claims 15 to 18 and 34 to 45, wherein the antibody-drug conjugate comprises or consists of the following structure or a regioisomer thereof:




wherein the wiggled line denotes any chemical bonding to the antibody.

47. The antibody-drug conjugate according to any one of claims 15 to 40, wherein the antibody-drug conjugate prior to antibody conjugation comprises CAS identifier 2754384-60-4.

48. The antibody according to any one of claims 1 to 14 or the antibody-drug conjugate according to any one of claims 15 to 47 for use as a medicament.

49. A pharmaceutical composition comprising the antibody according to any one of claims 1 to 14 or the antibody-drug conjugate according to any one of claims 15 to 47 and a pharmaceutically acceptable buffer, diluent, carrier, adjuvant or excipient.

50. The antibody according to any one of claims 1 to 14 or the antibody-drug conjugate according to any one of claims 15 to 47 or the pharmaceutical composition according to claim 49 for use in the treatment of a disease characterised by cells expressing uPAR, such as a cancer characterised by cells expressing uPAR.

51. The antibody, antibody-drug conjugate or composition for use according to claim 50, wherein the disease characterised by cells expressing uPAR is a cancer.

52. The antibody, antibody-drug conjugate or composition for use according to claim 51, wherein the cancer is selected from pancreatic adenocarcinoma (PAAD) such as pancreatic ductal adenocarcinoma (PDAC), bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSCC), acute myeloid leukemia (LAML), ovarian serous cystadenocarcinoma (OV), rectum adenocarcinoma (READ), sarcoma (SARC), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD).

53. The antibody, antibody-drug conjugate or composition for use according to claim 51, wherein the cancer is selected from pancreatic cancer, breast cancer, colon cancer, gastric cancer, esophageal cancer, hepatocellular cancer, prostate cancer, bladder cancer, mesothelioma, fibrosarcoma, and squamous cell carcinomas.

54. The antibody, antibody-drug conjugate or composition for use according to claim 51, wherein the cancer is breast cancer such as selected from triple-

negative breast cancer (TNBC), basal-like breast cancer (BLBC), HER-2 breast cancer, Luminal A type breast cancer and Luminal B type breast cancer.

55. The antibody, antibody-drug conjugate or composition for use according to claim 51, wherein the cancer is pancreatic cancer such as pancreatic ductal adenocarcinoma.

56. The antibody, antibody-drug conjugate or composition for use according to claim 51, wherein the cancer is mesothelioma such as selected from pleural mesothelioma, peritoneal mesothelioma, and pericardial mesothelioma.

57. The antibody, antibody-drug conjugate or composition for use according to any one of claims 51 to 56, wherein the cancer is metastatic cancer.

58. The antibody, antibody-drug conjugate or composition for use according to any one of claims 50 to 57, wherein administration is parenteral, for example, intravenously, intracerebroventricularly, intraarticularly, intra-arterially, intraperitoneally, intrathecally, intraventricularly, intrasternally, intracranially, intramuscularly or subcutaneously, or by infusion techniques.

59. The antibody, antibody-drug conjugate or composition for use according to any one of claims 50 to 58, wherein administration is intravenous.

60. The antibody, antibody-drug conjugate or composition for use according to any one of claims 50 to 59, wherein the antibody, antibody-drug conjugate or composition is administered in combination with one or more further agents, such as one or more further therapeutic agents.

61. The antibody, antibody-drug conjugate or composition for use according to any one of claims 50 to 60, wherein the cells expressing uPAR display uPAR overexpression, optionally wherein the cells expressing uPAR are tumour cells and/or tumour associated cells.

62. The antibody, antibody-drug conjugate or composition for use according to any one of claims 50 to 61, wherein the antibody, antibody-drug conjugate or

composition induces cell death and/or inhibits the growth and/or proliferation of the uPAR expressing cells.

63. The antibody, antibody-drug conjugate or composition for use according to any one of claims 50 to 61, wherein the antibody, antibody-drug conjugate or composition induces liberation of free cytotoxin from the uPAR expressing cells, leading to cell death and/or inhibition of the growth and/or proliferation of neighbouring cancer cells.

64. The antibody, antibody-drug conjugate or composition for use according to any one of claims 50 to 63, wherein the treatment is ameliorative or curative.

65. The antibody according to any one of claims 1 to 14, the antibody-drug conjugate according to any one of claims 15 to 47 or the pharmaceutical composition according to claim 49 for use in a method for inhibiting tumour progression in a subject.

66. The antibody according to any one of claims 1 to 14, the antibody-drug conjugate according to any one of claims 15 to 47 or the pharmaceutical composition according to claim 49 for use in a method for inhibiting, lowering or eliminating metastatic capacity of an uPAR expressing tumour in a subject.

67. A kit comprising the antibody according to any one of claims 1 to 14, the antibody-drug conjugate according to any one of claims 15 to 47 or the pharmaceutical composition according to claim 49, optionally further comprising means for administering the antibody or antibody-drug conjugate to a subject and/or instructions for use.

68. The antibody according to any one of claims 1 to 14, the antibody-drug conjugate according to any one of claims 15 to 47 or the pharmaceutical composition according to claim 49 for use in the manufacture of a medicament for treatment of a disease characterised by cells expressing uPAR, such as cancer.