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(54) Benævnelse: **Antistof-lægemiddelkonjugater rettet mod uPARAP**

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WO-A1-2010/111198
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LARS H ENGELHOLM ET AL: "Targeting a novel bone degradation pathway in primary bone cancer by inactivation of the collagen receptor uPARAP/Endo180", JOURNAL OF PATHOLOGY, vol. 238, no. 1, 30 January 2016 (2016-01-30), pages 120-133, XP055366841, GB ISSN: 0022-3417, DOI: 10.1002/path.4661
HENRIK J. JÜRGENSEN ET AL: "Complex Determinants in Specific Members of the Mannose Receptor Family Govern Collagen Endocytosis", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 289, no. 11, 14 March 2014 (2014-03-14), pages 7935-7947, XP055366853, US ISSN: 0021-9258, DOI: 10.1074/jbc.M113.512780
MARIA MELANDER ET AL: "The collagen receptor uPARAP/Endo180 in tissue degradation and cancer (Review)", INTERNATIONAL JOURNAL OF ONCOLOGY, 12 August 2015 (2015-08-12), XP055366849, GR ISSN: 1019-6439, DOI: 10.3892/ijo.2015.3120
JUSTIN STURGE: "Endo180 at the cutting edge of bone cancer treatment and beyond", JOURNAL OF PATHOLOGY, vol. 238, no. 4, 11 January 2016 (2016-01-11), pages 485-488, XP055366918, GB ISSN: 0022-3417, DOI: 10.1002/path.4673

DESCRIPTION

Field of invention

[0001] The present invention relates to molecular conjugates targeting the receptor uPARAP, in particular antibody-drug conjugates (ADCs) directed against uPARAP and their use in delivery of active agents to cells and tissues expressing uPARAP. The invention further relates to the use of said ADCs in the treatment of diseases involving uPARAP expressing cells, such as certain cancers.

Background

[0002] Urokinase-type Plasminogen Activator Receptor Associated Protein (uPARAP), also known as CD280, Endo180 and mannose receptor C type 2, is a member of the macrophage mannose receptor family of endocytic transmembrane glycoproteins. uPARAP is a membrane protein involved in matrix turnover during tissue remodelling, particularly the uptake and intracellular degradation of collagen.

[0003] The receptor uPARAP is upregulated in the tumour cells of specific cancers, including sarcomas and late-stage glioblastoma. Additionally, the receptor is most often upregulated in stromal cells surrounding solid tumours and some literature suggests a high expression of uPARAP in bone metastasis from prostate cancer (Caley et al., 2012, J. Pathol 5: 775-783). In healthy adult individuals, the receptor displays a restricted expression pattern (Melander et al., 2015, Int J Oncol 47: 1177-1188).

[0004] Antibody-drug conjugates (ADCs) are a new class of highly potent biopharmaceutical drug designed as a targeted therapy, in particular for the treatment of cancer. ADCs are complex molecules composed of an antibody (a whole mAb or an antibody fragment) linked, via a stable, chemical, linker that may possess labile bonds, to a biologically active drug or cytotoxic compound. By combining the unique targeting capabilities of antibodies with the cell-killing ability of cytotoxic drugs, antibody-drug conjugates allow sensitive discrimination between healthy and diseased tissue, based on expression of the antibody antigen. This means that, in contrast to traditional chemotherapeutic agents, antibody-drug conjugates actively target and attack cancer cells, so that healthy cells with little or no antigen expression are less severely affected. To date, three ADCs have received market approval and several ADCs are currently in clinical trials.

[0005] WO 2010/111198 discloses conjugates comprising an anti-uPARAP antibody and suggests use of such conjugates in the delivery of therapeutic agents to cells that express uPARAP.

[0006] 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 efficient treatments with increased specificity.

Summary

[0007] The present invention provides antibody-drug conjugates (ADCs) based on anti-uPARAP antibodies capable of binding to the N-terminal region of the uPARAP receptor. The ADCs as described herein are capable of specifically targeting cells and tissues expressing uPARAP, and have excellent *in vitro* and *in vivo* efficacy with no registered side effects.

[0008] In particular, the present disclosure relates to an antibody-drug conjugate according to claim 1 comprising: a. an antibody, wherein the antibody is selected from the group consisting of:i. an antibody or antigen-binding fragment thereof comprisingi) an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 1 or 9 or a sequence having at least 90% sequence identity thereto, andii) an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 5 or 10 or a sequence having at least 90% sequence identity thereto, wherein any sequence variance is outside the complementarity determining regions,ii. a humanised version of the antibody or antigen-binding fragment thereof of i.,iii. a chimeric version of the antibody or antigen-binding fragment thereof of i.,iv. an antibody or antigen-binding fragment thereof comprisingi) an immunoglobulin light chain variable region comprising the amino acid sequences of SEQ ID NOs: 2, 3 and 4, andii) an immunoglobulin heavy chain variable region comprising the amino acid sequences of SEQ ID NOs 6, 7 and 8,v. an antibody or antigen-binding fragment thereof comprisingi) an immunoglobulin light chain variable region comprising the amino acid sequences of SEQ ID NOs: 42, 43 and 44, andii) an immunoglobulin heavy chain variable region comprising the amino acid sequences of SEQ ID NOs 45, 46 and 47,vi. a humanised version of the antibody or antigen-binding fragment thereof of iv. or v.b. an active agent, wherein the active agent is selected from a therapeutic agent, a cytotoxic agent, a radioisotope and a detectable label, and c. a linker which links a) to b).

[0009] Furthermore, the present disclosure relates to the use of the ADC as defined above for the treatment of diseases and/or disorders involving expression of the uPARAP receptor.

Description of Drawings

[0010]

Figure 1. Schematic representation of the four protein family members of the Mannose receptor family, including uPARAP. All of the proteins have the same over-all domain composition with an N-terminal signal peptide followed by a cysteine-rich domain, a fibronectin type II domain (FN-II domain), 8-10 C-type lectin-like domains (CTLDs), a transmembrane

spanning region and a small cytoplasmic tail (Adapted from Melander et al., 2015 Int J Oncology 47: 1177-1188).

Figure 2. Schematic illustration of an uPARAP-directed ADC, in the form of a targeting antibody, conjugated to a maleimidocaproyl-valine-citrulline-p-aminobenzoyloxycarbonyl-monomethyl auristatin E (MC-VC-PAB-MMAE) linker-toxin construct. The targeting antibody is specific against the receptor uPARAP, which is found to be highly expressed in certain cancer types. The linker-toxin construct is attached by maleimide chemistry to thiols of free cysteines or reduced interchain disulphide bridges (N=1-10 toxins per antibody). The valine-citrulline linker region with the peptide/amide bond to the spacer entity is a substrate for lysosomal proteases such as cathepsin B, but is sufficiently stable in the extracellular environment to ensure release of the conjugated drug only when taken up by cells expressing the target antigen. The conjugated drug is a highly potent tubulin inhibitor in the form of monomethyl auristatin E (MMAE). As a unit (mAb-vc-MMAE), this ADC construct ensures specific delivery of the drug component only to cells expressing the uPARAP antigen, as well as intracellular release of the conjugated drug in these cells.

Figure 3: Cellular uptake of mAb 2h9, labeled with a fluorophore (AlexaFluor 647, AF647) using a method similar to the conjugation procedure described in the figure legend to figure 2 (partial reduction followed by reaction with a maleimide-derivatized AlexaFluor 647 reagent), in uPARAP-positive cell lines, measured by flow cytometry. MFI: Mean fluorescence intensity. Specificity ratio: Ratio of 2h9-AF647 / aTNP-AF647 signals, with aTNP being a non-targeted control mAb. These numbers demonstrate a specific uptake of 2h9-AF647, and confirm that mAb 2h9 is taken up by uPARAP-positive cells following such a conjugation method.

Figure 4: A. Reducing SDS-PAGE of a targeting antibody (2h9), a mAb-vc-MMAE ADC with a moderate drug-to-antibody ratio (DAR) of ~4-5, and a mAb-vc-MMAE ADC with a DAR of -8-10. It is seen that conjugated mAbs display reduced mobility in the gel, and that moderately conjugated ADC species are preferably conjugated via the mAb heavy chains, whereas the ADC with a higher DAR is conjugated via both the heavy- and the light chains. **B.** Reducing SDS-PAGE showing that incubation of ADCs with activated recombinant cathepsin B (+rh cathepsin B) reverts ADC gel mobility back to that of unmodified targeting antibody, and thus that the linker region is indeed cleavable by lysosomal proteases such as cathepsin B. **C.** ELISA analysis showing retained affinity of mAb 2h9 towards uPARAP following the reduction step of the conjugation procedure, as well as in ADC form. Altogether, these data show that ADC 2h9-vc-MMAE behaves as expected, in relation to gel mobility and affinity towards the target receptor following conjugation.

Figure 5: *In vitro* cell viability assays, based on exposure to the ADCs in a dilution series. The dilution series starts at 10 µg/mL ADC (mAb component), followed by a series of 4-fold dilutions of the ADCs. Cells were incubated for 72 hours, before being analyzed by colorimetric viability assay. Here, the assay shows a specific reduction in overall viability following incubation with uPARAP-directed ADC 2h9-vc-MMAE, in comparison to a non-targeted ADC (aTNP-vc-MMAE), in four cell lines expressing the target receptor (U937, THP-1, HT1080 and KNS42 cells), whereas a receptor-negative cell line (CHO-K1) remains unaffected. This

demonstrates a receptor-specific reduction in the viability of uPARAP-positive cell lines, following incubation with ADC 2h9-vc-MMAE.

Figure 6: Cell cycle distribution analysis of four uPARAP-positive cell lines (U937, THP-1, HT1080 or KNS42) following a 3-day incubation in the presence of 1µg/mL of uPARAP-directed ADC 2h9-vc-MMAE or control ADC aTNP-vc-MMAE, or 50nM free MMAE toxin. Since MMAE is a tubulin inhibitor, a drug effect may lead to an increase in the fraction of cells in either the Sub-G1 phase (ultimately leading to apoptosis), or the G2-M phase (inhibition of genomic segregation following DNA replication). A dash indicates a cell count too low to register, due to widespread cell death and disintegration. It is seen that all four cell lines display specific sensitivity towards uPARAP-directed ADC 2h9-vc-MMAE (and free MMAE), evident from the shift in cell cycle distribution towards the Sub-G1 and G2/M phases in these samples.

Figure 7: Competition assay, showing U937 cells being incubated for 3 days in the presence of 1µg/mL of uPARAP-directed ADC 2h9-vc-MMAE, in the simultaneous presence of different concentrations of unconjugated targeting antibody (2h9), another antibody targeting uPARAP (5f4), or the non-targeting control antibody (aTNP). It is seen that only a molar surplus (1+ µg/mL competing mAb) of non-conjugated targeting antibody 2h9 can compete for the effect of the ADC, thereby rescuing cells from ADC mediated cell death. Thereby, the interaction between uPARAP and the targeting antibody 2h9 is shown to be crucial for the observed cytotoxic effect.

Figure 8: It is shown that pre-incubating U937 cells in the presence of a broad-spectrum inhibitor of lysosomal proteases (E64D) leads to a complete abrogation of the cytotoxic effect of uPARAP-directed ADC 2h9-vc-MMAE. Thereby, lysosomal release of the conjugated drug is shown to be crucial for obtaining a cytotoxic effect.

Figure 9: *In vivo* testing of the efficacy of uPARAP-directed ADC 2h9-vc-MMAE in combating a uPARAP-positive, subcutaneous xenograft tumour, established by injection of the cell line U937 in CB17 SCID mice. The mice are treated by subcutaneous (s.c.) injection near the tumour with either uPARAP-directed ADC 2h9-vc-MMAE (N=10), control ADC aTNP-vc-MMAE (N=9), unconjugated mAb 2h9 (N=5), or a saline solution (PBS, N=5). All treatments are done in doses of 3 mg/kg/injection mAb component, as 4 doses total, given every 4 days. Day 0 marks the day of first injection, initiated once the tumour has reached a palpable size of 50-100 mm^3, and the graph shows the average tumour size across each treatment group. It is seen that treatment with ADC 2h9-vc-MMAE results in a drastic decrease in tumour growth, whereas all other treatment groups reach a point of sacrifice within 10-12 days after starting treatment. This demonstrates that uPARAP-directed ADC 2h9-vc-MMAE is efficient at inhibiting growth of a pre-established uPARAP-positive tumour *in vivo.* Furthermore, the data from the 2h9-vc-MMAE treated group represent a permanent cure rate of 50% (see also figure 10).

Figure 10: A more detailed look at the tumour growth of the 2h9-vc-MMAE treated group described in figure 9, showing that this group included mice suffering from non-complete treatment and tumour relapse, as well as mice that lost the tumour burden completely and showed no tumour relapse. Of the 10 mice treated with 2h9-vc-MMAE, 5 showed an almost

immediate relapse of tumour growth following treatment, quickly reaching a point of sacrifice, whereas the remaining 5 mice lost all signs of the tumour, and remained free from tumour growth for a period of 90 days, giving an overall permanent cure rate of 50% for 2h9-vc-MMAE treated mice following s.c. administration.

Figure 11: *In vivo* testing of the efficacy of uPARAP-directed ADC 2h9-vc-MMAE in combating a uPARAP-positive, subcutaneous xenograft tumour, established by injection of the cell line U937 in CB17 SCID mice. The mice are treated by intravenous (i.v.) injection via the tail veins, with either uPARAP-directed ADC 2h9-vc-MMAE (N=10), control ADC aTNP-vc-MMAE (N=10), unconjugated mAb 2h9 (N=5), or a saline solution (PBS, N=5). All treatments are done in doses of 5 mg/kg/injection mAb component, as 3 doses total, given every 4 days. Day 0 marks the day of first injection, once the tumour has reached a palpable size of 50-100 mm^3, and the graph shows the average tumour size across each treatment group. Under these conditions, treatment with uPARAP-directed ADC 2h9-vc-MMAE results in a complete abrogation of the tumour burden in all 10 mice, giving an overall permanent cure rate of 100% of the mice following intravenous administration of this ADC, further demonstrating the efficacy of ADC 2h9-vc-MMAE in combating solid uPARAP-positive tumours.

Figure 12: *In vitro* cell viability assays showing a specific reduction in overall viability following incubation with either uPARAP-directed ADC 2h9-vc-MMAE or uPARAP-directed ADC 5f4-vc-MMAE, in comparison to a non-targeted ADC (aTNP-vc-MMAE), in the U937 cell line. The data indicates that ADCs based on 5f4 have comparable efficacy to ADCs based on the 2h9 antibody.

Figure 13: Immunohistochemistry staining of different sarcomas (liposarcoma, myxofibrosarcoma, dermatofibrosarcoma protuberans (DFSP) and leiomyosarcoma (LMS). The staining method shows tissue expression of uPARAP as a dark reddish-brown color. Expression of uPARAP is evident in sections of malignant cancer (tumor) tissue, whereas sections of non-cancer tissue are devoid of uPARAP, demonstrating the increased expression levels of uPARAP, found in sarcomas. Scale bars: 20µm.

Figure 14: Different antibodies directed against the N-terminal part of uPARAP can be utilized for efficient drug delivery in an ADC format. ADCs with the composition mAb-vc-MMAE were prepared as described in the legend to Figure 2, using three different antibodies directed against epitopes within the three N-terminal domains of uPARAP (mAb 2h9, mAb 5f4 and mAb 9b7). For comparison, an ADC was prepared in the same manner but using an anti-uPARAP antibody directed against an epitope outside the N-terminal three domains (mAb 11c9). *In vitro* cell viability assays with U937 cells were then performed as described in the legend to Figure 5, using all of these ADCs. All ADCs lead to a specific reduction in overall cell viability but with the cellular sensitivity to 2h9-vc-MMAE, 5f4-vc-MMAE and 9b7-vc-MMAE being higher than the sensitivity to 11c9-vc-MMAE.

Figure 15: Different toxins can be used in an ADC format targeting the N-terminal part of uPARAP. ADCs with mAb 2h9 as the antibody component were prepared as described in the legend to Figure 2 but using the following linker-cytotoxin units instead of VC-PAB-MMAE: VC-

PAB-MMAF (with MMAF being monomethyl auristatin F, a carboxyl-variant of MMAE) and PEG4-va-PBD (with PEG4 referring to a polyethylenglycol spacer, va being valine-alanine and PBD referring to a dimeric pyrrolobenzodiazepine). The resulting ADCs (referred to as 2h9-vc-MMAF and 2h9-va-PBD respectively) were used for *in vitro* cell viability assays with U937 cells, performed as described in the legend to Figure 5. U937 cells displayed very strong sensitivity to 2h9-vc-MMAF and a more moderate sensitivity to 2h9-va-PBD.

Figure 16: An ADC with mAb 2h9 as the antibody component was prepared as described in the legend to Figure 2 but using the following linker-cytotoxin unit instead of VC-PAB-MMAE: PEG4-vc-Duocarmycin SA (with PEG4 referring to a polyethylenglycol spacer and vc being valine-citrulline). The resulting ADC (referred to as 2h9-vc-DuocSA) was used for *in vitro* cell viability assays with U937 cells, performed as described in the legend to Figure 5. U937 cells displayed a low but measurable sensitivity to 2h9-vc-DuocSA.

Figure 17: ADCs with mAbs 2h9 or aTNP as the antibody component were prepared as described in the legend to Figure 2, using the following linker-cytotoxin units: VC-PAB-MMAE or VC-PAB-MMAF. The resulting ADCs (referred to as 2h9-vc-MMAE, 2h9-vc-MMAF, aTNP-vc-MMAE and aTNP-vc-MMAF) were used for in vitro cell viability assays using human glioblastoma explants cells and performed as described in the legend to Figure 5. These glioblastoma explant cells showed a high degree of specific sensitivity towards uPARAP-directed ADCs, based on both the MMAE and the MMAF toxin.

Figure 18: A recombinant mAb 2h9 product, designated "2h9 cloned", was produced in CHO cells transfected with an expression vector including the DNA sequences encoding the light and the heavy chain of mAb 2h9 ([SEQ ID NO: 1] and [SEQ ID NO: 5], respectively). The reactivity of this product was analyzed in Western blotting and compared with mAb 2h9 produced by hybridoma cell culture ("2h9 original"). For Western blotting, a detergent cell lysate prepared from uPARAP-positive MG63 human osteosarcoma cells was analyzed, using identical concentrations of "2h9 cloned" and "2h9 original" as the primary antibodies. The two antibody products display identical reaction and both react specifically with the uPARAP protein. No reaction is seen in the absence of primary antibody (negative control).

Sequences

[0011]

SEQ ID NO	**Description**
SEQ ID NO: 1	mAb 2h9 Light Chain full length amino acid sequence
SEQ ID NO: 2	mAb 2h9 Light Chain full length CDR1
SEQ ID	mAb 2h9 Light Chain full length CDR2

SEQ ID NO	Description
NO: 3	
SEQ ID NO: 4	mAb 2h9 Light Chain full length CDR3
SEQ ID NO: 5	mAb 2h9 Heavy Chain full length amino acid sequence
SEQ ID NO: 6	mAb 2h9 Heavy Chain full length CDR1
SEQ ID NO: 7	mAb 2h9 Heavy Chain full length CDR2
SEQ ID NO: 8	mAb 2h9 Heavy Chain full length CDR3
SEQ ID NO: 9	Fab 2h9 Light Chain amino acid sequence 1-214
SEQ ID NO: 10	Fab 2h9 Heavy Chain amino acid sequence 1-224
SEQ ID NO: 11	mAb 5f4 Light Chain variable (VL) region amino acid sequence
SEQ ID NO: 12	mAb 5f4 Light Chain variable (VL) region CDR1
SEQ ID NO: 13	mAb 5f4 Light Chain variable (VL) region CDR2
SEQ ID NO: 14	mAb 5f4 Light Chain variable (VL) region CDR3
SEQ ID NO: 15	mAb 5f4 Heavy Chain variable (VH) region amino acid sequence
SEQ ID NO: 16	mAb 5f4 Heavy Chain variable (VL) region CDR1
SEQ ID NO: 17	mAb 5f4 Heavy Chain variable (VL) region CDR2
SEQ ID NO: 18	mAb 5f4 Heavy Chain variable (VL) region CDR3
SEQ ID NO: 19	Fab 9b7 Light Chain amino acid sequence 1-214
SEQ ID NO: 20	Fab 9b7 Light Chain amino acid sequence 8-214
SEQ ID NO: 21	Fab 9b7 Light Chain CDR1
SEQ ID NO: 22	Fab 9b7 Light Chain CDR2
SEQ ID	Fab 9b7 Light Chain CDR3

SEQ ID NO	Description
NO: 23	
SEQ ID NO: 24	Fab 9b7 Heavy Chain amino acid sequence 1-221
SEQ ID NO: 25	Fab 9b7 Heavy Chain amino acid sequence 9-221
SEQ ID NO: 26	Fab 9b7 Heavy Chain CDR1
SEQ ID NO: 27	Fab 9b7 Heavy Chain CDR2
SEQ ID NO: 28	Fab 9b7 Heavy Chain CDR3
SEQ ID NO: 29	Human uPARAP full length sequence (GenBank: AAI53885.1)
SEQ ID NO: 30	CysR domain as listed by NCBI (amino acids (aa) 46-161 of full length human uPARAP)
SEQ ID NO: 31	CysR domain as predicted by the SMART tool (simple modular architecture research tool) at EMBL (http://smart.embl-heidelberg.de/) {Schultz et al. Proc. Natl. Acad. Sci. USA, Vol. 95, pp. 5857-5864, May 1998} (amino acids (aa) 41-161 of full length human uPARAP)
SEQ ID NO: 32	FN-II domain as listed by NCBI (aa 181-228 of full length Human uPARAP)
SEQ ID NO: 33	FN-II domain as predicted by SMART (aa 180-228 of full length Human uPARAP)
SEQ ID NO: 34	CTLD-1 domain as listed by NCBI (aa 247-361 of full length Human uPARAP)
SEQ ID NO: 35	CTLD-1 domain as predicted by SMART (aa 235-360 of full length Human uPARAP)
SEQ ID NO: 36	CysR-FN-II-CTLD-1 as listed by NCBI (aa 46-361 of full length Human uPARAP)
SEQ ID NO: 37	CysR-FN-II-CTLD-1 as predicted by SMART (aa 41-360 of full length Human uPARAP)
SEQ ID NO: 38	CysR-FN-II as listed by NCBI (aa 46-228 of full length Human uPARAP)
SEQ ID NO: 39	CysR-FN-II as predicted by SMART (aa 41-228 of full length Human uPARAP)
SEQ ID NO: 40	FN-II-CTLD-1 as listed by NCBI (aa 181-361 of full length Human uPARAP)
SEQ ID NO: 41	FN-II-CTLD-1 as predicted by SMART (aa 180-360 of full length Human uPARAP)
SEQ ID	mAb 2h9 Light Chain Paratome-predicted ABR1

SEQ ID NO	Description
NO: 42	
SEQ ID NO: 43	mAb 2h9 Light Chain Paratome-predicted ABR2
SEQ ID NO: 44	mAb 2h9 Light Chain Paratome-predicted ABR3
SEQ ID NO: 45	mAb 2h9 Heavy Chain Paratome-predicted ABR1
SEQ ID NO: 46	mAb 2h9 Heavy Chain Paratome-predicted ABR2
SEQ ID NO: 47	mAb 2h9 Heavy Chain Paratome-predicted ABR3
SEQ ID NO: 48	mAb 5f4 Light Chain Paratome-predicted ABR1
SEQ ID NO: 49	mAb 5f4 Light Chain Paratome-predicted ABR2
SEQ ID NO: 50	mAb 5f4 Light Chain Paratome-predicted ABR3
SEQ ID NO: 51	mAb 5f4 Heavy Chain Paratome-predicted ABR1
SEQ ID NO: 52	mAb 5f4 Heavy Chain Paratome-predicted ABR2
SEQ ID NO: 53	mAb 5f4 Heavy Chain Paratome-predicted ABR3
SEQ ID NO: 54	mAb 9b7 Light Chain Paratome-predicted ABR1
SEQ ID NO: 55	mAb 9b7 Light Chain Paratome-predicted ABR2
SEQ ID NO: 56	mAb 9b7 Light Chain Paratome-predicted ABR3
SEQ ID NO: 57	mAb 9b7 Heavy Chain Paratome-predicted ABR1
SEQ ID NO: 58	mAb 9b7 Heavy Chain Paratome-predicted ABR2
SEQ ID NO: 59	mAb 9b7 Heavy Chain Paratome-predicted ABR3

[0012] Complementarity Determining Regions (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). Antigen binding regions (ABRs) according to the Paratome algorithm were also predicted as specified in the references Kunik et al. (2012a and b). The ABRs represent alternative CDRs of the antibodies disclosed herein.

[0013] Complete regions involved in antigen recognition and binding may deviate slightly from the specified CDRs and ABRs and all sequence data included in the variable regions or Fab fragments specified here are covered as potentially contributing to antigen binding. Methods or algorithms different from those employed here may be used for identification of potential binding/recognition regions. Therefore, in addition to the predicted CDRs as presented herein, this invention covers any amino acid sequences predicted to represent CDRs or ABRs in mAbs 2h9, 5f4 and 9b7 based on the respective Fab regions and variable regions (SEQ ID NOs: 9, 10, 11, 15, 20 and 25, respectively), using such methods or algorithms. Examples of additional methods and algorithms for the prediction of CDRs include, but are not limited to, the IMGT system (LeFranc et al., (2003)) .

[0014] Due to the position of primer regions during sequencing of the Fab 9B7 light and heavy chains some ambiguity is expected in the N-terminal region of these sequences. Thus, the first 7 amino acids of SEQ ID NO: 19 may not be exact. The same goes for amino acids 1-8 of SEQ ID NO: 24. SEQ ID NOs: 20 and 25 correspond to SEQ ID NOs: 19 and 24 respectively without the ambiguous N-terminal amino acids.

Detailed description

[0015] The antibody-drug conjugate targeting uPARAP of the present disclosure comprises

1. a) an antibody capable of binding to the cystein-rich domain (CysR), the Fibronectin type II (FN-II) domain and/or to the C-type lectin-like domain 1 (CTLD 1) of uPARAP,
2. b) an active agent, and
3. c) a linker which links a) to b) as defined in the claims.

[0016] In a particular aspect, the antibody-drug conjugate targeting uPARAP of the present disclosure comprises

1. a. an antibody, wherein the antibody is selected from the group consisting of:
    1. i. 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: 1 or 9 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: 5 or 10 or a sequence having at least 90%

sequence identity thereto, wherein any sequence variance is outside the complementarity determining regions,

ii. a humanised version of the antibody or antigen-binding fragment thereof of i.,

iii. a chimeric version of the antibody or antigen-binding fragment thereof of i.,

iv. an antibody or antigen-binding fragment thereof comprisingi) an immunoglobulin light chain variable region comprising the amino acid sequences of SEQ ID NOs: 2, 3 and 4, andii) an immunoglobulin heavy chain variable region comprising the amino acid sequences of SEQ ID NOs 6, 7 and 8,

v. an antibody or antigen-binding fragment thereof comprisingi) an immunoglobulin light chain variable region comprising the amino acid sequences of SEQ ID NOs: 42, 43 and 44, andii) an immunoglobulin heavy chain variable region comprising the amino acid sequences of SEQ ID NOs 45, 46 and 47,

vi. a humanised version of the antibody or antigen-binding fragment thereof of iv. or v.

2. b. an active agent, wherein the active agent is selected from a therapeutic agent, a cytotoxic agent, a radioisotope and a detectable label, and
3. c. a linker which links a) to b).

Antibody directed against uPARAP

[0017] The anti-uPARAP antibody of the present disclosure is internalised upon binding to uPARAP at the cell surface, thus allowing for intracellular actions of the active agent of the ADC complex. It is known from e.g. WO 2010/111198 that not all antibodies capable of binding to uPARAP are internalised at the same rate or in the same amount. Indeed, some anti-uPARAP antibodies are not internalised at all and are therefore not suitable for use in ADCs.

[0018] The uPARAP receptor consists of an N-terminal cysteine-rich domain (CysR), a fibronectin type II (FN-II) domain, and eight C-type lectin-like domains (CTLDs 1-8), cf. figure 1. Short amino acid sequences connect the individual domains. The data presented herein suggests that anti-uPARAP antibodies targeting the three most N-terminal domains of uPARAP are very efficient for use in ADCs.

[0019] Thus, the anti-uPARAP antibody of the present disclosure preferably binds to the N-terminal region of uPARAP, more preferably to an epitope located in the three most N-terminal domains of uPARAP, that is the cystein-rich domain, the fibronectin type II domain and/or C-type lectin-like domain 1, including the linker sequences connecting these domains of uPARAP.

[0020] Thus, the anti-uPARAP antibody of the present disclosure is capable of binding to a peptide comprising or consisting of the cystein-rich domain (CysR) (SEQ ID NO: 30 or 31), the fibronectin type II (FN-II) domain (SEQ ID NO: 32 or 33) and/or to the C-type lectin-like domain 1 (CTLD 1) (SEQ ID NO: 34 or 35) of uPARAP.

[0021] The cystein-rich domain, the fibronectin type II domain and the C-type lectin-like domain 1 including the linker sequences connecting these domains as listed by NCBI correspond to aa 46-361 of full length human uPARAP. Thus, in one embodiment the epitope for the anti-uPARAP antibody is located in aa 46-361 of SEQ ID NO: 29 (full length human uPARAP). In one embodiment, the anti-uPARAP antibody of the present disclosure binds to an epitope located in aa 31-365 of SEQ ID NO: 29, more preferably in aa 46-361 of SEQ ID NO: 29, corresponding to SEQ ID NO: 36 herein. SMART predicts CYSR-FN-II-CTLD1 including the linker sequences connecting these domains to aa 41-360 of SEQ ID NO: 29. Thus, in one embodiment the epitope for the anti-uPARAP antibody is located in aa 41-360 of SEQ ID NO: 29, corresponding to SEQ ID NO: 37 herein.

[0022] In one embodiment, the anti-uPARAP antibody of the present disclosure binds to the CysR domain and/or the CTLD-1 domain.

[0023] In one embodiment, the anti-uPARAP antibody of the present disclosure binds to the CysR domain, which is predicted by NCBI to consist of aa 46-161 of full length Human uPARAP, corresponding to SEQ ID NO: 30 herein, and by SMART to consist of aa 41-161 of full length Human uPARAP, corresponding to SEQ ID NO: 31 herein. I.e. in one embodiment it binds to an epitope located in aa 46-161 or 41-161 of SEQ ID NO: 29.

[0024] In one embodiment, the anti-uPARAP antibody of the present disclosure binds to the FN-II domain, which is predicted by NCBI to consist of aa 181-228 of full length Human uPARAP, corresponding to SEQ ID NO: 32 herein, and by SMART to consist of aa 180-228 of full length Human uPARAP, corresponding to SEQ ID NO: 33 herein. I.e. in one embodiment it binds to an epitope located in aa 181-228 or 180-228 of SEQ ID NO: 29.

[0025] In one embodiment, the anti-uPARAP antibody of the present disclosure binds to the CTLD-1 domain which is predicted by NCBI to consist of aa 247-361 of full length Human uPARAP, corresponding to SEQ ID NO: 34 herein, and by SMART to consist of aa 235-360 of full length human uPARAP, corresponding to SEQ ID NO: 35 herein. I.e. in one embodiment it binds to an epitope located in aa 247-361 or 235-360 of SEQ ID NO: 29.

[0026] In one embodiment, the anti-uPARAP antibody of the present disclosure is capable of binding to a peptide comprising or consisting of the CysR and FN-II domain including the linker sequences connecting these domains, which is predicted by NCBI to consist of aa 46-228 of full length human uPARAP, corresponding to SEQ ID NO: 38, and by SMART to consist of aa 41-228 of full length human uPARAP, corresponding to SEQ ID NO: 39 herein. I.e. in one embodiment it binds to an epitope located in aa 46-228 or 41-228 of SEQ ID NO: 29.

[0027] In one embodiment, the anti-uPARAP antibody of the present disclosure is capable of binding to a peptide comprising or consisting of the FN-II and CTLD-1 domain including the linker sequences connecting these domains, which is predicted by NCBI to consist of aa 181-361 of full length human uPARAP, corresponding to SEQ ID NO: 40 herein, and by SMART to consist of aa 180-360 of full length human uPARAP, corresponding to SEQ ID NO: 41 herein. I.e. in one embodiment it binds to an epitope located in aa 180-361 or 181-360 of SEQ ID NO: 29.

[0028] In one embodiment the anti-uPARAP antibody of the present disclosure is the mouse monoclonal IgG1κ antibody of clone 2.h.9:F12 commercially available from Merck Millipore (http://www.merckmillipore.com/DK/en/product/Anti-UPAR-Associated-Protein-Antibody%2C-clone-2.h.9%3AF12,MM NF-MAB2613?cid=BI-XX-BRC-P-GOOG-ANTI-B302-1075) or a functional fragment or variant thereof, such as a chimeric or humanised version thereof. Mouse monoclonal IgG1κ antibody clone 2.h.9:F12 is referred to herein as the "2h9" antibody or "mAb 2h9". The 2h9 antibody reacts with both human and mouse uPARAP and is therefore well suited for both preclinical and clinical studies.

[0029] Previous studies indicate that the epitope for the 2h9 antibody is located in the three most N-terminal domains of uPARAP, particularly in the CysR domain or the CTLD-1 domain. A soluble recombinant protein consisting of the three n-terminal domains of uPARAP (CysR, FN-II and CTLD-1) binds to immobilized 2h9 in a BIAcore setup, limiting the location of binding by mAb 2h9 to these three n-terminal domains (Jürgensen et al., 2011, JBC 286(37):32736-48). Furthermore, swapping the FN-II domain of uPARAP with the FN-II domain of other members of the same receptor family has no effect on binding of mAb 2h9, suggesting that the FN-II domain does not likely contain the epitope for mAb 2h9 (Jürgensen et al., 2014, JBC 289(11):7935-47). This effectively limits binding of mAb 2h9 to either the CysR domain, or the CTLD-1 domain.

[0030] The predicted CDRs of immunoglobulin light chain variable region of mAb 2h9 correspond to SEQ ID NOs: 2-4 and the predicted CDRs of immunoglobulin heavy chain variable region of mAb 2h9 correspond to SEQ ID NOs: 6-8.

[0031] In one embodiment the anti-uPARAP antibody of the present disclosure is an antibody corresponding to the 2h9 antibody or a functional fragment or variant thereof selected from the group consisting of:

1. a. an antibody or antigen-binding fragment thereof comprising
    1. i. an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 1 or 9 or a sequence having at least 70% sequence identity thereto, such as at least 80% sequence identity thereto, for example at least 90% sequence identity thereto, and/or
    2. ii. an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 5 or 10 or a sequence having at least 70% sequence identity thereto, such as at least 80% sequence identity thereto, for

example at least 90% sequence identity thereto,

2. b. an antibody or antigen-binding fragment thereof that binds to the same epitope as the antibody of a),
3. c. a humanised version of the antibody or antigen-binding fragment thereof of a), or a humanised version of the antibody or antigen-binding fragment thereof of b),
4. d. a chimeric version of the antibody or antigen-binding fragment thereof of a), or a chimeric version of the antibody or antigen-binding fragment thereof of b),
5. e. an antibody or antigen-binding fragment thereof comprising
    1. i. one or more of the amino acid sequences of SEQ ID NOs: 2, 3, 4, 6, 7 and 8, or
    2. ii. the amino acid sequences of SEQ ID NOs: 2, 3 and 4, and/or the amino acid sequences of SEQ ID NOs 6, 7 and 8,
6. f. an antibody or antigen-binding fragment thereof comprising
    1. i. one or more of the amino acid sequences of SEQ ID Nos: 42, 43, 44, 45, 46 and 47, or
    2. ii. the amino acid sequences of SEQ ID NOs: 42, 43 and 44, and/or the amino acid sequences of SEQ ID NOs 45, 46 and 47.

[0032] To preserve antigen recognition of the antibodies disclosed herein the sequence variance is usually not in the CDRs or ABRs. Thus, in a preferred embodiment, any sequence variation is located outside the CDRs or ABRs. All variant antibodies and antigen binding fragments disclosed herein retain the capability to bind to uPARAP.

[0033] For example, the antibody of the present disclosure may comprise

1. a. an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 1 or 9 or a sequence having at least 70% sequence identity thereto, such as at least 80% sequence identity thereto, for example at least 90% sequence identity thereto, and further comprising
    1. i. a CDR1 having an aa sequence according to SEQ ID NO: 2,
    2. ii. a CDR2 having an aa sequence according to SEQ ID NO: 3,
    3. iii. a CDR3 having an aa sequence according to SEQ ID NO: 4, and
2. b. an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 5 or 10 or a sequence having at least 70% sequence identity thereto, such as at least 80% sequence identity thereto, for example at least 90% sequence identity thereto, and further comprising
    1. i. a CDR1 having an aa sequence according to SEQ ID NO: 6,
    2. ii. a CDR2 having an aa sequence according to SEQ ID NO: 7,
    3. iii. a CDR3 having an aa sequence according to SEQ ID NO: 8,

    wherein any sequence variance is outside the CDRs.

[0034] Alternatively, the antibody of the present disclosure may comprise

1. a. an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 1 or 9 or a sequence having at least 70% sequence identity thereto, such as at least 80% sequence identity thereto, for example at least 90% sequence identity thereto, and further comprising
    1. i. an ABR1 having an aa sequence according to SEQ ID NO: 42,
    2. ii. an ABR 2 having an aa sequence according to SEQ ID NO: 43,
    3. iii. an ABR 3 having an aa sequence according to SEQ ID NO: 44, and
2. b. an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 5 or 10 or a sequence having at least 70% sequence identity thereto, such as at least 80% sequence identity thereto, for example at least 90% sequence identity thereto, and further comprising
    1. i. an ABR 1 having an aa sequence according to SEQ ID NO: 45,
    2. ii. an ABR 2 having an aa sequence according to SEQ ID NO: 46,
    3. iii. an ABR 3 having an aa sequence according to SEQ ID NO: 47,

    wherein any sequence variance is outside the ABRs.

[0035] By "antibody" we include substantially intact antibody molecules, 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.

[0036] By "antigen-binding fragment" we mean a functional fragment of an antibody that is capable of binding to uPARAP.

[0037] In one embodiment, the anti-uPARAP 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.

[0038] In one embodiment the anti-uPARAP antibody is a humanised or fully human monoclonal antibody or antigen-binding fragment thereof.

[0039] In one embodiment, the anti-uPARAP antibody of the present disclosure is a recombinant antibody.

[0040] The anti-uPARAP 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 antibody is an IgG monoclonal antibody. In one embodiment the antibody is IgG1κ.

[0041] In one embodiment the anti-uPARAP antibody is an antigen-binding fragment.

[0042] 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.

[0043] 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.

[0044] 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.

[0045] 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.

[0046] 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.

[0047] Methods of generating antibodies and antibody fragments 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.

[0048] 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.

[0049] It will be appreciated by persons skilled in the art that for human therapy or diagnostics, human or humanised antibodies are preferably used. Humanised forms of non-human (*e.g.* murine) antibodies are genetically engineered chimeric antibodies or antibody fragments having preferably minimal-portions derived from non-human antibodies. Humanised antibodies

include antibodies in which complementary determining regions (CDRs) of a human antibody (recipient antibody) are replaced by residues from a complementary determining region of a non-human species (donor antibody) such as mouse, rat of rabbit having the desired functionality. In some instances, Fv framework residues of the human antibody are replaced by corresponding non-human residues. Humanised antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanised antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the complementarity determining regions correspond to those of a non-human antibody and all, or substantially all, of the framework regions correspond to those of a relevant human consensus sequence. Humanised antibodies optimally also include at least a portion of an antibody constant region, such as an Fc region, typically derived from a human antibody.

[0050] Methods for humanising non-human antibodies are well known in the art. Generally, the humanised antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues, often referred to as imported residues, are typically taken from an imported variable domain. Humanisation can be essentially performed as described by substituting human CDRs with corresponding non-human CDRs. Accordingly, such humanised antibodies are chimeric antibodies, wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanised antibodies may be typically human antibodies in which some CDR residues and possibly some framework residues are substituted by residues from analogous sites in non-human antibodies.

[0051] Human antibodies can also be identified using various techniques known in the art, including phage display libraries.

[0052] Once suitable antibodies are obtained, they may be tested for antigen specificity, for example by ELISA.

Active agent

[0053] The anti-uPARAP ADC of the present disclosure comprises an active agent, i.e. a drug, which can be delivered intracellularly to cells expressing uPARAP on their surface. The active agent may e.g. be a therapeutic agent, a cytotoxic agent, a radioisotope or a detectable label. In a preferred embodiment the active agent is a therapeutic agent.

[0054] In one embodiment the active agent is a chemotherapeutic agent. Classes of chemotherapeutic agents include alkylating agents, anthracyclines, antimetabolites, anti-microtubule/anti-mitotic agents, histone deacetylase inhibitors, kinase inhibitors, peptide antibiotics, platinum-based antineoplastics, topoisomerase inhibitors and cytotoxic antibiotics.

[0055] In a preferred embodiment the active agent is a cytotoxic agent allowing for efficient

killing of the cells expressing uPARAP.

[0056] In one embodiment the active agent is an anti-mitotic agent, such as monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), a taxane (e.g. Paclitaxel or Docetaxel), a vinca alkaloid (e.g. Vinblastine, Vincristine, Vindesine or Vinorelbine), Colchicine or Podophyllotoxin.

[0057] In one embodiment, the cytotoxic agent is monomethyl auristatin E (MMAE). Because of its high toxicity, MMAE, which inhibits cell division by blocking the polymerization of tubulin, cannot be used as a single-agent chemotherapeutic drug. However, the combination of MMAE linked to an anti-CD30 monoclonal antibody (Brentuximab Vedotin, trade name Adcetris™) has been proven to be stable in extracellular fluid, cleavable by cathepsin and safe for therapy.

[0058] In one embodiment the cytotoxic agent is monomethyl auristatin F (MMAF). MMAF is an anti-microtubule/anti-mitotic agent and a carboxyl-variant of MMAE.

[0059] In one embodiment, the cytotoxic agent is a DNA-crosslinking agent, such as pyrrolobenzodiazepine or a dimeric pyrrolobenzodiazepine derivative.

[0060] In one embodiment, the cytotoxic agent is a DNA alkylating agent, such as Duocarmycin SA.

[0061] Examples of additional alkylating agents include thiotepa and CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analog topotecan (HYCAMTIN®), CPT-II (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogs); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogs, KW-2189 and CBI-TMI); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e. g., calicheamicin, especially calicheamicin gamma II and calicheamicin omega II; dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomycins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-

5-oxo-L-norleucine, doxorubicin (including ADRIAMYCIN®, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, doxorubicin HCl liposome injection (DOXIL®) and deoxy doxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate, gemcitabine (GEMZAR®), tegafur (UFTORAL®), capecitabine (XELODA®), an epothilone, and 5-fluorouracil (5-FU); folic acid analogs such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex; razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoids, e.g., paclitaxel (TAXOL®), albumin-engineered nanoparticle formulation of paclitaxel (ABRAXANE.TM.), and doxetaxel (TAXOTERE®); chloranbucil; 6-thioguanine; mercaptopurine; methotrexate; a platinum analog such as cisplatin and carboplatin; vinblastine (VELBAN®); platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); oxaliplatin; leucovovin; vinorelbine (NAVELBINE®); novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); a retinoid such as retinoic acid; pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN®) combined with 5-FU and leucovovin.

[0062] Anti-hormonal agents act to regulate, reduce, block, or inhibit the effects of hormones that can promote the growth of cancer, and are often administered as systemic, or whole-body treatment. They may be hormones themselves. Examples include antiestrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), raloxifene (EVISTA®), droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LYI 17018, onapristone, and toremifene (FARESTON®); anti-progesterones; estrogen receptor down-regulators (ERDs); agents that function to suppress or shut down the

ovaries, for example, luteinizing hormone-releasing hormone (LHRH) agonists such as leuprolide acetate (LUPRON® and ELIGARD®), goserelin acetate, buserelin acetate and tripterelin; other anti-androgens such as flutamide, nilutamide and bicalutamide; and aromatase inhibitors such as, for example, 4(5)-imidazoles, aminoglutethimide, megestrol acetate (MEGASE®), exemestane (AROMASIN®), formestanie, fadrozole, vorozole (RIVISOR®), letrozole (FEMARA®), and anastrozole (ARIMIDEX®). In addition, bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), etidronate (DIDROCAL®), NE-58095, zoledronic acid/zoledronate (ZOMETA®), alendronate (FOSAMAX®), pamidronate (AREDIA®), tiludronate (SKELID®), or risedronate (ACTONEL®); as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); siRNA, ribozyme and antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation; vaccines such as THERATOPE® vaccine and gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; topoisomerase 1 inhibitor (e.g., LURTOTECAN®); rmRH (e.g., ABARELIX®); lapatinib ditosylate (an ErbB-2 and EGFR dual tyrosine kinase small-molecule inhibitor also known as GW572016); COX-2 inhibitors such as celecoxib (CELEBREX®; 4-(5-(4-methylphenyl)-3-(trifluoromethyl)- IH-pyrazol-1-yl) benzenesulfonamide; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0063] In one embodiment, the active agent is a nucleotide, such as an oligonucleotide, for example an siRNA or a miRNA.

[0064] 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, i.e. there will be between 1 and 10 drug units per antibody molecule. In one embodiment, the DAR is between 2 and 8, for example between 3 and 6, such as 4 or 5.

Linker

[0065] 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. For example, Brentuximab Vedotin includes an enzyme-sensitive cleavable linker that delivers the potent and highly toxic antimicrotubule agent monomethyl auristatin E (MMAE), a synthetic antineoplastic agent, to cells.

[0066] Trastuzumab Emtansine, another approved ADC, is a combination of the microtubule-formation inhibitor mertansine (DM-1), a derivative of the Maytansine, and antibody

Trastuzumab (Herceptin™, Genentech/Roche), attached by a stable, non-cleavable linker.

[0067] 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.

[0068] For both types of linker, drug release is crucial for obtaining a cellular effect. Drugs which are able to freely diffuse across cell membranes may escape from the targeted cell and, in a process called "bystander killing," also attack neighbouring cells, such as cancer cells in the vicinity of the uPARAP expressing target cell.

[0069] In a preferred embodiment the ADC targeting uPARAP as disclosed herein comprises a linker that links the anti-uPARAP antibody and the active agent. The linker may be cleavable or non-cleavable. In one embodiment the linker is a cleavable linker allowing for intracellular release of the active agent inside the uPARAP expressing cells.

[0070] 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).

[0071] The linker may e.g. be a polypeptide linker, a peptide linker or nucleic acid linker.

[0072] In particular embodiments 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 nonlimiting example the linker is a peptide selected from protamine, a fragment of protamine, (Arg)9, biotin-avidin, biotin-streptavidin and antennapedia peptide. Other non-nucleotide linkers include alkyl or aryl chains of about 5 to about 100 atoms. In some embodiments the linker is a nucleotide linker.

[0073] In one 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.

[0074] 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, .

[0075] In one embodiment the ADC of the present disclosure further comprises a spacer. The spacer may for example connect the linker and the active agent. In one embodiment, the spacer is paraaminobenzoic acid (PAB).

[0076] In one embodiment the spacer is or includes a polyethylenglycol spacer, such as a PEG4 spacer.

[0077] In one embodiment 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 amino acid side chain and a reactive attachment group in the linker precursor. In one 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, azides or alkynes.

[0078] In one embodiment the ADC of the present disclosure comprises an anti-uPARAP antibody as disclosed herein and the linker-drug complex Vedotin. Vedotin is a linker-drug complex comprising the cytotoxic agent MMAE, a spacer (paraaminobenzoic acid), a cathepsin-cleavable linker (Valine-citrulline dipeptide) and an attachment group consisting of caproic acid and maleimide. Vedotin is MC-VC-PAB-MMAE. Brentuximab Vedotin (trade name Adcetris™) is an example of an FDA-approved ADC comprising Vedotin.

[0079] In one embodiment, the ADC of the present disclosure comprises an anti-uPARAP antibody as disclosed herein and a linker-spacer-toxin unit being VC-PAB-MMAF.

[0080] In one embodiment, the ADC of the present disclosure comprises an anti-uPARAP antibody as disclosed herein and a linker-spacer-toxin unit being PEG4-VA-PBD.

[0081] In one embodiment, the ADC of the present disclosure comprises an anti-uPARAP antibody as disclosed herein and a linker-spacer-toxin unit being PEG4-VC-DuocarmycinSA.

[0082] In one embodiment, the ADC of the present disclosure comprises a linker-drug complex as described in US 2006/074008, which is incorporated by reference in its entirety.

[0083] The linker-drug construct may e.g. be attached to the anti-uPARAP antibody by maleimide chemistry to thiols of reduced interchain or intrachain disulphide bridges.

Therapeutic use

[0084] The ADCs directed against uPARAP as described herein are useful for the delivery of active agents, such as therapeutic or cytotoxic agents to cells expressing uPARAP and thus for the treatment of a range of diseases and disorders characterized by uPARAP expression, in particular uPARAP overexpression.

[0085] In one embodiment, the present disclosure provides a pharmaceutical composition comprising an effective amount of a anti-uPARAP ADC, as described herein, together with a pharmaceutically acceptable buffer, diluent, carrier, adjuvant or excipient.

[0086] 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 lyophilised, e.g. through freeze drying, spray drying, spray cooling, or through use of particle formation from supercritical particle formation.

[0087] By "pharmaceutically acceptable" we mean a non-toxic material that does not decrease the effectiveness of the anti-uPARAP 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).

[0088] The term "buffer" is intended to mean an aqueous solution containing an acid-base mixture with the purpose of stabilising pH. Pharmaceutically acceptable buffers are well known in the art.

[0089] The term "diluent" is intended to mean an aqueous or non-aqueous solution with the purpose of diluting the agent in the pharmaceutical preparation.

[0090] 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.

[0091] 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.

[0092] 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.

[0093] 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.

[0094] 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. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art.

[0095] 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 multidose 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.

[0096] In one embodiment the ADCs of the present disclosure are administered intravenously.

[0097] In one embodiment the ADCs of the present disclosure are administered

subcutaneously.

[0098] In one embodiment the ADCs of the present disclosure are administered intracranially or intracerebrally.

[0099] The pharmaceutical compositions will be administered to a patient in a pharmaceutically effective amount. A 'therapeutically effective amount', or 'effective amount', or 'therapeutically effective', as used herein, refers to that amount which 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. A therapeutically effective amount can be determined by the ordinarily skilled medical or veterinary worker based on patient characteristics, such as age, weight, sex, condition, complications, other diseases, *etc.,* as is well known in the art. The administration of the pharmaceutically effective dose can be carried out both by single administration in the form of an individual dose unit, or else several smaller dose units, and also by multiple administrations of subdivided doses at specific intervals. Alternatively, the dose may be provided as a continuous infusion over a prolonged period.

[0100] It will be appreciated by persons skilled in the art that the ADCs targeting uPARAP described herein may be administered alone or in combination with other therapeutic agents. For example, the ADCs targeting uPARAP 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, platinum-based antineoplastics, etoposide, taxanes, topoisomerase inhibitors, antiproliferative immunosuppressants, corticosteroids, sex hormones and hormone antagonists, cytotoxic antibiotics and other therapeutic agents.

[0101] In one embodiment the ADC 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.

[0102] In one embodiment the present disclosure provides a kit comprising an ADC targeting uPARAP as described herein or a pharmaceutical composition comprising same. The kit may optionally further comprise means for administering the ADC to a subject and instructions for

use.

[0103] In one embodiment the present disclosure relates to a method for delivery of an active agent to a uPARAP-expressing cell in a subject comprising administering to the subject a uPARAP-directed ADC or a composition comprising a uPARAP-directed ADC as described herein, such that the active agent is delivered to said cell.

[0104] In one embodiment the present disclosure relates to the uPARAP-directed ADC or a composition comprising said uPARAP-directed ADC as described herein, for use in the delivery of an active agent to a uPARAP-expressing cell in a subject, comprising administering to the subject a uPARAP-directed ADC or a composition comprising a uPARAP-directed ADC as described herein, such that the active agent is delivered to said cell.

[0105] In one embodiment the present disclosure relates to a method for treatment of a disease or disorder characterised by cells expressing uPARAP in a subject, comprising administering to the subject a uPARAP-directed ADC or a composition comprising a uPARAP-directed ADC as described herein to said subject.

[0106] In one embodiment the present disclosure relates to the uPARAP-directed ADC or a composition comprising said uPARAP-directed ADC as described herein for use in the treatment of a disease or disorder characterised by cells expressing uPARAP.

[0107] In one embodiment the present disclosure relates to a method for inhibiting the growth of a cell expressing uPARAP *in vivo* or *in vitro* comprising administering a uPARAP-directed ADC or a composition comprising a uPARAP-directed ADC as described herein. This inhibition of growth may include cell death or may include growth inhibition without cell death.

[0108] In a particularly preferred embodiment the uPARAP-expressing cell is a tumour cell and/or a tumour associated cell and the present disclosure relates to a method for treatment of cancer in a subject, comprising administering to the subject the uPARAP-directed ADC or a composition comprising a uPARAP-directed ADC as described herein to said subject.

[0109] 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.

[0110] In one embodiment the present disclosure relates to a method for inhibiting tumour progression in a subject, comprising administering to the subject a uPARAP-directed ADC or a composition comprising a uPARAP-directed ADC as described herein to said subject. This inhibition of tumor progression may include complete or incomplete eradication of tumors, or may include growth arrest without cell death.

[0111] In one embodiment the present disclosure relates to a method for inhibiting, lowering or eliminating metastatic capacity of a tumour in a subject, comprising administering to the subject

a uPARAP-directed ADC or a composition comprising a uPARAP-directed ADC as described herein to said subject.

[0112] In one embodiment the tumour cells express or overexpress uPARAP.

[0113] In one embodiment the tumour associated cells express or overexpress uPARAP.

[0114] In one embodiment the present disclosure provides a method for inducing cell death and/or inhibiting the growth and/or proliferation of cells expressing uPARAP, comprising the step of administering to the individual an effective amount of an ADC targeting uPARAP as described herein, or a pharmaceutical composition comprising an ADC targeting uPARAP as described herein.

[0115] The treatment preferably induces cell death and/or inhibits the growth and/or proliferation of the uPARAP expressing cells, such as tumour cells or tumour associated cells.

[0116] In one embodiment the treatment is ameliorative.

[0117] In one embodiment the treatment is curative.

[0118] In one embodiment the present disclosure provides an ADC targeting uPARAP as described herein for the preparation of a medicament for inducing cell death and/or inhibiting the growth and/or proliferation of cells expressing uPARAP, such as tumour cells or tumour associated cells.

[0119] The expression and role of uPARAP in cancer has been investigated by several research groups; cf. review by Melander et al (Melander et al., 2015, Int J Oncol 47: 1177-1188) and article by Engelholm et al (Engelholm et al., 2016, J. Pathol. 238, 120-133).

[0120] In one embodiment the cancer is a solid tumour, wherein the tumour cells and/or the tumour associated cells express uPARAP.

[0121] In one embodiment the cancer is a solid tumour, wherein the tumour cells express uPARAP.

[0122] Examples of cancers characterized by overexpression of uPARAP include, but are not limited to, sarcoma, including osteosarcoma (Engelholm et al., 2016, J Pathol 238(1): 120-33) as well as other sarcomas, glioblastoma (Huijbers et al., 2010, PLoS One 5(3):e9808), prostate cancer and bone metastases from prostate cancer (Kogianni et al., 2009, Eur J Cancer 45(4): 685-93), breast cancer and in particular "basal like" breast cancer (Wienke et al., 2007, Cancer Res 1;67(21): 10230-40), and head- and neck cancer (Sulek et al., 2007, J Histochem Cytochem 55(4): 347-53).

[0123] In one embodiment the cancer is sarcoma, such as osteosarcoma, liposarcoma,

myxofibrosarcoma, dermatofibrosarcoma protuberans (DFSP) and/or leiomyosarcoma (LMS).

[0124] In one embodiment the cancer is glioblastoma.

[0125] In one embodiment the cancer is a solid tumour, wherein the tumour associated cells express uPARAP. When uPARAP is expressed by tumour associated cells, the therapeutic effect is believed to be mediated via the so-called "by-stander" effect and/or via reduction and/or elimination of stromal cell-mediated stimulation of tumour growth and dissemination.

[0126] Examples of cancers characterized by overexpression of uPARAP in the tumour associated cells include but are not limited to breast cancer (Schnack et al., 2002, Int J Cancer 10;98(5): 656-64), head- and neck cancer (Sulek et al., 2007, J Histochem Cytochem 55(4): 347-53) and multiple other solid malignant tumours.

[0127] In one embodiment, the cancer is not a solid tumour. For instance, the ADC of the present disclosure may e.g. be used for the treatment of uPARAP-expressing leukemia, for example, from the macrophage-monocyte lineage.

[0128] In other embodiments, the disease or disorder characterised by cells expressing uPARAP is not cancer.

[0129] uPARAP is involved in bone growth and homeostasis (Madsen et al., 2013, PLoS One 5;8(8): e71261). Thus, in one embodiment the ADC of the present disclosure may be used for the treatment of a disease characterized by bone degradation, wherein the bone degradation is mediated by non-malignant cells, such as osteoporosis.

[0130] Due to its role in collagen accumulation, a role for uPARAP has also been shown in fibrosis (Madsen et al., 2012, J Pathol 227(1):94-105). Thus, in one embodiment the ADC of the present disclosure may be used for the treatment of fibrosis, for example of kidney, lung and liver.

[0131] In one embodiment the ADC of the present disclosure may be used for the treatment of diseases and disorders associated with macrophages, including atherosclerosis and chronic inflammation.

References

[0132]

Kabat, E. A., Wu, T. T., Bilofsky, H., Reid-Miller, M., Perry, H. (1983) Sequence of proteins of immunological interest. Bethesda: National Institute of Health.

Kabat, E. A., Wu, T. T., Perry, H., Gottesman, K. and Foeller, C. (1991) Sequences of proteins

of immunological interest. Fifth Edition. NIH Publication No. 91-3242.

Wu, T.T., Kabat, E. A. (2008) Pillars article: an analysis of the sequences of the variable regions of Bence Jones proteins and myeloma light chains and their implications for antibody complementarity. J. Exp. Med. 132, 211-250. J. Immunology 180, 7057-7096.

Dunbar, J., Deane, C. M. (2016) ANARCI: antigen receptor numbering and receptor classification. Bioinformatics, 32, 298-300.

Lefranc MP, Pommié C, Ruiz M, Giudicelli V, Foulquier E, Truong L, Thouvenin-Contet V, Lefranc G. (2003) IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains. Dev Comp Immunol. 27, 55-77.

Kunik V, Ashkenazi S, Ofran Y. (2012a) Paratome: an online tool for systematic identification of antigen-binding regions in antibodies based on sequence or structure. Nucleic Acids Res. 40(Web Server issue):W521-4. doi: 10.1093/nar/gks480. Epub 2012 Jun 6.

Kunik V, Peters B, Ofran Y. (2012b) Structural consensus among antibodies defines the antigen binding site. PLoS Comput Biol. 8(2):e1002388.

Example 1: *In vitro* and *in vivo* efficacy of ADCs directed against the N-terminal region of uPARAP

Materials and methods

Preparation and evaluation of mAb-vc-MMAE ADCs

[0133] Monoclonal antibodies (mAbs) against uPARAP or against trinitrophenol (TNP) were generated and produced using hybridoma technique after immunization of mice, according to established methods known in the art. In the case of mAbs against uPARAP, the host mice for immunization were gene deficient with respect to uPARAP, leading to antibodies reactive with both the human and the murine antigen. ADCs were prepared by a commonly employed conjugation method, described previously in the art (Doronina et al. 2003 Nature biotechnology 21(7):778-84; Francisco et al., 2003. Blood 102(4):1458-65; Hamblett et al., 2004. Clinical cancer research 10(20):7063-70).

[0134] Antibodies were subjected to mild reduction by a 10 minute incubation at 37°C in the presence of 10mM DTT in a 50mM sodium borate, 50mM NaCl, pH 8.0 buffer at 5 mg/mL concentration, followed by removal of DTT by buffer exchange using 30kDa NMWL centrifugal filters to fresh PBS pH 7.4 with 1mM EDTA, then adjusted to 2 mg/mL concentration. This was

followed by immediate conjugation to a 5-10 times molar surplus of maleimidocaproyl-valine-citrulline-p-aminobenzoyloxycarbonyl-monomethyl auristatin E (MC-VC-PAB-MMAE, i.e. Vedotin), dissolved in water-free DMSO to a final DMSO content of 10% v/v during conjugation for 2 hours at 37°C. The resulting mAb-vc-MMAE ADCs were purified by gel filtration on PD-10 desalting columns. The average drug-to-antibody ratio (DAR) of the resulting ADCs was determined based on the absorbance ratio of purified conjugate samples at λ=248nm and λ=280nm. Unmodified mAbs display an A_{248nm}/A_{280nm} ratio of 0.43, and the A_{MAX} at λ=248nm of MMAE gives rise to a higher A_{248nm}/A_{280nm} ratio for mAb-vc-MMAE ADCs, which has been demonstrated to reflect the DAR of the resulting ADCs (Hamblett et al., 2004. Clinical cancer research 10(20):7063-70; Sanderson et al., 2005. Clinical Cancer Research 11:843-852).

Cell lines

[0135] U937, THP-1 and HT1080 cells were all obtained from ATCC. KNS42 cells were kindly provided by Lara Perryman, Biotech Research and Innovation Centre (BRIC), University of Copenhagen. CHO-K1 cells were obtained from Invitrogen. All cells were maintained in appropriate medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin, in a 37°C, 5% CO_2 atmosphere incubator.

SDS-PAGE analysis of conjugate species

[0136] Reducing SDS-PAGE was performed by running a 4-12% NuPAGE Bis-Tris SDS-PAGE gel, loading 5µg of total protein per lane, reduced by boiling for 3 minutes in sample buffer in the presence of 40mM DTT. The gels were stained using a standard 0.1% coomassie blue stain. For cathepsin B linker cleavage assay, samples were treated with recombinant human (rh) Cathepsin B according to manufacturer's instructions, using 100ng of activated rhCathepsin B to 20µg ADC (mAb component), in a 25mM MES, pH 5.0 buffer, and incubation at 37°C overnight.

ELISA analysis of uPARAP-binding of mAbs

[0137] A 96-well ELISA plate was coated with 25ng/well of a soluble truncated uPARAP protein containing the first 3 N-terminal domains of human uPARAP, with intact epitope for mAb 2h9. Untreated mAbs (2h9 or aTNP), same mAbs subjected to the reduction procedure of conjugation (see above), or ADCs 2h9-vc-MMAE or aTNP-vc-MMAE, were then employed as a primary antibody, followed by a HRP-conjugated rabbit antimouse Ig secondary antibody. Finally an o-phenylenediamine dihydrochloride-containing substrate solution was added, and the color reaction was stopped by adding 1M H_2SO_4. Plates were read at 492 nm using a plate reader.

In vitro cytotoxicity of ADCs - Cell viability assay

[0138] Cells tested were seeded at low density (20-25% confluence, generally 5-10×10^3 cells per well) in a 96 well plate in 90µL of medium, and incubated overnight. The next day, mAb-vc-MMAE conjugates based on mAb 2h9, mAb 5f4 or non-targeted control mAb aTNP were prepared as a serial dilution (1:4) in PBS and added in volumes of 10µL to each well, with a final maximum ADC concentration of 10µg/mL mAb component. Cells were incubated for 72 hours, before 20µL of CellTiter 96 AQueous One Solution Cell Proliferation Assay (MTS, Promega) was added, and incubated for an appropriate time for formation of color (usually 1 hour). The plates were then read at 490nm, with background subtraction at 630nm, using a plate reader.

In vitro cytotoxicity of ADCs - Cell cycle analysis

[0139] Cell cycle analysis was performed using a Nucleocounter NC-3000 system (ChemoMetec Denmark), using the manufacturers standard protocol for analyzing the cell cycle distribution of a population of cells, based on the DNA content of each cell. The percentage of cells in Sub-G1, G1, S, or G2/M-phases of the cell cycle was established from histogram analysis using the NucleoView NC-3000 software.

Receptor competition and lysosomal protease inhibition

[0140] For receptor competition assay, receptor depletion assay, and assay for inhibition of lysosomal proteases, U937 cells were seeded as for a cell viability assay (see above). For receptor competition assay, a constant 2h9-vc-MMAE concentration of 1µg/mL mAb component was kept in all wells, and the unmodified competition mAb was simultaneously added in a dilution series (1:2) starting at a concentration of 8µg/mL competitive mAb. Cells were then subjected to a 72 hour cytotoxicity cell viability assay (see above). For the assay of inhibition of lysosomal proteases, U937 cells were preincubated with 20µM of E64D protease inhibitor for 2 hours, before starting a 72 hour cytotoxicity cell viability assay (see above).

Animal experiments

[0141] All animal experiments were performed under legal approval from The Danish Veterinary and Food Administration. All reagents and cell lines used for animal experiments were tested negative for the presence of murine viruses, bacteria, mycoplasma and fungi. Animals received standard of care, and were sacrificed upon any of the following signs: loss of more than 10% of body weight, visible distress or illness, compromised food- or water intake or

defecation, signs of severe inflammation in the vicinity of tumours, or tumour growth which exceeded a volume of 1000mm^3 or compromised the free movement of the animals. Tumour growth was measured using electronic calipers, and tumour volumes were calculated using the formula Volume=(L×W^2)/2, with L being the longest dimension of the tumour, and W being the width in the perpendicular dimension.

Treatment of a subcutaneous uPARAP-positive U937 xenograft tumour model in mice by s.c. injection

[0142] For tumour establishment, mice were shaved at the flank, and received a subcutaneous injection of 1×10^6 U937 cells, and then closely monitored in order to observe the development of solid tumours. Upon formation of palpable tumours with a volume of 50-100mm^3, the mice started treatment in one of four treatment groups: 2h9-vc-MMAE (N=10), aTNP-vc-MMAE (N=9), unmodified mAb 2h9 (N=5) or PBS vehicle control (N=5). All treatments were given as a total of 4 subcutaneous doses of 3 mg/kg mAb component in the tumour area, at 4 days intervals. Injections were performed under brief isoflurane anesthesia to avoid risks for the animal handler. During treatment, the tumours were evaluated every two days, until reaching a point of sacrifice. Mice which fully lost any tumour burden were checked two times a week for a period of 3 months after ending treatment.

Treatment of a subcutaneous uPARAP-positive U937 xenograft tumour model in mice by intravenous injection

[0143] For tumour establishment, mice were shaved at the flank, and received a subcutaneous injection of 1×10^6 U937 cells, and then closely monitored in order to observe the development of solid tumours. Upon formation of palpable tumours with a volume of 50-100mm^3, the mice started treatment in one of four treatment groups: 2h9-vc-MMAE (N=10), aTNP-vc-MMAE (N=10), unmodified mAb 2h9 (N=5) or PBS vehicle control (N=5). All treatments were given as a total of 3 intravenous doses of 5 mg/kg mAb component in the tail veins of the mice, at 4 days intervals. During treatment, the tumours were evaluated every two days, until reaching a point of sacrifice. Mice which fully lost any tumour burden were checked two times a week for a period of 3 months after ending treatment.

Statistics

[0144] All samples were done in triplicates. Error bars: Standard deviation.

Results and conclusions

[0145] The collagen receptor uPARAP is upregulated in the tumour cells of specific cancers, including sarcomas and late-stage glioblastoma. Additionally, the receptor is most often upregulated in stromal cells surrounding solid tumours. In healthy adult individuals, the receptor displays a restricted expression, thus making it a potential target for ADC therapy.

[0146] For this purpose, we selected a monoclonal antibody, 2h9, obtained after immunization of a uPARAP gene-deficient mouse, and prepared a uPARAP-directed ADC (2h9-vc-MMAE) using a well-established conjugation method. The targeting antibody 2h9 was shown to tolerate the conjugation procedure well, with negligible loss of affinity. The resulting ADC was shown to be highly specific in killing or inducing growth arrest in uPARAP-positive cells *in vitro,* with U937 cells being the most sensitive cell line tested. uPARAP is a constitutively recycling receptor, directing its cargo to the lysosomal compartment. We found that ADC efficiency in highly sensitive cells such as U937 cells was completely dependent on linker cleavage, since uPARAP-dependent cytotoxicity was abrogated after inhibition of lysosomal cathepsins with E64D. Therefore, we suggest that the lysosomal capacity for cleavage of the linker contributes to differences in ADC sensitivity between different cell types, in collaboration with overall differences in sensitivity towards the conjugated cytotoxin.

[0147] For *in vivo* studies, we utilized a fast-growing subcutaneous xenograft tumour model with U937 cells in CB17 SCID mice. Using this model, ADC 2h9-vc-MMAE was found to be highly efficient at eradicating solid U937 tumours *in vivo.* Following treatment by local subcutaneous administration, 5 mice remained tumour-free 90 days after finishing the treatment regimen, hence constituting a 50% cure rate. More importantly, following treatment by intravenous administration, we observed a potent effect resulting in a 100% cure rate. Notably, this eradication of tumours was obtained without any evident adverse effects upon regular inspection of the treated mice. Importantly, the 2h9 antibody is reactive against both human and murine uPARAP, a cross-reactivity enabled by the use of a uPARAP-deficient mouse for immunization when raising the antibody. Therefore, in this xenograft model, in addition to beneficial anti-tumoural effects, any potential detrimental side effects on the host would be revealed, but no signs of detrimental effects were seen.

[0148] The epitope for the 2h9 antibody is located within the first three N-terminal domains of uPARAP, more particularly in either the CysR domain or CTLD-1. *In vitro* studies presented herein indicate that another ADC comprising an anti-UPARAP antibody targeting the first three N-terminal domains of uPARAP, namely 5f4, is as efficient as ADCs comprising the 2h9 antibody. The epitope for the 5f4 antibody is in the FN-II domain of uPARAP. Thus, we hypothesize that ADCs comprising anti-uPARAP antibodies directed against epitopes within the first three N-terminal domains of uPARAP are particularly efficient as ADCs.

[0149] In conclusion, the data presented here very strongly support the notion of the collagen receptor uPARAP as a versatile target in ADC cancer therapy based on expression pattern and molecular function. Furthermore, these data show that ADCs comprising antibodies directed against the first three N-terminal domains of uPARAP, such as ADC 2h9-vc-MMAE, are highly

efficient for targeting of uPARAP-expressing cells *in vitro* and *in vivo.*

Example 2: *In vitro* efficacy of MMAE-based ADCs

[0150] In addition to the ADCs of Example 1, the following MMAE ADCs were generated: 9b7-vc-MMAE and 11c9-vc-MMAE.

[0151] mAb 2h9, mAb 5f4 and mAb 9b7 are directed against epitopes within the three N-terminal domains of uPARAP, while mAb 11c9 is an anti-uPARAP antibody directed against an epitope outside the N-terminal three domains of uPARAP.

[0152] *In vitro* cell viability assays with U937 cells were performed as described in Example 1, using all of these ADCs. All ADCs lead to a specific reduction in overall cell viability but with the cellular sensitivity to 2h9-vc-MMAE, 5f4-vc-MMAE and 9b7-vc-MMAE being significantly higher than the sensitivity to 11c9-vc-MMAE (Figure 14).

[0153] Thus, the inventors conclude that ADCs comprising anti-uPARAP antibodies capable of binding to epitopes within the three most N-terminal domains of uPARAP are very efficient ADCs.

Example 3: *In vitro* efficacy of ADCs comprising different linkers, spacers and toxins

[0154] Different toxins can be used in an ADC format targeting the N-terminal part of uPARAP. ADCs with mAb 2h9 as the antibody component were prepared as described above but using the following linker-cytotoxin units instead of VC-PAB-MMAE:

- VC-PAB-MMAF (with MMAF being monomethyl auristatin F, a carboxyl-variant of MMAE)
- PEG4-va-PBD (with PEG4 referring to a polyethylenglycol spacer, va being valine-alanine and PBD referring to a dimeric pyrrolobenzodiazepine)
- PEG4-vc-Duocarmycin SA (with PEG4 referring to a polyethylenglycol spacer and vc being valine-citrulline)

[0155] The resulting ADCs (referred to as 2h9-vc-MMAF, 2h9-va-PBD and 2h9-vc-DuocSA, respectively) were used for *in vitro* cell viability assays with U937 cells, performed as described above. U937 cells displayed very strong sensitivity to 2h9-vc-MMAF, a more moderate sensitivity to 2h9-va-PBD and a low but measurable sensitivity to 2h9-vc-DuocSA. The results are shown in figures 15 and 16.

Example 4: *In vitro* efficacy of ADCs on human glioblastoma explant cells

[0156] The ADCs 2h9-vc-MMAE and 2h9-vc-MMAF were tested by *in vitro* cell viability assays, performed as described in Example 1, for their capacity to specifically kill human glioblastoma explant cells. Glioblastoma explant cells are e.g. described in Staberg et al., 2017, Cell Oncol. 40: 21-32. These cells displayed a very strong and specific sensitivity towards both ADC 2h9-vc-MMAE, as well as ADC 2h9-vc-MMAF, thus demonstrating high efficacy of these ADCs in combating human glioblastoma cells. The results are shown in figure 17.

Example 5: Recombinant antibody

[0157] The protein product encoded by a synthetic DNA, comprising [SEQ ID NO: 1] (light chain of monoclonal antibody 2h9 against uPARAP) and [SEQ ID NO: 5] (heavy chain of the same antibody), was expressed in CHO cells. The resulting recombinant antibody product was purified and was shown by Western blotting to specifically recognize uPARAP in the same manner as monoclonal antibody 2h9 produced by hybridoma cell culture (figure 18).

REFERENCES CITED IN THE DESCRIPTION

Cited references

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- WO2010111198A **[0005]** **[0017]**
- US2006074008A **[0082]**

Non-patent literature cited in the description

- **CALEY et al.**J. Pathol, 2012, vol. 5, 775-783 **[0003]**

- **MELANDER et al.**Int J Oncol, 2015, vol. 47, 1177-1188 [0003]
- **MELANDER et al.**Int J Oncology, 2015, vol. 47, 1177-1188 [0010]
- **SCHULTZ et al.**Proc. Natl. Acad. Sci. USA, 1998, vol. 95, 5857-5864 [0011]
- **JÜRGENSEN et al.**JBC, 2011, vol. 286, 3732736-48 [0029]
- **JÜRGENSEN et al.**JBC, 2014, vol. 289, 117935-47 [0029]
- Remington's Pharmaceutical SciencesMack Publishing Company19900000 [0087]
- Pharmaceutical ExcipientsPharmaceutical Press20000000 [0087]
- **MELANDE et al.**Int J Oncol, 2015, vol. 47, 1177-1188 [0119]
- **ENGELHOLM et al.**J. Pathol., 2016, vol. 238, 120-133 [0119]
- **ENGELHOLM et al.**J Pathol, 2016, vol. 238, 1120-33 [0122]
- **HUIJBERS et al.**PLoS One, 2010, vol. 5, 3e9808- [0122]
- **KOGIANNI et al.**Eur J Cancer, 2009, vol. 45, 4685-93 [0122]
- **WIENKE et al.**Cancer Res 1, 2007, vol. 67, 2110230-40 [0122]
- **SULEK et al.**J Histochem Cytochem, 2007, vol. 55, 4347-53 [0122] [0126]
- **SCHNACK et al.**Int J Cancer 10, 2002, vol. 98, 5656-64 [0126]
- **MADSEN et al.**PLoS One 5, 2013, vol. 8, 8e71261- [0129]
- **MADSEN et al.**J Pathol, 2012, vol. 227, 194-105 [0130]
- **KABAT, E. A.WU, T. T.BILOFSKY, H.REID-MILLER, M.PERRY, H.**Sequence of proteins of immunological interestBethesda: National Institute of Health19830000 [0132]
- **KABAT, E. A.WU, T. T.PERRY, H.GOTTESMAN, K.FOELLER, C.**Sequences of proteins of immunological interest, 1991, 913242- [0132]
- **WU, T.T.KABAT, E. A.**Pillars article: an analysis of the sequences of the variable regions of Bence Jones proteins and myeloma light chains and their implications for antibody complementarityJ. Exp. Med., 2008, vol. 132, 211-250 [0132]
- J. Immunology, vol. 180, 7057-7096 [0132]
- **DUNBAR, J.DEANE, C. M.**ANARCI: antigen receptor numbering and receptor classificationBioinformatics, 2016, vol. 32, 298-300 [0132]
- **LEFRANC MPPOMMIÉ CRUIZ MGIUDICELLI VFOULQUIER ETRUONG LTHOUVENIN-CONTET VLEFRANC G**IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domainsDev Comp Immunol, 2003, vol. 27, 55-77 [0132]
- **KUNIK VASHKENAZI SOFRAN Y**Paratome: an online tool for systematic identification of antigen-binding regions in antibodies based on sequence or structureNucleic Acids Res, 2012, [0132]
- **KUNIK VPETERS BOFRAN Y**Structural consensus among antibodies defines the antigen binding sitePLoS Comput Biol, 2012, vol. 8, 2e1002388- [0132]
- **DORONINA et al.**Nature biotechnology, 2003, vol. 21, 7778-84 [0133]
- **FRANCISCO et al.**Blood, 2003, vol. 102, 41458-65 [0133]
- **HAMBLETT et al.**Clinical cancer research, 2004, vol. 10, 207063-70 [0133] [0134]
- **SANDERSON et al.**Clinical Cancer Research, 2005, vol. 11, 843-852 [0134]
- **STABERG et al.**Cell Oncol., 2017, vol. 40, 21-32 [0156]

PATENTKRAV

1. Antistof-lægemiddelkonjugat rettet mod uPARAP omfattende:

a. et antistof, hvor antistoffet er valgt fra gruppen bestående af:

i. et antistof eller antigenbindende fragment deraf omfattende

i) en variabel region i en let immunoglobulinkæde, der omfatter eller består af aminosyresekvensen ifølge SEQ ID NO: 1 eller 9 eller en sekvens med mindst 90% sekvensidentitet dermed, og

ii) en variabel region i en tung immunglobulinkæde omfattende eller bestående af aminosyresekvensen ifølge SEQ ID NO: 5 eller 10 eller en sekvens med mindst 90% sekvensidentitet dermed, hvor en eventuel sekvensvarians er uden for de komplementaritetsbestemmende regioner,

ii. en humaniseret version af antistoffet eller det antigenbindende fragment deraf ifølge i.,

iii. en kimærisk version af antistoffet eller det antigen-bindende fragment deraf ifølge i.,

iv. et antistof eller antigenbindende fragment deraf omfattende

i) en variabel region i en let immunoglobulinkæde, der omfatter aminosyresekvenserne ifølge SEQ ID NO: 2, 3 og 4, og

ii) en variabel region i en tung immunoglobulinkæde omfattende aminosyresekvenserne ifølge SEQ ID NOs 6, 7 og 8,

v. et antistof eller antigenbindende fragment deraf omfattende

i) en variabel region i en let immunoglobulinkæde omfattende aminosyresekvenserne ifølge SEQ ID NO: 42, 43 og 44, og

ii) en variabel region i en tung immunoglobulinkæde omfattende aminosyresekvenserne ifølge SEQ ID NOs 45, 46 og 47,

vi. en humaniseret version af antistoffet eller det antigenbindende fragment deraf ifølge iv. eller v.,

b. et aktivt middel, hvor det aktive middel er valgt blandt et terapeutisk middel, et cytotoksisk middel, en radioisotop og en detekterbar markør, og

c. en linker, som forbinder a) til b).

2. Antistof-lægemiddelkonjugat ifølge krav 1, hvor antistoffet er valgt blandt et museantistof, et kimærisk antistof, et humant antistof, et humaniseret antistof, et humaniseret antigenbindende fragment, et Fab-fragment, et Fab'-fragment, et F(ab')2-fragment, et

Fv, et enkeltkædet antistof (SCA), såsom et scFv, den variable del af de tunge og/eller lette kæder deraf og et Fab-miniantistof.

3. Antistof-lægemiddelkonjugat ifølge et hvilket som helst af de foregående krav, hvor antistoffet er et monoklonalt antistof.

4. Antistof-lægemiddelkonjugat ifølge et hvilket som helst af de foregående krav, hvor det aktive middel er et kemoterapeutisk middel, såsom et kemoterapeutisk middel valgt fra gruppen bestående af alkyleringsmidler, anthracycliner, antimetabolitter, anti-mikrotubuli/anti-mitotiske midler, histondeacetylaseinhibitorer, kinaseinhibitorer, peptidantibiotika, platinbaserede antineoplastika, topoisomeraseinhibitorer og cytotoksiske antibiotika.

5. Antistof-lægemiddelkonjugat ifølge et hvilket som helst af de foregående krav, hvor det aktive middel er

a. et antimitotisk middel valgt fra gruppen bestående af monomethylauristatin E (MMAE), monomethylauristatin F (MMAF), en taxan (f.eks. Paclitaxel eller Docetaxel), et vincaalkaloid (f.eks. Vinblastin, Vincristin, Vindesin eller Vinorelbin), Colchicin og Podophyllotoxin, eller

b. et DNA-tværbindingsmiddel valgt fra gruppen bestående af pyrrolobenzodiazepin og et dimert pyrrolobenzodiazepinderivat, eller

c. et alkyleringsmiddel, såsom Duocarmycin SA.

6. Antistof-lægemiddelkonjugat ifølge et hvilket som helst af de foregående krav til anvendelse som medikament.

7. Farmaceutisk sammensætning omfattende et antistof-lægemiddelkonjugat ifølge et hvilket som helst af kravene 1 til 5 og en farmaceutisk acceptabel buffer, fortyndingsmiddel, bærer, adjuvans eller excipiens.

8. Antistof-lægemiddelkonjugat ifølge et hvilket som helst af kravene 1 til 5 eller farmaceutisk sammensætning ifølge krav 7 til anvendelse i en fremgangsmåde til behandling af en sygdom, der er karakteriseret ved celler, der udtrykker uPARAP, såsom hvor den sygdom, der er karakteriseret ved celler, der udtrykker uPARAP, er valgt blandt cancer, en knoglenedbrydningssygdom, såsom osteoporose, fibrose, og makrofagassocierede

sygdomme eller lidelser, såsom aterosklerose eller kronisk inflammation.

9. Antistof-lægemiddelkonjugat eller farmaceutisk sammensætning til anvendelse ifølge krav 8, hvor sygdommen er cancer.

10. Antistof-lægemiddelkonjugat eller farmaceutisk sammensætning til anvendelse ifølge krav 9, hvor canceren er sarkom, såsom osteosarkom, liposarkom, myxofibrosarkom, dermatofibrosarcoma protuberans (DFSP) og/eller leiomyosarkom (LMS).

11. Antistof-lægemiddelkonjugat eller farmaceutisk sammensætning til anvendelse ifølge krav 9, hvor canceren er glioblastom.

12. Antistof-lægemiddelkonjugat eller farmaceutisk sammensætning til anvendelse ifølge krav 9, hvor canceren er prostatacancer eller knoglemetastaser fra prostatacancer.

13. Antistof-lægemiddelkonjugat eller farmaceutisk sammensætning til anvendelse ifølge krav 9, hvor canceren er brystcancer.

14. Antistof-lægemiddelkonjugat eller farmaceutisk sammensætning til anvendelse ifølge krav 9, hvor canceren er hoved- og halscancer.

15. Antistof-lægemiddelkonjugat eller farmaceutisk sammensætning til anvendelse ifølge krav 9, hvor canceren er leukæmi.

16. Kit, der omfatter et antistof-lægemiddelkonjugat ifølge et hvilket som helst af kravene 1 til 5 eller en farmaceutisk sammensætning ifølge krav 7, og som eventuelt yderligere omfatter midler til administration af antistof-lægemiddelkonjugatet til et individ og/eller brugsanvisninger.

DRAWINGS

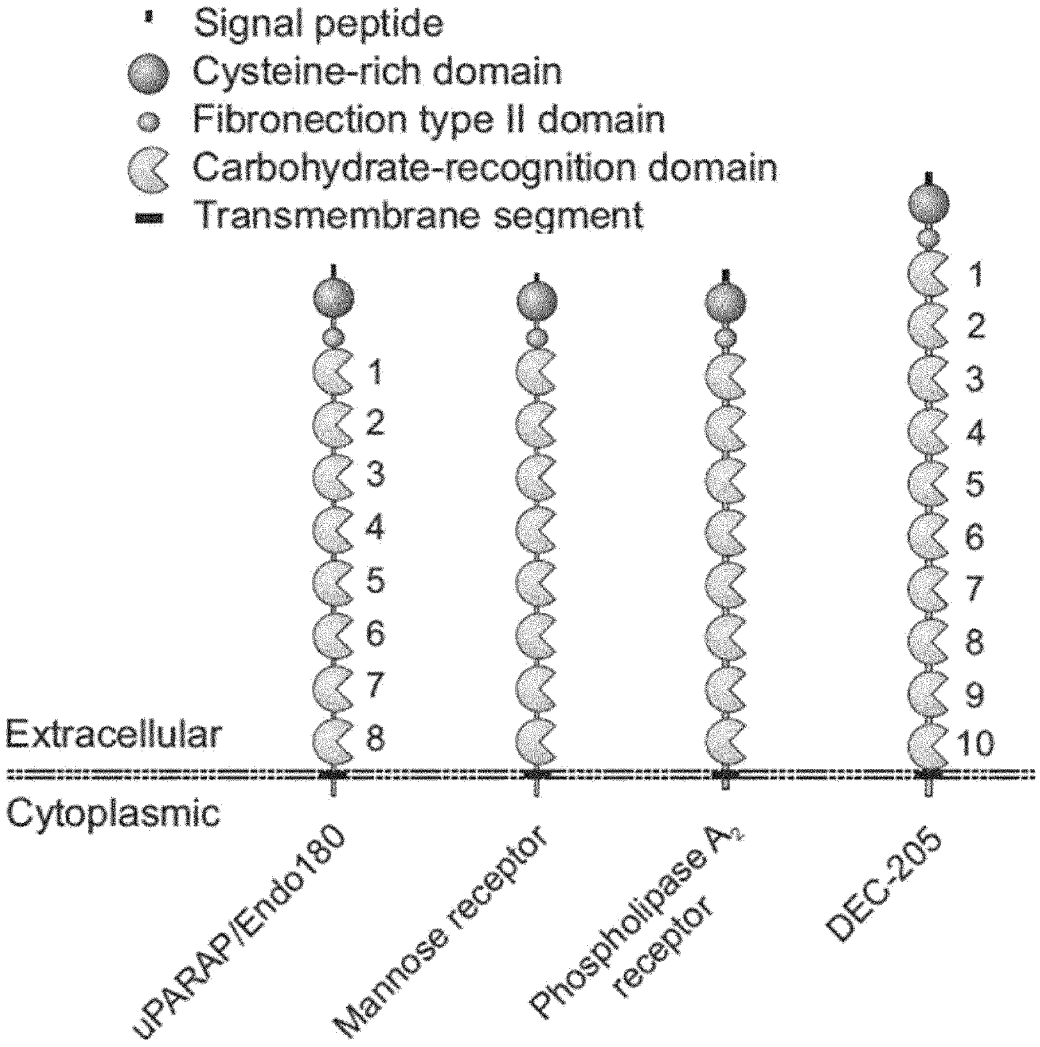

Fig. 1

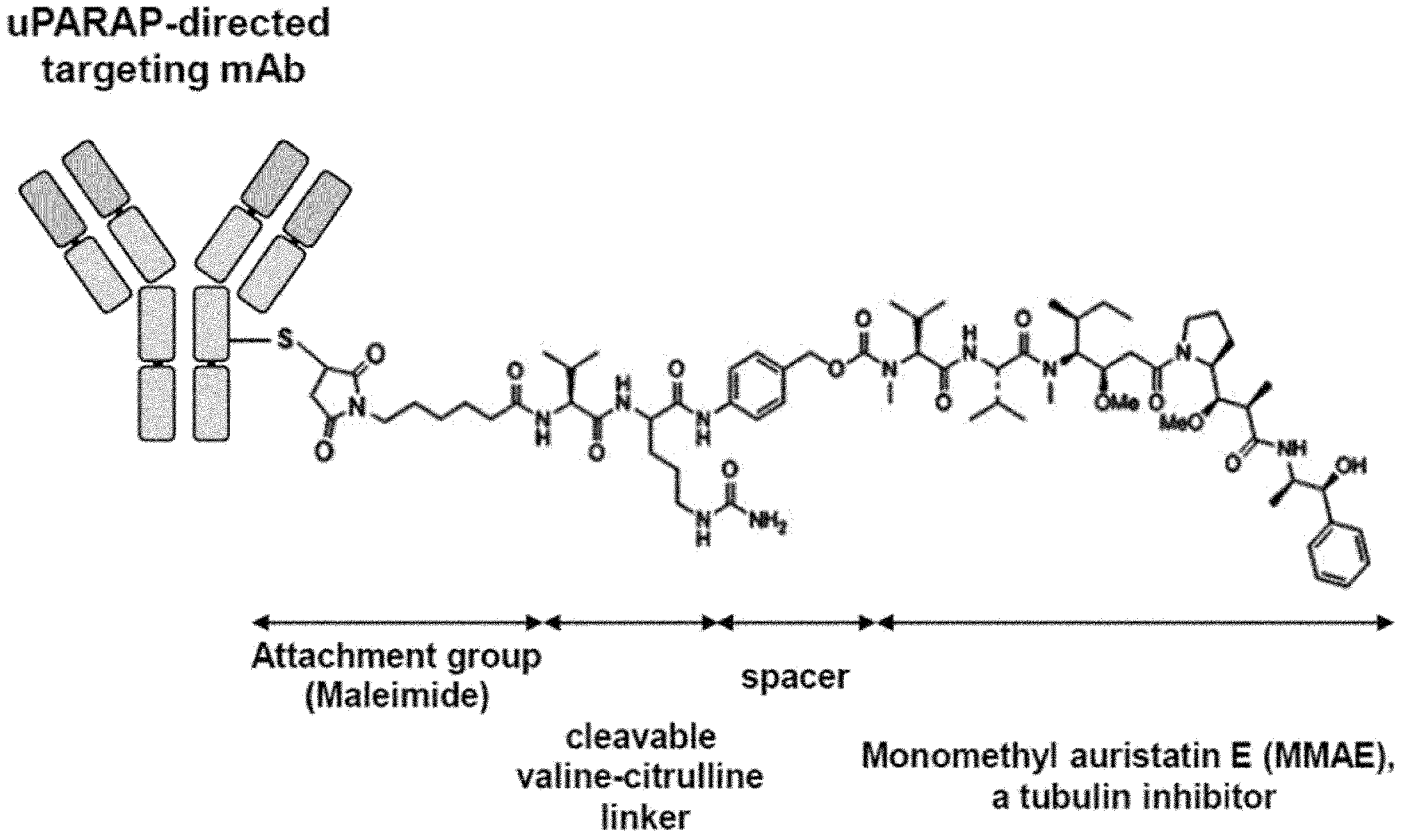

Fig. 2

	2h9-AF647 signal (MFI)	specificity ratio
uPARAP-positive cell lines		
KNS42	203356	35,4
HT 1080	118637	12,2
THP-1	81591	13,9
U937	11262	6,9
uPARAP-negative control cell line		
CHO-K1	2883	1,2

Fig. 3

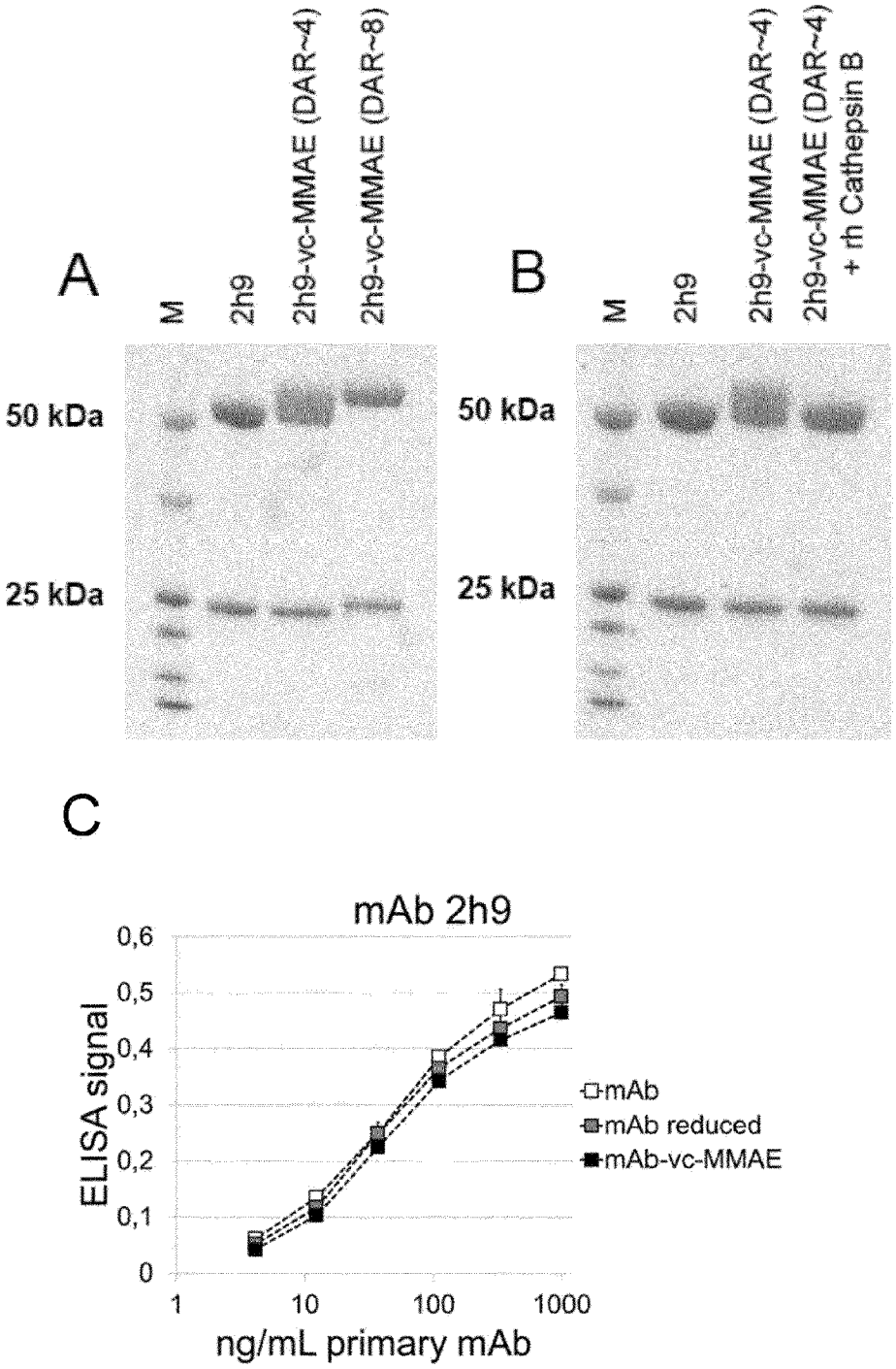
A
M
2h9
2h9-vc-MMAE (DAR~4)
2h9-vc-MMAE (DAR~8)
50 kDa
25 kDa
B
M
2h9
2h9-vc-MMAE (DAR~4)
2h9-vc-MMAE (DAR~4) + rh Cathepsin B
50 kDa
25 kDa
C
mAb 2h9
ELISA signal
0,6
0,5
0,4
0,3
0,2
0,1
0
1
10
100
1000
ng/mL primary mAb
mAb
mAb reduced
mAb-vc-MMAE

Fig. 4

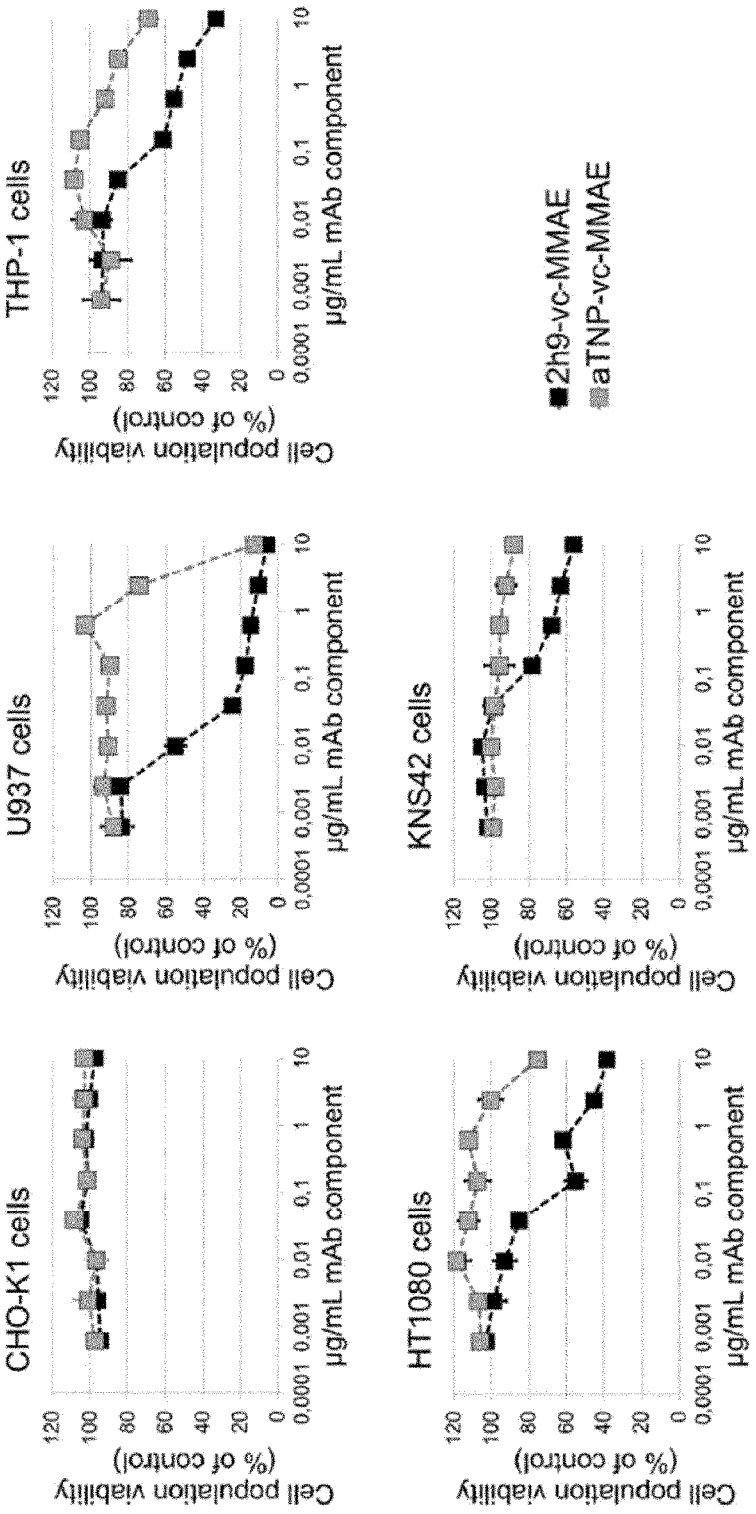
CHO-K1 cells
U937 cells
THP-1 cells
HT1080 cells
KNS42 cells
Cell population viability (% of control)
µg/mL mAb component
0,0001 0,001 0,01 0,1 1 10
0 20 40 60 80 100 120
2h9-vc-MMAE
aTNP-vc-MMAE

Fig. 5

Cell cycle phase distribution (%)

U937 cells

Treatment	Sub-G1	G1	S	G2/M
Control	6,0	62,9	25,6	5,5
2h9-vc-MMAE	-	-	-	-
aTNP-vc-MMAE	5,2	63,8	24,8	6,2
MMAE	-	-	-	-

THP-1 cells

Treatment	Sub-G1	G1	S	G2/M
Control	21,7	51,7	13,2	13,4
2h9-vc-MMAE	32,8	21,4	18,9	26,7
aTNP-vc-MMAE	19,8	49,7	18,1	12,3
MMAE	-	-	-	-

HT1080

Treatment	Sub-G1	G1	S	G2/M
Control	14,4	59,8	15,7	9,3
2h9-vc-MMAE	31,0	38,4	14,3	15,4
aTNP-vc-MMAE	13,5	59,1	13,6	13,1
MMAE	-	-	-	-

KNS42

Treatment	Sub-G1	G1	S	G2/M
Control	4,6	77	11,3	6,8
2h9-vc-MMAE	26,8	19,3	13,2	38,9
aTNP-vc-MMAE	4,1	76	12,2	7,3
MMAE	28,8	15,8	17,1	36,4

Fig. 6

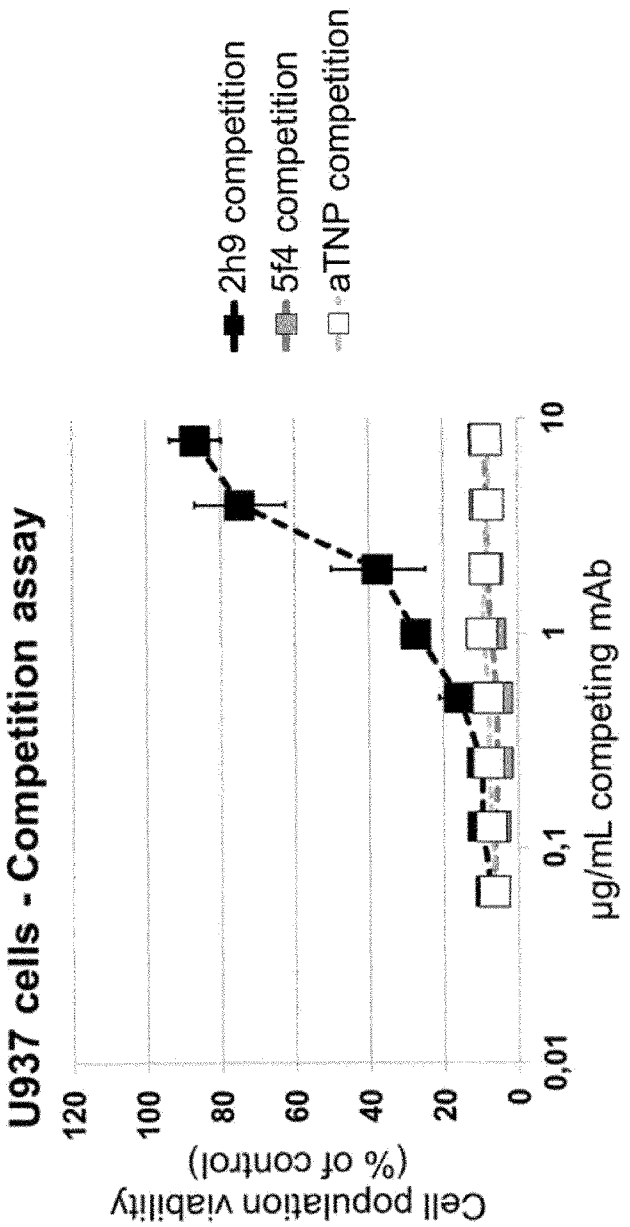
U937 cells - Competition assay
Cell population viability
(% of control)
120
100
80
60
40
20
0
0,01
0,1
1
10
µg/mL competing mAb
2h9 competition
5f4 competition
aTNP competition

Fig. 7

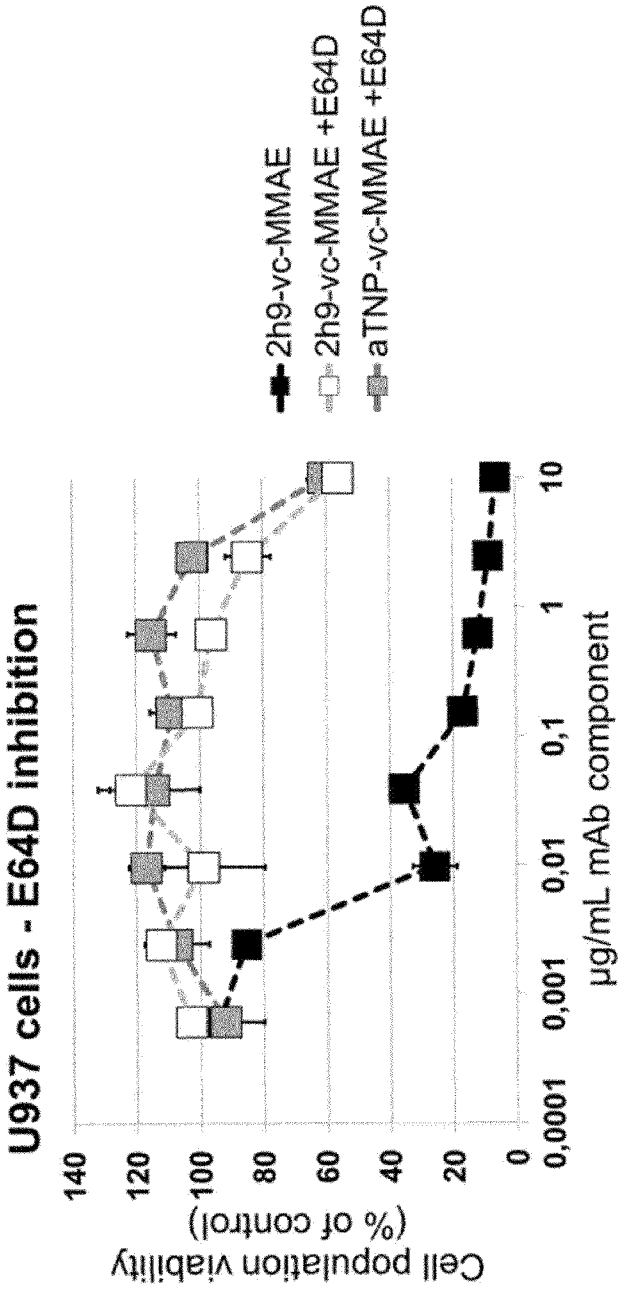
U937 cells - E64D inhibition
2h9-vc-MMAE
2h9-vc-MMAE +E64D
aTNP-vc-MMAE +E64D
Cell population viability
(% of control)
140
120
100
80
60
40
20
0
0,0001
0,001
0,01
0,1
1
10
µg/mL mAb component

Fig. 8

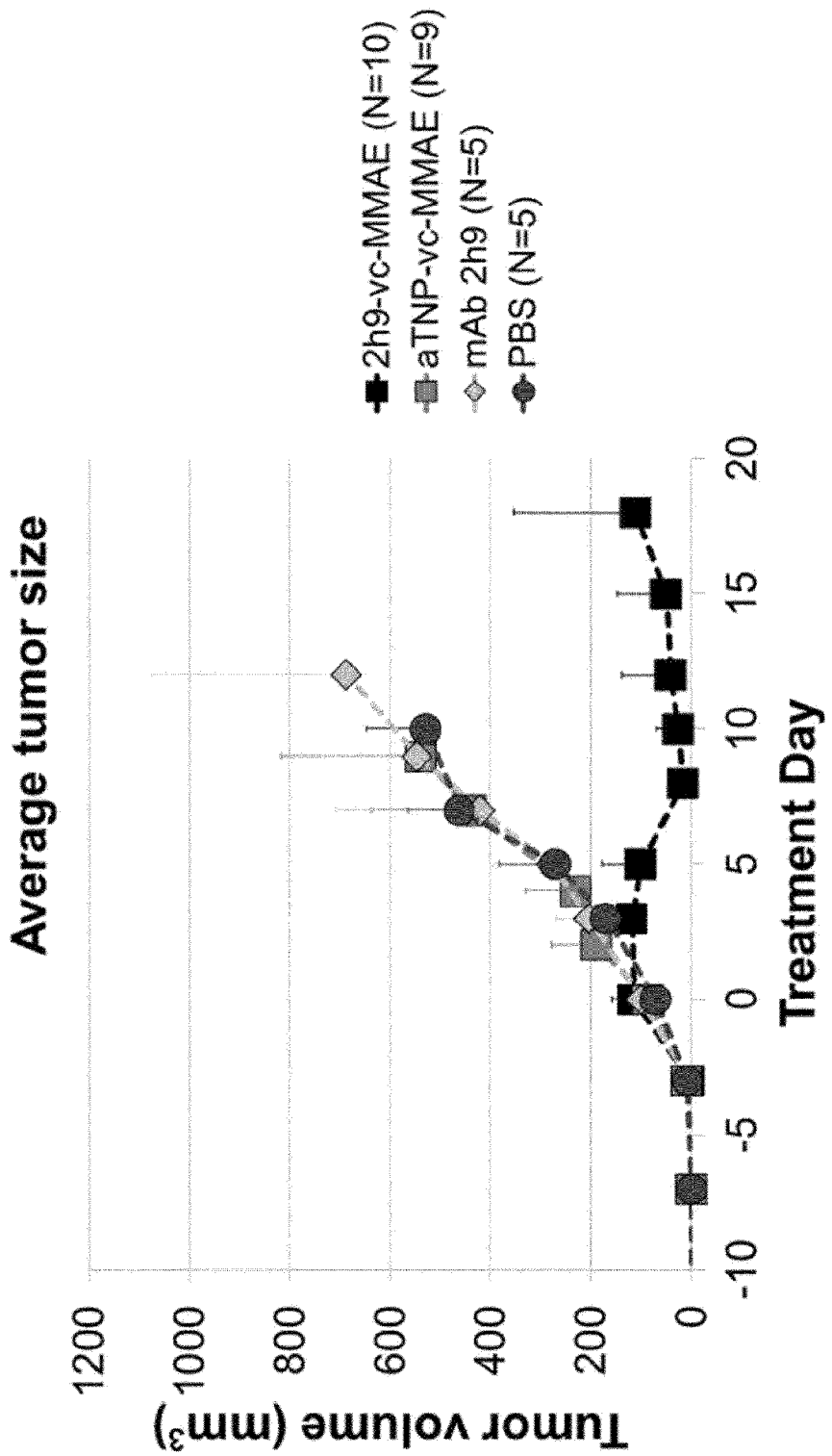
Average tumor size
Tumor volume (mm³)
1200
1000
800
600
400
200
0
-10
-5
0
5
10
15
20
Treatment Day
2h9-vc-MMAE (N=10)
aTNP-vc-MMAE (N=9)
mAb 2h9 (N=5)
PBS (N=5)

Fig. 9

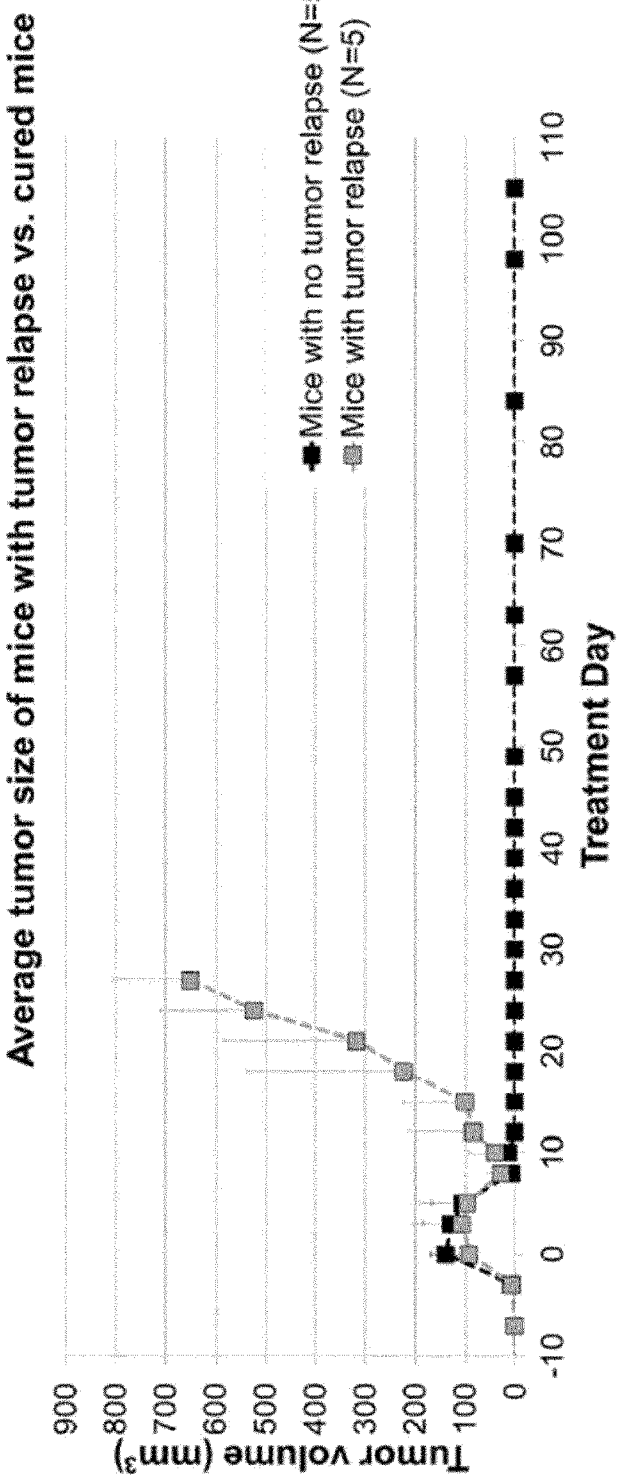
Average tumor size of mice with tumor relapse vs. cured mice
Tumor volume (mm³)
900
800
700
600
500
400
300
200
100
0
-10
0
10
20
30
40
50
60
70
80
90
100
110
Treatment Day
Mice with no tumor relapse (N=5)
Mice with tumor relapse (N=5)

Fig. 10

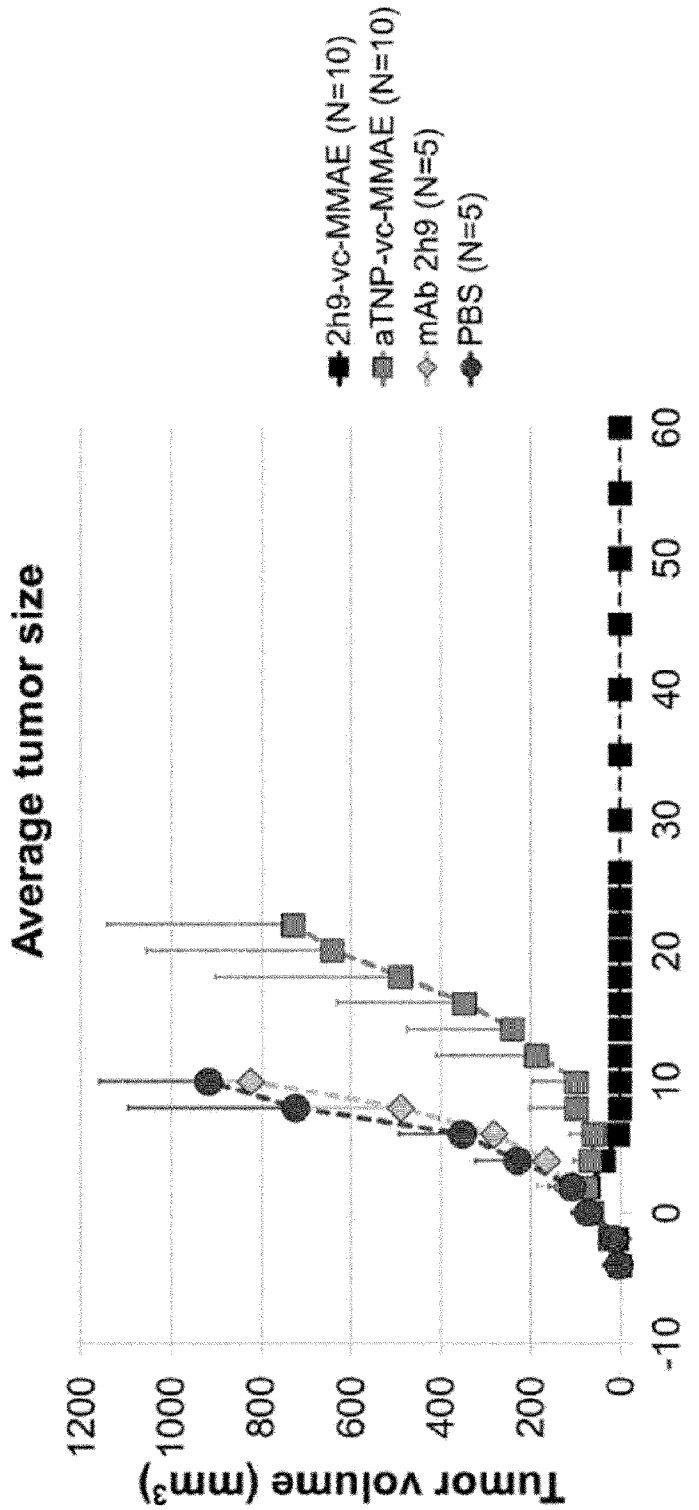
Average tumor size
Tumor volume (mm³)
1200
1000
800
600
400
200
0
-10
0
10
20
30
40
50
60
Treatment Day
2h9-vc-MMAE (N=10)
aTNP-vc-MMAE (N=10)
mAb 2h9 (N=5)
PBS (N=5)

Fig. 11

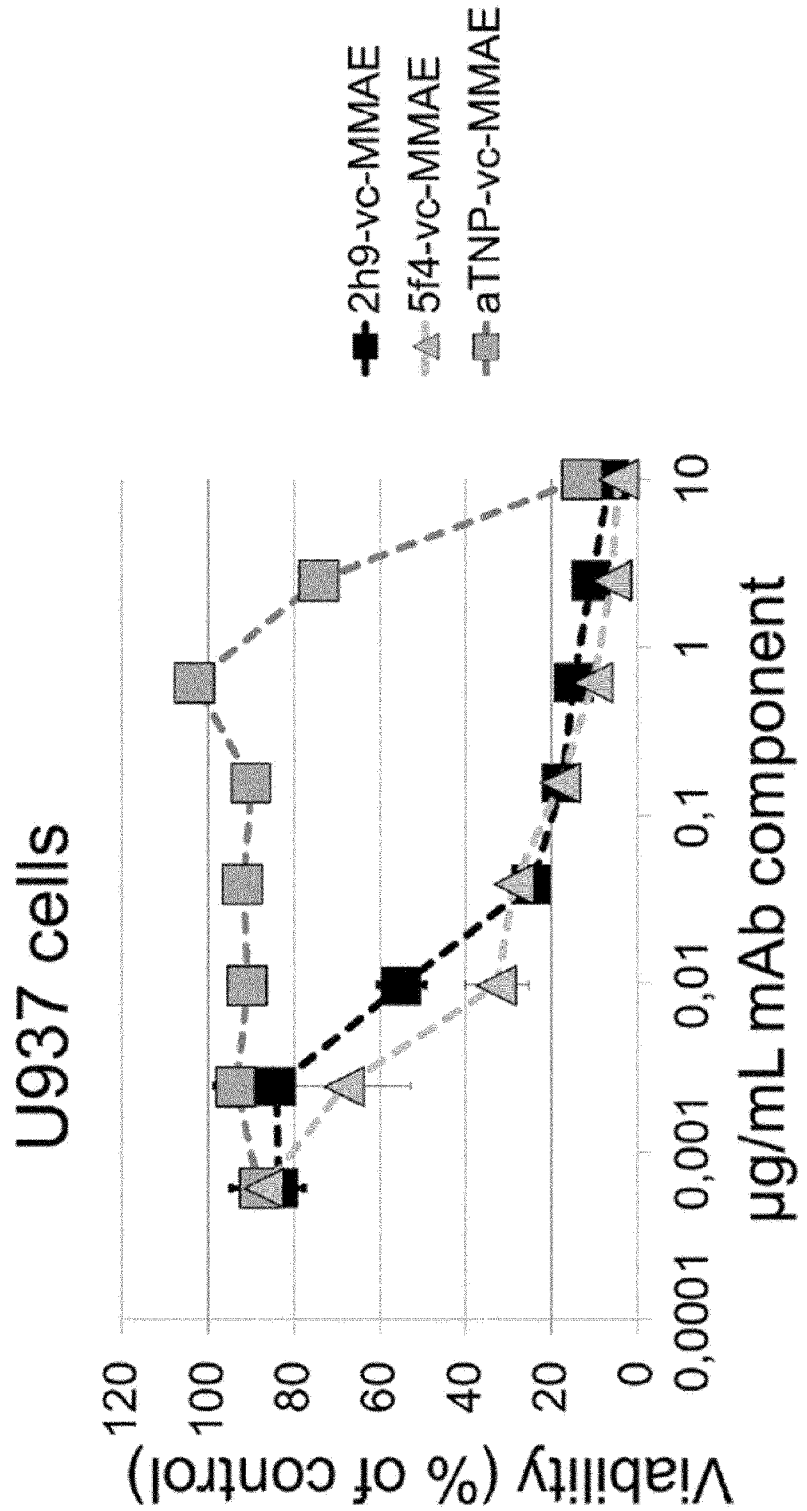
U937 cells
Viability (% of control)
120
100
80
60
40
20
0
0,0001
0,001
0,01
0,1
1
10
µg/mL mAb component
2h9-vc-MMAE
5f4-vc-MMAE
aTNP-vc-MMAE

Fig. 12

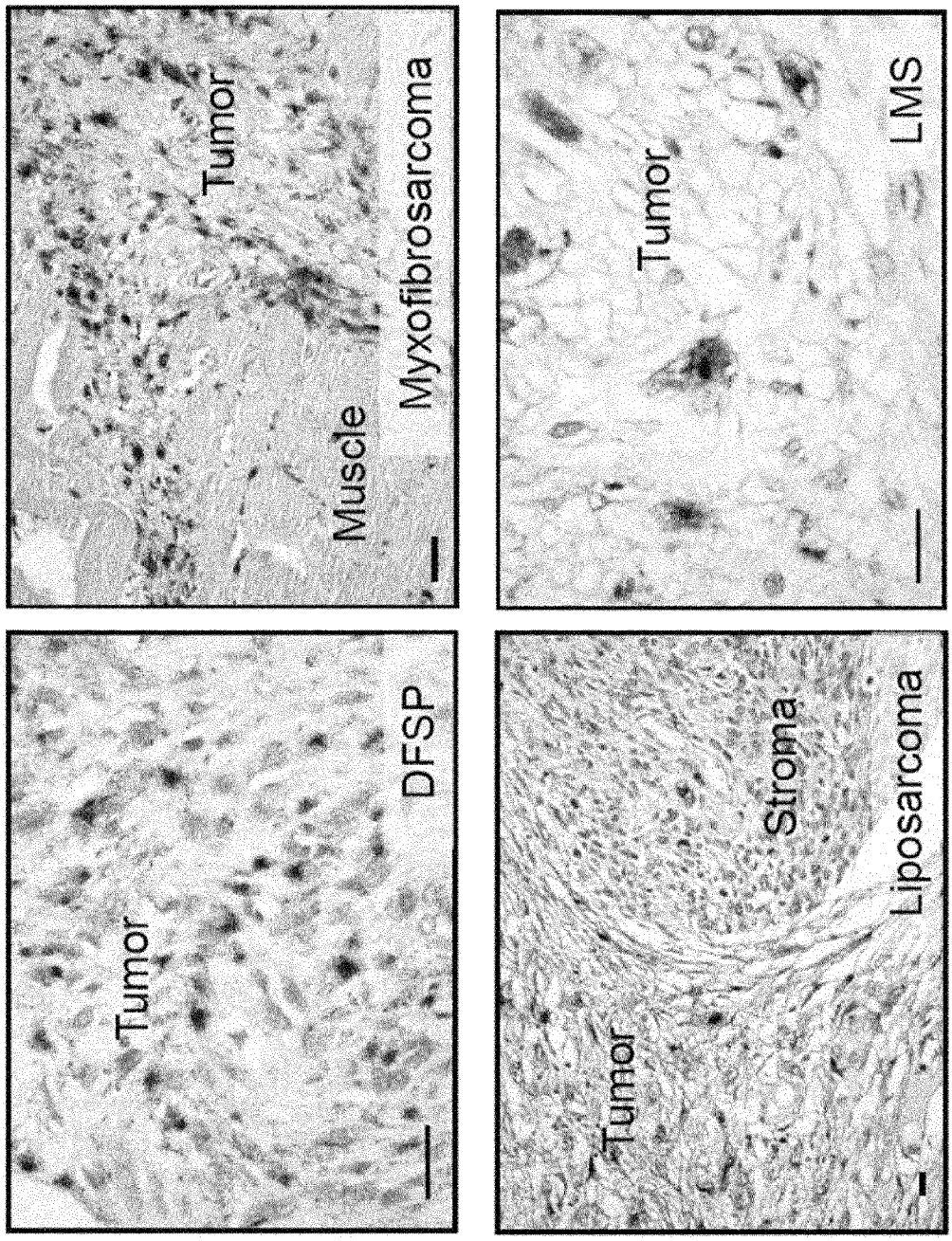
Tumor
DFSP
Tumor
Muscle
Myxofibrosarcoma
Tumor
Stroma
Liposarcoma
Tumor
LMS

Fig. 13

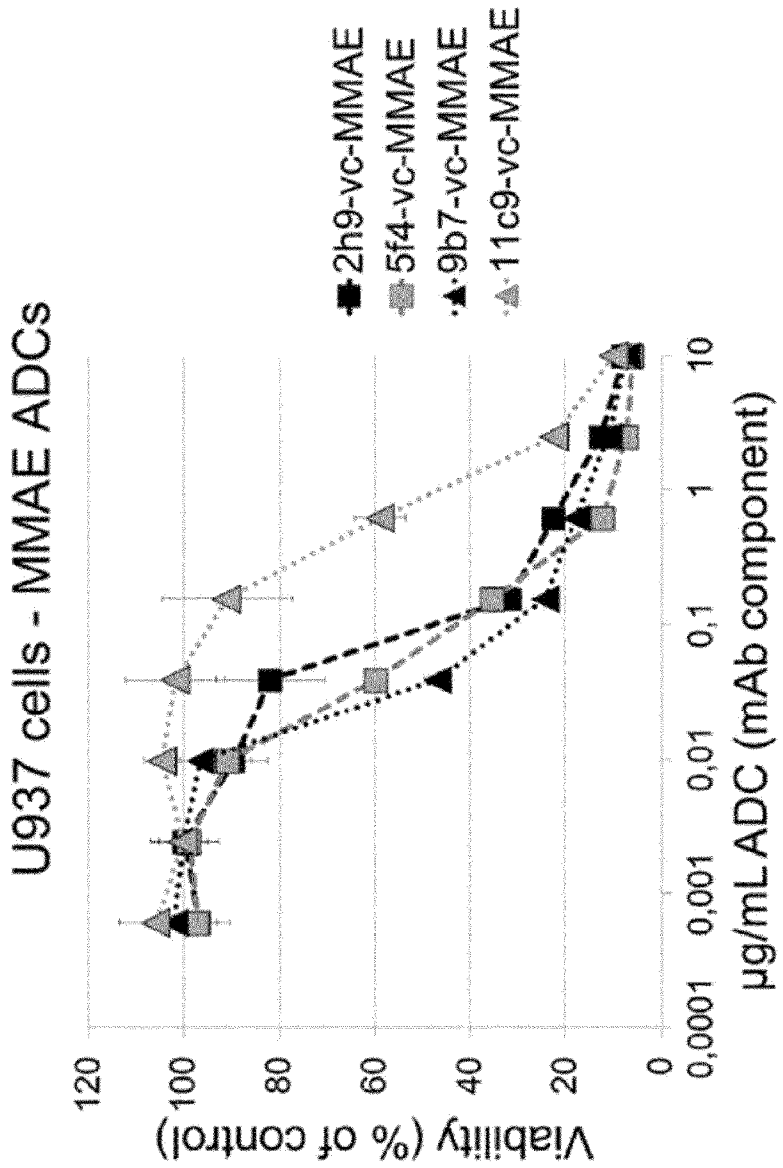
U937 cells - MMAE ADCs
2h9-vc-MMAE
5f4-vc-MMAE
9b7-vc-MMAE
11c9-vc-MMAE
Viability (% of control)
120
100
80
60
40
20
0
0,0001
0,001
0,01
0,1
1
10
µg/mL ADC (mAb component)

Fig. 14

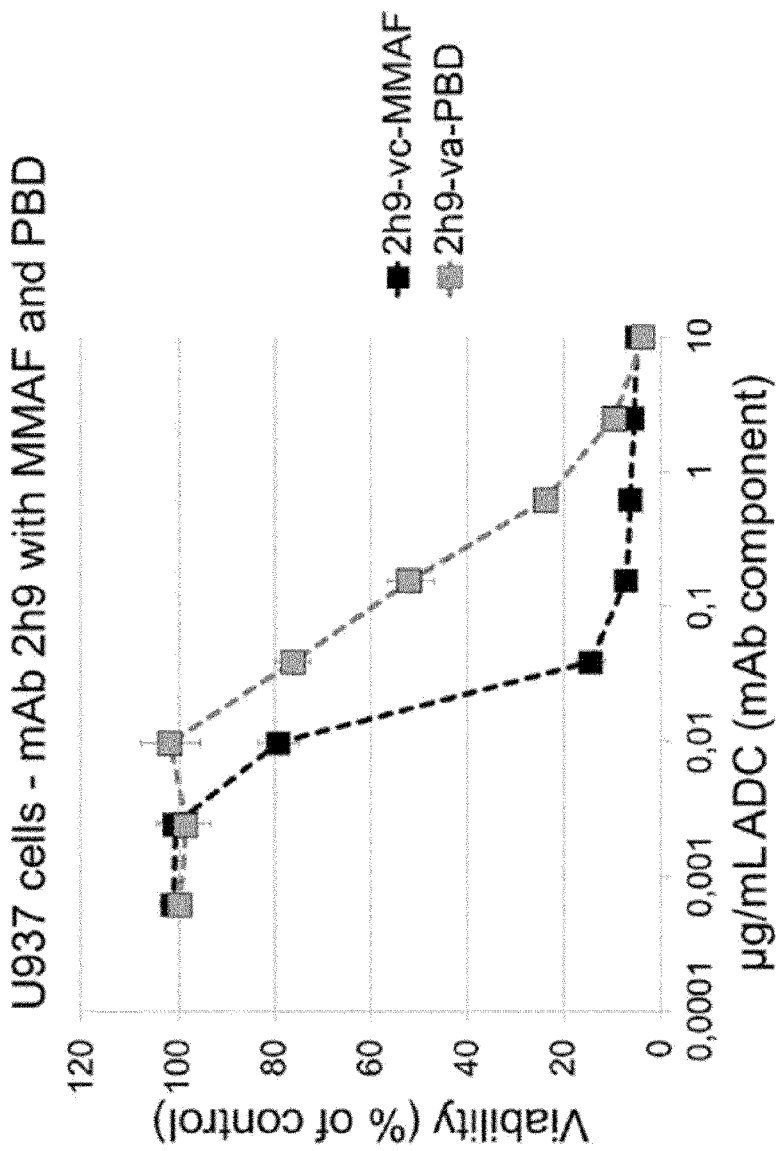
U937 cells - mAb 2h9 with MMAF and PBD
2h9-vc-MMAF
2h9-va-PBD
Viability (% of control)
120
100
80
60
40
20
0
0,0001
0,001
0,01
0,1
1
10
µg/mL ADC (mAb component)

Fig. 15

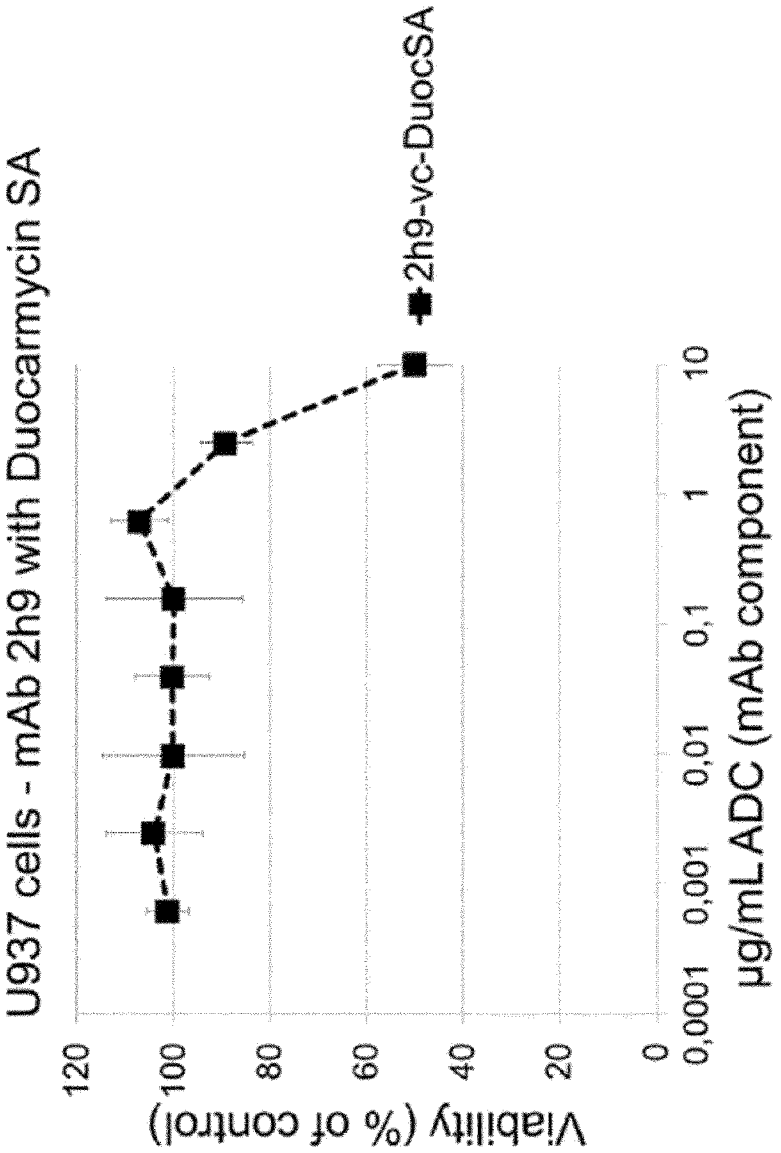
U937 cells - mAb 2h9 with Duocarmycin SA
2h9-vc-DuocSA
Viability (% of control)
120
100
80
60
40
20
0
0,0001
0,001
0,01
0,1
1
10
µg/mL ADC (mAb component)

Fig. 16

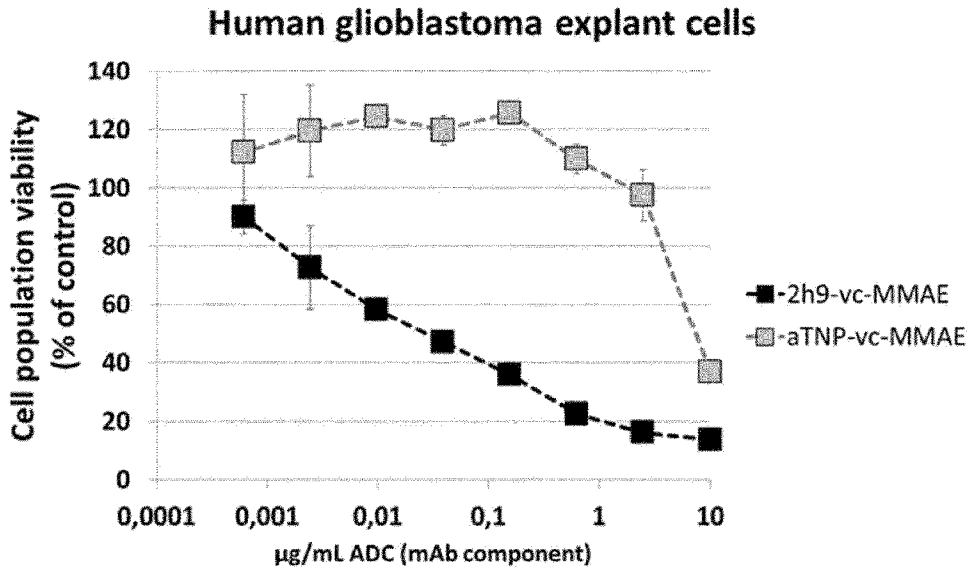

Fig. 17a

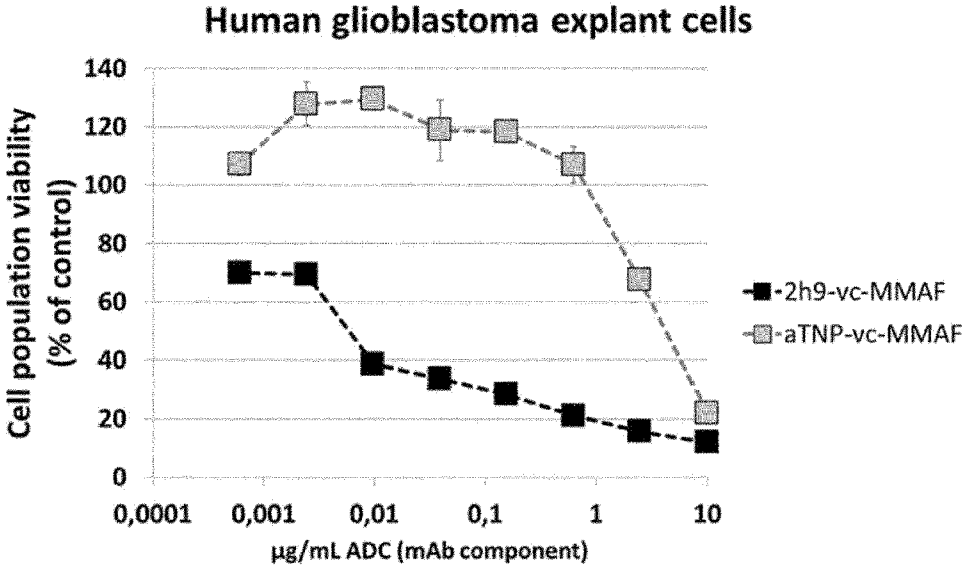

Fig. 17b

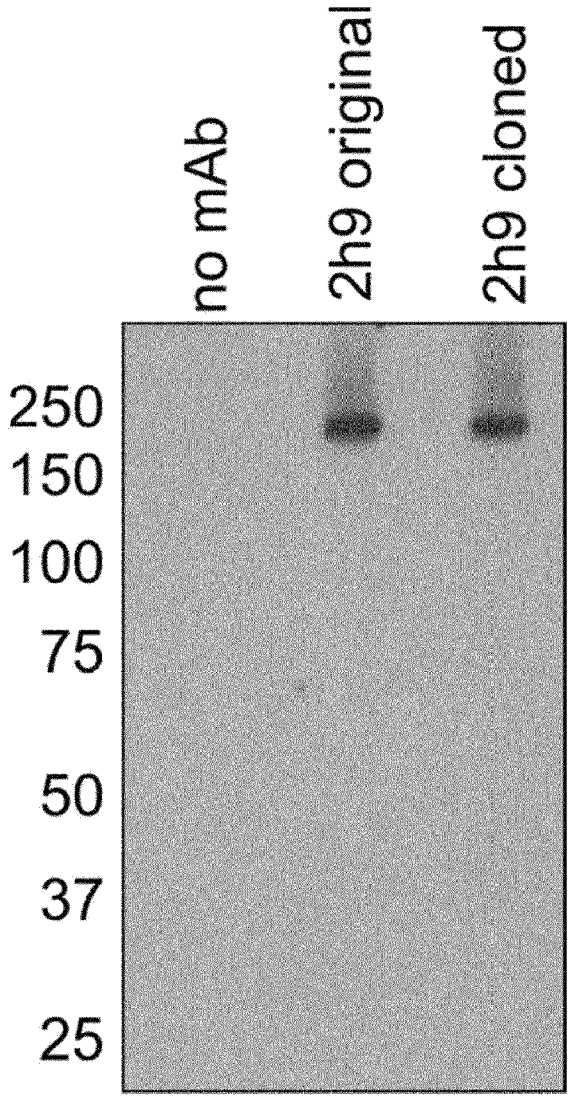

Fig. 18