IN VITRO ASSESSMENT OF FUNCTIONAL RESPONSE TO PD1 BLOCKADE

In recent years, a novel type of cancer immunotherapy has been developed using specific monoclonal antibodies targeting immune checkpoint molecules, such as the programmed death-1 (PD1) receptor/PD-ligand 1 (PDL1) interaction. The mechanisms underlying the antitumor activity of immune checkpoint inhibitors are thought to involve a restoration of T-cell activation, which is initially impaired by tumor suppression mechanisms.

Indeed, avoiding recognition by the immune system is one of the tricks used by tumor cells to sustain their uncontrolled proliferation. Among other mechanisms, through the expression of inhibitory ligands such as PDL1 and the engagement of the T-cell expressing PD1 / PDL1 axis, cancer cells impair T-cell activation and become invisible for the host immune system, thereby undergoing immune escape. Modulation of the immune system response to promote effector functions and tackle tumor progression is thus key for the development of innovative therapeutic strategies in Immuno-Oncology.

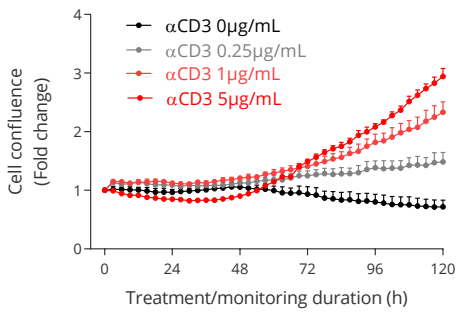
Through a series of characterization experiments, this study sought to establish whether cytokine monitoring, especially IFNγ release, can represent a relevant predictive marker to evaluate PBMC proliferation/activation promotion upon PD1 blockade. As explained here, we demonstrated that the quantification of human IFNγ enables rapid and accurate functional characterization of anti-PD1 antibodies. Associated with a real-time live cell imaging approach, the HTRF-based IFNγ assay commercialized by Cisbio™ empowers the Immuno-Oncology drug discovery programs.

ASSESSMENT OF PBMC PROLIFERATION BY LIVE-CELL IMAGING

Real-time live cell imaging of αCD3-induced PBMC proliferation

PBMCs were stimulated with increasing concentrations of anti-CD3 antibody, for TCR ligation. A real time live cell imaging experiment was used to monitor PBMC confluence overtime (as a surrogate of cell proliferation) and determine PBMC response efficacy to anti-CD3 stimulation.

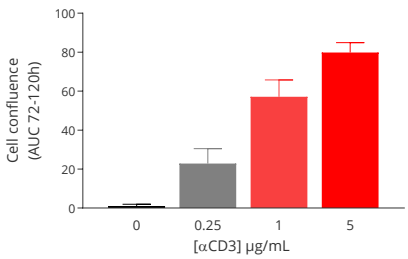
The graph opposite shows a kinetic monitoring of the cell confluence over a 120h period. PBMC proliferation is dose-dependently triggered upon anti-CD3 stimulation and is clearly evidenced after 72h post-treatment.

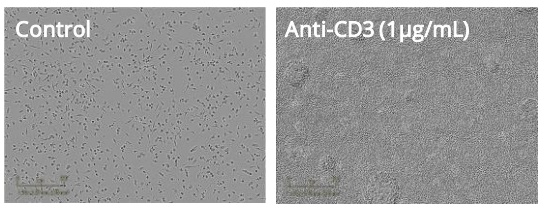


The dose-dependent anti-CD3-induced PBMC proliferation is still highlighted on the figure opposite, which displays the area under curve calculated over the 72-120h time window.

Bottom images are shown to illustrate immune cell proliferation after 120h treatment with 1µg/mL anti-CD3 (right) compared to control (left).

Altogether, real-time live cell imaging allows for evidencing PBMC proliferation induced by anti-CD3 stimulation overtime and in a dose-dependent manner.

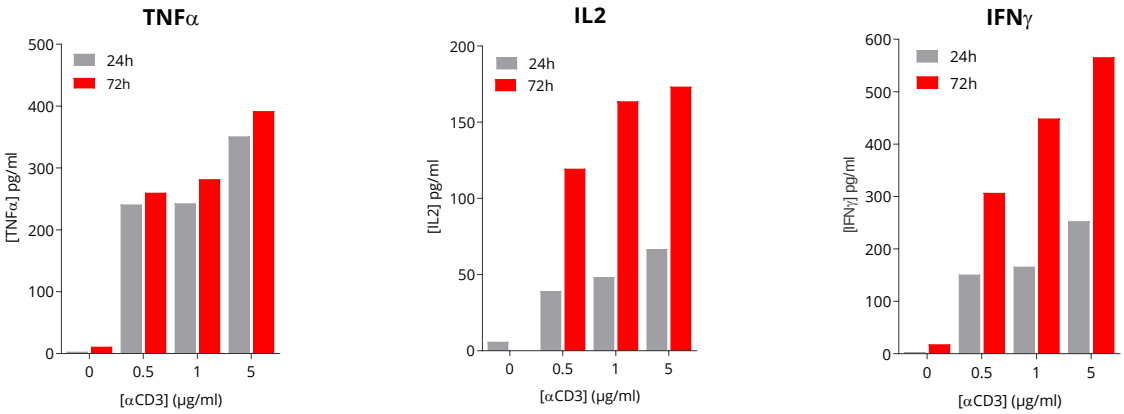




ASSESSMENT OF PBMC PROLIFERATION BY CYTOKINE RELEASE

Time-course quantification of IFNγ, IL2 and TNFα released by αCD3-stimulated PBMC

PBMCs were stimulated with increasing concentrations of anti-CD3 antibody. Cell supernatants were collected at 24h and 72h, and key inflammatory cytokines were quantified by HTRF methodology (Cisbio™ assays).



As shown on the above graphs, while TNFα secretion is efficiently induced at 24h, even at the lowest tested concentration of anti-CD3 and remains almost stable up to 72h, released IFNγ and IL2 levels are dose-dependently increased and continue to accumulate following anti-CD3 treatment as detected at 24h and 72h, respectively.
In parallel to live-cell imaging, the quantification of key cytokines enables to assess earlier biological events that prefigure for PBMC proliferation/activation.
Thus cytokine quantification, especially IL2 and IFNγ are valuable surrogate tools to ascertain the PBMC activation.

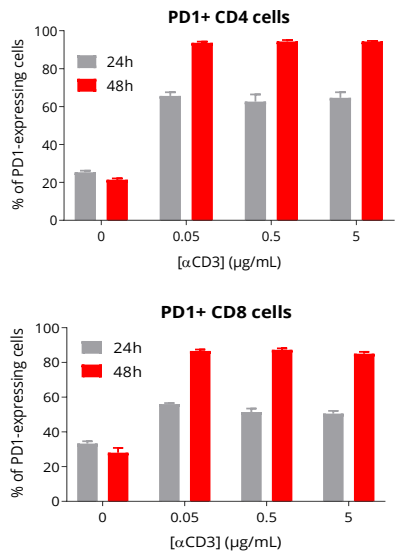
ASSESSMENT OF PD1 EXPRESSION BY FLOW CYTOMETRY

PD1 expression in αCD3-stimulated PBMCs

PD1/PDL1 axis is physiologically involved in maintaining immune homeostasis and protecting against excessive immune response. The expression of PD1 is thus tightly and dynamically regulated.
Here we wanted to assess the expression level of PD1 upon anti-CD3 stimulation.

To this end, healthy donor PBMCs were activated with increasing anti-CD3 concentrations either for 24h (grey bars) or 48h (red bars), and then stained for PD1. The percentage of PD1 expressing CD4+ T and CD8+ T cells was analyzed and determined by flow cytometry.

As shown in the graphs, an increase of PD1-positive CD4 and CD8 lymphocytes proportions was observed after 24h stimulation with 0.05µg/mL anti-CD3. At 48h, more than 80% of activated T cells (both CD4 and CD8) were positive for PD1 expression.



These results suggest that PD1/PDL1 axis takes place rapidly after immune cell activation, thus promoting an efficient counter-regulation mechanism. It also implies that targeting PD1/PDL1 axis could improve the anti-CD3-induced PBMC proliferation.

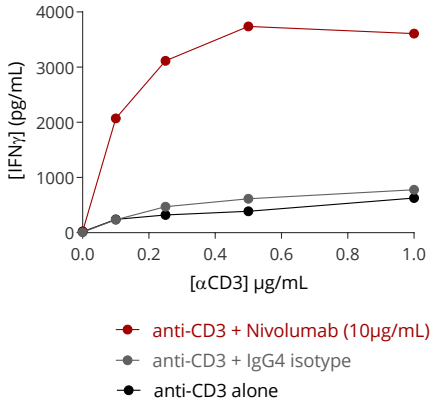
PD1 BLOCKADE ENHANCES αCD3-INDUCED PBMC PROLIFERATION

αCD3-induced PBMC proliferation triggers IFNγ release and is enhanced by PD1 blockade

Since an increase of PD1 expression level was previously demonstrated in activated CD4 and CD8 lymphocytes, we hypothesized that PD1-PDL1 blockade would improve PBMC proliferation induced by anti-CD3 stimulation.

PBMCs were thus treated for 72h with increasing concentrations of anti-CD3 antibody alone (black curve) or with irrelevant antibody (grey curve) or with Nivolumab (red curve). Then IFNγ release was quantified using HTRF methodology (Cisbio™ assays).

The results clearly establish a dose-dependent increase of released IFNγ levels, further exacerbated in the presence of Nivolumab.

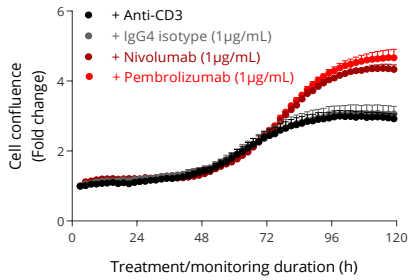


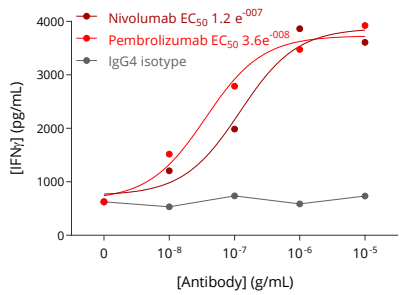
Nivolumab- and Pembrolizumab-mediated PD1 blockade promote αCD3-induced PBMC proliferation & activation

A real time live cell imaging experiment was conducted to monitor PBMC confluence overtime and determine efficacy of combining anti-CD3 and anti-PD1 antibodies, Nivolumab or Pembrolizumab.

The kinetic monitoring of cell confluence confirms that PBMCs rapidly proliferate under anti-CD3 activation. Interestingly, this anti-CD3-induced proliferative effect is specifically enhanced by PD1 blockade.

Furthermore, IFNγ level was also shown to be increased under combination of anti-CD3 and Nivolumab or Pembrolizumab. Indeed, as evidenced in the left graph, treatment of anti-CD3-activated PBMC with increasing concentrations of each of anti-PD1 antibodies for 72h resulted in a significant dose-dependent increase of IFNγ release.





CONCLUSION

On the whole, the data presented here allowed for highlighting the accuracy and reliability of combined *in vitro* approach based on live cell imaging and human IFNγ released level quantification as surrogate predictive index for the robust assessment of functional responses to anti-PD1 antibodies.

Associated with real-time live cell imaging, the global approach also delivers supportive and highly-informative kinetic phenotypic monitoring. It thus constitutes a specialized *in vitro* immune cell activation assay to evaluate, in general, candidate biologics for their immunomodulatory properties of immune cell activation, thereby empowering Immuno-Oncology drug discovery programs.

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