Development of *In vitro* potency assay to assess the effect of T-Cells to kill cancer cells

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Background:

With the rising numbers of phase I/II clinical trials using CAR-T cell therapies, there is an urgent need to reach a global consensus on the definition of "potency." The emphasis is to manufacture functional therapeutics eliciting a consistent clinical response. We have developed a novel potency assay to assess the function of cellular therapeutics to kill cancer cells. This *in vitro* assay quantifies the potency of autologous and allogeneic products by overcoming the current drawbacks associated with the bioluminescence, radiolabeled, and enzymatic assays. This assay could also be modeled to screen the potency of several allogeneic products engineered to kill cancer cells isolated from a patient**.**

Methods, Results and Conclusions:

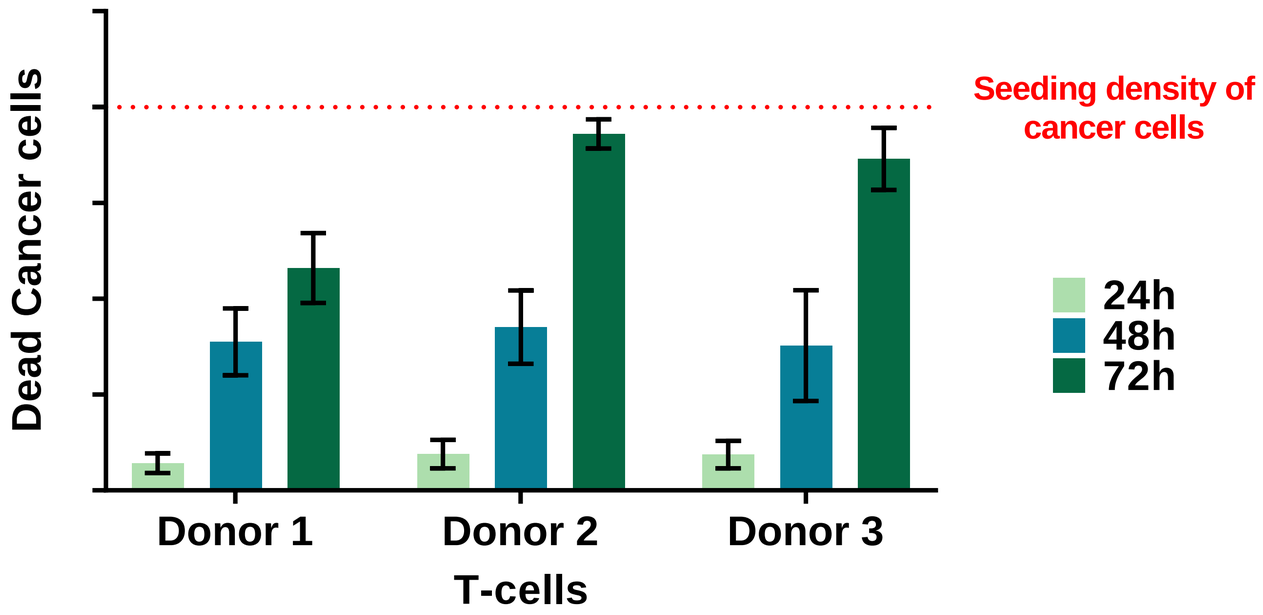
Study design/ Methods:

T-cells were isolated from three buffy coats derived from healthy donors, co-cultured with cancer cells, and the effect of allogeneic T-cells killing the cancer cells was evaluated. RA-1 (Ramos) cells, B-lymphocytes isolated from Burkitt's Lymphoma patient, were used as a model cancer cell line. The potency of T-cells was measured at different time points (24,48 and 72 hours), and the increase in the number of dead cancer cells was quantified. Flow cytometry was used to identify subsets of T-cells such as cytotoxic T-cells (Tc) and helper T-cells (Th) involved in killing the RA-1 cells. RA-1 cells treated with doxorubicin was used as a control. The release of TNF-α and IFN-ɤ by T-cells was also quantified as an adjunct measure of the T-cell killing assay.

Results:

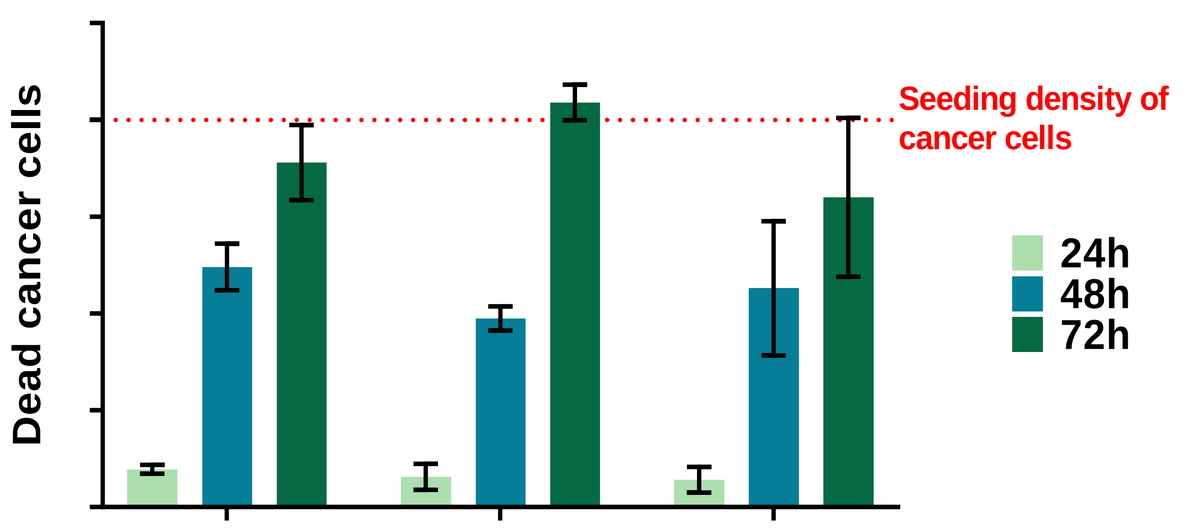
Static assay: T-cells killing cancer cells (RA.1)

Ratio of effector to target cells - 5:1

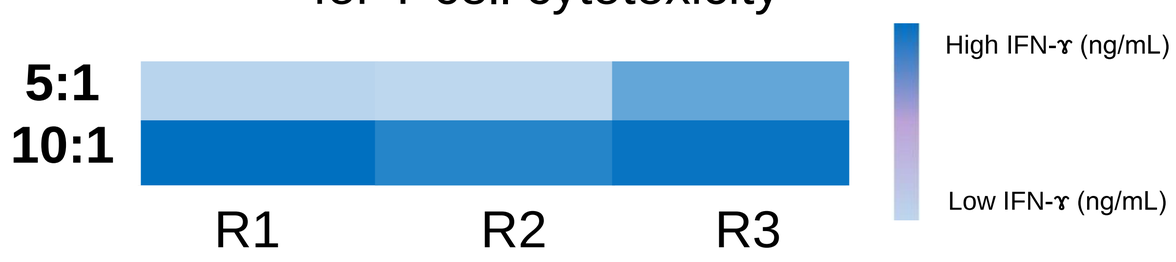


High rate of killing observed at 72 hours

Ratio of effector to target cells - 10:1

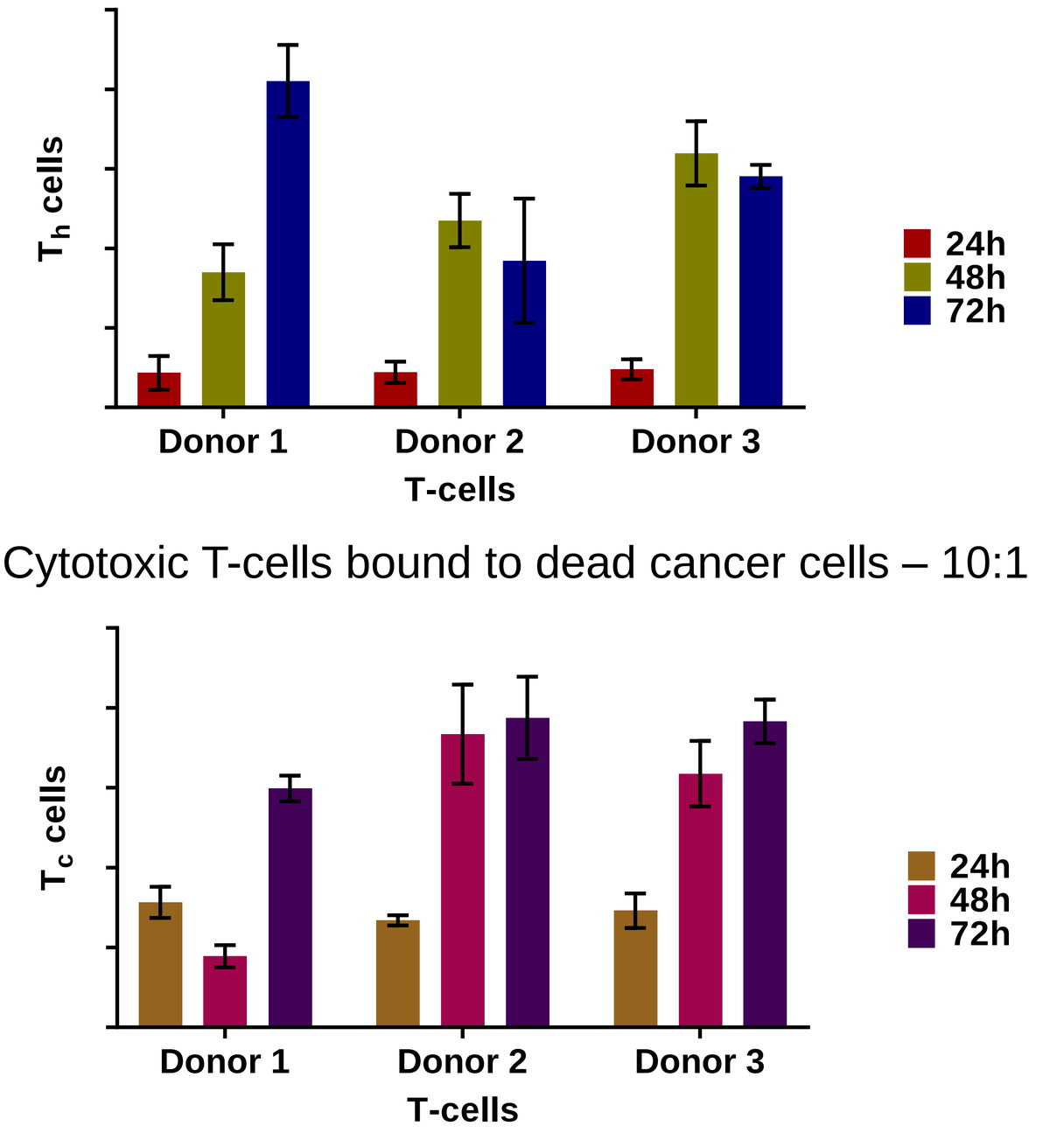


The release of IFN-ɤ by T-cells as an adjunct measure for T-cell cytotoxicity



Inconsistent expression of IFN-ɤ

Helper T-cells bound to dead cancer cells – 10:1



Cytotoxic T-cells bound to dead cancer cells – 10:1

Conclusions:

The function of T-cells to kill cancer cells was quantified using our potency assay. This assay can be modified to simulate other tumor models, such as lung cancer, ovarian cancer, etc. This potency assay can also be modified to test the function of acellular therapeutics like extracellular vesicles and exosomes.