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703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Generation of Hinge-Modified Human CD19 Chimeric Antigen Receptor (CAR) T Cells for the Treatment of CD19-Low Leukemia

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CAR T cell therapy targeting CD19 has emerged to show spectacular success in the treatment of B leukemias and lymphomas. This strategy is mainly achieved by potentiating T-cells to recognize and target tumor cells. However, CD19 CAR T cell therapy can result in neurotoxicity [1] and cytokine-associated toxicity as well as relapse with antigen dim or negative disease [2]; therefore, strategies to mitigate these side effects are needed.

Recent trials in adults with lymphoma using fully human CAR (Hu19-28) T cells resulted in the secretion of lower levels of cytokines and conferred lower neurologic toxicity. [3] To assess the potential responsiveness of this Hu19-28 CAR T cells against B-acute lymphocytic leukemia (B-ALL), T cells engineered with the Hu19-28 CAR were compared with murine FMC63-based CARs. Hu19-28 and FMC63-based CAR T cells exhibited a rapid increase in IFNg secretion in response to the CD19 $^{+}$ NALM6 ALL cell line (CD19 ^{High}). Interestingly, and consistent with the lower toxicity of Hu19-28 CAR T cells in patients with lymphoma, IFNg secretion by Hu19-28 CAR T cells in response to a CD19 ^{Low} NALM6 line was significantly reduced as compared to FMC63-28 CAR T cells. Hu19-28 CAR T cells also exhibited high *in vitro* cytotoxicity against CD19 ^{High} NALM6. Notably though, Hu19-28 CAR T cells demonstrated lower *in vitro* cytotoxicity against CD19 ^{Low} NALM6 as compared to FMC63-28 CAR T cells. These important differences were also detected *in vivo*; both Hu19-28 and FMC63-28 CAR T cells eradicated CD19 ^{High} leukemia in an NSG mouse model but only the latter efficiently controlled the *in vivo* growth of CD19 ^{Med} leukemia.

As we and others have shown that the hinge domain of the CAR plays a critical role in its function, especially against low antigen density tumors [4,5,6], we evaluated the impact of the hinge on the function of the Hu19-28 CAR T cells. Compared with the initial construct with a CD8a hinge length of 54 amino acids (Hu19-8H ^{54}-28), Hu19 constructs with 45 (Hu19-H ^{45}-28), 25 (Hu19-H ^{25}-28), and 13 (Hu19-8H ^{13}-28) amino acids were generated. All CAR constructs were highly expressed at the surface of transduced T lymphocytes and were efficient in the killing of CD19 ^{High} leukemia. However, only the Hu19-H ^{25}-28 and Hu19-8H ^{13}-28 CAR constructs exhibited high cytotoxicity against CD19 ^{Low} leukemia (**Figure 1**). Together these data support the preclinical evaluation of Hu19-28 CAR constructs with shorter CD8a hinge lengths for the treatment of pediatric patients with relapsed/refractory ALL.

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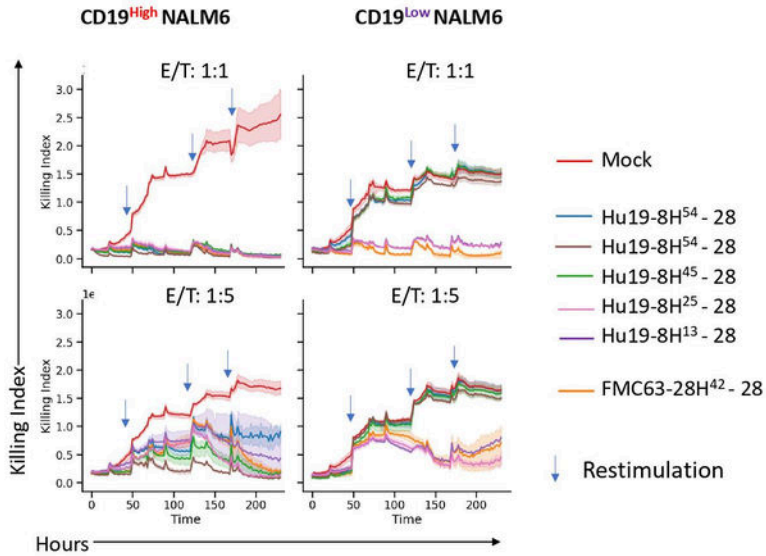

Figure 1. Hu19-CAR T with truncated CD8 hinges exhibit high *ex vivo* cytotoxicity against $CD19^{Low}$ leukemia. Healthy donor T lymphocytes were transduced with HuCD19 CARs with different size CD8α hinges as well as the canonical FMC63 murine CAR with a CD28α hinge. The indicated CART and untransduced T cells (mock) were co-cultured with GFP+ NALM6 lines harboring high ($CD19^{High}$, left) or low ($CD19^{Low}$, right) levels of CD19 at either a 1:1 or 1:5 effector/target (E/T) ratios and repeated NALM6 stimulations every 48h-72h as depicted by the arrows. *Ex-vivo* cytotoxicity was monitored using the Incucyte™ system over 200 hours and killing index over time is presented.

Figure 1

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