Next-Generation Off-the-Shelf CAR T-Cells: A Novel Platform to Enable Comprehensive Elimination of Aberrant Effector Cells for the Treatment of Autoimmune Diseases in the Absence of Conditioning Chemotherapy

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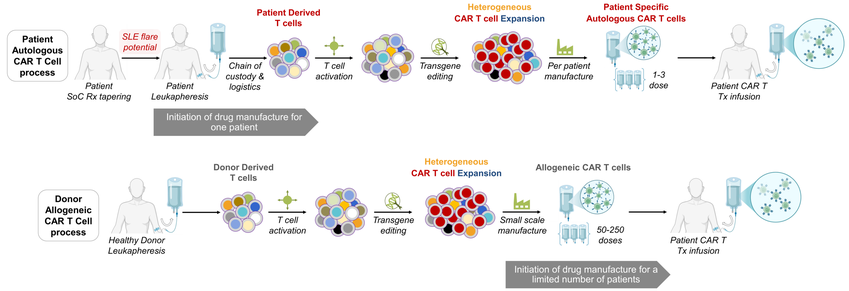
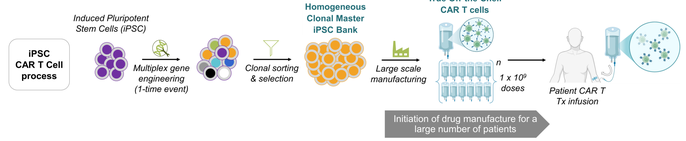
INTRODUCTION

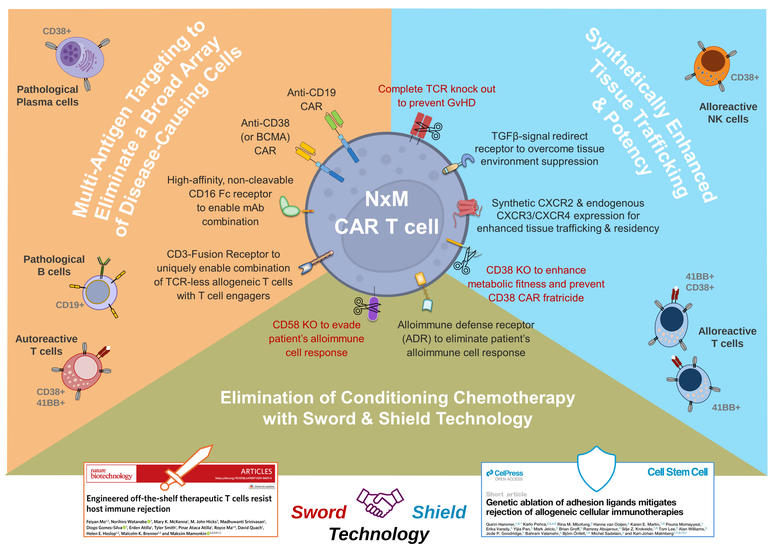
- Autologous anti-CD19 chimeric antigen receptor (CAR) T-cell therapy has revolutionized the treatment of CD19-expressing hematologic malignancies and has demonstrated success in patients with systemic lupus erythematosus (SLE).
- Widespread application of autologous CAR T-cell therapy faces many challenges including, but not limited to, the need for patient leukapheresis, drug product manufacture inconsistency, and the requirement for patient hospitalization to monitor for common adverse events such as cytokine release syndrome (CRS) & immune effector cell-associated neurotoxicity syndrome (ICANS).
- An off-the-shelf CAR T-cell therapy that is scalable, available on-demand without the need for leukapheresis, and can be given in the out-patient setting would greatly improve patient accessibility. Furthermore, the ability to eliminate multiple pathological immune cell types, without requiring intense CCT, would extend therapeutic application and maximize patient safety.

CURRENT CAR T CELL THERAPY PARADIGMS





NEXT GENERATION MULTI-ANTIGEN TARGETING (NxM) CAR T CELL



RESULTS: NEXT GENERATION MULTI ANTIGEN TARGETING CAR19x38 T CELL (NxM) ACTIVATION SPECIFICTY & POTENCY

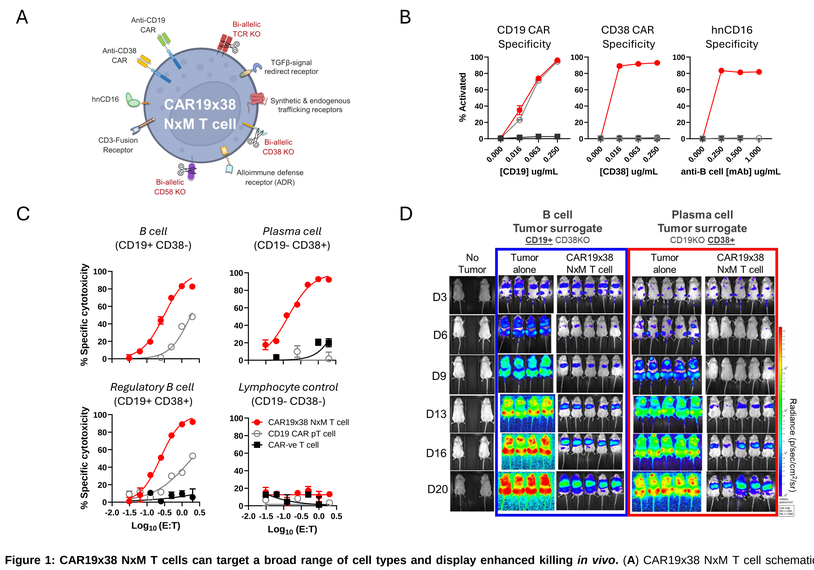


Figure 1: CAR19x38 NxM T cells can target a broad range of cell types and display enhanced killing *in vivo*. (**A**) CAR19x38 NxM T cell schematic highlighting synthetic edit profile. (**B**) Incubation of CAR19x38 NxM T cells with immobilized antigens ± monoclonal antibody for 24 hours followed by surface activation marker evaluation via flow cytometry. (**C**) CAR19x38 NxM T cells, and relevant controls, were co-cultured with NALM6 B cell tumors containing no, single, or dual deletions of CD19 and CD38 antigens in a 24-hour cytotoxicity assay over a range of E:T. (**D**) NSG mice were inoculated with 1E5 CD38 KO (CD19+/CD38 KO) or CD19 KO (CD19 KO/CD38+) NALM6 tumors and treated with 2E6 CAR19x38 NxM T cells 3 days after. Representative images depicting tumor burden are shown across time (days).

RESULTS: SWORD & SHIELD TECHNOLOGY ENHANCES ALLO-PERSITENCE AND ALLEVIATES THE NEED FOR LYMPHO-CONDITIONING

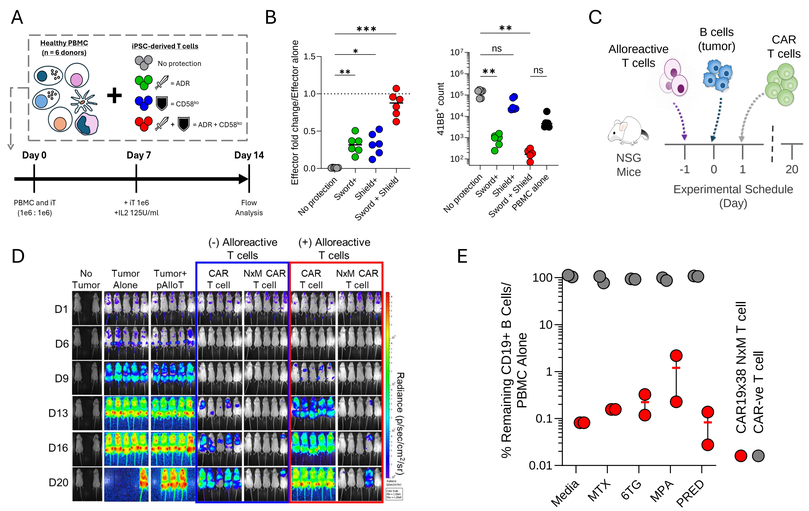


Figure 2: *Sword and Shield* technology protects SoC therapy compatible NxM CAR T cells from alloimmune cell elimination. (**A**) Mixed lymphocyte reaction (MLR) schematic. (**B**) NxM CAR T cell variant fold changes (left) normalized to cell alone controls and total 4-1BB+ alloreactive (patient anti-drug) immune cells (right) on day 14 MLR. One-Way ANOVA with multiple comparison was used to determine statistical significance; *p<0.05; **p<0.001; ***p<0.0001; and ns = not significant. (**C**) *In vivo* schematic and (**D**) tumor burden quantification in mice inoculated with β2M-deficient NALM6 B cell tumors treated with CAR T cells (no sword & shield technology) or NxM CAR T cells ± primed alloreactive T cells. (**E**) NxM CAR T cell anti-B cell potency evaluation ± physiological concentrations of standard of care (SoC) autoimmune drugs over a 24-hour cytotoxicity assay.

RESULTS: EFFECTIVE BROAD & DEEP MULTI-LINEAGE IMMUNE CELL ELIMINATION IN AN IMMUNE REPLETE ALLO-ENVIRONMENT

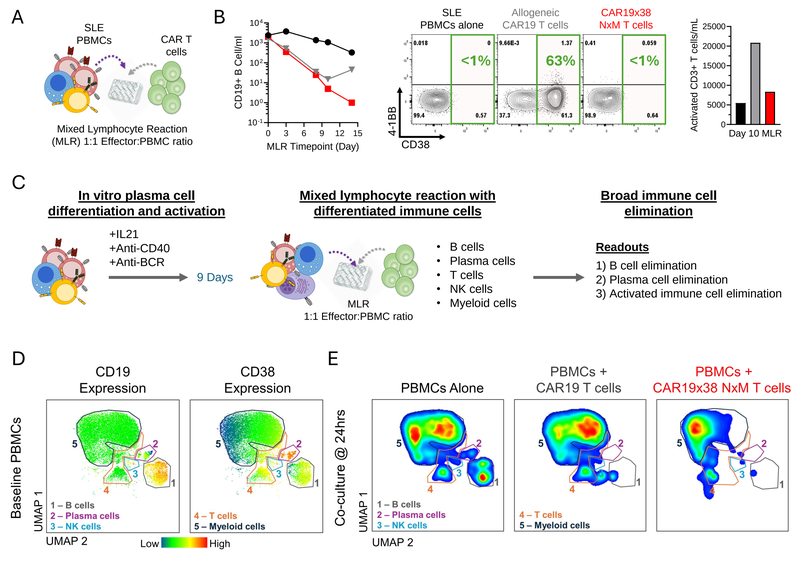


Figure 3: CAR19x38 NxM T cells eliminate SLE patient derived B cells and plasma cells whilst preventing the emergence of patient alloreactive immune responses. (**A**) *In vitro* schematic of SLE patient derived PBMC:allogeneic CAR19 primary T cells or CAR19x38 NxM T cell MLR. (**B**) CD19+ B cell elimination kinetic (left) and representative flow cytometry plots highlighting the percentage (middle) and total number (right) of remaining alloreactive 4-1BB+ CD38+ patient T cells on MLR day 10. (**C**) *In vitro* 9-day plasma cell differentiation schematic. Following the emergence of plasma cells (CD38+, CD138+, CD19 low), the PBMCs were co-cultured with CAR19 T cells or CAR19x38 NxM T cells for 72-hours at a 1:1 ratio. (**D**) PBMC CD19 and CD38 antigen expression at baseline determined by Uniform Manifold Approximation and Projection (UMAP) and FlowSOM meta-clustering. (**E**) Proportion of patient immune cell subsets remaining following 24 hour co-culture with CAR19 T cells or CAR19x38 NxM T cells.

CONCLUSIONS & ACKNOWLEDGMENTS

To maximize patient access and expand CAR T-cell therapy into multiple autoimmune and hematological disease indications, we have developed a versatile platform that allows for the creation of clonal, multiplexed-precision engineered induced pluripotent stem cells (iPSC) and their differentiation into next-generation multi-antigen targeting (NxM) CAR T-cell therapies.

1. **Multi-antigen targeting via dual CAR (CD19 x CD38 or CD19 x BCMA), hnCD16 and CD3FR expression:** NxM CAR T cells are engineered with the versatility to eliminate pathological cells through the engagement of one or multiple activating receptors, alone or in combination with monoclonal antibodies and T cell engagers, that synergize to enhance potency against a broad range of immune cells that constitutively or transiently express select disease antigens.
2. **Ability to resist patient derived alloimmune responses:** The combination of an allo-defence receptor (ADR) and the genetic disruption of CD58 (*Sword & Shield technology*) allows NxM CAR T cells to circumvent the need for conditioning chemotherapy, enhances therapeutic outcomes, and maximizes patient access by enabling outpatient treatment.
3. **Does not require lymphodepleting chemotherapy and can be combined with SoC maintenance therapy:** Sword & Shield technology provides NxM CAR T cells the ability to exert functional persistence in an intact immune environment and support clinical activity in combination with SoC therapeutic regimens.
4. **Express chemokine receptors that maximize tissue potency:** The expression of an array of endogenous and synthetically expressed chemokine receptors enhances NxM CAR T cell activity at central and peripheral sites of pathological B cell, plasma cell and T cell activity (refer to Fate Tx platform presentations below for additional data).

CAR19x38 NxM T cells (*termed FT839*) can be manufactured at large scale in a cost-effective manner, administered off-the-shelf to broadly reach patients, and used to target multiple pathogenic cell types for the treatment of autoimmune and hematologic diseases.

FT819 Oral Presentation
Dr Vaneet Sandhu
OP0032

FT522 Poster Presentation
Dr Lilly Wong
POS1402

Fate Therapeutics
Platform Presentations