

induce target cell cytotoxicity and that cytotoxicity did not correlate with antigen expression levels. For example, CD3-based bispecific antibodies targeting HLA-DR or CD138 were not able to induce cytotoxicity in spite of intermediate to high HLA-DR and CD138 expression levels (Engelberts et al. (2020) Ebiomedicine 52:102625). Few

5 T-cell redirecting therapies have reached late-stage clinical development, possibly due to significant toxicity, manufacturing problems, immunogenicity, and low response rates in solid tumors. In particular, toxicity may occur when the T-cell engager includes a CD3 binding arm as a result of uncontrolled immune activation and cytokine release.

10 Thus, while significant progress has been made, there is still a need for novel PSMA antibodies that are therapeutically effective yet have acceptable toxicity. Such novel PSMA antibodies should also have appropriate pharmacokinetic and pharmacodynamic properties and optimally be manufacturable with high yield and purity. Furthermore, stable formations of such antibodies are needed in which minimal

15 degradation and aggregation occurs.

These needs are addressed by the present invention.

Summary of the invention

The present ~~invention~~disclosure provides novel antibodies for PSMA-based therapy.

20 Bispecific antibodies were constructed in which PSMA-binding regions were combined with binding regions capable of binding a V γ 9V δ 2 T cell receptor and thus engaging $\gamma\delta$ T cells. Surprisingly, the bispecific antibodies were able to mediate activation of autologous V γ 9V δ 2 T cells, including tumor-infiltrating V γ 9V δ 2 T cells, and to induce killing of patient-derived tumor cells in the presence of autologous PBMC-derived

25 V γ 9V δ 2 T cells with very high potency. These activities were also observed using tumor cell lines at low effector cell ($\gamma\delta$ T cell) to target cell (tumor cell) ratios, which is important, because $\gamma\delta$ T cells are only a subpopulation of T cells in humans which can vary in numbers. Normal healthy tissue, on the other hand, was not affected, indicating the potential of these antibodies for an efficacious yet safe cancer

treatment. In addition, studies with whole human blood indicate that in spite of the high potency on target cells, the antibody only induced low levels of cytokine release, suggesting a low risk of cytokine release syndrome.

Furthermore, a novel bispecific VHH-human-Fc-containing antibody format was developed which, when produced in mammalian host cells, yielded a highly homogenous and pure product. Moreover, the format had suitable pharmacokinetic properties for use in medical treatment and a suitable stable formulation for this product was developed. Without being bound by any specific theory, the molecular size and decreased hydrodynamic radius of the format may be highly suitable for tumor penetration, yet avoid renal filtration and ensure, via binding to FcRn, protection from degradation.

Accordingly, in a first aspectexample, the inventiondisclosure provides a multispecific antibody comprising a first antigen-binding region capable of binding human PSMA and a second antigen-binding region capable of binding a human V γ 9V δ 2 T cell receptor.

In one aspect, the present disclosure refers to a multispecific antibody comprising a first antigen-binding region capable of binding human PSMA and a second antigen-binding region capable of binding a human V γ 9V δ 2 T cell receptor, wherein the first antigen-binding region comprises a CDR1 of SEQ ID NO: 14, a CDR2 of SEQ ID NO: 15, and a CDR3 of SEQ ID NO: 16, and wherein the first antigen-binding region comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 3.

In further aspectexamples, the inventiondisclosure relates to pharmaceutical compositions comprising the antibodies of the inventiondisclosure, uses of the antibodies of the inventiondisclosure in medical treatment, and to nucleic acid constructs, expression vectors for producing antibodies of the inventiondisclosure and to host cells comprising such nucleic acid constructs or expression vector.

In one aspect, the present disclosure refers to a pharmaceutical composition comprising a multispecific antibody disclosed herein and a pharmaceutically-acceptable excipient.

In one aspect, the present disclosure refers to the multispecific antibody disclosed herein for use as a medicament.

In one aspect, the present disclosure refers to use of the multispecific antibody disclosed herein in the manufacture of a medicament for the treatment of cancer.

In one aspect, the present disclosure refers to use of the multispecific antibody disclosed herein in the manufacture of a medicament for the treatment of prostate

cancer.

In one aspect, the present disclosure refers to use of the multispecific antibody disclosed herein in the manufacture of a medicament for the treatment of cancers in which PSMA is expressed on the tumor neo-vasculature or tumor-associated endothelial cells of primary or metastatic tumors including from colorectal cancer, lung cancer, breast cancer, endometrial and ovarian cancer, gastric cancer, renal cell cancer, urothelial cancer, hepatocellular cancer, oral squamous cancer, thyroid tumors and glioblastomas.

In one aspect, the present disclosure refers to use of the multispecific antibody disclosed herein in the manufacture of a medicament for the treatment of adenoid cystic carcinoma of the head and neck.

In one aspect, the present disclosure refers to a nucleic acid construct encoding the multispecific antibody disclosed herein.

In one aspect, the present disclosure refers to an expression vector comprising a nucleic acid construct disclosed herein.

In one aspect, the present disclosure refers to a host cell, such as a mammalian host cell, preferably a CHO cell comprising a nucleic acid construct disclosed herein or an expression vector disclosed herein.

In one aspect, the present disclosure refers to a method for production of a multispecific antibody disclosed herein, preferably a multispecific antibody disclosed herein, by (co-)expression of one or more nucleic acid constructs encoding the multispecific antibody in a suitable host cell, such as a mammalian cells, preferably a CHO cell, followed by purification of the produced recombinant antibody.

Further ~~aspects and embodimentsexamples~~ of the ~~invention~~disclosure are described below.

Brief description of the drawings

Figure 1: Sequence alignment of humanization variants of the anti-PSMA VHH JVZ-007. LV1044 is the original llama-derived sequence; the other sequences are humanized variants.

Figure 2: Schematic representation of the half-life extended format of the bispecific VHHs. Fc silencing mutations are indicated by the asterisks. The sequence of the modified hinge is indicated next to the figure, as well as the CH3 mutations that confer the KiH mediated hetero-dimerization. HC: heavy chain.