IL-7 and CCL19 Expression in Specific Peptide Enhanced Affinity Receptor T-cells Targeting MAGE-A4 Display Improved Survival and Ability to Induce Migration of Immune Cells

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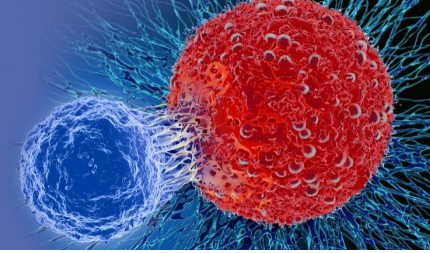


Introduction

- Specific peptide enhanced affinity receptor (SPEAR) T-cells are engineered with T-cell receptors (TCRs) designed to target tumor cells with specific antigens presented on their surface by human leukocyte antigen (HLA) molecules.
- SPEAR T-cell first-generation and next-generation products are in early-stage to late-stage clinical development with responses across multiple solid tumor types,[1,2] and further enhancements are being investigated preclinically.
- Here we describe a next-generation SPEAR T-cell therapy (ADP-A2M4N7X19; **Figure 1**), targeting the melanoma-associated antigen A4 (MAGE-A4) tumor antigen peptide presented on HLA-A*02:01, that utilizes "Proliferation-Inducing and Migration-Enhancing" (PRIME) technology to secrete interleukin 7 (IL-7) and C-C motif chemokine ligand 19 (CCL19).
- IL-7 plays a role in stimulating proliferation and supporting the survival of T-cells, and CCL19 induces the migration of immune cells.[3]
  - Unmodified T-cells are incapable of producing IL-7 or CCL19, relying on other cell types to produce these elements.
- The addition of IL-7 and CCL19 is hypothesized to enhance proliferative and survival capabilities, as well as infiltration of other immune cells into MAGE-A4-positive tumors.
  - Together these enhancements may lead to the formation of tertiary lymphoid structures within the tumor.
- We confirmed that the IL-7 and CCL19 constructs did not negatively impact critical functionality of ADP-A2M4N7X19.
  - There were no differences in potency and memory T-cell phenotype between ADP-A2M4N7X19 and T-cells expressing the MAGE-A4-targeted TCR alone (ADP-A2M4; data not presented here).
- Here we evaluate the biological efficacy of ADP-A2M4N7X19 by determining the functional effects of IL-7 and CCL19 co-expression upon stimulation with MAGE-A4 in vitro.

Methods

T-cell Expansion as a Function of IL-7 Production

- ADP-A2M4, ADP-A2M4N7X19, and non-transduced (ntd) T-cells from 3 donors were stimulated weekly with irradiated A375 (MAGE-A4 positive) cells without or with 20 ng/mL exogenous recombinant human IL-7 (rhIL-7; **Figure 2**, top and bottom panels, respectively).
- Absolute cell counts (CD45+ T-cells/mL) were performed every 7 days and used to calculate fold change from the known density of cells seeded.

Antigen Dependency of ADP-A2M4N7X19 Proliferation

- ADP-A2M4, ADP-A2M4N7X19, and ntd T-cells from 2 donors were stimulated weekly with irradiated A375 (MAGE-A4 positive) cells with or without 20 ng/mL exogenous rhIL-7 (data not shown).
- On day 7, antigen was removed (**Figure 3**, vertical dashed line).
- Absolute cell counts (CD45+ T-cells/mL) were performed every 7 days and used to calculate fold change from the known density of cells seeded.

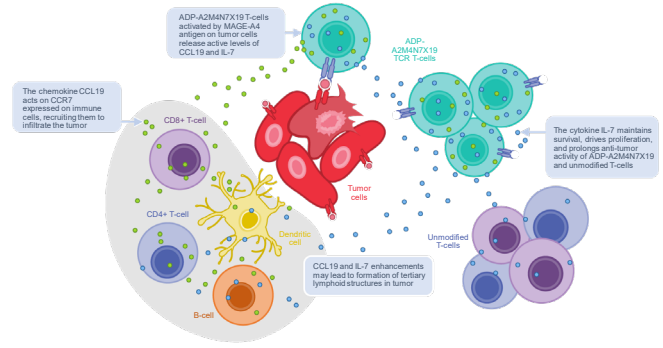
Cytokine Release in Response to Repeated Antigen Stimulation

- ADP-A2M4, ADP-A2M4N7X19, and ntd T-cells from 3 donors were stimulated weekly with irradiated A375 (MAGE-A4 positive) cells.
- Supernatants were collected on Days 1, 7, 8, 14, 15, 21, 22, and 28, and cytokine levels (pg/mL) determined on supernatants (**Figure 4**).

Improvement in Local T-cell Migration by CCL19

- Immune cell migration toward ADP-A2M4N7X19 was assessed by a trans-well migration assay.
  - ADP-A2M4, ADP-A2M4N7X19, ADP-A2M4IL7 (T-cells expressing the MAGE-A4–targeted TCR along with IL-7), and ntd T-cells from 3 donors were incubated with NCI-H1755 (MAGE-A4 positive) cells in the lower chamber for 48 hours.
  - The upper chamber was pre-seeded with primary dermal microvascular endothelial cells, followed by the addition of fluorescently stained mature dendritic cells.
  - rhCCL19 was added to independent wells as a positive control.
  - Migration was measured in a plate reader over 6 hours (**Figure 5**).

Figure 1. ADP-A2M4N7X19: T-cells Expressing IL-7 and CCL19

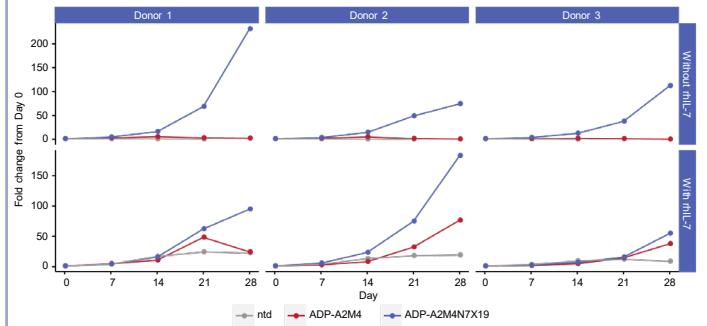


CCL19, C-C motif chemokine ligand 19; CCR7, C-C motif chemokine receptor 7; IL-7, interleukin 7.

Results

- ADP-A2M4N7X19 proliferate after repeated antigen stimulations, while ADP-A2M4 showed little proliferation upon antigen stimulation without IL-7 (**Figure 2**, top).
- Exogenous rhIL-7 resulted in expansion of both ADP-A2M4 and ADP-A2M4N7X19 T-cells, with weak expansion of ntd T-cells (**Figure 2**, bottom).

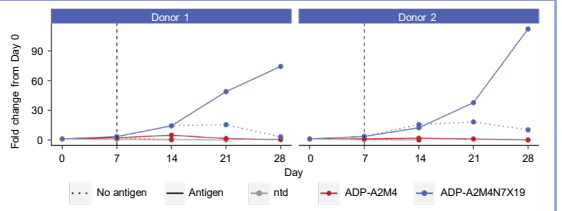
Figure 2. IL-7 Signaling Improves Survival and Proliferation of ADP-A2M4N7X19 T-cells



ntd, non-transduced; rhIL-7, recombinant human interleukin 7.

- Removal of antigen resulted in the decline of ADP-A2M4N7X19 T-cell populations in both donors (**Figure 3**). This is an important observation to support the safety of ADP-A2M4N7X19.
- Exogenous rhIL-7 had minimal effect on T-cell persistence after antigen removal (data not shown), suggesting that ADP-A2M4N7X19 T-cells require both antigen exposure and IL-7 signaling (not IL-7 alone) for survival.

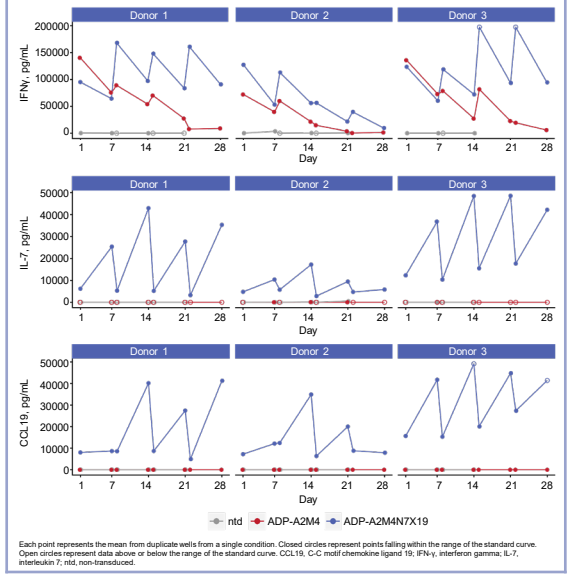
Figure 3. Survival of ADP-A2M4N7X19 T-cells is Dependent Upon IL-7 Signaling and Antigen Exposure



IL-7, interleukin 7; ntd, non-transduced.

- Interferon gamma (IFNγ) levels were consistently higher for ADP-A2M4N7X19 T-cells after repeated stimulation (**Figure 4**).
- ADP-A2M4N7X19 had sustained IL-7 and CCL19 production throughout the duration of the restimulation assay, in line with IFNγ data (**Figure 4**).

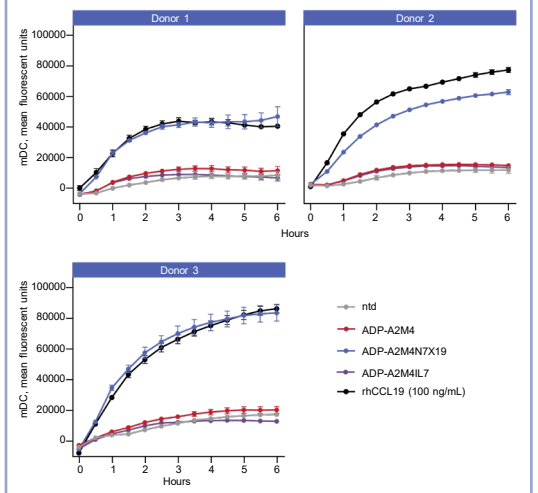
Figure 4. Sustained Cytokine Secretion by ADP-A2M4N7X19 T-cells Following Repeated Antigen Stimulation



Each point represents the mean from duplicate wells from a single condition. Closed circles represent points falling within the range of the standard curve. Open circles represent data above or below the range of the standard curve. CCL19, C-C motif chemokine ligand 19; IFNγ, interferon gamma; IL-7, interleukin 7; ntd, non-transduced.

- CCL19 production conferred the ability of ADP-A2M4N7X19 to induce immune cell migration compared to ADP-A2M4 and ADP-A2M4IL7 upon T-cell stimulation (**Figure 5**).

Figure 5. ADP-A2M4N7X19 T-cells Induce Mature Dendritic Cell Migration as a Consequence of CCL19 Production Following Repeated Antigen Stimulation



rhCCL19, recombinant human C-C motif chemokine ligand 19; mDC, mature dendritic cells; ntd, non-transduced.

Conclusions

- Transduction of SPEAR T-cells with PRIME technology increases T-cell engraftment, functionality, and improves potential for immune cell infiltration into the tumor, which is hypothesized to lead to improved anti-tumor activity in the clinic.
- ADP-A2M47X19 T-cells need both antigen exposure and IL-7 signaling for survival, suggesting that increased T-cell activity will only occur in the presence of MAGE-A4 antigen.
- Based on results presented here, a Phase 1 clinical trial will be initiated with ADP-A2M4N7X19 in multiple indications.

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Abbreviations Used in Text
CCL19, C-C motif chemokine ligand 19; HLA, human leukocyte antigen; IFNγ, interferon gamma; IL-7, interleukin 7; MAGE-A4, melanoma-associated antigen A4; ntd, non-transduced; PRIME, "Proliferation-Inducing and Migration-Enhancing"; SPEAR, specific peptide enhanced affinity receptor; TCR, T-cell receptor.

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