(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 December 2024 (12.12.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/251839 A1

(51) International Patent Classification:
C07K 16/28 (2006.01) *A61P 35/00* (2006.01)

(21) International Application Number:
PCT/EP2024/065505

(22) International Filing Date:
05 June 2024 (05.06.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
23177313.6 05 June 2023 (05.06.2023) EP

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

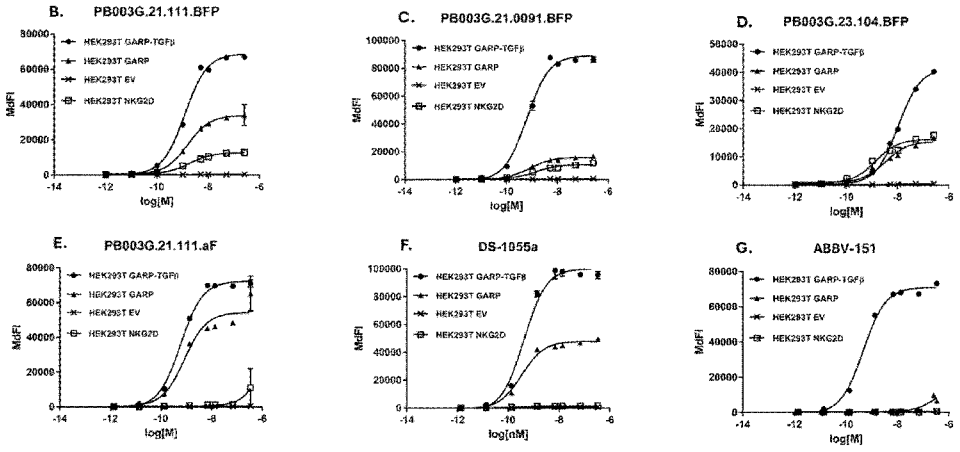
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE,

(54) Title: ANTI-GARP ANTIBODIES AND METHODS OF USE

Fig. 1

A.

	GARP		GARP-TGFb1		NKG2D	
	Molecular studies (KD) [nM]	Cell-based assay (EC50) [nM]	Molecular studies (KD) [nM]	Cell-based assay (EC50) [nM]	Molecular studies (KD) [nM]	Cell-based assay (EC50) [nM]
PB003G.21.0111.aF	9 ± 3	0.85	292 ± 11	0.60	N/A	N/A
PB003G.21.0111.BFP	9 ± 3	1.60	345 ± 7.8	1.24	0.3	1.88
PB003G.21.0091.BFP	N/D*	0.81	256 ± 25	0.64	0.36	1.44
PB003G.23.0104.BFP	N/D*	3.35	1170 ± 132	10.79	0.36	1.41
DS-1055a	6 ± 2	0.34	7.0 ± 0.9	0.42	N/A	N/A
ABBV-151	No binding	No binding	7.0	0.46	N/A	N/A



(57) Abstract: The present invention relates to an antibody capable of binding GARP, or a target-binding fragment or derivative thereof retaining target binding capacities.

[Continued on next page]

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SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

Anti-GARP antibodies and methods of use

Reference to sequence listing submitted as a compliant xml 1.0 format File (.xml)

Pursuant to the EFS-Web legal framework and 37 CFR §§ 1.821-825 (see MPEP § 2442.03(a)), Rule 30 EPC, and § 11 PatV, an electronic sequence listing compliant with WIPO standard ST.26 in the form of an XML 1.0 format file is submitted concurrently with the instant application, and the entire contents of the sequence listing are incorporated herein by reference. For the avoidance of doubt, if discrepancies exist between the sequences mentioned in the specification and the electronic sequence listing, the sequences in the specification shall be deemed to be the correct ones.

Field of the invention

The present invention relates to antibodies and fragments or derivatives thereof that bind to GARP and/or GARP/TGFβ, and methods of using the same.

Incorporation by Reference

All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes. In the event that there are any inconsistencies between the teachings of one or more of the references incorporated herein and the present disclosure, the teachings of the present specification are intended.

Background

The tumor microenvironment (TME) is comprised of cancer cells, stromal tissue (e.g. blood vessels, fibroblasts, immune cells and signalling molecules) and extracellular matrix. The TME is a major determinant for tumor progression, tumor metastasis and response to therapy. An important aspect of the TME that supports tumor progression, tumor metastasis and response to therapy is immunosuppression. Many factors may contribute to immunosuppression in the tumor microenvironment, including loss of ligands on tumor cells required for immune cell triggering, upregulation of ligands on tumor cells that trigger so-called immunosuppressive receptors on immune cells, as well as the presence of subsets of immunosuppressive immune cells, such as regulatory T cells (Treg), that actively suppress immune effector cells in the TME.

Technical Problem

It is an object of the present invention to provide new therapeutic modalities that reduce immunosuppression in the tumor environment, and/or directly stimulate the killing of tumor cells by cytotoxic lymphocytes.

It is an object of the present invention to provide a new and improved therapeutic approach to treat cancer.

These and other problems are solved by the embodiments set forth in the independent claims herein. The dependent claims disclose preferred embodiments.

Brief description of the figures

Figures 1A-1G depict binding to GARP and GARP-TGFβ1 complex, as well as to NKG2D in case of bifunctional fusion proteins (BFPs) comprised of an IgG molecule fused to ULBP2. The table in Fig. 1A summarizes affinities (KD values) measured in molecular binding studies using surface plasmon resonance (SPR), and binding to cells evaluated by measuring EC50 with flow cytometry. All studied compounds showed affinities in a low nanomolar range for GARP, similar to DS-1055a, but in contrast to ABBV-151 which showed no binding to GARP. All compounds showed lower affinity to GARP-TGFβ1 complex, than to GARP, or in comparison

to antibodies having the VH/VL sequences of DS-1055a (Daiichi Sankyo, Satoh et al 2021) or ABBV-151 (AbbVie, Tolcher et al 2022). These antibodies will for convenience, be nicknamed DS-1055a and ABBV-151 herein.

Binding to cell-expressed targets showed as EC50 were in a comparable order of magnitude for most compounds (between studied compounds, and in comparison with DS-1055a and ABBV-151), except for PB003G.23.0104.BFP which showed slightly lower binding affinity. Binding to NKG2D was confirmed for all BFPs (*N/D – not determined due to very low off-rate). Figures 1B-1G show results of cell-based assays using HEK293 expressing GARP, GARP-TGFβb1 or NKG2D. All studied compounds (PB003G.21.0111.aF (1B), PB003G.21.0091.BFP (1C), PB003G.23.0104.BFP (1D), PB003G.21.0111.BFP (1E), and DS1055a (1F)) showed strong, dose-dependent binding to HEK293T cells expressing GARP-TGFβ1 complex, and binding to cells expressing GARP. ABBV-151 showed binding only to GARP-TGFβb1 complex expressing cells (1G). All bifunctional fusion proteins showed binding to NKG2D (1B-1D).

Figures 2A–2C depict comparison of Fc affinities to FcyRIIIA and ADCC potential between native (fucosylated) and afucosylated PB003G.21.0111 IgG1 (PB003G.21.0111.aF). The table in Fig. 2A shows affinities of Fc for the ADCC-inducing receptor FcyRIIIA, which is present in humans in 2 variants (176V and 176F). Afucosylation of PB003G.21.0111 resulted in 12- and 10-fold increase of affinity for FcyRIIIA (176V) and FcyRIIIA (176F), respectively. KD values were measured using surface plasmon resonance (SPR). Increased affinity for FcyRIIIA translates into higher ADDC potential of PB003G.21.0111 IgG1 towards GARP-TGFββ1 expressing target cells. The comparison of ADCC induced by native (fucosylated) and afucosylated PB003G.21.0111 IgG1 (PB003G.21.0111.aF) is shown in Figures 2B and 2C. The afucosylated Fc domain of anti-GARP PB003G.21.0111.aF resulted in significant increase of ADCC induction potential of against GARP-expressing Raji cells (2B), and GARP-TGFβ1-expressing Raji cells (2C) when compared to PB003G.21.0111 IgG1 in its native (fucosylated) form. ADCC assay was performed using NK cells as effector cells; cell lysis was measured using flow cytometry.

Figures 3A-3B depict PB003G.21.0091.BFP and PB003G.21.0111.aF potential to block GARP-TGFb1 complex formation (3A), and to inhibit downstream TGFβ1 signaling (3B). (3A) - Cells preincubated with PB003G.21.0091.BFP or PB003G.21.0111.aF were not able to bind latent TGFβ1, showing that binding of either of the molecules blocks the ability of GARP to

complex TGFβ1. The assay was performed using flow cytometry. (3B) Incubation of cells bearing GARP-TGFβ1 complex with PB003G.21.0091.BFP or PB003G.21.0111.aF inhibits TGFβ1 downstream signaling measured as the level of phosphorylated SMAD in Western blot. This result suggests that both molecules block TGFβ1 maturation and release from the complex with GARP.

Figures 4A-4H depict the potential of the compounds to induce antibody-dependent cellular cytotoxicity (ADCC) against cancer cells and Tregs. ADCC assays were performed using NK cells isolated from healthy donors. Half-effective concentrations (EC50) calculated for induction of ADCC by the compounds against cancer cells are summarized in the table (Figure 4A). Figures 4B-4E depict representative results showing dose-dependent NK-mediated killing of Raji-GARP cells (4B), Raji-GARP-TGFβ1 cells (4C), L428 cells with native expression of GARP-TGFβ1 complex (4D), and Tregs (4E) induced by PB003G.21.0111.aF, PB003G.21.0111.BFP, PB003G.21.0091.BFP, PB003G.23.0104.BFP and control DS1055a. ADCC induction EC50 for PB003G.21.0091.BFP against Tregs was estimated at approximately 1 nM. The analysis was performed using flow cytometry. Figures 4F-4H depict NK cells activation upon treatment with studied PB003G compounds. NK cells activation was monitored using activation biomarkers – proinflammatory cytokines TNFα (4F), INFγ (4G) and the degranulation marker CD107a (4H). All compounds caused statistically significant increase of the activation markers, proving that compounds activate NK cells in the presence of target cells.

Figures 5A-5C depict anti-tumor efficacy of PB003G.21.0111.aF in models bearing human tumors in the humanized mice. CD34+ humanized NSG-IL15 mice with engrafted Raji-GARP-TGFβ1 tumor (5A) or HT-29 tumor (5B). Mice were treated with PB003G.21.0111.aF (10 mg/kg i.v.) for 3 weeks, leading to statistically significant 39% inhibition of tumor growth in comparison to vehicle control in both models. In Raji-GARP-TGFβ1 tumor model the number of CD8+ cytotoxic T cells (CTLs) was estimated in tumor tissue using flow cytometry. It was observed that in PB003G.21.0111.aF-treated mice the frequency of CTLs was increased (5C).

Figures 6A-6B depict safety analysis of PB003G.21.0111.aF treatment in HT-29 tumor model in mice. Safety was assessed by monitoring of mice body weight (5A), and graft vs. host disease (GvHD) symptoms (5B). There were no statistical differences between mice treated with PB003G.21.0111.aF compared to DS-1055a or vehicle control.

Figure 7 depicts analysis of binding to platelets. Platelets are the predominant cell population expressing GARP-TGFβ1 complex under physiological conditions. Therefore, platelets can potentially bind anti-GARP antibodies. Platelets were defined as alive CD41+CD42b+CD45- cells using flow cytometry. Data represents a fold increase of MdFI normalized to MdFI of isotype control. All tested PB003G compounds showed very low level of binding to platelets, while DS-1055a and ABBV-151 showed significantly higher binding. Bars represent mean with SD calculated based on the data from six independent platelets donors. Statistical analysis was done using two-way analysis of variance (ANOVA; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).

Figure 8 depicts schematic structure of bifunctional fusion protein (BFP) that is an anti-cancer target antibody fused within both Fab`s arms with ULBP2, immunoligand for NKG2D receptor. In one embodiment, the anti cancer antibody is an anti GARP antibody. In other embodiments, the antibody has increased ADCC e.g. as being afucosylated, preferably in the IgG1 format.

Figure 9 depicts the comparison of the potential to induce antibody-dependent cellular cytotoxicity (ADCC) against cancer cells (Raji-AVB8) between anti-AVB8 antibody in two formats: IgG1 (ADWA) and bifunctional fusion protein with NKG2D binding domain (ADWA_BFP). BFP format enhanced the ADCC potential of the AVB8-binding antibody. ADCC assays were performed using IL-15 stimulated NK cells isolated from healthy donors.

DETAILED DESCRIPTION OF THE INVENTION

According to a first aspect of the invention, an antibody capable of binding GARP, or a target-binding fragment or derivative thereof retaining target binding capacities, is provided which comprises the heavy chain/light chain variable domain (HCVD/LCVD) pairs set forth in the following:

- SEQ ID NO: 3 and 4,
- SEQ ID NO: 5 and 6, and/or
- SEQ ID NO: 7 and 8.

The antibody having the VH/VL sequences of SEQ ID NOs: 3 and 4 is called PB003G.21.0091, PB003G.21.0091.BFP, or 091.bfp herein.

The antibodies having the VH/VL sequences of SEQ ID NOs: 5 and 6 are called PB003G.21.0111, PB003G.21.0111.aF, PB003G.21.0111.BFP, or 0111.aF herein.

The antibody having the VH/VL sequences of SEQ ID NOs: 7 and 8 is called PB003G.23.0104, PB003G.23.104.BFP herein.

Afucosylated variants of these antibodies carry the tag "aF", while bifunctional variants carry the tag "BFP".

The term "GARP" as used herein, relates to glycoprotein A repetitions predominant. GARP (UniProt identifier: Q14392) is encoded by the *Lrrc32* gene and plays important roles in cell-surface docking and activation of latent TGFβ.

The antibodies according to the invention target the glycoprotein A repetitions predominant (GARP) or GARP-transforming growth factor beta 1 (TGFβ1) complex, which either prevents release of active TGFβ1, or directly kills the tumor cells or regulatory T cells expressing the target, potentially leading to antitumor activity.

In one embodiment, said antibody of fragment or derivative thereof is, or is derived from, a monoclonal antibody.

As used herein, the term "monoclonal antibody (mAb)" shall refer to an antibody composition having a homogenous antibody population, i.e., a homogeneous population consisting of a whole immunoglobulin, or a fragment or derivative thereof retaining target binding capacities. Particularly preferred, such antibody is selected from the group consisting of IgG, IgD, IgE, IgA and/or IgM, or a fragment or derivative thereof retaining target binding capacities.

As used herein, the term "fragment" shall refer to fragments of such antibody retaining target binding capacities, e.g.

- a CDR (complementarity determining region)

- a hypervariable region,
- a variable domain (Fv)
- an IgG or IgM heavy chain (consisting of VH, CH1, hinge, CH2 and CH3 regions)
- an IgG or IgM light chain (consisting of VL and CL regions), and/or
- a Fab and/or $F(ab)_2$.

As used herein, the term "derivative" shall refer to protein constructs being structurally different from, but still having some structural relationship to, the common antibody concept, e.g., scFv, Fab and/or $F(ab)_2$, as well as bi-, tri- or higher specific antibody constructs, and further retaining target binding capacities. All these items are explained below.

Other antibody derivatives known to the skilled person are Diabodies, Camelid Antibodies, Nanobodies, Domain Antibodies, bivalent homodimers with two chains consisting of scFvs, IgAs (two IgG structures joined by a J chain and a secretory component), shark antibodies, antibodies consisting of new world primate framework plus non-new world primate CDR, dimerized constructs comprising CH3+VL+VH, and antibody conjugates (e.g. antibody or fragments or derivatives linked to a toxin, a cytokine, a radioisotope or a label). These types are well described in the literature and can be used by the skilled person on the basis of the present disclosure, without adding further inventive activity.

Methods for the production of a hybridoma cell are disclosed in Köhler & Milstein (1975).

Methods for the production and/or selection of chimeric or humanised mAbs are known in the art. For example, US6331415 by Genentech describes the production of chimeric antibodies, while US6548640 by Medical Research Council describes CDR grafting techniques and US5859205 by Celltech describes the production of humanised antibodies.

Methods for the production and/or selection of fully human mAbs are known in the art. These can involve the use of a transgenic animal which is immunized with the respective protein or peptide, or the use of a suitable display technique, like yeast display, phage display, B-cell display or ribosome display, where antibodies from a library are screened against human iRhom2 in a stationary phase.

In vitro antibody libraries are, among others, disclosed in US6300064 by MorphoSys and US6248516 by MRC/Scripps/Stratagene. Phage Display techniques are for example disclosed in US5223409 by Dyax. Transgenic mammal platforms are for example described in EP1480515A2 by TaconicArtemis.

IgG, IgM, scFv, Fab and/or $F(ab)_2$ are antibody formats well known to the skilled person. Related enabling techniques are available from the respective textbooks.

As used herein, the term "Fab" relates to an IgG/IgM fragment comprising the antigen binding region, said fragment being composed of one constant and one variable domain from each heavy and light chain of the antibody

As used herein, the term "$F(ab)_2$" relates to an IgG/IgM fragment consisting of two Fab fragments connected to one another by disulfide bonds.

As used herein, the term "scFv" relates to a single-chain variable fragment being a fusion of the variable regions of the heavy and light chains of immunoglobulins, linked together with a short linker, usually serine (S) or glycine (G). This chimeric molecule retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of a linker peptide.

According to one embodiment, the antibody or fragment or derivative thereof has an enhanced potential to induce ADCC, relative to a naturally occurring antibody or fragment or derivative.

The main strategy to enhance the capacity of an IgG to induce ADCC is to alter the Fc portion of the antibody to increase binding affinity to the activating FcγRIIIA via site-directed mutagenesis, changing Fc domain glycosylation, and/or preventing Fc domain fucosylation. Creation of IgG variants with improved binding to activating FcγR by mutagenesis has been an effective strategy for increasing ADCC efficiency of IgG antibodies (Shields et al 2000, Tang et al 2007, Zahavi et al 2018, the contents of all of which are incorporated herein by reference for enablement purposes). In addition to modification of Fc residues, asymmetrical engineering of the Fc portion to create heterodimers of different heavy chains yielded more stable antibodies with enhanced ADCC functionality (Liu et al 2013, the content of which is incorporated herein by reference for enablement purposes).

According to one embodiment, the antibody or fragment or derivative thereof comprises an afucosylated Fc domain.

Afucosylated antibodies are monoclonal antibodies engineered such that the oligosaccharides in the Fc region of the antibody do not have any fucose sugar units. When antibodies are in afucosylated IgG format, their capacity to induce antibody-dependent cellular cytotoxicity (ADCC) is increased.

Technically, afucosylation can be accomplished by either

- inhibiting addition of fucose to the already existing sugar chain, e.g., by overexpressing th enzyme GnTIII (see e.g. Davies et al 2001), or by knocking out FUT8 (Fucosyltransferase 8, Yamane-Ohnuki et al 2004)*)*
- removing fucoses that has already been added to the existing sugar chain chemically or enzymatically,
- redirecting fucose synthesis by a heterologous enzyme that depletes the fucose pool inside the cell (GlyMaxx technology by ProBioGen, see also Chung et al, 2012), and/or
- using an expression system that does not add fucose residues into the sugar chains, like e.g. a Ciliate base expression system (see e.g., EP2542575A1).

In one embodiment, the antibodies according to the invention were produced by expression in FUT8-KO CHO (Chinese hamster ovary) cell line containing a knock-out of the FUT8 gene. The antibodies thus produced lack fucose, and are, as such, afucosylated. In the examples shown herein, the afucosylated antibodies according to the invention were produced in that way. It should be noted, however, that similar effects are to be expected for antibodies according to the present invention afucosylated by other approaches, as e.g. described above.

A published anti-GARP antibody is ABBV-151 (Tolcher et al 2022) manufactured by AbbVie. Another commercially available anti-GARP antibody is DS-1055a (Satoh et al 2021) manufactured by Daiichi Sankyo.

The inventors have yet shown herein that the antibodies according to the invention have an improved functional profile relative to DS-1055 and ABBV-151, in particular regarding effector functions (e.g. ADCC) and/or the blocking of GARP-TGFβ1 complex formation and TGF β1 downstream signalling.

Table 1: Summary of some characteristics of the antibody according to the invention and prior art

Feature	PB003G.21.0111.aF	PB003G.21.0091_BFP	DS-1055	ABBV-151
Binds naked GARP	Yes	Yes	Yes	No
Binds complex of GARP-TGFβ1	Yes	Yes	Yes	Yes
Blocks GARP-latent TGFβ1 complex formation	Yes	Yes	No	No
Inhibits TGFβ1 maturation	Yes	Yes	No	Yes
ADCC on cancer cells	Yes	Yes	Yes	No
ADCC on Tregs	Yes	yes	Yes	No
Off-target binding to platelets	very weak	very weak	Yes	Yes

As such, the respective bifunctional molecules have the same improved functional profile relative to DS-1055 and ABBV-151, in particular regarding effector functions/ADCC and/or the blocking of GARP-TGFβ1 complex formation.

According to another aspect of the invention, a bifunctional molecule is provided comprising

- a binding domain that is capable of binding a cancer antigen, and
- a binding domain that is capable of binding NKG2D

The term "NKG2D" (UniProt identifier: P26718) as used herein relates to an activating receptor (transmembrane protein) belonging to the NKG2 family of C-type lectin-like receptors. NKG2D is encoded by *KLRK1* (killer cell lectin like receptor K1) gene which is located in the

NK-gene complex (NKC). In humans, it is expressed by NK cells, γδ T cells and CD8+ αβ T cells.

Generally, the activating receptor NKG2D is peculiar in its capability to bind to numerous and highly diversified MHC class I-like self-molecules. These ligands are poorly expressed on normal cells but can be induced on damaged, transformed or infected cells, with the final NKG2D ligand expression resulting from multiple levels of regulation. Although redundant molecular mechanisms can converge in the regulation of all NKG2D ligands, different stimuli can induce specific cellular responses, leading to the expression of one or few ligands. A large body of evidence demonstrates that NK cell activation can be triggered by different NKG2D ligands, often expressed on the same cell, suggesting a functional redundancy of these molecules. However, since a number of evasion mechanisms can reduce membrane expression of these molecules both on virus-infected and tumor cells, the co-expression of different ligands and/or the presence of allelic forms of the same ligand guarantee NKG2D activation in various stressful conditions and cell contexts. Noteworthy, NKG2D ligands can differ in their ability to down-modulate NKG2D membrane expression in human NK cells supporting the idea that NKG2D transduces different signals upon binding various ligands. Moreover, whether proteolytically shed and exosome-associated soluble NKG2D ligands share with their membrane-bound counterparts the same ability to induce NKG2D-mediated signalling is still a matter of debate (Zingoni et al, 2018).

Using a bifunctional molecule which comprise a binding domain that is capable of binding NKG2D provides the option to engage and activate respective immune cells, as to attack cancer cells which are bound by the respective other binder of the bifunctional molecule. As such, bifunctional molecules which comprise a binding domain that is capable of binding NKG2D offer advantages to the well- established bispecific T cell engagers ("biTEs") which comprise an anti-CD3 binder that engages T cells only, and only those that express CD3.

According to embodiments thereof,

a) the binding domain that is capable of binding a cancer antigen is an antibody or target-binding fragment or derivative thereof, and/or
b) the binding domain that is capable of binding NKG2D is a ligand of NKG2D, optionally ULBP2 or an active fragment thereof.

The term "ULBP2" (UniProt identifier: P26718) as used herein relates to UL16 binding protein 2, which is a GPI-anchored cell surface glycoprotein encoded by the *ULBP2* gene located on the chromosome 6. ULBP2 is related to MHC class I molecules, but its gene maps outside the MHC locus. The domain structure of ULBP2 differs significantly from those of conventional MHC class I molecules. It does not contain the α3 domain and the transmembrane segment. ULBP2 is thus composed of only the α1α2 domain which is linked to the cell membrane by the GPI anchor.

Glycosylphosphatidylinositol (GPI) in short, is a phosphoglyceride that can be attached to the C-terminus of a protein during posttranslational modification. The resulting GPI-anchored proteins play key roles in a wide variety of biological processes. GPI is composed of a phosphatidylinositol group linked through a carbohydrate-containing linker (glucosamine and mannose glycosidically bound to the inositol residue) and via an ethanolamine phosphate (EtNP) bridge to the C-terminal amino acid of a mature protein. he extracellular fragment of ULBP2 devoid of the GPI anchor.

The term "a cancer antigen", as used herein, refers to any molecule (e.g., protein, polypeptide, peptide, lipid, carbohydrate, etc.) solely or predominantly expressed or overexpressed on the surface of a tumor cell or cancer cell, or cell in the tumor microenvironment, such that the antigen is associated with the tumor or cancer. The cancer antigen can additionally be expressed by normal, non-tumor, or non-cancerous cells. However, in such cases, the expression of the cancer antigen by normal, non-tumor, or non-cancerous cells is not as robust as the expression by tumor or cancer cells. In this regard, the tumor or cancer cells can overexpress the antigen or express the antigen at a significantly higher level, as compared to the expression of the antigen by normal, non-tumor, or non-cancerous cells. Also, the cancer antigen can additionally be expressed by cells of a different state of development or maturation. For instance, the cancer antigen can be additionally expressed by cells of the embryonic or fetal stage, which cells are not normally found in an adult host. Alternatively, the cancer antigen can be additionally expressed by stem cells or precursor cells, which cells are not normally found in an adult host.

According to embodiments, the binding domain that is capable of binding a cancer antigen binds to at least one target selected from the group shown in the following table:

Table 2: Examples of cancer antigens that can be used in the bifunctional molecule as disclosed herein

cancer antigen	cancer indication (example)	Molecule (example)
CD19	Precursor B-cell acute lymphoblastic leukemia	blinatumomab
CD20	Relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma	ibritumomab
	Chronic lymphocytic leukemia	obinutuzumab
	Chronic lymphocytic leukemia	ocrelizumab
	B-cell non-Hodgkin's lymphoma	ofatumumab
	Follicular lymphoma	rituximab
CD22	Precursor B-cell acute lymphoblastic leukemia	inotuzumab
CD30	Hodgkin lymphoma	brentuximab
CD33	Acute myeloid leukemia	gemtuzumab
CD52	B-cell chronic lymphocytic leukemia	alemtuzumab
CTLA-4	Metastatic melanoma	ipilimumab
EGFR	Metastatic colorectal carcinoma	cetuximab
	Metastatic squamous non-small cell lung carcinoma	necitumumab
	Metastatic colorectal cancer	panitumumab
HER2	Metastatic breast cancer	ado-trastuzumab
	Metastatic breast cancer	pertuzumab
	Metastatic breast cancer	trastuzumab
	HER2-overexpressing breast cancer, metastatic gastric or gastroesophageal junction adenocarcinoma	
PD-1	Metastatic squamous non-small cell lung carcinoma	nivolumab
	Metastatic melanoma	pembrolizumab
	Metastatic melanoma	
PDGFRA	Soft tissue sarcoma	olaratumab
PD-L1	Urothelial carcinoma	atezolizumab
	Urothelial carcinoma	durvalumab
	Metastatic Merkel cell carcinoma	avelumab
	Urothelial carcinoma	
SLAMF7	Multiple myeloma	elotuzumab
VEGF	Metastatic colorectal cancer	bevacizumab
VEGFR2	Gastric cancer	ramucirumab
AV8B (integrin)	squamous cell carcinoma, mammary cancer, colon cancer, and prostate cancer	ADWA-11
GARP		DS-1055, ABBV-151, this application

Such bifunctional molecule can also be equipped with an enhanced potential to induce ADCC, relative to a naturally occurring antibody or fragment or derivative, as discussed elsewhere herein. For that purpose, in particular the Fc domain of the antibody that binds to a cancer antigen can be modified accordingly. ADCC and immune cell engagement via NKG2D binding complement each other synergistically in their cancer cell-killing effector functions.

According to embodiments,

- ULBP2 comprises, or consists of, the amino acid sequence according to SEQ ID NO: 1, and/or
- the binding domain that is capable of binding a cancer antigen is the anti-GARP antibody or fragment or derivative according to the above description.

Such anti-GARP antibody or fragment or derivative according to the above description comprises the heavy chain/light chain variable domain (HCVD/LCVD) pairs set forth in the following:

- SEQ ID NO: 3 and 4,
- SEQ ID NO: 5 and 6, and/or
- SEQ ID NO: 7 and 8.

An anti-AVB8 antibody or fragment or derivative according to the above description comprises the heavy chain/light chain variable domain (HCVD/LCVD) pairs according to SEQ ID NO: 9 and 10.

In one embodiment, ULBP2 comprises, or consists of, the amino acid sequence according to SEQ ID NO: 1. In one embodiment, the active fragment of ULBP2 comprises, or consists of, the amino acid sequence according to SEQ ID NO: 2.

In one embodiment of the bifunctional molecule according to the above description, the binding domain that is capable of binding NKG2D is fused, directly or via flexible linker to the N-terminus of the VH or VL domain of the antibody capable of binding GARP is, or the target-binding fragment or derivative thereof.

Such linker has, for example, following amino acid sequence: $(GGGGS)_4$ (SEQ ID NO: 19)

In one embodiment, the binding domain that is capable of binding NKG2D is fused to the N-terminus of the VL domain of the antibody capable of binding GARP, or the target-binding fragment or derivative thereof.

In one embodiment of the antibody or target-binding fragment or derivative thereof, or the bifunctional molecule, the binding domain that is capable of binding GARP is in the format of an IgG antibody.

In one embodiment, the bifunctional molecule comprises a pair of (i) an anti-GARP antibody heavy chain and (ii) an active fragment of ULBP2 fused to the N-terminus of an anti-GARP antibody light chain, which pair is selected from the group consisting of

- SEQ ID NO: 13 and 14,
- SEQ ID NO: 15 and 16,, and/or
- SEQ ID NO: 17 and 18.

In one embodiment, the bifunctional molecule comprises a pair of (i) an anti-AVB8 antibody heavy chain and (ii) an active fragment of ULBP2 fused to the N-terminus of an anti- AVB8 antibody light chain as set firth in SEQ ID NO: 11 and 12.

According to another aspect of the invention, a target binding molecule is provided that

(i) competes for binding to GARP with the antibody or target-binding fragment or derivative thereof, or the bifunctional molecule, according to the above description,

(ii) or binds to the same epitope of GARP as the antibody or target-binding fragment or derivative thereof, or the bifunctional molecule, according to the above description,

In one embodiment, said target binding molecule is an antibody or target-binding fragment or derivative thereof, or a bifunctional molecule, as defined elsewhere herein.

As used herein, the term "competes for binding" is used in reference to target binding molecule with an activity which binds to the same substrate as does the antibody or target-binding fragment or derivative thereof, or the bifunctional. The efficiency (e.g., kinetics or thermodynamics) of binding the target molecule may be the same as or greater than or less than the efficiency substrate binding by the antibody or target-binding fragment or derivative thereof, or the bifunctional molecule. For example, the equilibrium binding constant (Kj) for binding to the substrate may be different. The term "K_m" as used herein refers to the Michaelis-Menton

constant for an enzyme and is defined as the concentration of the specific substrate at which a given enzyme yields one-half its maximum velocity in an enzyme catalysed reaction

As used herein, the term "binds to the same epitope" with reference to two or more binding molecules means that the molecules bind to the same segment of amino acid residues, as determined by a given method. Techniques for determining whether an antibody binds to the same epitope as another antibody include, for example, epitope mapping methods, such as, x-ray analyses of crystals of antigen: antibody complexes which provides atomic resolution of the epitope and hydrogen/deuterium exchange mass spectrometry (HDX-MS). Other methods monitor the binding to antigen fragments or mutated variations of the antigen where loss of binding due to a modification of an amino acid residue within the antigen sequence is often considered an indication of an epitope component. In addition, computational combinatorial methods for epitope mapping can also be used. These methods rely on the ability of the antibody of interest to affinity isolate specific short peptides from combinatorial phage display peptide libraries.

According to another aspect of the invention, the use of the antibody or target-binding fragment or derivative thereof, or the bifunctional molecule, or the target binding molecule according to the above description (for the manufacture of a medicament) is provided in the treatment of a human or animal subject

- being diagnosed for,
- suffering from or
- being at risk of developing

a neoplastic disease, or for the prevention of such condition.

This language is deemed to encompass both the swiss type claim language accepted in some countries (in this case, brackets are deemed absent) and EPC2000 language (in this case, brackets and content within the brackets is deemed absent).

According to another aspect of the invention, a pharmaceutical composition comprising the antibody or target-binding fragment or derivative thereof, or the bifunctional molecule,

according to the above description, and optionally one or more pharmaceutically acceptable excipients, is provided.

According to another aspect of the invention, a combination comprising (i) the antibody or target-binding fragment or derivative thereof, or the bifunctional molecule, according to the above description, and (ii) one or more therapeutically active compounds, is provided.

According to another aspect of the invention, a method for treating or preventing a neoplastic disease is provided, which method comprises administration, to a human or animal subject, of the antibody or target-binding fragment or derivative thereof, or the bifunctional molecule, or the target binding molecule, or the pharmaceutical composition, or the combination according to the above description, in a therapeutically sufficient dose is provided.

Examples

While the invention has been illustrated and described in detail in the drawings and foregoing description, such illustration and description are to be considered illustrative or exemplary and not restrictive; the invention is not limited to the disclosed embodiments. Other variations to the disclosed embodiments can be understood and effected by those skilled in the art in practicing the claimed invention, from a study of the drawings, the disclosure, and the appended claims. In the claims, the word "comprising" does not exclude other elements or steps, and the indefinite article "a" or "an" does not exclude a plurality. The mere fact that certain measures are recited in mutually different dependent claims does not indicate that a combination of these measures cannot be used to advantage. Any reference signs in the claims should not be construed as limiting the scope.

All amino acid sequences disclosed herein are shown from N-terminus to C-terminus.

Materials & Methods

Affinities of antibodies to GARP, GARP-TGFβ1, and NKG2D using surface plasmon resonance (SPR)

The affinities of antibodies to GARP and GARP-TGFβ1 were determined using surface plasmon resonance in a BIACORE 8K instrument (Cytiva). Anti human IgG (Fc) antibody was immobilized on a CM5 chip by direct amine coupling according to the supplier's instruction to capture selected binders in BFP and afucosylated IgG1 formats. Human GARP or GARP-TGFβ1 complex were injected as analytes over the chip surface in serial dilutions. Sensor surfaces were regenerated after each binding cycle with 10 mM glycine-HCl, pH 2.1. Generated data with double reference subtraction (reference cell subtracted and blank subtracted) were analyzed using BIA evaluation software (Cytiva). Association (kon) and dissociation (koff) rate constants were evaluated from global fitting based on a 1:1 Langmuir binding model and the equilibrium dissociation constant was calculated from the equation: KD = koff / kon.

The affinities of antibodies to NKG2D were determined using surface plasmon resonance in a BIACORE 8K instrument (Cytiva). Anti human IgG (Fc) antibody was immobilized on a CM5 chip by direct amine coupling according to the supplier's instruction to capture selected binders in BFP format. Serial dilution (50 – 0.78 nM) of human NKG2D/His (Sino Biological) was injected as analyte. Sensor surfaces were regenerated after each binding cycle with 10 mM glycine-HCl, pH 2.1. Generated data was analyzed as described above.

Binding to cells using flow cytometry

For cell-binding studies HEK239T cells were used transfected with GARP, GARP-TGFβ1, NKG2D or empty vector (EV) as a control. Barcoding was performed to analyze two or more cell lines in one sample in flow cytometry. Different cell lines were barcoded with fluorescent labels using CellTrace™ system (Invitrogen). Following barcoding antibodies stainings were performed. For anti-GARP staining human LAP (TGF-beta 1) Alexa Fluor® 647-conjugated antibody (R&D Systems) and human LRRC32/GARP Alexa Fluor® 647-conjugated antibody (R&D Systems) were used (incubated for 30 min on ice). For staining with tested antibodies serial dilutions were prepared for final concentrations of 40, 20, 10, 5, 0.5, 0.05, 0.005 and 0.0005 µg/mL. Cells were incubated for 30 min on ice. For secondary antibody staining cells were washed and incubated with Allophycocyanin (APC) AffiniPure Goat Anti-Human IgG, Fcγ fragment specific (Jackson ImmunoResearch) antibody (0.4 µL per sample) for 30 min on ice. Following incubation cells were washed with DPBS + 0.5% FBS, resuspend in 200 µL DPBS containing SYTOX Blue (Invitrogen) diluted 1:1000 for live/dead staining and stored on ice until analysis. Samples were analyzed on Attune Cytometer equipped with CytKick autosampler Flow Cytometer (Thermo Scientific, USA), and Attune™ Automatic Software

v.5.1.1. 20000 events were captured in control samples and 20000 events were captured for tested samples in "cells of interest" gate after discarding Sytox Blue positive dead cells and doublets. EC50 values were calculated using nonlinear regression (curve fit) function and the log(agonist) vs. response – 3 parameters equation.

Affinity evaluation of antibodies for FcγRIIIA using surface plasmon resonance (SPR)

The affinities of a PB003G.21.0111 and PB003G.21.0111.aF for FcyRIIIA (176V and 176F) were determined using surface plasmon resonance in a BIACORE 8K instrument (Cytiva). Recombinant, biotinylated FcγRIIIA/His-Avi (176V and 176F) were injected over the SA chip (Cytiva) surface. Serial dilutions of antibodies were injected in single-cycle kinetics mode. The sensor surface was regenerated after each binding cycle with 10 mM glycine-HCl, pH 3.0 (Cytiva). Generated data with double reference subtraction (reference cell subtracted and blank subtracted) were analyzed using Biacore Insight Evaluation Software v4.0 (Cytiva). Association (kon) and dissociation (koff) rate constants were evaluated based on a 1:1 Langmuir binding model and the equilibrium dissociation constant was calculated from the equation: KD = koff / kon.

ADCC assay (NK-mediated killing)

Effector cell isolation

NK cells were isolated from peripheral blood mononuclear cells (PBMCs) obtained from healthy donor buffy coats. PBMC were first isolated by density centrifugation with SepMate™-50 PBMC Isolation Tubes (Stemcell™). The layer of PBMCs was transferred to a fresh 50 mL conical tube and incubated with Red Blood Cell Lysis Buffer (Biolegend, 420302) at RT for 10 minutes. Tubes were filled up with DPBS and centrifuged (350g, 5 min). After washing PBMCs were subjected to NK cell isolation by immunomagnetic negative selection with NK Cell Isolation Kit (Miltenyi Biotec, 130-092-657) according to manufacturer's protocol. Isolation purity and NK cells phenotyping cells was performed by flow cytometry (Cytek Northern Lights (NL-00020)). Viability of NK cells were evaluated with LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit (Invitrogen™, L34966, 1:1000). Following markers were tested with antibodies: CD3 (APC-H7 Mouse Anti-Human CD3, BD Biosciences, 560176), CD16 (Alexa Fluor® 647 Mouse Anti-Human CD16, BD Biosciences, 557710), CD56 (Brilliant Violet 605TM anti-human CD56 (NCAM), BioLegend®, 362538), CD69 (BV711 Mouse Anti-Human CD69, BD Biosciences, 563836), CD107a (PE Mouse Anti-Human CD107a, BD

Pharmingen™, 555801), NKG2D (BV421 Mouse Anti-Human CD314 (NKG2D), BD Biosciences, 743558) according to manufacturer's protocol.

In experiments using Raji-AVB8 cells, NK cells were incubated overnight with an addition of IL15 in culture medium (5 ng/ml).

Measurements of ADCC towards tumor cells

Target cells (Raji-GARP and Raji-GARP-TGFβ1, Raji-AVB8, or L428) were stained with CFSE for separation from effector cells on flow cytometry and their viability was assessed with LIVE/DEAD™ Fixable Violet Dead Cell Stain Kit. PB003G.21.0111 and PB003G.21.0111.aF were added to cells at the serial dilution. NK cells isolated on the previous day were mixed with target cells in tha ratio of 10:1 (effector to target). Cells were incubated for 4 hours, following by cell lysis detection with Live/Dead™ Fixable Violet Dead Cell Stain Kit solution (1:1000 in DPBS, Invitrogen™, L34964) and incubated at 4°C for 20 min. Cells were fixed with 4% paraformaldehyde solution (Thermo Scientific™, J19943.K2) for 10 min at 4°C. Following fixation, cell pellets were suspended in FACS buffer and subjected for flow cytometry analysis. Percentage of target cells killing vs. antilog concentrations were plotted. The data are presented as means with standard deviation (error bars). For statistical analysis, the ordinary one-way ANOVA with Dunnett's multiple comparisons test was applied.

Measurements of ADCC towards Tregs

Treg cells were isolated from PBMCs from the same donor as NK cells by immunomagnetic two-step procedure with human CD4+CD25+ Regulatory T Cell Isolation Kit (Miltenyi Biotec, 130-091-301) according to the manufacturer's protocol. Tregs cells were seeded in a density 200,000 cells/well on a 96-well plate, mixed with Dynabeads™ Human T-Activator CD3/CD28 for T Cell Expansion and Activation (Gibco, 11161D) in ratio 1:1 and cultured for two days in IMDM (Gibco™, 31980030). Isolation purity and phenotyping of Tregs was performed by flow cytometry (Cytek Northern Lights (NL-00020)). Viability of Tregs cells was checked with LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit (Invitrogen™, L34966, 1:1000). Following markers were tested with antibodies: CD4 (PerCP/Cyanine5.5 anti-human CD4 Antibody, Biolegend, 300529), CD25 (BV605 Mouse Anti-Human CD25, BD Horizon, 562660), CD45 (Brilliant Violet 785™ anti-human CD45 Antibody, BioLegend®, 304048), CD127 (BV650 Mouse Anti-Human CD127, BD Horizon, 563225), GARP (PE/Cy7 anti-human GARP, BioLegend, 352508), FoxP3 (Alexa Fluor® 647 Mouse anti-Human FoxP3, BD Pharmingen, 560045) according to the manufacturer's protocol.

Dynabeads™ were separated from cells with a magnet (Miltenyi Biotec) and discarded. The cells were labeled with 0.1 µM of CellTrace™ CFSE Proliferation Kit (Invitrogen™, C34554). Target cells (10,000 cells per well) were seeded into conical bottom 96-well plate, and incubated with serial dilutions of studied antibodies for 30 min at 37°C, 5% CO2 for pre-coating. NK cells isolated on the previous day were added to final ratio of effector cells to target cells 10:1.After overnight incubation, LIVE/DEAD™ Fixable Violet Dead Cell Stain Kit (1:1000 in DPBS, Invitrogen™, L34964) was used to estimate cell lysis level. Cells were incubated with anti-GARP antibody (BD Pharmingen, 562341). Cells were fixed with 4% paraformaldehyde (Thermo Scientific™, J19943.K2) for 10 min at 4°C. Following fixation, 100 µL of FACS buffer was used for flow cytometry analysis on Cytek Northern Lights (NL-00020) flow cytometer. Relative percentage of cell lysis was calculated by subtracting target viability control from experimental lysis values. Data was presented as relative percentage lysis against indicated antibody concentration (nM) on a log10 scale. Graphs presenting dose-response curves were fitted by 4-parameters nonlinear regression equation. Data were analyzed using FlowJo™ v10.8.1 Software (BD Life Sciences). Percentage of target cells killing vs. antilog concentrations were plotted. The data is presented as a mean with standard deviation (error bars) from at least two technical replicates. Due to donor-dependent variation between biological repetitions there is no statistical analysis.

Degranulation assay

Target cells were preincubated for 30 min with tested molecules at 50 nM. Then PBMCs were added to the E:T ratio 1:1. BD GolgiStop™, BD GolgiPlug™ and anti-CD107a antibody were added to culture for 4 h. Analysis was done using flow cytometry as described below.

Surface marker and intracytoplasmic cytokine staining

Degranulating and cytokine secreting NK cells were analyzed by multi-parametric surface markers and intracellular cytokine staining. After the coincubation of PBMCs with target cells, all cells were washed with and stained with viability marker, anti-CD56 (Clone 5.1H11) anti-CD16 (Clone 3G8) anti-CD3 (Clone SK7) for 25 min at 37°C. Samples were fixed and permeabilized according to manufacturer's directions (Fixation/Permeabilization Kit, BD). After washing, cells were stained with intracellular anti-IFN-γ (Clone B27) and anti-TNFα antibodies (Clone MAb11) for an additional 60 min. Data was acquired on a Cytek Flow Cytometer and analysis was done using FlowJo X v.10.0. Results are presented as mean values

of percentages and standard deviation. Differences in the means were calculated using a two-way ANOVA test. Significance is indicated as follows: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001, ****P ≤ 0.0001.

Blockage of GARP-TGFβ1 complex formation assay

Raji-GARP cells were incubated with tested antibodies and controls in serial dilutions (final concentrations 0.2 ng/mL to 50 µg/mL), and latent TGFβ1 (15 µg/mL) in full RPMI medium. After incubation (37 °C, 5% CO2, 60 min), After washing cells were stained for 30 minutes at room temperature with anti-LAP-AF647 antibody for latent TGFβ1 detection on cells. Samples were acquired (20.000 events) using Attune NxT Flow Cytometer equipped with CytKick Max autosampler. Data analysis was performed with Attune Cytometric Software ver 4.3.002.1. MdFI vs. log concentrations were plotted. Non-linear curve fitting (inhibition vs log agonist, three parameters) was applied.

Detection of SMAD2 phosphorylation by Western blot for TGFβ1 downstream signaling evaluation

Jurkat and Jurkat β8-GARP cells (1×10^6 cells/well) were stimulated with anti-CD3 (OCT3 clone, BioLegend, final concentration 1 µg/mL) and soluble anti-CD28 (BD Biosciences, final concentration 1 µg/mL). Stimulations were done simultaneously to incubation with tested antibodies and controls: PB003G.21.0091.BFP, PB003G.21.0111.aF, DS-1055, ABBV-151, and IgG1 isotype control. Cell lysates were collected after 24 h and prepared for Western blot. 4-20% Mini-Protean TGX Gel (BioRad) was used for SDS-PAGE electrophoresis. After transfer, the membrane incubated overnight with anti-pSMAD2 antibody (1:1000 dilution in 5% BSA/TBST). Secondary antibody solution (anti-rabbit IgG-HRP, R&D Systems, diluted 1:1000 in TBST/5% BSA) was applied for 1 hour in RT. Bands were detected using Immobilon Western Chemiluminescent HRP Substrate and ChemiDoc MP (BioRad). After image acquisition membranes were washed and incubated with anti-GAPDH antibody for loading control. For densitometric analysis, ImageLAb v. 6.0.1 build 34 was used.

In vivo efficacy using Raji-GARP-TGFβ1 tumor model

The anti-tumor efficacy, safety, and immune-activation by PB003G.21.0111.aF was evaluated in Raji-GARP-TGFβ1 tumor-bearing CD34+ hematopoietic stem cell (HSC) humanized NSG-hIL15 mice at Jackson Laboratories (Bar Harbor, USA). Raji-GARP-TGFβ1 cells ($2x10^6$) were

inoculated subcutaneously into the right flank/shoulder of CD34+ HSC humanized NSG-hIL15 mice. When the average tumor volume reached around 150-180- mm^3, the mice were randomized according to HSC donor, and tumor volume. The mice were treated with intravenous injections of vehicle, PB003G.21.0111.aF , or DS-1055 at 10 mg/kg body weight (bw), biweekly for 6 doses. The tumor volume and mice weights were measured 2-3 times a week. 2-way ANOVA with Bonferroni's multiple comparison was used for statistical analysis.

***In vivo* efficacy using HT-29 tumor model**

The anti-tumor efficacy of PB003G.21.0111.aF was evaluated in HT29 colon carcinoma tumor-bearing CD34+ hematopoietic stem cell (HSC) humanized NCG-hIL15 mice at GemPharmatech (Nanjing, China). HT29 cells ($5x10^6$) were inoculated subcutaneously into the right flank/shoulder of CD34+ HSC humanized NCG-hIL15 mice. When the average tumor volume reached around 80 mm3, the mice were randomized according to HSC donor, humanization level, and tumor volume. The mice were treated with intravenous injections of vehicle, PB003G.21.0111.aF , or DS-1055 at 10 mg/kg body weight (bw), biweekly for 6 doses. The tumor volume and mice weights were measured 2-3 times a week.

Binding to platelets *in vitro* using flow cytometry

Platelets were isolated from the buffy coats obtained from healthy donors by density centrifugation. PBMC were first isolated by density centrifugation with SepMate™-50 PBMC Isolation Tubes (Stemcell™). The layer of PBMCs was transferred to a fresh 50 mL conical tube and incubated with Red Blood Cell Lysis Buffer (Biolegend, 420302) at RT for 10 minutes. Tubes were filled up with DPBS and centrifuged (350g, 5 min). After washing and resuspension PBMCs were centrifuged (350 g, 5 min), and supernatant was kept as a platelet-rich fraction. Platelet-rich fraction was then centrifuged at 3200 g, 5 min. and resuspended in Tyrode's solution.

5×10^6 cells from 200 µL platelet-rich fraction in Tyrode's solution was incubated with biotinylated compounds (10 µg/mL) and stained with viability marker (LIVE/Dead™ Fixable Violet - LIVE/Dead™ 405 nm Stain , ThermoFisher Scientific, Cat# L34955), anti-CD41 (BioLegend, Clone HIP8, Cat# 303725), anti-CD42b (BioLegend, Clone HIP1, Cat# 303920), anti-CD45 (BioLegend, Clone HI30, Cat# 563716) for 20 min at 37°C. After washing, cells were centrifuged at 3000g, 5 min. and then stained with FITC Streptavidin (BioLegend, Cat# 405202) for an additional 15 min. After washing cells were centrifuged at 3000 g 5 min and

resuspended in final of 100 µL. Data was acquired on a Cytek Flow Cytometer and analysis was done using FlowJo X v.10.0. Results are presented as mean values and standard deviation of fold change of FITC MdFI.

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Sequences

The following sequences form part of the disclosure of the present application. A WIPO ST26 compatible electronic sequence listing is provided with this application, too. For the avoidance of doubt, if discrepancies exist between the sequences in the following table and the electronic sequence listing, the sequences in this table shall be deemed to be the correct ones.

In some cases, the signal peptides may be encompassed in the reproduced sequences. In such case, the sequences shall be deemed disclosed with and without signal peptides. A readily available tool to identify signal peptides in a given protein sequence is SignalP - 6.0 provided by Dansk Technical University under https://services.healthtech.dtu.dk/service.php?SignalP. Optionally, where applicable, the signal peptide is underlined. The same applies for sequences that comprise a His tag, which shall be deemed disclosed with and without His tag.

Table 3: Sequences

SEQ	name	type	AA sequence
1	ULBP2 full length	ULBP2 full length with signal peptide	MAAAAATKILLCLPLLLLLSGWSRAGRADPHSLCYDITVIPKFRPGPRWCAVQGQVDEKT FLHYDCGNKTVTPVSPLGKKLNVTTAWKAQNPVLREVVDILTEQLRDIQLENYTPKEPLT LQARMSCEQKAEGHSSGSWQFSFDGQIFLLFDSEKRMWTTVHPGARKMKEKWENDKVVAM SFHYFSMGDCIGWLEDFLMGMDSTLEPSAGAPLAMSSGTTQLRATATTLILCCLLIILPC FILPGI
2	ULBP2 active fragment	ULBP2 active fragment	GRADPHSLCYDITVIPKFRPGPRWCAVQGQVDEKTFLHYDCGNKTVTPVSPLGKKLNVTT AWKAQNPVLREVVDILTEQLRDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDG QIFLLFDSEKRMWTTVHPGARKMKEKWENDKVVAMSFHYFSMGDCIGWLEDFLMGMDSTL EPSAGAPLAMS
3	PB003.21.0091 (optionally with	VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSIYAISWVRQAPGQGLEWMGGIIPLNYAQKF QGRVTITADESTSTAYMELSSLRSEDTAVYYCAWGSGWDNPFDYWGQGTLVTVSS
4		VL	DIQMTQSPSSLSASVGDRVTITCRASQTISGYLNWYQQKPGKAPKLLIYAASSLQSGVPS RFSGSGSGTDFTLTISSLQPEDFATYYCLQTVSTPYTFGQGTKVEIK

	afucosylated Fc domain)		
5	PB003.21. 0111 (optionally with afucosylated Fc domain)	VH	EVQLLESGGGLVQPGGSLRLSCAASGFPFSDYAMSWVRQAPGKGLEWVSAISGGGGTTYY ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKAGAYSTGFDSWGQGTLVTVSS
6		VL	DIQMTQSPSSLSASVGDRVTITCRASQTIINYLNWYQQEPGKAPKLLIYTASRLQSGVPS RFSGSGSGTDFTLTISSLQPEDFATYYCQQSFATPYTFGGGTKVEIK
7	PB003.23. 0104 (optionally with afucosylated Fc domain)	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYAMGWVRQAPGKGLEWVSSISGSGGSTYQ ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTTGGGWTLGPFDYWGQGTLVTVSS
8		VL	DIQMTQSPSSLSASVGDRVTITCRSSQNIGSYLNWYQQKPGKAPKLLIYGASILQTGVPS RFSGSRSGTDFTLTISSLQPEDFTTYYCQQSYNFPLTFGGGTKVEIK
9	AWDA anti integrin antibody	VH	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDYYMNWVRQAPGKGLEWVGWIDPDQGNTIYEP KFQGRFTISADTSKNSAYLQMNSLRAEDTAVYYCARRLLMDYWGQGTLVTVSS
10		VL	DIQMTQSPSSLSASVGDRVTITCRSTKSLSHFNGNTYLFWYQQKPGKAPKRLIYYMSSLASG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSLEYPFTFGGGTKVEIK
11	AWDA anti integrin antibody BFP	HC	**EVQLVESGGGLVQPGGSLRLSCAASGFNIKDYYMNWVRQAPGKGLEWVGWIDPDQGNTIYEP KFQGRFTISADTSKNSAYLQMNSLRAEDTAVYYCARRLLMDYWGQGTLVTVSS**ASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
12		LC plus ULBP2	GRADPHSLCYDITVIPKFRPGPRWCAVQGQVDEKTFLHYDCGNKTVTPVSPLGKKLNVTTAW KAQNPVLREVVDILTEQLRDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDGQIFL LFDSEKRMWTTVHPGARKMKEKWENDKVVAMSFHYFSMGDCIGWLEDFLMGMDSTLEPSAGA PLAMSGGGGSGGGGSGGGGSGGGGS**DIQMTQSPSSLSASVGDRVTITCRSTKSLSHFNGNTY LFWYQQKPGKAPKRLIYYMSSLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSLEY PFTFGGGTKVEIK**RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS GNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
13	PB003.21. 0091.BFP	HC	**QVQLVQSGAEVKKPGSSVKVSCKASGGTFSIYAISWVRQAPGQGLEWMGGIIPLNYAQKF QGRVTITADESTSTAYMELSSLRSEDTAVYYCAWGSGWDNPFDYWGQGTLVTVSS**ASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK
14		LC plus ULBP2	GRADPHSLCYDITVIPKFRPGPRWCAVQGQVDEKTFLHYDCGNKTVTPVSPLGKKLNVTT AWKAQNPVLREVVDILTEQLRDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDG QIFLLFDSEKRMWTTVHPGARKMKEKWENDKVVAMSFHYFSMGDCIGWLEDFLMGMDSTL EPSAGAPLAMSGGGGSGGGGSGGGGSGGGGS**DIQMTQSPSSLSASVGDRVTITCRASQTI SGYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCL QTVSTPYTFGQGTKVEIK**RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC
15	PB003.21. 0111.BFP	HC	**EVQLLESGGGLVQPGGSLRLSCAASGFPFSDYAMSWVRQAPGKGLEWVSAISGGGGTTYY ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKAGAYSTGFDSWGQGTLVTVSS**A STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK

16		LC plus ULBP2	GRADPHSLCYDITVIPKFRPGPRWCAVQGQVDEKTFLHYDCGNKTVTPVSPLGKKLNVTT AWKAQNPVLREVVDILTEQLRDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDG QIFLLFDSEKRMWTTVHPGARKMKEKWENDKVVAMSFHYFSMGDCIGWLEDFLMGMDSTL EPSAGAPLAMSGGGGSGGGGSGGGGSGGGGS**DIQMTQSPSSLSASVGDRVTITCRASQTI INYLNWYQQEPGKAPKLLIYTASRLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ QSFATPYTFGGGTKVEIK**RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC
17	PB003.23.0104.BFP	HC	**EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYAMGWVRQAPGKGLEWVSSISGSGGSTYQ ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTTGGGWTLGPFDYWGQGTLVTVSS** ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
18		LC plus ULBP2	GRADPHSLCYDITVIPKFRPGPRWCAVQGQVDEKTFLHYDCGNKTVTPVSPLGKKLNVTT AWKAQNPVLREVVDILTEQLRDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDG QIFLLFDSEKRMWTTVHPGARKMKEKWENDKVVAMSFHYFSMGDCIGWLEDFLMGMDSTL EPSAGAPLAMSGGGGSGGGGSGGGGSGGGGS**DIQMTQSPSSLSASVGDRVTITCRSSQNI GSYLNWYQQKPGKAPKLLIYGASILQTGVPSRFSGSRSGTDFTLTISSLQPEDFTTYYCQ QSYNFPLTFGGGTKVEIK**RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC
19	Linker sequence 1		GGGGSGGGGSGGGGSGGGGS

BFP stands for bifunctional fusion protein, af stands for afucosylated

What is claimed is:

1. An antibody capable of binding GARP, or a target-binding fragment or derivative thereof retaining target binding capacities, which comprises the heavy chain/light chain variable domain (HCVD/LCVD) pairs set forth in the following:

    - SEQ ID NO: 3 and 4,
    - SEQ ID NO: 5 and 6, and/or
    - SEQ ID NO: 7 and 8.

2. The antibody or fragment according to claim 1, which is or is derived from a monoclonal antibody.

3. The antibody or fragment according to any one of the aforementioned claims, which antibody or fragment or derivative has an enhanced potential to induce ADCC.

4. The antibody or fragment according to any one of the aforementioned claims, which comprises an afucosylated Fc domain

5. A bifunctional molecule comprising

    - a binding domain that is capable of binding a cancer antigen, and
    - a binding domain that is capable of binding NKG2D

6. The bifunctional molecule according to claim 5, wherein

    a) the binding domain that is capable of binding a cancer antigen is an antibody or target-binding fragment or derivative thereof,
    b) the binding domain that is capable of binding NKG2D is a ligand of NKG2D, optionally ULBP2 or an active fragment thereof.

7 The bifunctional molecule according to any one of claims 5 or 6, wherein the binding domain that is capable of binding a cancer antigen binds to at least one target selected from the group set forth in table 2.

8 The bifunctional molecule according to anyone of claims 5 - 7, wherein

- ULBP2 comprises, or consists of, the amino acid sequence according to SEQ ID NO: 1, and/or
- the binding domain that is capable of binding a cancer antigen is the antibody or fragment ort derivative according to claim 1.

9 The bifunctional molecule according to any one of claims 5 - 8, wherein the active fragment of ULBP2 comprises, or consists of, the amino acid sequence according to SEQ ID NO: 2.

10 The bifunctional molecule according to any one of claims 5 - 9, wherein the binding domain that is capable of binding NKG2D is fused, directly of via a flexible linker, to the N-terminus of the VH or VL domain of the antibody capable of binding GARP, or to the target-binding fragment or derivative thereof.

11 The bifunctional molecule according to any one of claims 5 - 9, wherein the binding domain that is capable of binding NKG2D is fused to the N-terminus of the VL domain of the antibody capable of binding GARP, or the target-binding fragment or derivative thereof.

12 The antibody or target-binding fragment or derivative thereof, or the bifunctional molecule, according to any one of the aforementioned claims, wherein the binding domain that is capable of binding GARP is in the format of an IgG antibody.

13 The bifunctional molecule according to according to any one of claims 5 - 11, which comprises a pair of (i) an anti-GARP antibody heavy chain and (ii) an active fragment of ULBP2 fused to the N-terminus of an anti-GARP antibody light chain, which pair is selected from the group consisting of

- SEQ ID NO: 13 and 14,
- SEQ ID NO: 15 and 16, and/or
- SEQ ID NO: 17 and 18.

14 A target binding molecule that

(i) competes for binding to GARP with the antibody or target-binding fragment or derivative thereof, or the bifunctional molecule, according to any one of the aforementioned claims,

(ii) or binds to the same epitope of GARP as the antibody or target-binding fragment or derivative thereof, or the bifunctional molecule, according to any one of the aforementioned claims.

15 The target binding molecule of claim 14, which is an antibody or target-binding fragment or derivative thereof, or a bifunctional molecule.

16 Use of the antibody or target-binding fragment or derivative thereof, or the bifunctional molecule, or the target binding molecule according to any one of claims 1 – 15 (for the manufacture of a medicament) in the treatment of a human or animal subject

- being diagnosed for,
- suffering from or
- being at risk of developing

a neoplastic disease, or for the prevention of such condition.

17 A pharmaceutical composition comprising the antibody or target-binding fragment or derivative thereof, or the bifunctional molecule, according to any one of the aforementioned claims, and optionally one or more pharmaceutically acceptable excipients.

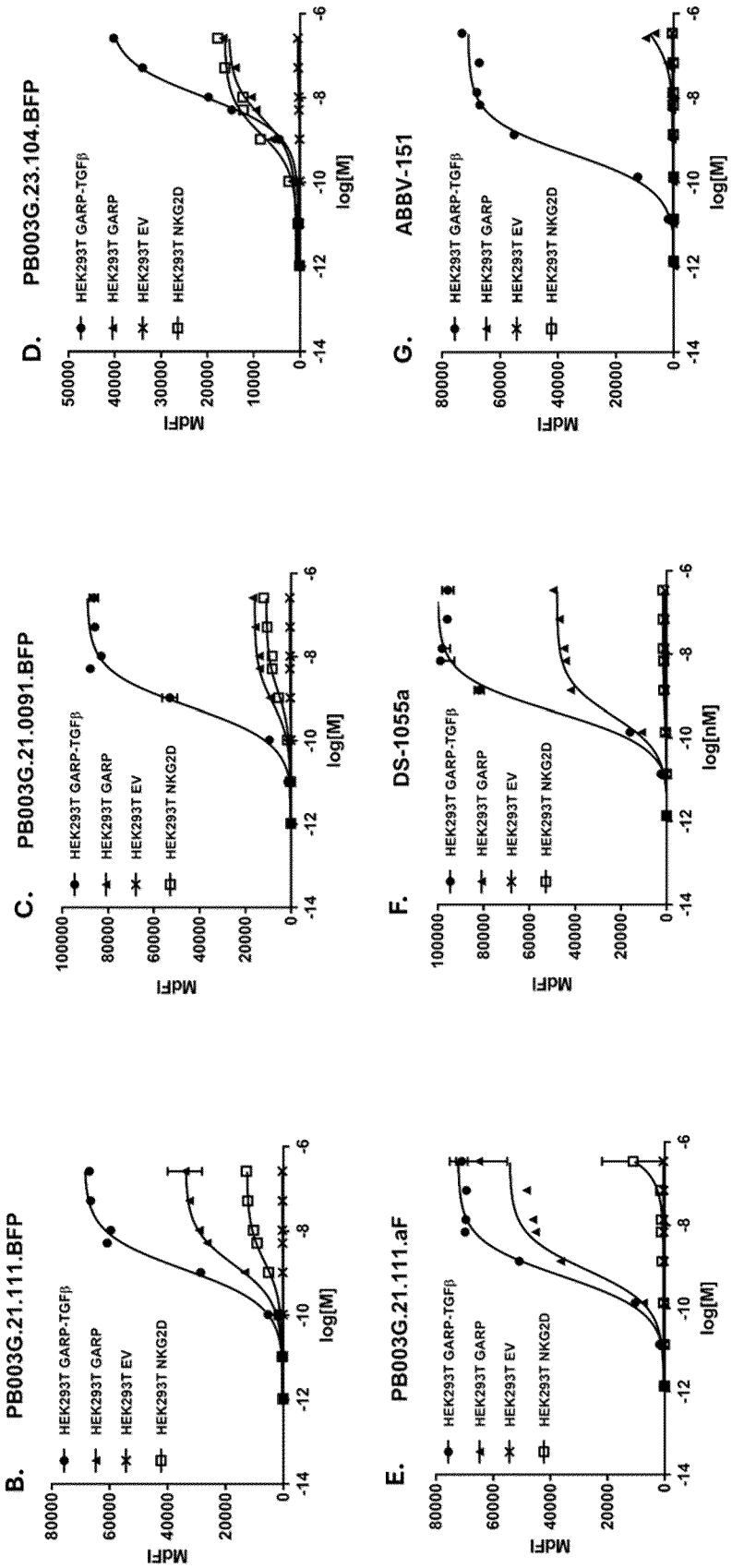
18 A combination comprising (i) the antibody or target-binding fragment or derivative thereof, or the bifunctional molecule, according to any one of the aforementioned claims, and (ii) one or more therapeutically active compounds.

19 A method for treating or preventing a neoplastic disease, which method comprises administration, to a human or animal subject, of the antibody or target-binding fragment or derivative thereof, or the bifunctional molecule, or the target binding molecule according to any one of claims 1 – 15, the pharmaceutical composition according to claim 17, or the combination according to claim 18, in a therapeutically sufficient dose.

A.

	GARP		GARP-TGFb1		NKG2D	
	Molecular studies (KD) [nM]	Cell-based assay(EC50) [nM]	Molecular studies (KD) [nM]	Cell-based assay(EC50) [nM]	Molecular studies (KD) [nM]	Cell-based assay(EC50) [nM]
PB003G.21.0111.aF	9 ± 3	0.85	292 ± 11	0.60	N/A	N/A
PB003G.21.0111.BFP	9 ± 3	1.60	345 ± 7.8	1.24	0.3	1.88
PB003G.21.0091.BFP	N/D*	0.81	256 ± 25	0.64	0.35	1.44
PB003G.23.0104.BFP	N/D*	3.35	1170 ± 132	10.79	0.36	1.41
DS-1055a	6 ± 2	0.34	7.0 ± 0.9	0.42	N/A	N/A
ABBV-151	No binding	No binding	7.0	0.48	N/A	N/A

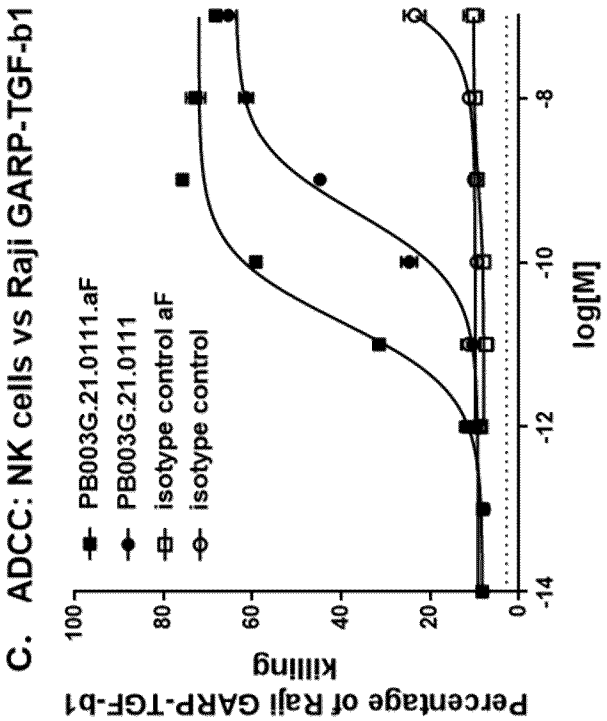
Fig. 1


B.
PB003G.21.111.BFP
C.
PB003G.21.0091.BFP
D.
PB003G.23.104.BFP
E.
PB003G.21.111.aF
F.
DS-1055a
G.
ABBV-151
HEK293T GARP-TGFβ
HEK293T GARP
HEK293T EV
HEK293T NKG2D
MdFI
log[M]
log[nM]

A.

	FcγRIIIA (176V)		FcγRIIIA (176F)	
	Affinity (KD) [nM]	KD fold difference	Affinity (KD) [nM]	KD fold difference
PB003G.21.0111.aF	119	12.3	665	10
PB003G.21.0111 (native) IgG1	9.70		61	
DS-1055	10.50	N/A	72	N/A

Fig. 2


C. ADCC: NK cells vs Raji GARP-TGF-b1
PB003G.21.0111.aF
PB003G.21.0111
isotype control aF
isotype control
Percentage of Raji GARP-TGF-b1 killing
100
80
60
40
20
0
-14
-12
-10
-8
log[M]

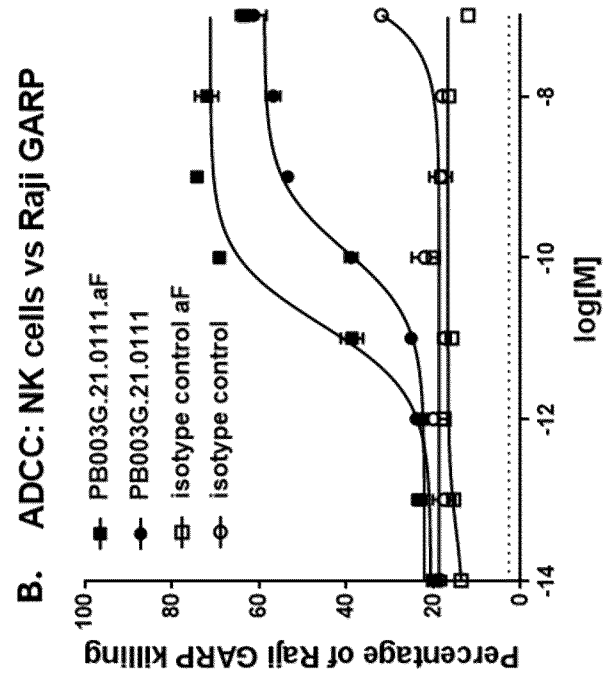

B. ADCC: NK cells vs Raji GARP
PB003G.21.0111.aF
PB003G.21.0111
isotype control aF
isotype control
Percentage of Raji GARP killing
100
80
60
40
20
0
-14
-12
-10
-8
log[M]

Fig. 2 ctd'

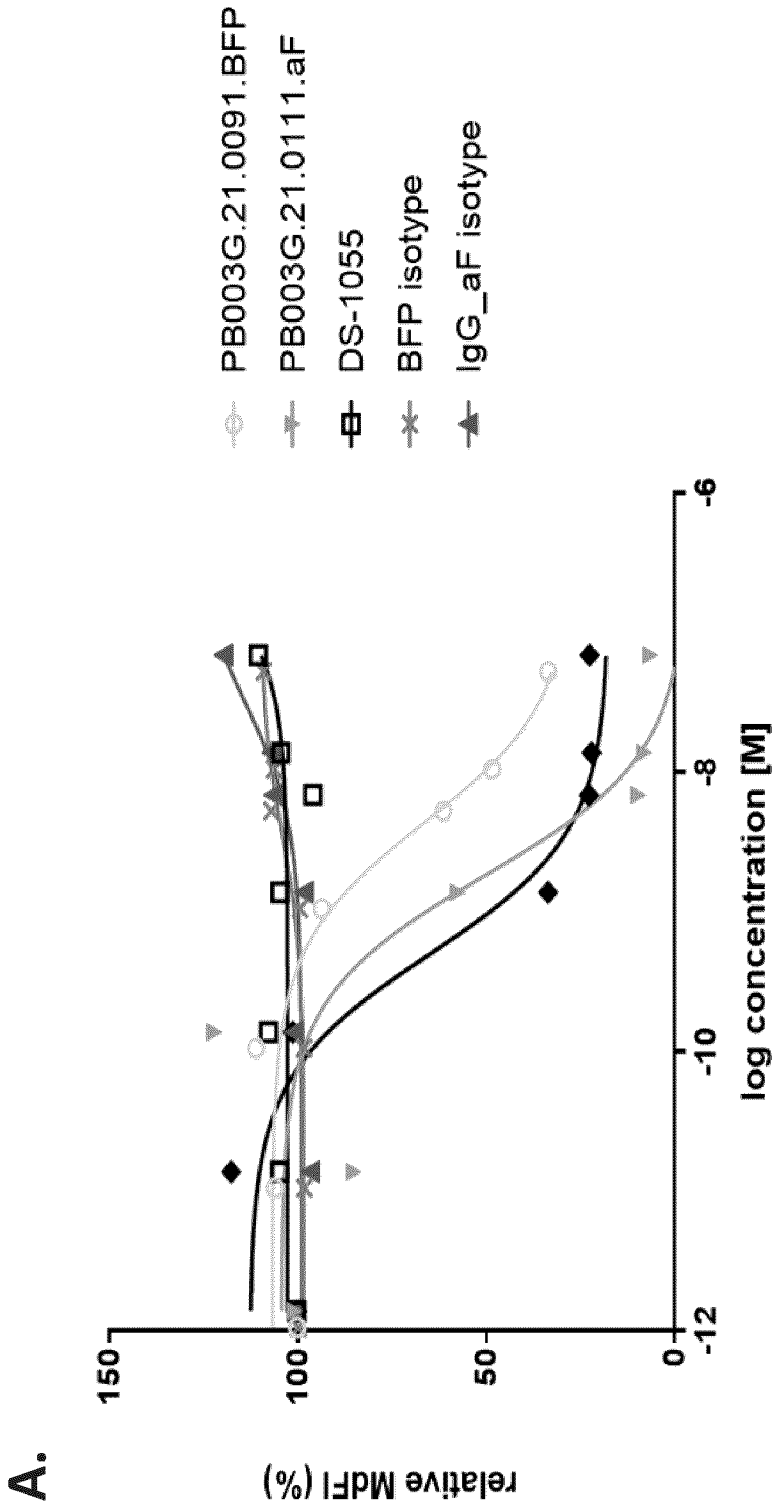

A.
relative MdFI (%)
150
100
50
0
-12
-10
-8
-6
log concentration [M]
PB003G.21.0091.BFP
PB003G.21.0111.aF
DS-1055
BFP isotype
IgG_aF isotype

Fig. 3

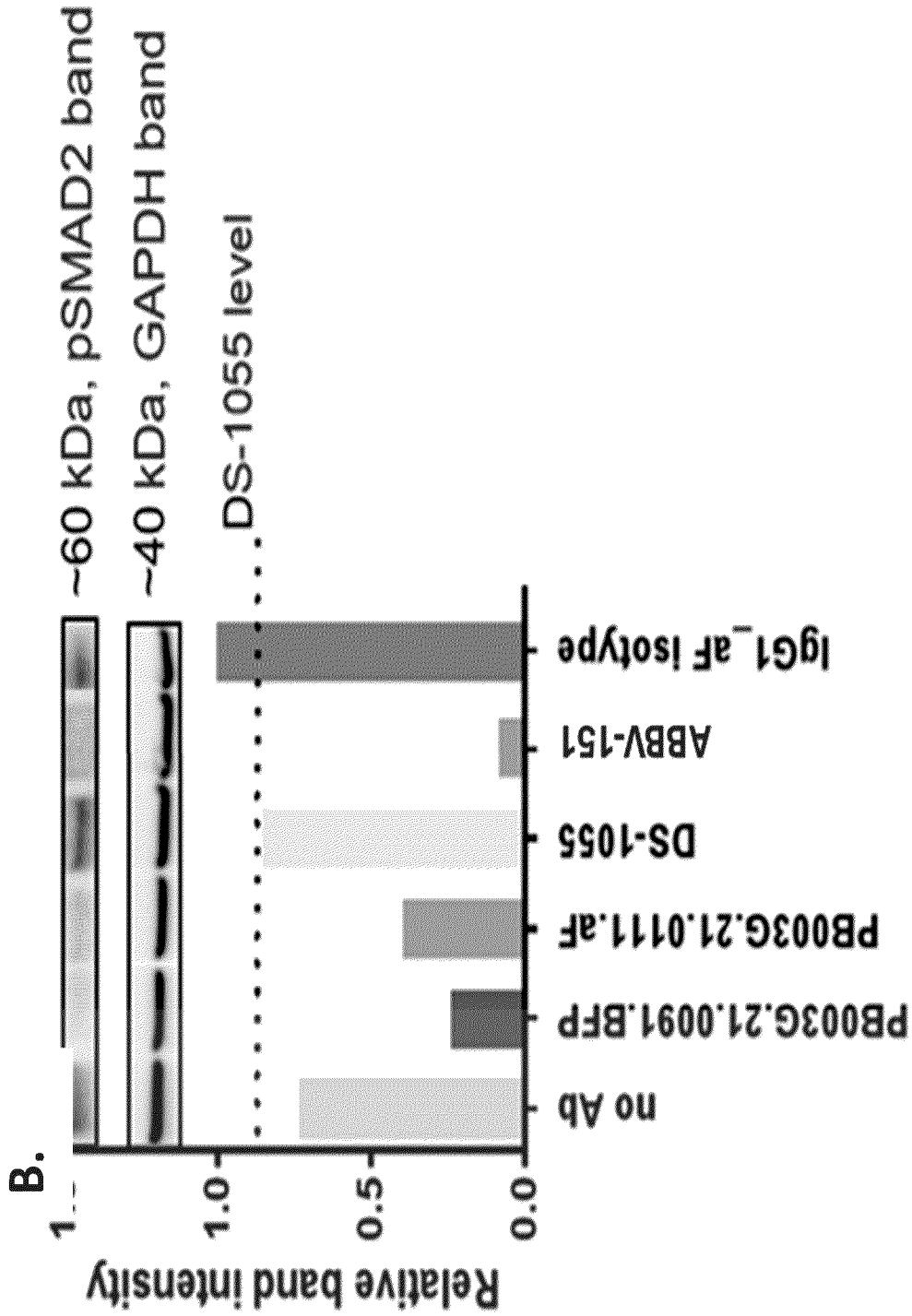

B.
~60 kDa, pSMAD2 band
~40 kDa, GAPDH band
DS-1055 level
Relative band intensity
1.0
0.5
0.0
no Ab
PB003G.21.0091.BFP
PB003G.21.0111.aF
DS-1055
ABBV-151
IgG1_aF isotype

Fig. 3 ctd'

A.

	EC50 [nM] Raji GARP-TGFβ1	EC50 [nM] Raji GARP	EC50 [nM] L428
PB003G.21.0111.aF	0.03 ± 0.025	0.039 ± 0.046	0.033 ± 0.015
PB003G.21.0111.BFP	0.04 ± 0.02	0.032 ± 0.02	0.068 ± 0.04
PB003G.21.0091.BFP	0.008 ± 0.004	0.04 ± 0.02	0.14 ± 0.095
PB003G.23.0104.BFP	0.4 ± 0.1	0.26 ± 0.16	0.345 ± 0.145
DS-1055a	0.007 ± 0.0025	0.007 ± 0.007	0.003 ± 0.001

Fig. 4

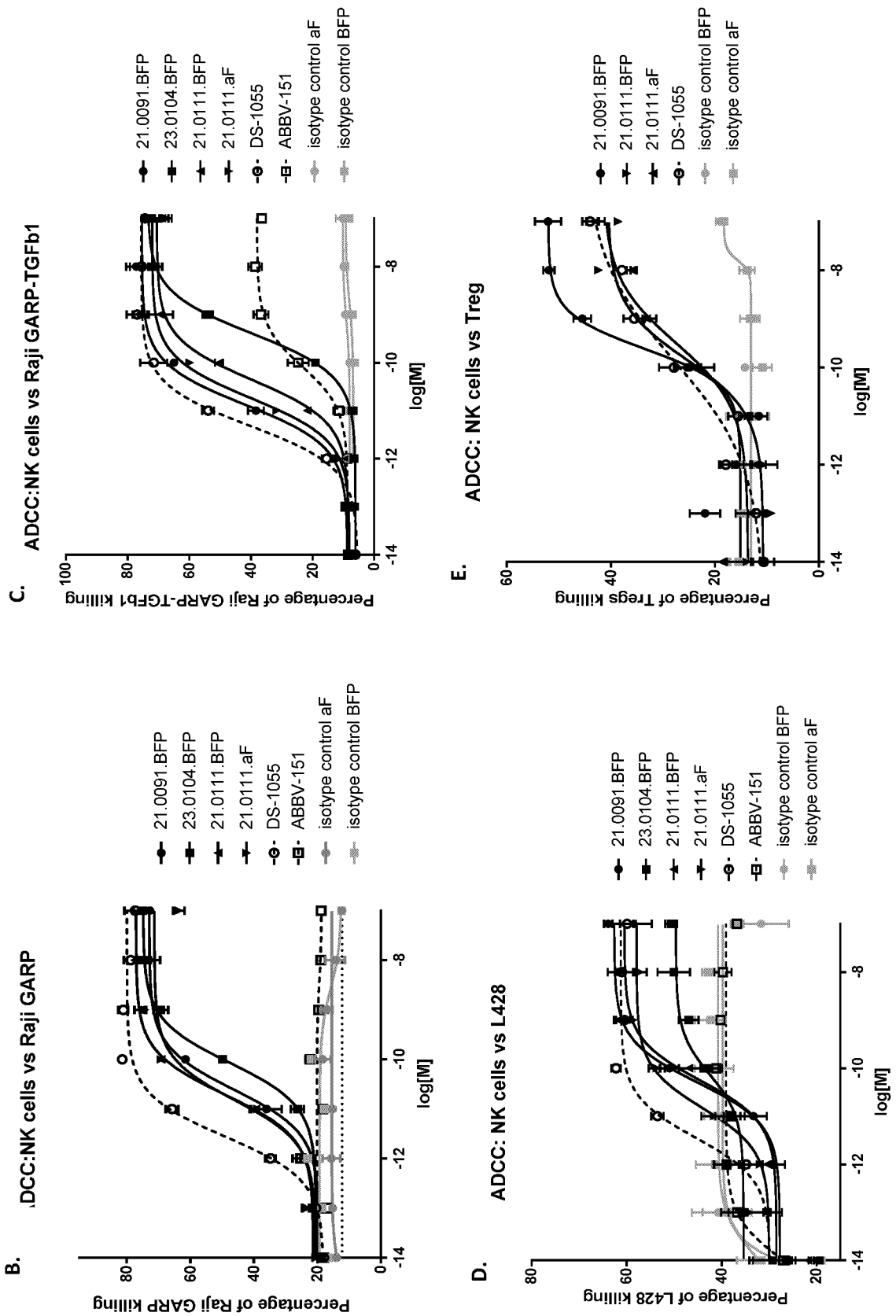

B.
ADCC:NK cells vs Raji GARP
Percentage of Raji GARP killing
log[M]
C.
ADCC:NK cells vs Raji GARP-TGFb1
Percentage of Raji GARP-TGFb1 killing
log[M]
D.
ADCC: NK cells vs L428
Percentage of L428 killing
log[M]
E.
ADCC: NK cells vs Treg
Percentage of Tregs killing
log[M]
21.0091.BFP
23.0104.BFP
21.0111.BFP
21.0111.aF
DS-1055
ABBV-151
isotype control BFP
isotype control aF

Fig. 4 ctd'

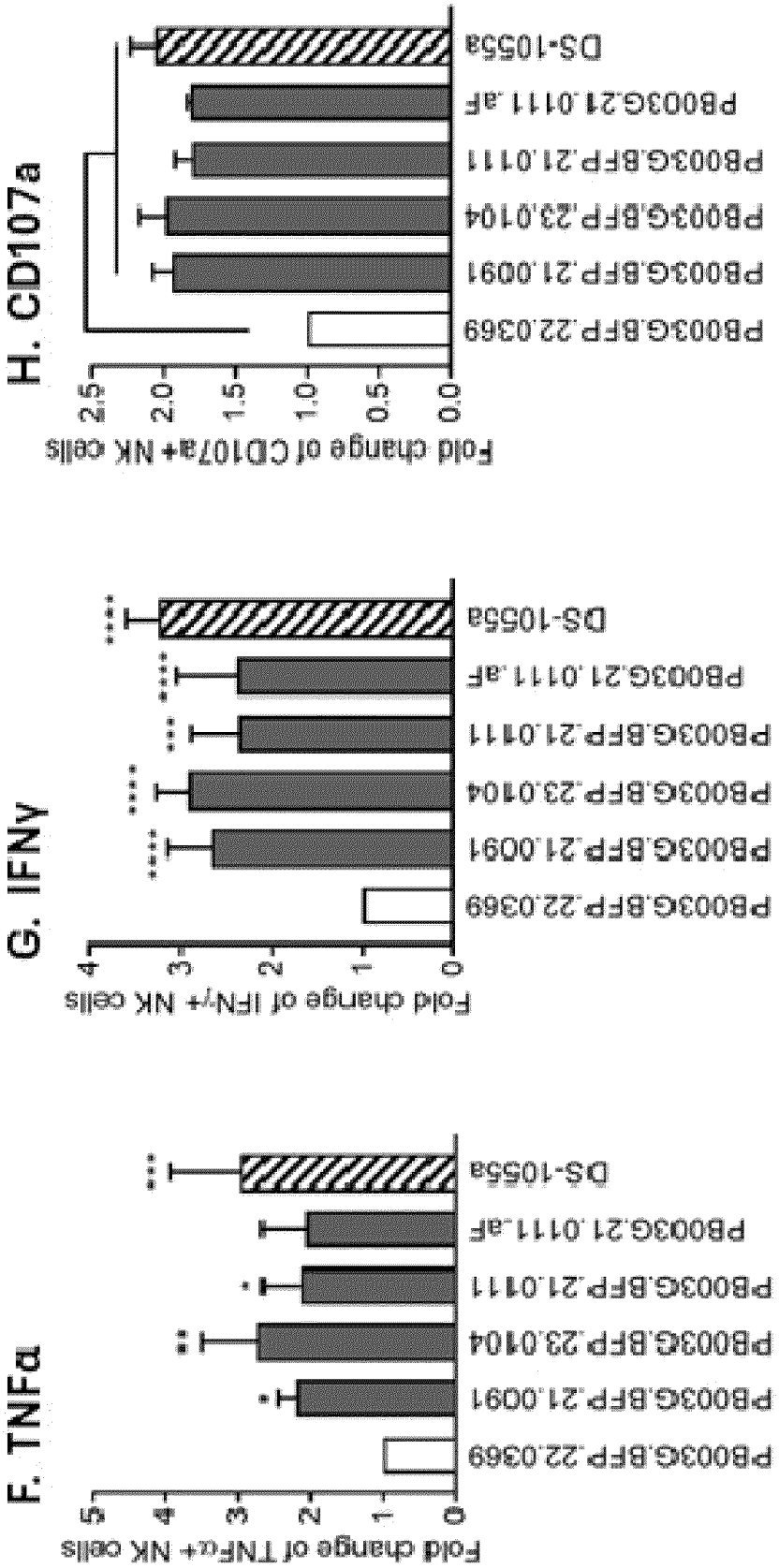

H. CD107a
Fold change of CD107a+ NK cells
0.0
0.5
1.0
1.5
2.0
2.5
PB003G.BFP.22.0369
PB003G.BFP.21.0091
PB003G.BFP.23.0104
PB003G.BFP.21.0111
PB003G.21.0111.aF
DS-1055a
G. IFNγ
Fold change of IFNγ+ NK cells
0
1
2
3
4
PB003G.BFP.22.0369
PB003G.BFP.21.0091
PB003G.BFP.23.0104
PB003G.BFP.21.0111
PB003G.21.0111.aF
DS-1055a
F. TNFα
Fold change of TNFα+ NK cells
0
1
2
3
4
5
PB003G.BFP.22.0369
PB003G.BFP.21.0091
PB003G.BFP.23.0104
PB003G.BFP.21.0111
PB003G.21.0111.aF
DS-1055a

Fig. 4 ctd'

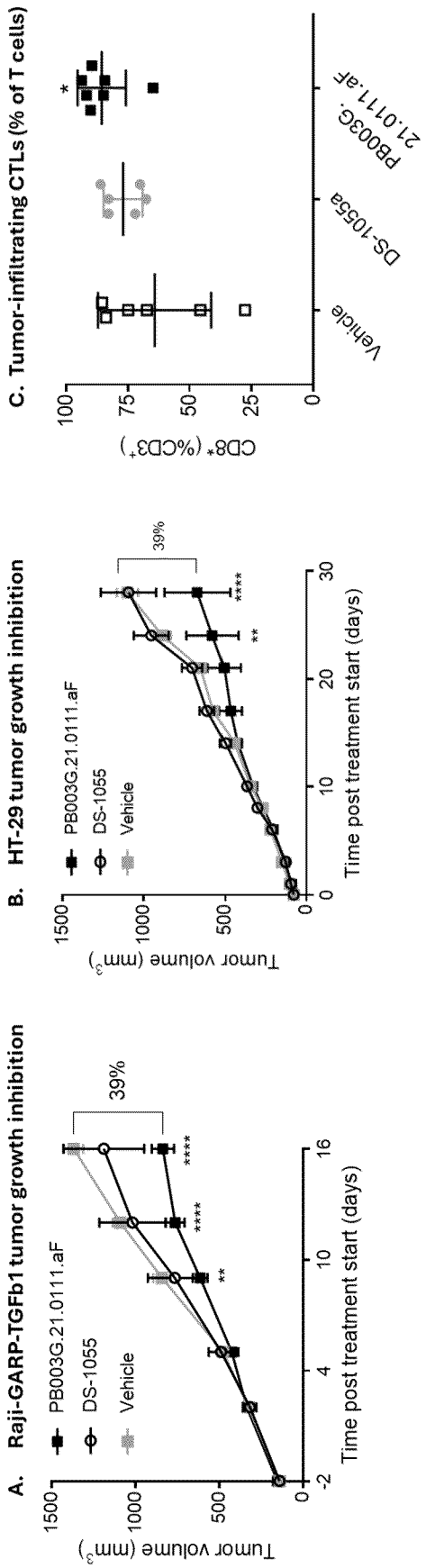

A. Raji-GARP-TGFb1 tumor growth inhibition
PB003G.21.0111.aF
DS-1055
Vehicle
Tumor volume (mm³)
Time post treatment start (days)
39%
B. HT-29 tumor growth inhibition
C. Tumor-infiltrating CTLs (% of T cells)
CD8⁺ (%CD3⁺)
Vehicle
DS-1055a
PB003G.21.0111.aF

Fig. 5

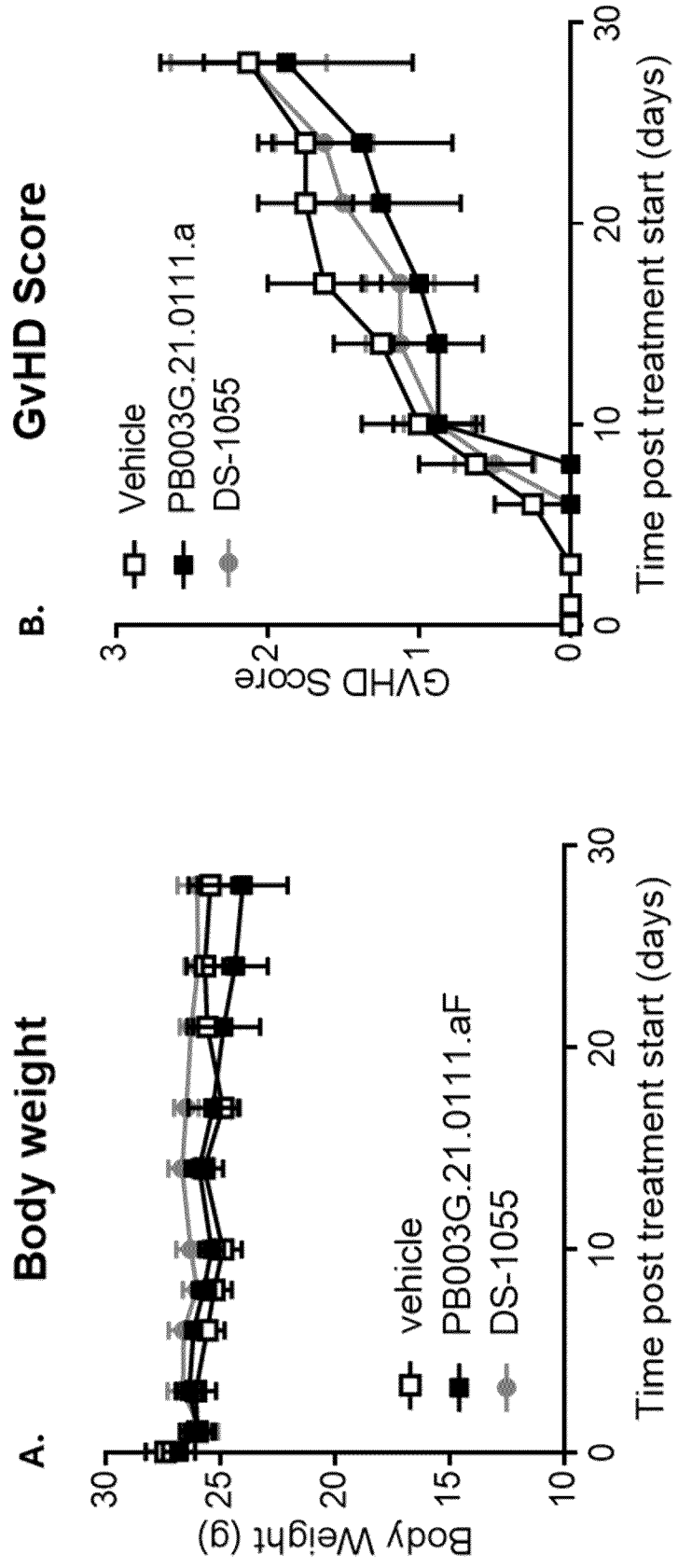

A.
Body weight
Body Weight (g)
30
25
20
15
10
0
10
20
30
Time post treatment start (days)
vehicle
PB003G.21.0111.aF
DS-1055
B.
GvHD Score
GVHD Score
3
2
1
0
0
10
20
30
Time post treatment start (days)
Vehicle
PB003G.21.0111.a
DS-1055

Fig. 6

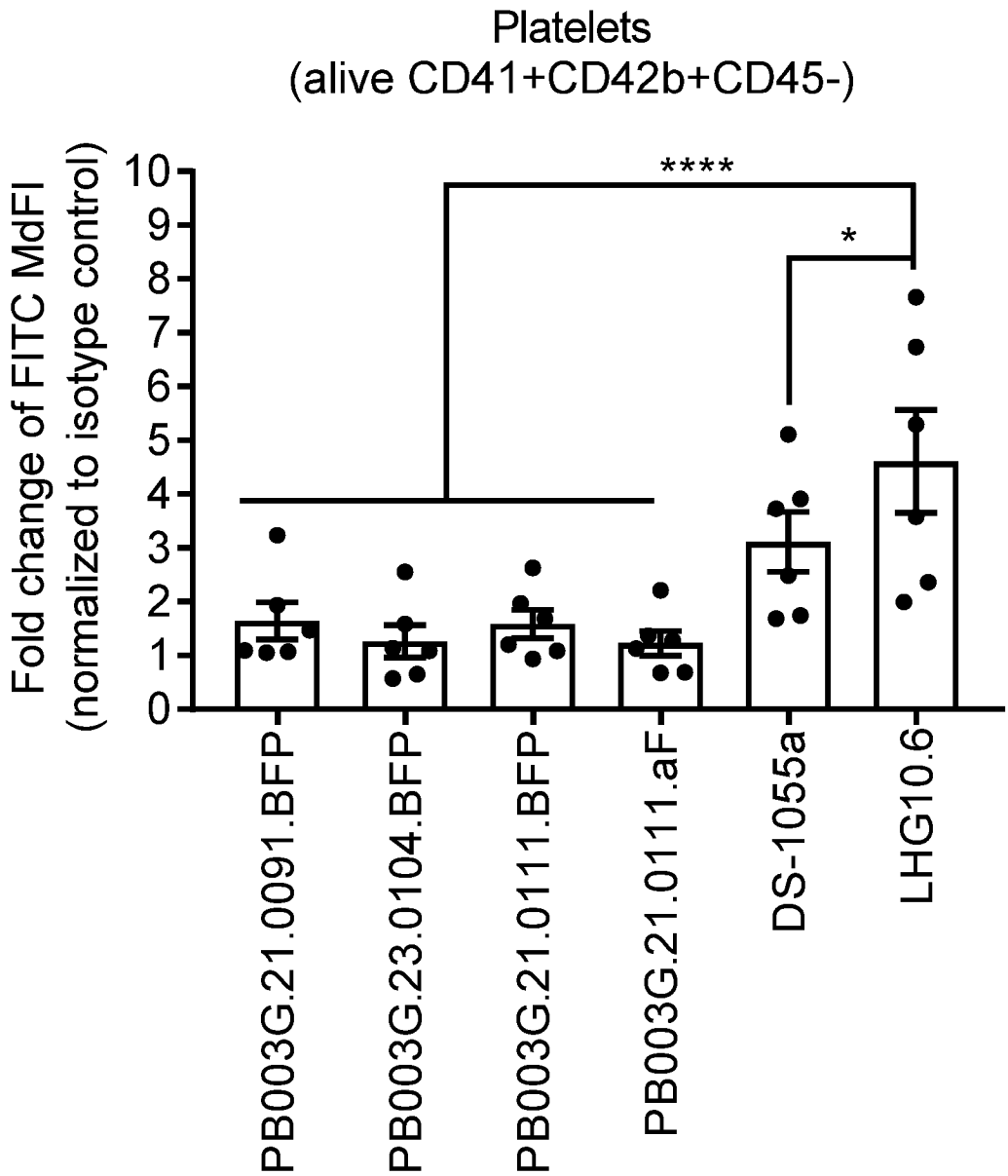

Platelets
(alive CD41+CD42b+CD45-)
Fold change of FITC MdFI
(normalized to isotype control)
0
1
2
3
4
5
6
7
8
9
10
****
*
PB003G.21.0091.BFP
PB003G.23.0104.BFP
PB003G.21.0111.BFP
PB003G.21.0111.aF
DS-1055a
LHG10.6

Fig. 7

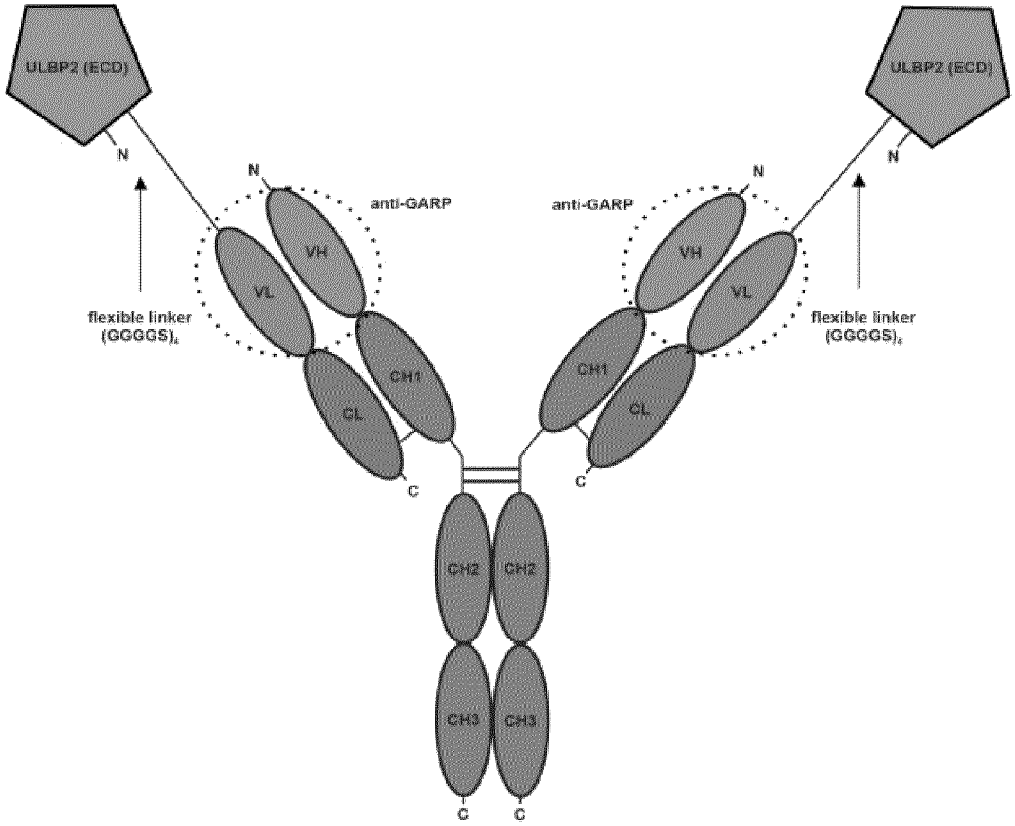

ULBP2 (ECD)
ULBP2 (ECD)
N
N
N
N
anti-GARP
anti-GARP
VH
VL
VH
VL
flexible linker
(GGGGS)4
flexible linker
(GGGGS)4
CH1
CL
CH1
CL
C
C
CH2
CH2
CH3
CH3
C
C

Fig. 8

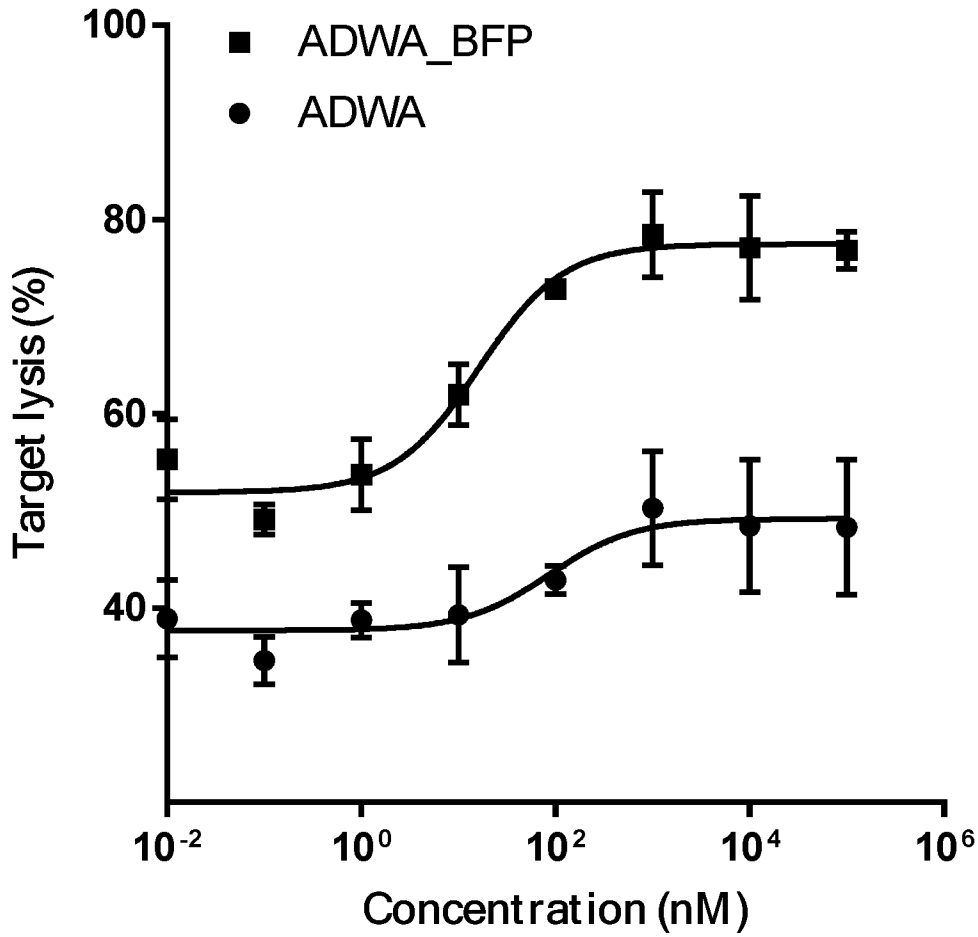

ADWA_BFP
ADWA
Target lysis (%)
100
80
60
40
10-2
100
102
104
106
Concentration (nM)

Fig. 9

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/065505

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, FSTA, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2022/206753 A1 (SHANDONG SIMCERE BIOPHARMACEUTICAL CO LTD [CN] ET AL.) 6 October 2022 (2022-10-06)	1-4, 14-19
Y	the whole document see in particular abstract; examples, claims	5-13

X	WO 2015/015003 A1 (UNIV CATHOLIQUE LOUVAIN [BE]; LUDWIG INST FOR CANCER RES LTD [CH]) 5 February 2015 (2015-02-05)	1-4, 14-19
Y	the whole document see in particular examples, claims	5-13

X	WO 2017/173091 A1 (MUSC FOUND FOR RES DEV [US]) 5 October 2017 (2017-10-05)	1-4, 14-19
Y	see in particular Ex. 4, 6, Table 1 the whole document	5-13

	-/--	

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 September 2024	16/09/2024

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European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Sirim, Pinar

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 832 747 A1 (UNIV CATHOLIQUE LOUVAIN [BE]; LUDWIG INST FOR CANCER RES LTD [CH]) 4 February 2015 (2015-02-04)	1-4, 14-19
Y	the whole document see in particular Examples	5-13

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A	see in particular abstract; Fig. 1-3	1-4,14, 15

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A	WO 2019/057805 A1 (MERCK PATENT GMBH [DE]) 28 March 2019 (2019-03-28) the whole document see in particular Fig. 1E, 1I, Examples, Table 1, SEQ ID NOs. 50, 52, 61	1-19

A	WO 2017/214151 A1 (UNIV NORTHWESTERN [US]) 14 December 2017 (2017-12-14) the whole document see in particular Fig. 1, C and D; Ex. 1	1-19

A	WO 2020/264321 A1 (UNIV JOHNS HOPKINS [US]) 30 December 2020 (2020-12-30) the whole document see in particular Fig. 1, Examples	1-19

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Leonard Elissa K. ET AL: "Engineered cytokine/antibody fusion proteins improve delivery of IL-2 to pro-inflammatory cells and promote antitumor activity", bioRxiv, 4 May 2023 (2023-05-04), XP093200742, DOI: 10.1101/2023.05.03.539272 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10187205/pdf/nihpp-2023.05.03.539272v2.pdf the whole document figure 1	1-19
A	JAKUB TOMALA ET AL: "Chimera of IL-2 Linked to Light Chain of anti-IL-2 mAb Mimics IL-2/anti-IL-2 mAb Complexes Both Structurally and Functionally", ACS CHEMICAL BIOLOGY, vol. 8, no. 5, 17 May 2013 (2013-05-17), pages 871-876, XP055369414, ISSN: 1554-8929, DOI: 10.1021/cb3007242 the whole document see in particular abstract; Fig. 1B, Table 1	1-19

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2024/065505

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2024/065505

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