Snapshot of the interaction between T cells and cancer cells for the identification of novel immune checkpoints

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Abstract

T cells kill infected cells by recognizing foreign proteins such as viral peptides presented on the surface of infected cells. However, T cells cannot kill highly mutated cancers. Cancers inhibit T cell function through the expression of surface regulatory proteins such as PD-L1. The blockade of these regulatory proteins led to unprecedented clinical benefits for patients who responded. Current methods cannot comprehensively characterize the regulatory proteins present at the junction of cancer cells and T cells (the immunological synapse). With advances in proximity labeling enzymes, we can fuse these enzymes to the immunological synapse and take a snapshot of all the nearby regulatory proteins. We hypothesize that the immunological synapse of cancer cells encompasses uncharacterized immunoregulatory proteins that can be targeted for therapy. To test this, we have cloned, introduced, and validated known cancer antigens (MART-1 and NY-ESO-1) in cancer cell lines. We will compare the immunological synapse formed between these cancer cells and Jurkat T cells.

T-cell Inhibitors Cause T-cell Dysfunction

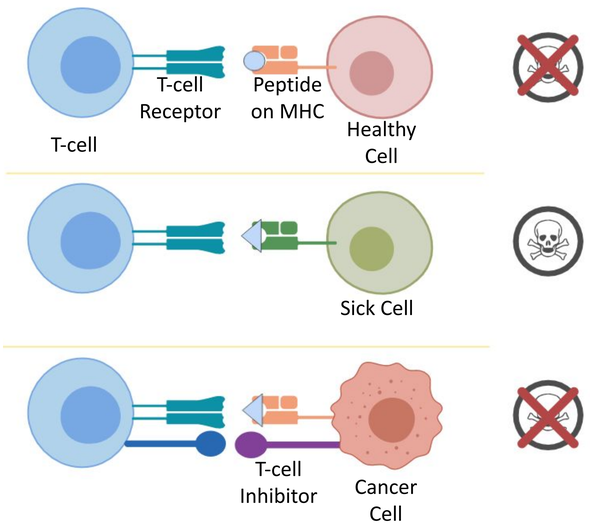


Figure 1: T-cells recognize foreign peptides on surfaces of cells. As a result they kill infected cells while leaving the healthy ones alone. Cancer cells present immune checkpoints which inhibits T-cells from killing[1].

Methods: Gibson Assembly

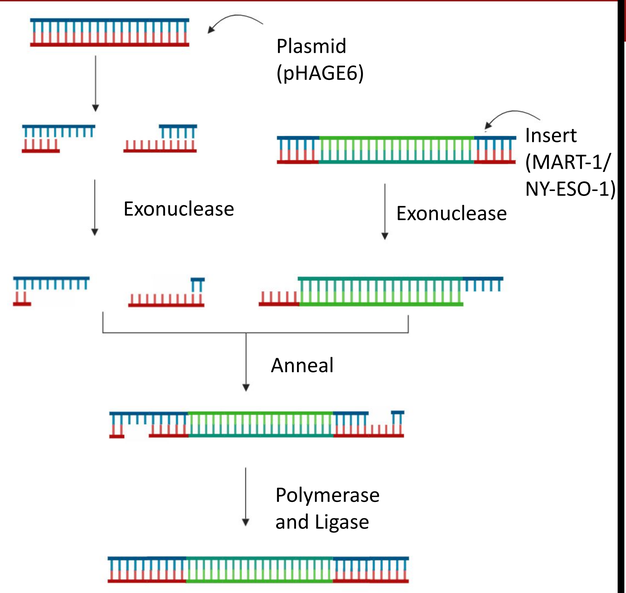


Figure 2: Gibson Assembly was used to combine our gene of interest (MART-1 and NY-ESO-1) with a cut plasmid (pHAGE-6)[2].

Transfection of Cancer Cells with MART-1 and NY-ESO-1

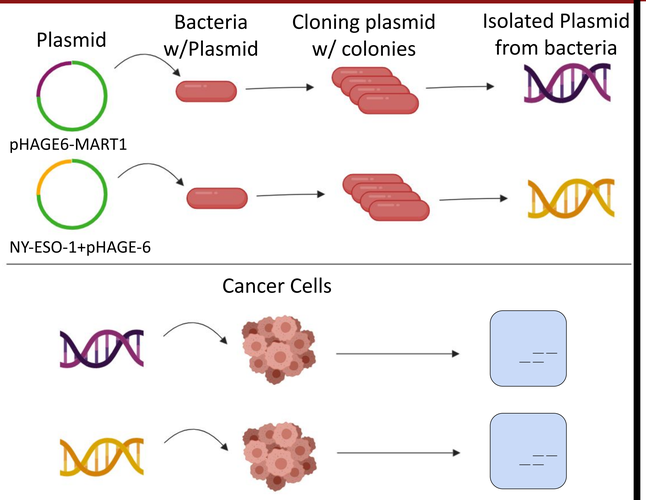
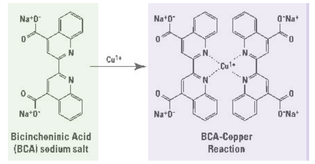


Figure 3: pHAGE6 plasmids containing MART-1 and NY-ESO-1 plasmids were amplified in bacteria. The isolated plasmid was used for transfection. Protein expression was confirmed using a Western Blot.

Protein Isolation from Transfected Cells



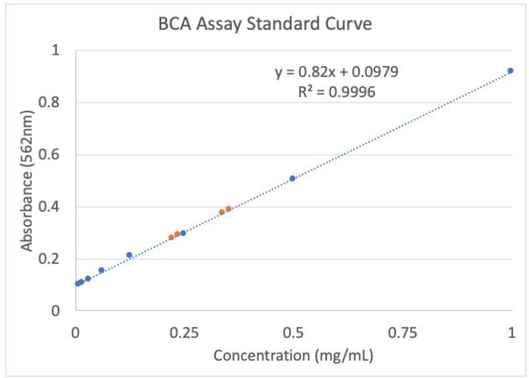


Figure 4: Cells were lysed in urea lysis buffer and concentration was quantified by BCA Assay. Orange dots are the collected protein samples and the blue dots are the standards with BSA.

Validation of MART-1 and NY-ESO-1 Expression in Transfected Cells

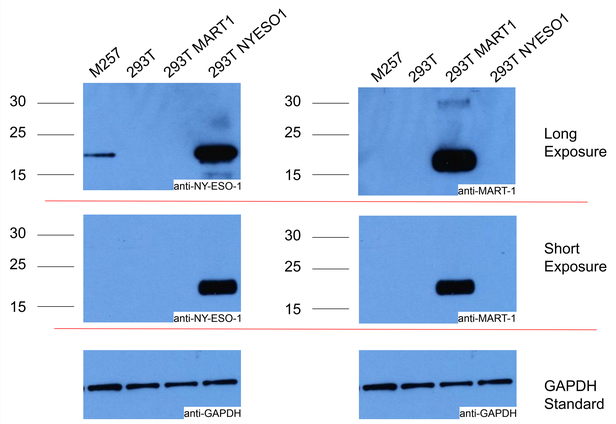


Figure 5: Our Western Blot shows that the MART-1 and NY-ESO-1 is expressed in the corresponding transfected 293T cell sample. Our GAPDH standard also shows that we loaded equal amounts of protein.

Conclusion & Future Direction

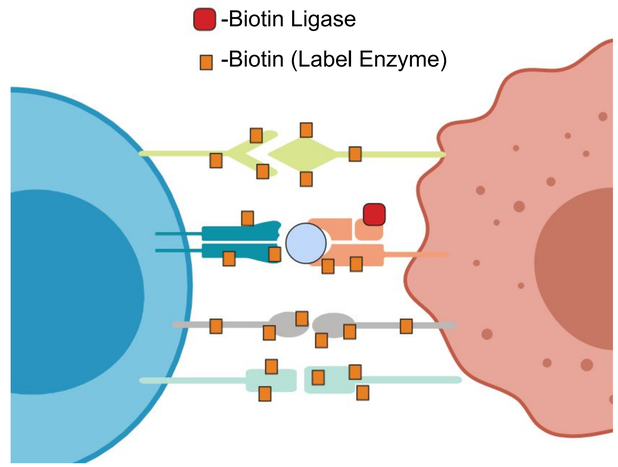


Figure 6: Functional validation will be done with co-culture with Jurkat T cells[3]. Biotin Ligase would label protein interactions between our cancer cells and T cells. This would reveal previously unseen reactions that could be tested for cancer treatment.

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References

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2. D. G. Gibson et al., Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat Methods 6, 343-345 (2009).
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4. T. C. Branon et al., Efficient proximity labeling in living cells and organisms with TurboID. Nat Biotechnol 36, 880-887 (2018).