Using an *In vitro* exhausted T cell assay to screen immuno-oncology candidates

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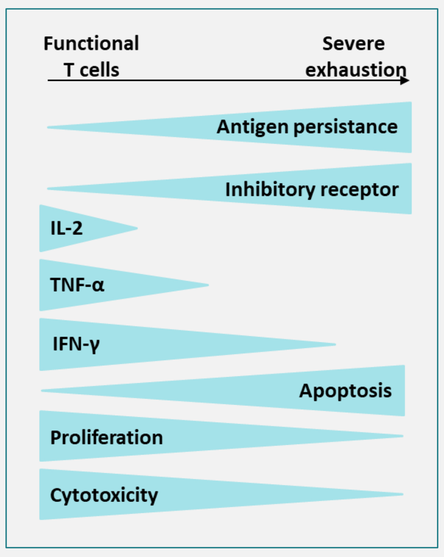
Introduction

During the last years, significant advancement has been made in the clinical application of cancer immunotherapies. Molecules directed against immune checkpoints and other agonists show great promise for the treatment of a variety of malignancies. Next to the lymphocyte checkpoint modulators, a wide range of therapeutics with the potential to reverse the tumor-induced suppression are under development. This increasing interest in the tumor microenvironment drives the focus on new bioassays to represent all the players of the cancer immune response. Early functionality screening of antibody-based checkpoint modulators can be performed by *in vitro* assays using primary immune cells. T cell exhaustion can be used to evaluate the potential of new therapeutic candidates to reverse the exhaustion of the T cells and facilitate the anti-tumour immune response during early development stages. However, a significant proportion of these biotherapeutics induce an unwanted immune response which can influence pharmacokinetics, efficacy and safety. Early *in silico* and *in vitro* risk assessment of unwanted immunogenicity can reduce the attrition rate.

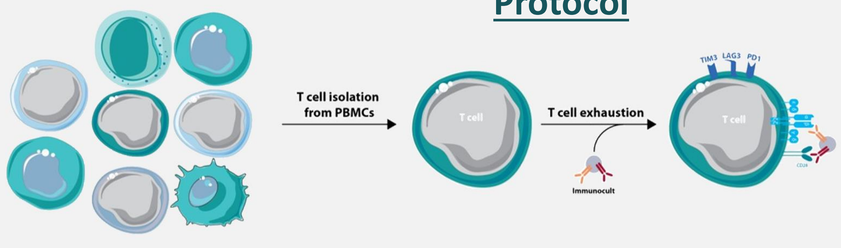
T cell exhaustion assay



The antigen persistence in the microenvironment in situations such as chronic viral infections (e.g., LCMV model) or in the tumor microenvironment induces the chronic stimulation of T cells. This persistent stimulation leads to the exhaustion of T cells, characterized by a decrease in their activation.

Here, we developed an *in vitro* T cell exhaustion assay to evaluate the capacity of new immuno-oncology candidates to reverse the exhaustion.
In this model, T cells are stimulated several times with a CD3/CD28 tetramer and then tested in a co-culture in the presence of test products.

Protocol

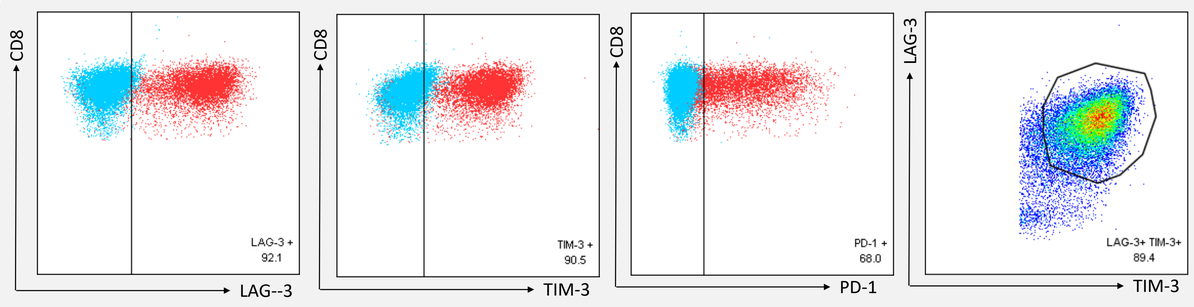


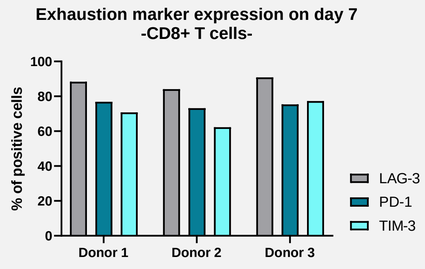
- ✓ **Pan-T, CD4⁺ or CD8⁺ T cells**
- ✓ **Multiple rounds of stimulation**
- ✓ **CD3/CD28**

1. Kahan *et al.* 2019. Viruses. 2019 Jan;11(2) https://doi.org/10.3390/v11020156

T cell exhaustion phenotype

Exhaustion marker expression

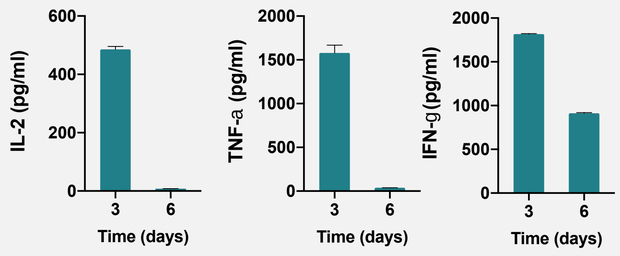


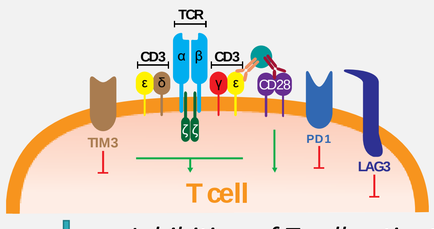


Here, we evaluated the expression of PD-1, TIM-3, LAG-3 and the double positive population LAG-3⁺TIM-3⁺ on exhausted pan-T cells by flow cytometry (surface staining). The expression of surface markers is expected to increase and remain stable in exhausted T cells. These data represent the expression in the CD8⁺ T cell population after 7 days of exhaustion.

The red population corresponds to the positive cells while the blue population corresponds to the FMO control. The expression on day 0 of those markers is limited, except for PD-1 where we can observe up to 10 % of positive cells on freshly isolated T cells. After 24h of stimulation, the expression of PD-1, TIM-3 and LAG-3 increases up to 20-40 %. On exhausted T cells, the expression increases until reaching 70 to 90 % of positive cells. Those exhaustion markers can be co-expressed.

Cytokine secretion



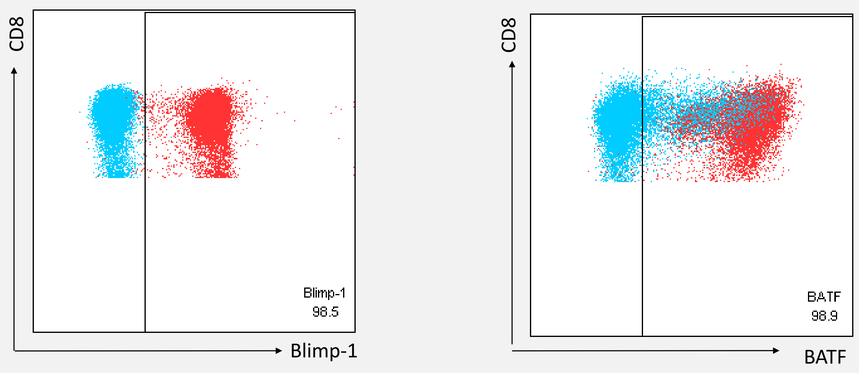


Inhibition of T cell activation and cytokines secretion

The phenotype of exhaustion is described by an increased expression of the exhaustion markers, as shown above, and is confirmed by a loss of effector function represented by a disappearance/decrease of cytokines' secretion, proliferation and cytotoxicity. Here, we evaluated the secretion of IL-2, TNF-α and IFN-γ in a pan-T cell exhaustion assay after one stimulation (day 3) and after two stimulations (day 6). The secretion of effector cytokines is expected to decrease in exhausted T cells. Multiple rounds of stimulation suppress the IL-2 and TNF-α secretion and reduce the IFN-γ secretion.

Exhaustion Transcription factors

Transcription factors expression

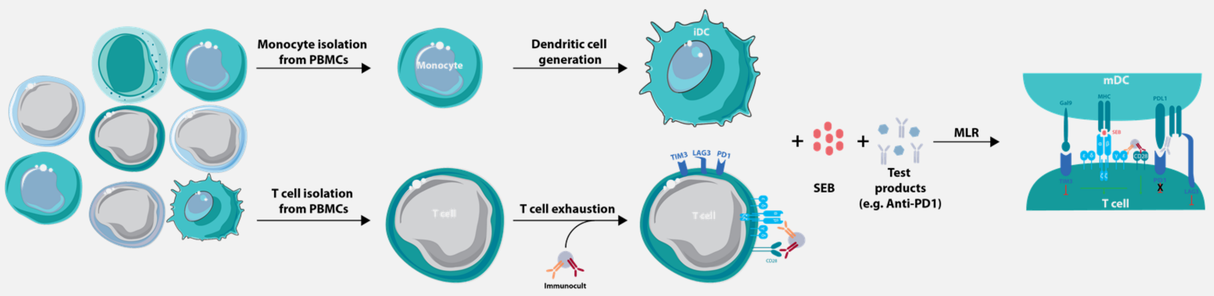


The exhaustion phenotype is driven by the expression of various transcription factors that are implicated in different pathways, for example responsible for directing the expression of the inhibitory receptor PD-1. Here, we evaluated the expression of BATF and Blimp-1 on the exhausted pan-T cells by flow cytometry (intracellular staining). The expression of these transcription factors is expected to increase and remain stable in exhausted T cells. These data represent the expression of Blimp-1 and BATF in the CD8⁺ T cell population after 7 days of exhaustion. The red population corresponds to the positive cells while the blue population corresponds to the FMO control. The expression of those markers at day 0 is limited for Blimp-1 and BATF. After 7 days of *in vitro* exhaustion, the expression of those transcription factors was superior to 80 %.

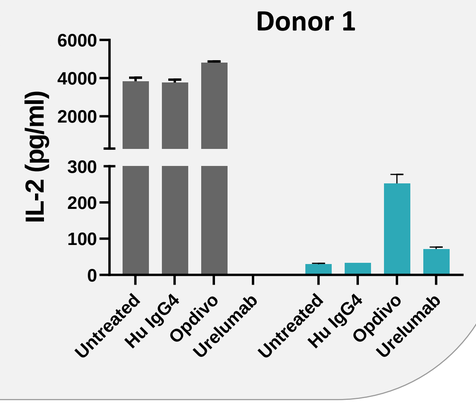
2.Kahn *et al.* 2019. Nature. 2019 Jul;571(7764):211-218. doi: 10.1038/s41586-019-1325-x

Screening immuno-oncology candidates using T cell exhaustion

To screen the efficiency of new therapeutics, a co-culture assay was developed. The exhausted pan-T cells were co-cultured in the presence of autologous immature dendritic cells and SEB superantigen at a suboptimal concentration. The non-exhausted T cells are used as control population. In this case study, two test products were evaluated: a clinical batch of an anti-PD-1, OPDIVO® and the anti-CD137 antibody, Urelumab at 10 µg/ml each.



The IL-2 secretion was measured after 3 days of co-culture. The exhaustion of the T cells resulted in a decrease in IL-2 secretion in the exhausted (blue bar) compared to non-exhausted T cells (grey bar). The treatment with OPDIVO (anti-PD1) was able to reverse partially the loss of T cell function as well as to increase the IL-2 secretion in the non-exhausted T cells. Urelumab (anti-CD137) tends to increase the IL-2 secretion.



Conclusion

These experiments help advance our understanding of the tumour microenvironment and optimize the therapeutic effects of new drugs, design better clinical trials and ultimately discover relevant biomarkers. *In vitro* functional assays, such as T cell exhaustion, is a helpful tool to screen the immuno-modulatory and functional properties of new leads in the early development phase. Additionally, unwanted immunogenicity can be assessed using T cell activation and proliferation assays.