

CART-engineered Marrow-infiltrating Lymphocytes (MILs™) are more polyfunctional than their matched peripheral blood counterparts

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Background

WindMIL Therapeutics is developing Marrow-infiltrating Lymphocytes (MILs™), a novel form of adoptive T cell therapy composed of bone marrow-derived, patient-autologous, polyclonal CD4 and CD8 T cells [1]. Genetically unmodified MILs™ have demonstrated antitumor activity in patients with multiple myeloma [2] and are being developed for several other tumor types. Distinguishing features of T cells from bone marrow compared to T cells from peripheral blood lymphocytes (PBLs) include their memory phenotype, inherent tumor antigen-specificity, higher CD8:CD4 ratio and ability to persist long-term [3]. Based on these differences, we hypothesized that MILs™ would provide a more robust platform for CAR-T therapy compared to PBLs. We have previously shown that CAR-modified MILs™ (CAR-MILs™) demonstrate superior killing of tumor target cells in vitro compared to CAR-T cells generated from patient-matched PBLs (CAR-PBLs) [4]. In this study, we compared, at the single cell level, functionality of patient-matched CAR-MILs™ and CAR-PBLs following antigen-specific in vitro stimulation.

Methods

CAR-MILs™ and CAR-PBLs engineered to express a BCMA-specific, 4-1BB/CD3z-signaling CAR were produced using cryopreserved lymphocytes from the bone marrow and blood of six patients with multiple myeloma. CD4 and CD8 T cells isolated from the CAR-MILs™ and CAR-PBLs products were stimulated with K562 cells transduced with either BCMA (K562-BCMA) or nerve growth factor receptor (K562-NGFR) at a ratio of 1:2 for 20 hrs. After 20 hrs of co-culture, T cells were enriched and loaded into IsoCode chips containing ~12,000 microchambers pre-patterned with a 32-plex antibody array. Protein secretion from 1000-2000 single T cells per product was detected by a fluorescence ELISA-based assay and single cell polyfunctional profiles analyzed using IsoPeak (IsoPlexis).

Results

CD4 and CD8 T cells from both CAR-MILs™ and CAR-PBLs demonstrated an antigen-specific increase in polyfunctionality (secretion of 2+ cytokines per cell) and polyfunctional strength index (PSI) in response to BCMA stimulation compared to NGFR control. When compared to CAR-PBLs, CAR-MILs™ demonstrated increased polyfunctionality and increased PSI in both CD4 and CD8 T cells. The enhanced PSI in CAR-MILs™ was predominated by effector, stimulatory and chemoattractive proteins associated with antitumor activity including Granzyme B, IFN γ , IL-8, MIP1a and MIP1b. Coincidentally, increased PSI and enhanced secretion of these same proteins was reported to be associated with improved clinical responses in patients with Non-Hodgkin lymphoma treated with CD19-specific CAR-T therapy [5].

Conclusion

Based on these data and the inherent antitumor properties of MILs™, we speculate that CAR-MILs™ would be more potent and effective than currently approved CAR-T products derived from PBLs.

References

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