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Establishment of In vitro Human T cell Exhaustion Model to Enable High Throughput

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Background

Immune surveillance was defined as three phases during tumorigenesis: elimination, equilibrium and escape. During the escape phase, cancer cell acquire a series of mechanism to evade the recognition by immune system, including reduce the function of antigen presenting cells, recruitment of suppressive cells, induce T cell exhaustion, etc. The exhaustion phenotype of T cells were frequently observed in various cancer types and exhibit as reduced effector cytokine production, decrease in proliferation and cytolytic activity and overexpression of inhibitory receptors. Restoring T cell exhaustion phenotype become the inspiring filed for cancer therapy and PD1 blocking antibody further encouraging the concept of intervention on T cell exhausted phenotype in tumor microenvironment.

In this study, we utilize human primary T cell to establish the in vitro model of T cell exhaustion. Subsequent functional study reveal that the induced exhausted T cell (Tex) display significantly weak response towards dendritic cell activation compare to normal T cell (Tnorm). We take advantage of the model to validate the function of PD1 antibody and demonstrate that blocking of PD1 could partially restore the function of Tex, which suggesting there are still other regulators involved in mediating T cell exhaustion beyond PD1.

Methods

Induction of T cell Exhaustion

Total T/CD4+ T/ CD8+ T cell was isolated from PBMC using negative selection kit and stimulated with T-Activator CD3/CD28 Dynabeads at the ratio of 1:1.

Cells were counted (supernatant collected), washed to remove original beads, and re-stimulated with a fresh batch of Dynabeads every 2 days.

T cell was analyzed of PD1, LAG3, TIM3, and Ki67.

Supernatant was analyzed for IFNγ and IL-2.

T cell/DC cell MLR:

Tfresh and Tex were labeled with CellTrace™ Far red and co-culture with allogeneic DC cells in the Ratio of 10:1 (T: DC) for 4 days.

T cell was analyzed by FACS and supernatant was analyzed for IFNγ and IL-2 by ELISA.

Results

Repeated Dynabeads stimulation of T cell results in exhausted phenotype indicated by increase of inhibitory immune checkpoints and also decrease of cytokine secretion.

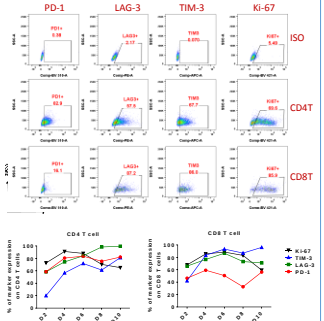

Figure 1 In vitro-generated Tex highly expressed multiple inhibitory checkpoint markers including PD-1, LAG-3, and TIM-3.

Supernatant of T cell with repeated stimulation with Dynabeads showed upregulation of IL-2 and IFNγ on day2 and reduction of IL-2 and IFNr start from day4.

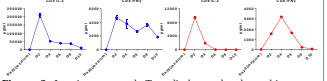

Figure 2 In vitro-generated Tex display reduced cytokine secretion.

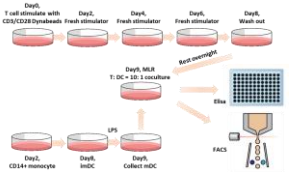

Figure 3 Experimental scheme of Tex generation and followed up MLR.

Tex display reduced response towards allogeneic DC compared to Tfresh in the MLR assay.

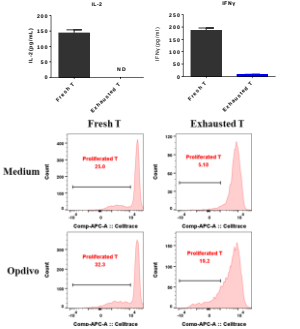

Figure 4 Tex display reduced response in MLR assay by reduced of proliferation and also IL-2 and IFNγ secretion.

MLR assay of Tex and Tfresh in the presence or absence of anti-PD1 antibody or anti-TIGIT antibody suggest that anti-PD1 could partially restore the exhaustion phenotype of T cell.

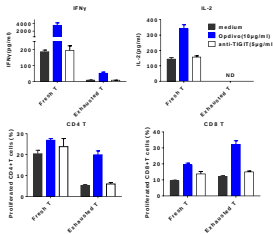

Figure 5 Effect of Opdivo and anti-TIGIT antibody in MLR assay. Opdivo could partially reverse T cell exhaustion phenotype by increase of IFNγ and also promote T cell proliferation.

Summary

- T cell exhaustion phenotype could be achieved by repeated stimulation by Dynabeads for 10 days indicated by increased expression of inhibitory immune checkpoints and decrease of functional cytokine secretion.
- Opdivo can partially restore T cell exhaustion phenotype by promoting cytokine production and also increase of T cell proliferation in MLR assay while anti-TIGIT treatment show no significant effect in MLR assay.

Reference

- Dunsford, et al. Immuno-Oncology. Humana, New York, NY, 2020. 89-101.
- Wu, Lei, et al. "Blockade of TIGIT/CD155 signaling reverses T-cell exhaustion and enhances antitumor capability in head and neck squamous cell carcinoma." Cancer immunology research (2019).