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Blood 142 (2023) 3459–3460

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Optimal, Off-the-Shelf, CAR-iNKT Cell Platform-Based Immunotherapy for Multiple Myeloma

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Autologous CAR-T cell immunotherapy targeting BCMA confers significantly improved progression free survival as compared to standard-of-care in relapsed/refractory multiple myeloma. However, despite >50% complete remission rates, patients continue to relapse. Of note, in current licenced BCMA CAR-T products, 4-1BB comprises the co-stimulatory domain of a 2 nd generation CAR.

Expression of CD1d by myeloma plasma cells (PC) lends itself to immunotherapy with the CD1d-restricted, glycolipid-reactive iNKT cells which can be deployed 'off-the-shelf' without causing aGVHD. We hypothesised that 'off-the-shelf' anti-BCMA CAR-iNKT would be an alternative platform for the treatment of MM. We sought to identify the CAR design that would function optimally in conjunction with iNKT cells. To this end, we designed several different second (28z, 4-1BBz, OX40z) and third (28z-4-1BBz, 28z-OX40z) generation BCMA CARs and tested their anti-myeloma activity.

Lentiviral transduction efficiency of iNKT cells was comparable for all five CARs and all five CAR-iNKT were able to kill two BCMA-expressing but not BCMA knock out myeloma cell lines in short- and long-term cytotoxicity assays. The level of cytotoxicity varied with donor, timing of the assay and cell lines, with the only consistent pattern seen with the 3 rdgeneration CD28-4-1BB CAR. This showed the least reactivity at low E:T ratios in both 4 and 16hrs assays for both donors tested. Testing cytotoxicity against primary myeloma PC (n=3) showed that the 3 rd gen CARs were less active than the three 2 nd gen counterparts.

Short term proliferation assays using Incucyte Zoom demonstrated a higher proliferative potential of CD28z CAR-iNKT cells with CD28z-4-1BBz CAR showing the least proliferation. In a longer term 35-day assay, following stimulation with the iNKT cell ligand aGalCer, we found that CD28z CAR-iNKT cells showed the highest expansion.

To further probe functional differences between the five CARs, we tested the corresponding CAR-iNKT cells in acoustic force avidity assays. These highlighted CD28z CAR-iNKT cells as having the highest avidity amongst CAR-iNKT cells with untransduced iNKT cells the lowest **(**Fig. 1A **).** Using confocal microscopy, we explored how these differences in avidity impacted the ability of CAR-iNKT cells to shape the immune synapse (IS) and engage the cytolytic apparatus. We found that while conjugates of untransduced iNKT cells with myeloma cells all fell in the distal centrosome group, of the five CARs, CD28z and 4-1BBz CAR-iNKT conjugates recorded the highest and the lowest respectively proximal and docked conjugates, i.e., those signifying imminent or active discharge of the cytolytic granules. To delve further into the mechanism(s) of higher avidity and ability to established IS by CD28z CAR-iNKT, we compared the transcriptomes of CD28z vs 4-1BBz CAR-iNKT from three donors. We found 64 genes differentially expressed (14 up- and 50 down-regulated in CD28z CAR-iNKT, padj<0.05). Amongst

the upregulated genes we identified, currently investigating genes that encode proteins that were previously implicated in the strength and duration of IS.

To investigate the *in vivo* correlates of these functional assays, we deployed a xenograft myeloma model using MM1.S luciferase-expressing cells **(**Fig. 1B **)**. All five CAR iNKT cells were i.v transferred to myeloma-bearing mice at the limiting dose of 10^6 CAR+ cells/mouse and disease burden was monitored by bioluminescence. In line with the functional assays, we found that mice receiving CD28z CAR-iNKT cells demonstrated the lowest disease burden and survived the longest ($p<0.01$). While disease burden and survival of animals receiving CD28z-4-1BBz CAR-iNKT was the same as controls, 4-1BBz CAR-iNKT-treated mice showed significantly higher disease burden and shortened survival as compared to CD28z CAR-iNKT. Further disease burden assessment by flow-cytometry at sacrifice showed that CD28z CAR-iNKT-treated animals had the lowest ($p<0.01$) frequency of myeloma cells in their peripheral blood, bone marrow and spleen.

Finally, in ongoing xenograft assays, we find that compared to 10^6 28z BCMA CAR-iNKT and $5x10^6$ untransduced iNKT, $5x10^6$ 28z BCMA CAR-iNKT cells maintain better disease control ($p=0.008$, day7).

We conclude that CD28z by enhancing effector-target avidity and strength of IS constitutes the optimal CAR design for clinical development of CAR-iNKT therapy of multiple myeloma.

Disclosures No relevant conflicts of interest to declare.

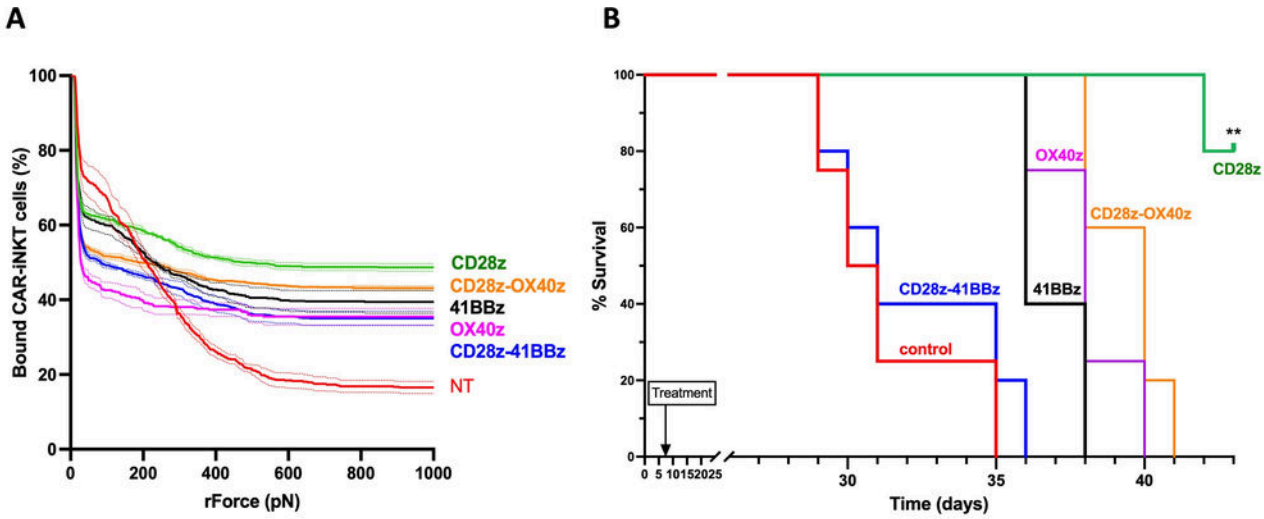

Figure 1. Higher avidity and *in vivo* efficacy of CD28z CAR-iNKT cells.

A. Acoustic force avidity assays measuring fraction of CAR-iNKT cells with co-stimulatory domains as shown that remain bound to the target myeloma MM1.S cells upon application of variabale acoustic force (in picoNewtons).

B. Kaplan-Meier survival curve of NSG mice injected with $7x10^6$ MM1.S-luciferase cells and treated seven days later with iNKT cells transduced with an anti-BCMA CAR containing one of five different co-stimulatory domains as shown. Disease burden was monitored by bioluminescence (** $p<0.01$; unpaired t-test).

Figure 1

https://doi.org/10.1182/blood-2023-189905