ELSEVIER

Blood 144 (2024) 4785–4786

The 66th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

702.CAR-T CELL THERAPIES: BASIC AND TRANSLATIONAL

Development of Allogeneic Dual-Targeting CD19/BCMA CAR-T Cell Therapeutics Using ALL-in-One Site-Specific Integration Technology for Autoimmune Diseases

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Background: Chimeric antigen receptor (CAR) T cells have been approved for treating patients with B cell malignancies and multiple myeloma. Autologous anti-CD19 and anti-BCMA CAR-T cells have shown significant efficacy in autoimmune disease indications. Developing allogeneic CAR-T for autoimmune diseases is highly attractive due to increased accessibility, cost-effectiveness, and a higher probability of success given the relatively low disease burden. Allogeneic CAR-T cells offer a significantly lower cost of goods (COG), approximately 1/10th of autologous CAR-T, and a much higher production yield, ranging from 100-1000 doses per batch. These cells are derived from healthy donor T cells, avoiding potential abnormalities in T cells from autoimmune disease patients. The higher safety requirement as the use of CAR T cells for indications outside hematology and oncology is considered, site-specific integration of CAR with minimal gene editing provides an ideal platform for manufacturing allogeneic CAR-T. Additionally, since memory B cells with CD19 markers and long-lived plasma cells expressing BCMA are implicated in certain autoimmune diseases like systemic lupus erythematosus (SLE), dual-targeting CD19 and BCMA allogeneic CAR-T is expected to achieve deeper remission and higher efficacy.
Methods: HY034 is an allogeneic CD19/BCMA CAR-T cell that simultaneously targets B cell CD19 and the long-lived plasma cell BCMA surface antigens. We used a non-viral, site-specific integration technology platform (PrecisionGENE, PrecisionGEnetic Engineering) for manufacturing. A CAR with proprietary humanized anti-CD19 and anti-BCMA in a loop structure was inserted into the TRAC locus, eliminating TCR$\alpha\beta$ expression to prevent graft-versus-host disease (GvHD). To increase the persistence of allogeneic CAR-T in vivo, a human virus-derived protein is co-expressed with the CAR to down-regulate certain MHC molecules and avoid attack by host T cells and NK cells. The CAR-mediated and target-dependent activity of HY034 against both CD19+ and BCMA+ target cell lines was tested for in vitro cytotoxicity, cytokine production, and proliferation through multiple rounds of antigen stimulation, as well as the in vivo xenograft model. The functionality of immune evasion was tested by in vitro assays simulating host T and NK cell-mediated rejections.
Results: Various formats of humanized CD19 and BCMA scFvs, including tandem and loop formats with different orientations, were tested to ensure dual targeting of CD19 and BCMA antigens. The optimal configuration in the loop format was used to build HY034. In the membrane protein array (MPA) assay, the loop-formatted scFv demonstrated highly specific binding toward human CD19 and human BCMA antigens, with no detectable off-target binding. HY034 showed similar binding to both antigens, equivalent CAR-mediated cytotoxicity, cytokine production, and proliferation against both CD19+ NALM6 cell line and BCMA+ MM.1S cell line, and they were comparable to the parental single-antigen CAR-T. In vivo, HY034 effectively controlled the tumor growth of CD19+ NALM6 and BCMA+ MM.1S cells in the xerograph model. To ensure product safety with minimal off-target editing, we used site-specific integration technology and the ALL-IN-ONE strategy, with the CAR co-expressing a viral protein to reduce HvG inserted into the TRAC site. Analysis of MHC class I and II expression in HY034 revealed down-regulation of HLA-A and HLA-B, while HLA-E and HLA-DRDPDQ expression remained unaffected. In co-culture with allogeneic T and NK cells, HY034 mitigated allogeneic recognition and rejection responses. In co-culture with allogeneic healthy donor PBMCs, HY034 eliminated primary B cells and proliferated in a CAR-dependent manner, reduced the rejection by the alloreactive T cells and NK cells.
Conclusion: HY034 is a novel CD19/BCMA dual targeting allogeneic CAR-T product by site-specific integration technology and ALL-IN-ONE strategy to ensure the product safety. The preclinical data for HY034 demonstrate the efficacy and safety necessary for advancing to first-in-human studies.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2024-199874