A CD19/CD3 bispecific TandAb, AFM11, recruits T cells to potently and safely kill CD19+ tumor cells in pre-clinical models

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affimed unique antibody therapeutics

1. Introduction

We engineered a humanized, bispecific tetravalent antibody with two binding sites for CD19 and two binding sites for CD3, the CD19/CD3 RECRUIT-TandAb® AFM11, for the treatment of CD19+ B cell malignancies, such as Non Hodgkin Lymphoma (NHL) and Acute Lymphoblastic Leukemia (ALL).

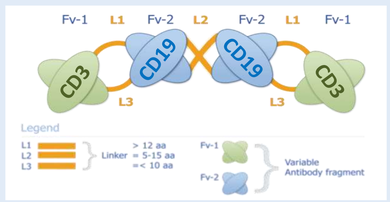
CD19 is expressed from early B cell development through terminal differentiation into plasma cells, and is therefore an attractive target for the development of therapeutic antibodies to treat B cell malignancies. Clinical proof of concept for T cell recruitment was demonstrated by the bispecific CD19/CD3 BiTE blinatumomab in ALL.

The advantages of the TandAb® technology, relative to other approaches, include: (i) a sub/low-pM potency due to high affinity binding to both, T cells and tumor cells, (ii) a stable, off-the shelf product, and (iii) a half-life allowing for bolus infusion. We evaluated in vitro efficacy and safety of AFM11 using CD19+ cell lines, and in vivo efficacy in a murine NOD/scid xenograft model reconstituted with human PBMC. Further, we used standard preclinical IND enabling assays to evaluate tissue cross reactivity, PK and toxicological profile.

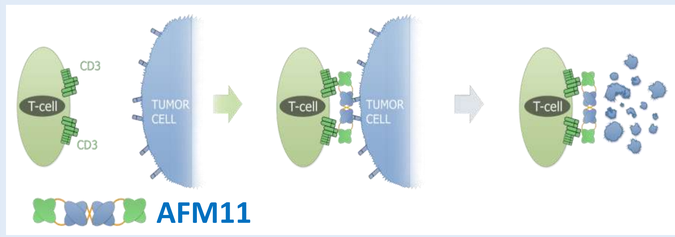
2. RECRUIT-TandAbs – tetravalent, bispecific antibodies

a. TandAb Features
- comprised of scFv domains
- expressed as a single gene product
- linkers favor intermolecular head-to-tail homodimerization

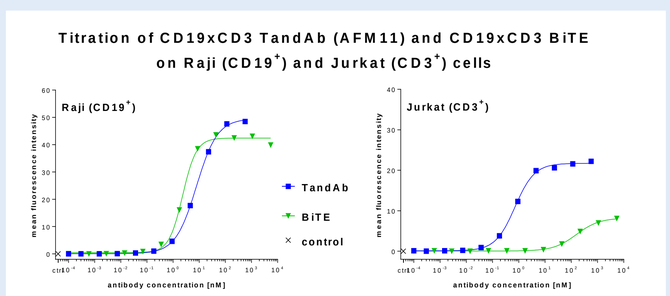
b. TandAb Properties
- recruitment of NK or T cells as effector cells
- potent cytotoxicity against target cells
- no off-target cytotoxicity
- no Fc-associated side effects
- bivalent binding to each target
- drug-like properties (production and stability)
- favorable half-life



3. AFM11 is a CD3 RECRUIT-TandAb engaging T cells

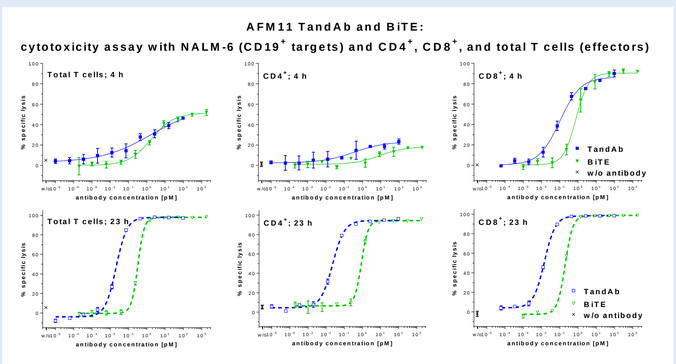


4. AFM11 TandAb displays high affinity to CD3 and CD19



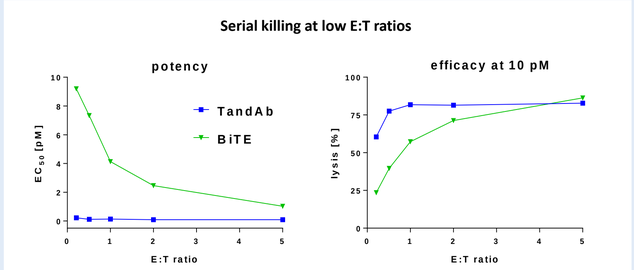
- High affinity to CD3+ (K_D: 0.7 nM) and CD19+ (K_D: 7 nM) cells
- Similar affinity of the AFM11 TandAb and the BiTE to CD19
- Affinity to CD3+ cells of AFM11 TandAb is 2 orders of magnitude higher compared to BiTE

5. AFM11 TandAb facilitates cytotoxicity via CD4+ and CD8+ T cells



- CD8+ T cells facilitate AFM11-mediated cytotoxicity with faster kinetics than CD4+ T cells
- Both CD8+ and CD4+ T cells facilitate cytotoxicity of AFM11
- AFM11 is more potent than CD19/CD3 BiTE

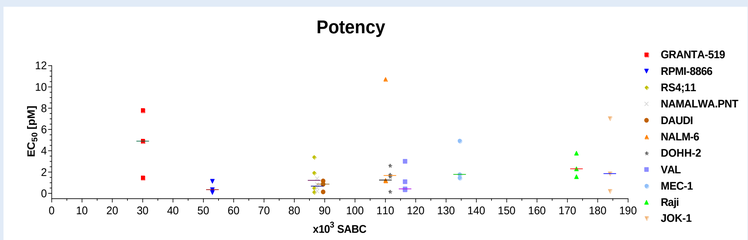
6. AFM11 TandAb displays high cytotoxicity and serial killing



FACS-based cytotoxicity assays were performed with NALM-6 target cells and human T cells as effector cells at the indicated E:T ratios in the presence of serial dilutions of AFM11 TandAb or BiTE. For each E:T ratio, EC_{50} and lysis values were determined after 23 h incubation and plotted.

- AFM11 TandAb exhibits higher efficacy and potency than BiTE in the course of the cytotoxic assay and at different E:T ratios
- Similar efficacy and potency at various E:T ratios suggests that AFM11 TandAb facilitates serial killing of tumor target cells by T cells

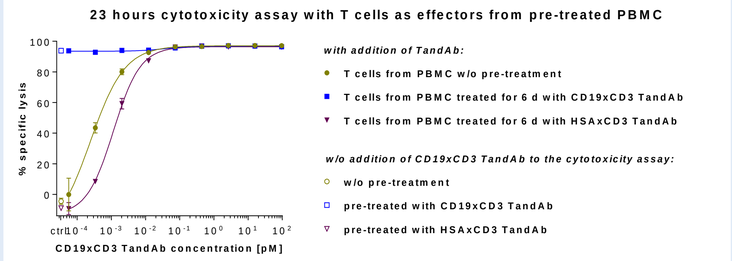
7. AFM11 TandAb potency does not correlate with CD19 density



4 hour calcein-release assays were performed with human PBMC as effector cells on the indicated CD19+ target cell lines expressing different levels of CD19 in the presence of increasing concentrations of AFM11. EC_{50} values were calculated and plotted as a function of the SABC (standardized antibody binding capacity, SABC) determined with anti-CD19 mAb HD37.

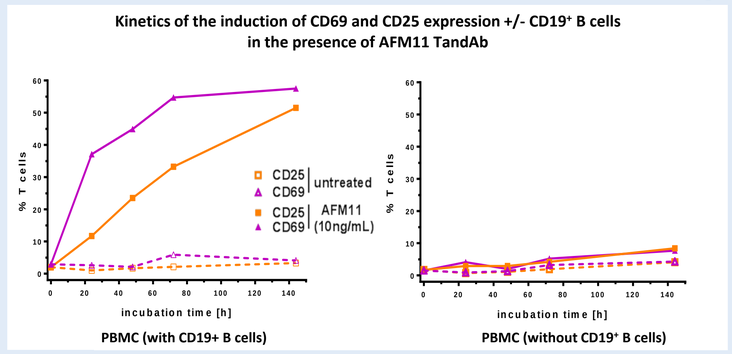
8. T cells retain cytotoxicity after stimulation of PBMC with AFM11

Human PBMC → Incubation for 7 days +/- AFM11 (1 µg/mL) +/- HSAxCD3 (1 µg/mL) → Enrichment of T cells → Cytotoxicity assay

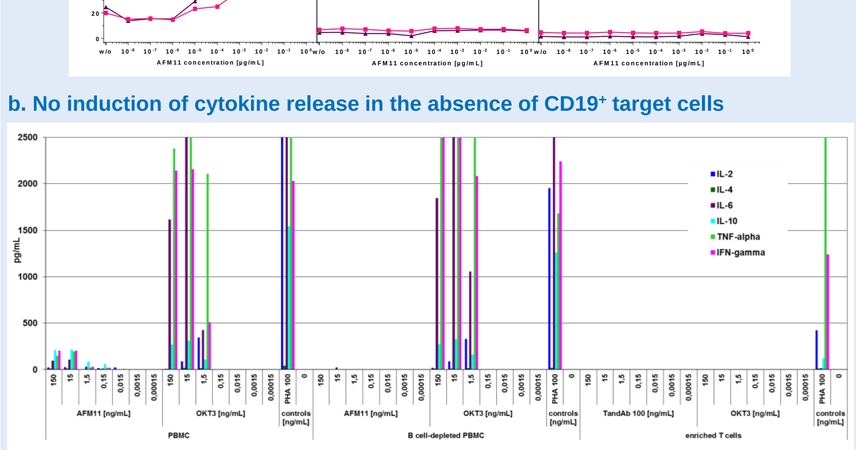


9. No off-target activity

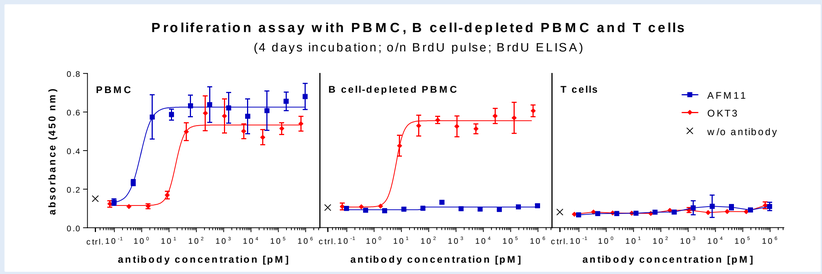
a. No induction of activation markers on T cells in the absence of CD19+ target cells



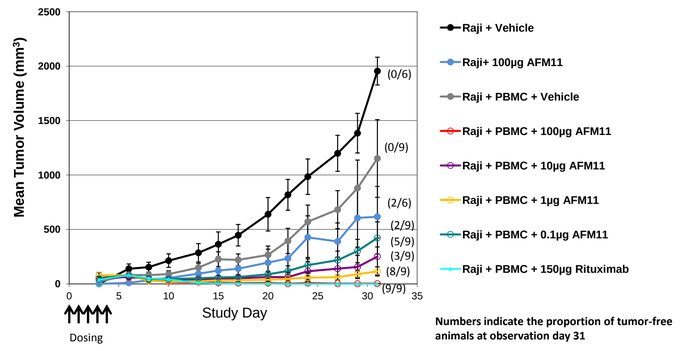
b. No induction of cytokine release in the absence of CD19+ target cells

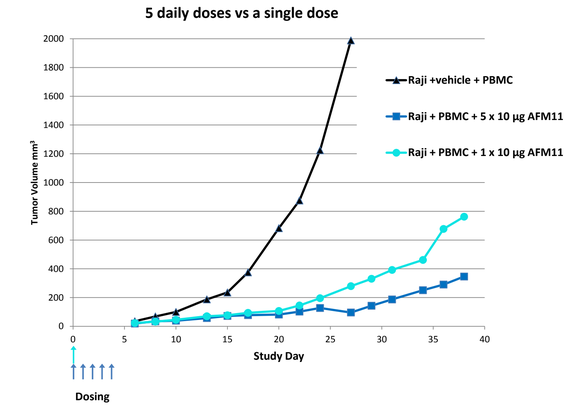


c. No induction of cell proliferation in the absence of CD19+ target cells



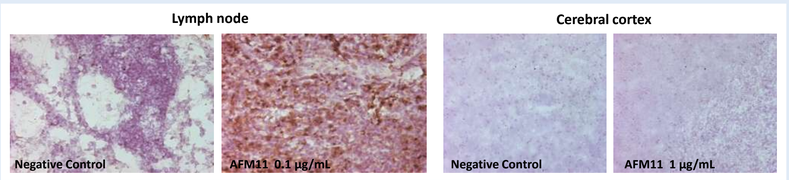
10. Xenograft Burkitt Lymphoma model: highly efficacious protection by AFM11 TandAb





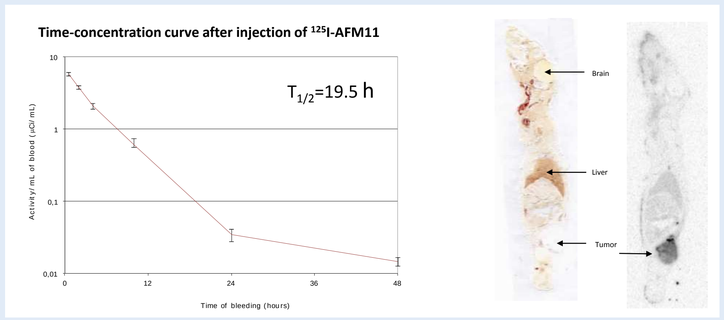
- Potent, dose-dependent growth inhibition of CD19+ Raji tumors in an *in vivo* xenograft model
- Similar efficacy of a single 0.5 mg/kg dose and 5 daily injections

11. Tissue crossreactivity study



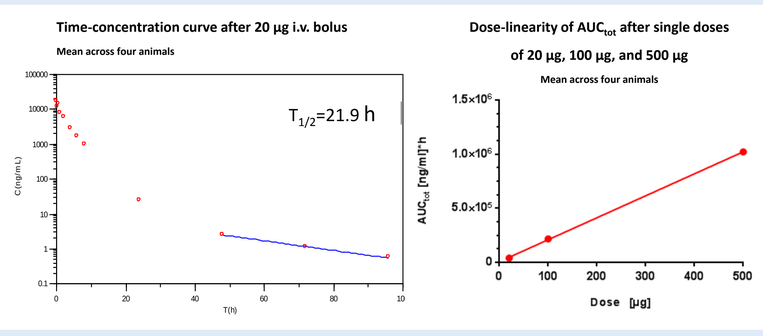
- Strong specific cell surface staining of lymphoid cells in lymph nodes
- No staining of CD3-/CD19- tissues

12. Autoradiography study with 125I-AFM11 in a Raji xenograft model in NOD/scid mice



- Autoradiography of animals from the 24 h time point
- Accumulation of 125I-AFM11 in the tumor

13. Excellent PK properties of AFM11 in CD1 mice



14. Summary/Conclusion

- Excellent potency and efficacy characteristics *in vitro*
- Potent anti-tumor activity *in vivo*
- Promising safety characteristics:
  - no off-target activity *in vitro*
  - no unspecific accumulation in a xenograft model in mice
  - specific staining of positive tissues in a tissue crossreactivity study
- PK properties that do not require administration by continuous infusion
- Excellent drug-like properties (data not shown)
- Phase I study initiated in patients with relapsed and/or refractory Non-Hodgkin Lymphoma (NHL)