

ANTI-CD163 ANTIBODIES WITH IMPROVED ADCC CAPABILITIES**Technical field of the invention**

The present invention relates to improved antibodies targeting CD163. In particular, the present invention relates to improved antibodies targeting CD163
5 with improved capabilities in relation to inducing Antibody-Dependent Cell-mediated Cytotoxicity (ADCC).

Background of the invention

Tumor-associated macrophages (TAM) are the most abundant immune cells found
10 in solid tumors and their important contributions to tumor progression are well documented (1). Besides their trophic functions, supporting angiogenesis, invasion and metastasis, TAM have also been suggested to inhibit T cell proliferation and activation via release of the immune-suppressive cytokine IL-10 (2) and the local depletion of arginine (3) and tryptophan (4), on which T cells are
15 highly dependent. The important functions of macrophages in relation to tumor progression have already attracted substantial interest in developing new therapeutic strategies for targeting TAM. Strategies currently in clinical trials include, blocking of the chemokine CCL2, or its receptor CCR2, which inhibits TAM recruitment by neutralizing the mobilization of bone marrow-derived monocytes,
20 and targeting the macrophage growth factor receptor CSF1R (M-CSFR; c-FMS; CD115) (5). While CCL2/CCR2 blockade only targets the recruitment of monocyte-derived macrophages, CSF1 plays an essential role in both the survival and differentiation of tissue-resident macrophages as well as maturation of monocyte-derived macrophage (6). Indeed, although clinical data are still limited, reduced
25 numbers of TAM has been reported after treatment with monoclonal antibody (mAb) therapy against CSF1R, with interesting therapeutic effects in tenosynovial giant cell tumors (7, 8).

Despite a strong association between TAM accumulation and poor clinical outcome
30 in the vast majority of clinical studies, certain reports associate TAM numbers or accumulation of specific TAM subsets with a good prognosis. One example is the frequency of HLA-DR⁺ TAM that has been associated with beneficial outcomes in several studies (9, 10), potentially reflecting their roles in orchestrating protective

immune responses (11). To this extend, recent studies using paired single cell analysis by mass cytometry and RNA sequencing, have revealed an unprecedented level of diversity within the tumor-infiltrating myeloid cell (TIM) compartment in lung adenocarcinoma and renal cell carcinoma (RCC) patients (12, 13). In the case of RCC, 17 distinct TAM phenotypes were documented¹². Although we still lack a deeper understanding about the functions of different TAM subsets and their respective contributions to tumor progression, it is tempting to speculate that a selective targeting of TAM subsets that abrogates tumor-promoting mechanisms while preserving innate immune functions may promote anti-tumor immunity and could offer significant clinical benefits.

Expression of CD163 by TAM has been shown to be a particularly strong indicator of poor prognosis in several different cancers. CD163 is a macrophage and monocyte specific transmembrane protein that functions as a scavenger receptor for haptoglobin-hemoglobin complexes, formed upon intravascular haemolysis (14). Expression of CD163 is induced by tumor-promoting cytokines such as IL-6 and IL-10, whereas inflammatory stimuli, including lipopolysaccharide (LPS), TNFa and IFNg, lead to a rapid downregulation of expression and removal of membrane bound CD163 via proteolytic shedding (15, 16). This, together with the generation of anti-inflammatory heme metabolites from hemoglobin scavenging, has led to the association of CD163⁺ macrophages with anti-inflammatory functions (16).

It has recently been shown that tumor-associated macrophages expressing CD163 are key players in tumor progression and also in the observed resistance to immunotherapy (17). Particularly, it was showed that specific depletion CD163⁺ tumor associated macrophages in murine melanoma models resistant to standard immunotherapy leads to a massive recruitment of leukocytes and especially cytotoxic T cells. This influx drove an anti-tumor immune response and ultimately tumor regression. This finding has been corroborated in three additional murine tumor models. Importantly, it has been shown that specific depletion of CD163⁺ tumor-associated macrophages is more effective than an alternative approach, currently in clinical development, based on pan-depletion of tumor-associated macrophages.

CD163 has also commonly been used as a marker that is characteristic for the M2 population of macrophages (70,71), but it has also been suggested that CD163+ macrophages form a distinct subpopulation of M2 macrophages (22).

5 Targeting CD163, in particular when expressed on (immune) cells, has also been suggested as a therapeutic approach for the treatment of various inflammatory diseases. (40, 41). In particular, as further described herein, specific depletion of CD163+ macrophages may be relevant in the treatment of inflammatory or fibrotic indications, where macrophages are known to play a pivotal role in
10 regulating immune activity (42, 43).

Other diseases in which CD163, CD163+ macrophages and/or M2 macrophages are involved (and in particular, diseases and disorders that can be prevented or treated by depletion of CD163+ macrophages using the antibodies described
15 herein) will be clear to the skilled person based on the disclosure herein, and for example and without limitation include:

Inflammatory and Autoimmune Diseases, such as selected from the group consisting of Rheumatoid arthritis, Systemic lupus erythematosus (SLE), Multiple
20 sclerosis, Inflammatory bowel disease (IBD), Psoriasis, Scleroderma, Macrophage activation syndrome (MAS), and hemophagocytic lymphohistiocytosis (HLH).

Cancers, such as selected from the group consisting of Breast cancer, Lung cancer, Colorectal cancer, Prostate cancer, Ovarian cancer, Head and neck
25 cancers, melanoma, Pancreatic cancer, Gastric cancer, Kidney cancer, Brain cancer, Hematological cancers, and Sarcomas.

Fibrotic Diseases, such as selected from the group consisting of Pulmonary fibrosis, Liver fibrosis (cirrhosis), Cardiac fibrosis post-myocardial infarction, Renal
30 fibrosis in chronic kidney disease, Irradiation-induced fibrosis, and Skin fibrosis.

Metabolic Disorders, such as selected from the group consisting of Obesity, Type 2 diabetes mellitus, Non-alcoholic fatty liver disease (NAFLD), and Metabolic syndrome.

Cardiovascular diseases, such as selected from the group consisting of Atherosclerosis, Myocardial infarction (heart attack), Heart failure, and Hypertension.

- 5 Neurological Disorders, such as selected from the group consisting of Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis (ALS), Traumatic brain injury, Stroke, and Multiple system atrophy.

- Respiratory Diseases, such as selected from the group consisting of Chronic
10 obstructive pulmonary disease (COPD), Asthma, Interstitial lung diseases (e.g., idiopathic pulmonary fibrosis), and Allergic rhinitis.

Pregnancy-Related Conditions, such as selected from the group consisting of Preeclampsia and Placental insufficiency.

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Dermatological Disorders, such as selected from the group consisting of Atopic dermatitis, Eczema, and Allergic contact dermatitis.

- Gastrointestinal Diseases, such as selected from the group consisting of Celiac
20 disease, Gastritis, and Peptic ulcer disease.

Other Conditions, such as selected from the group consisting of Systemic vasculitis, Gout and Anemia of chronic disease.

- 25 Depleting CD163 expressing macrophages can be accomplished by different methods.

A possibility is to use an antibody-drug conjugate targeting a toxin to CD163 expressing cells, and thereby killing them. However, use of toxins may be associated with adverse effects, and especially for CD163 which is found in high
30 levels on Kupffer cells, it may lead to disposition of toxin to the liver.

An alternative could be to use an antibody that binds CD163 on the cell surface and mediates antibody dependent cellular cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP), i.e. killing of CD163 expressing cells by immune cells.

Hence, an improved antibody targeting CD163 would be advantageous, and in particular, a more efficient and/or reliable antibody targeting CD163 would be advantageous.

5 Summary of the invention

An antibody that should exert antibody dependent cellular cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP) must be exposed on the surface of the cells expressing the antigen after binding the antigen for as long a period as possible (18). This makes targeting internalization receptors (such as CD163) 10 challenging, since the target and hence the antibody will be endocytosed, and an antibody bound to the target dissociated intracellularly. Therefore other means of inducing cell killing would be advised when targeting internalization receptors, as seen for targeting SIGLEC (19) or HER-2 (20). This is especially true for antibody binding a receptor with a high endocytosis capacity, as CD163 (21-23). This 15 makes targeting CD163 with the aim to induce ADCC or ADCP killing of CD163 expressing cells difficult and counter-intuitive and will prompt a skilled person to seek other means for cell killing.

Several factors have an influence on the capabilities of antibodies to work as medicaments, in particular antibodies designed to conduct ADCC. At least the 20 following features have an impact on the efficiency of the antibody, in particular when the antibody is not coupled to a drug:

- Availability of the epitope on target cells
- The time the epitope-antibody binding is available on the target cells
- The equilibrium dissociation constant (K_D) between the antibody and its 25 antigen
- The efficiency of the antibody to conduct ADCC
- The efficiency of the antibody to conduct ADCP
- Level of antibody aggregation
- Thermal stability of the antibody

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In here the following surprising results are presented:

- **Example 2** shows that only a few of known CD163 antibodies exhibit extended surface exposure on CD163 expressing cells. This means that even an internalizing receptor potentially can be used as target for ADCC,

contrary to common general knowledge (21-23), if the correct antibody is used.

- **Example 3** shows that OS001 surprisingly exhibits very low sensitivity to soluble CD163, which is found in plasma in mg/L concentrations, meaning that OS001 and derivatives are especially well suited for in vivo use.
- **Example 4** shows that these surprising results may be explained with a higher affinity of OS001 for trimeric CD163 (the conformation exposed on the cell surface) compared to monomeric CD163 (the conformation found in blood). ORV3 also exhibits prolonged surface exposure but is very sensitive to soluble CD163 and cell binding is easily outcompeted. In summary, OS001 exhibits stronger binding for trimeric CD163 than monomeric CD163, which is the cause for its lower sensitivity of CD163 cell binding to soluble CD163, since CD163 is a trimer on the cell surface and a monomer in solution.
- **Example 5** shows that the epitopes bound by ORV3, 15-5-20 and 15-5-30 are non-overlapping with OS001.
- **Example 6** shows that OS001 is further an ideal mAb for therapeutic use since CD163 binding is not inhibited by the CD163 ligand hemoglobin-haptoglobin.
- **Examples 7-8 and 10** show that after testing a range of techniques for increasing ADCC we can see that afucosylation is surprisingly significantly better than the other approaches, despite it not being reported to be superior to other approaches in the literature. This superiority is especially clear when looking at the clinically relevant human monocyte derived macrophages, where we decrease EC50 more than 100-fold (literature mentions 2-40-fold). This means that afucosylation for some reason is a surprisingly superior approach for OS001. Further, both OS001 and its variants and ORV3 can exhibit ADCC signaling in a CD16 reporter system when using recombinant cells expressing CD163, however, when using the more clinically relevant human monocyte derived macrophages expressing CD163 as target we see that ORV3 surprisingly does not exhibit ADCC signaling, whereas OS001 and the afucosylated variants OS017, OS022, OS023 and OS024 do.
- **Examples 9 and 11** show that albeit afucosylation is reported to decrease the stability of antibodies, measured as the unfolding melting temperature

(24), for OS001 we surprisingly do not observe such an effect, T melt is unchanged. Further corroborating the surprising suitedness of afucosylation as ADCC enhancement technology for OS001.

- **Example 12** shows that an identified substitution of a single amino acid residue in the HC of OS001 (D72E, outside the CDRs) leads to lower levels of aggregate formation formed during expression and initial purification. This increases the druggability of the antibody.
- **Example 13** shows that a further identified substitution of a single amino acid residue in the LC CDR3 of OS001 (G89Q) leads to increased affinity of the antibody for CD163. This substitution can be combined with the HC substitution (D72E) and keep improved affinity.
- **Example 14** demonstrates that novel OS variants exhibit ADCC activity.
- **Example 15** shows the effect of CD163+ macrophage depletion on pro-inflammatory cytokine production.

In sum, surprisingly, CD163 binding antibodies have been identified, which are considered superior for inducing efficient ADCC killing of CD163 expressing cells, and this antibody is further improved.

The preferred identified improved antibodies targeting CD163 according to the invention can thus be grouped as:

- Specifically selected afucosylated anti-CD163
- Specifically selected afucosylated anti-CD163 + G89Q
- Specifically selected afucosylated anti-CD163 + D72E
- Specifically selected afucosylated anti-CD163 + G89Q + D72E
- Specifically selected "regular glycosylated" anti-CD163 + G89Q
- Specifically selected "regular glycosylated" anti-CD163 + D72E
- Specifically selected "regular glycosylated" anti-CD163 + G89Q + D72E

Thus, an object of the present invention relates to the provision of improved CD163 targeting antibodies for medical uses. In particular, it is an object of the present invention to provide improved CD163 targeting antibodies that solves the above-mentioned problems of the prior art with induction of ADCC.

Thus, one aspect of the invention relates to an antibody targeting CD163 comprising

- a light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - 5 ○ a CDR 2 region according to SEQ ID NO: 20; and
 - a CDR 3 region according to SEQ ID NO: 21 or 25;
- a heavy chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 22;
 - a CDR 2 region according to SEQ ID NO: 23; and
 - 10 ○ a CDR 3 region according to SEQ ID NO: 24;

wherein said antibody is afucosylated.

These afucosylated antibodies with the respective CDR regions include the antibody versions **OS017, OS022, OS023 and OS024**.

15 Another aspect of the present invention relates to an antibody targeting CD163 comprising

- a light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and
 - 20 ○ a CDR 3 region according to SEQ ID NO: 21;
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 15;
 - the heavy chain variable region comprising
 - 25 ▪ a CDR 1 region according to SEQ ID NO: 22;
 - a CDR 2 region according to SEQ ID NO: 23; and
 - a CDR 3 region according to SEQ ID NO: 24;

with the proviso that the heavy chain variable region comprises 72E;

(This version includes **OS018**);

30 or

- a light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and
 - a CDR 3 region according to SEQ ID NO: 25 (LC: G89Q);

- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 15 (HC: D72E);
with the proviso that the heavy chain variable region comprises
- 5 ▪ a CDR 1 region according to SEQ ID NO: 22;
 ▪ a CDR 2 region according to SEQ ID NO: 23; and
 ▪ a CDR 3 region according to SEQ ID NO: 24;
- with the proviso that the heavy chain variable region comprises 72E;
10 (This version includes **OS020**);
or
- a light chain variable region comprising
- 15 ○ a CDR 1 region according to SEQ ID NO: 19;
 ○ a CDR 2 region according to SEQ ID NO: 20; and
 ○ a CDR 3 region according to SEQ ID NO: 25 (LC: G89Q);
- a heavy chain variable region comprising
- 20 ○ a CDR 1 region according to SEQ ID NO: 22;
 ○ a CDR 2 region according to SEQ ID NO: 23; and
 ○ a CDR 3 region according to SEQ ID NO: 24 (wt).
 (This version includes **OS019**)
- Thus, these regular glycosylated antibodies encompass at least **OS018, OS019, and OS020**.

25 Yet another aspect of the present invention is to provide a vector encoding the antibody targeting CD163 according to the invention.

Yet an aspect of the invention relates to a cell expressing the antibody targeting CD163 according to the invention, and/or a cell comprising the vector according to
30 the invention.

A further aspect relates to a composition comprising the antibody targeting CD163 according to the invention, and one or more physiologically acceptable carriers, excipients and/or diluents.

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Yet another aspect of the invention relates to a combination comprising the antibody targeting CD163 according to the invention and an immune checkpoint inhibitor.

- 5 An aspect of the invention relates to the antibody targeting CD163 according to the invention, and/or composition according to the invention and/or combination according to the invention, for use as a medicament.

A related aspect relates to the antibody targeting CD163 according to the
10 invention, and/or composition according to the invention and/or combination according to the invention, for use in the treatment or alleviation of cancer.

Brief description of the figures

Figure 1

- 15 Figure 1 shows surface exposure screen for different known anti-CD163 antibodies. Most commercially available CD163 antibodies bind CD163 expressed on a cell surface poorly (25), we therefore tested a range of commercially available and hybridoma based antibodies developed and selected for binding to CD163 on the cell surface for their surface exposure on CD163 expressing cells as
20 a function of time. The hybridoma based antibodies were a kind gift from Associate Professor Yaseelan Palarasah, University of Southern Denmark. We also compared them to the commercially available antibodies Mac2-158, Mac2-48, R-20 and Ki-M8.
The figure shows staining for IgG bound to the cell surface after incubation for 15-
25 360 minutes.

Figure 2

Figure 2 shows surface exposure for humanized OS001 and ORV3 after binding to CD163 expressing cells as a function of time, as detected by a secondary antibody.

30 Figure 3

Figure 3 shows Surface plasmon resonance study of sCD163 effect on binding of antibodies to immobilized CD163. Comparing effect of soluble CD163 on binding of OS001 (circle), ORV3 (square), 15-5-20 (up-triangle), and 15-5-30 (down

triangle). Response at plateau was normalized relative to response at plateau with no sCD163 present.

Figure 4

Figure 4 shows **(A)** binding of ORV3 and OS001 (both at 1 µg/ml) to human macrophages expressing CD163 (M2c macrophages). Inhibition is calculated with binding in the absence of soluble CD163 as reference. **(B)** Inhibition of CD163 mAb binding to CD163 expressing macrophages using constant concentration of sCD163 of 2 µg/ml and varying mAb concentration.

Figure 5

Figure 5 shows SPR study of binding of 25 nM monomeric CD163 or trimeric CD163 to the CD163 binding mAbs OS001 and ORV3 bound on a Protein G SPR sensor chip.

Figure 6

Figure 6 shows mAb epitope binding. **(A)** First buffer or 100 µg/ml mAb over the chip with immobilized CD163 followed by 5 µg/ml OS001. **(B)** first buffer or 100 µg/ml mAb over the chip with immobilized CD163 followed by 5 µg/ml ORV3.

Figure 7

Figure 7 shows **(A)** Lower inhibition (<25%) of HbHp binding to CD163 on sensor when sensor is preincubated with OS001, whereas binding of HbHp was inhibited more (> 50%) when preincubated with ORV3. **(B)** Preincubating CD163 on sensor with HbHp inhibited binding of antibody substantially less for OS001 than for ORV3.

Figure 8

Figure 8 shows representative titrations of ADCC signalling in reporter system using recombinant cells expressing CD163 as target cells in order to identify preferred method for enhancing ADCC. **(A-C)** ADCC high CD163 (V158). **(D-F)** ADCC low CD163 (F158) for selected antibodies.

Figure 9

Figure 9A-C show representative titrations of ADCP signalling in reporter system using recombinant cells expressing CD163 as target cells in order to identify preferred method for enhancing ADCP for selected antibodies.

Figure 10

Figure 10 shows representative titrations of ADCC signalling in reporter system using human monocyte derived macrophages as target cells. **(A-C)** ADCC high CD163 (V158). **(D-F)** ADCC low CD163 (F158) for selected antibodies.

5 Figure 11

Figure 11 shows a comparison of ADCC induced signalling of OS001 and ORV3 on either **(A+B)** recombinant FlpIn293 cells; or **(C+D)** Human monocyte derived CD163 expressing macrophages (hMDM).

Figure 12

- 10 Figure 12 shows **(A)** titration of antibody induced PMBC mediated macrophage depletion using the two CD163 mAbs OS001 and the ADCC enhanced afucosylated OS017, **(B)** lack of effect of ADCC inert/Fc-silenced construct OS021.

Figure 13

- 15 Figure 13 shows **(A-C)** Titration of effect of OS001, OS017, OS022, OS023 and ORV3 compared to effect of the Fc-silenced CD163 mAb OS022 on macrophage inhibition of T cell proliferation in a patient with 5% NK PBMC cell count, shown as change in replication index. **(D)** Comparison of effect of adding different antibodies at 5 µg/ml and controls on T cell proliferation measured as expansion index, (*= $p < 0.05$, **= $p < 0.01$).

20 Figure 14

Figure 14 shows aggregate levels as percentage of total IgG after expression and initial capture purification step, for antibodies with and without the D72E mutation in the heavy chain, ***: $p < 0.001$ (unpaired t test (two tailed)).

Figure 15

- 25 Figure 15 shows representative surface plasmon resonance sensorgrams of the binding of antibodies to CD163 immobilized CD163. **(A)** OS001, OS018, OS019, and OS020. **(B)** OS017, OS022, and OS023. **(C)** Fab HC wt/LC wt (as found in OS001 and OS017), Fab HC D72E/LC wt (as found in OS018 and OS022), Fab HC wt/LC G89Q (as found OS019 and OS024), and Fab HC D72E /LC G89Q (as found
30 in OS020 and OS023).

Figure 16

Figure 16 shows that the improved sequence constructs OS022 (top) and OS023 (bottom) retain the ability to exert ADCC mediated effects to a similar level as OS017 in ADCC reporter assay.

5 Figure 17

Figure 17 shows the amount of IFN γ released to supernatant after incubating with different CD163 mAbs or PBS control (UT). (**: $p < 0.01$), as measured in the PBMC based depletion assay described in Example 15.

10 The present invention will now be described in more detail in the following.

Detailed description of the invention

Definitions

Prior to discussing the present invention in further details, the following terms and
15 conventions will first be defined:

CD163

As used herein, the term "CD163" (Cluster of Differentiation 163) also known as "M130", "MM130" or "SCAR11" has its general meaning in the art and refers to a protein that in humans is encoded by the *CD163* gene [Gene ID: 9332]. CD163 is
20 exclusively expressed in monocytes and macrophages. It functions as an acute phase-regulated receptor involved in the clearance and endocytosis of hemoglobin/haptoglobin complexes by macrophages, and may thereby protect tissues from free hemoglobin-mediated oxidative damage. This protein may also function as an innate immune sensor for bacteria and inducer of local
25 inflammation, as for example mentioned in the review by Kowal et al. [40]:
"Altogether, studies strongly support the notion that membrane-bound CD163 functions as a receptor for endogenous and exogenous ligands, thereby participating in the initiation and/or perpetuation of the inflammatory response."
The molecular size is 130 kDa. The receptor belongs to the scavenger receptor
30 cysteine rich family type B and consists of a 1048 amino acid residues extracellular domain, a single transmembrane segment and a cytoplasmic tail with several splice variants. An exemplary human amino acid sequence is represented by SEQ ID NO: 26. The extracellular domain of CD163 ranges from the amino acid

residue at position 42 to the amino acid residue 1050 in SEQ ID NO: 26. There are, however, different splice variants of CD163. This sequence (SEQ ID NO: 26) is uniprot seq Q86VB7-1, which is designated as the main variant. But variants Q86VB7-2, Q86VB7-3 and Q86VB7-4 are also described. The extracellular parts
5 are similar, so they also bind OS001 and ORV3 in a similar manner.

Tumor associated macrophage (TAM)

As used herein, the term "tumor associated macrophage" or "TAM" has its general meaning in the art and is intended to describe a type of cell belonging to the macrophage lineage. They are found in close proximity or within tumor masses.
10 TAMs are derived from circulating monocytes or resident tissue macrophages, which form the major leukocytic infiltrate found within the stroma of many tumor types. Accordingly, the term "CD163+ tumor associated macrophages" refers to a subset of TAM characterized by the expression of CD163. In some embodiments, the population of CD163+ tumor associated macrophages of the present invention
15 is further characterized by the expression of and immune-modulatory cytokines such as IL10, Ido1 and Lgals1.

Cancer

As used herein, the term "cancer" has its general meaning in the art and includes, but is not limited to, solid tumors and blood-borne tumors. The term cancer
20 includes diseases of the skin, tissues, organs, bone, cartilage, blood and vessels. The term "cancer" further encompasses both primary and metastatic cancers. Some examples of cancers that may be treated by administration of at least one antibody of the invention include those listed on pages 4 to 6 of WO
2020/058372.

25 Melanoma

As used herein, "melanoma" refers to a condition characterized by the growth of a tumor arising from the melanocytic system of the skin and other organs. Most melanocytes occur in the skin, but are also found in the meninges, digestive tract, lymph nodes and eyes. When melanoma occurs in the skin, it is referred to as
30 cutaneous melanoma. Melanoma can also occur in the eyes and is called ocular or intraocular melanoma. Melanoma occurs rarely in the meninges, the digestive tract, lymph nodes or other areas where melanocytes are found. 40-60 % of melanomas carry an activating mutation in the gene encoding the serine-threonine protein kinase B-RAF (BRAF). Among the BRAF mutations observed in

melanoma, over 90 % are at codon 600, and among these, over 90 % are a single nucleotide mutation resulting in substitution of glutamic acid for valine (BRAFFV600E).

In some embodiments, the subject suffers from melanoma.

5 Treatment

As used herein, the term "treatment" or "treat" refer to both prophylactic or preventive treatment as well as curative or disease modifying treatment, including treatment of patient at risk of contracting the disease or suspected to have contracted the disease as well as patients who are ill or have been diagnosed as
10 suffering from a disease or medical condition, and includes suppression of clinical relapse. The term "treatment" as used herein also includes administration to a patient having a medical disorder or who ultimately may acquire the disorder, in order to prevent, cure, delay the onset of, reduce the severity of, or ameliorate one or more symptoms of a disorder or recurring disorder, or in order to prolong
15 the survival of a patient beyond that expected in the absence of such treatment. By "therapeutic regimen" is meant the pattern of treatment of an illness, e.g., the pattern of dosing used during therapy. A therapeutic regimen may include an induction regimen and a maintenance regimen. The phrase "induction regimen" or "induction period" refers to a therapeutic regimen (or the portion of a therapeutic
20 regimen) that is used for the initial treatment of a disease. The general goal of an induction regimen is to provide a high level of drug to a patient during the initial period of a treatment regimen. An induction regimen may employ (in part or in whole) a "loading regimen", which may include administering a greater dose of the drug than a physician would employ during a maintenance regimen,
25 administering a drug more frequently than a physician would administer the drug during a maintenance regimen, or both. The phrase "maintenance regimen" or "maintenance period" refers to a therapeutic regimen (or the portion of a therapeutic regimen) that is used for the maintenance of a patient during treatment of an illness, e.g., to keep the patient in remission for long periods of
30 time (months or years). A maintenance regimen may employ continuous therapy (e.g., administering a drug at regular intervals, e.g., weekly, monthly, yearly, etc.) or intermittent therapy (e.g., interrupted treatment, intermittent treatment, treatment at relapse, or treatment upon achievement of a particular predetermined criteria [e.g., pain, disease manifestation, etc.]).

Immune checkpoint inhibitor

As used herein, the term "immune checkpoint inhibitor" has its general meaning in the art and refers to any compound inhibiting the function of an immune inhibitory checkpoint protein. As used herein the term "immune checkpoint

5 protein" has its general meaning in the art and refers to a molecule that is expressed by T cells in that either turn up a signal (stimulatory checkpoint molecules) or turn down a signal (inhibitory checkpoint molecules). Immune checkpoint molecules are recognized in the art to constitute immune checkpoint pathways similar to the CTLA-4 and PD-1 dependent pathways (see e.g. Pardoll,

10 2012. Nature Rev Cancer 12:252-264; Mellman et al., 2011. Nature 480:480-489). Examples of inhibitory checkpoint molecules include A2AR, B7-H3, B7-H4, BTLA, CTLA-4, CD277, IDO, KIR, PD-1, LAG-3, TIM-3 and VISTA. Inhibition includes reduction of function and full blockade. Preferred immune checkpoint inhibitors are antibodies that specifically recognize immune checkpoint proteins. A

15 number of immune checkpoint inhibitors are known and in analogy of these known immune checkpoint protein inhibitors, alternative immune checkpoint inhibitors may be developed in the (near) future. The immune checkpoint inhibitors include peptides, antibodies, nucleic acid molecules and small molecules. Examples of immune checkpoint inhibitor includes PD-1 antagonist,

20 PD-L1 antagonist, PD-L2 antagonist CTLA-4 antagonist, VISTA antagonist, TIM-3 antagonist, LAG-3 antagonist, IDO antagonist, KIR2D antagonist, A2AR antagonist, B7-H3 antagonist, B7-H4 antagonist, and BTLA antagonist.

Combination

As used the terms "combination" and "combination therapy" are interchangeable

25 and refer to treatments comprising the administration of at least two compounds administered simultaneously, separately or sequentially. As used herein the term "co-administering" as used herein means a process whereby the combination of at least two compounds is administered to the same patient. The at least two compounds may be administered simultaneously, at essentially the same time, or

30 sequentially. The at least two compounds can be administered separately by means of different vehicles or composition. The at least two compounds can also be administered in the same vehicle or composition (e.g. pharmaceutical composition). The at least two compounds may be administered one or more times and the number of administrations of each component of the combination

35 may be the same or different.

- Such combination therapies, in which administration of one or more antibodies of the invention is suitably (meaning, in suitable doses according to a suitable dosage regimen) combined with administration of one or more other active
- 5 principles, depending on the disease or disorder to be treated. A combination therapy will usually at least provide an additive effect. It is also within the scope of the invention that the use of a combination or combination therapy may result in a synergistic effect.
- 10 For example, as further described herein, when the antibodies of the invention are to be used for the treatment of cancer, it is envisaged that the antibodies of the invention may suitably be used in combination with one or more checkpoint inhibitors (including those referred to herein), such as those mentioned herein and those mentioned on pages 7 to 8 of WO 2020/058372.
- 15 When the antibodies of the invention are to be used for the treatment of inflammatory diseases (including fibrosis associated with an inflammatory disease), it is envisaged that the antibodies of the invention may suitably be used in combination with one or more other anti-inflammatory drugs and/or
- 20 immunomodulatory agents, such as the anti-inflammatory drugs and/or immunomodulatory agents referred to in paragraph [0274] of WO 2022/159349. Other suitable anti-inflammatory drugs or treatments that can be combined with administration of the antibodies of the invention will be clear to the skilled person, and the treating physician will be able to suitably combine (i.e. into suitable
- 25 combined treatment and/or dosage regimen) treatment with such other anti-inflammatory drugs or treatments with treatment with an antibody of the invention, depending on factors of such as the specific disease to be treated, its progression and the condition of the patient to be treated.
- 30 When the antibodies of the invention are to be used for the treatment of fibrosis (which, as mentioned herein, may be primary or secondary fibrosis), it is envisaged that the antibodies of the invention may suitably be used in combination with one or more other anti-fibrotic drugs or treatments known per se, such as those mentioned in paragraph [0273] of WO 2022/159349. Other
- 35 suitable anti-fibrotic drugs or treatments that can be combined with

administration of the antibodies of the invention will be clear to the skilled person, and the treating physician will be able to suitably combine (i.e. into suitable combined treatment and/or dosage regimen) treatment with such other anti-fibrotic drugs or treatments with treatment with an antibody of the invention, 5 depending on factors of such as the specific disease to be treated, its progression and the condition of the patient to be treated.

Monoclonal antibody

The term "monoclonal antibody" refers to an antibody obtained from a population 10 of substantially homogeneous antibodies, wherein each monoclonal antibody will typically recognize a single epitope on the antigen. The term "monoclonal" is not limited to any particular method for making the antibody. For example, monoclonal antibodies of the invention may be made by the hybridoma method as described in Kohler et al. Nature 256, 495 (1975) or may be isolated from phage 15 libraries using the techniques as described herein.

Antigen binding domain

The term "antigen binding domain" or "antigen binding region" or "fragment or derivative thereof" refers to that portion of the selective binding agent (such as an 20 antibody molecule), which contains the amino acid residues that interact with an antigen and confer on the binding agent its specificity and affinity for the antigen. Preferably, the antigen binding region will be of human origin. In other embodiments, the antigen binding region can be derived from other animal species, in particular domestic animal and rodents such as rabbit, rat or hamster.

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As used herein, the term "specificity" has its usual meaning in the art (reference is for example made to the standard handbooks and to paragraph n) on pages 53-56 of WO 08/020079) and generally refers to the number of different types of antigens or antigenic determinants to which a particular antigen-binding molecule 30 or antigen-binding protein (such as an antibody of the invention) can bind. The specificity of an antigen-binding protein can be determined based on affinity and/or avidity, as described on pages 53-56 of WO 08/020079 (incorporated herein by reference), which also describes some preferred techniques for measuring binding between an antigen-binding molecule (such as an antibody of 35 the invention) and the pertinent antigen. Typically, antigen-binding proteins will

bind to their antigen with a dissociation constant (KD) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter (i.e. with an association constant (KA) of 10^5 to 10^{12} liter/moles or more, and preferably 10^7 to 10^{12} liter/moles or more and more preferably 10^8 to 10^{12} liter/moles). Any KD value greater than 10^4 mol/liter (or any KA value lower than 10^4 M⁻¹) liters/mol is generally considered to indicate non-specific binding. Preferably, a monovalent immunoglobulin sequence of the invention will bind to the desired antigen with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM. Specific binding of an antigen-binding protein to an antigen or antigenic determinant can be determined in any suitable manner known per se, including, for example, Scatchard analysis and/or competitive binding assays, such as radioimmunoassays (RIA), enzyme immunoassays (EIA) and sandwich competition assays, and the different variants thereof known per se in the art; as well as the other techniques mentioned herein. As will also be clear to the skilled person, and as described on pages 53-56 of WO 08/020079, the dissociation constant may be the actual or apparent dissociation constant. Methods for determining the dissociation constant will be clear to the skilled person, and for example include the techniques mentioned on pages 53-56 of WO 08/020079.

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Effective amount

The terms "effective amount" and "therapeutically effective amount" when used in relation to an antibody or antigen binding domain, fragment or derivative thereof, immunoreactive with CD163, refer to an amount of a selective binding agent that is useful or necessary to support an observable change in the level of one or more biological activities of CD163.

Sequence identity

In the context of the present invention, the term "sequence identity" or "homologue" indicates a quantitative measure of the degree of homology between two amino acid sequences or between two nucleic acid sequences. If the two sequences to be compared are not of equal length, they must be aligned to give the best possible fit, allowing the insertion of gaps or, alternatively, truncation at the ends of the polypeptide sequences or nucleotide sequences. The sequence

identity can be calculated as $\frac{(N_{ref} - N_{dif})100}{N_{ref}}$, wherein Ndif is the total number of non-

identical residues in the two sequences when aligned and wherein Nref is the number of residues in one of the sequences. Hence, the DNA sequence AGTCAGTC will have a sequence identity of 75% with the sequence AATCAATC (Ndif=2 and Nref=8). A gap is counted as non-identity of the specific residue(s), i.e. the DNA
5 sequence AGTGTC will have a sequence identity of 75% with the DNA sequence AGTCAGTC (Ndif=2 and Nref=8).

With respect to all embodiments of the invention relating to amino acid sequences or nucleotide sequences, the percentage of sequence identity between one or more sequences may also be based on alignments using the clustalW software
10 (<http://www.ebi.ac.uk/clustalW/index.html>) with default settings. For nucleotide sequence alignments these settings are: Alignment=3Dfull, Gap Open 10.00, Gap Ext. 0.20, Gap separation Dist. 4, DNA weight matrix: identity (IUB). For amino acid sequence alignments, the settings are as follows: Alignment=3Dfull, Gap Open 10.00, Gap Ext. 0.20, Gap separation Dist. 4, Protein weight matrix:
15 Gonnet.

Alternatively, nucleotide sequences may be analysed using programme DNASIS Max and the comparison of the sequences may be done at
<http://www.paralign.org/>. This service is based on the two comparison algorithms called Smith-Waterman (SW) and ParAlign. The first algorithm was published by
20 Smith and Waterman (1981) and is a well-established method that finds the optimal local alignment of two sequences. The other algorithm, ParAlign, is a heuristic method for sequence alignment; details on the method are published in Rognes (2001). Default settings for score matrix and Gap penalties as well as E-values were used.

25 K_D

In the present context, the terms "K_D" or "K_D value" refer to the equilibrium dissociation constant between the antibody and its antigen (CD163). The K_D value relates to the concentration of antibody (the amount of antibody needed for a particular experiment) and so the lower the K_D value (lower concentration) and
30 thus the higher the affinity of the antibody. In the present context, K_D is measured by surface plasmon resonance technology using a Biacore T200 instrument.

Antibody-dependent cell-mediated cytotoxicity (ADCC)

As used herein the term "antibody-dependent cell-mediated cytotoxicity" or "ADCC" refer to a cell-mediated reaction in which non-specific cytotoxic cells (e.g., Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody
5 on a target cell and subsequently cause lysis of the target cell. While not wishing to be limited to any particular mechanism of action, these cytotoxic cells that mediate ADCC generally express Fc receptors (FcRs).

As further described herein, in one specific aspect of the invention, an antibody of
10 the invention may be modified in order to increase (its ability to effect) ADCC, and such modified antibodies with increased ADCC form a further aspect of the invention. Suitable methods of techniques for increasing ADCC of an antibody of the invention will be clear to the skilled person based on the disclosure herein, and may for example and without limitation include afucosylation and/or the
15 introduction of one more suitable modifications to the Fc portion that increase ADCC, i.e. compared to the original/corresponding "non-modified" (as defined herein) antibody of the invention. Other suitable techniques known per se for increasing ADCC of an antibody may also be used. Reference is again made to the prior art cited herein; see for example refs. (54) to (58).

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Antibody-dependent cellular phagocytosis (ADCP)

Antibody-dependent cellular phagocytosis (ADCP) is a key MOA for many antibody therapies. It is defined as a highly regulated process in which an antibody eliminates binding target and initiates phagocytosis by linking its Fc domain to a
25 specific receptor on the phagocytic cell. Unlike ADCC, ADCP can mediate monocytes, macrophages, neutrophils and dendritic cells via FcγRIIa, FcγRI and FcγRIIIa, where FcγRIIa (CD32a) on macrophages represents the major pathway. To meet this need, Creative Biolabs has launched a primary cell-based ADCP assay service.

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As further described herein, in one specific aspect of the invention, an antibody of the invention may be modified in order to increase (its ability to effect) ADCP, and such modified antibodies with increased ADCP form a further aspect of the invention. Suitable methods of techniques for increasing ADCP of an antibody of
35 the invention will be clear to the skilled person based on the disclosure herein,

and may for example and without limitation include the introduction of one more suitable modifications to the Fc portion that increase ADCP, i.e. compared to the original/corresponding "non-modified" (as defined herein) antibody of the invention. Other suitable techniques known per se for increasing ADCP of an antibody may also be used. Reference is again made to the prior art cited herein; see for example refs. (56) and (57).

Complement-dependent cytotoxicity (CDC)

As used herein the term "complement-dependent cytotoxicity" or "CDC" refers to the ability of a molecule to initiate complement activation and lyse a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule (e.g., an antibody) complexed with a cognate antigen. To assess complement activation, a CDC assay, e.g., as described in Gazzano-Santaro et al., J. Immunol. Methods, 202: 163 (1996), may be performed.

As further described herein, in one specific aspect of the invention, an antibody of the invention may be modified in order to increase (its ability to effect) CDC, and such modified antibodies with increased CDC form a further aspect of the invention. Suitable methods of techniques for increasing CDC of an antibody of the invention will be clear to the skilled person based on the disclosure herein, and may for example and without limitation include the introduction of one more suitable modifications to the Fc portion that increase CDC, i.e. compared to the original/corresponding "non-modified" (as defined herein) antibody of the invention. Other suitable techniques known per se for increasing CDC of an antibody may also be used. Reference is again made to the prior art cited herein; see for example refs. (54), (55) and (59).

Plasma half-life

The "plasma half-life" (or "half-life" for short) of an antibody of the invention can generally be defined as described in paragraph o) on page 57 of WO 08/020079 and as mentioned therein refers to the time taken for the serum concentration of the antibody to be reduced by 50%, in vivo, for example due to degradation of the sequence or compound and/or clearance or sequestration of the sequence or compound by natural mechanisms. The half-life of an antibody of the invention

can be determined in any manner known per se, such as by pharmacokinetic analysis. Suitable techniques will be clear to the person skilled in the art, and may for example generally be as described in paragraph o) on page 57 of WO 08/020079. As also mentioned in paragraph o) on page 57 of WO 08/020079, the half-life can be expressed using parameters such as the $t_{1/2}$ -alpha, $t_{1/2}$ -beta and the area under the curve (AUC). Reference is for example made to the Experimental Part below, as well as to the standard handbooks, such as Kenneth, A et al: Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists and Peters et al, Pharmacokinetic analysis: A Practical Approach (1996). Reference is also made to "Pharmacokinetics", M Gibaldi & D Perron, published by Marcel Dekker, 2nd Rev. edition (1982). The terms "increase in half-life" or "increased half-life" as also as defined in paragraph o) on page 57 of WO 08/020079 and in particular refer to an increase in the $t_{1/2}$ -beta, either with or without an increase in the $t_{1/2}$ -alpha and/or the AUC or both.

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As further described herein, in one specific aspect of the invention, an antibody of the invention may be modified in order to increase its half-life, and such modified antibodies with increased half-life form a further aspect of the invention. Suitable methods of techniques for increasing the half-life of an antibody of the invention will be clear to the skilled person based on the disclosure herein, and may for example and without limitation include the introduction of one more modifications to the Fc portion that increase half-life, i.e. compared to the original/corresponding "non-modified" (as defined herein) antibody of the invention.

25

Generally, antibodies of the invention that have been modified in order to increase their half-life (i.e. by means of one of the methods and techniques referred to herein) may have a half-life that is increased with more than 1 hour, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the half-life of the corresponding "non-modified" (as defined herein) antibody of the invention (i.e. not containing the half-life extending modifications).

Preferably, an antibody of the invention that has been modified in order to increase its half-life will preferably have a half-life that is at least 2 times,

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preferably at least 4 times, such as at least 5 times, for example at least 8 times or more, greater than the half-life of the corresponding non-modified antibody of the invention.

5 Fc region

As used herein "Fc region" includes the polypeptides comprising the constant region of an antibody excluding the first constant region immunoglobulin domain. Thus, Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE
10 and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM Fc may include the J chain. For IgG, Fc comprises immunoglobulin domains Cgamma2 and Cgamma3 (Cγ2 and Cγ3) and the hinge between Cgamma1 (Cγ1) and Cgamma2 (Cγ2). Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to comprise residues C226 or
15 P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat et al. (1991, NIH Publication 91-3242, National Technical Information Service, Springfield, Va.).

The "EU index as set forth in Kabat" refers to the residue numbering of the human
20 IgG1 EU antibody as described in Kabat et al. supra. Fc may refer to this region in isolation, or this region in the context of an antibody, antibody fragment, or Fc fusion protein. An Fc variant protein may be an antibody, Fc fusion, or any protein or protein domain that comprises an Fc region. Particularly preferred are proteins comprising variant Fc regions, which are non-naturally occurring variants of an Fc
25 region. The amino acid sequence of a non-naturally occurring Fc region (also referred to herein as a "variant Fc region") comprises a substitution, insertion and/or deletion of at least one amino acid residue compared to the wild type amino acid sequence. Any new amino acid residue appearing in the sequence of a variant Fc region as a result of an insertion or substitution may be referred to as a
30 non-naturally occurring amino acid residue. Note: Polymorphisms have been observed at a number of Fc positions, including but not limited to Kabat 270, 272, 312, 315, 356, and 358, and thus slight differences between the presented sequence and sequences in the prior art may exist.

When the Fc portion that is present in the antibodies of the invention is a non-naturally occurring (i.e. synthetic or semi-synthetic) Fc domains, it may in particular be an Fc portion/domain that contains one or more suitable mutations that confer one or more desired properties to the Fc portion and/or to the

5 antibodies of the invention that contains said Fc portion (i.e. compared to an antibody of the invention in which the Fc portion does not contain said mutation(s). Examples of such mutations and their associated properties (such as, without limitation, modulated effector functions or altered half-life) be clear to the skilled person. Reference is for example made to Foss et al. (52), to the review by

10 Wang et al. (53) and the further references cited therein.

In the antibodies of the invention, such mutations may be selected based upon factors such as the desired properties to be conferred on the antibodies of the invention and the intended use(s) of the antibodies of the invention, and such

15 selection will be within the skill of the artisan, also based on the further disclosure herein.

For example, in one aspect of the invention, such mutations may be chosen to increase the plasma half-life of the antibodies of the invention (i.e. compared to

20 the corresponding the antibodies of the invention without said mutations). Again, suitable mutations (which will usually be such that they will increase the affinity of the Fc portion for the neonatal Fc-receptor) will be clear to the skilled person based on the disclosure herein. Reference is again also made to Foss et al. (52) and Wang et al. (53) and to the further references cited therein.

25

Some mutations that according to the invention can in particular be used for increasing the plasma half-life are:

- Introduction of the mutations M252Y/S254T/T256E in the Fc portion (heavy
- 30 chain), for which reference is also made to reference (44). Some preferred, but non-limiting examples of antibodies of the invention include the antibodies OS025, OS026, OS060 and OS061 listed in Table 13;
- Introduction of the mutation M428L/N434S in the Fc portion (heavy chain), for which reference is also made to reference (45). Some preferred, but non-

limiting examples of antibodies of the invention include the antibodies OS027, OS028, OS062 and OS063 listed in Table 13;

- Introduction of the mutation H433K/N434F in the Fc portion (heavy chain), for which reference is also made to reference (46). Some preferred, but non-limiting examples of antibodies of the invention include the antibodies OS029, OS030, OS064 and OS065 listed in Table 13;
- Introduction of the mutation M428L/N434A in the Fc portion (heavy chain), for which reference is also made to reference (47). Some preferred, but non-limiting examples of antibodies of the invention include the antibodies OS031, OS032, OS066 and OS067 listed in Table 13;
- Introduction of the mutation L309D/Q311H/N434S in the Fc portion (heavy chain), for which reference is also made to reference (48). Some preferred, but non-limiting examples of antibodies of the invention include the antibodies OS033, OS034, OS068 and OS069 listed in Table 13;
- Introduction of the mutation T250Q/M428L in the Fc portion (heavy chain), for which reference is also made to reference (49). Some preferred, but non-limiting examples of antibodies of the invention include the antibodies OS0035, OS036, OS070 and OS071 listed in Table 13;
- Introduction of the mutation Q311R/M428L in the Fc portion (heavy chain), for which reference is also made to reference (50). Some preferred, but non-limiting examples of antibodies of the invention include the antibodies OS037, OS038, OS072 and OS073 listed in Table 13;
- Introduction of the mutation N434A in the Fc portion (heavy chain), for which reference is also made to reference (51). Some preferred, but non-limiting examples of antibodies of the invention include the antibodies OS039, OS040, OS075 and OS075 listed in Table 13.

Some representative antibodies of the invention containing such Fc mutations (named OS025 to OS040 and OS050 to OS074) are listed in Table 13, and these antibodies form another preferred but non-limiting aspect of the invention.

The LC sequences (SEQ ID NOs: 27 and 28, which have the same sequences as SEQ ID NO:1 and SEQ ID NO:2, respectively) and HC sequences (SEQ ID NOs: 29 to 44) that are present in these antibodies (and that are representative for LC and

HC sequences that may be present in antibodies of the invention that contain Fc mutations) are listed in Table 14.

In yet another preferred but non-limiting aspect of the invention, the invention
5 relates to an antibody of the invention (as further defined herein) in which the LC
sequence is one of SEQ ID NOs: 27 and 28 or in which the HC sequence is one of
SEQ ID NOs: 29 to 44. In a specific but non-limiting aspect, the invention relates
to an antibody of the invention (as further defined herein) in which the LC
sequence is one of SEQ ID NOs: 27 and 28 and in which the HC sequence is one
10 of SEQ ID NOs: 29 to 44.

In yet another preferred but non-limiting aspect of the invention, the invention
relates to an antibody of the invention (as further defined herein) in which the LC
sequence is one of SEQ ID NOs: 1 and 2 (= SEQ ID NOs: 27 and 28) or in which
15 the HC sequence is one of SEQ ID NOs: 3 to 16 or 29 to 44. In another specific
but non-limiting aspect, the invention relates to an antibody of the invention (as
further defined herein) in which the LC sequence is one of SEQ ID NOs: 27 and 28
and in which the HC sequence is one of SEQ ID NOs: 3 to 16 or 29 to 44.

20 In an embodiment according to the invention, the antibody targeting CD163
comprises one or more modifications in the heavy chain variable region selected
from the group consisting of T250Q, M252Y, S254T, T256E, L309D, Q311H,
Q311R, N434S, M428L, H433K, N434S, N434F, N434A and combinations thereof.

25 In another embodiment according to the invention, the antibody targeting CD163
comprises one or more modifications in the heavy chain variable region selected
from the group consisting of

- M252Y, S254T and T256E;
- M428L and N434S;
- 30 • H433K and N434F;
- M428L and N434A;
- L309D, Q311H and N434S;
- T250Q and M428L;
- Q311R and M428L ; and
- 35 • N434A.

The above antibodies and modifications are explained above and further outlined in Tables 13 and 14.

In yet an embodiment, the antibody targeting CD163 comprises a heavy chain variable region selected from the group consisting of SEQ ID Nos: 29-44, preferably in combination with a light chain variable region selected from SEQ ID NO: 27 or 28. It is to be understood that these antibodies may be afucosylated or having normal focusylation.

10 Fc receptor or FcR

The terms "Fc receptor" or "FcR" are used to describe a receptor that binds to the Fc region of an antibody. The primary cells for mediating ADCC, NK cells, express FcγRIII, whereas monocytes express FcγRI, FcγRII, FcγRIII and/or FcγRIV. FcR expression on hematopoietic cells is summarized in Ravetch and Kinet, Annu. Rev. Immunol., 9:457-92 (1991). To assess ADCC activity of a molecule, an in vitro ADCC assay, such as that described in U.S. Pat. No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecules of interest may be assessed in vivo, e.g., in an animal model such as that disclosed in Clynes et al., Proc. Natl. Acad. Sci. (USA), 95:652-656 (1998). As used herein, the term "Effector cells" are leukocytes which express one or more FcRs and perform effector functions. The cells express at least FcγRI, FcγRII, FcγRIII and/or FcγRIV and carry out ADCC effector function. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils.

In some embodiments, the antibody suitable for depletion of CD163+ TAM is a full-length antibody. In some embodiments, the full-length antibody is an IgG1 antibody. In some embodiments, the full-length antibody is an IgG3 antibody.

30 Afucosylation

Afucosylation is a biochemical phenomenon that involves the alteration of glycoproteins by the removal of fucose residues from their carbohydrate structures.

Fucose is a monosaccharide (a simple sugar) that can be found in various glycans, which are sugar molecules attached to proteins. The absence of fucose residues on certain glycoproteins can have significant biological implications, especially in the immune system.

5 Afucosylation primarily impacts the complex-type N-glycans on glycoproteins. These glycoproteins are often involved in cell signalling, immune responses, and various cellular processes.

One of the most noteworthy consequences of afucosylation is its effect on antibodies. Antibodies, also known as immunoglobulins, play a crucial role in our
10 immune system by recognizing and binding to specific antigens, such as pathogens or abnormal cells. This includes B-cell activation and response. The removal of fucose from the antibody's glycan structure results in what are known as afucosylated antibodies (Pereira NA et al. Afucosylated anti-cancer antibodies with enhanced antibody-dependent cellular cytotoxicity).

15 Therapeutic antibodies with low fucose content may show higher ADCC (Antibody-Dependent Cellular Cytotoxicity), i.e. elevated killing activity against tumorigenic and infected cells.

Afucosylated antibodies according to the invention may be produced using the
20 GlymaxX® technology developed by ProBioGen. Here, antibody plasmids are co-transfected with GDP-6-deoxy-D-lyxo-4-hexulose reductase in order to redirect the intracellular fucose synthesis pathway and produce afucosylated glycan patterns. See also example 2.

Alternatively, they can be expressed in cells where FUT8, the enzyme that add
25 fucose to the glycan structure, has been knocked-out, thus yielding afucosylated antibodies (26-28).

Normal/regular glycosylated antibodies

Antibodies are glycoproteins, and all IgG based antibodies are glycosylated on Asn297. The N-glycan structure linked to Asn297 is generally a biantennary
30 complex oligosaccharide and while the exact structure may vary depending on antibody and expression system, a re-current structure is a fucose sugar near the stem of the structure (29). For the use in the present text, we will discriminate between glycosylation patterns of IgG molecules expressed in cells not being engineered for glycosylation pattern (normal/regular glycosylated) and of IgG

molecules expressed in cell lines that do not put fucose on the glycan structure of IgGs, thus expressing afucosylated IgGs (30).

Non-modified antibodies

In the present context, the term "non-modified" antibody refers to an antibody
5 which has not been modified compared to the modified antibody it is compared to.
For example, a corresponding non-modified antibody compared to the
afucosylated antibodies of the invention, is an antibody having a normal/regular
glycosylation pattern.

Also, a corresponding non-modified antibody compared to the antibodies of the
10 invention having a normal/regular glycosylation pattern, but being sequence
optimized (e.g., by having the G89Q and/or D72E mutation), is an antibody not
being sequence optimized.

Also, with respect to any modifications that can be made to an antibody of the
invention (including but not limited to those mentioned herein, such as
15 afucosylation or making one or more specific mutations, such as mutations to the
Fc portion of an antibody of the invention), although most such modifications are
mentioned herein with respect to achieving a particular effect (such as increasing
half-life, ADCC, ADCP or CDC), it should be noted that it is also within the scope
of the invention that a particular modification (or a particular combination of such
20 modifications) results in improving two or more desired properties of an antibody
of the invention.

Bispecific and multispecific antibodies

Bispecific antibodies are antibodies (or antibody-based proteins or constructs) that
25 contain two distinct binding domains or binding unit so that they can bind to two
different antigens (or to two different epitopes on the same antigen, in which case
they are also referred to as "biparatopic" antibodies) or that have been otherwise
engineered so that they can bind to two different antigens. Similarly, multispecific
antibodies are antibodies (or antibody-based proteins or constructs) that contain
30 two or more (such as two, three or four) distinct binding domains so that they can
bind to two or more (such as two, three or four) different antigens (which may
also suitably include two or more different epitopes on the same antigen) or that
have been otherwise engineered so that they can bind to two different antigens.

As further described herein, the invention also relates to multispecific (and in particular bispecific) antibodies comprising a first binding domain that is capable of binding (and in particular, of specifically binding) to CD163 and at least one other (i.e. a second) binding domain that is capable of binding (and in particular, of specifically binding) to another antigen (i.e. different from CD163), which bispecific antibodies are as further described herein.

In particular, the invention relates to such multispecific (and in particular bispecific) antibodies, in which the second binding domain is capable of binding (and in particular, of specifically binding) to another protein or antigen that is expressed on the surface of a macrophage. Examples of such proteins or antigens will be clear to the skilled person and for example, and preferably, can be one of TREM2, CD206, CSF-1R, CD169, SIGLEC7/9, CLEVER1 or MARCO. Reference is for example made to refs. (68) and (69).

15

Thus, in one specific aspect, the invention relates to such multispecific (and in particular bispecific) antibodies, in which the binding domain that is capable of binding to CD163 comprises:

- a light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and
 - a CDR 3 region according to SEQ ID NO: 21 or 25;
- a heavy chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 22;
 - a CDR 2 region according to SEQ ID NO: 23; and
 - a CDR 3 region according to SEQ ID NO: 24.

In a more specific aspect, the invention relates to such bispecific antibodies, in which the binding domain that is capable of binding to CD163 comprises:

- a light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and
 - a CDR 3 region according to SEQ ID NO: 21 or 25;
- a heavy chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 22;

- a CDR 2 region according to SEQ ID NO: 23; and
- a CDR 3 region according to SEQ ID NO: 24;

and in which the second binding domain is capable of binding (and in particular, of specifically binding) to another protein or antigen that is expressed on the surface

5 of a macrophage.

In a particular aspect, the invention relates to such multispecific (and in particular bispecific) antibodies, in which the binding domain that is capable of binding to CD163 comprises:

- 10 • a light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and
 - a CDR 3 region according to SEQ ID NO: 21 or 25;
- a heavy chain variable region comprising
 - 15 ○ a CDR 1 region according to SEQ ID NO: 22;
 - a CDR 2 region according to SEQ ID NO: 23; and
 - a CDR 3 region according to SEQ ID NO: 24;

and in which the second binding domain is capable of binding (and in particular, of specifically binding) to one of TREM2, CD206, CSF-1R, CD169, SIGLEC7/9,

20 CLEVER1 or MARCO.

In another specific aspect, the invention relates to such multispecific (and in particular bispecific) antibodies, in which the binding domain that is capable of binding to CD163 comprises:

- 25 • a light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and
 - a CDR 3 region according to SEQ ID NO: 21;
- a heavy chain variable region comprising the amino acid sequence of SEQ
30 ID NO: 15, or sequences having at least 90% sequence identity, such as at
least 95% sequence identity to SEQ ID NO: 15;
 - the heavy chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 22;
 - a CDR 2 region according to SEQ ID NO: 23; and
 - 35 ▪ a CDR 3 region according to SEQ ID NO: 24;

with the proviso that the heavy chain variable region comprises 72E;

or

- a light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - 5 ○ a CDR 2 region according to SEQ ID NO: 20; and
 - a CDR 3 region according to SEQ ID NO: 25 (LC: G89Q);
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 15 (HC: D72E);

10 with the proviso that the heavy chain variable region comprises

- a CDR 1 region according to SEQ ID NO: 22;
- a CDR 2 region according to SEQ ID NO: 23; and
- a CDR 3 region according to SEQ ID NO: 24;

with the proviso that the heavy chain variable region comprises 72E;

15 or

- a light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and
 - 20 ○ a CDR 3 region according to SEQ ID NO: 25 (LC: G89Q);
- a heavy chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 22;
 - a CDR 2 region according to SEQ ID NO: 23; and
 - a CDR 3 region according to SEQ ID NO: 24 (wt).

25 For example and without limitation, such bispecific antibodies may have heavy and light chain variable regions which are (essentially) as described herein for the antibodies **OS018, OS019, and OS020 of the invention.**

In a more specific aspect, the invention relates to such multispecific (and in particular bispecific) antibodies, in which the binding domain that is capable of binding to CD163 comprises:

- a light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and
 - 35 ○ a CDR 3 region according to SEQ ID NO: 21;

- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 15;

 - the heavy chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 22;
 - a CDR 2 region according to SEQ ID NO: 23; and
 - a CDR 3 region according to SEQ ID NO: 24;

with the proviso that the heavy chain variable region comprises 72E;
- or
- a light chain variable region comprising

 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and
 - a CDR 3 region according to SEQ ID NO: 25 (LC: G89Q);

• a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 15 (HC: D72E);

with the proviso that the heavy chain variable region comprises

 - a CDR 1 region according to SEQ ID NO: 22;
 - a CDR 2 region according to SEQ ID NO: 23; and
 - a CDR 3 region according to SEQ ID NO: 24;

with the proviso that the heavy chain variable region comprises 72E;
- or
- a light chain variable region comprising

 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and
 - a CDR 3 region according to SEQ ID NO: 25 (LC: G89Q);

• a heavy chain variable region comprising

 - a CDR 1 region according to SEQ ID NO: 22;
 - a CDR 2 region according to SEQ ID NO: 23; and
 - a CDR 3 region according to SEQ ID NO: 24 (wt);

and in which the second binding domain is capable of binding (and in particular, of specifically binding) to another protein or antigen that is expressed on the surface of a macrophage. For example and without limitation, such bispecific antibodies may have heavy and light chain variable regions which are (essentially) as

described herein for the antibodies **OS018, OS019, and OS020 of the invention.**

In a particular aspect, the invention relates to such multispecific (and in particular 5 bispecific) antibodies, in which the binding domain that is capable of binding to CD163 comprises:

- a light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and
 - 10 ○ a CDR 3 region according to SEQ ID NO: 21;
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 15;
 - the heavy chain variable region comprising
 - 15
 - a CDR 1 region according to SEQ ID NO: 22;
 - a CDR 2 region according to SEQ ID NO: 23; and
 - a CDR 3 region according to SEQ ID NO: 24;

with the proviso that the heavy chain variable region comprises 72E;

or

- 20 • a light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and
 - a CDR 3 region according to SEQ ID NO: 25 (LC: G89Q);
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 15 (HC: D72E);
- 25 with the proviso that the heavy chain variable region comprises
 - a CDR 1 region according to SEQ ID NO: 22;
 - a CDR 2 region according to SEQ ID NO: 23; and
 - 30 ▪ a CDR 3 region according to SEQ ID NO: 24;

with the proviso that the heavy chain variable region comprises 72E;

or

- a light chain variable region comprising
- 35
 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and

- a CDR 3 region according to SEQ ID NO: 25 (LC: G89Q);
 - a heavy chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 22;
 - a CDR 2 region according to SEQ ID NO: 23; and
- 5 ○ a CDR 3 region according to SEQ ID NO: 24 (wt);
- and in which the second binding domain is capable of binding (and in particular, of specifically binding) to one of TREM2, CD206, CSF-1R, CD169, SIGLEC7/9, CLEVER1 or MARCO. For example and without limitation, such bispecific antibodies may have heavy and light chain variable regions which are (essentially) as
- 10 described herein for the antibodies **OS018**, **OS019**, and **OS020** of the invention.

The bispecific and multispecific antibodies provided by the invention may also be modified in order to increase their half-life (i.e. compared to the corresponding “non-modified” bispecific antibody), for example by means of afucosylation, by

15 means of suitably introducing one or more mutations in the Fc region of the bispecific antibody (if the bispecific antibody contains an Fc region that can be mutated/modified for increased half-life) or by another suitable method or technique referred to herein.

20 The bispecific and multispecific antibodies provided by the invention may also be modified in order to increase (their ability to effect) ADCC, ADCP and/or CDC (and in particular ADCC), for example by means of one of the methods and techniques described herein for the monospecific antibodies of the invention, such as suitably introducing one or more mutations in the Fc region of the bispecific antibody that

25 increase ADCC, ADCP and/or CDC, respectively (if the bispecific antibody contains an Fc region that can be mutated/modified for increased ADCC, ADCP and/or CDC, respectively).

In one particular aspect, a bi- or multispecific antibody of the invention may

30 include a binding domain or binding unit that is a so-called immune cell engager, i.e. a binding domain, binding unit or other suitable moiety that is capable of binding to an immune effector cell (such as a T-cell or NK-cell) and that in particular is capable of binding to an immune effector cell such that at least one biological function of said immune effector cell is activated or increased. As will be

35 clear to the skilled person, usually, such a binding domain or binding unit will be

such that it is capable of binding to an activating receptor on said immune effector cell. Reference is for example made to the reviews cited herein as references (62), (64), (65) and (66) as well as for example reference (63).

- 5 For example, suitable binding domains that can function as immune cell engagers may bind to activating receptors on T-cells such as CD3, CD19 or CD20 or to activating receptors on NK-cells such as CD16a or CD56. Based upon the disclosure and references cited herein (such as references (62) to (66), the skilled person will be able to select binding domains or binding units that are suitable for
10 this purpose and suitably include such a suitable binding domain or binding unit into a bispecific or multispecific antibody of the invention.

Other terms used herein

Terms that are not explicitly defined herein will have their common meaning in the art, for which reference is made to the standard handbooks as well as for
15 example WO 2020/058372 and WO 2021/016128.

Afucosylated anti-CD163 antibodies

As outlined in the example section, the inventing team have identified versions of anti-CD163 antibodies, which have superior properties in relation to the induction
20 of ADCC. Without being bound by theory, the effect could be explained by a unique epitope on CD163, allowing the antibody to bind efficiently to trimers of CD163, whereas binding to monomers of CD163 is limited (see example 5).

Thus, an aspect of the invention relates to an antibody targeting CD163
25 comprising

- a light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and
 - a CDR 3 region according to SEQ ID NO: 21 (wt) or 25 (G89Q);
- 30 • a heavy chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 22;
 - a CDR 2 region according to SEQ ID NO: 23; and
 - a CDR 3 region according to SEQ ID NO: 24;

wherein said antibody is afucosylated.

These afucosylated antibodies with the respective CDR regions includes the antibody versions **OS017, OS022, OS023 and OS024**. "wt" indicates the sequence of OS001 from which these afucosylated antibodies are derived. "G89Q" indicates that for the CDR3 region in the light chain region a "G" to "Q" mutation
5 at position 89 has been introduced compared to OS001. The statements in brackets are not to be seen as limiting for the embodiments, and are only inserted for information purposes.

The afucosylated antibodies may be further defined by their variable regions with
10 some extent of variation. Thus, in an embodiment, the antibody targeting CD163 comprises,

- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 1, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 1 (wt); and
15
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 4, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 4 (wt);

with the proviso that the light chain variable region comprises 89G.

20 This version includes **OS017**.

In an embodiment, the antibody targeting CD163 comprises,

- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 1, or sequences having at least 90% sequence identity, such as at
25 least 95% sequence identity to SEQ ID NO: 1 (wt); and
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 15 [HC: D72E];

30 with the proviso that the heavy chain variable region comprises 72E.

This version includes **OS022**. "D72E" indicates that for the heavy chain region a "D" to "E" mutation at position 72 has been introduced compared to OS001.

In an embodiment, the antibody targeting CD163 comprises

- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 2 (LC: G89Q); and
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 15 (HC: D72E);

with the proviso that the light chain variable region comprises 89Q; and
with the proviso that the heavy chain variable region comprises 72E.

10 This version includes **OS023**.

In an embodiment, the antibody targeting CD163 comprises

- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 2 (LC: G89Q); and
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 4, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 4 (wt);

20 with the proviso that the light chain variable region comprises 89Q.

This version includes **OS024**.

The afucosylated antibodies may also be defined by their specific variable regions.
Thus, in an embodiment, the antibody targeting CD163 comprises

- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 1 (wt); and
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 4 (wt); **[OS017]**

or

- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 1 [wt]; and
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15 [HC: D72E]; **[OS022]**

or

- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2 [LC: G89Q]; and
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15 [HC: D72E]; **[OS023]**

5 or

- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2 [LC: G89Q]; and
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 4 [wt]; **[OS024]**

10

It is noted that for the light chain variable regions according to the invention (such as SEQ ID NOs: 1, 2, 27 and 28), the amino acid fragment 1-29 is a signal peptide, which may be removed during production of the final antibody and the uses thereof. Further, the indicated numbering such as 89Q is counted without this signal peptide.

Similarly, for the heavy chain variable regions according to the invention (SEQ ID NOs: 3-16 or 29-44, including SEQ ID NO: 15), the amino acid fragment 1-19 is a signal peptide, which may be removed during production of the final antibody and the subsequent uses thereof. Further, the indicated numbering such as 72E is counted without this signal peptide.

The skilled person is well aware of the use of signal peptides during antibody production and may also select different signal peptides and achieve a similar effect.

Thus, in embodiments of the invention the light chain variable region comprises the amino acid sequence of SEQ ID NO: 1, 2, 27 or 28 without amino acid 1-29, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 1, 2, 27 or 28 without amino acid 1-29.

In other embodiments of the invention the heavy chain variable region comprises the amino acid sequence of any of SEQ ID NO's: 3-16 or 29-44 without amino acid 1-19 or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO's: 3-16 or 29-44 without amino acid 1-19.

In a preferred embodiment, the heavy chain variable region comprises the amino acid sequence of any SEQ ID NO: 15 without amino acid 1-19, or a sequence having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO's: 3-16 or 27-44 without amino acid 1-19.

5

It is to be understood that for sequences without the signal peptide, sequence similarities are counted for sequences without signal peptides.

It is also to be understood that the embodiments relating to heavy and light chain variable regions without signal peptides also apply to the regular glycosylated anti-CD163 antibodies further outlined below.

Regular glycosylated anti-CD163 antibodies

As outlined in the example section, the inventing team has also identified modified versions of specific anti-CD163 antibodies with improved properties. These antibodies may be regular glycosylated. Thus, another aspect of the invention relates to an antibody targeting CD163 comprising

- a light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and
 - a CDR 3 region according to SEQ ID NO: 21 (wt);
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 15 (HC: D72E);
 - the heavy chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 22;
 - a CDR 2 region according to SEQ ID NO: 23; and
 - a CDR 3 region according to SEQ ID NO: 24.

with the proviso that the heavy chain variable region comprises 72E
(This version includes **OS018**);

or

- a light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and
 - a CDR 3 region according to SEQ ID NO: 25 (LC: G89Q);

- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 15 (HC: D72E); with the proviso that the heavy chain variable region comprises

5 ▪ a CDR 1 region according to SEQ ID NO: 22;

 ▪ a CDR 2 region according to SEQ ID NO: 23; and

 ▪ a CDR 3 region according to SEQ ID NO: 24;;

with the proviso that the heavy chain variable region comprises 72E (This version includes **OS020**);

10 or
- a light chain variable region comprising

 ○ a CDR 1 region according to SEQ ID NO: 19;

 ○ a CDR 2 region according to SEQ ID NO: 20; and

15 ○ a CDR 3 region according to SEQ ID NO: 25 (LC: G89Q);

• a heavy chain variable region comprising

 ○ a CDR 1 region according to SEQ ID NO: 22;

 ○ a CDR 2 region according to SEQ ID NO: 23; and

 ○ a CDR 3 region according to SEQ ID NO: 24 (wt).

20 (This version includes **OS019**)

These regular glycosylated anti-CD163 antibodies may be further defined by their variable regions with some variance. Thus, in an embodiment, the antibody targeting CD163, comprises

- 25 • a light chain variable region comprising the amino acid sequence of SEQ ID NO: 1, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 1 (LC: G89Q); and
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at
- 30 least 95% sequence identity to SEQ ID NO: 15 (HC: D72E);
- with the proviso that the light chain variable region comprises 89G; and
- with the proviso that the heavy chain variable region comprises 72E.
- This version includes **OS018**.

35 In an embodiment, the antibody targeting CD163, comprises

- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 2 (LC: G89Q); and
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 15 (HC: D72E);
with the proviso that the light chain variable region comprises 89Q; and
with the proviso that the heavy chain variable region comprises 72E.
This version includes **OS020**.

10

In an embodiment, the antibody targeting CD163, comprises

- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 1, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 2 (LC: G89Q); and
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 4, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 4 (wt);
with the proviso that the light chain variable region comprises 89Q.
This version includes **OS019**.

20

These regular glycosylated anti-CD163 antibodies may also be defined by their specific variable regions. Thus, in an embodiment, the antibody targeting CD163 comprises

- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 1 (wt); and
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15 (HC: D72E); (This version includes **OS018**)

or

- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2 (LC: G89Q); and
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15 (HC: D72E); (This version includes **OS020**)

or

- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2 (LC: G89Q); and

- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 4 (wt). (This version includes **OS019**)

As described above, not all of the antibodies must be afucosylated to show improved properties. For example, the G89Q variant, the D72E and the variant with the combination of G89Q and D72E show improved properties without being afucosylated. For example, example 13 shows that these variants have increased affinity for CD163. Thus, in an embodiment, the antibodies have regular/normal glycosylation, such as not being afucosylated.

10

The antibodies may be provided in different formats. Thus, in an embodiment, the antibody is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, an antibody, wherein the heavy chain and the light chain are connected by a flexible linker, an Fv molecule, an antigen binding fragment, a Fab fragment, a Fab' fragment, a F(ab')₂ molecule, a fully human antibody, a humanized antibody, and a chimeric antibody.

In yet an embodiment, the antibody targeting CD163 is a F(ab')₂ molecule.

20 In yet another embodiment, the antibody is humanized, preferably a humanized monoclonal antibody.

Albeit the antibodies may induce ADCC, it may be speculated to attach a cytotoxic moiety to increase the efficacy as a drug. Thus, in an embodiment, the antibody targeting CD163 is coupled to a detectable label or a substance having (cyto)toxic or therapeutic activity. Suitable cytotoxic moieties (which can generally be defined as a moiety which *"is capable of inducing cell death in vivo or in vitro, for example when administered to a patient"* (see for example WO 2011/039510, page 30) and suitable linkers and techniques that can be used to link such moieties to an antibody of the invention will be clear to the skilled person (reference is for example made to the reviews cited as references (60) and (61)), and for example but without limitation include the cytotoxic moieties that have already been suggested in the art for antibody-drug conjugates comprising binding/targeting moiety directed towards CD163. Reference is for example made to WO 2011/039510, pages 31-32 and to WO 2020/058372, pages 25 to 29.

However, since the antibodies according to the invention can induce ADCC there may not be a need for coupling cytotoxic moieties. Thus, in an embodiment, the antibody targeting CD163 is free from a cytotoxic moiety and/or not being an
5 antibody-drug conjugate.

Example 13 shows that the antibodies have strong affinity for CD163. Thus, in an embodiment, the antibody targeting CD163 has a K_D value to CD163 below $1 \cdot 10^{-7}$, such as below $1 \cdot 10^{-8}$, or such as below $1 \cdot 10^{-9}$ M, or such as in the range $1 \cdot 10^{-7}$
10 to $1 \cdot 10^{-12}$ M, such as in the range $1 \cdot 10^{-7}$ to $1 \cdot 10^{-10}$ M. Preferably, said K_D is lower than for the corresponding non-modified antibody (OS001). Similar or slightly higher K_D may also be acceptable, since other factors may be more important.

15 Example 12 shows that certain variants of the antibodies show lower levels of aggregation than the antibodies from which they were derived. In particular, the variants containing the HC D72E mutation shows surprisingly low levels of aggregation. Thus, in an embodiment, the antibody targeting CD163 is primarily in a monomeric form, such as having lower levels of aggregation than the
20 corresponding non-modified antibody, such as OS001, such as below 10% aggregation, such as below 8% such as below, preferably 4%, more preferably below 2% aggregation.

Level of aggregation can be reduced by purification. An advantage of the HC D72E mutation is that this task is easier and less will be lost. Thus, in an embodiment,
25 the level of aggregation is determined after purification, such as after protein A and/or protein G purification.

As previously explained (see also examples 7, 8, 10 and 11), the antibodies according to the invention is able to effectively induce ADCC. Thus, in an
30 embodiment, the antibody targeting CD163 is capable of inducing ADCC, preferably to a higher extent than the corresponding non-modified antibody, such as OS001, such as at least 5 times higher, such as at least 10 times higher than the corresponding non-modified antibody, preferably at least 50 times higher and even more preferably at least 100 times higher than the corresponding non-
35 modified antibody. Preferably ADCC induction is measured in the CD16 reporter

assay described in example 8 using human monocyte derived macrophages as target cells.

Albeit the antibodies can induce ADCC, it may also be important that the antibodies maintain the ability to induce ADCP. As shown in example 7, the antibodies according to the invention can indeed mediate ADCP. Thus, in an embodiment, the antibody targeting CD163 is capable of inducing ADCP, preferably to a level similar to the corresponding non-modified antibody, such as OS001, such as at least to a level of 70% of the corresponding non-modified antibody, such as at least 75%, preferably at least 80%. Preferably ADCP induction is measured as measured in the CD32 (ADCP) reporter assay described in example 7.

Example 9 shows that the antibodies have a good thermal stability. Thus, in an embodiment, the antibody targeting CD163 has a thermal stability similar to or higher than the corresponding non-modified antibody, such as OS001, such as at least to a level of 70% of the corresponding non-modified antibody, such as OS001, such as at least 75%, preferably at least 80%.

Example 4 shows that the antibodies according to the invention bind stronger to trimers of CD163 than to monomers of CD163. This is considered an important feature of the antibodies. Thus, in an embodiment, said antibody is able to bind to trimers of CD163 with a higher affinity than monomers of CD163, such as giving a higher signal for the trimeric CD163 than the monomeric CD163, such as at least 50% higher signal, such as at least 100% higher signal, preferably when binding is studied on the SPR as described in example 4.

Thus, without being limited to any explanation or hypothesis, for antibodies that are to be used for therapeutic purposes, and in particular for the treatment of cancer, it may be preferable to use (monoclonal) antibodies against CD163 that bind CD163 in its trimeric form (and in particular in the trimeric form that is present on the surface of a cell, such as the surface of a TAM) with a higher affinity than they bind the monomeric form of CD163 that is present in circulation.

As can be seen from the further disclosure herein, in one specific aspect, the invention provides such (monoclonal) antibodies. See e.g. Example 4 and Figure 5.

- 5 In an embodiment, the antibody targeting CD163 according to the present invention comprises one or more modifications that improves ADCC.

According to a further aspect of the invention, these antibodies of the invention are used in the treatment of cancer or other diseases (as further described
10 herein). According to yet another aspect of the invention, these antibodies of the invention are used for depletion of TAMs expressing (trimeric) CD163 on their surface, in particular by means of ADCC or other mechanisms of cell depletion (as further described herein).

- 15 Thus, without being limited to any particular theory, mechanism or hypothesis, according to one preferred aspect of the invention, the invention relates to antibodies binding CD163 that are such that they bind CD163 that is present on the cell surface with higher affinity than CD163 that is present in circulation (i.e. in blood or plasma).

20

According to a specific aspect, the antibodies of according to this aspect of the invention are preferably such that they bind the trimeric form of CD163 that is present on the surface of a cell (and in particular the trimeric form CD163 that is present on the surface of a TAM) with higher affinity than they bind to monomeric
25 CD163.

According to an embodiment, the antibodies of the invention are such that they bind trimers of CD163 with a higher affinity than monomers of CD163, as determined by SPR (for example using the SPR method that is used in example
30 4).

According to another embodiment, the antibodies of the invention are such that they bind on an epitope that is present on the trimeric form of CD163 which epitope is not formed, or is not accessible, when CD163 is in its monomeric form.
35

According to a further aspect, the antibodies of the invention are such that they bind on an epitope that is present on the trimeric form of CD163, which epitope is formed by at least two, such as two or three, of the CD163 molecules that form the CD163 trimer.

5

According to yet another aspect, the antibodies of the invention are such that they bind on an epitope that is present on the trimeric form of CD163, which epitope is formed by two, such as two or three, of the CD163 molecules that form the CD163 trimer.

10

According to yet another aspect, the antibodies of the invention are such that they bind on an epitope that is present on the trimeric form of CD163, which epitope is formed by all three of the CD163 molecules that form the CD163 trimer.

15 According to yet another aspect, the antibodies of the invention are such that they bind to an epitope on trimeric CD163 that is (essentially) not recognized by the known CD163 antibodies ORV3, 15-5-20 and 15-5-30.

According to yet another specific but non-limiting aspect, the antibodies of the
20 invention are such that they (essentially) do not compete for binding to trimeric CD163 with the antibody ORV3.

According to yet another aspect, the antibodies of the invention are such that they (essentially) do not compete for binding to trimeric CD163 with the antibodies
25 ORV3, 15-5-20 and 15-5-30.

According to yet another aspect, the antibodies of the invention are such that at least 50% of the antibodies remain bound to trimeric CD163 in the presence of monomeric (i.e. soluble) CD163, in particular at a physiologically relevant
30 concentration of sCD163, such as around 2 µg/ml of sCD163. In particular, the antibodies of the invention are such that at least 50% of the antibodies remain bound to trimeric CD163 that has been immobilized on a carrier (such as a carrier that can be suitably used in a method for measuring binding in the presence of sCD163) in the presence of monomeric (i.e. soluble) CD163, in particular at a
35 physiologically relevant concentration of sCD163, such as around 2 µg/ml of

sCD163. This can for example be determined by means of the method used in Example 5.

According to yet another aspect, the antibodies of the invention are such that they
5 are able to bind to trimers of CD163 with a higher affinity than monomers of CD163, such as giving a higher signal for the trimeric CD163 than the monomeric CD163, such as at least 50% higher signal, such as at least 100% higher signal, preferably when binding is studied on the SPR as described in example 4.

10 Preferably the antibody binds trimeric CD163 with an affinity that is 5 times higher than for the monomer, alternatively 10 times higher, 20 times higher or 50 times higher.

According to yet another aspect of the invention, which is as further described
15 herein, the antibodies of the invention are further such that they comprise an Fc portion that, compared to a naturally occurring Fc portion, has been modified so as to have improved ADCC capabilities (as further described herein). Examples of such modifications are those that are referred to herein and other suitable modifications will be clear to the skilled person based on the disclosure herein.

20

Vector

An aspect of the invention relates to a vector or vectors encoding the antibody targeting CD163 according to the invention. In one embodiment, LC and HC are encoded on the same vector. In another embodiment, the LC is expressed from
25 one vector and the HC is expressed from another vector.

Cell

Yet an aspect of the invention relates to a cell expressing the antibody targeting CD163 according to the invention, and/or a cell comprising the vector or vectors
30 according to the invention.

Composition

The antibodies according to the invention may form part of a composition. Thus, a further aspect relates to a composition comprising the antibody targeting CD163

according to the invention, and one or more physiologically acceptable carriers, excipients and/or diluents.

In an embodiment, the composition is a pharmaceutical composition.

5

Combination

The antibodies according to the invention may form part of a combination. Thus, an yet another aspect of the invention relates to a combination comprising the antibody targeting CD163 according to the invention and an immune checkpoint
10 inhibitor, which combination may in particular be used for the treatment of cancer. Suitable checkpoint inhibitors will be clear to the skilled person and for example and without limitation may include checkpoint inhibitors that have been suggested in the art for use in combination with antibodies, antibody-drug
conjugates or other active principles targeting CD163. Reference is for example
15 made to WO 2020/058372, pages 7 to 10, where some suitable examples are listed.

In an embodiment, the immune checkpoint inhibitor is selected from the group consisting of PD-1 antagonists, PD-L1 antagonists, PD-L2 antagonists, CTLA-4
20 antagonists, VISTA antagonists, TIM-3 antagonists, LAG-3 antagonists, IDO antagonists, KIR2D antagonists, A2AR antagonists, B7-H3 antagonists, B7-H4 antagonists, and BTLA antagonists.

It is also envisaged that, depending on factors such as the particular cancer to be
25 treated and its progression, that the antibodies of the invention may suitably be combined (i.e. as part of a combination therapy) with other drugs or active principles used for the treatment of cancer and/or with other cancer treatments, such as one or more of surgery, chemotherapy, radiation therapy, cryotherapy, hormonal therapy, immunotherapy and cytokine therapy.

30

Medical uses

An aspect of the invention relates to the antibody targeting CD163 according to the invention, and/or composition according to the invention and/or combination according to the invention, for use as a medicament.

35

Use in cancer

A related aspect relates to the antibody targeting CD163 according to the invention, and/or composition according to the invention and/or combination according to the invention, for use in the treatment or alleviation of cancer.

5

In an embodiment, the cancer is selected from the group consisting of solid tumours and blood-borne tumours.

- In another embodiment, the cancer is selected from the group consisting of
- 10 cancer cells from the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestinal tract, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, prostate, skin, stomach, testis, tongue, or uterus. In addition, the cancer may specifically be of the following histological type, though it is not limited to these: neoplasm, malignant; carcinoma; carcinoma, undifferentiated;
- 15 giant and spindle cell carcinoma; small cell carcinoma; papillary carcinoma; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and
- 20 cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial polyposis coli; solid carcinoma; carcinoid tumor, malignant; bronchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma;
- 25 granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous; adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma;
- 30 papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; Paget's disease, mammary; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma w/squamous metaplasia; thymoma, malignant; ovarian stromal tumor, malignant;
- 35 thecoma, malignant; granulosa cell tumor, malignant; and robblastoma, malignant;

Sertoli cell carcinoma; Leydig cell tumor, malignant; lipid cell tumor, malignant; paraganglioma, malignant; extra-mammary paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; malignant melanoma in giant

5 pigmented nevus; epithelioid cell melanoma; blue nevus, malignant; sarcoma; fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma; liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; mullerian mixed tumor; nephroblastoma; hepatoblastoma; carcinosarcoma; mesenchymoma,

10 malignant; brenner tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant; dysgerminoma; embryonal carcinoma; teratoma, malignant; struma ovarii, malignant; choriocarcinoma; mesonephroma, malignant; hemangiosarcoma; hemangioendothelioma, malignant; kaposi's sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma; osteosarcoma;

15 juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant; mesenchymal chondrosarcoma; giant cell tumor of bone; Ewing's sarcoma; odontogenic tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic fibrosarcoma; pinealoma, malignant; chordoma; glioma, malignant; ependymoma; astrocytoma; protoplasmic astrocytoma; fibrillary

20 astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma; ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor; meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor, malignant; malignant lymphoma; Hodgkin's disease;

25 Hodgkin's lymphoma; paragranuloma; malignant lymphoma, small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular; mycosis fungoides; other specified non-Hodgkin's lymphomas; malignant histiocytosis; multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma cell leukemia; erythroleukemia;

30 lymphosarcoma cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; and hairy cell leukemia.

Example 10 shows ADCC mediated depletion of CD163 expressing macrophages,

35 Thus, in an embodiment, the antibody targeting CD163 is administered to a

subject at a therapeutically effective amount capable of depleting the population of CD163+ tumor associated macrophages.

In a related embodiment, the antibody targeting CD163 is administered to a
5 subject at a therapeutically effective amount capable of depleting the population of CD163+ tumor associated macrophages and increasing the amount of tumor infiltrating CD8+ T cells.

In yet an embodiment, the cancer is resistant to checkpoint therapy.
10

In yet a further embodiment, the cancer is characterized by a low tumour infiltration of CD8+ T cells and/or a high tumour infiltration of CD163+ tumour associated macrophages. In the present context "low tumour infiltration of CD8+ T cells" refers e.g. to lower CD8+ T cells than average patients in the same cancer
15 group. Also, in the present context "a high tumour infiltration of CD163+ tumour associated macrophages" refers e.g. to an CD163+ count higher than average patient in same patient group.

Use in inflammatory diseases and fibrotic diseases

20 Given the role that (macrophages expressing) CD163 is known to play in inflammatory and fibrotic diseases, it is expected that the antibodies of the invention may also find use in the treatment of inflammatory diseases. Such inflammatory diseases may be acute or chronic inflammatory diseases (including fibrotic diseases) and may also be auto-immune diseases (and in particular,
25 chronic auto-immune diseases), such as one of the auto-immune diseases mentioned in paragraph [009] of in WO2022/159349.

It is also envisaged that the antibodies of the invention may be used in the prevention or reduction of the damage that inflammatory diseases may cause to
30 cells or tissues of the human body. For example, it is envisaged that the antibodies of the invention maybe used to prevent fibrosis, reduce and/or ameliorate fibrosis or reduce the progression of fibrosis. Thus, it is also envisaged that the antibodies of the invention may be used in the prevention or treatment of fibrotic diseases, in particular to prevent or delay, to alleviate, or to arrest or
35 inhibit the development of fibrosis.

- Examples of inflammatory and/or fibrotic diseases that can be treated and/or prevented using the antibodies described herein will be clear to the skilled person based on the further disclosure herein, and for example and without limitation
- 5 include the diseases mentioned in the review by Kowal et (40) and the diseases and disorders mentioned in WO2022/159349 (41, see for example paragraphs [0009] to [0011], paragraphs [0235] to [0240] and paragraphs [0261] to [0274]).
- 10 In one specific but non-limiting embodiment the condition or disorder to be treated may be selected from the group consisting of: arthritic diseases (such as rheumatoid arthritis, spondylitis, osteoarthritis); chronic inflammatory bowel disease (IBD, such as Crohn's disease, ulcerative colitis); peridontitis; psoriasis; asthma; systemic lupus erythematosus; multiple sclerosis; autoimmune chronic
- 15 inflammatory diseases; IgG4-related diseases, mixed connective tissue disease; autoimmune liver disease (such as biliary cirrhosis); sepsis; hemophagocytic syndrome; liver disease; liver failure; hepatitis; atherosclerosis; diabetes, obesity, non-alcoholic fatty liver disease, joint inflammation, inflammation-induced cartilage destruction, macrophage activation syndrome, hemophagocytic
- 20 lymphohistiocytosis, pulmonary fibrosis, scleroderma, colon stenosis, as well as conditions presenting with primary or secondary organ fibrosis including liver, renal, skin and lung.

It will also be clear to the skilled person that fibrosis may occur on its own (also

25 called "primary fibrosis" or "idiopathic fibrosis") or as a consequence or result of an underlying disease or disorders (with such fibrosis that is associated with another, underlying disease also being referred to as "secondary" fibrosis), and it is generally within the scope of the invention that the antibodies of the invention may be used for the prevention and treatment of primary fibrosis, for the

30 prevention and treatment of secondary fibrosis, for the prevention and treatment of an underlying disease (and in particular an underlying inflammatory disease) that causes or is otherwise associated with secondary fibrosis, and/or for the prevention and treatment of both secondary fibrosis and the underlying disease with which it is associated (and all of the foregoing is included in the terminology

35 "prevention and/or treatment of fibrosis" as used herein).

In each of these cases, the fibrosis may in particular be fibrosis that is associated with the presence of M2 macrophages (or an increased level of M2 macrophages, in particular in the affected tissues) and/or fibrosis that may be prevented or
5 treated (as defined herein) by depletion of CD163+ macrophages, in particular in the affected tissues.

As is known to the skilled person, fibrosis can occur in many tissues within the body, typically as a result of inflammation or damage. Common sites of fibrosis
10 include the lungs, liver, kidneys, brain, and heart. Accordingly, the antibodies of the invention may be used for the prevention and/or treatment of fibrosis (and in particular, fibrosis associated with an undesired and/or deleterious presence or activity of macrophages, such as M2 macrophages and in particular CD163+ macrophages) in any part, tissue or organ in which fibrosis may occur, such as
15 the lungs, skin, connective tissues, liver, heart, kidney, retina, and other tissues (in particular soft tissues). Other examples of organs, tissues or parts of the body where fibrosis may occur, as well as the type (s) of fibrosis that may occur in such organs, tissues or parts of the body, will be clear to the skilled person. Reference is for example made to the further disclosure and references cited herein, such as
20 paragraphs [0266] to [0272] of the International application WO 2022/159349. For example and without limitation, the antibodies of the invention may be used for the prevention and/or treatment of fibrosis of the lung, such as fibrothorax, pulmonary fibrosis including cystic fibrosis and idiopathic pulmonary fibrosis (idiopathic meaning 'of unknown cause'), radiation-induced lung injury (following
25 radiation therapies commonly used to treat cancer); fibrosis of the liver including cirrhosis; fibrosis of the kidney fibrosis including glomerulosclerosis, tubulointerstitial fibrosis, tubular atrophy and dilation as well as and rarefaction of the glomerular and peritubular capillaries; fibrosis of the brain; fibrosis of the heart including interstitial fibrosis and replacement fibrosis; as well as other
30 fibrotic conditions such as arterial stiffness; arthrofibrosis (for example affecting the knee, shoulder or other joints); chronic kidney disease; Crohn's disease and other fibrotic diseases affecting the intestine; Dupuytren's contracture (for example affecting the hands or fingers); keloid fibrosis and other fibrotic diseases affecting the skin such as nephrogenic systemic fibrosis; lipedema (characterised
35 by increased nodular and fibrotic adipose tissue, typically in lower limbs);

mediastinal fibrosis (i.e. affecting soft tissue of the mediastinum); myelofibrosis (i.e. affecting bone marrow); myofibrosis (i.e. affecting skeletal muscles); Peyronie's disease (i.e. affecting the penis); progressive massive fibrosis (i.e. affecting the lungs, for example as a result of pneumoconiosis); retroperitoneal
5 fibrosis (i.e. affecting soft tissue of the retroperitoneum); fibrotic forms of adhesive capsulitis (shoulder); as well as scleroderma/systemic sclerosis (i.e. affecting the skin or lungs).

Thus, a related aspect of the invention relates to the antibody targeting CD163
10 according to the invention, and/or composition according to the invention and/or combination according to the invention, for use in the treatment or alleviation of an inflammatory disease.

Another related aspect of the invention relates to the antibody targeting CD163
15 according to the invention, and/or composition according to the invention and/or combination according to the invention, for use in the treatment or alleviation of a fibrotic disease.

Yet another related aspect of the invention relates to the antibody targeting
20 CD163 according to the invention, and/or composition according to the invention and/or combination according to the invention, for use in the treatment or alleviation of an autoimmune disease.

Other aspects

25 In a further aspect the invention relates to a method for treating or alleviating a subject suffering from cancer, the method comprising administering to the subject an antibody and/or composition and/or combination according to the invention.

In a further aspect the invention relates to a method for preventing or treating a
30 disease or disorder in subject, where said disease or disorder can be prevented or treated by depleting M2 macrophages in said subject, the method comprising administering to the subject an antibody and/or composition and/or combination according to the invention.

In a further aspect the invention relates to a method for depleting M2 macrophages in a subject, the method comprising administering to the subject an antibody and/or composition and/or combination according to the invention.

- 5 In a further aspect the invention relates to a method for preventing or treating a disease or disorder in subject, where said disease or disorder can be prevented or treated by depleting CD163+ macrophages in said subject, the method comprising administering to the subject an antibody and/or composition and/or combination according to the invention.

10

In a further aspect the invention relates to a method for depleting CD163+ macrophages in a subject, the method comprising administering to the subject an antibody and/or composition and/or combination according to the invention.

- 15 In each of the preceding and other aspects of the invention, the M2 macrophages or CD163+ macrophages may be tissue-resident or infiltrating macrophages.

The subject may in particular be a subject having increased/elevated levels of M2 macrophages and in particular CD163+ macrophages (i.e. in at least one part,

- 20 tissue or organ of the body) and/or having a level of such macrophages that is undesirably high (again, in at least one part, tissue or organ of the body).

In another aspect, the antibodies of the invention may be used in the prevention or treatment of a diseases or disorder that is caused by and/or associated with an

- 25 increased/elevated level of M2 macrophages and in particular CD163+ macrophages (i.e. in at least one part, tissue or organ of the body) and/or with a level of such macrophages that is undesirably high (again, in at least one part, tissue or organ of the body).

- 30 As will be clear to the skilled person, in order to diagnose whether a disease or disorder that is caused by and/or associated with an increased, elevated and/or undesired level of M2 macrophages (and in particular CD163+ macrophages) is present in a subject, one or more suitable diagnostic methods and techniques may be used. Methods and techniques that are suitable for this purpose will be clear to
35 the skilled person (also based on the disclosure herein), and may depend on

factors such as the particular disease to be diagnosed and the part(s), tissue(s) organ(s) of the body or cells of the body that are suspected to have increased, elevated and/or undesired levels of such macrophages and/or be affected by the disease to be diagnosed. As will also be clear to the skilled person, such methods and techniques may involve measuring levels of one or more suitable markers and/or the use of suitable imaging techniques (i.e. suitable for measuring/determining the level of M2 macrophages and in particular CD163 expressing macrophages).

10 In one particular aspect, such diagnostic methods and techniques may involve determining the level of soluble CD163 (i.e. in a sample of blood or another suitable bodily fluid) and/or determining the amount of CD163 expressing cells, for example in a part, tissue or organ of the body that is suspected to have increased, elevated and/or undesired levels of CD163 expressing cells and/or that

15 is suspected to be affected by the disease to be diagnosed. Such techniques for determining the amount of CD163 expressing cells may for example involve determining the amount of such cells in a biopsy sample that has been obtained from such part, tissue or organ of the body (for example, using techniques of histochemistry/immunohistochemistry known per se, see for example page 12 of

20 WO 2020/058372) or may for example involve scanning or imaging said part, tissue or organ of the body for the presence and/or the level of CD163 expressing cells present therein. As will also be clear to the skilled person, and depending on the specific technique used, the latter will often involve the use of a tag, marker or tracer that will allow the CD163 expressing cells to be marked, tagged,

25 labelled, visualized/imaged, sorted or otherwise determined or measured (i.e. using a suitable technique known per se, usually depending on the specific tag or marker used). Suitable tags, markers and tracers will be clear to the skilled person and can for example be as described on pages 30 to 31 of WO 2011/039510, in paragraphs [0291], [0292] and [0386] to [0393] of WO

30 2021/016128, on pages 11-13 of WO 2020/058372 or in WO 2024/156888. Generally, and as further described in these references, such an agent will usually consist of a binding domain or binding unit (such as an antibody-derived binding unit such as a Fab fragment or VHH or even a full-sized antibody) that comprises or is linked to a detectable label or moiety (which can be as described in these

35 references).

In a preferred aspect of the invention, which can in particular be suitable for detecting, imaging, measuring or sorting CD163 expressing cells (i.e. vs soluble CD163), such a binding domain or binding unit can be such that it preferably
5 binds (i.e. is specific for, as defined herein) CD163 that is expressed on cells vs soluble CD163. As will be clear to the skilled person based on the disclosure herein, such a binding domain or binding unit that preferably binds to CD163 on a cell may in particular be such that it preferably binds (i.e. is specific for) to a CD163 trimer compared to soluble CD163 and/or CD163 in monomeric form. For
10 example and without limitation, such a binding domain or binding unit may be an antibody of the invention (or a CD163 binding fragment thereof, such as a Fab fragment) or another antibody or antibody-based fragment that preferably binds (i.e. is specific for) trimeric CD163.

15 As will also be clear to the skilled person, the methods and techniques mentioned herein for measuring or determining the presence and/or levels of CD163 can not only be used for the purposes of diagnosis, but also to determine, monitor or follow the progress of the disease and/or to determine, monitor or follow the efficacy of any treatment that is applied to a subject in order to prevent or treat
20 (as defined herein) a disease or disorder that is caused by and/or associated with an increased, elevated and/or undesired level of M2 macrophages (and in particular CD163+ macrophages). It is also within the scope of the invention that (the data or results obtained from) such methods and techniques will be used as guidance by the treating physician in order to determine, set, adapt or modify the
25 treatment that is applied to the subject to be treated (including the agent(s) and/or dosage(s) used, the method of administration, the duration of treatment and/or other aspects of the treatment regimen). As will be clear to the skilled person, within the context of the present invention, such treatment will generally involve the administration or use of at least one antibody of the invention.

30

In a specific aspect, such methods and techniques will be used to measure, follow or determine the depletion of M2 macrophages (and in particular CD163+ macrophages), in particular from at least one part, tissue or organ of the body that has or is suspected to have increased, elevated and/or undesired levels of

CD163 expressing cells and/or that is or is suspected to be affected by the disease to be prevented or treated.

Thus, in a further aspect the invention relates to a method for preventing or
5 treating a disease or disorder in subject, where said disease or disorder can be prevented or treated by depleting M2 macrophages in said subject, the method comprising administering to the subject an antibody and/or composition and/or combination according to the invention, wherein the depletion of such M2 macrophages is measured, followed or determined by one of the methods and
10 techniques described herein.

In a further aspect the invention relates to a method for depleting M2 macrophages in a subject, the method comprising administering to the subject an antibody and/or composition and/or combination according to the invention,
15 wherein the depletion of such M2 macrophages is measured, followed or determined by one of the methods and techniques described herein.

In a further aspect the invention relates to a method for preventing or treating a disease or disorder in subject, where said disease or disorder can be prevented or
20 treated by depleting CD163+ macrophages in said subject, the method comprising administering to the subject an antibody and/or composition and/or combination according to the invention, wherein the depletion of such CD163+ macrophages is measured, followed or determined by one of the methods and techniques described herein.

25 In a further aspect the invention relates to a method for depleting CD163+ macrophages in a subject, the method comprising administering to the subject an antibody and/or composition and/or combination according to the invention, wherein the depletion of such CD163+ macrophages is measured, followed or
30 determined by one of the methods and techniques described herein.

According to a specific aspect of the invention, said depletion of M2 macrophages (and in particular CD163 macrophages) is measured, followed or determined by means of a suitable method or technique that involves the use of an agent that
35 comprises a binding domain or binding unit that is specific for CD163 that is

expressed on a cell (i.e. vs soluble CD163) and a detectable label or moiety (as further described herein). In this aspect, said binding domain or binding unit may in particular be a binding domain or binding unit that is specific for trimeric CD163 (i.e. vs monomeric and/or soluble CD163) as further described herein. As also
5 described herein, said binding domain or binding unit may be an antibody or an antigen-binding fragment thereof and may in particular be an antibody of the invention or an antigen-binding fragment thereof.

Such an agent comprising an antibody of the invention (or an antigen part or
10 fragment thereof) and a detectable label (as further described herein) forms a further aspect of the invention.

It should also be noted that, while it is assumed that one of the main mechanisms by which the antibodies of the invention exert their favourable effect on the
15 prevention and/or treatment of the diseases and disorders mentioned herein is via the depletion of macrophages (and in particular M2 macrophages and more in particular CD163+ macrophages), and in particular via depletion by means of ADCC, ADCP and/or CDC (and more in particular by means of ADCC), the invention in its broadest sense is not limited to any specific explanation,
20 hypothesis or mechanism, and it is not excluded from the scope of the invention that other mechanisms or explanations play a role or make a contribution, such as, for example and without limitation, reduction of the level of one or more anti-inflammatory factors that are expressed, secreted or produced by one or more populations of macrophages (such as by M2 macrophages and in particular by
25 CD163+ macrophages, for example one or more of TGF- β , PDGF, VEGF, IGF-1, Galectin-3 or IL-10); macrophage repolarization or reprogramming and/or other means and mechanisms of modulating the activity of one or more macrophages (such as the activity of M2 macrophages and in particular of CD163+ macrophages), in particular in the parts, cells, tissues or organs that are affected
30 by (the undesired and/or deleterious effect(s) of) said macrophages, such as, for example and without limitation, the pro-fibrotic effects in or on fibrotic tissues or (in case of CD163+ TAMs) the pro-tumor effects in or on (solid) tumors.

It is also not excluded from the invention in its broadest sense that, in addition to
35 their action on M2 macrophages (and in particular CD163+ macrophages), the

antibodies of the invention may also act on (and/or effect their favorable effect(s) by interacting with) other macrophages that are involved in the disease or disorder to be prevented or treated (such as, for example in the case of fibrosis, CD16+ macrophages).

5

As further described herein, in one specific aspect, the macrophages may be tumor-associated macrophages ("TAMs"), in particular M2 TAMs and/or CD163+ TAMs, in particular when the antibodies are used in the prevention and/or treatment of cancer.

10

It should be noted that embodiments and features described in the context of one of the aspects of the present invention also apply to the other aspects of the invention. For example, embodiments/aspects relating to the regular glycosylated antibodies may also apply to the embodiments/aspects relating to the

15 afucosylated antibodies and vice versa.

All patent and non-patent references cited in the present application, are hereby incorporated by reference in their entirety.

20 The invention will now be described in further details in the following non-limiting examples.

Examples

Example 1: Expression of antibodies

25 **Aim of study**

Expression of IgG1 antibody variants.

Materials and Methods

The antibodies with pairing of light chain (LC) and heavy chain (HC) as listed Table 1 were expressed. The Fab and CDR sequence of OS001 was taken from

30 (31), whereas the sequence for the antibody ORV3 was taken from (32). Kabat based CDR sequences of OS001 are given as SEQ ID NO's: 19-24 and the optimized Kabat based CDR sequence of LC CDR 3 of OS019, OS020, OS023 and OS024 is given as SEQ ID NO: 25.

Table 1: Overview of different combinations of plasmid for LC and HC for producing mAbs described in the examples.

Antibody	subclass	LC sequence	HC sequence	Glycosylation
OS000	IgG4	SEQ ID NO: 1	SEQ ID NO: 3	Wild type
OS001	IgG1	SEQ ID NO: 1	SEQ ID NO: 4	Wild type
OS002	IgG1 FT	SEQ ID NO: 1	SEQ ID NO: 5	Wild type
OS003	IgG1 IQ	SEQ ID NO: 1	SEQ ID NO: 6	Wild type
OS004	IgG1 AAA	SEQ ID NO: 1	SEQ ID NO: 7	Wild type
OS005	IgG1 VLPLL	SEQ ID NO: 1	SEQ ID NO: 8	Wild type
OS006	IgG1 DLE	SEQ ID NO: 1	SEQ ID NO: 9	Wild type
OS007	IgG1 DAE	SEQ ID NO: 1	SEQ ID NO: 10	Wild type
OS008	IgG1 GASDALIE	SEQ ID NO: 1	SEQ ID NO: 11	Wild type
OS009	IgG1 DE	SEQ ID NO: 1	SEQ ID NO: 12	Wild type
OS010	IgG1 MS	SEQ ID NO: 1	SEQ ID NO: 13	Wild type
OS016	IgG1 FTDE	SEQ ID NO: 1	SEQ ID NO: 14	Wild type
OS017	IgG1	SEQ ID NO: 1	SEQ ID NO: 4	Afucosylated
OS018	IgG1 HC D72E	SEQ ID NO: 1	SEQ ID NO: 15	Wild type
OS019	IgG1 LC G89Q	SEQ ID NO: 2	SEQ ID NO: 4	Wild type
OS020	IgG1 HC D72E LC G89Q	SEQ ID NO: 2	SEQ ID NO: 15	Wild type
OS021	IgG1 silent	SEQ ID NO: 1	SEQ ID NO: 16	Wild type
OS022	IgG1 HC D72E	SEQ ID NO: 1	SEQ ID NO: 15	Afucosylated
OS023	IgG1 HC D72E LC G89Q	SEQ ID NO: 2	SEQ ID NO: 15	Afucosylated
OS024	IgG1 LC G89Q	SEQ ID NO: 2	SEQ ID NO: 4	Afucosylated
ORV3	IgG1 format	SEQ ID NO: 17	SEQ ID NO: 18	Wild type

HC: Heavy chain variant; LC: Light chain variant.

- 5 For constructs OS001-OS016, OS021 and ORV3 expression was performed in ExpiCHO (ThermoFisher) to produce antibodies with wild-type glycosylation pattern. Briefly, DNA encoding the listed sequences were synthesized and cloned in pcDNA 3.4 Topo vector at ThermoFisher, and expression performed according to manufacturer's instructions. Subsequently, all antibodies were purified using
- 10 MabSelect SuRe Sepharose (Cytiva), loading protein in cell supernatant, washing column with 10 column volumes PBS pH 7.4 and step elution with 0.1 M Glycine pH 3, fractions were immediately neutralized with 1/10th volume of Tris-HCl pH 9.0. Pooled antibody fractions were buffer exchanged into PBS pH 7.4 on Sephadex G-25 (Cytiva). Antibody concentration was performed using Amicon®

Ultra Centrifugal Filters with a 30 kD molecular weight cut off. All proteins were tested to be essentially endotoxin free (<1 EU/mg) and purity confirmed by SDS-PAGE.

5 Afucosylated antibodies and OS018, OS019 and OS020 were produced at Evitria AG (Switzerland) as follows:

Cloning and production of proteins (Evitria):

The corresponding cDNAs were cloned into Evitria's vector system using conventional (non-PCR based) cloning techniques. The Evitria vector plasmids

10 were gene synthesized. Plasmid DNA was prepared under low-endotoxin conditions based on anion exchange chromatography. DNA concentration was determined by measuring the absorption at a wavelength of 260 nm. Correctness of the sequences was verified with Sanger sequencing.

Suspension-adapted CHO K1 cells (originally received from ATCC and adapted to
15 serum-free growth in suspension culture at Evitria) were used for production. The seed was grown in eviGrow medium, a chemically defined, animal-component free, serum-free medium. Cells were transfected with plasmids using eviFect, Evitria's custom-made, proprietary transfection reagent, and cells were grown after transfection in eviMake2, an animal-component free, serum-free medium.

20 Supernatant was harvested by centrifugation and subsequent filtration (0.2 µm filter). Afucosylated antibodies were produced using the GlymaxX® technology developed by ProBioGen. Here, antibody plasmids were co-transfected with GDP-6-deoxy-D-lyxo-4-hexulose reductase to redirect the intracellular fucose synthesis pathway and produce afucosylated glycan patterns.

25

Purification of IgG (Evitria):

The antibody was purified using MabSelect™ SuRe™ with Dulbecco's PBS (Lonza BE17-512Q) as wash buffer, 0.1 M Glycine pH 3.5 as elution buffer and 1 M Tris HCl as neutralisation buffer (pH 9). Buffer exchange was performed by dialysis

30 using Pierce Slide-A-Lyzer™ G2 Dialysis Cassettes with a 2K molecular weight cut off. Antibody concentration was performed using Amicon® Ultra Centrifugal Filters with a 30 kD molecular weight cut off. All proteins were tested to be essentially endotoxin free (<1 EU/mg) and purity confirmed by SDS-PAGE.

Results and conclusions

All antibodies were being expressed in reasonable and comparable amounts (data not shown).

Example 2: Selection of antibody for ADCC

Aim of study

5 An antibody able to exert antibody dependent cellular cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP) must be exposed on the surface of the cells expressing the antigen for a certain period of time. Prolonging exposure of mAb on the surface increases ADCC response mediated by NK cells (18). This makes targeting of internalization receptors (like CD163) challenging, 10 since they will be endocytosed, and an antibody bound to the target dissociated. Therefore, other means of inducing cell killing is advised when targeting internalization receptors, as seen for targeting SIGLEC (19) or HER-2 (20). This is especially true for an antibody binding a receptor with a high endocytosis capacity, as CD163 (21-23).

15 As previously mentioned, this makes targeting CD163 with the aim to induce ADCC or ADCP killing of CD163 expressing cells difficult and counter intuitive.

Surprisingly it was observed that a murine antibody against human CD163 exhibited staining of CD163 expressing cells even after prolonged incubation (preliminary data - not shown). This suggests that mediating ADCC against a cell 20 expressing CD163 with CD163 as target might be possible. It was therefore decided to screen the time-extended surface exposure of a range of antibodies binding human CD163. Binding levels of mAb at the surface after prolonged incubation of CD163 expressing cells at 37°C with unlabeled murine mAbs by staining for presence of murine mAb on the cell surface was tested. The goal 25 being to identify the preferred mAb for targeting CD163 cells in patients to mediate ADCC killing of CD163 expressing cells.

Materials and methods

Antibodies were either commercially available (Mac2-158 and Mac2-48 (IQ Products, the Netherlands), R-20 (Novus Biologicals, USA) and Ki-M8 (Biozol, 30 Germany) or a kind gift from Associate Professor Yaseelan Palarasah, University of Southern Denmark. The latter antibodies were generated by immunizing mice with human CD163 domain 1-5, isolating hybridomas and selecting antibodies

exhibiting binding to CD163 on the cell surface, as screened by incubation at 4°C of CHO cells expressing human CD163 recombinantly with purified mAbs.

Staining and flow cytometry:

Recombinant FlpIn293 cells expressing full length human CD163 were cultured in complete DMEM supplemented with 10%FCS, 2 mM L-Glutamine, Pen/Strep (cDMEM) and on the day of the experiments harvested using 10 mM EDTA, 2 gm/ml Lidocaine-HCl. For measuring surface exposure of bound antibody, FlpIn293 hCD163 cells were resuspended in cDMEM at 10⁷ cells/ml and incubated with 1 ug/ml mAb for 30 min on ice. Following cells were washed three times in cDMEM and finally resuspended in cDMEM at the original cell concentration and incubated at 37C for the indicated time. At timepoint 0, 5, 15, 30, 60, 120 and 240 min, 100 µl antibody-stained cell suspension was transferred to a V-bottom 96 well plate where it was stained with ATTO488 conjugated polyclonal secondary anti mouse IgG for 20 min in FACS buffer (2% FCS, 0.09 mM NaN₃) on ice before fluorescent intensity was measured on a NovoCyte Quanteon Flow cytometer (Agilent)

Results

As can be seen from Figure 1 only four mAbs; 15-5-20, 15-5-30, Mac2-158 and mac2-48 exhibited prolonged surface binding, substantiating that this is a rare and surprising property of the mAbs. Here it is worth noticing that Mac2-158 and Mac2-48 are two different maturation clones of a basically identical antibody, only differing in a few amino acid residues in their sequence (31).

We next tested if the prolonged surface exposure was also observed for the humanized version of Mac2-158, when expressed in a humanized format, humanization described in (31). Here we test the humanized CD163 antibody in an IgG1 format called OS001.

The humanized Mac2-158 variant OS001 exhibits prolonged surface exposure (Figure 2). We further tested a CD163 antibody (ORV3) in clinical testing and found that it also exhibited prolonged surface exposure (Figure 2).

Conclusion

The antibody Mac2-158 exhibits surprisingly long exposure after binding to the internalization receptor CD163. It binds with characteristics optimal for inducing

ADCC using CD163 as a target and the properties are conserved when humanizing the antibody. The humanized IgG1 version of this mAb, OS001, as well as antibodies 15-5-20 and 15-5-30 (after humanization) could therefore be potential starting points for mAb development of ADCC inducing mAbs.

5 **Example 3: Effect of soluble CD163 on binding to CD163 on a biosensor surface**

Aim of study

CD163 can shed from the surface of CD163-expressing cells after proteolytic cleavage (15, 16, 33) leading to the presence of soluble CD163 in the blood in the 10 mg/L range. This soluble CD163 (sCD163) may act as a sink and in effect preclude binding of a CD163 mAb to the cell surface, meaning that a therapeutic antibody will be less effective. Further, since the levels of sCD163 can vary between patients and even for the same patients depending on disease state or complicating conditions, such as infections or even cancer (34), effect of a 15 therapeutic CD163 antibody may vary. Therefore, it is preferable that the cell surface binding of a CD163 antibody for therapeutic use exhibits limited sensitivity to sCD163.

We therefore tested the effect of adding sCD163 to CD163 antibodies on the binding to CD163 immobilized on a surface plasmon resonance sensor chip 20 (Biacore (Cytiva)), and on antibody binding to CD163 expressing human monocyte derived macrophages (M2c). We tested the three CD163 antibodies identified in Example 1 and 2 and a fully human CD163 antibody described elsewhere (ORV3) (32). We tested for effect of sCD163 upon binding to CD163 on a surface plasmon resonance sensor chip and for the two humanized antibodies in 25 IgG1 format on binding to M2c.

Materials and methods

Surface plasmon resonance

CD163 was expressed recombinantly and purified as described previously (35). Binding of antibodies to CD163 immobilized on a biosensor chip with increasing 30 ratios of sCD163 added was studied by surface plasmon resonance (SPR) on a Biacore T200 instrument (Cytiva) essentially as described (35). Briefly, CD163 was immobilized on a CM5 chip and CD163 mAb in running buffer (10 mM HEPES,

150 mM NaCl, 3 mM CaCl₂, and 0.05% Surfactant P-20, pH 7.4) was injected with or without sCD163, with sCD163 ranging from 0 to 8-fold molar concentration relative to antibody (mixed prior to injection). The density of human CD163 domain 1-9 on the flow cell was in the range 0.017-0.025 pmol/mm² and the chip
5 was regenerated by two cycles of injection of 10 µL 100mM phosphoric acid. Data were analyzed using the BIAcore T200 Evaluation Software 3.1. All experiments were as minimum performed in independent triplicates of duplicates.

M2c binding

M2c macrophages were differentiated from monocytes purified from buffy coats
10 by culturing them for 7 days in RPMI1640 supplemented with 10% FBS, 2 mM L-Glutamine and 10 ng/ml MCSF + 10 ng/ml IL-10. Macrophages were harvested by adding 10 mM EDTA in 1X PBS and incubating for 15 minutes followed by flushing the petri dish. The cells were pelleted (4°C, 400g, 5 min) and resuspended in FACS buffer without EDTA to a concentration of 1-2*10⁶ cells/mL. The cells
15 were plated in 96-well plate (v-bottom) and pelleted by centrifugation (4°C, 400g, 5 min). Antibodies were labelled using the Zenon Human IgG labeling kit (Invitrogen) with Alexa Fluor 488. 2 µg mAb was mixed with 10 µL component A from the kit, incubated for 5 min at RT, blocked with 10 µL component B, and incubated for 5 min at RT.
20 Macrophages were stained in FACS buffer without EDTA mixed with 2 µg/mL sCD163 SRCR1-5, human IgG, Oligo Block, Brilliant stain buffer and labelled antibody at different concentrations in a total volume of 50 µL. After 30 minutes of incubation at 4°C, the cells were collected by centrifugation (4°C, 400g, 5 min) and resuspended in FACS buffer. SYTOX Dead Cell Stain (Invitrogen) was added
25 before running the samples on a ID7000 Spectral Flow Cytometer (Sony).

Results

As can be seen from Figure 3, OS001 is substantially less sensitive to soluble CD163 in its binding to CD163 immobilized on a sensor chip than the other antibodies tested (ORV3, 15-5-20, 15-5-30).
30 Further, as seen on Figure 4A, when using a constant concentration of antibody and increasing the concentration of soluble CD163 inhibition of binding of OS001 to M2c (macrophages expressing CD163) is much lower than observed for ORV3.

Surprisingly, when using a constant and physiologically relevant concentration of sCD163 (2 µg/ml) (34) more than 50% binding of OS001 can be observed even at the lowest concentration (3 ng/ml, corresponding to a 667 fold excess of sCD163) (Figure 4B). Contrary, for ORV3 cell binding is competed out already at a 2:1 ratio 5 (Figure 4B).

Conclusion

Of the mAbs tested OS001 is by far less sensitive to soluble CD163, and very surprisingly cannot be competed below 50% binding even at very high ratios of sCD163 to mAb. This makes OS001, and derivatives thereof, ideal candidates for 10 clinical use if one wants to mediate ADCP or ADCC killing of CD163 expressing cells. It also means that the other mAbs identified to have prolonged surface extension (15-5-20, 15-5-30 and ORV3) cannot be used, and that a selection procedure involving both screening for surface exposure and sensitivity to soluble CD163 is needed.

15 **Example 4: Binding of monomeric and trimeric CD163 to antibody**

Aim of Study

In has been found that CD163 forms a trimer at high concentrations and at the cell surface, whereas it is a monomer in the concentration range found for soluble CD163 in blood/plasma. It was speculated that the different sensitivities towards 20 soluble CD163 may be due to different propensities to bind either monomeric or trimeric CD163. Since binding to CD163 on the cell implies binding a trimeric version of CD163, a mAb to be used for targeting CD163 expressing cells for ADCC mediated eradication should bind trimeric CD163 with higher affinity than it binds monomeric CD163.

25 **Methods**

Surface plasmon resonance (SPR) experiments were carried out on a Biacore 3000 using 10 mM HEPES, pH 7.5, 150 mM NaCl, 2 mM CaCl₂ and 0.05% Tween 20 as running buffer. Binding analysis was performed at 25 °C, and data were collected at a rate of 1 Hz. Using the BIAevaluation 4.1.1 software (Cytiva), 30 recorded signals from the active flow cell were double referenced, i.e. the signal from the in-line reference flow cell was subtracted as was the signal from a blank run (0 nM analyte).

Monoclonal antibodies OS001, OS020 and V3 were captured in the active flow cell of a protein G-coupled CM5 sensor chip, to a level of 200 RU. Next, monomeric human CD163_1-5 or trimeric CD163_1-9_CC, both at a concentration of 25 nM, was injected in both the active and reference flow cells for 2 min followed by a dissociation phase of 5 min. At the end of each cycle, both flow cells were regenerated by a 30 s injection of 10 mM glycine pH 1.5.

Results

As can be seen from Figure 5, OS001 surprisingly binds trimeric CD163 with higher affinity than monomeric CD163, for which it exhibits poor affinity. For ORV3, which is sensitive to soluble CD163 for binding CD163 on the cell surface, we see that ORV3 in binding of monomeric and trimeric CD163 exhibits a higher affinity for monomeric CD163.

Conclusion

For developing an antibody for clinical use for targeting CD163 expressing cells, it is mandatory to bind trimeric CD163 with higher affinity than monomeric CD163. This is a novel finding and a novel property of OS001, clearly showing that it is surprisingly well-suited for clinical development for CD163 cell-targeting.

Example 5: Epitope binding

Aim of study

To better understand the role of specific epitopes in the ability to exhibit prolonged exposure, low sensitivity to sCD163 and ability to induce ADCC, we set out to examine overlap of epitopes between antibodies. We used surface plasmon resonance to investigate pairwise inhibition of CD163 binding of the different mAbs.

Materials and methods

Surface plasmon resonance

CD163 was expressed recombinantly and purified as described previously (35).

Binding of antibodies to CD163 immobilized on a biosensor chip was studied by surface plasmon resonance (SPR) on a Biacore T200 instrument (Cytiva) essentially as described (35). Briefly, CD163 was immobilized on a CM5 chip and CD163 mAb in running buffer (10 mM HEPES, 150 mM NaCl, 3 mM CaCl₂, and

0.05% Surfactant P-20, pH 7.4). First either buffer or 100 µg/ml of an antibody was injected with followed by injection of either buffer or 5 µg/ml antibody. The density of human CD163 domain 1-9 on the flow cell was in the range 0.017-0.025 pmol/mm² and the chip was regenerated by two cycles of injection of 10 µL 5 100mM phosphoric acid. Data was analyzed using the BIAcore T200 Evaluation Software 3.1. All experiments were as minimum performed in independent triplicates of duplicates.

Results

As can be seen from Figure 6, saturating the sensor chip with ORV3 (V3) did not 10 impede binding of OS001 to CD163. However, saturating with OS001 led to a minor inhibition in binding ORV3 (V3) to CD163. Since we only observe minor inhibition in one configuration of the assay, the two epitopes are in all likelihood not overlapping but binding of OS001 to its CD163 epitope may pose some sterically hindrance for access of ORV3 to the ORV3 epitope, but without OS001 15 actually interacting with the ORV3 epitope.

Preliminary data further indicates that the CD163 antibodies 15-5-20 and 15-5-30 only partly inhibit OS001 binding, indicating that the OS001 epitope is unique (data not shown).

Conclusion

20 The epitopes of ORV3, 15-5-20 and 15-5-30 for CD163 are considered non-overlapping with the epitope for OS001.

Example 6: Effect of the CD163 ligand HbHp on mAb CD163 binding

Aim of study

The best described physiological role of CD163 is the binding and uptake of the 25 complex between hemoglobin (Hb) and haptoglobin (Hp) that is formed after the lysis of red blood cells (14). It is desirable that a CD163 binding therapeutic antibody does not interfere with the interaction between HbHp and CD163 for two reasons. First, hemolysis leads to the release of Hb and thus formation of the HbHp complex. Ideally, degree of receptor binding of a therapeutic mAb and thus 30 its effect should not be affected by (local) hemolysis. Second, Hb can exert toxic damage to tissue, and its rapid removal is desirable. Thus, using a therapeutic mAb that impedes the binding of HbHp to its clearance receptor may be

envisioned to increase the risk of Hb mediated oxidative damage. Further, conserved HbHp binding can be argued to be especially important in treatment of cancer since local hemolysis is known to take place in tumors and iron from Hb is an important iron source for cancer cells to enable continued division.

5 We therefore wanted to investigate if OS001 inhibited binding of HbHp to CD163.

Materials and methods

The influence of OS001 and ORV3 on the binding of haptoglobin-hemoglobin (HpHb) to CD163 was analyzed by Bio-Layer Interferometry using the Octet RED96e system (Pall ForteBio). Biotinylated CD163 (10µg/ml, NHS-PEG4-biotin in
10 3:1 ratio) was captured by pre-hydrated SAX biosensors (Pall ForteBio) in BLI running buffer (10 mM HEPES, 150 mM NaCl, 5 mM CaCl₂, 0.1% BSA, 0.05% Tween 20, pH 7.4). Baseline was generated in for 60 s in BLI running buffer. Association of 0.33µM KN2NRY, 0.33µM ORV3 or 1µM HpHb (pre-incubated for 15 min in an equimolar ratio of 1µM each) was measured for 500 s followed by a
15 second association step for 500 s containing 0.33µM OS001 +/- 0.3µM HpHb, 0.33µM ORV3 +/- 0.3µM HpHb or just 0.3µM HpHb as indicated on the sensorgrams. Finally, dissociation was measured for 500 s in BLI running buffer. All sensorgrams were aligned using the last 2 s of baseline and a Savitzky-Golay filter was applied to smoothen the data. The experiment was repeated in three
20 independent experiments.

Results

As can be seen from Figure 7 the impact of HbHp upon binding to CD163 immobilized on a BLI sensor is more than a factor 2 lower for OS001 than ORV3 (V3), making OS001 the preferred antibody.

Conclusion

Very limited inhibition of CD163 binding of OS001 by HbHp substantiates its usefulness as an antibody for mediating ADCC or ADCP to CD163 expressing cells, and that local hemolysis can be ignored and that the mAb does not interfere with iron clearing.

30 Example 7: ADCC and ADCP effect in reporter assay

Aim of study

Based on the previous obtained data (see examples 1-6), OS001 was chosen as the ideal CD163 binding antibody for potentially generating an ADCC response against CD163 expressing cells, we wanted to investigate if the binding abilities of the mAb were sufficient to give it ability to bind CD163 expressing cells and induce ADCC signaling on cells expressing CD16 (FcγRIIIA).

We tested different techniques for increasing ADCC response, either by site directed mutagenesis of specific amino acid residues of the Fc-region or by engineering of the glycosylation (26-28). Thus, the antibodies below were tested:

The ADCC signaling was tested in a reporter assay system monitoring CD163 mediated signal induction in reporter cells expressing CD16. A fluorescent signal was induced in the reporter cells after binding of CD16 on the reporter cell surface to IgG1 bound to a cell line expressing the target, here human CD163. Two variants of CD16 were tested, CD16 V158 and CD16 F158, corresponding to two different alleles found in the human population. V158 is generally exhibiting a higher ADCC response rate than F158 (36). A good response to both alleles is essential for an ADCC inducing therapeutic mAb, and increasing response to the weak receptor is especially important to be able to generate effect in all patients irrespective of their CD16 allele. In addition, the ADCP capabilities of the mAbs were also tested in a similar reporter assay where reporter cells expressed CD32 instead of CD16.

Methods

The target cells, FlpIn293 cells expressing human CD163, were cultivated in complete DMEM medium (cDMEM). Jurkat effector cells (InvivoGen, jk1l-nfat-cd16, jk1l-nfat-cd16low, jk1l-nfat-cd32) were cultivated as described in the manufacturer protocol. Jk1l-nfat-cd16 cells expressed the V158 allele (mediating a generally higher response) and the jk1l-nfat-cd16low expressed the CD16 F158 allele (mediating a generally lower response).

Co-cultivation was run in assay buffer (IMDM, L-glut, 4% IgG-depleted FBS). One day before co-cultivation, the target cells were harvested using TrypLE (Gibco), resuspended in cDMEM, and plated at 20,000 cells/well in a clear, tissue-treated 96-well plate (Thermo Scientific). On the day of co-cultivation, effector cells were centrifuged (300g, 5 min, RT), washed with 1X PBS, and centrifuged (300g, 5 min, RT) before being resuspended in assay buffer. Supernatant was removed

from the 96-well plate, and antibody and 150,000 effector cells were added to the wells in a total volume of 75 μ L of assay buffer. The plate was incubated in a 37°C, 5% CO₂ incubator overnight. On day of analysis, 20 μ L supernatant from each plate was moved to a white, 96-well plate (Greiner). 50 μ L QUANTI-luc (InvivoGen, rep-glc2), prepared as described by the manufacturer, was added to the supernatant immediately before measuring the luminescence using a plate reader (PerkinElmer, EnSpire Multimode Plate Reader).

Results

As can be seen from Figures 8, and 9, OS001 surprisingly mediates ADCC and ADCP signaling in the reporter assay, which would not be assumed for an antibody binding an internalization receptor (21-23). The two negative controls OS000 (IgG4 format) and OS021 (ADCC-silent) do not give a signal in the assay, confirming that the signaling is indeed mediated by interaction with CD16 which mediates ADCC effector functions.

Further, it can be seen from Figures 8 and 9 as well as Table 2, that OS017 (generated by afucosylation expression technology) surprisingly has a significant higher change in EC₅₀ compared to the other ADCC enhancing technologies. This was not expected from previous studies on other antibodies, where the DLE construct, as found in OS006, has been reported to be superior to other approaches, and afucosylation only on par with the DE construct (OS009) and LPLIL (OS005) (26). Also, it can be seen that the increased ADCC effect is observed for both the CD16 V158 and F158 allele. These two alleles have been reported to mediate a generally higher (V158) or lower (F158) ADCC response in humans in different systems of ADCC targets and antibodies (36).

Table 2: Relative EC₅₀ values for novel constructs (normalized to OS001) in reporter assay for ADCC and ADCP effect. Relative EC₅₀ given as (EC₅₀ concentration OS001/EC₅₀ concentration OSXXX) meaning that a high value indicates superior effect. The CD163 expressing cell was a recombinant cell line (FlpIn293-hCD163)

Construct number	Construct description	Relative EC50 ADCC V158 (high)	Relative EC50 ADCC F158 (low)	Relative EC50 ADCP
OS000	IgG4	0	0	n.d
OS001	IgG1	1	1	1
OS002	IgG1 FT	0.9	1.5	0.8

OS003	IgG1 IQ	1.5	1.3	0.7
OS004	IgG1 AAA	1.6	3.5	0.5
OS005	IgG1 VLPLL	2.2	5.4	1.4
OS006	IgG1 DLE	5.9	2.5	0.6
OS007	IgG1 DAE	4.0	5.6	0.5
OS008	IgG1 GASDALIE	4.5	3.2	0.5
OS009	IgG1 DE	7.6	7.0	0.6
OS010	IgG1 MS	1.4	1.2	0.6
OS016	IgG1 FTDE	3.7	4.0	0.5
OS017	IgG1 aFuc	11.2	17.6	0.8

aFuc: Afucosylation

In addition, none of the constructs exhibited superior ADCP signaling, so therefore mAbs not significantly decreasing ADCP signaling were considered for further development. Increased ADCP would have been expected for some of the

5 constructs (26-28).

Conclusion

Afucosylation (OS017) is the superior approach for increasing ADCC effectiveness of the CD163 binding mAb OS001, also better than other techniques reported to give higher increase in ADCC effectiveness.

- 10 OS005, OS006 and OS009 also exhibited increased ADCC albeit at a lower level.
None of the constructs exhibited increased ADCP signaling.

Example 8: ADCC effector assay on human monocyte derived macrophages

15 Aim of study

Since ADCC signaling may depend on density of the target on the cell, we also wanted to compare preferred constructs for inducing ADCC signaling with human monocyte derived macrophages expressing CD163 (M2c), since these have a higher density of CD163 on the cell surface than the recombinant cell line. The

- 20 ADCC signaling was tested in a reporter assay system monitoring CD163 mediated signal induction in reporter cells expressing CD16. A fluorescent signal was induced in the reporter cells after binding of CD16 on the reporter cell surface to IgG1 bound to a cell line expressing the target, here human macrophages expressing human CD163. Two variants of CD16 were tested, CD16 V158 and

CD16 F158, corresponding to two different alleles found in the human population. V158 is generally exhibiting a higher ADCC response rate than F158 (36).

Methods

Experiments were performed essentially as described in detail in example 7 using
5 M2c macrophages as target cells and again using Jk^{tl}-nfat-cd16 and jk^{tl}-nfat-cd16^{low} cells, for the V158 and F158 CD16 allele, respectively. M2c was obtained by differentiation from monocytes purified from buffy coats by culturing them for 7 days in RPMI1640 supplemented with 10% FBS, 2 mM L-Glutamine and 10 ng/ml MCSF + 10 ng/ml IL-10.

10 **Results**

As can be seen from Table 2 and Table 3, ADCC enhancement is further potentiated by testing on cells with a high expression level of CD163, here M2c macrophages. All the constructs exhibiting enhanced ADCC in example 7 also exhibited enhanced ADCC here. Again OS017, ADCC enhancement by
15 afucosylation, was most efficient, as can be seen from Figure 10 and Table 3.

Table 3: Relative EC50 ADCC induction in two reporter assay models studying either binding to the CD163 V158 variant (higher response rate) or CD16 F158 (lower response rate) (normalized to OS001)

Antibody		Relative EC50 ADCC (CD16 V158)	Relative EC50 ADCC (CD16 F158)
OS001	IgG1	1	1
OS005	IgG1 VLPLL	23	13
OS006	IgG1 DLE	26	71
OS009	IgG1 DE	23	12
OS017	IgG1 aFuc	39	111

20 Further, ADCC was increased more than a factor 100 in the CD16 F158 system when using afucosylation (OS017) to increase ADCC signaling of OS001. This is substantially and surprisingly higher than the 2-40 fold increased described in biologically relevant in vitro systems for other antibody target systems (30).

Figure 11A shows that ORV3 can also induce ADCC in recombinant cells
25 expressing CD163, albeit at a lower level than OS001. However, ORV3 does not have the ability to induce ADCC signaling towards human monocyte derived macrophages expressing CD163 (Figure 11B). This surprisingly shows that

prolonged surface exposure, which both OS001 and ORV3 exhibit, is not enough to be able to induce ADCC. Probably antigen density affects effective ADCC ability in a more complex setting, and surprisingly acts in two different directions for the two mAbs. Therefore, selection of optimal mAb for inducing ADCC and technique
5 for increasing ADCC signaling is by no means trivial.

Conclusion

Afucosylation is the superior approach for increasing ADCC effectiveness of the CD163 binding mAb OS001, also better than other techniques reported to give higher increase in ADCC effectiveness. Contrary to some of other methods
10 afucosylation exhibited consistent improvement in ADCC whether using the CD163 V158 or F158 variant, making it especially useful for therapeutic use, since the CD16 F158 patients will be in most need of a boost of ADCC activity. This points to OS017 and OS006 as the more interesting alternatives, but OS017 being superior, as opposed to what would be expected based on reports in the field
15 (26).

OS005, OS006 and OS009 also exhibited increased ADCC albeit at a lower level.

Example 9 Thermal unfolding/stability of ADCC enhanced constructs

Aim of study

For an antibody to be druggable it needs to be stable so that it can be provided in
20 high concentrations and stored over a longer time before patient use. We therefore tested the thermal unfolding pattern of the engineered OS001 variants.

Material and methods

This experiment was conducted using the Protein Thermal Shift™ Dye Kit (Applied Biosystems™). Initially, the antibody concentrations were adjusted to 0.5 µg/mL.
25 Working on ice, 25 µL antibody dilution was mixed with 25 µL 1X PBS and 25 µL Protein Thermal Shift Buffer. 15 µL of this was moved to a MicroAmp Fast Optical 96-well Reaction Plate and mixed with 5 µL 4X Protein Thermal Shift Dye. The plate was sealed and centrifuged (1000 rpm, 1 min, 4°C) before running the plate in a QuantStudio 3 Real-Time PCR system using QuantStudio Design and Analysis
30 Desktop Software (v. 1.4). A melt curve was run in two steps (1) 1.6°C/s, 25.0°C, 2 min, (2) 0.05°C/s 99.0°C, 2 min. Filter type 'Coupled' was used and melt curve filter m2(558+11).

Results

Thermal unfolding of the tested constructs generated melting profiles that were fitted to models of protein unfolding are summarized in Table 4.

Table 4: Thermal unfolding analysis of antibodies screened for ADCC activity.

5 Temperatures are given in °C.

Antibody	Unfold start	T max 1 st peak	T max 2nd peak
OS001	65	73	78
OS002	65	78	78
OS003	65	73	78
OS004	55	70	78
OS005	65	75	78
OS006	46	57	78
OS007	46	58	78
OS008	45	58	78
OS009	45	58	78
OS010	57	67	78
OS016	46	61	78
OS017	65	74	78

As can be seen, some of the changes to increase ADCC signaling ability in OS001 led to substantial decreases in T unfold, especially for the first unfolding peak. This was to a certain degree to be expected for some of the constructs (37).

10 However, for some of the constructs (OS002, OS003, OS005 and OS017) we observed no changes in thermal unfolding pattern. This is especially surprising regarding OS017, since it has previously been reported that afucosylation leads to lower thermal stability of the mAb (24).

Conclusion

15 The surprisingly conserved stability of OS017 combined with its surprising superior efficacy in inducing ADCC points to it being a special superior construct for developing an ADCC inducing CD163 mAb with OS001 as the starting point.

Example 10 PBMC mediated depletion of CD163+ macrophages *in vitro*

Aim of study

The dependency of ADCC enablement of receptor density on cell surface prompted us to further compare potency in a more complex system. We studied selected antibodies for their ability to mediate cytotoxicity of human PBMCs towards CD163 expressing macrophages. The goal also being to demonstrate that the effect was dependent on Fc-receptor engagement.

Materials and methods

Macrophages purified from buffy coats were loosed by adding 10 mM EDTA and incubating for 15 minutes followed by flushing the petri dish. The macrophages were resuspended in cRPMI and TCM supplemented with 10 ng/mL IL-10 and 10 ng/mL m-CSF to a concentration of 2×10^5 cells/mL. After 1 day of incubation at 37°C in 5% CO₂ incubator, the effector cells (PBMCs) were thawed and resuspended in media containing RPMI 1640 (10% FCS, Pen/Strep, L-glutamine) supplemented with 1000X b-mercaptoethanol, 100X NEAA, 100X sodium pyruvate, 10 ng/ml IL-15 and 50U/ml IL-2 to a concentration of 9×10^6 cells/mL. The supernatant was removed from the macrophages and 25 µL of the effector cells, equal to 240,000 cells, were added to all wells, except for the macrophage control, the media control and the macrophage lysis control. In the case of the control wells, 25 µL of the same suspension media that was used for the effector cells is added, although without the effector cells themselves. 25 µL of the antibody dilution series was added to all wells, except the controls. Instead, 25 µL of media containing RPMI 1640 (10% FCS, Pen/Strep, L-glutamine) was added to these wells. 7.5 µL of Lysis solution from the CytoTox Non-Radioactive Cytotoxicity Assay (Promega, G1780) was added to macrophages alone as a maximum LDH release control. The plate was incubated in a 37°C, 5% CO₂ incubator overnight. On the day of analysis, 50 µL of supernatant from each plate was moved to a white F-bottom 96-well plate (Greiner, 655098). 50 µL of CytoTox reagent (Promega, G1780) was added to each sample aliquot. The plate was covered from light and incubated 30 min at room temperature. 50 µL of stop-solution (Promega, G1780) was added to each well before measuring absorbance using a plate reader (PerkinElmer, Enspire Multimode Plate reader). For calculating the percent cytotoxicity, the average absorbance value for the culture medium background was subtracted from the experimental values, the macrophage spontaneous values and the macrophage + effector cells spontaneous values. For the macrophage maximum LDH release control, the

average of the macrophage spontaneous values was subtracted. In the end percent cytotoxicity was calculated as:

$$\% \text{ Cytotoxicity} = \frac{(\text{Experimental value} - \text{Macrophage and effector cells spontaneous})}{\text{Macrophage maximum} - \text{macrophage spontaneous}}$$

Results

- 5 On Figure 12 the titrations of PBMC mediated depletion of CD163+ macrophages are depicted (see also Table 5). As negative control an ADCC silenced OS001 variant, OS021, was included. As can be seen, OS021 only has marginal effect, clearly supporting the role of Fc-receptor engagement in the effector-function of the CD163 mAbs.
- 10 **Table 5.** EC50 values for different antibodies inducing PBMC mediated cytotoxicity in CD163 expressing macrophages. Also shown the maximum induced cytotoxicity, compared to all CD163-expressing cells.

mAb	EC50 (ng/ml)	Max induced cytotoxicity (%)
OS001	3.2	25
OS009	2.8	40
OS017	2.6	46

15 **Conclusion**

Even in a complex setting afucosylation significantly increases the Fc-effector function of OS001, as demonstrated by the lower EC50 of **OS017** and increased span in signal. Also, ADCC mediated depletion of CD163 expressing macrophages is possible after targeting CD163, an internalization receptor.

Example 11 Effect of CD163+ macrophage depletion on T cell proliferation

Removing CD163 expressing tumor associated macrophages ultimately will have the effect to allow influx of T cells into a tumor and to prevent inhibition of T cell activity and proliferation mediated by CD163+ TAMs (17, 38, 39). We tested this in assay demonstrating inhibition of T cell proliferation by M2c macrophages.

Materials and Methods

Macrophages purified from buffy coats were loosed by adding 10 mM EDTA and incubating for 15 minutes followed by flushing the petri dish. The macrophages were resuspended in T cell medium (1000X b-mercaptoethanol, 100X NEAA, 100X sodium pyruvate) to a final concentration of 3×10^5 cells/mL.

- 5 Each vial of frozen PBMCs was thawed in pre-warmed T cell medium, pelleted by centrifugation (300g, 20°C, 5 min), and resuspended in 2 mL 100 µg/mL DNase 1 solution. After 15 minutes of incubation, the cells were resuspended at 10×10^6 cells/mL in T cell medium containing 1000X CytoTrack Red Dye (Bio-Rad). The cells were incubated for 15 minutes protected from light, and pelleted by
- 10 centrifugation (300g, 20°C, 5 min). After a subsequent washing step, the PBMCs were resuspended in T cell medium to a final concentration of 6×10^6 cells/mL. Antibody dilutions were prepared in 1X PBS.

- In a 24-well plate, 200 µL/well assay medium (T cell medium supplemented with 2500X m-CSF, 5000X IL-15, 10000X IL-2, and 33.3X Dynabeads (Gibco)) was
- 15 mixed with 100 µL/well macrophages, 100 µL/well PBMCs, and 16 µL antibody dilution. After 3 days of incubation at 37°C in 5% CO₂ incubator, the supernatant was transferred to Eppendorf-tubes placed in a magnetic rack. From this supernatant was moved to a V-bottom FACS plate. The cells were pelleted by centrifugation (400g, 5 min, 4° C) and resuspended in 100 µL staining mix (FACS
- 20 buffer, OligoBlock, anti-human IgG, and Abs). After 30 minutes of incubation at 4°C in the dark, the cells were pelleted by centrifugation (400g, 5 min, 4°C) and resuspended in 100 µL FACS buffer. SYTOX Cell Dead Stain was added before running the plate on the SONY ID7000 Spectral Flow Cytometer.

Results

- 25 In a patient with a NK cell count in the PBMCs of 5% both OS001 and OS017 increased T cell proliferation, otherwise inhibited by M2c macrophages (Figure 13A). There was virtually no effect of adding an ADCC silenced version of the antibody (OS021), demonstrating that Fc receptor engagement is needed for effective macrophage neutralization and restoration of T cell proliferation.
- 30 Further, at 5 µg/ml OS017 can offset the inhibition of macrophages on T cell proliferation, whereas there was no effect of the CD163 mAb ORV3 (Figure 13C).

Conclusion

ADCC mediating CD163 mAb **OS017** can effectively cancel the effect of macrophage inhibition on T cell proliferation.

Example 12: Development of OS001 variant with less tendency to aggregate formation in expression and capture step

5 **Aim of the study**

For an antibody to be used in therapy controlled and limited degree of aggregation is crucial, since aggregated antibody can exhibit different activity than monomeric and, more importantly, be immunogenic. Initial aggregates formed during expression and capture-purification step (protein A based MabSelect SuRe) can
10 may to a certain degree be removed in the subsequent purification step, but this is tedious, costly and lowers the overall yield, therefore minimal formation of aggregate in the early production steps is highly desirable.

We observed that OS001 and OS017 (same Fab sequence) displayed a relative high level of aggregate formation, there was no influence on fucosylation on
15 aggregation formation level. To reduce amount of aggregate present after MabSelecet SuRe capture, we generated and expressed Fab sequence variants to see if we could lower the tendency to formation of aggregates.

Materials and Methods

Amount of aggregate was determined by analytical size exclusion chromatography
20 with an Agilent AdvanceBio SEC column (300A 2.7 µm 7.8 x 300 mm) and DPBS as running buffer at 0.8 ml/min.

Plasmid (pcDNA 3.4 TOPO) containing the heavy chain variant HC-D72E was synthesized at ThermoFisher (Germany).

Antibodies were expressed at Evitria, either in afucosylated or standard
25 glycosylation format. Expressed as described in example 1.

Results

As can be seen from Figure 14 and Table 6, we consistently found lower levels of aggregate after expression and MabSelect SuRe capture of OS001 variants with the HC mutation D72E. Name of antibodies with the HC D72E mutation is **OS018**,
30 **and OS020** (standard glycosylation) and **OS022 and OS023** (both afucosylated).

Table 6: Shows the aggregation level of individual constructs.

Variant	Aggregate level after Protein A purification	HC
OS017	6.7%	Wildtype
OS018	1.2%	D72E
OS019	5.6%	Wildtype
OS020	1.8%	D72E
OS022	1.1%	D72E
OS023	1.7%	D72E

Conclusion

The heavy chain variant D72E surprisingly lead to a lower level of aggregates
5 formed after initial purification step. This is beneficial since aggregate can
compromise product safety and increase cost for purification. Antibodies with the
heavy chain variant D72E are therefore superior for further development of a
therapeutic antibody targeting CD163.

Example 13: selection of OS001 variant with increased CD163 affinity

10 **Aim of study**

As a rule of thumb, high affinity for the target is desirable for a therapeutic
antibody. During the screening of different sequence variants of OS001 to reduce
tendency of aggregation during expression and initial purification step we also
tested CD163 affinity of the generated constructs to see if we could identify
15 constructs with increased CD163 affinity.

Materials and methods

Plasmid (pcDNA 3.4 TOPO) containing the heavy chain variant HC-D72E and the
light chain variant LC-G89Q were synthesized at ThermoFisher (Germany).
Expressed as described in example 1.

20 Fab fragments of the generated antibodies were produced as follows:

10 mg antibody at 1.3 mg/ml in PBS was added 1 M Na-Acetate pH 6 to a final
concentration of 100 mM Na-Acetate, then added freshly prepared Cys to 10 mM,
EDTA to 1 mM and papain to a final concentration 0.01 mg/ml. The mixture was
incubated at 30 C overnight, then added iodo-acetamide to 75 mM and incubated
25 at 30 C for 30 minutes. Undigested antibody was removed by passing mixture over
a 1 ml MabSelect SuRe column and collecting flow-through. The purified Fab
fragment was dialyzed into PBS pH 7.4 using an Amicon® Ultra Centrifugal Filters

with a 3 kDa molecular weight cut off, whereafter purity was confirmed by SDS-PAGE.

Affinity for CD163 was determined using surface plasmon resonance. CD163 was expressed recombinantly and purified as described previously (35). Binding of antibodies to CD163 immobilized on a biosensor chip was studied by surface plasmon resonance (SPR) on a Biacore T200 instrument (Cytiva) essentially as described (35). Briefly, CD163 was immobilized on a CM5 chip and CD163 mAb in running buffer (10 mM HEPES, 150 mM NaCl, 3 mM CaCl₂, and 0.05% Surfactant P-20, pH 7.4) was injected in concentrations ranging from 0.03 to 10 µg/ml. The density of human CD163 domain 1-9 on the flow cell was in the range 0.017-0.025 pmol/mm². The chip was regenerated by two cycles of injection of two times 10 µL 100 mM phosphoric acid. Data was analysed using the BIAcore T200 Evaluation Software 3.1 fitting to a 1:1 kinetic interaction model. All experiments were performed in independent triplicates of duplicates.

15 **Results**

The full-length antibody constructs produced and tested as well as their CD163 binding parameters are summarized in Table 7 and 8, for wild type glycosylation and afucosylation, respectively. Sensorgrams are shown in Figure 15.

Table 7: Overview of novel OS001 (wild type glycosylation) derivates and their binding parameters for binding human CD163 as studied by surface plasmon resonance.

Construct name	Description	k _a (10 ⁵ /Ms)	k _d (10 ⁻⁴ /s)	K _D (nM)
OS001	HC: wt (SEQ ID NO: 4) LC: wt (SEQ ID NO: 1) Glyc: wt	4.0 ± 0.8	2.6 ± 0.1	0.64 ± 0.04
OS018	HC: D72E (SEQ ID NO: 15) LC: wt (SEQ ID NO: 1) Glyc: wt	6.5 ± 0.3	3.3 ± 0.1	0.51 ± 0.03
OS019	HC: wt (SEQ ID NO: 4) LC: G89Q (SEQ ID NO: 2) Glyc: wt	5.2 ± 0.4	2.1 ± 0.1	0.41 ± 0.02
OS020	HC: D72E (SEQ ID NO: 15) LC: G89Q (SEQ ID NO: 2) Glyc: wt	4.7 ± 0.5	2.2 ± 0.1	0.47 ± 0.03

Glyc.: Glycosylation type; HC: Heavy chain variant; LC: Light chain variant.

Table 8: Overview of novel OS017 derivates (afucosylated variants) and their binding parameters for binding human CD163 as studied by surface plasmon resonance.

Construct name	Description	k_a ($10^5/Ms$)	K_d ($10^{-4}/s$)	K_D (nM)
OS017	HC: wt (SEQ ID NO: 4) LC: wt (SEQ ID NO: 1) Glyc: afucosylated	4.4 ± 0.2	4.5 ± 0.1	1.0 ± 0.03
OS022	HC: D72E (SEQ ID NO: 15) LC: wt (SEQ ID NO: 1) Glyc: afucosylated	5.2 ± 0.7	4.9 ± 0.1	0.95 ± 0.11
OS023	HC: D72E (SEQ ID NO: 15) LC: G89Q (SEQ ID NO: 2) Glyc: afucosylated	4.6 ± 0.1	3.7 ± 0.1	0.80 ± 0.02

Glyc.: Glycosylation type; HC: Heavy chain variant; LC: Light chain variant.

5 Introduction of the mutation G89Q in the light chain of OS001 leads to lower off-rates from CD163. G89 is part of the CDR of the LC of OS001. This is accompanied by lowered dissociation constants.

When studying Fab fragments of the IgGs (Table 9), it can be seen that the improved affinity demonstrated by the Fab constructs shows that the changes to
10 the CDR (L89Q) leads to an increased affinity that is not based on avidity, but genuine increased 1:1 affinity. The HC mutation D72E also increases the affinity, albeit not as much as LC G89Q. This increase is especially surprising since HC D72E is not part of the CDR.

Table 9: Overview of OS001 derived Fab fragments and variants and their
15 binding parameters for binding human CD163 as studied by surface plasmon resonance.

Construct name	Description	k_a ($10^5/Ms$)	K_d ($10^{-4}/s$)	K_D (nM)
Fab OS001 OS017	HC: wt (SEQ ID NO: 4) LC: wt (SEQ ID NO: 1)	2.5 ± 0.4	9.7 ± 1.7	39 ± 3
Fab OS018 OS022	HC: D72E (SEQ ID NO: 15) LC: wt (SEQ ID NO: 1)	2.2 ± 0.6	5.8 ± 1.8	27 ± 1
Fab OS019 OS024	HC: wt (SEQ ID NO: 4) LC: G89Q (SEQ ID NO: 2)	3.0 ± 0.6	5.5 ± 1.2	18 ± 1
Fab OS020 OS023	HC: D72E (SEQ ID NO: 15) LC: G89Q (SEQ ID NO: 2)	1.2 ± 0.1	1.8 ± 0.1	15 ± 1

D72E is not part of the CDR of the mAb, but it is a vernier position. Introduction of the mutation D72E in the heavy chain of OS001 and OS017 led to higher on-rate rates, of the full-length antibodies, i.e. more rapid binding to target.

- 5 However, the D72E mutation slightly increased dissociation rate, overall leading to a non-significant effect on affinity as compared to OS001. Surprisingly, the increased dissociation rate is more than counteracted by combining it with LC G89Q, meaning that the combination of LC G89Q and HC D72E exhibited the highest affinity when studying Fab fragments, and also exhibited higher affinity
10 than wt IgG1.

Conclusion

The changes of LC G89Q and HC D72E both increase affinity of the antibody for CD163, and they can be combined to generate an afucosylated antibody with increased CD163 affinity, namely OS023.

15 Example 14: Demonstration that novel OS variants exhibit ADCC activity

Aim of the Study

- To demonstrate that the novel sequence variants OS022 and OS023 of OS001 showing improved druggability and affinity for CD163 (as described in example 11 and 12) still can exert an ADCC effect on CD163 cells. We therefore tested the
20 construct in the reporter assay using human monocyte derived macrophages (described in example 7), as well as the of T cell proliferation (described in example 10).

Materials and methods

See examples 8 and 11 for detailed description of methods.

25 Results

- As can be seen from Figure 16, OS022 and OS023 are as potent as OS017 in mediating ADCC effects in the reporter assay using human monocyte derived macrophages as target. Also, it can be seen from Figure 13, that they are as efficient as OS017 in the T cell proliferation assay. Any differences observed in the
30 study were not significant.

Conclusion

OS022 and OS023 have retained ADCC mode of action ability. Due to their higher affinity for the target (as shown in example 13) they are considered to be superior in an *in vivo* setting, where target is less available, as well as their improved expression characteristics (lower level of aggregates formed, as shown in example 12) make them easier and cheaper to produce for therapeutic use.

Example 15: Effect of CD163+ macrophage depletion on pro-inflammatory cytokine production

Aim of Study

- 10 As removing CD163 expressing tumor associated macrophages resulted in increased T cell proliferation we next asked if this also resulted in increased production of the inflammatory cytokine IFN γ . We tested this in the PBMC based depletion assay by measuring IFN γ level in supernatant by ELISA.

Materials and Methods

- 15 Monocyte-derived macrophages prepared from monocytes purified from buffy coats were loosed by adding 10 mM EDTA and incubating for 15 minutes followed by flushing the petri dish. The macrophages were resuspended in RPMI-1640 medium to a final concentration of 3×10^5 cells/mL.

- Each vial of frozen PBMCs was thawed in pre-warmed RPMI-1640 medium,
20 pelleted by centrifugation (300g, 20°C, 5 min), and resuspended in 2 mL 100 μ g/mL DNase 1 solution. After 15 minutes of incubation, the cells were resuspended at 3×10^6 cells/mL in medium containing 50 ng/mL IL-15, and 100 ng/mL M-CSF. Antibody dilutions were prepared in 1X PBS. In a 24-well plate, 100 μ L/well macrophages, 100 μ L/well PBMCs, and 16 μ L antibody dilution and 284 μ L
25 RPMI-1640 were mixed and incubation at 37°C in 5% CO₂ incubator. After 24hrs, the supernatant was transferred to 15 ml falcon tubes and remaining cells were pelleted by centrifugation (400g, 5 min, 4° C) after which supernatant was harvested. Subsequently IFN γ levels were estimated using the human IFN-gamma DuoSet ELISA kit (RnD Systems) in accordance with manufactures instructions.

30

Results

Depletion of macrophages using OS017 or OS023 resulted in high and significant (p<0.01 for OS017 and OS023 compared to other treatments) increase in IFN γ in culture supernatant, whereas there was virtually no effect of adding an ADCC silenced version of the antibody (OS021) or another CD163 antibody ORV3 (Figure 17), demonstrating that macrophage depletion using OS017 or OS023 leads to high increase in IFN γ production of remaining immune cells.

Conclusion

ADCC mediating CD163 mAbs **OS017 and OS023** can effectively lead to increased production of IFN γ . This will presumably lead to an increased immune activity in the tumors.

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Sequence listings

Table 10: Antibody sequences

SEQ ID NO	Name	Sequence
1	NRY LC OS001- OS018, OS022	MDMRVPAMDMRVPAQLLGLLLLWLPGARCDIVMTQSPSSLSASVGDRVITTC RASQSVSSDVAFQQKPGKSPKPLIYY YASNRY SGVPSRFGSGSGTDFTLTI SSLQAEDFAVYFCGQDYTSPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTL TLISKADYEKKVYACEVTHQGLSSPVTKSFNRGEC
2	NRY G89Q LC OS019, OS020, OS023, OS024	MDMRVPAMDMRVPAQLLGLLLLWLPGARCDIVMTQSPSSLSASVGDRVITTC RASQSVSSDVAFQQKPGKSPKPLIYY YASNRY SGVPSRFGSGSGTDFTLTI SSLQAEDFAVYFCGQDYTSPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTL TLISKADYEKKVYACEVTHQGLSSPVTKSFNRGEC
3	KN2 IgG4 HC OS000	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWMWIRQFPGNKLEWMGY ITYSGSTYYNPSLKS RVTISVDTSKNQFSLKLSS VTAADTATYYCVSGTYFDYWGQGTTLTVSSASTKGPSVFPLAPCSRSTSES TAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYTCNVNHNKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPP KPKDITLMISRTPEVTCVVVDVSDPEVQFNWYVDGVEVHNAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSD GSGFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSL
4	KN2 IgG1 HC OS001, OS017, OS019, OS024	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWMWIRQFPGNKLEWMGY ITYSGSTYYNPSLKS RVTISVDTSKNQFSLKLSS VTAADTATYYCVSGTYFDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYTCNVNHNKPSNTKVDKRVESKYGPPCPSCPAPELLGGPSVFL FPPKPKDITLMISRTPEVTCVVVDVSDPEVQFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSGFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK
5	KN2- IgG1- FT HC OS002	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWMWIRQFPGNKLEWMGY ITYSGSTYYNPSLKS RVTISVDTSKNQFSLKLSS VTAADTATYYCVSGTYFDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYTCNVNHNKPSNTKVDKRVESKYGPPCPSCPAPELLGGPSVFL FPPKPKDITLMISRTPEVTCVVVDVSDPEVQFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSGFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK
6	KN2- IgG1- IQ HC OS003	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWMWIRQFPGNKLEWMGY ITYSGSTYYNPSLKS RVTISVDTSKNQFSLKLSS VTAADTATYYCVSGTYFDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYTCNVNHNKPSNTKVDKRVESKYGPPCPSCPAPELLGGPSVFL FPPKPKDITLMISRTPEVTCVVVDVSDPEVQFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSGFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK

7	KN2-IgG1-AAA HC OS004	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITS SDY AWNWIRQFPGNKLEWMG YITYSGSTYYNPSLKS RVTISVDTSKNQFSLKLSS VTAADTATYYCVSGTY Y FDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLMI S RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNYARVSVSLTVLHQDWLNGKEYKCKVSNKALPAPIA A ATISKAKGQPREPQ VYITLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK
8	KN2-IgG1-VLPLL HC OS005	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITS SDY AWNWIRQFPGNKLEWMG YITYSGSTYYNPSLKS RVTISVDTSKNQFSLKLSS VTAADTATYYCVSGTY Y FDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL LPPKPKDTLMI S RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP EE QYNST L RVVSVSLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYITLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPL V L DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK
9	KN2-IgG1-DLE HC OS006	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITS SDY AWNWIRQFPGNKLEWMG YITYSGSTYYNPSLKS RVTISVDTSKNQFSLKLSS VTAADTATYYCVSGTY Y FDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP V FL FPPKPKDTLMI S RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVSLTVLHQDWLNGKEYKCKVSNKALPL P PEKTISKAKGQPREP QVYITLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK
10	KN2-IgG1-DAE HC OS007	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITS SDY AWNWIRQFPGNKLEWMG YITYSGSTYYNPSLKS RVTISVDTSKNQFSLKLSS VTAADTATYYCVSGTY Y FDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLAGP V FL FPPKPKDTLMI S RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVSLTVLHQDWLNGKEYKCKVSNKALPAPE E EKTISKAKGQPREP QVYITLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK
11	KN2-IgG1-GASDA LIE HC OS008	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITS SDY AWNWIRQFPGNKLEWMG YITYSGSTYYNPSLKS RVTISVDTSKNQFSLKLSS VTAADTATYYCVSGTY Y FDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLAGP V FL FPPKPKDTLMI S RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVSLTVLHQDWLNGKEYKCKVSNKALPL P PEKTISKAKGQPREP QVYITLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK
12	KN2-IgG1-DE HC OS009	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITS SDY AWNWIRQFPGNKLEWMG YITYSGSTYYNPSLKS RVTISVDTSKNQFSLKLSS VTAADTATYYCVSGTY Y FDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP V FL FPPKPKDTLMI S RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVSLTVLHQDWLNGKEYKCKVSNKALPAPE E EKTISKAKGQPREP QVYITLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK

13	KN2-IgG1-MS HC OS010	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWNWIRQFPGNKLEWMGY ITYSGSTYYNPSLKS RVTISVDTSKNQFSLKLSS VTAADTATYYCVSGTYFDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNMALPAPI SK TISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
14	KN2-IgG1-FTDE HC OS016	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWNWIRQFPGNKLEWMGY ITYSGSTYYNPSLKS RVTISVDTSKNQFSLKLSS VTAADTATYYCVSGTYFDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLAGPSVFL FPPKPKDTLMISRTPEVTCVVDV EE EDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKV T NKALPAP E EKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
15	KN2 D72E HC OS018, OS020, OS022, OS023	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWNWIRQFPGNKLEWMGY ITYSGSTYYNPSLKS RVTISVDTSKNQFSLKLSS VTAADTATYYCVSGTYFDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI E KTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
16	KN2 LALAPG HC OS021	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWNWIRQFPGNKLEWMGY ITYSGSTYYNPSLKS RVTISVDTSKNQFSLKLSS VTAADTATYYCVSGTYFDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFL FPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL G APIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
17	ORV3 LC	MDMRVPAMDMRVPAQLLGLLLWLPGARCDIQMTQSPSSLSASVGRVTITC RASQSI SRYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIT SSLQPEDFATYYCQQSYSTQRGAFGGQTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDESTYLSST LTLSKADYEKKHVYACEVTHQGLSSPVTKSFNRGEC
18	ORV3 HC	MKHLWFFLLLVAAPRWVLSQVQLVESGPGGVQPGRSRLRLSCAASGFTFSSYV MHWVRQAPGKGLEWVAVISEDGSNKYEADSVKGRFTISRDN SKNTLYLQMNS LRAEDTAVYYCARENVRPYDFWRGYNSEYYYYGLDVGQGTTVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK

LC: Light Chain; HC: Heavy Chain; CDR 1: *Italic*; CDR 2: **Bold**; CDR 3: Underline;
Gray: mutations. Amino acid fragment 1-29 in the light chain SEQ ID Nos (SEQ ID
NOs: 1 and 2) is a signal peptide, and the indicated numbering such as 89Q is

counted without this signal peptide. The amino acid fragment 1-19 in the heavy chain SEQ ID NOs: (SEQ ID NOs: 3-16) is a signal peptide, and that indicated numbering such as 72E is counted without this signal peptide.

Table 11: Sequences – CDRs

SEQ ID NO	Name	Sequence
19	LC CDR 1 - Kabat	RASQSVSSDVA
20	LC CDR 2- Kabat	YASNRYs
21	LC CDR 3- Kabat	GQDYTSPrT
22	HC CDR 1- Kabat	SDYAWN
23	HC CDR 2- Kabat	YITYSgSTYYNPSLKS
24	HC CDR 3- Kabat	GTYYFDY
25	LC CDR 3- Kabat (optimized)	QQDYTSPrT

5 LC: Light Chain; HC: Heavy Chain

Table 12: CD163 Sequence

SEQ ID NO	Sequence
26	MSKLRMVLLEDSGSADFRRHFNLSPFTITVVLLLSACFVTSSLGGTDKELRLVDGENKC SGRVEVKVQEWGTVCNNGWSMEAVSVICNQLGCPTAIKAPGWANSAGSGRIWMDHVSC RGNESALWDCKHDGWGKHSNCTHQQDAGVTCSDGSNLEMLRTRGGNMCSGRIEIKFQGRW GTVCDDNFNIDHASVICRQLECGSAVSFSGSSNFGESGPIWFDDLICNGNESALWNCKH QGWGKHNCDHAEDAGVICSKGADLSLRLVDGVTECSGRLEVRFQGEWGTICDDGWDSYDA AVACKQLGCPTAVTAIGRVNASKGFGHIWLDSVSCQGHEPAIWQCKHHEWGKHYCNHNED AGVTCSDGSDLELRLRGGGSRCAGTVEVEIQRLLKGVCDRGWGLKEADVVCRQLGCGSAL KTSYQVYSKIQATNTWFLSSCNGNETSLWDCKNWQWGGLTCDHYEEAKITCSAHREPRL VGGDIPCSGRVEVKHGDTWGSICDSDFSLEAASVLCRELQCGTVVSILGGAHFGEGNGQI WAEEFQCEGHESHLSLCPVAPRPEGTCSHSRDVGVVCSRYTEIRLVNGKTPCEGRVELKT LGAWGSLCNSHWDIEDAHVLCQQLKCGVALSTPGGARFGKGNQIWRHMFHCTGTEQHMG DCPVTALGASLCPSEQVASVICSGNQSQTLSSCNSSSLGPTRPTIPEESAVACIESGQLR LVNGGGRCAGRVEIYHEGSWGTICDDSWDLSDAHVVCRQLGCGEAINATGSAHFEGGTGP IWLDEMCKNGKESRIWQCHSHGWGQQNCRHKEDAGVICSEFMSLRLTSEASREACAGRLE VFYNGAWGTVGKSSMSETTVGVVCRQLGCADKGKINPASLDKAMSIPMWVDNVQCPKGPD TLWQCPSSEPWEKRLASPSEETWITCDNKIRLQEGPTSCSGRVEIWHGGSWGTVCDDSWDL DDAQVVCQQLGCGPALKAFKEAEFGQGTGPIWLNEVKCKGNESSLWDCPARRWGHSECGH KEDAAVNCTDISVQKTPQKATTGRSSRQSSFIAVGILGVVLLAIFVALFFLTTKRRRQRQR LAVSSRGENLVHQIQYREMNSCLNADDLDLMNSSENSHESADFSAAELISVSKFLPISGM EKEAILSHTEKENGNL

The extracellular domains are shown as underlined.

10 Table 13: representative antibodies of the invention

Antibody	subclass	LC sequence	HC sequence	Glycosylation
OS025	IgG1	SEQ ID NO: 27	SEQ ID NO: 29	Afucosylated
OS026	IgG1	SEQ ID NO: 27	SEQ ID NO: 30	Afucosylated
OS027	IgG1	SEQ ID NO: 27	SEQ ID NO: 31	Afucosylated
OS028	IgG1	SEQ ID NO: 27	SEQ ID NO: 32	Afucosylated

OS029	IgG1	SEQ ID NO: 27	SEQ ID NO: 33	Afucosylated
OS030	IgG1	SEQ ID NO: 27	SEQ ID NO: 34	Afucosylated
OS031	IgG1	SEQ ID NO: 27	SEQ ID NO: 35	Afucosylated
OS032	IgG1	SEQ ID NO: 27	SEQ ID NO: 36	Afucosylated
OS033	IgG1	SEQ ID NO: 27	SEQ ID NO: 37	Afucosylated
OS034	IgG1	SEQ ID NO: 27	SEQ ID NO: 38	Afucosylated
OS035	IgG1	SEQ ID NO: 27	SEQ ID NO: 39	Afucosylated
OS036	IgG1	SEQ ID NO: 27	SEQ ID NO: 40	Afucosylated
OS037	IgG1	SEQ ID NO: 27	SEQ ID NO: 41	Afucosylated
OS038	IgG1	SEQ ID NO: 27	SEQ ID NO: 42	Afucosylated
OS039	IgG1	SEQ ID NO: 27	SEQ ID NO: 43	Afucosylated
OS040	IgG1	SEQ ID NO: 27	SEQ ID NO: 44	Afucosylated
OS060	IgG1	SEQ ID NO: 28	SEQ ID NO: 29	Afucosylated
OS061	IgG1	SEQ ID NO: 28	SEQ ID NO: 30	Afucosylated
OS062	IgG1	SEQ ID NO: 28	SEQ ID NO: 31	Afucosylated
OS063	IgG1	SEQ ID NO: 28	SEQ ID NO: 32	Afucosylated
OS064	IgG1	SEQ ID NO: 28	SEQ ID NO: 33	Afucosylated
OS065	IgG1	SEQ ID NO: 28	SEQ ID NO: 34	Afucosylated
OS066	IgG1	SEQ ID NO: 28	SEQ ID NO: 35	Afucosylated
OS067	IgG1	SEQ ID NO: 28	SEQ ID NO: 36	Afucosylated
OS068	IgG1	SEQ ID NO: 28	SEQ ID NO: 37	Afucosylated
OS069	IgG1	SEQ ID NO: 28	SEQ ID NO: 38	Afucosylated
OS070	IgG1	SEQ ID NO: 28	SEQ ID NO: 39	Afucosylated
OS071	IgG1	SEQ ID NO: 28	SEQ ID NO: 40	Afucosylated
OS072	IgG1	SEQ ID NO: 28	SEQ ID NO: 41	Afucosylated
OS073	IgG1	SEQ ID NO: 28	SEQ ID NO: 42	Afucosylated
OS074	IgG1	SEQ ID NO: 28	SEQ ID NO: 43	Afucosylated
OS075	IgG1	SEQ ID NO: 28	SEQ ID NO: 44	Afucosylated

Table 14: Representative light chain and heavy chain sequences that can be present in the antibodies of the invention. SEQ ID NO: 27 corresponds to SEQ ID NO: 1 and SEQ ID NO: 28 corresponds to SEQ ID NO: 2.

SEQ ID NO	Name	Sequence
27	NRY LC	MDMRVPAMDMRVPAQLLGLLLLWLPGARCDIVMTQSPSSLSASVGDRVITICRASQSVSSDVAWFQQKPGKSPKPLIYYASNRYSGVPSRFSGSGSGTDFTLTISLQAEDFAVYFCGQDYTSPTFTGGGKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
28	NRY G89Q LC	MDMRVPAMDMRVPAQLLGLLLLWLPGARCDIVMTQSPSSLSASVGDRVITICRASQSVSSDVAWFQQKPGKSPKPLIYYASNRYSGVPSRFSGSGSGTDFTLTISLQAEDFAVYFCQQDYTSPTFTGGGKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
29	KN2 IgG1 M252Y, S254T, T256E	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDYAWNWIRQFPGNKLEWMGYITYSGSTYYNPSLKSRTVISVDTSKNQFSLKLSSVTAADTATYYCVSGTYYFDYWGGTTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPPELLGGPSVFL

		FPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVL DSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNYHTQKSLSLSPGK
30	KN2 D72E M252Y, S254T, T256E	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWNWIRQFPGNKLEWMGYITYSGSTYYNPSLKSRTISVDTSKNQFSLKLSS VTAADTATYYCVSGTYYFDYWGGQTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVL DSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNYHTQKSLSLSPGK
31	KN2 IgG1 M428L, N434S	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWNWIRQFPGNKLEWMGYITYSGSTYYNPSLKSRTISVDTSKNQFSLKLSS VTAADTATYYCVSGTYYFDYWGGQTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVL DSDGSFFLYSKLTVDKSRWQQGNVSCSVLHEALHSHYHTQKSLSLSPGK
32	KN2 D72E, M428L, N434S	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWNWIRQFPGNKLEWMGYITYSGSTYYNPSLKSRTISVDTSKNQFSLKLSS VTAADTATYYCVSGTYYFDYWGGQTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVL DSDGSFFLYSKLTVDKSRWQQGNVSCSVLHEALHSHYHTQKSLSLSPGK
33	KN2 IgG1 H433K, N434F	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWNWIRQFPGNKLEWMGYITYSGSTYYNPSLKSRTISVDTSKNQFSLKLSS VTAADTATYYCVSGTYYFDYWGGQTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVL DSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALKFHYHTQKSLSLSPGK
34	KN2 D72E, H433K, N434F	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWNWIRQFPGNKLEWMGYITYSGSTYYNPSLKSRTISVDTSKNQFSLKLSS VTAADTATYYCVSGTYYFDYWGGQTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVL DSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALKFHYHTQKSLSLSPGK
35	KN2 IgG1 M428L, N434A	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWNWIRQFPGNKLEWMGYITYSGSTYYNPSLKSRTISVDTSKNQFSLKLSS VTAADTATYYCVSGTYYFDYWGGQTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP

		QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFFLYSKLTVDKSRWQQGNVFSCSVLHEALHHAHYTQKSLSLSPGK
36	KN2 D72E, M428L, N434A	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWMWIRQFPGNKLEWMGYITYSGSTYYNPSLKSRVTISVETSKNQFSLKLSS VTAADTATYYCVSGTYYFDYWGGQTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFFLYSKLTVDKSRWQQGNVFSCSVLHEALHHAHYTQKSLSLSPGK
37	KN2 IgG1 L309D, Q311H, N434S	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWMWIRQFPGNKLEWMGYITYSGSTYYNPSLKSRVTISVDTSKNQFSLKLSS VTAADTATYYCVSGTYYFDYWGGQTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVQHHDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFFLYSKLTVDKSRWQQGNVFSCSVLHEALHSHYHTQKSLSLSPGK
38	KN2 D72E, L309D, Q311H, N434S	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWMWIRQFPGNKLEWMGYITYSGSTYYNPSLKSRVTISVETSKNQFSLKLSS VTAADTATYYCVSGTYYFDYWGGQTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVQHHDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFFLYSKLTVDKSRWQQGNVFSCSVLHEALHSHYHTQKSLSLSPGK
39	KN2 IgG1 T250Q, M428L	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWMWIRQFPGNKLEWMGYITYSGSTYYNPSLKSRVTISVDTSKNQFSLKLSS VTAADTATYYCVSGTYYFDYWGGQTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL FPPKPKDQLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFFLYSKLTVDKSRWQQGNVFSCSVLHEALHSHYHTQKSLSLSPGK
40	KN2 D72E, T250Q, M428L	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWMWIRQFPGNKLEWMGYITYSGSTYYNPSLKSRVTISVETSKNQFSLKLSS VTAADTATYYCVSGTYYFDYWGGQTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL FPPKPKDQLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFFLYSKLTVDKSRWQQGNVFSCSVLHEALHSHYHTQKSLSLSPGK
41	KN2 IgG1 Q311R, M428L	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWMWIRQFPGNKLEWMGYITYSGSTYYNPSLKSRVTISVDTSKNQFSLKLSS VTAADTATYYCVSGTYYFDYWGGQTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFFLYSKLTVDKSRWQQGNVFSCSVLHEALHSHYHTQKSLSLSPGK

42	KN2 D72E, Q311R, M428L	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWNWIRQFPGNKLEWMGY ITYSGSTYYNPSLKS RVTISVETSKNQFSLKLSS VTAADTATYYCVSGTYFDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVLHEALHNHYTQKSLSLSPGK
43	KN2 IgG1 N434A	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWNWIRQFPGNKLEWMGY ITYSGSTYYNPSLKS RVTISVDTSKNQFSLKLSS VTAADTATYYCVSGTYFDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EA LHAHYTQKSLSLSPGK
44	KN2 D72E, N434A	MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGYSITSDY AWNWIRQFPGNKLEWMGY ITYSGSTYYNPSLKS RVTISVETSKNQFSLKLSS VTAADTATYYCVSGTYFDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EA LHAHYTQKSLSLSPGK

LC: Light Chain; HC: Heavy Chain; CDR 1: *Italic*; CDR 2: **Bold**; CDR 3: Underline;
Gray: mutations. Amino acid fragment 1-29 in the light chain SEQ ID Nos (SEQ ID
NOs: 27 and 28) is a signal peptide, and the indicated numbering such as 89Q is
5 counted without this signal peptide. The amino acid fragment 1-19 in the heavy
chain SEQ ID NOs: (SEQ ID NOs: 29-44) is a signal peptide, and that indicated
numbering such as 72E is counted without this signal peptide.

Items

1. An antibody targeting CD163 comprising
 - a light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - 5 ○ a CDR 2 region according to SEQ ID NO: 20; and
 - a CDR 3 region according to SEQ ID NO: 21 or 25;
 - a heavy chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 22;
 - a CDR 2 region according to SEQ ID NO: 23; and
 - 10 ○ a CDR 3 region according to SEQ ID NO: 24;

wherein said antibody is afucosylated.
2. The antibody targeting CD163 according to item 1 comprising,
 - a light chain variable region comprising the amino acid sequence of SEQ ID
15 NO: 1, or sequences having at least 90% sequence identity, such as at
 least 95% sequence identity to SEQ ID NO: 1; and
 - a heavy chain variable region comprising the amino acid sequence of SEQ
ID NO: 4, or sequences having at least 90% sequence identity, such as at
least 95% sequence identity to SEQ ID NO: 4;
 - 20

with the proviso that the light chain variable region comprises 89G;
or

 - a light chain variable region comprising the amino acid sequence of SEQ ID
NO: 1, or sequences having at least 90% sequence identity, such as at
25 least 95% sequence identity to SEQ ID NO: 1; and
 - a heavy chain variable region comprising the amino acid sequence of SEQ
ID NO: 15, or sequences having at least 90% sequence identity, such as at
least 95% sequence identity to SEQ ID NO: 15;- 30 with the proviso that the heavy chain variable region comprises 72E;
or
- a light chain variable region comprising the amino acid sequence of SEQ ID
NO: 2, or sequences having at least 90% sequence identity, such as at
least 95% sequence identity to SEQ ID NO: 2; and

- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 1;
with the proviso that the light chain variable region comprises 89Q; and
 - 5 with the proviso that the heavy chain variable region comprises 72E;
or
 - a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 2; and
 - 10 • a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 4, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 4;
with the proviso that the light chain variable region comprises 89Q.
- 15 3. The antibody targeting CD163 according to item 1 or 2, comprising
- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 1; and
 - a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 4;
- 20 or
- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 1; and
 - a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15;
- 25 or
- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2; and
 - a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15;
- 30 or
- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2; and
 - a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 4.

4. An antibody targeting CD163 comprising
- a light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and
 - 5 ○ a CDR 3 region according to SEQ ID NO: 21;
 - a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 15];
- 10 ○ the heavy chain variable region comprising
- a CDR 1 region according to SEQ ID NO: 22;
 - a CDR 2 region according to SEQ ID NO: 23; and
 - a CDR 3 region according to SEQ ID NO: 24;
- 15 with the proviso that the heavy chain variable region comprises 72E;
- or
- a light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and
 - 20 ○ a CDR 3 region according to SEQ ID NO: 25;
 - a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 15;
- with the proviso that the heavy chain variable region comprises
- 25
- a CDR 1 region according to SEQ ID NO: 22;
 - a CDR 2 region according to SEQ ID NO: 23; and
 - a CDR 3 region according to SEQ ID NO: 24;;
- with the proviso that the heavy chain variable region comprises 72E;
- or
- 30
- a light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and
 - a CDR 3 region according to SEQ ID NO: 25;
 - a heavy chain variable region comprising
- 35
- a CDR 1 region according to SEQ ID NO: 22;
 - a CDR 2 region according to SEQ ID NO: 23; and

- a CDR 3 region according to SEQ ID NO: 24.

5. An antibody targeting CD163 according to item 4, comprising

- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 1, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 1; and
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 15;

10

with the proviso that the light chain variable region comprises 89G; and

with the proviso that the heavy chain variable region comprises 72E;

or

- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 2; and
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 15;

20 with the proviso that the light chain variable region comprises 89Q; and with the proviso that the heavy chain variable region comprises 72E;

or

- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 1, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 2; and
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 4, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 4;

with the proviso that the light chain variable region comprises 89Q.

30

6. The antibody targeting CD163 according to any of items 4-5, comprising

- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 1; and
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15;

35

105

or

- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2; and
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15;

5

or

- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2; and
- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 4.

10

7. The antibody according to any of items 4-6, which has regular glycosylation, such as not being afucosylated.

- 15 8. The antibody targeting CD163 according to any of the preceding items, wherein the antibody is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, an antibody, wherein the heavy chain and the light chain are connected by a flexible linker, an Fv molecule, an antigen binding fragment, a Fab fragment, a Fab' fragment, a F(ab')₂ molecule, a fully human antibody, a humanized antibody, and a chimeric antibody.

20

9. The antibody targeting CD163 according to any of the preceding items, wherein the ligand is a F(ab')₂ molecule.

- 25 10. The antibody targeting CD163 according to any of the preceding items, wherein the antibody is humanized, preferably a humanized monoclonal antibody.

11. The antibody targeting CD163 according to any of the preceding items, coupled to a detectable label or a substance having toxic or therapeutic activity.

30

12. The antibody targeting CD163 according to any of the preceding items, having K_D value to CD163 below 1*10⁻⁷, such as below 1*10⁻⁸⁹, or such as below 1*10⁻⁹ M, or such as in the range 1*10⁻⁷ to 1*10⁻¹² M, such as in the range 1*10⁻⁷ to 1*10⁻¹⁰ M, preferably wherein said K_d is lower than for the corresponding non-

35 modified antibody.

13. The antibody targeting CD163 according to any of the proceeding items, being primarily in a monomeric form, such as having lower levels of aggregation than the corresponding non-modified antibody (OS001), such as below 10%
5 aggregation, such as below 8% such as below, preferably 4%, more preferably below 2% aggregation, preferably the level of aggregation is determined after purification, such as after protein A and/or protein G purification.
- 10 10. The antibody targeting CD163 according to any of the proceeding items, being capable of inducing ADCC, preferably to a higher extent than the corresponding non-modified antibody, such as OS001, such as at least 5 times higher, such as at least 7 times higher, preferably at least 10 times higher than the corresponding antibody, more preferably, at least 50 times higher and even more preferably at least 100 times higher than the corresponding non-modified antibody, preferably
15 ADCC induction is measured in the CD16 reporter assay described in example 8 using human monocyte derived macrophages as target cells.
- 20 11. The antibody targeting CD163 according to any of the proceeding items, being capable of inducing ADCP, preferably to a level similar to the corresponding non-modified antibody, such as OS001, such as at least to a level of 70% of the corresponding non-modified antibody, such as OS001, such as at least 75%, preferably at least 80%, preferably ADCP induction is measured as measured in the CD32 (ADCP) reporter assay described in example 7.
- 25 12. The antibody targeting CD163 according to any of the proceeding items, having a thermal stability similar to or higher than the corresponding non-modified antibody, such as OS001, such as at least to a level of 70% of the corresponding non-modified antibody, such as at least 75%, preferably at least 80%, preferably as measured in the CD32 (ADCP) reporter assay described in
30 example 7.
13. The antibody targeting CD163 according to any of the proceeding items, wherein said antibody is able to bind to trimers of CD163 with a higher affinity than monomers of CD163, such as giving a higher signal for the trimeric CD163
35 than the monomeric CD163, such as at least 50% higher signal, such as at least

100% higher signal, preferably when binding is studied on the SPR as described in example 4.

14. The antibody targeting CD163 according to any of the proceeding items, being
5 free from a cytotoxic moiety and/or not being an antibody-drug conjugate.

14.01. The antibody targeting CD163 according to any of the proceeding items,
wherein the light chain variable region comprises the amino acid sequence of SEQ
ID NO: 1, 2, 27 or 28 without amino acid 1-29 (signal peptide), or sequences
10 having at least 90% sequence identity, such as at least 95% sequence identity to
SEQ ID NO: 1, 2, 27 or 28 without amino acid 1-29 (signal peptide).

14.02. The antibody targeting CD163 according to any of the proceeding items,
wherein the heavy chain variable region comprises the amino acid sequence of
15 any of SEQ ID NO's: 3-16 or 29-44 without amino acid 1-19 (signal peptide) or
sequences having at least 90% sequence identity, such as at least 95% sequence
identity to SEQ ID NO's: 3-16 or 29-44 without amino acid 1-19 (signal peptide).

14.03. The antibody targeting CD163 according to any of the proceeding items,
20 wherein the heavy chain variable region comprises the amino acid sequence of
any SEQ ID NO: 15 without amino acid 1-19 (signal peptide), or a sequence
having at least 90% sequence identity, such as at least 95% sequence identity to
SEQ ID NO's: 3-16 or 27-44 without amino acid 1-19 (signal peptide).

25 15. A vector or vectors encoding the antibody targeting CD163 according to any of
the preceding items, such as LC and HC being encoded on the same vector or
such as the LC is expressed from one vector and the HC is expressed from
another vector.

30 16. A cell expressing the antibody targeting CD163 according to any of items 1-
14, and/or a cell comprising the vector(s) according to item 15.

17. A composition comprising the antibody targeting CD163 according to any of
items 1-14, and one or more physiologically acceptable carriers, excipients and/or
35 diluents.

18. The composition according to item 17, being a pharmaceutical composition.

19. A combination comprising the antibody targeting CD163 according to any of
5 items 1-4 and an immune checkpoint inhibitor.

20. The combination according to item 19, wherein the immune checkpoint
inhibitor is selected from the group consisting of PD-1 antagonists, PD-L1
antagonists, PD-L2 antagonists, CTLA-4 antagonists, VISTA antagonists, TIM-3
10 antagonists, LAG-3 antagonists, IDO antagonists, KIR2D antagonists, A2AR
antagonists, B7-H3 antagonists, B7-H4 antagonists, and BTLA antagonists.

21. The antibody targeting CD163 according to any of item 1-14, and/or
composition according to any of items 17-18 and/or combination according to any
15 of items 19-20, for use as a medicament.

22. The antibody targeting CD163 according to any one of items 1-14, and/or the
composition according to any of items 17-18 and/or combination according to any
of items 19-20, for use in the treatment or alleviation of cancer.

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23. The antibody targeting CD163 according to any one of items 1-14, and/or the
composition according to any of items 17-18 and/or combination according to any
of items 19-20, for use according to any of items 21-22, wherein the cancer is
selected from the group consisting of solid tumours and blood-borne tumours.

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24. The antibody targeting CD163 according to any one of items 1-14, and/or the
composition according to any of items 17-18 and/or combination according to any
of items 19-20, for use according to any of items 21-23, being administering to a
subject at a therapeutically effective amount capable of depleting the population
30 of CD163+ tumor associated macrophages.

25. The antibody targeting CD163 according to any one of items 1-14, and/or the
composition according to any of items 17-18 and/or combination according to any
of items 19-20, for use according to any of items 21-24, being administering to a
35 subject at a therapeutically effective amount capable of depleting the population

of CD163+ tumor associated macrophages and increasing the amount of tumor infiltrating CD8+ T cells.

26. The antibody targeting CD163 according to any one of items 1-14, and/or the
5 composition according to any of items 17-18 and/or combination according to any of items 19-20, for use according to any of items 21-25, wherein the cancer is resistant to checkpoint therapy.

27. The antibody targeting CD163 according to any one of items 1-14, and/or the
10 composition according to any of items 17-18 and/or combination according to any of items 19-20, for use according to any of items 21-25, wherein the cancer is characterized by a low tumour infiltration of CD8+ T cells and/or a high tumour infiltration of CD163+ tumour associated macrophages.

15 28. A method for treating or alleviating a subject suffering from cancer, the method comprising administering to the subject an antibody and/or composition and/or combination according to the invention.

29. The antibody targeting CD163 according to any one of items 1-14, and/or the
20 composition according to any of items 17-18 and/or combination according to any of items 19-20, wherein the (monoclonal) antibody binds CD163 in its trimeric form, preferably the trimeric form that is present on the surface of a cell, such as the surface of a TAM, with a higher affinity than it binds the monomeric form of CD163 that is present in circulation.

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30. The antibody targeting CD163 according to any one of items 1-14, and/or the composition according to any of items 17-18 and/or combination according to any of items 19-20, for use in the treatment of cancer.

30 31. The antibody targeting CD163 according to any one of items 1-14, and/or the composition according to any of items 17-18 and/or combination according to any of items 19-20, for use for depletion of TAMs expressing (trimeric) CD163 on their surface, such as by means of ADCC.

32. The antibody targeting CD163 according to any one of items 1-14, and/or the composition according to any of items 17-18 and/or combination according to any of items 19-20, wherein the antibody binds CD163 that is present on the cell surface with higher affinity than CD163 that is present in circulation, such as in
5 blood or plasma.

33. The antibody targeting CD163 according to any one of items 1-14, and/or the composition according to any of items 17-18 and/or combination according to any of items 19-20, wherein the antibody binds the trimeric form of CD163 that is
10 present on the surface of a cell, and in particular the trimeric form CD163 that is present on the surface of a TAM, with higher affinity than they bind to monomeric CD163.

34. The antibody targeting CD163 according to any one of items 1-14, and/or the
15 composition according to any of items 17-18 and/or combination according to any of items 19-20, wherein the antibody binds trimers of CD163 with a higher affinity than monomers of CD163, in particular as determined by Surface plasmon resonance (SPR), such as using the SPR method that is used in example 4.

20 35. The antibody targeting CD163 according to any one of items 1-14, and/or the composition according to any of items 17-18 and/or combination according to any of items 19-20, wherein the antibody binds on an epitope that is present on the trimeric form of CD163 which epitope is not formed, or is not accessible, when CD163 is in its monomeric form.

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36. The antibody targeting CD163 according to any one of items 1-14, and/or the composition according to any of items 17-18 and/or combination according to any of items 19-20, wherein the antibody binds on an epitope that is present on the trimeric form of CD163, which epitope is formed by at least two, such as two or
30 three, of the CD163 molecules that form the CD163 trimer.

37. The antibody targeting CD163 according to any one of items 1-14, and/or the composition according to any of items 17-18 and/or combination according to any of items 19-20, wherein the antibody binds on an epitope that is present on the

trimeric form of CD163, which epitope is formed by all three of the CD163 molecules that form the CD163 trimer.

38. The antibody targeting CD163 according to any one of items 1-14, and/or the composition according to any of items 17-18 and/or combination according to any of items 19-20, wherein the antibody binds to an epitope on trimeric CD163 that is (essentially) not recognized by the known CD163 antibodies ORV3, 15-5-20 and/or 15-5-30.
39. The antibody targeting CD163 according to any one of items 1-14, and/or the composition according to any of items 17-18 and/or combination according to any of items 19-20, wherein the antibody (essentially) do not compete for binding to trimeric CD163 with the antibody ORV3.
40. The antibody targeting CD163 according to any one of items 1-14, and/or the composition according to any of items 17-18 and/or combination according to any of items 19-20, wherein the antibody does not compete for binding to trimeric CD163 with the antibodies ORV3, 15-5-20 and/or 15-5-30.
41. The antibody targeting CD163 according to any one of items 1-14, and/or the composition according to any of items 17-18 and/or combination according to any of items 19-20, wherein at least 50% of the antibodies remain bound to trimeric CD163 in the presence of monomeric (i.e. soluble) CD163, such as at a physiologically relevant concentration of sCD163, such as around 2 µg/ml of sCD163.
42. The antibody targeting CD163 according to any one of items 1-14, and/or the composition according to any of items 17-18 and/or combination according to any of items 19-20, wherein at least 50% of the antibodies remain bound to trimeric CD163 that has been immobilized on a carrier, such as a carrier that can be suitably used in a method for measuring binding in the presence of sCD163, in the presence of monomeric (i.e. soluble) CD163, such as a physiologically relevant concentration of sCD163, such as around 2 µg/ml of sCD163.

43. The antibody targeting CD163 according to any one of items 1-14, and/or the composition according to any of items 17-18 and/or combination according to any of items 19-20, wherein the antibody are such that they are able to bind to trimers of CD163 with a higher affinity than monomers of CD163, such as giving a higher signal for the trimeric CD163 than the monomeric CD163, such as at least 50% higher signal, such as at least 100% higher signal, preferably when binding is determined using SPR, preferably as described in example 4.
44. The antibody targeting CD163 according to any one of items 1-14, and/or the composition according to any of items 17-18 and/or combination according to any of items 19-20, wherein the antibody binds trimeric CD163 with an affinity that is 5 times higher than for the monomer, alternatively 10 times higher, 20 times higher or 50 times higher.
45. The antibody targeting CD163 according to any one of items 1-14, and/or the composition according to any of items 17-18 and/or combination according to any of items 19-20, wherein the antibody comprises an Fc portion that, compared to a naturally occurring Fc portion, has been modified so as to have improved ADCC capabilities, such as being an antibody according to item 1.

Claims

1. An antibody targeting CD163 comprising
- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 1, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 1;
 - the light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and
 - a CDR 3 region according to SEQ ID NO: 21;
 - a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 15;
 - the heavy chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 22;
 - a CDR 2 region according to SEQ ID NO: 23; and
 - a CDR 3 region according to SEQ ID NO: 24.
- with the proviso that the heavy chain variable region comprises 72E;
- or
- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 2;
 - the light chain variable region comprising
 - a CDR 1 region according to SEQ ID NO: 19;
 - a CDR 2 region according to SEQ ID NO: 20; and
 - a CDR 3 region according to SEQ ID NO: 25;
 - a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 15;
- with the proviso that the heavy chain variable region comprises
- a CDR 1 region according to SEQ ID NO: 22;
 - a CDR 2 region according to SEQ ID NO: 23; and
 - a CDR 3 region according to SEQ ID NO: 24;
- with the proviso that the heavy chain variable region comprises 72E.

2. An antibody targeting CD163 according to claim 1, comprising
- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 1, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 1; and
 - a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 15;
- with the proviso that the light chain variable region comprises 89G; and
with the proviso that the heavy chain variable region comprises 72E;
- or
- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 2; and
 - a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, or sequences having at least 90% sequence identity, such as at least 95% sequence identity to SEQ ID NO: 15;
- with the proviso that the light chain variable region comprises 89Q; and
with the proviso that the heavy chain variable region comprises 72E.
3. The antibody targeting CD163 according to any of claims 1-2, comprising
- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 1; and
 - a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15;
- or
- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2; and
 - a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15.

4. The antibody targeting CD163 according to any of the preceding claims, comprising
- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2; and
 - 5 • a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15.
5. The antibody according to any of the preceding claims, being afucosylated.
- 10 6. The antibody targeting CD163 according to any of the preceding claims, having K_D value to CD163 below $1 \cdot 10^{-7}$, such as below $1 \cdot 10^{-8}$, or such as below $1 \cdot 10^{-9}$ M, or such as in the range $1 \cdot 10^{-7}$ to $1 \cdot 10^{-12}$ M, such as in the range $1 \cdot 10^{-7}$ to $1 \cdot 10^{-10}$ M, preferably, wherein said K_D is lower than for the corresponding non-modified antibody;
- 15 and/or
- being primarily in a monomeric form, such as having lower levels of aggregation than the corresponding non-modified antibody, such as below 10% aggregation, such as below 8% such as below, preferably 4%, more preferably below 2%
- 20 aggregation,
- and/or
- being capable of inducing ADCC, preferably to a higher extent than the corresponding non-modified antibody, such as at least 5 times higher, such as at least 7 times higher, preferably at least 10 times higher than the corresponding antibody, more preferably, at least 50 times higher and even more preferably at least 100 times higher;
- 25 and/or
- 30 having a thermal stability similar to or higher than the corresponding non-modified antibody, such as at least to a level of 70% of the corresponding non-modified antibody, such as at least 75%, preferably at least 80%;
- and/or

being able to bind to trimers of CD163 with a higher affinity than monomers of CD163.

7. The antibody targeting CD163 according to any of the preceding claims 1-2,
5 comprising one or more modifications in the heavy chain variable region selected from the group consisting of T250Q, M252Y, S254T, T256E, L309D, Q311H, Q311R, N434S, M428L, H433K, N434S, N434F, N434A and combinations thereof.

8. The antibody targeting CD163 according to claim 7, comprising one or more
10 modifications in the heavy chain variable region selected from the group consisting of

- M252Y, S254T and T256E;
- M428L and N434S;
- H433K and N434F;
- 15 • M428L and N434A;
- L309D, Q311H and N434S;
- T250Q and M428L;
- Q311R and M428L ; and
- N434A.

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9. The antibody targeting CD163 according to any of the proceeding claims, being coupled to a detectable label or a substance having toxic or therapeutic activity

and/or

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the antibody targeting CD163 according to any of the proceeding claims, comprising one or more modifications that improves ADCC.

10. A vector or vectors encoding the antibody targeting CD163 according to any of
30 claims 1-9; and/or a cell expressing the antibody targeting CD163 according to any of claims 1-9.

11. A composition comprising the antibody targeting CD163 according to any of
claims 1-9, and one or more physiologically acceptable carriers, excipients and/or
35 diluents.

12. A combination comprising the antibody targeting CD163 according to any of claims 1-9 and an immune checkpoint inhibitor.

5 13. The combination according to claim 12, wherein the immune checkpoint inhibitor is selected from the group consisting of PD-1 antagonists, PD-L1 antagonists, PD-L2 antagonists, CTLA-4 antagonists, VISTA antagonists, TIM-3 antagonists, LAG-3 antagonists, IDO antagonists, KIR2D antagonists, A2AR antagonists, B7-H3 antagonists, B7-H4 antagonists, and BTLA antagonists.

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14. The antibody targeting CD163 according to any of claim 1-9, and/or composition according to claim 11 and/or combination according to claim 12 or 13 for use as a medicament.

15 15. The antibody targeting CD163 according to any one of claims 1-9, and/or the composition according to claim 11 and/or combination according to claim 12 or 13 for use in treatment or alleviation of cancer.

16. The antibody targeting CD163 according to any one of claims 1-9, and/or the
20 composition according to claim 11 and/or combination according to claim 12 or 13 for use in treatment or alleviation of inflammatory diseases.

17. The antibody targeting CD163 according to any one of claims 1-9, and/or the composition according to claim 11 and/or combination according to claim 12 or 13
25 for use in treatment or alleviation of fibrotic diseases.