# SARDRICS

# Simplifying Progress

# Evaluation of Checkpoint Inhibitor Therapies using a Mixed Lymphocyte Reaction (MLR) Assay

against

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## Summary & Impact

- Checkpoint inhibitor antibodies target receptors in the synapse between T cells and antigen-presenting cells, such as dendritic cells (DCs), to increase T cell activation and enhance the T cell response against cancer.
- Common side effects of these immunotherapeutics are due to inflammation, in which an over-activation of the immune response can result in T cell attack of healthy body cells.
- A mixed lymphocyte reaction (MLR) assay, which mimics the T cell:DC synapse, can be used to evaluate these drugs *in vitro*.
- Here we present an assay for guantification of T cell response

in MLR in 96- or 384-well plates using the iQue<sup>®</sup> advanced flow cytometry platform.

- Samples of cells and supernatants are analyzed using kits from the iQue<sup>®</sup> portfolio for measurement of T cell phenotypes, proliferation and cytokine release.
- These data exemplify the power of iQue<sup>®</sup> to generate pharmacological data for checkpoint inhibitor effects, with potential to enhance drug discovery or research applications.
- Sartorius outsourcing services can work with you to assess your molecules method of action using our platform MLR assay.

# Pembrolizumab (anti-PD-1) induces activation and inflammation



#### iQue 3<sup>®</sup> System



iQue 3<sup>®</sup> Advanced Flow Cytometer An advanced flow cytometry platform with a patented sampling method allowing for rapid sample acquisition to deliver fast actionable results... Capable of handling 96 and 384-well plates.

## Assay Concept



Sartorius Reagents and Consumables A suite of reagents, kits and protocols for cell health and function screening



iQue Forecyt<sup>®</sup> Software Fully integrated, easy-to-use data analysis software combines plate level analytics with novel data visualization tools. Import pre-set gating templates for instantaneous readouts.



- T cells co-cultured for 6 days with DCs (3:1 ratio) had 3x greater expression of activation marker CD25 (dot plots) compared to T cells alone. Activation was further enhanced by Pembrolizumab (anti-PD-1) in a concentration dependent manner.
- Release of activatory and inflammatory cytokines also increased with Pembrolizumab concentration.
- The temporal release profile differed between cytokines with TNFα, IL-2, IFNy release decreasing from day 2 to 6, whilst inflammatory cytokines, CCL2 and CCL3, saw an increase in production over time.
- EC<sub>50</sub> values were similar across the assay outputs, ranging from 0.3 μg/mL for CD25 expression to 0.5 μg/mL for IL-2 and TNFα release (day 2) to 1.0  $\mu$ g/mL for day 6 CCL2 and CCL3 production.

## Donor pairs differ in sensitivity to activation by anti-CTLA4



#### Abs targeting the PD-1/PD-L1 pathway increase cytokine release



- Analysis of CD4+ T cells co-cultured with DCs using the iQue<sup>®</sup> Human T Cell Activation Kit showed enhancement in activation marker expression and cytokine release compared to T cells alone.
- The addition of checkpoint inhibitor molecules Nivolumab (anti-PD-1), Pembrolizumab (anti-PD-1), Durvalumab (anti-PD-L1) induced a concentration dependent increase in IFNy and TNF $\alpha$ .
- These data were generated by Sartorius outsourcing services using a platform assay as part of the immuno-oncology package.



Scan the QR code to find out more about Sartorius outsourcing services on the iQue<sup>®</sup>.

#### Heterogeneity in donor's HLA haplotype induces T cell activation





#### Combining checkpoint inhibitors induces synergistic effects



- High HLA mismatch T:DC ratio 9:1 3:1 4.5:1T cells Dyna-9.1 4 5.1 only beads CD25
- DCs were co-cultured with CD4+ T cells from two different donors (one with a similar HLA profile to the DCs (low mismatch)) and one with a largely different HLA profile (high mismatch)) at a range of T:DC ratios.
- Cells were analyzed after 5 days using the iQue<sup>®</sup> Human T Cell Activation Kit.
- High HLA mismatch induced greater activation marker expression (CD25), T cell proliferation and cytokine release(not shown) during MLR. This indicated T cell recognition of 'non-self' antigens and induction of the immune response.



#### Dexamethasone (a corticosteroid) impacts cytokine release

- Corticosteroids are often used as a first line treatment for checkpoint inhibitor-induced adverse inflammatory events.
- T cell and DC co-cultures were incubated with Dexamethasone (1 μM) and/or Pembrolizumab (10 μg/mL) and analyzed on day 4 using the iQue<sup>®</sup> Human Inflammation Panel Kit.
- Concentrations of IFNy, CCL3, and IL-6 (not shown) decreased in the presence of Dexamethasone.
- Contrastingly, CXCL10 and CCL2 (not shown) release increased. Further investigation is needed to fully profile the drug's effects.