One-Step Construction of Allogeneic CAR-NK Cells Preventing Rejection and Mediating Enhanced Anti-tumor Responses

Fuguo Liu[1,2], Mubin Tarannum[2], Yingjie Zhao[1], James Ham[1], Rizwan Romee[2], Jianzhu Chen[1]

1. Koch Institute for Integrative Cancer Research at MIT, 2. Dana-Farber Cancer Institute, Harvard Medical School

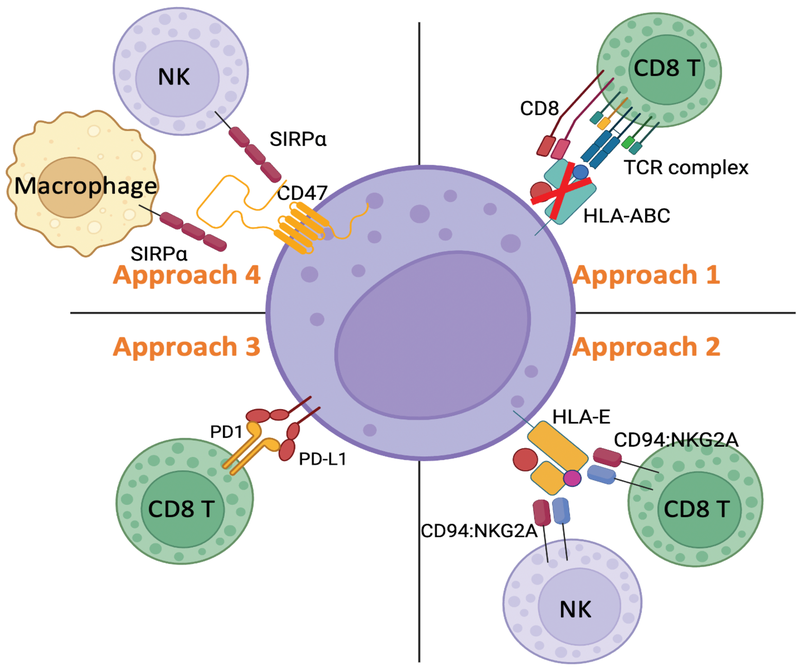
Introduction & Goal

Adoptive cell therapy is promising in treating multipe types of cancers. Currently, in adoptive cell therapy, we predominantly use autologous CAR cells. However, this approach is costly and sometimes yields insufficient effector cells. Also, the effector cells from patients may display exhausted phenotypes. Comparing to autologous CAR cell therapy, allogeneic CAR cell therapy has some potential advantages, such as immediate availability of CAR cells for treatment, and the cost can be reduced by using industrialized process.

However, to enable the use of allogeneic CAR cells, we need to overcome two hurdles. First is to avoid graft versus host disease (GVHD). Allogeneic NK cells don't mediate allo-reaction, hence, they don't induce GVHD. This is an advantage of allogeneic NK cells to be used clinically. Second is to avoid rejection of CAR NK cells by the recipient's immune system, and this is the focus of my study.

Goal: develop a technology that will enable the use of allogeneic CAR NK cells in adoptive cell therapy by avoiding recipient's immune cell killing.

Strategies



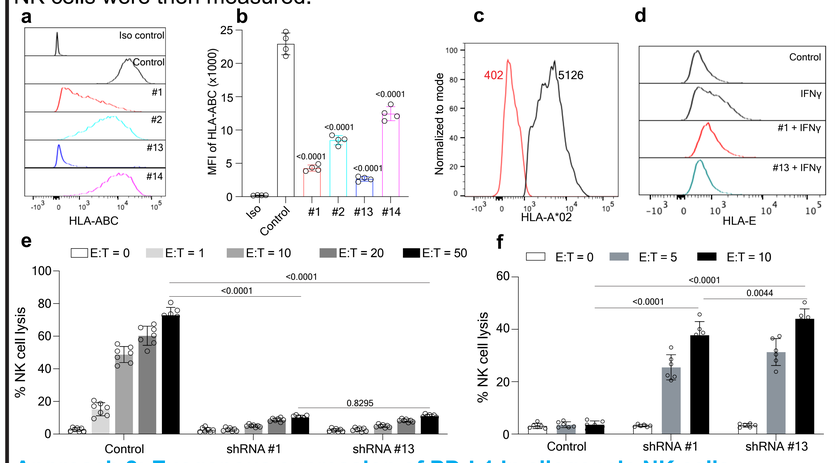
Key findings

- Combination of HLA-ABC knockdown and PD-L1/SCE overexpression inhibits allorejection of NK cells in vitro and in vivo
- Combination of HLA-ABC knockdown, CD19 CAR/mesothelin CAR, and PD-L1/SCE overexpression achieved by one single transduction of immune effector cells
- HLA-ABC reduced, PD-L1/SCE overexpressed CD19 CAR NK cells escape allorejection and arrest CD19⁺ tumor growth in vivo
- PD-L1 and SCE enhance NK cell cytotoxicity
- This technology can be applied to other effector cell platform

Results

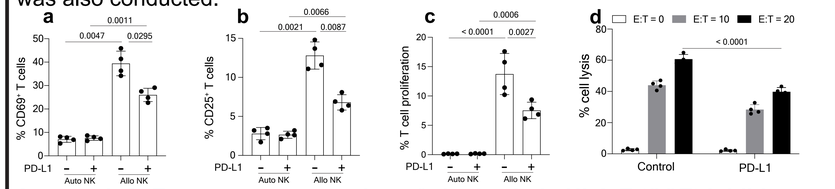
Approach 1: HLA-ABC reduced NK cells escape from host CD8⁺ T cell killing but subject to host NK cell killing

NK cells were transduced with lentivectors that express different shRNAs to downregulate HLA-ABC. HLA-ABC reduced allogeneic NK cells were sorted and cocultured with host CD8⁺ T cells or host NK cells. Survival of HLA-ABC reduced NK cells were then measured.



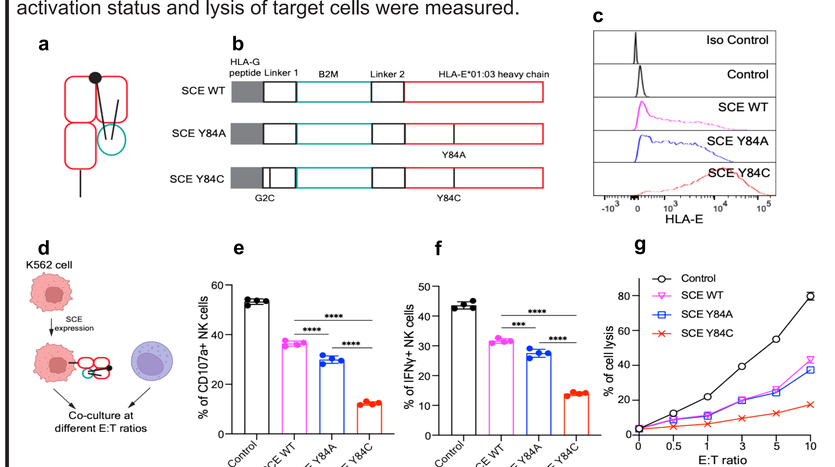
Approach 2: Exogenous expression of PD-L1 in allogeneic NK cells moderately inhibits host CD8⁺ T cell responses

NK cells with or without PD-L1 overexpression were cocultured with allogeneic T cells. CD69+ T cells and T cell proliferation were measured. CD8 T cell killing assay was also conducted.



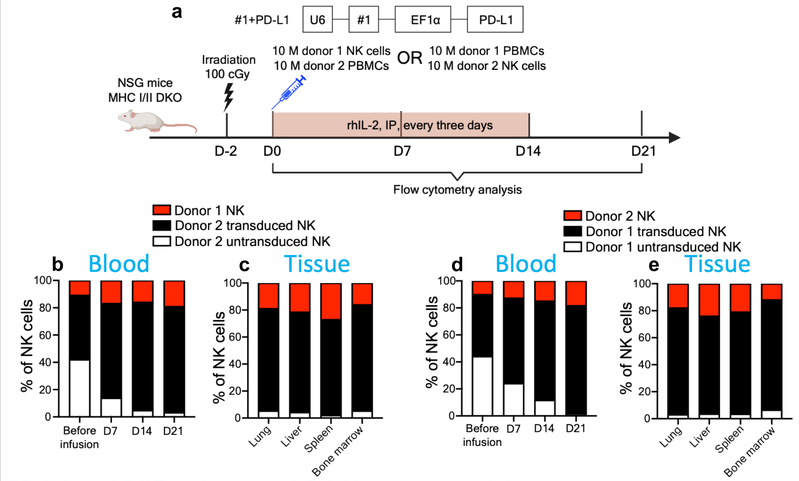
Approach 2: Exogenous expression of single chain HLA-E (SCE) inhibits killing of allogeneic NK cells by host NK cells

Single chain HLA-E (SCE) incorporates HLA-E*01:03 heavy chain, B2M, and G peptide into a single molecule. We constructed plasmids that express three different SCE variants: SCE WT, SCE Y84A, and SCE Y84C. SCE variants were overexpressed on K562 cells. Primary NK cells were used to kill K562 cells with or without SCE overexpression.NK cell activation status and lysis of target cells were measured.



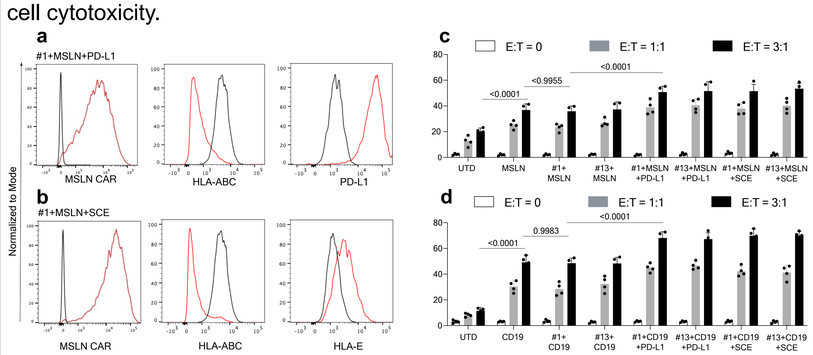
Combination of HLA-ABC knockdown and PD-L1/SCE overexpression inhibits allorejection of NK cells in vivo

HLA-ABC reduced and PD-L1/SCE overexpressed NK cells and allogeneic PBMCs were infused into NSG MHC I/II DKO mice. The percentages of NK cell subpopulations in blood and tissues were monitored.



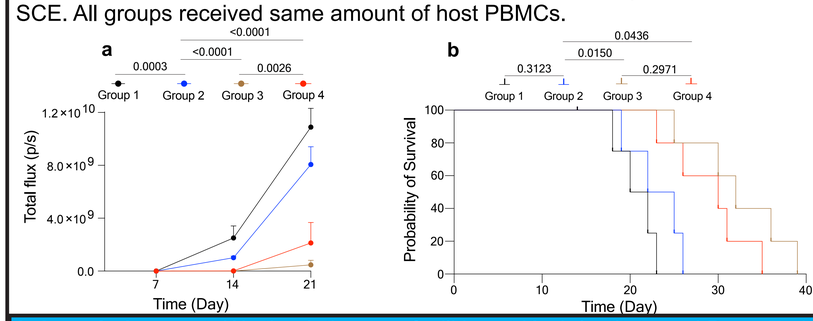
PD-L1 and SCE enhance CAR NK cell cytotoxicity

Combination of HLA-ABC knockdown, CD19 CAR/mesothelin CAR, and PD-L1/SCE overexpression can be achieved by one single transduction of NK cells. Unexpectedly, we found that PD-L1 and SCE overexpression enhanced CAR NK cell cytotoxicity.



HLA-ABC reduced, PD-L1/SCE overexpressed CD19 CAR NK cells escape allorejection and arrest CD19⁺ tumor growth in vivo

Allogeneic CD19 CAR NK cells and host PBMCs were adoptively transferred into NSG MHC I/II DKO mice two days after inoculation of Raji-luciferace cells. Tumor burden and survival of mice were monitored. Group 1: Allo UTD NK; Group 2: Allo CD19 CAR NK; Group 3: Allo #1+CD19CAR+PD-L1 NK; Group 4: #1+CD19CAR+SCE. All groups received same amount of host PBMCs.



Acknowledgement: We thank the core facilities at Koch Institute and Dana-Farber Cancer Institute.