T CELL ENGAGING BISPECIFIC ANTIBODIES GENERATED FROM A NOVEL, LARGE AND DIVERSE CD3 PANEL DEMONSTRATE FUNCTIONALITY UNCOUPLED FROM AFFINITY

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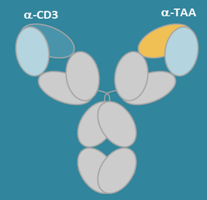


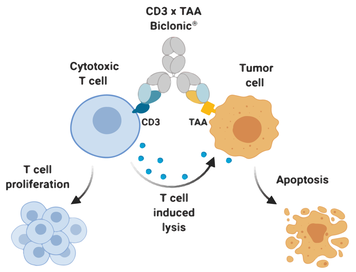
INTRODUCTION

T cell engaging bispecific antibodies (TCEs) recruit and activate T cells to specifically lyse tumor cells.

TCEs have demonstrated clinically meaningful responses in different hematological malignancies but their clinical application may be limited by toxicities associated with cytokine release syndrome (CRS).

TCEs could potentially be improved for therapeutic use by utilizing CD3 binding domains that optimally balance proliferation, tumor cell killing and cytokine release of TCE-activated T cells.

Large and diverse anti-CD3 antibody panels are challenging to generate.

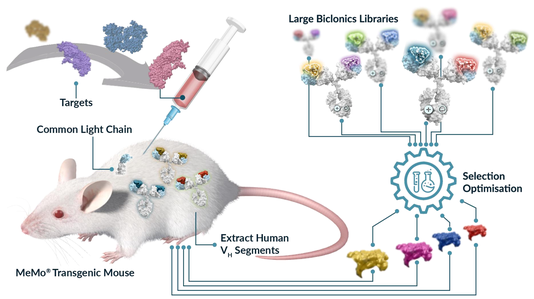


OBJECTIVE

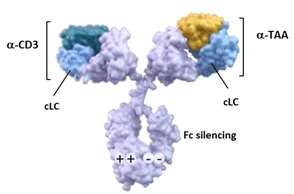
We aimed to generate and functionally characterize a novel, large and diverse common light chain CD3 antibody panel for the discovery and optimization of T cell engaging antibodies.

METHODS

Human common light chain (cLC) Fabs binding CD3 were generated by immunization of MeMo® mice with TCR/CD3 receptor complex expressing lipoparticles and T cells and by subsequent panning of immune phage libraries, constructed from high titer mice. MeMo® is a transgenic mouse containing a human variable heavy chain repertoire and a common human light chain.



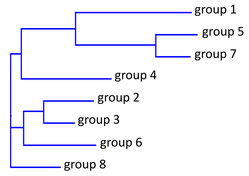
The CD3 Fab panel and two Fabs targeting two tumor associated antigens were formatted together as TAAxCD3 full-length IgG1 human bispecific antibodies with Fc effector function silenced (TCE Biclonics®) and evaluated in functional assays in which tumor cells were co-cultured with resting human T cells as effector cells. Biclonics® are bispecific human IgG1 molecules comprising two cLC and two heavy chains with different variable regions. Charge engineering in the CH3 regions directs preferential bispecific heterodimer formation.

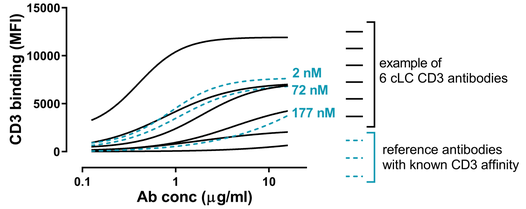


RESULTS

Large common light chain CD3 antibody panel

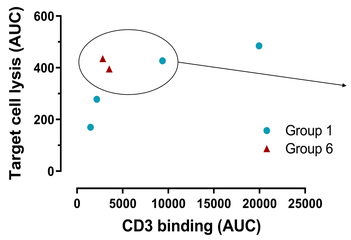
- We generated a panel of more than 180 unique cLC anti-CD3 antibodies, derived from MeMo®
- The CD3 panel comprised eight distinct groups, analysis predicts each CD3 Fab group originates from a unique B cell clone.
- Anti-CD3 binding affinity for entire panel was evaluated in monovalent format (TAAxCD3 Biclonics®) by flow cytometry using HBL-ALL cells and compared to monovalent binding reference antibodies with known affinities
- The antibodies showed affinities for CD3 in the low pM to high nM range.

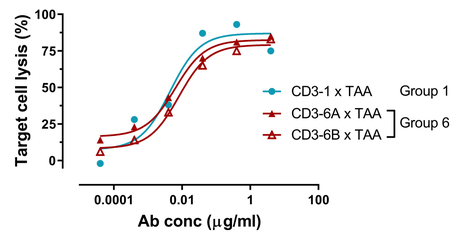




CD3 affinity and CD3xTAA Biclonics® activity are uncoupled

- We paired representative variants from each CD3 Fab group with a TAA Fab, tested the CD3xTAA Biclonics® for CD3 binding in flow cytometry, and evaluated them for their capacity to lyse target cells in a T cell cytotoxicity assay.
- Interestingly, Biclonics® from two groups bound to CD3 with different binding affinity to CD3 showed however relatively equivalent cytolytic activity.





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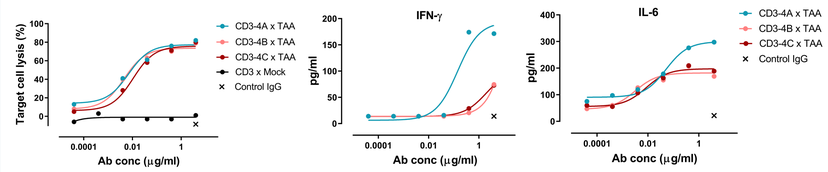
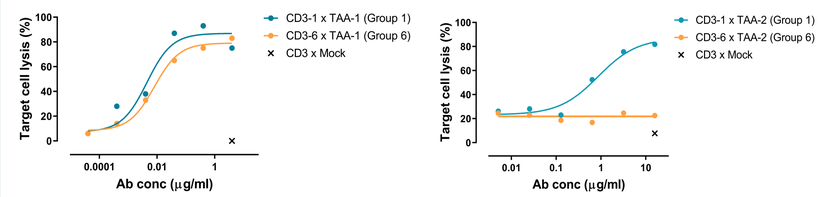
CD3 induced lysis and cytokine release can be uncoupled

- Several Biclonics® with variants within a CD3 Fab group had similar cytolysis but significantly lower IFN-γ and IL-6 release, demonstrating that CD3 induced lysis can be uncoupled from cytokine production.



Optimal activity of CD3 Fab can be TAA specific

- Representative variants from four CD3 Fab groups were combined with two different tumor targeting Fabs (TAA1 and TAA2) to generate two sets of TCE Biclonics®: CD3xTAA1 and CD3xTAA2.
- Three out of four CD3 Fab groups induced TAA dependent cytolysis in combination with TAA1 as well as TAA2
- CD3 Fabs from group 6 demonstrated effective tumor cell killing when combined with TAA1 but not when combined with target TAA2



CONCLUSIONS

1. **A novel, large and diverse cLC CD3 Fab panel for TCE Biclonics® format provides opportunities to identify TCEs with optimal activity as TCE activity that cannot be predicted from CD3 affinity alone:**
   - CD3 affinity and CD3xTAA TCE activity can be dis-linked
   - CD3 induced lysis can be uncoupled from cytokine release
   - Activity of CD3 Fab can be tailored to specific TAA.

2. **A broad therapeutic window for a TCE may be optimally achieved by empirical evaluation of CD3 and TAA Fab arm panels in CD3xTAA IgG format using a high throughput screening setup.**