IgG2a-formatted 4-1BB agonism combined with S100A9 inhibition enhances T cell activation and tumor control in a preclinical model of multiple myeloma

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INTRODUCTION

Relapse in multiple myeloma (MM) is driven by an immunosuppressive bone marrow (BM) microenvironment enriched in:

- Dysfunctional T and NK cells
- Myeloid-derived suppressor cells

4-1BB agonism enhances T cell activation and shows promise in solid tumors, but its impact in MM is not well characterized.

Tasquinimod (TQ), a clinical stage S100A9 inhibitor, may overcome myeloid immunosuppression (NCT04405167; phase Ib/IIa RRMM).

AIM

Evaluate the combination of a 4-1BB agonist and TQ to overcome the immunosuppressive tumor microenvironment and enhance anti-myeloma immune activity.

METHOD

Two 4-1BB agonist clones were compared in the 5TGM1 model

- Clone LOB12.3 (IgG1k)
- Clone 3H3 (IgG2a)

Lead 4-1BB agonist was combined with TQ in the 5TGM1 model

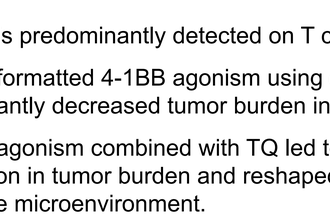
- Tumor load analysis
- Immune phenotyping

Ex vivo 4-1BB urelumab monotherapy was tested on BM samples

- Tumor load analysis
- Immune phenotyping

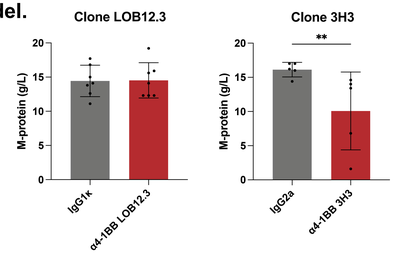
RESULTS

Figure 1: 4-1BB is a therapeutically targetable co-stimulatory marker on T and NK cells in MM-bearing mice.



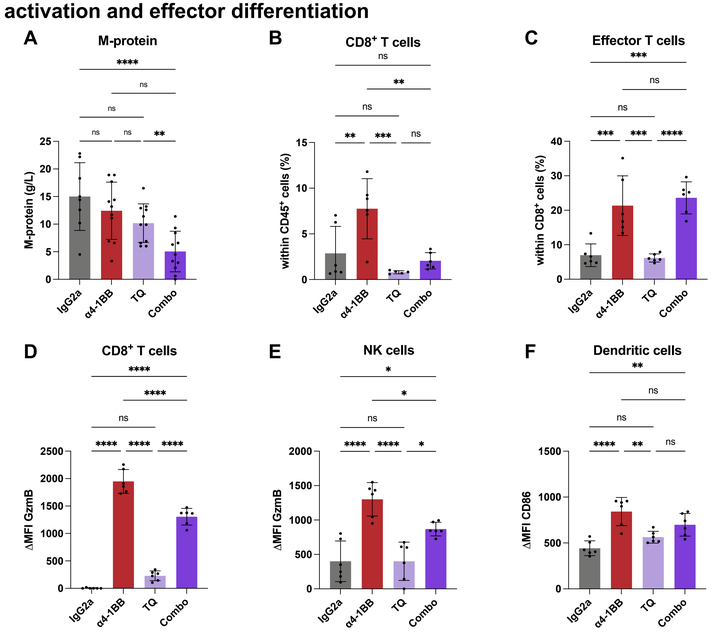
Dot plots showing the expression of murine 4-1BB gene *Tnfrsf9* in BM within the murine 5T33 scRNA-seq dataset.

Figure 2: Differential antitumor activity of 4-1BB agonist clones LOB12.3 and 3H3 in 5TGM1 MM model.



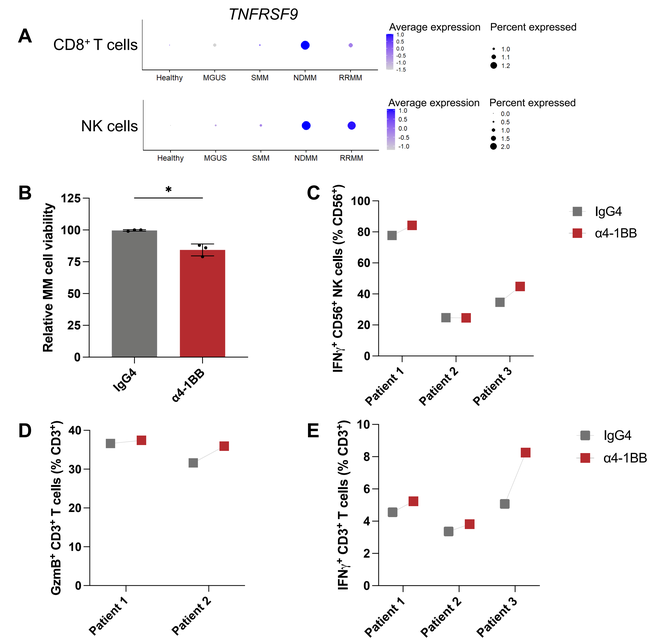
Serum M-protein analysis following treatment with α4-1BB clone LOB12.3 (n=7 per group) and clone 3H3 (n=5 per group).
Mann-Whitney U test: **p<0.01

Figure 3: Co-targeting T cell and myeloid compartments using α4-1BB and tasquinimod enhances anti-MM activity by granzyme B-mediated T cell activation and effector differentiation



Serum M-protein analysis following treatment with α4-1BB clone 3H3 and TQ, n<8 per group **(A)**. CD8⁺ T cells **(B)**, CD8⁺ CD44⁺ CD62L⁻ cells determined as effector T cells **(C)**, ΔMFI of granzyme B in CD8⁺ T cells **(D)** and NK cells **(E)**, ΔMFI of CD86 in dendritic cells **(F)** in BM of mice following treatment with α4-1BB clone 3H3 and TQ, n=6 per group.
One-way ANOVA: ns: p≥0.05, *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001

Figure 4: 4-1BB is expressed on T and NK cells in MM patients and its targeting enhances immune cell activation



Dot plots showing expression of human 4-1BB gene *TNFRSF9* in T and NK cells across different MM stages from publicly available datasets (GSE124310 and GSE161801) **(A)**. Relative MM cell viability after 72h *ex vivo* treatment with isotype control IgG4 or urelumab **(B)**. Paired scatter plots of interferon-γ in CD56⁺ NK cells **(C)** interferon-γ **(D)** and granzyme B **(E)** in CD3⁺ T cells after 72h *ex vivo* treatment; n=3. Mann-Whitney U test: *p<0.05

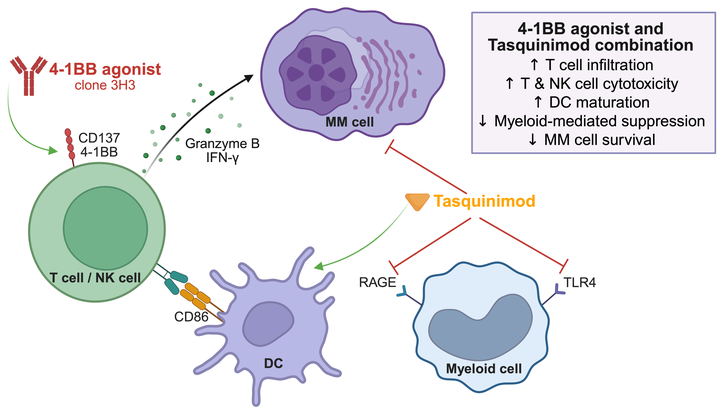
CONCLUSIONS

4-1BB is predominantly detected on T cells and NK cells.

IgG2a-formatted 4-1BB agonism using clone 3H3 significantly decreased tumor burden in 5TGM1 mice.

4-1BB agonism combined with TQ led to a significant reduction in tumor burden and reshaped the tumor immune microenvironment.

Ex vivo 4-1BB monotherapy enhanced immune cell activation in newly diagnosed MM patients.



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