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Transient epigenetic reprogramming enhances T-cell proliferation and tumor clearance

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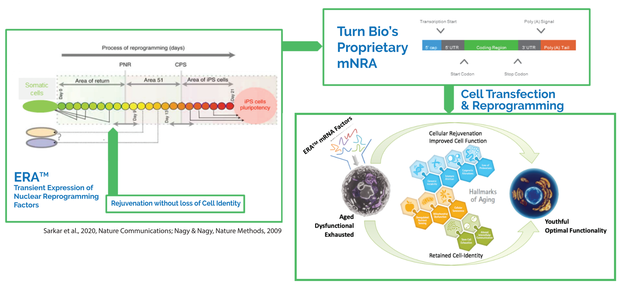
Introduction

Turn Biotechnologies is advancing its Epigenetic Reprogramming of Aging (ERA™) mRNA Technology as a novel approach to rejuvenate T-cells. ERA™ modulates a cell's epigenome, pushing the cell towards a more functional phenotype without interfering with cellular identity. T-cell aging, dysfunction, and exhaustion are epigenome driven changes in gene expression that are reversible events [1-4]. By resetting the epigenomic landscape to a more regulated and functional state, ERA™ Technology offers the tremendous opportunity to CAR-T cell manufacturing by its ability to induce stem memory T cell (Tscm)-like phenotype while mitigating T cell dysfunction and exhaustion. These ERA-induced effects enhance CAR-T cell proliferation and tumor cell killing. Importantly, ERA-treated CAR-T cells with superior efficacy and proliferative capacity allow for effective dosing with less cells, enabling storage of remaining cells from a single manufacturing process for treatment of relapse disease. These ERA™-mediated improvements represent a significant advancement in CAR-T cell therapy by enhancing clinical efficacy and safety, ultimately facilitating reduced costs and superior accessibility to CAR-T cell therapies for cancer patients.



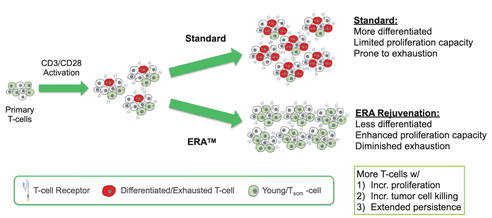
Sarkar et al., 2020, Nature Communications; Nagy & Nagy, Nature Methods, 2009

ERA™ is a cellular rejuvenation technology mediated by transient expression of epigenetic reprogramming factors. The biological activity of ERA™ factors promotes a rapid and multifaceted amelioration of cellular aging through the resetting of the epigenetic clock to a more youthful and functional state.

Results

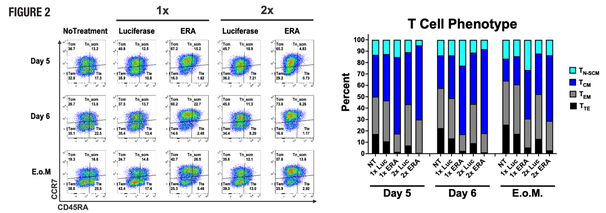
ERA™ incorporation into T cell manufacturing enhances Tscm and Tcm populations

FIGURE 1



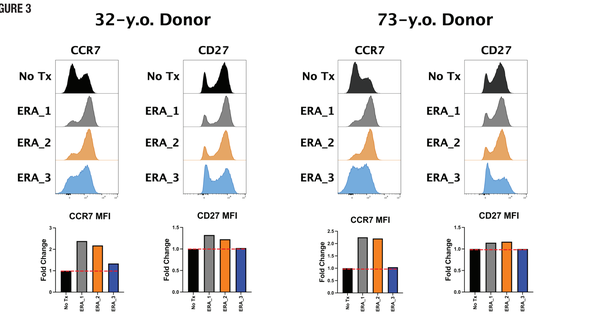
ERA™ Incorporation into T cell Manufacturing. ERA™ mRNA cocktail is transfected by electroporation into CD3/CD28-activated T cells. ERA™ treatment is transient expression of reprogramming factors and will reduce T cell dysfunction and exhaustion while preserving and enhancing the young / Tscm T cell population, yielding a superior drug product.

FIGURE 2



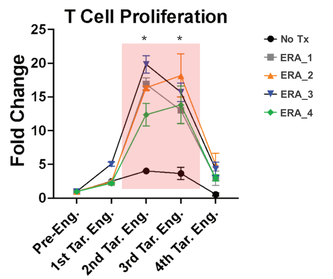
ERA™ treatment enhances Tscm and Tcm populations during T cell Manufacturing. Increased CCR7 expression indicates ERA™ treatment enhances central memory (Tcm) and stem memory T cells (Tscm) -like phenotype. Increased % Tcm and Tscm population will lead to higher proliferation and longer persistence in patients. 1x ERA and 2x ERA indicated different doses of ERA™ treatment. 1x Luc (Luciferase) and 2x Luc are the corresponding control samples with similar mock mRNA doses. Day indicates after the first ERA™ treatment. EoM: End of Manufacturing. TN-SCM (T Naïve-Stem Cell Memory), TCM (T central memory), TEM (T Effector memory), TE (T Effector).

FIGURE 3



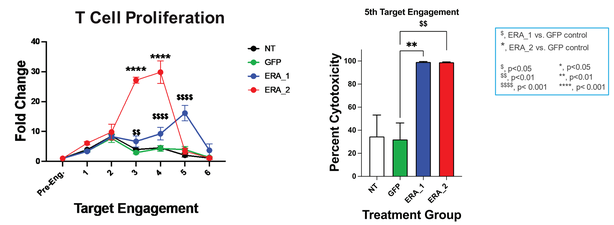
ERA™ treatment enhances expression of CCR7 and CD27 in T-cell regardless of donor age. Both 32-year-old donor and 73-year-old donor showed increased CCR7 and CD27 expression after ERA™ treatment. ERA_1, ERA_2, and ERA_3 are different doses and versions of ERA™ mRNA cocktail.

FIGURE 6



ERA™ treatment enhances T-cell proliferation during target cell killing (Donor: 63-year-old). ERA™ treated T-cells can proliferate 3-5 times more compared to No-treatment T-cells. ERA_1, ERA_2, ERA_3, ERA_4, and ERA_5 are different doses and versions of ERA™ mRNA cocktail. ERA vs. NT * p<0.01

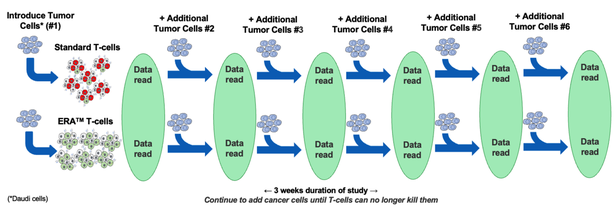
FIGURE 7



ERA™ treatment enhances T-cell proliferation and cytotoxicity in younger (32-year-old) donor. ERA™ treated T-cells from 32-year-old donor can proliferate 3-4 times more compared to No-treatment T-cells. ERA_1 and ERA_2 are different doses and versions of ERA™ mRNA cocktail.

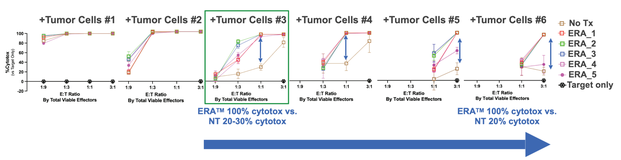
ERA™ increases T cell proliferation and cytotoxic activity while mitigating exhaustion

FIGURE 4



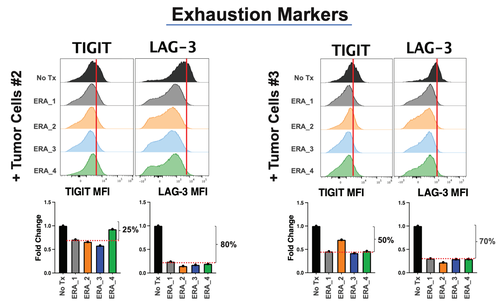
Schematics of a long-term killing assay. Daudi target cells are engaged with T-cells in the presence of CD19-CD3 bispecific antibody. Different ratios (1:9, 1:3, 1:1, and 3:1) of Effector to Target (E:T) employed during the experiment. Effector and target cells are cocultured for 3 or 4 days per engagement.

FIGURE 5



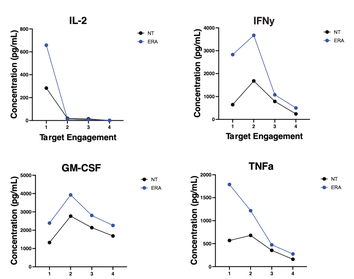
ERA™ treatment enhances T-cell mediated cytotoxicity from a 63-year-old donor. Different Effector to Target ratios (1:9, 1:3, 1:1, and 3:1) were used during the experiment. Effector and target cells are cocultured for 3 or 4 days per engagement. Starting at the 3rd addition of tumor cells, ERA-treated T-cells (63-year-old donor) compared to non-treated T-cells (same donor) have increased efficiency in killing tumor cells and cytotoxicity remains high after several additions of tumor cells indicating extended capacity to kill tumor cells. ERA_1, ERA_2, ERA_3, ERA_4, and ERA_5 are different doses and versions of ERA™ mRNA cocktail. ERA vs. NT $ p<0.05

FIGURE 8



ERA™ treatment protects T-cell from exhaustion and reduces T-cell exhaustion marker expression. ERA™ treated T-cells have reduced expression of TIGIT and LAG-3 compared to No-treatment T-cells at the end of 2nd and 3rd tumor cells engagement. ERA_1, ERA_2, ERA_3, and ERA_4 are different doses and versions of ERA™ mRNA cocktail.

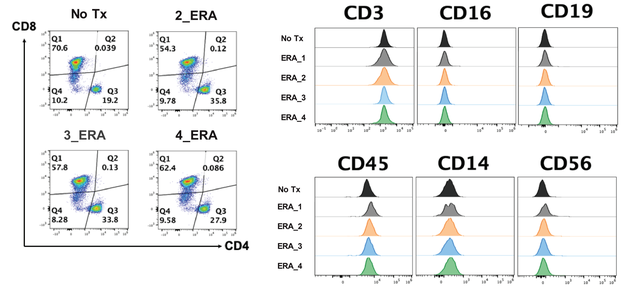
FIGURE 9



ERA™ treatment induces T-cell to produce more IL-2, GM-CSF, IFN-γ, and TNF-α. Increased IL-2 and GM-CSF production enhances T-cell proliferation and more IFN-γ and TNF-α production improves T-cell mediated cytotoxicity.

ERA™ safely maintains cell identity

FIGURE 10



ERA™ treatment is safely maintains T-cell identity. Different doses and versions of ERA™ mRNA cocktails does not influence a T-cell marker (CD3) or change the expression of other immune cell markers (CD16, CD19, CD14, and CD56), indicating a high safety profile. ERA_1, ERA_2, ERA_3, and ERA_4 are different doses and versions of ERA™ mRNA cocktail.

Conclusions

Incorporating ERA™ mRNA Technology into T cell manufacturing (compared to control cells):

- Enhances Tscm and Tcm populations.
- Increases expression of CCR7 and CD27 in T cells, regardless of donor age.
- Increases tumor cell killing of T-cells from both young and old donors.
- Increases T cell proliferation (3 - 5 times more) from both young and old donors.
- Both protects against and reduces exhaustion.
- Induces increased production/secretion of IL-2, GM-CSF, IFN-γ, and TNF-α.
- Maintains T-cell identity, indicating a high safety profile.

These ERA™-induced effects can potentially translate into the clinic as greater efficacy and extended persistence to prevent and treat disease relapse.

Discussion

The benefits of ERA™ incorporation into CAR-T cell manufacturing has the potential to:

- Significantly improve both clinical efficacy and safety while expanding patient access.
- By reducing the number of cells needed per therapeutic dose, ERA™ is also expected to provide relapse patients the opportunity to receive an additional dose without the cost associated with a full CAR-T cell manufacturing process.
- We anticipate that ERA™ technology can be applicable to a wide range of CAR-T cell therapies targeting different tumor antigens and different types of cancers, including solid tumors.
- Notably, ERA™ technology could be introduced into current manufacturing workflows without disruption of established standard operating procedures.

Ultimately, application of the ERA™ technology to CAR-T cell products is expected to result in a superior immunotherapy in terms of both efficacy and durable response as well as answering the unmet need for relapse patients without additional manufacturing.

REFERENCES: 1) Goronzy, J.J., et al. Journal of leukocyte biology 104 (4): 691–699 (2018); 2) Sen D.R. et al. Science. 2016; 354:1165–1169.; 3) ZZeng Z. et al. Cancer Biol. Med. 17, 923–936 (2020) ; 4) Yang, R. et al. Genome Biol 21, 2 (2020); 5) Sarkar, T.J. et al. Nat. Comm. 11,1545 (2020); 6) Nagy, A. and Nagy, K. Nature Methods. 7, 1 (2009)