analysis. We used MM cell lines with different expression of target ligands: U266 and ARP-1, BCMAhigh and NKG2DLhigh; XG-1, BCMAhigh and NKG2DLlow; NCI H929 R20, NKG2DLlow. K562, BCMAnegative and NKG2DLhigh, a leukemia cell line. **Results:** NKG2D-CAR NK-92MI cells consistently showed much higher in vitro antitumor activity than the parental line NK-92MI against U266 (84 ± 2% vs 40.7 ± 4% at a 1:1 E:T ratio), ARP-1 (82.1 ± 4% vs 21.3 ± 9.2% at a 16:1 E:T ratio), XG-1 (67.9 ± 9% vs 18.5 ± 4% at a 16:1 E:T ratio) and NCI H929 R20 (50.9 ± 6% vs 23.7 ± 2% at a 16:1 E:T ratio) cell lines. Next, we compared cytotoxicity between NKG2D and BCMA-CAR NK-92MI against U266 (84 ± 2% vs 91.9 ± 3% at a 1:1 E:T ratio), ARP-1 (82.1 ± 4% vs 80.4 ± 3.7% at a 16:1 E:T ratio), XG-1 (67.9 ± 9% vs 89.9 ± 2% at a 16:1 E:T ratio) and K562 (94 ± 3% vs 25.74 ± 4% at a 1:1 E:T ratio) cell lines. Strikingly, there were no significant differences between NKG2D-CAR NK and the gold standard BCMA-CAR against MM cell lines with high and similar BCMA and NKG2DL expression. In addition, correlation between target ligands expression on the tumor and efficacy of both CARs was also shown. None of the CAR NK-92MI studied populations showed toxicity against PBMCs from healthy donors and in vivo MM orthotopic xenograft mouse model experiments are ongoing. **Conclusions:** We have generated two novel and stable CAR NK-92 immunoproducts that improve the oncolytic efficacy of the parental cell line. Indeed, NKG2D-CAR cells are as equally efficient as BCMA-CAR cells to eradicate diverse MM cells. To summarize, all these data show the feasibility to use this 'off-the-shelf' approach for MM.

Keywords:
CAR NK-92
Chimeric Antigen Receptor
NKG2D-CAR

Tracks:
Immunotherapeutic Approaches to MM

OAB-036

In vivo efficacy of BCMA-iNKT-CAR is enhanced by NT-I7, a long-acting IL-7

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B-cell maturation antigen (BCMA) targeted chimeric antigen receptor-T (CAR-T) cells generate short-lived responses in multiple myeloma (MM) with high risk of cytokine release syndrome and neurotoxicity. Expressing CAR proteins on alternative cell types could lead to a less toxic cure. Invariant natural killer T-cells (iNKTs) express a monomorphic T cell receptor that recognizes glycolipids displayed by the MHC-like molecule CD1d. iNKTs do not cause graft versus host disease (GvHD), making them a useful alternative cell type for an allogeneic, "off the shelf" CAR product, including a BCMA-CAR . Prior to testing efficacy of BCMA-iNKT-CAR we generated and tested BCMA-CAR-T. Human T-cells were transduced with a lentivirus encoding a 3rd generation CAR targeting BCMA or CD19 (negative control). In vivo efficacy was tested by engrafting 5X105 MM.1S cells (BCMA+; expressing luciferase and GFP; MM.1S-CG) i.v. into NSG mice (day 0) followed by treatment with 2x10-6 BCMA-CAR-T or CD19-CAR-Ts (day 28). All BCMA-CAR-T (n=7) treated mice were tumor free at day 150, while all CD19-CAR-T treated mice died of tumor (median survival 40 days; n=4), demonstrating our BCMA-CAR is active and useful for testing in iNKTs. iNKTs isolated from normal human PBMCs were stimulated with matched donor irradiated negative fraction PBMCs. We generated BCMA-iNKT-CAR and CD19-iNKT-CAR (CD19-iCAR) and found efficient killing of MM.1S-CG by BCMA-iCAR and negligible killing by CD19-iCAR in Chr51 killing assays. Since iNKT's express the IL-7R, we used NT-I7 (a long-acting IL-7) to enhance expansion and efficacy of BCMA-iCARs. Mice were engrafted as above (day 0) and treated with 10X106 1) BCMA-iCAR/vehicle (VEH; n=10) 2) CD19-iCAR/VEH (n=5) 3) BCMA-iCAR/NT-I7 (n=10) 4) CD19-iCAR/NT-I7 (n=5) or 5) nothing. Median survival of mice treated with CD19-iCAR/VEH and CD19-iCAR NT-I7 was 45 and 49 days, respectively. Median survival of BCMA-iCAR/VEH treated mice was 163 days while 7 of 10 BCMA-iCAR/NT-I7 treated mice lived >200 days with no tumor detected by BLI. BCMA-iCAR/NT-I7 treated mice had an avg. of 92 iNKT+CAR+/μL blood on day 62 and 0 by day 90; no CARs were detected in BCMA-iCAR/VEH treated mice at either time point. Thus, NT-I7 enhanced BCMA-iCAR antitumor efficacy and prolonged iNKT-CAR cell survival in vivo. To assess long lived functional BCMA-iCARs, we re-challenged the 7 living BCMA-iCAR/NT-I7 mice with 5X105 MM.1S-CG cells and treated them with VEH (n=3) or NT-I7 (NT-I7#2; n=4). Two of four NT-I7#2 treated mice were tumor free 5 weeks later while NSG control (n=5) and VEH had high tumor burden, demonstrating NT-I7's potential anti-tumor effect. Future experiments focus on repeating preliminary results and testing CS1 (SLAMF7), a priority CAR-T candidate, on iNKTs and CS1 CRISPR/cas9 gene edited iNKTs. iNKTs hold promise as an alternative cell source for off-the-shelf CAR therapies and combination with NT-I7 extended CAR-cell and overall survival in a MM mouse model.

Keywords:
B-cell maturation antigen
Chimeric Antigen Receptor
iNKT

Tracks:
Immunotherapeutic Approaches to MM

OAB-037

Single cell Dissection of Resistance to anti-BCMA CAR-T cell Therapy

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