Hijacking hematopoietic stem cell homing machinery to enhance bone marrow residency and antitumor durability of chimeric antigen receptor T cells

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Background
Although chimeric antigen receptor (CAR) T therapies gained substantial initial response rate in relapsed/refractory haematological malignancies, primary resistance and frequent relapses underlie the major causes of treatment failure. Current attempts augmenting CAR T expansion, persistence or exhaustion have been proposed yet the clinical impacts are still unresolved. With evolving evidence supporting cruciality of CAR T bone marrow residency of where malignant cells resided for effective tumor eradication, this study introduces a novel approach to synthetically redirect and potentiate CAR T marrow infiltration by adopting inherent homing machinery of hematopoietic stem cells (HSCs) and potentially mediate treatment outcome.

Methods
Exemplifying with leukemia, trafficking trajectories of anti-CD19 CAR were captured using NSG xenograft pre-transplanted with leukemic cell lines. CAR T abundance and leukemia burden in major hematopoietic organs (bone marrow, peripheral blood, spleen) were characterized by flow cytometry at designated timepoints post-CAR infusion. Marrow infiltration capacity of CAR T and HSCs were measured with in vitro migration assay and adhesion assay against marrow-rich chemokine SDF-1. Marrow homing potential of CAR T cells co-transduced with HSCs homing factors was evaluated with xenograft, supported with clonal tracking by single-cell RNA sequencing.

Results
A three-phase cascade for CAR T cell trafficking was discovered comprising consecutive intervals of 'homing', "expansion" and "persistence". Importantly, CAR T ingrestion of only 0.05% to the marrow within the first three days post-infusion is sufficient to clear 94.0% of local and systemic leukemia. Additionally, the levels of marrow-residing CAR T cells ($r=-0.86$), but not in other hematopoietic organs (blood: $r=0.07$; spleen: $r=-0.36$), showed a strong reciprocal association with tumor load, highlighting the importance of marrow residency to tumor control. Functional analysis of CAR T cells showed inferior migration towards SDF-1 (chemotactic index: 3.2 vs. 15.7) and adhesion to marrow-derived stroma compared with HSCs. Noteworthy, CAR T cells armored with HSC homing factors were preferentially redirected to marrow resulted in 1.4-8.5-fold increase in infiltration at day 3. Single-cell RNA sequencing further revealed low expression of HSC homing factors, indicating an intervenable window to enhance CAR trafficking.

Conclusions
This study not only add significant impetus onto the fundamental CAR biology, but also deliver the next-generation, homing-armored CAR T cells to overcome therapy resistance in hematologic malignancies

Future Plans
Post-infusion CAR T cells' fate will be mapped at single-cell resolution, aiming to uncover the dynamics of clonality along trafficking cascade and identify new potential targets for CAR T fitness improvement.