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ORAL ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Precision Targeting of the Malignant Clone in Diffuse Large B Cell Lymphoma Using Chimeric Antigen Receptor T Cells Against the Clonotypic IGHV4-34 B Cell Receptor

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Introduction: CD19-directed Chimeric Antigen Receptor T cell (CART19) immunotherapy has revolutionized the treatment of B cell lymphoma. However, most CART19-treated patients either fail to respond or show disease progression after an initial response. In fact, up to 30% of lymphoma relapses following CART19 show loss of CD19 expression. In addition, several patients who respond well to CART19 display complete loss or severe reduction of their normal B cell repertoire, leaving them exposed to recurrent infections, limited response to vaccines, and requiring prophylactic measures such as intravenous immunoglobulins.

A significant portion of mature B cell malignancies express B cell receptors (BCR) that use the same immunoglobulin heavy variable gene: IGHV4-34. In particular, ~30% of activated B cell (ABC) diffuse large B cell lymphomas (DLBCL), ~35% of primary central nervous system lymphomas, ~65% of vitreoretinal lymphomas, and ~35% of hairy cell leukemia variant express IGHV4-34. This suggests that the IGHV4-34 heavy chain is critical for driving the disease by delivering cell survival and proliferation signals, and multiple studies have shown that BCR signaling is required for ABC-DLBCL survival. However, while highly enriched in several types of B cell lymphomas, IGHV4-34 expressing B cells compose only ~5% of the normal B cell repertoire of healthy individuals.

Therefore, we hypothesized that anti-IGHV4-34 CAR T cells would be highly effective and safe against B cell malignancies, as they would: (i) efficiently recognize IGHV4-34+ lymphoma cells while sparing normal B cells; and (ii) target a tumor driver that is essential for lymphoma cell survival (**Fig 1A**).

*Methods and Results:*We developed a novel CAR construct (CD8-41BB-CD3z) targeting the IGHV4-34+ BCR (CART4-34) using a single-chain variable fragment (scFv) derived from the 9G4 rat monoclonal antibody. We used tumor B cells that endogenously (HBL1) or exogenously (Jeko1, Bonna12, Mec1) express the IGHV4-34 BCR. Using cytotoxicity, cytokine secretion and proliferation assays *in vitro* we showed that CART4-34 specifically target all IGHV4-34+ tumor B cells tested while sparing IGHV4-34- tumor B cells and, most importantly, healthy B cells. However, while the initial CART4-34 showed strong cytotoxicity towards IGHV4-34+ cells in short-term *in vitro* assays, they were significantly inferior to CART19 in *in vivo* xenograft models.

We hypothesized that the poor performance of CART4-34 compared to CART19 was due to the unique challenge of targeting the membrane-distal portion of the BCR, 18 nm from the plasma membrane. In contrast, CART19 targets an epitope of CD19 located just 5 nm from the membrane (**Fig 1B**). Therefore, to improve immune synapse (IS) formation, we designed new CAR constructs with smaller extracellular domains by replacing the original CD8 hinge (44 amino acids (aa)) with either: (i) a short version of the IgG4 hinge (12aa); or (ii) a G4S linker (5aa). We found that both CART4-34 (IgG4) and CART4-34 (G4S)

exhibited significantly improved cytotoxicity towards IGHV4-34+ tumor cells in short-term and long-term *in vitro* assays in all IGHV4-34+ cell lines tested, while maintaining specificity. Importantly, in *in vivo* human xenograft models of IGHV4-34+ lymphoma (HBL1), CART4-34 with short hinges showed significantly improved tumor control comparable to, or better than, CART19 (**Fig 1B**). We also found that while CART19-resistant lymphoma cells had complete loss of CD19 surface expression, CART4-34-resistant tumor cells retained high expression of IgM, suggesting a reduced likelihood of antigen-negative escape owing to the critical role of the BCR for lymphoma B cell survival.
Mechanistically, we found that while the original CART4-34 (CD8 hinge) formed IS with striking morphological differences compared to IS formed by CART19, the IS formed by CART4-34 with short hinges highly resembled those formed by CART19, providing a rationale for the improved anti-tumor potency of short-hinge CART4-34.
*Conclusion:*We implemented a novel paradigm for the treatment of B cell malignancies: the specific targeting of the malignant clone, while sparing the remainder of the healthy B cell repertoire. We designed and optimized a best-in-class anti-IGHV4-34 CAR T cell product with potent anti-tumor effects and minimal toxicity towards IGHV4-34-negative B cells. The activity of CART4-34 will be investigated in a Phase I clinical trial.

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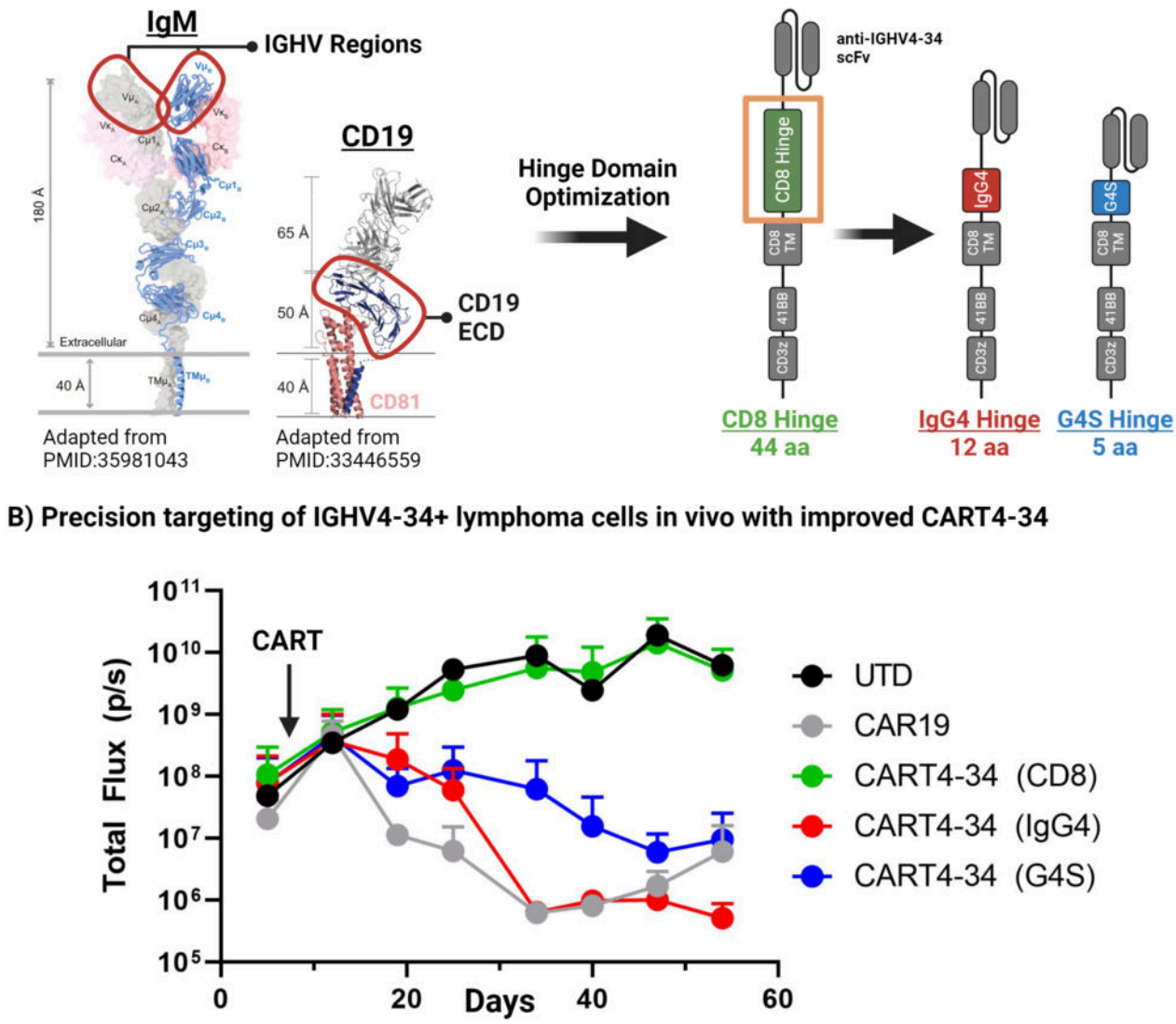

Figure 1. Precision Targeting of the Malignant Clone in Diffuse Large B Cell Lymphoma using Chimeric Antigen Receptor T Cells against the Clonotypic IGHV4-34 B Cell Receptor. A) Cryo-EM structures of the BCR (adapted from PMID:35981043) and CD19 (adapted from PMID:33446559), showing the distance between the plasma membrane and the IGHV regions (180 Angstroms, 18nm) or the CD19 Extracellular Domain (ECD) (50 Angstroms, 5nm), respectively. To address this challenge, we designed novel anti-IGHV4-34 CAR constrcuts that replaced the CD8 hinge domain (44aa) with either (i) the short IgG4 hinge (12aa) or (ii) a G4S linker (5aa). **B)** We injected $5x10^6$ HBL1 (Luciferase+) cells subcutaneously into the right flank of immuno-compromised mice on Day 0. On Day 6, $1.5x10^6$ of the indicated CART (UTD: untransduced control; CART19: anti-CD19 CART; CART4-34: anti-IGHV4-34 CART with the indicated Hinge Domain in parenthesis). Tumor burden was monitored weekly by bioluminescence imaging. TM: Transmembrane domain.

Figure 1

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