Next-Generation Off-the-Shelf CAR T-Cell Therapies for Conditioning-Free Treatment of a Broad Spectrum of Autoimmune Diseases and Hematologic Malignancies

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In collaboration with

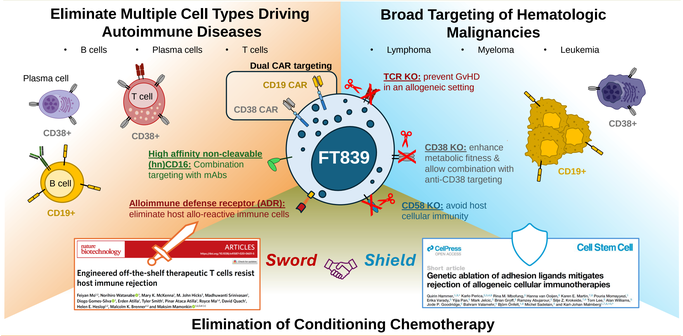
INTRODUCTION

Anti-CD19 chimeric antigen receptor (CAR) T-cell therapy has revolutionized the treatment of CD19-expressing hematologic malignancies and has now demonstrated profound responses in patients affected with autoimmune disease, including systemic lupus erythematosus (SLE).

Widespread application of autologous CAR T-cell therapy faces many challenges including, but not limited to, the need for patient apheresis, the risk of failure of drug product manufacture, and the requirement to hospitalize patients to monitor and treat common adverse events such as cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). In addition, patients are pre-treated with intense conditioning chemotherapy (CCT) consisting of cyclophosphamide (Cy) and fludarabine (Flu), which increases the risk of severe infection and secondary malignancies, and limits patient access and reach.

An off-the-shelf CAR T-cell therapy that is scalable, available on-demand without apheresis, and can be administered in the out-patient setting would greatly improve patient accessibility. In addition, the use of less intense, or the elimination of, Cy / Flu conditioning chemotherapy may maximize patient safety and tolerability.

Next Generation multi-antigen targeting (NxM) CAR T Cells



CONCLUSIONS

To maximize patient access and expand CAR T-cell therapy to multiple disease indications, we developed a platform that allows for the creation of clonal, multiplexed-engineered induced pluripotent stem cells and their differentiation into next-generation multi-antigen targeting (NxM) CAR T-cell therapies. Off-the-shelf NxM CAR T cells incorporate novel synthetic functional elements designed to effectively eliminate a range of aberrant cells while evading recognition by alloreactive immune cells.:

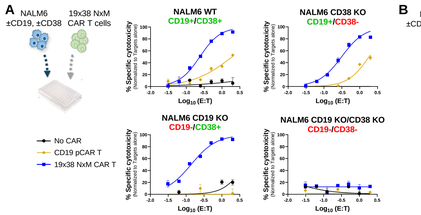
1. **Multi-antigen targeting via dual CAR expression:** NxM CAR T cells are engineered with the versatility to specifically eliminate cell lines with variable antigen expression and display enhanced elimination of lines expressing multiple targeted antigens.
2. **Resistance to immune cell-mediated depletion:** ADR/CD58KO-edited (*Sword & Shield*) NxM CAR T cells exhibit resistance against immune cell-mediated depletion by evading and selectively targeting 4-1BB-positive alloreactive immune cells. Addition of CD38 CAR provides supplemental resistance by eliminating CD38+ alloreactive immune cells.
3. **Pan-hematologic malignancy targeting and enhanced anti-tumor activity:** 19x38 NxM CAR T cells specifically eliminate cell lines that originate from a variety of hematologic malignancies and display enhanced anti-tumor efficacy through multi-antigen targeting.
4. **Simultaneous targeting of multiple cell types that drive autoimmune diseases:** 19x38 NxM CAR T cells eliminate B cells, Plasma cells, and T cells that contribute to autoimmune diseases while preventing the emergence and proliferation of alloreactive T cells.

NxM 19x38 CAR T cells, **FT839**, can be manufactured at 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 diseases and hematologic malignancies.

RESULTS

Next-generation multi-antigen targeting CAR T cells expressing anti-CD19 and -CD38 CARs (19x38 NxM CAR T cells) eliminate cells with variable antigen expression *in vitro* and drive enhanced and durable efficacy *in vivo* against xenograft models of leukemia

19x38 NxM CAR T cells eliminate targets at low E:T ratio with variable antigen expression *in vitro*



Single dose 19x38 NxM CAR T cells control aggressive leukemia xenografts with enhanced targeting of tumors expressing both CD19 and CD38 in *in vivo* stress model

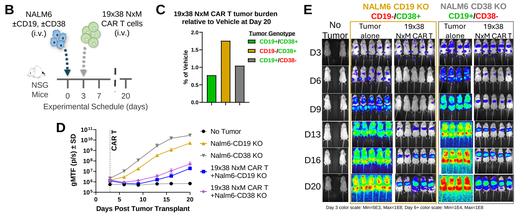
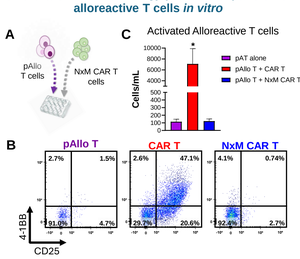


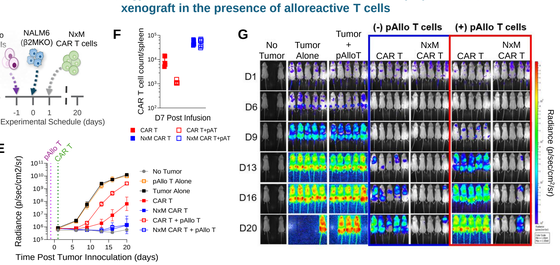
Figure 1: Dual CAR-expressing NxM CAR T cells target a broad range of cell types and display enhanced target killing *in vivo*. (**A**) *In vitro* schematic; NxM CAR T cells expressing both anti-CD19 and anti-CD38 CARs (19x38 NxM CAR T cells, FT839), NxM T cells without CAR expression (No CAR), or CD19 CAR expressing primary T cells (CD19 pCAR T) were challenged with NALM6 lines with single, combination, or no expression of CD19 and CD38 antigens in a 24-hour cytotoxicity assay over a range of E:T (2:1 to 1:32). (**B**) *In vivo* schematic; mice were inoculated with disseminated xenograft WT (CD19+CD38+), CD19 KO (CD19-/CD38+), or CD38 KO (CD19+/CD38-) NALM6 leukemia B cells and treated with 19x38 NxM CAR T cells. (**C**) Average tumor burden of 19x38 NxM CAR T treated mice at Day 20 post inoculation normalized to average tumor burden of respective tumor alone mice. (**D**) Bioluminescence-based tumor quantification; geometric mean of total flux per group (p/s) was measured to monitor tumor growth in mice. (**E**) Representative images depicting tumor burden.

Sword & Shield Technology eliminates the need for pre-treatment with intense conditioning chemotherapy through selective targeting and evasion of alloreactive (anti-drug) immune cells

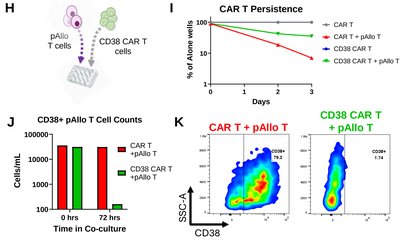
Sword & Shield* Technology prevents expansion of alloreactive T cells *in vitro



***Sword and Shield* Technology allows for the durable elimination of a B cell lymphoblastic leukemia xenograft in the presence of alloreactive T cells**



19x38 NxM CAR T cells eliminate CD38+ alloreactive T cells



19x38 NxM CAR T cells supplement *Sword & Shield* Technology

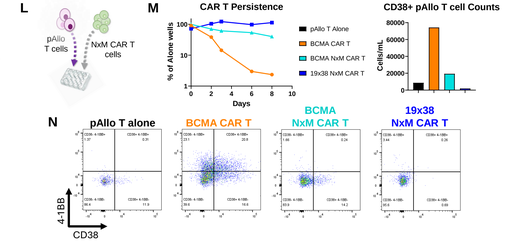


Figure 2: *Sword and Shield* Technology protects NxM CAR T cells from alloreactive immune cell depletion and anti-CD38 CAR enhances resistance against allorejection. (**A**) Schematic of *in vitro* mixed lymphocyte reaction (MLR) consisting of primed alloreactive T cells (pAllo T cells) and CAR T cells or NxM CAR T cells that contain ADR and CD58 KO technology. pAllo T cells are generated following repeated exposure to donor-specific T cells. (**B**) Representative flow plots showing pAllo T cell activation after 72 hrs in co-culture. (**C**) Absolute cell counts of activated pAllo T cells (4-1BB+, CD25+) across three unique pAllo T cell donors after 72 hrs in co-culture; One-way ANOVA with Holm-Sidak correction; *p<0.05. (**D**) *In vivo* schematic, (**E**) bioluminescence-based tumor burden quantification in mice inoculated with disseminated xenograft β2M-deficient NALM6 leukemia B cells treated with CAR T cells or NxM CAR T cells in the presence or absence of pAllo T cells. (**F**) Absolute CAR T cell counts among total splenocytes on Day 7. (**G**) Representative images depicting tumor burden. (**H**) Schematic of *in vitro* MLR consisting of pAllo T cells and anti-CD38 CAR T cells. (**I**) Percentage of CAR T cells remaining after 72 hr co-culture with pAllo T cells normalized to CAR T cell alone wells. (**J**) Absolute cell counts of CD38+ pAllo T cells at start and end of 72-hour co-culture with anti-CD38 CAR T cells. (**K**) Representative flow cytometry plots of CD38+ pAllo T cells after 72 hrs in co-culture. (**L**) Schematic of *in vitro* MLR consisting of 1:1 ratio of pAllo T cells to BCMA CAR T, BCMA NxM CAR T, or 19x38 NxM CAR T cells. (**M**) Percentage of CAR T cells remaining at selected timepoints over 8 days of co-culture with pAllo T cells compared to CAR T cell alone wells (left). Absolute cell counts of CD38+ pAllo T cells after 72 hrs in co-culture (right). (**N**) Representative flow cytometry plots of 4-1BB and CD38 surface expression on pAllo T cells after 72-hours in co-culture.

19x38 NxM CAR T broadly targets hematologic malignancies with enhanced clearance of myeloma and lymphoma cell lines

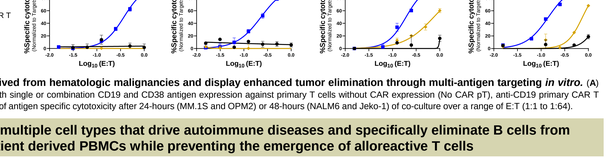
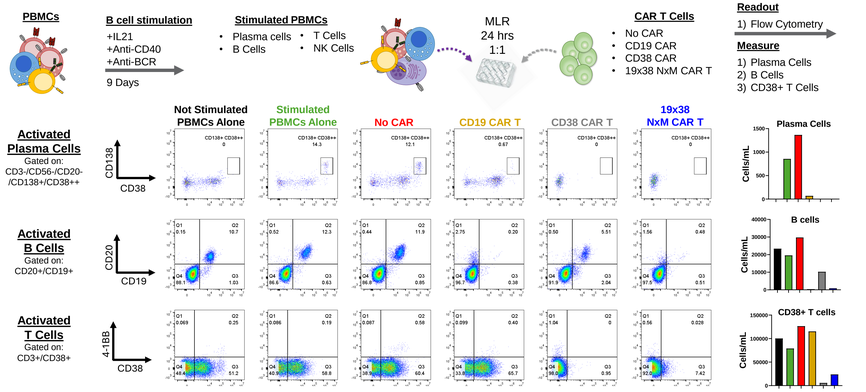


Figure 3: 19x38 NxM CAR T cells eliminate cell lines derived from hematologic malignancies and display enhanced tumor elimination through multi-antigen targeting *in vitro*. (**A**) Left, schematic of *in vitro* challenge of multiple malignant cell types with single or combination CD19 and CD38 antigen expression against primary T cells without CAR expression (No CAR pT), anti-CD19 primary CAR T cells (CD19 pCAR T), or 19x38 NxM CAR T cells. Right, quantification of antigen specific cytotoxicity after 24-hours (MM.1S and OPM2) or 48-hours (NALM6 and Jeko-1) of co-culture over a range of E:T (1:1 to 1:64).

NxM CAR T cells simultaneously target multiple cell types that drive autoimmune diseases and specifically eliminate B cells from autoimmune disease patient derived PBMCs while preventing the emergence of alloreactive T cells

19x38 NxM CAR T cells simultaneously eliminate hyper-activated plasma cells, B cells, and T cells *in vitro*



NxM CAR T cells target and eliminate Systemic Lupus Erythematosus (SLE) PBMC derived B cells whilst simultaneously preventing the emergence of alloreactive (anti-drug) T cell activation and proliferation

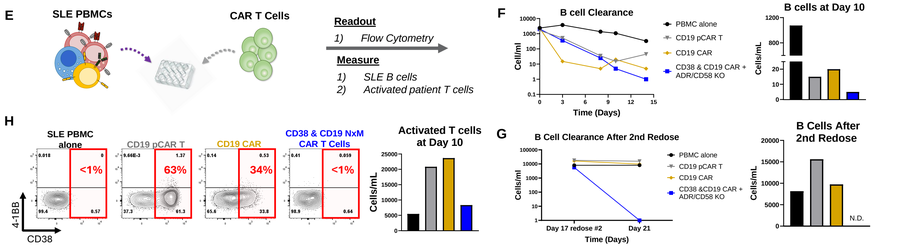


Figure 4: NxM CAR T cells simultaneously target multiple cell types that drive autoimmune diseases and eliminate B cells derived from a patient with autoimmune disease while preventing alloreactive responses from patient T cells. (**A**) *In vitro* schematic; B cell maturation was stimulated in PBMCs with IL21, anti-CD40, and anti-BCR treatment over 9 days. After B cell maturation, the PBMCs were placed in an MLR against No CAR, single CAR (anti-CD19 or anti-CD38 CAR), or 19x38 NxM CAR T cells for 24-hours at a ratio of 1:1. (**B**) Activated plasma cells, (**C**) activated B cells, and (**D**) activated T cells were identified by flow cytometry and absolute amounts were quantified in each condition after 24-hours of MLR co-culture. (**E**) *In vitro* schematic of SLE derived PBMC MLR in co-culture with donor mismatched (allogeneic) anti-CD19 primary CAR T cells, anti-CD19 CAR T cells, or anti-CD38 x anti-CD19 NxM CAR T cells. (**F**) Tracking of B cell clearance over 14 days of co-culture (left) and total CD19+ B cell counts following 10 days in co-culture (right). (**G**) Tracking of total B cells from Day 17 to Day 21 of MLR co-culture upon second round of B cell rechallenge at Day 17 (first round of rechallenge at Day 14) (left). Remaining total B cells at day 21 of MLR co-culture following second round of rechallenge (right). (**H**) Representative flow cytometry plots of SLE PBMC derived T cells at 10 days of MLR co-culture and the percentage expression of 4-1BB and CD38 surface activation markers (left). Total SLE donor derived activated T cell counts following 10 days of co-culture (right).