In vivo delivery of CD3-directed CD19-CAR lentivectors leads to the generation of CAR-T and NK-like (CAR-TaNK) cells capable of complete ablation of B cells in the blood, bone marrow, and tissue in NSG-SGM3 CD34+ humanized mice

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Abstract

Introduction: Direct *in vivo* delivery of lentiviral vectors (LV) to generate CD19 CAR+ cells without the need for *ex vivo* preparation represents a promising approach to transform autologous CAR therapy into an off-the-shelf treatment. In these studies, direct administration of a new LV encoding a CD19 CAR into humanized NOD *scid* gamma (NSG) mice expressing human IL-3, GM-CSF, and SCF (NSG-SGM3) resulted in a dose-dependent elimination of B cells in the peripheral blood, peritoneal fluid, bone marrow, and tissue of treated mice.

Methods: CD3-directed LV encoding a CD19 CAR with a novel synthetic driver element was manufactured utilizing a 25L clinical scale suspension-based process. NSG-SGM3 mice transplanted with human CD34+ cells from cord blood were injected with LV doses (1E6 TU, 1E7 TU, or 5E7 TU) intraperitoneally (IP) or 1E7 TU intravenously (IV). Quantification of CD19 CAR+ cells and CD20+ B cells in peripheral blood, peritoneal fluid, and bone marrow was assessed by flow cytometry. Additionally, immunohistochemical analysis was performed to evaluate the tissue-resident human B cells and for any other histopathological observations following test article administration.

Results: All CD34+ humanized NSG-SGM3 mice were confirmed to exhibit efficient human hematopoietic engraftment by flow cytometry prior to test article administration (peripheral blood hCD45: 68.9% ± 4.93%; hCD19+ B cells: 53.7% ± 5.11%). Direct LV administration at the 1E7 TU and 5E7 TU doses demonstrated a dose-dependent reduction in circulating human B cells compared to the control and 1E6 TU dose ($p < 0.05$). The synthetic driver elements co-expressed with the CAR led to the formation of unique CD3+ CD8+ CD56+ T and NK-like (TaNK) CD19 CAR+ cells in circulation. The 1E7 TU IP dose demonstrated ablation of B cells (total cells/μL) in peripheral blood (control: 9.67 ± 3.72 vs. LV treated: 0.157 ± 0.117), intraperitoneal fluid (control: 0.322 ± 0.244 vs. LV treated: 0.038 ± 0.029), bone marrow (control: 9.16± 1.83 vs. LV treated: 0.734 ± 0.864), and splenic tissue. Both IP and IV routes of administration showed significant B cell depletion at the 1E7 TU dose. However, complete B cell elimination in splenic tissue was only observed at the 5E7 TU dose. Non-treated CD34+ humanized NSG-SGM3 mice exhibited hepatic portal inflammation and moderate graft-versus-host disease (GVHD) in the colon. Interestingly, mice treated with LV encoding CD19 CAR exhibited decreased inflammatory pathology, suggesting potential B cell involvement in the inflammatory response in this model.

Conclusion: In this study, direct *in vivo* delivery of LV encoding CD19 CAR resulted in the generation of functionally active CD19 CAR TaNK cells capable of eliminating target B cells in peripheral blood, peritoneal fluid, bone marrow, and tissue.

Introduction

Direct Vector Injection

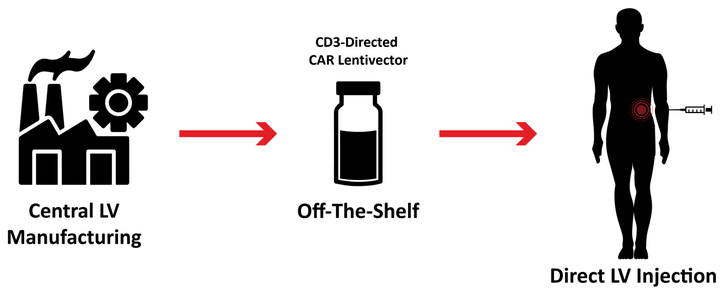
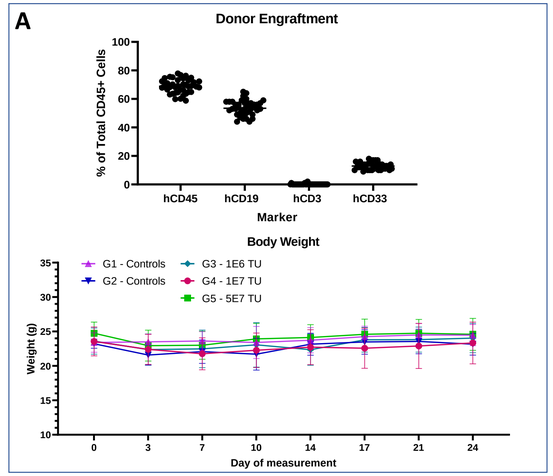


Fig. 1: Humanized Mice & CAR-TaNKs



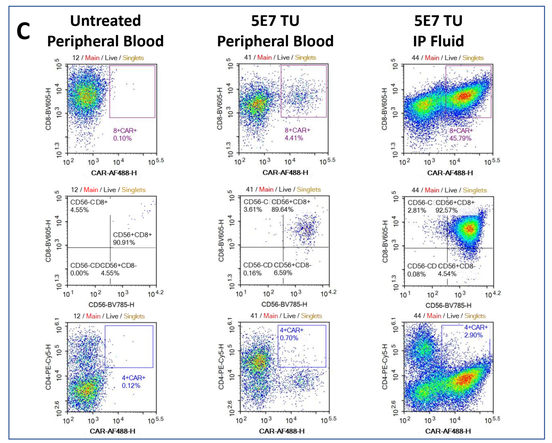
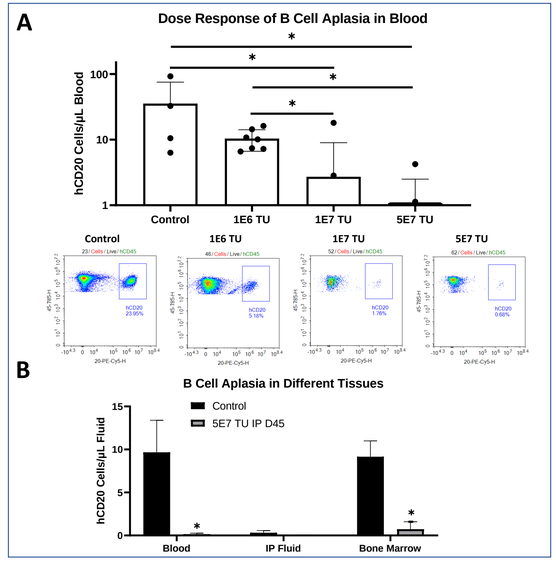


Figure 1: (**A**) Donor engraftment of human B cells (CD19), T cells (CD3), and myeloid cells (CD33) in Hu-CD34+ NSG-SGM3 mice (N=40). (**B**) The mean body weight of mice in the different dose groups was not significantly different from the control group, indicating that the drug did not cause any significant changes in mouse body weight (N=4-7, mean +/- SD). (**C**) Representative flow cytometry plots show the identification of CD8+ CD56+ CAR+ T cells possessing NK-like features (TaNK) with co-expression of both CD8a and CD56 on the same cell. Similar CAR-TaNK phenotype are observed in both peripheral blood and IP fluid.

Fig. 2: B Cell Depletion in Blood & Tissues



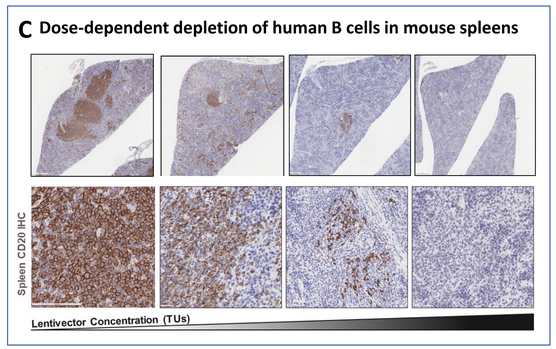


Figure 2: (**A**) Analysis of circulating B cells (hCD20) in peripheral blood 28-days post-injection of increasing doses of CD3-directed CD19 CAR LV demonstrated a dose-dependent B-cell depletion (N=4-7, mean +/- SD. * = p < 0.05). Representative hCD20 flow cytometry plots included. (**B**) Injection of 5E7 TU/mouse resulted in complete ablation of B-cells in peripheral blood, IP fluid, and bone marrow (N=4-7, mean +/- SD. * = p < 0.05) by D45 compared to control groups. (**C**) Immunohistochemical analysis demonstrated a dose-dependent depletion of B-cells (hCD20) in spleens of mice at D45.

Fig. 3: Liver and Colon Histology

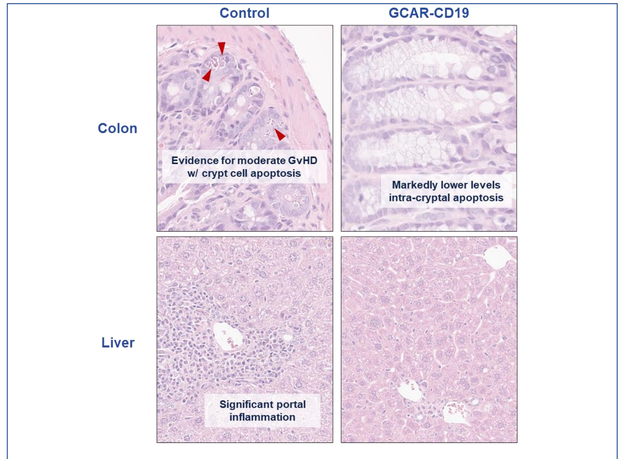


Figure 3: H&E histology of colon (top) and liver (bottom) at D45 from non-treated (control) and treated mice (GCAR-CD19). Hu-CD34+ NSG-SGM3 mice show significant colitis with crypt cell apoptosis (arrowheads) consistent with moderate GvHD. These mice also display significant portal inflammation in the liver (left). The mice treated with direct *in vivo* injection of GCAR-CD19 LV display reduced inflammatory pathology with decreased levels of crypt cell apoptosis and decreased portal inflammation.

Conclusions

- The Hu-CD34+ NSG-SGM3 mouse strain demonstrates robust engraftment of human B cells and represents a target-rich model to test *in vivo* administration of CD19 CAR lentivectors.
- CD3-targeting of CAR lentivectors *in vivo* leads to the successful formation of CAR+ cells that display a unique CD3+CD8+CD56+ CAR-TaNK cell phenotype.
- CAR+ cells generated *in vivo* following direct injection of lentivectors can expand and functionally deplete B-cells in a dose-dependent manner in peripheral blood and tissues, such as the spleen.
- Hu-CD34+ NSG-SGM3 mice display significant inflammation including GvHD in various organs, such as the colon and liver. Following direct *in vivo* injection of the CD19-CAR LV, histologic examination revealed a decrease in inflammation in the colon and liver
- *In vivo* delivery of CD3-targeted lentivectors represents a promising platform that can lead to an off-the-shelf approach for autologous CAR-T therapies

References

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