Understanding the Geometry and Valency of Bispecific Antibodies in the Optimization of Tumor-Dependent Activation of 4-1BB

Abstract: 1737

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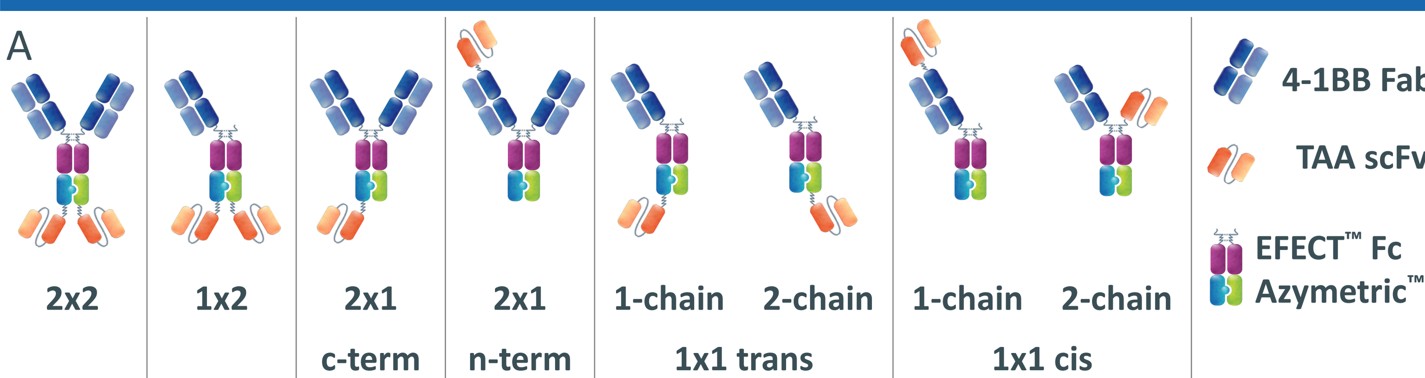
Introduction

4-1BB is a TNF family receptor expressed on the surface of tumor-infiltrating T cells. Activation of 4-1BB enhances the activation, metabolism and function of tumor-infiltrating T cells and promotes tumor regression. The clinical development of several anti-4-1BB antibodies have been limited by liver toxicity and lack of activity. To address these clinical liabilities with targeting 4-1BB, we designed bispecific 4-1BB x tumor associated antigen (TAA) antibodies to selectively activate T cells within the tumor microenvironment.

To understand the impact of antibody format and valency on potency of T cell activation, we generated a panel of 4-1BBxTAA bispecific antibodies in different formats with the Azymetric™ and EFECT™ platforms. We compared potency and maximal activity of these constructs in co-culture assays with tumor cells and T or Jurkat reporter cells, in comparison to urelumab, a monospecific 4-1BB antibody, as a clinical benchmark. The Azymetric™ platform allowed the production and evaluation of unique antibody formats, from which an extensive structure activity relationship analysis was performed. We also examined the accessible epitope space by sampling the possible paratope binding conformations of the molecule. From this we determined the geometric limits of antigen engagement.

Azymetric™ platform allows development of antibody formats to investigate structure-activity relationship



B

Characteristic	Value
Transient Expression	42 mg/L
Stable Pool Expression	450 mg/L
Aggregates post Protein A Purification (UPLC-SEC)	9.7%
Aggregates final (UPLC-SEC)	1.6%
Purity (LC/MS)	99.6%
Accelerated Stability (14 days @ 37C)	0% change
Acid Stability (pH 4 for 1 hr)	0% change

C

Post protein A purification

Post SEC purification

AU

Minutes

Figure 1: (A) Azymetric™ formats[1] can be generated rapidly and efficiently to enable structure-function investigation of 4-1BB activation. All antibodies also incorporated EFECT™ mutations[2] in the CH2 domain of the antibody to prevent interaction with FcγR. (B) Production characteristics and (C) UPLC-SEC plots of lead 2x1 c-term 4-1BBxFRα Azymetric™ format, showing the ability to produce pure, stable bispecific proteins at research scale.

Optimal format of 4-1BBxTAA bispecific antibodies contain bivalent anti-4-1BB with distal TAA binding domains

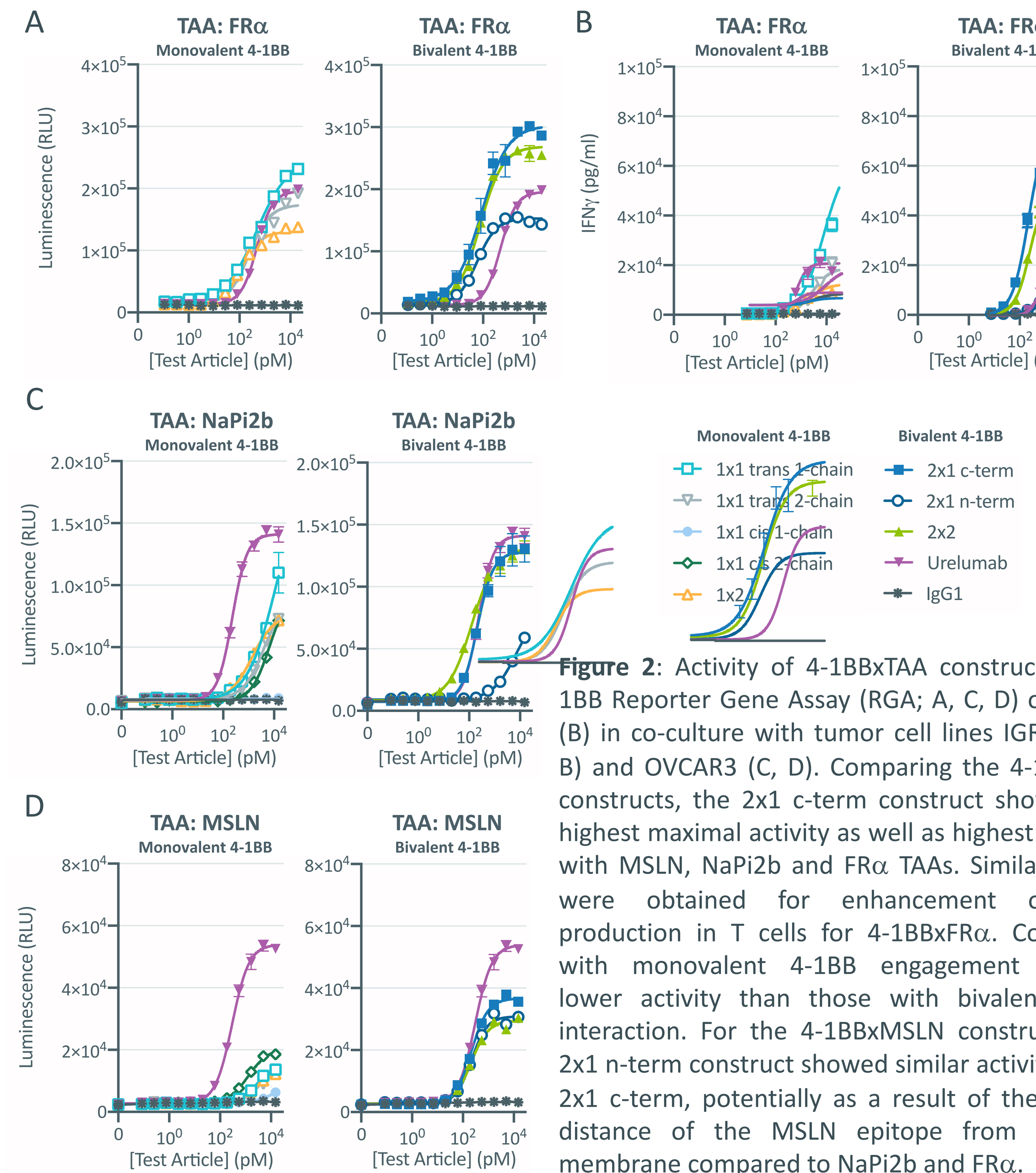


Figure 2: Activity of 4-1BBxTAA constructs on 4-1BB Reporter Gene Assay (RGA; A, C, D) or T cells (B) in co-culture with tumor cell lines IGROV1 (A, B) and OVCAR3 (C, D). Comparing the 4-1BBxTAA constructs, the 2x1 c-term construct showed the highest maximal activity as well as highest potency with MSLN, NaPi2b and FRα TAAs. Similar results were obtained for enhancement of IFNγ production in T cells for 4-1BBxFRα. Constructs with monovalent 4-1BB engagement showed lower activity than those with bivalent 4-1BB interaction. For the 4-1BBxMSLN constructs, the 2x1 n-term construct showed similar activity to the 2x1 c-term, potentially as a result of the greater distance of the MSLN epitope from the cell membrane compared to NaPi2b and FRα.

4-1BBxTAA activity is dependent on TAA expression; activity exceeds Urelumab on FRα^{mid} cells

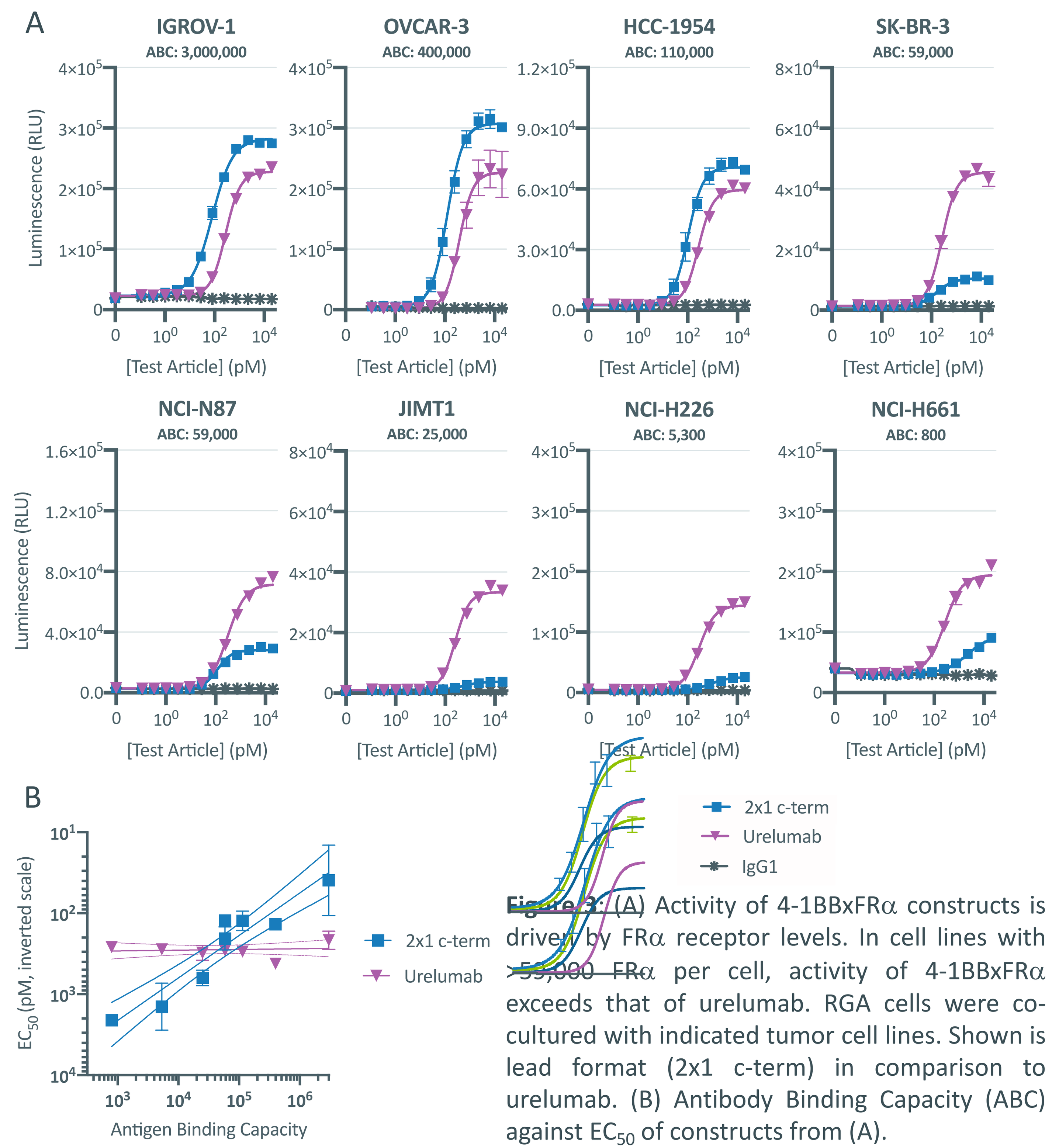


Figure 3: (A) Activity of 4-1BBxFRα constructs is driven by FRα receptor levels. In cell lines with >59,000 FRα per cell, activity of 4-1BBxFRα exceeds that of urelumab. RGA cells were co-cultured with indicated tumor cell lines. Shown is lead format (2x1 c-term) in comparison to urelumab. (B) Antibody Binding Capacity (ABC) against EC_{50} of constructs from (A).

Investigation of geometry of 4-1BBxTAA 2x1 c-terminal fusions indicates interparatope distance similar to immune synapse

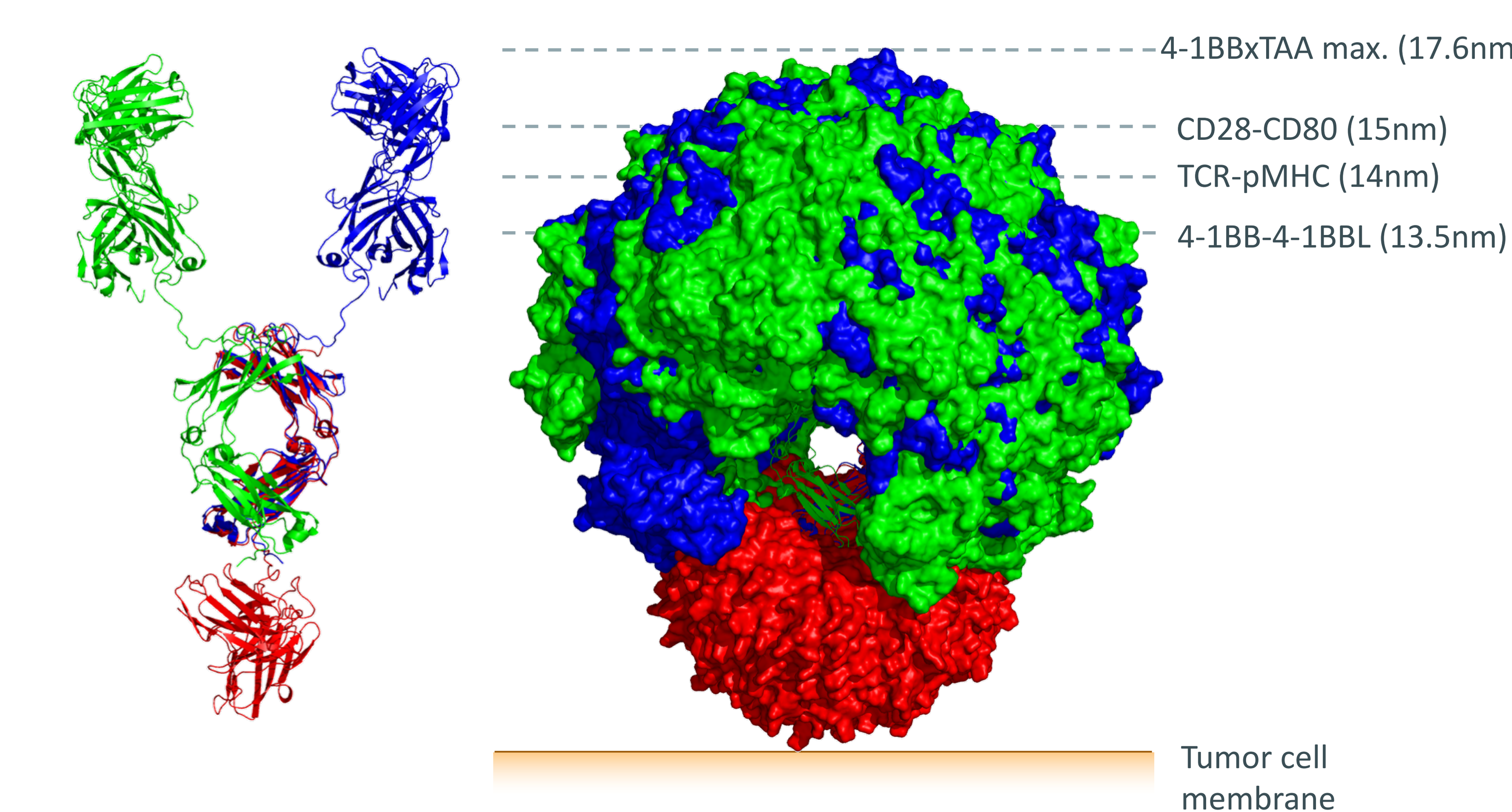


Figure 5: (A) Ribbon model of a 2x1 c-term 4-1BBxTAA construct. (B) The volume of space accessible to each of the three paratopes as determined using a computational model. The space accessible to the two 4-1BB Fabs are colored green and blue, with the scFv paratope coloured red. The distance between the 4-1BB arms and TAA arm are similar to that of key protein complexes in the immune synapses. Marked are distances between key proteins in the immune synapse (CD28-CD80 and TCR-pMHC) as well as 4-1BB-4-1BBL. Not shown are CD45 (28-50nm) and CD148 (47-55nm) which are excluded from the synapse.

Lead 2x1 c-term 4-1BBxFRα format shows FRα-dependent tumor growth inhibition and immune activation in mice

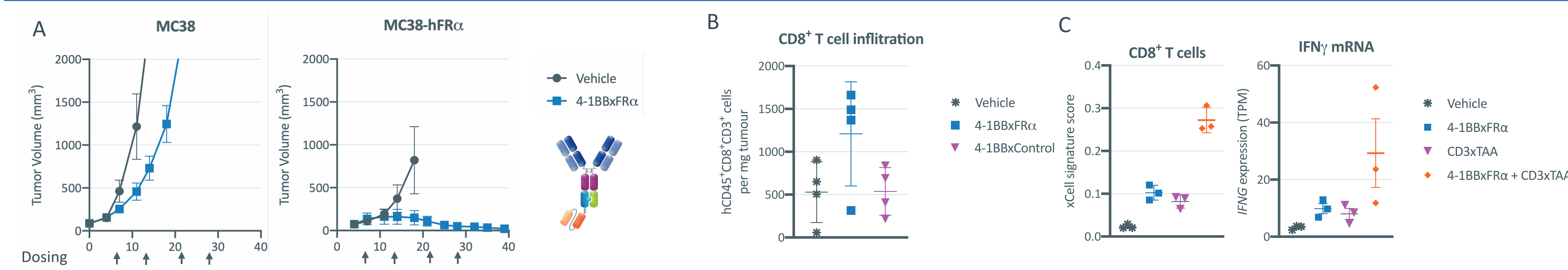


Figure 4: (A) MC38 cells were transduced with human FRα (hFRα) and cloned by limiting dilution. Wild type and cloned hFRα cells were implanted in C57BL/6 h4-1BB mice and randomized to indicated treatments when mean tumor volume of reached ~80mm³. All test articles were administered at 10 mg/kg i.v., once per week for four weeks. (B) Mice with subcutaneous IGROV1 tumors were transplanted with PBMC from healthy donors and test articles injected. On day 41 post-randomisation, tumors were taken, digested and the numbers of hCD45+CD8+CD3+ T cells per mg tumor examined. 4-1BBxControl constructs were also used with a virus-specific arm in place of the TAA arm. (C) OVCAR3 tumor chunks were implanted into mice which were then engrafted with healthy donor PBMC. At the end of the experiment, tumors were removed and analysed by RNAseq. Shown is xCell score corresponding with CD8+ T cells, as well as IFNγ response in the tumor. Increases of CD8+ T cells and IFNγ mRNA in the tumors of mice dosed with CD3xTAA and 4-1BBxFRα suggest that this combination could be productive. B and C show responding donors.

Conclusion

We describe the development of a set of bispecific antibodies for the conditional agonism of 4-1BB which allow activation of T cells in the tumor. The optimal format for 4-1BBxTAA bispecifics was investigated, with the 2x1 format with two 4-1BB binding Fabs and single TAA binding scFv on the c-term of the Fc showing superiority to other formats across three different TAA. We then examined the dependence of activity on TAA receptor number on the tumor cell. Cells expressing greater than 59,000 receptors per cell showed greater activity than urelumab, suggesting a potential advantage in both safety and activity. We then investigated the lead 2x1 c-term 4-1BBxTAA format in a mouse *in vivo* efficacy model and demonstrated FRα-dependent tumor growth inhibition and a trend towards immune activation and increases in TILs.

This work represents an investigation of 4-1BBxTAA formats which would be difficult to engineer without an efficient, flexible bispecific technology such as Azymetric™. We identified a series of 4-1BBxTAA bispecific agonist antibody formats which were transferable between multiple TAAs. These formats also allowed optimization of activity and selectivity to promote maximal therapeutic index and efficacy, key factors which are potentially able to contribute to improved clinical outcomes.

References

[1]Spreter, T et al, Improving biophysical properties of a bispecific antibody scaffold to aid developability MAbs. 2013; 5(5): 646–654.
[2] US20190248897
[3]Aran, D., Hu, Z. & Butte, A.J. xCell: digitally portraying the tissue cellular heterogeneity landscape. Genome Biol 18, 220 (2017).

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