Trispecific Antibodies for Selective CD16A-directed NK-Cell Engagement in Multiple Myeloma

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Abstract

Development of antibody scaffolds to directly engage cytotoxic effector cells for therapeutic applications is limited by the scarcity of surface antigens which are expressed exclusively on tumor cells and show limited or no expression on non-malignant cells. We have therefore designed a novel antibody format to selectively retarget effector cell cytotoxicity to tumor cells co-expressing two surface antigens. NK-cells play an important role in the innate immune response to multiple myeloma (MM) and are known to contribute to the efficacy of novel therapeutics. We, therefore, utilized a MM-based model system to generate proof-of concept data demonstrating antibody-mediated NK-cell retargeting to cell lines co-expressing two MM-expressed surface antigens with increased selectivity ('dual-targeting').

B-cell maturation antigen (BCMA/CD269) is widely considered to be a promising target antigen for antibody-based therapies of MM due to its almost universal expression on patient myeloma cells and its restricted surface expression on cells outside of the haematological lineage. However, low levels of expression on healthy tissue, including skin, has been reported, which may result in side effects of BCMA-targeted antibody therapies due to effector cell activation in these organs. CD200 is a second MM-expressed surface antigen found on malignant plasma cells of the majority of patients. To increase selectivity of antibody-induced, effector cell-mediated cytotoxicity towards malignant tissue, we developed a trispecific antibody format capable of selectively engaging NK-cells through bivalent binding to CD16A (FcγRIIIa) and monovalent binding to both BCMA and CD200. Using an *in vitro* model system, we demonstrated that binding to BCMA+/CD200+ cell lines and the resulting increase in avidity leads to preferential lysis of antigen double-positive cells compared with antigen single-positive cells. These data suggest that dual-targeting may eventually be used to increase the therapeutic window compared to approaches targeting only one antigen. In addition to the MM-based model system used here, the novel trispecific antibodies we have developed may be adapted to alternative target combinations within MM or in other tumor indications. Moreover, they could be used to target phenotypically distinct tumor cell clones to induce deeper and more prolonged antitumor responses or to retarget other effector cell populations, such as T-cells, with increased selectivity and enhanced safety in the absence of exclusively tumor-expressed target antigens.

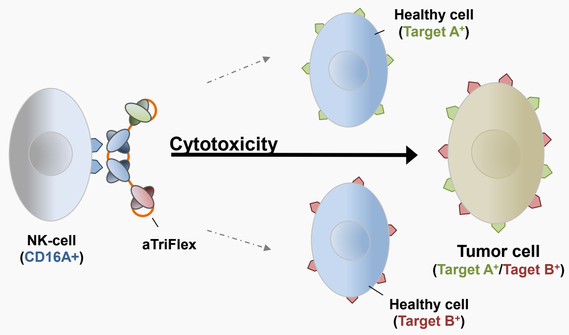
Key results

1. **A novel trispecific CD16A-directed antibody format was developed to selectively retarget NK-cell cytotoxicity to two tumor expressed surface antigens**
2. ***In vitro* proof-of-concept data suggest increased selectivity of NK-cell-mediated target cell lysis using dual-targeting trispecifics**
3. **Trispecific antibodies may allow novel targeting approaches in multiple myeloma**

Dual-targeting: Concept and MoA

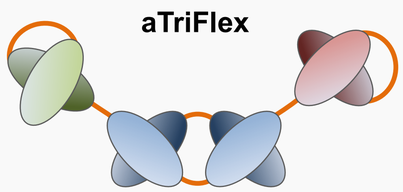
1) Bivalent NK-cell engagement via CD16A (FcγRIIIa)
2) Monovalent, low affinity binding to antigen single-positive cells
3) Increased avidity upon bivalent target cell interaction

> Preferential lysis of antigen double-positive cells



aTriFlex - A novel trispecific CD16A-directed antibody format

Antibody design



- Asymmetric, trispecific Flexibody
- **Bivalent for CD16A (FcγRIIIa)**
- **Monovalent for two tumor antigens**
- Heterodimer, ~100kDa (theor.)
- Engineered diabody-like core module to enforce chain heterodimerization

anti-CD16A Fv | anti-BCMA Fv | anti-CD200 Fv | $(GGS)_n$ linker

Expression and purification

- Two trispecifics incorporating low affinity anti-BCMA and anti-CD200 domains were generated
- Expressed in stably-transfected CHO cells
- Purification by IMAC/pSEC

	Heterodimer in CCS [%]	Heterodimer in CCS [mg/L]	Final purity (SEC) [%]
aTriFlex_105	53	22	92.2
aTriFlex_115	93	225	96.2

CCS: Cell culture supernatant

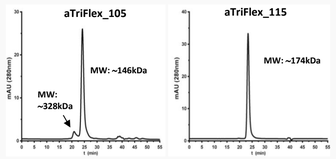


Figure 1. Size-exclusion chromatography (SEC) of purified aTriFlex_105 and aTriFlex_115. Proteins were resolved on a Superdex 200 Increase 10/300 GL (GE) column and molecular weight was assessed by comparison of elution profiles with molecular weight markers

Characterization of anti-CD16A core module

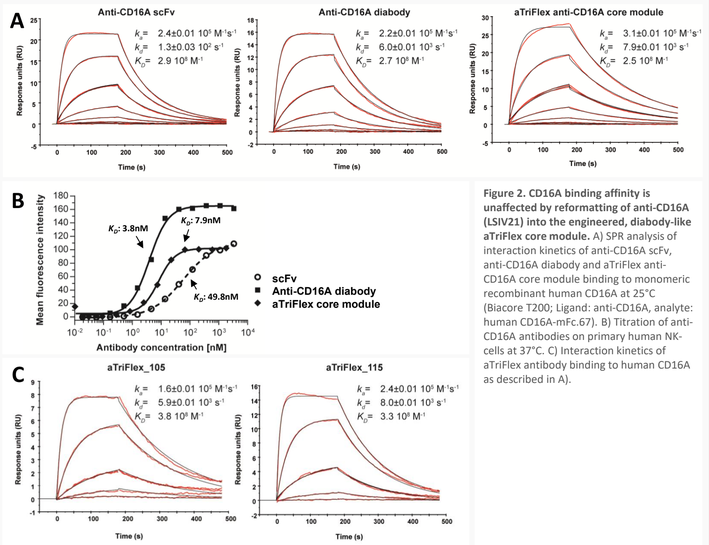
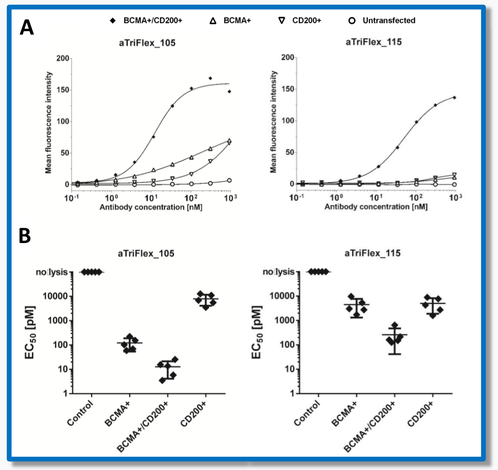


Figure 2. CD16A binding affinity is unaffected by reformatting of anti-CD16A (LSIV21) into the engineered, diabody-like aTriFlex core module. A) SPR analysis of interaction kinetics of anti-CD16A scFv, anti-CD16A diabody and aTriFlex anti-CD16A core module binding to monomeric recombinant human CD16A at 25°C (Biacore T200; Ligand: anti-CD16A, analyte: human CD16A-mFc.67). B) Titration of anti-CD16A antibodies on primary human NK-cells at 37°C. C) Interaction kinetics of aTriFlex antibody binding to human CD16A as described in A).

Selective targeting of BCMA+/CD200+ cells *in vitro*

- **Dual-targeting of BCMA and CD200 increased aTriFlex avidity on antigen double-positive cells**
- **Up to 20-fold increased cytotoxic *in vitro* potency towards BCMA+/CD200+ cells**



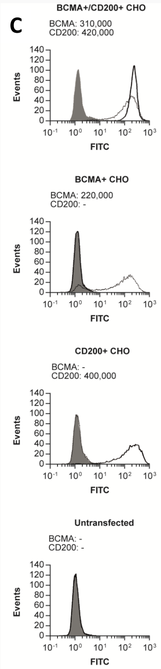


Figure 3. Dual-targeting of antigen double-postive cells using trispecific aTriFlex antibodies. A) Titration of aTriFlex antibodies on stably-transfected CHO cells expressing BCMA, CD200 or both at 37°C. As control, antibodies were titrated on antigen-negative cells (untransfected) B) *In vitro* potency of aTriFlex antibodies in cytotoxicity assays using primary human NK-cells as effectors (E) and stably-transfected CHO cells as targets (T) at an E:T ratio 5:1 (Calcein-release assay, 4h incubation). C) Quantification of BCMA and CD200 surface expression on stably-transfected CHO cells used in A) and B). Values represent specific antigen binding capacities (SABC) of MRC OX-104 (anti-CD200) and ANC3B1 (anti-BCMA) antibodies as determined by flow cytometry and QIFIKIT analysis.

Disclosures

- T.G., M.W., C.H., U.R., K.E., I.F. and M.T. are full-time employees of Affimed GmbH.
- R.G. and V.M. are full-time employees of AbCheck s.r.o.

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