Abstract No

8045

AFM26 is a novel, highly potent BCMA/CD16A-directed bispecific antibody for high affinity NK-cell engagement in multiple myeloma

Thorsten Gantke[1], Uwe Reusch[1], Christian Kellner[2], Kristina Ellwanger[1], Ivica Fucek[1], Michael Weichel[1], Matthias Peipp[2] and Martin Treder[1,#]

[1] Affimed GmbH, Im Neuenheimer Feld 582, 69120 Heidelberg, Germany, [2] Christian-Albrechts-University of Kiel, Division of Stem cell Transplantation and Immunotherapy, Arnold-Heller Straße 3, 24105 Kiel, Germany

Abstract

Background: Despite recent advances in the treatment of multiple myeloma (MM), novel therapies are needed to achieve long-lasting remissions in a greater number of patients. Natural killer (NK) cells play a key role in the immune response to MM and have been implicated in the clinical efficacy of current standard of care interventions, including IMiDs, proteasome inhibitors, recently approved immunotherapies and autologous stem cell transplantation (ASCT). Numerous strategies are being developed to enhance the natural NK-cell cytotoxicity against myeloma cells, which is frequently dysregulated in MM. Approaches include modulation of activity, through cytokine stimulation or immune checkpoint targeting, and adoptive transfer of culture expanded NK-cells in ASCT-eligible MM. While highly attractive, these approaches are non-targeted, as they rely on the natural cytotoxicity of NK-cells, and may benefit from antigen-specific retargeting and effector activation. AFM26 is a novel tetravalent, bispecific antibody designed to specifically enhance NK-cell anti-MM activity by redirecting NK-cell cytotoxicity to cells expressing B-cell maturation antigen (BCMA/CD269), an antigen expressed on MM cells. **Methods:** NK-cell engagement and cytotoxicity of AFM26 towards MM cell lines and freshly isolated tumor cells from MM patients was characterized *in vitro* and compared with classical antibody formats. **Results:** AFM26 engages NK-cells with superior avidity (K_D: 1-2nM) through bivalent interaction with CD16A (FcγRIIIa) and demonstrates extended cell surface retention that is not affected by high level serum IgG, which is particularly relevant in MM. Importantly, AFM26 does not induce NK-cell depletion but selectively elicits potent and efficacious lysis of MM cells *in vitro*. **Conclusions: AFM26, the first-in-class BCMA-targeting NK-cell engager, is a promising candidate to enhance NK-cell activity and confer tumor-specificity to NK-cells in MM. AFM26 is well differentiated from classical antibody formats and appears to be suitable to target BCMA(+) disease as a single agent or in combination with cellular NK-cell therapy.**

Key Points:

AFM26, a first-in-class NK-cell engager targeting BCMA

1. **Potently stimulates NK-cell-mediated cytotoxicity towards myeloma cells, including cells expressing very low levels of BCMA**
2. **May have a superior safety profile compared to BCMA/CD3 T-cell engagers**
3. **Engages NK-cells with high avidity in presence of high level serum IgG and irrespective of CD16A polymorphism**
4. **Does not induce NK-cell depletion**

High avidity NK-cell engagement

- **Bivalent engagement of NK-cells through CD16A**

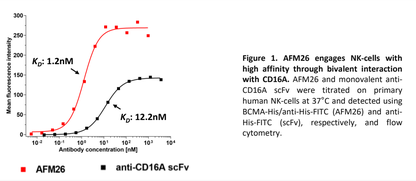


Figure 1. AFM26 engages NK-cells with high affinity through bivalent interaction with CD16A. AFM26 and monovalent anti-CD16A scFv were titrated on primary human NK-cells at 37°C and detected using BCMA-His/anti-His-FITC (AFM26) and anti-His-FITC (scFv), respectively, and flow cytometry.

- **NK-cell binding activity superior to IgG_1 and Fc-engineered IgG_1**
- **Largely unaffected by polyclonal serum IgG**

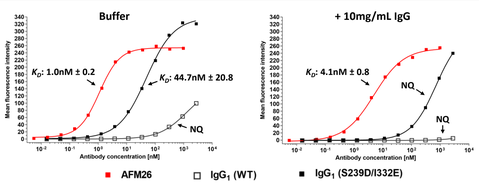


Figure 2. AFM26 exhibits superior NK-cell binding activity compared with wild-type (WT) and Fc-engineered IgG1 (S239D/I33E) and is largely unaffected by polyclonal IgG. Antibodies were titrated on primary human NK-cells at 37°C in presence (right panel) or absence (left panel) of 10mg/mL polyclonal IgG (Gammanorm, Octapharma) followed by detection using BCMA-His/anti-His-FITC and flow cytometry. K_D values are mean ± SD of three independent experiments. NQ: not quantifiable.

- **Prolonged NK-cell surface retention that is not compromised by polyclonal IgG**

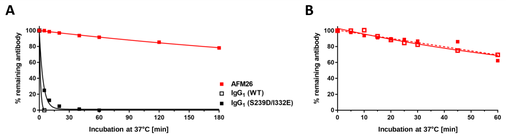


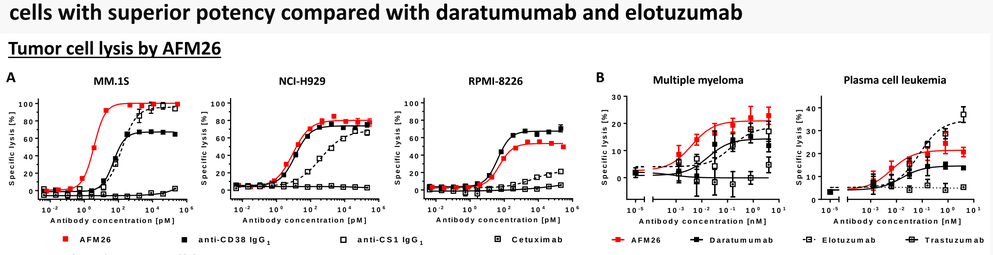
Figure 3. AFM26 exhibits markedly longer cell surface retention on NK-cells than wild-type (WT) and Fc-engineered (S239D/I332E) IgG1 that is unaffected by polyclonal IgG. A) AFM26 and IgGs were added to primary human NK-cells at 100µg/mL and 400µg/mL, respectively, before removal of unbound antibody by washing and incubation in 10mL PBS (2% FCS, 0.1% NaN3) at 37°C. Remaining surface-bound antibody was quantified by detection with BCMA-His/anti-His-FITC and flow cytometry. B) AFM26 NK-cell surface retention in presence and absence of 10mg/mL polyclonal IgG (Gammanorm, Octapharma). Remaining antibody was detected as in A).

- **Interaction with CD16A is independent of receptor polymorphism at 158 (V/F)**

AFM26 potently induces NK-cell-mediated lysis of BCMA(+) target cell lines and primary myeloma tumor cells *in vitro*

- **Despite the low expression of BCMA on MM tumor cells, AFM26 induces lysis of primary tumor cells with superior potency compared with daratumumab and elotuzumab**

Tumor cell lysis by AFM26



AFM26 binding to cell lines

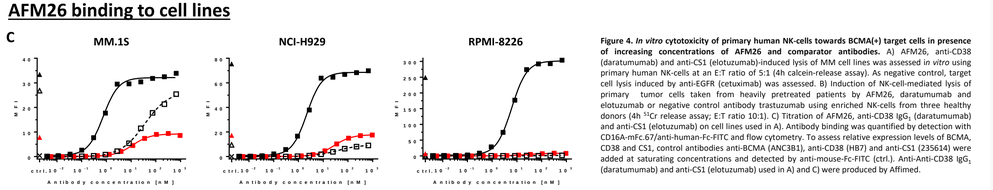


Figure 4. *In vitro* cytotoxicity of primary human NK-cells towards BCMA(+) target cells in presence of increasing concentrations of AFM26 and comparator antibodies. A) AFM26, anti-CD38 (daratumumab) and anti-CS1 (elotuzumab)-induced lysis of MM cell lines was assessed *in vitro* using primary human NK-cells at an E:T ratio of 5:1 (4h calcein-release assay). As negative control, target cell lysis induced by anti-EGFR (cetuximab) was assessed. B) Induction of NK-cell-mediated lysis of primary tumor cells taken from heavily pretreated patients by AFM26, daratumumab and elotuzumab or negative control antibody trastuzumab using enriched NK-cells from three healthy donors (4h ^{51}Cr release assay; E:T ratio 10:1). C) Titration of AFM26, anti-CD38 IgG_1 (daratumumab) and anti-CS1 (elotuzumab) on cell lines used in A). Antibody binding was quantified by detection with CD16A-mFc.67/anti-human-Fc-FITC and flow cytometry. To assess relative expression levels of BCMA, CD38 and CS1, control antibodies anti-BCMA (ANC3B1), anti-CD38 (HB7) and anti-CS1 (235614) were added at saturating concentrations and detected by anti-mouse-Fc-FITC (ctrl.). Anti-Anti-CD38 IgG_1 (daratumumab) and anti-CS1 (elotuzumab) used in A) and C) were produced by Affimed.

AFM26 shows similar efficacy compared to a BCMA/CD3 BiTE and may offer a superior safety profile

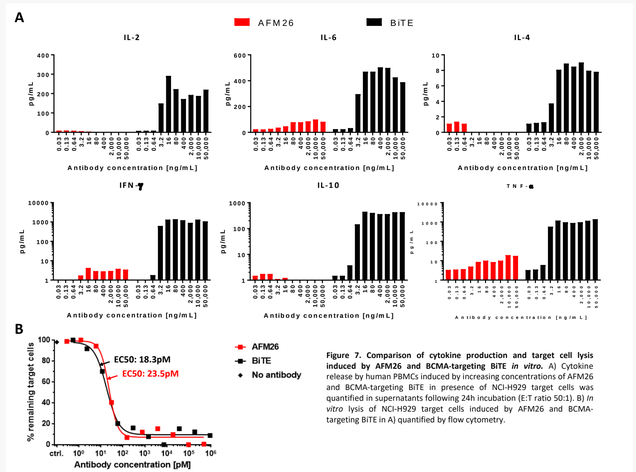


Figure 7. Comparison of cytokine production and target cell lysis induced by AFM26 and BCMA-targeting BiTE *in vitro*. A) Cytokine release by human PBMCs induced by increasing concentrations of AFM26 and BCMA-targeting BiTE in presence of NCI-H929 target cells was quantified in supernatants following 24h incubation (E:T ratio 50:1). B) *In vitro* lysis of NCI-H929 target cells induced by AFM26 and BCMA-targeting BiTE in A) quantified by flow cytometry.

AFM26 retains potent activity towards target cells expressing very low levels of BCMA

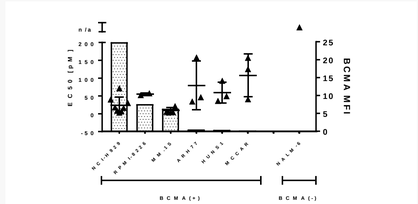


Figure 5. Potency of AFM26 induction of NK-cell-mediated target cell lysis *in vitro* in relation to BCMA expression levels on MM cell lines. Summary of EC50 values of AFM26-induced target cell lysis determined in *in vitro* cytotoxicity assays conducted as in Fig. 4A (left axis). Data are mean ± SD. BCMA mean fluorescence intensity (MFI) as quantified by flow cytometry indicated as bars (right axis).

AFM26 does not induce NK-cell lysis *in vitro* in contrast to daratumumab and elotuzumab

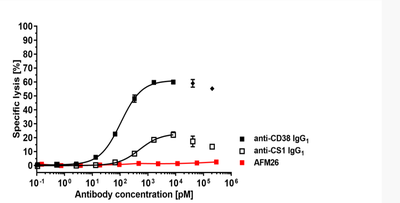


Figure 6. AFM26 does not deplete NK-cells *in vitro*. Antibody-induced lysis of NK-cell lysis was assessed *in vitro* using primary human NK-cells (calcein-release assay, 4h incubation). Anti-CD38 IgG1 (daratumumab) and anti-CS1 (elotuzumab) IgG_1 were produced by Affimed.

Conclusions and Outlook

- **AFM26 promises to fully unlock NK-cell cytotoxicity in myeloma as single agent or in combination with adoptively transferred NK-cells.**
- **AFM26 may address the medical need of MRD positivity after high-dose therapy (HDT)/ASCT in first-line MM treatment:**
  1. **NK-cells are the first lymphocyte population to reappear after HDT/ASCT.**
  2. **Adoptive transfer of large numbers of NK-cells is a feasible, potentially safe and widely applicable strategy to provide a highly active effector population in combination with ASCT.**

[#] Corresponding author