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Blood 142 (2023) 454–455

The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

Bclxl Prevents Progressive Exhaustion in BCMA CAR T Cells with Maintenance of High TCF1 Expressing T Cells

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Although BCMA targeted CAR T cell therapies are highly effective in the treatment of multiple myeloma (MM), patients with high disease burden have not fully benefited from this therapy with shorter disease remissions despite initial responses. It is postulated that high disease burden results in chronic antigenic stimulation of CAR T cells with the induction of exhaustion-associated gene signatures and CAR T cells contraction, limiting their *in vivo* potency. We have previously reported that *BCL2L1* (gene encoding for BCLxL) armoring of BCMA CAR T cells enhanced CAR T cells functional fitness and improved proliferation and cytotoxicity after chronic antigenic stimulation *in vitro*, as well as superior tumor clearance in xenograft models of MM. In the current study we aimed to define the molecular mechanisms that mediates BCL2L1-armored CAR T cell and examine the effect of BCLxL variants on CAR T cells fitness.

In *in vivo* studies, NSG mice were systemically injected with OPM2 cells (stably transduced with firefly luciferase) and led to develop large MM disease burden, treatment with BCLxL armored (BCMA_BCL2L1) vs unarmored CARs (BCMA_CAR) or control T cells completely eliminated the MM disease and significantly improved animal survival. In animals treated with armored CARs (BCMA_BCL2L1) no disease recurrence was noted 180 days post treatment (in contrast to unarmored CARs where disease recurrence was noted within 60 days). BCLxL also improved CARs persistence, with armored CAR T cells being detectable in the marrow of these mice 7 and 14 days post treatment. Of note, BCLxL armored CAR T cells did not demonstrate uncontrolled proliferation as they were no longer detectable on day 120 post-infusion. Furthermore, CITEseq profiling on harvested human T cells 7 days post CAR T cells infusion to diseased mice revealed a differential T cell repertoires and phenotypes between BCMA_CAR and BCMA_BCL2L1 CARs. While unarmored CARs were significantly enriched for exhausted T cells (CD8_Tex), BCMA_BCL2L1 CAR T were enriched with early activated non-exhausted CD8 T cells, precursor exhausted T cells (CD8_Tpex) and naïve CD4 and CD8 T cells. Importantly, single cell genes set enrichment analysis (ssGSEA) revealed a differential metabolic signature with higher oxidative phosphorylation in BCMA_BCL2L1 CAR T cells compared to unarmored CARs. These findings suggested that armoring CAR T cells with BCL2L1 maintain CAR T cells in an activated and precursor exhausted T cell states with improved mitochondrial and metabolic fitness, preventing terminal T cells exhaustion.

To further evaluate the impact of BCLxL in suppressing BCMA CAR T cells' exhaustion, we engineered BCMA CAR T cells with armored expression of two BCLxL mutants (BCLxL $^{\Delta 26\text{-}83}$ or BCLxL $^{\Delta 46\text{-}83}$) harboring large or small deletions involving a BCLxL regulatory loop. These mutants were previously reported to enhance BCLxL function *(Chang et al The EMBO Journal 1997,16:968-977)*. Under chronic antigenic stimulation (CAS) or hypoxic conditions, wild type or mutants BClxL overexpression in BCMA CAR T cells resulted in higher proliferation rates compared to unarmored CARs. Compared to wild type BClxL, BCLxL $^{\Delta 26\text{-}83}$ and BCLxL $^{\Delta 46\text{-}83}$ mutants demonstrated higher proliferation capacity post CARs or under hypoxic conditions. Intriguingly, majority of BCLxL-overexpressing BCMA CAR-T cells, particularly BCMA CAR-T $^{BCLxL\_\Delta 26\text{-}83}$ cells, maintained a central memory (T_{CM}) phenotype following a long co-culture with MM tumor cells with lower levels of exhaustion markers. Importantly BCLxL-overexpressing BCMA CAR-T cells had substantially higher level of TCF1 with lower TOX/TOX2 expression compared to unarmored BCMA CAR T cells. TCF1 ^{high} population was also enriched in unstimulated BCLxL-overexpressing BCMA CAR T cells suggesting a positive regulatory impact of BCL2L1 on TCF1 expression, either through direct or indirect pathways. Expectedly, TCF1 ^{high} BCMA CAR-T cells overexpressing a BCLxL were mainly associated with the naive/T_{CM} phenotype.

In summary, we have identified a role for BCLxL and two mutant variants lacking a loop regulatory domain (BCLxL $^{\Delta 26\text{-}83}$ or BCLxL $^{\Delta 46\text{-}83}$), in maintenance of BCMA CAR T cells in central memory and precursor exhausted states with retained high TCF1 expression, even under hypoxic and chronic antigenic stimulation conditions. These studies further support the clinical development of a BCL2L1 armored CAR T cells for the treatment of MM.

Disclosures Boise: *Abbvie:* Consultancy; *Astra Zeneca:* Consultancy, Honoraria. **Neri:** *Sanofi:* Consultancy, Honoraria; *Pfizer:* Consultancy, Honoraria; *BMS:* Consultancy, Honoraria; *Janssen:* Consultancy, Honoraria. **Bahlis:** *Abbvie:* Consultancy, Honoraria, Other: member of steering committee; *Karyopharm therapeutics:* Honoraria, Membership on an entity's Board of Directors or advisory committees; *Celgene:* Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding; *Takeda:* Honoraria, Membership on an entity's Board of Directors or advisory committees; *BMS:* Consultancy, Honoraria; *GSK:* Consultancy, Other: member of steering committee; *Forus:* Consultancy, Honoraria; *Takeda:* Consultancy; *Genentech/Roche:* Honoraria; *Amgen:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees; *Sanofi:* Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding; *Pfizer:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding; *Janssen:* Honoraria, Membership on an entity's Board of Directors or advisory committees, Other: IRC member and chair, Research Funding.

https://doi.org/10.1182/blood-2023-189747