Combination Immunotherapy with NY-ESO-1 Specific CAR+ T cells and T-Cell Vaccine Improves Anti-Myeloma Effect

Krina K. Patel[1], Simon Olivares[2], Harjeet Singh[2], Lenka V. Hurton[2], Mary Helen Huls[3], Muzaffar H. Qazilbash[1], Partow Kebriaei[1], Richard E. Champlin[1] and Laurence JN Cooper[2,4]

[1]Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX;
[2]Division of Pediatrics, The University of Texas MD Anderson Cancer Center, Houston, TX., [3]Intrexon Corporation, Germantown, MD; [4]ZIOPHARM Oncology, Boston, MA

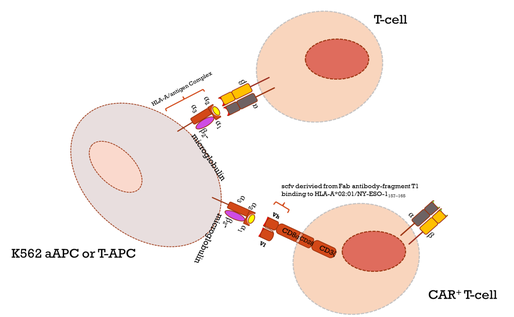
MAIN CONCLUSION

WE DEMONSTRATE THE FEASIBILITY OF GENERATING T-APC THAT PROPAGATE NY-ESO-1-SPECIFIC CAR+ T CELLS, INCREASE T-CELL PERSISTENCE *IN VIVO,* AND IMPROVE ANTI-MYELOMA EFFECT.

INTRODUCTION

- **Multiple Myeloma**
  - Systemic plasma cell malignancy
  - NY-ESO-1, a tumor-associated antigen (TAA)
    - Expressed by majority of patients with high risk or relapsed multiple myeloma
    - Absent expression on healthy cells/tissues
  - Prognosis:
    - Overall survival less than 2 years concordant with high risk disease
    - Incurable in standard risk and high risk disease despite combination of novel therapies including Bortezomib/IMiDs/steroids and autologous transplant with lenalidomide maintenance
- **T cells expressing chimeric antigen receptor (CAR)**
  - Early clinical trials in myeloma patients have shown promise
  - High risk patients with myeloma in urgent need of effective novel therapies
  - CAR can recognize peptide processed from NY-EOS-1 in context of HLA A2[1]
- **T cells expressing T-cell receptor (TCR)**
  - Clinical trials in synovial sarcoma and multiple myeloma demonstrate that T cells expressing NY-ESO-1-specific TCR have anti-tumor effects
  - Affinity-modified TCR can recognize peptide processed from NY-ESO-1 in context of HLA A2[2]
- **Cancer vaccine**
  - T cells as antigen presenting cells (T-APC)
    - Generation and manipulation of clinical grade T-APC to present TAA has a path for clinical translation.
- **Premise**
  - Enforced expression of membrane-bound IL-15 (mIL15) on the surface of NY-ESO-1 presenting T-APC by non-viral gene transfer using *Sleeping Beauty* (SB) system improves persistence of T cells[3]
  - Autologous T-APC expressing NY-ESO-1 can activate and numerically expand antigen-specific effector T cells as effectively as our control activating and propagating cells (AaPC) derived from genetically modified K-562 cells.
  - The NY-ESO-1 specific effector T cells will persist longer *in vivo* when infused with T-APC versus alone and will lead to improved myeloma control.

HYPOTHESIS



STUDY RATIONALE

- **To Improve Treatment Outcome**
  - Adoptively transfer genetically modified T cells expressing CAR (or T-cell receptor, TCR) specific for NY-ESO-1 with autologous T-APC expressing NY-ESO-1 for improved persistence and anti-tumor effect

RESULTS

Generation of T-APC

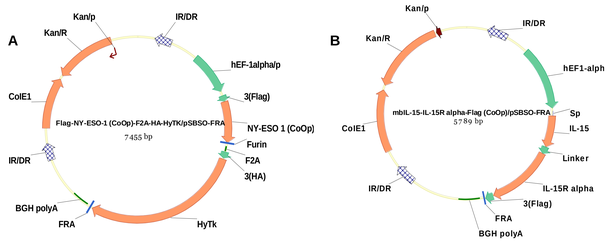


Figure 1: SB transposons as DNA plasmids encoding (A) NY-ESO-1 co-expressed with fusion gene of hygromycin phosphotransferase (Hy) and thymidine kinase (TK) from HSV-1 (HyTK) and (B) mIL15. Both HyTK and mIL15 have epitope tags for detection.

Characterization of T-APC

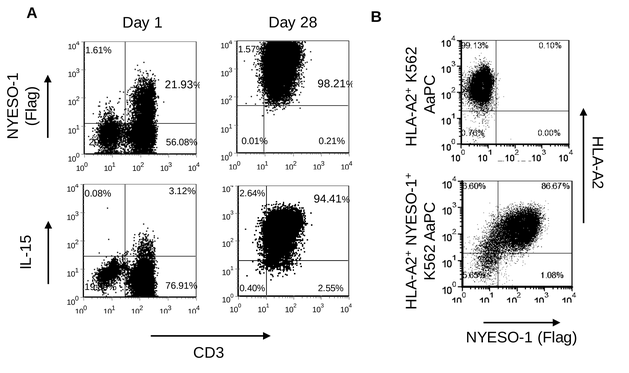


Figure 2: Characterization of T-APC (A) Expression of NY-ESO-1 (based on FLAG tag) and mIL15 on T-APCs on Day 1 (day after electroporation) and Day 28 (after sustain propagation on OKT3-loaded K562 AaPC in presence of cytocidal concentration of hygromycin B). (B) Expression of NYESO-1 (Flag) on HLA-A2+ K562 AaPC. (C) Propagation of T-APC expressing NYESO-1 alone (T-APC NYESO-1) or in combination with membrane bound IL-15 (mIL-15) (T-APC NYESO-1+mIL-15+) propagated on OKT3-loaded K562 AaPC. (D) Expression of co-stimulatory molecules (HLA-DR, IL-15, CD2, CD11a, CD50, CD52, CD54, CD58, CD70, CD83, CD86, CD137L) on T-APC NYESO-1+ mIL15+.

Generation of NY-ESO-1-specific effector T cells

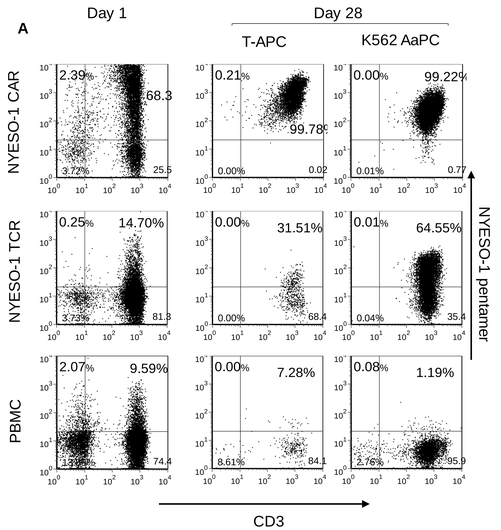
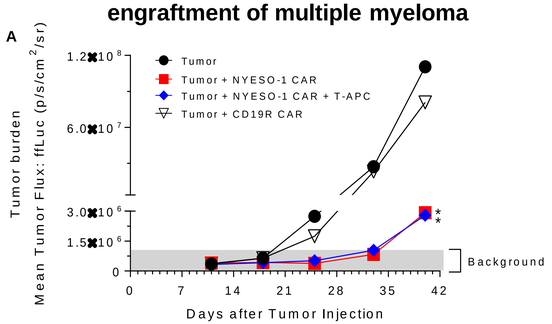


Figure 3: Characterization of NYESO-1 specific T cells. (A) Expression of NY-ESO-1-specific HLA A2-restricted CAR and TCR on CD3+ T cells before and after 28-day sustained proliferation on autologous HLA A2+ T-APC and K-562-derived AaPC that were genetically modified to express NY-ESO-1. "PBMC" are non genetically modified (control) T cells. (B) Specific killing (by chromium release assay) of HLA A2+ NY-ESO-1+ (CD19neg) U266 multiple myeloma cells with NY-ESO-1-specific CAR+ T cells compared to NY-ESO-1neg EL4 cells. Mean ± SD of triplicates is shown.

In vivo efficacy: NY-ESO-1 CAR+ T cells delays engraftment of multiple myeloma



Improved persistence of NYESO-1-specific CAR+ T cells in the presence of T-APC

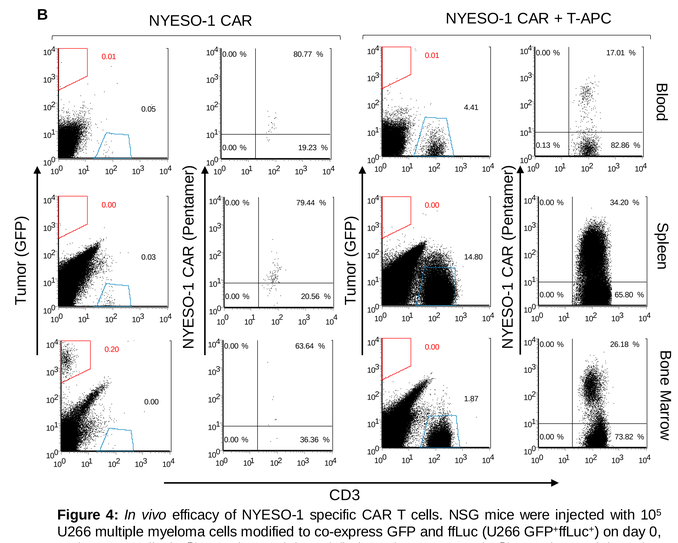


Figure 4: *In vivo* efficacy of NYESO-1 specific CAR T cells. NSG mice were injected with 10^5 U266 multiple myeloma cells modified to co-express GFP and ffLuc (U266 GFP+ffLuc+) on day 0, and CAR T cells (10^7/mouse) were injected (i.v.) on day 1, T-APC (10^7/mouse) were injected to the appropriate groups on day 1 and day 5. Tumor flux was calculated from serial bioluminescent imaging (BLI) from ffLuc+U266 using D-Luciferin substrate. (A) Tumor flux over time is shown. Background BLI is highlighted in gray. (B) At the end of the experiment, tissues (blood, bone marrow, spleen) were harvested from mice and evaluated for the presence of tumor (GFP), T cells (CD3) and NYESO-1-specific CAR (pentamer).

CONCLUSIONS

- SB system can be used to generate NY-ESO-1+ T-APC from peripheral blood mononuclear cells
- NY-ESO-1+ T-APC can propagate NY-ESO-1-specific CAR+ or TCR+ T cells
- Propagated CAR+ T cells exhibit redirected specificity for NY-ESO-1+ U266 multiple myeloma
- Co-administration of NY-ESO-1+ T-APC with NY-ESO-1 CAR+ T cells leads to improved persistence of effector T cells.

FUTURE DIRECTIONS

- Continue evaluation of CAR T cells in combination with T-APC vaccine *in vivo*
- Consider clinical trial using the combination of CAR T cells and T-APC vaccine in high risk and/or relapsed refractory multiple myeloma patients

REFERENCES

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CONFLICT OF INTEREST

Patel: *Ziopharm Oncology:* Equity Ownership, Patents & Royalties; *Intrexon:* Equity Ownership, Patents & Royalties. **Olivares:** *Ziopharm Oncology:* Equity Ownership, Patents & Royalties; *Intrexon:* Equity Ownership, Patents & Royalties. **Singh:** *Ziopharm Oncology:* Equity Ownership, Patents & Royalties; *Immatics:* Equity Ownership, Patents & Royalties; *Intrexon:* Equity Ownership, Patents & Royalties. **Hurton:** *Ziopharm Oncology:* Equity Ownership, Patents & Royalties; *Intrexon:* Equity Ownership, Patents & Royalties. **Huls:** *Ziopharm Oncology:* Equity Ownership, Patents & Royalties; *Intrexon:* Employment, Equity Ownership, Patents & Royalties. **Qazilbash:** *Celgene:* Membership on an entity's Board of Directors or advisory committees; *Takeda:* Membership on an entity's Board of Directors or advisory committees; *Amgen:* Membership on an entity's Board of Directors or advisory committees. **Cooper:** *City of Hope:* Patents & Royalties; *Intrexon:* Equity Ownership; *Ziopharm Oncology:* Employment, Equity Ownership, Patents & Royalties; *Targazyme, Inc.,:* Equity Ownership; *Immatics:* Equity Ownership; *Sangamo BioSciences:* Patents & Royalties; *MD Anderson Cancer Center:* Employment; *Miltenyi Biotec:* Honoraria.