Antibody Analytics

An *in vitro* T cell exhaustion model for characterization of multi-specific biologics

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Introduction

Most studies investigating T cell exhaustion employ mouse models of chronic viral infections (e.g. LCMV), which are difficult or expensive to perform and, on most occasions, do not allow the assessment of human-targeting biologics.

Traditional T cell activation assays employing polyclonal stimuli (e.g. SEB-activation), do not generate exhausted T cells and are not suitable to assess whether a compound has an impact on T cell exhaustion.

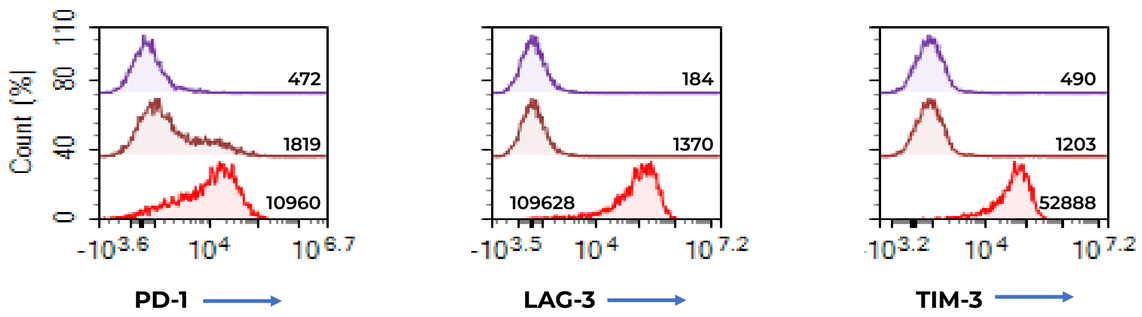
There is an unmet need for a convenient biologically relevant *in vitro* model of human T cell exhaustion. Antibody Analytics have developed an *in vitro* model of chronic antigen stimulation that results in T cells with phenotypical and functional characteristics of exhaustion.

This model can be employed for the screening of immuno-modulatory molecules, bispecific T cell engagers and multi-functional molecules.

Generation of Exhausted T cells

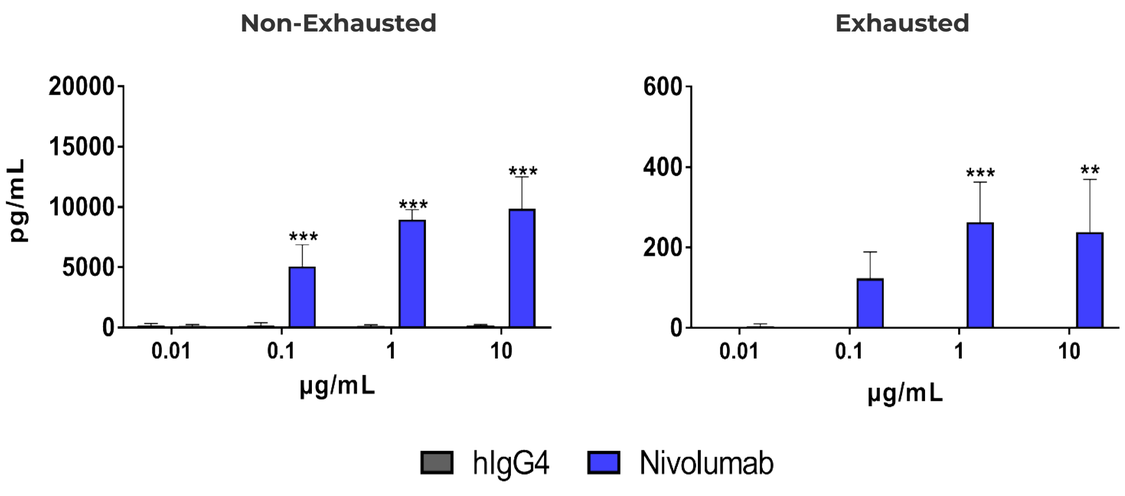
Isolated T cells were stimulated several times with antibodies targeting the TCR and costimulatory molecules. The generated phenotype of the exhausted T cells was assessed by flow cytometry and secondary functional assays

In vitro Tex cells express high levels of coinhibitory receptors



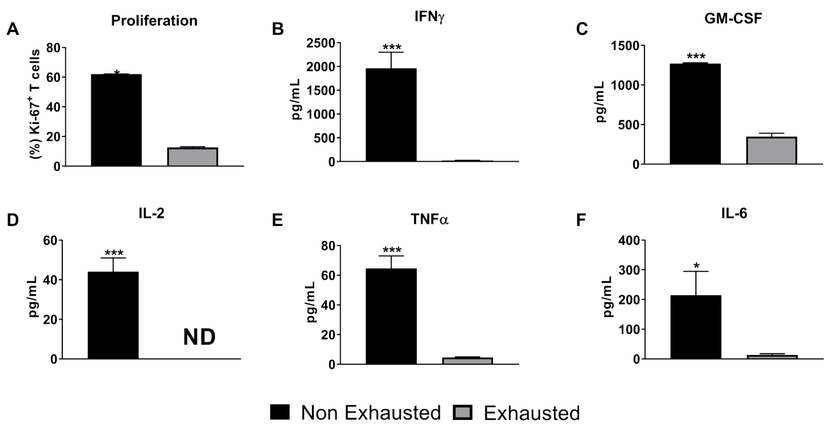
CD3+ T cell were either stimulated once or multiple times through their TCR and costimulatory molecules and the expression of several co-inhibitory and costimulatory molecules was assessed by flow cytometry. Similar resulted were observed in CD4+ and CD8+ isolated T cells and in multiple donors.

PD-1 blockade partially restores Tex cell functionality

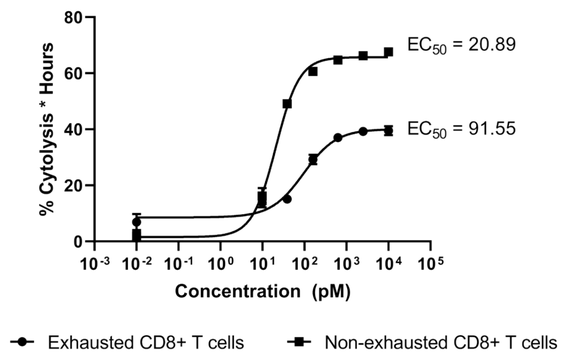


Freshly isolated (non-exhausted) or exhausted T cells were used in a mixed lymphocyte reaction (MLR) in the presence of various concentrations of the PD-1 targeting antibody Nivolumab. IFNγ production was measured after 5 days by ELISA.

In vitro generated Tex cells are functionally impaired

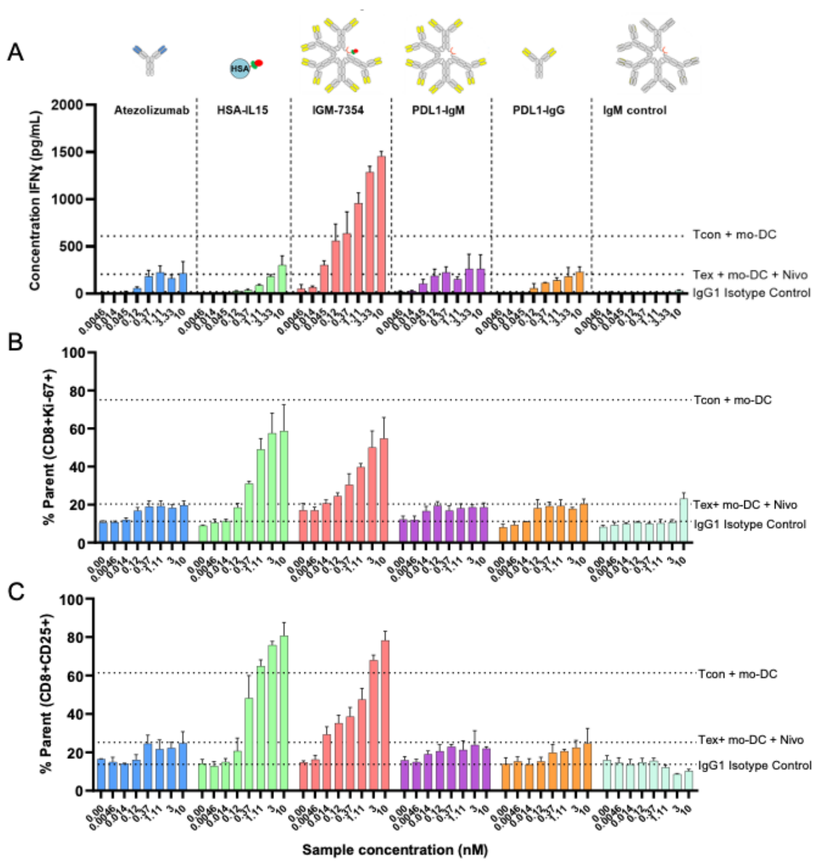


In vitro generated Tex cells have diminished ability to produce IFNγ relative to non-exhausted (freshly isolated) T cells in a secondary T cell re-stimulation assay.



In vitro generated CD8+ Tex cells have diminished ability to induce target cell cytolysis mediated by a bispecific a T cell engager.

A bifunctional antibody that targets PD-L1 and activates the IL-15 signaling pathway potently reverses T cell exhaustion



Treatment with IGM-7354 promoted increased secretion of IFNγ (A), proliferation (B) and T cell activation (C) by *in vitro* generated Tex cells in a MLR assay

Summary

- Robust generation of human in vitro Tex cells
- *In vitro* generated Tex express high levels of co-inhibitory molecules
- *In vitro* generated Tex cells are functionally impaired and have reduced ability to mediated effector functions
- PD-1 blockade partially restores functionality of Tex cells
- IGM-7354, an IgM-based bifunctional biologic, reverses T cell exhaustion more potently than anti-PD-L1 antibodies or a non-targeted IL-15 fusion molecule alone