Development and validation of a medium-throughput phenotypic screening platform for the identification of novel immuno-oncology targets

Léa Legrand, Marie-Claire Letellier, Ariane Scoumanne, João Marchante, Virginie Raballi, Murielle Martin, Stefano Crosignani, Christophe Quêva, Michel Deheux, Sandra Cauwenberghs, Jakub M. Świercz

ABSTRACT

Immunetherapy is a major breakthrough in cancer treatment. Although recent strategies, including immune-checkpoint blockade have demonstrated therapeutic benefit in patients with advanced or recurrent cancers, further improvements are needed to maximize treatment efficacy. In the tumor microenvironment, in addition to tumor-infiltrating lymphocytes, several immune-suppressive mechanisms occur, including immune cell exhaustion, regulatory T cells, myeloid-derived suppressor cells, and tumor-associated macrophages. Understanding the mechanisms of human immunosuppression, particularly in the tumor microenvironment, is essential to identify new therapeutic approaches.

In order to shed light on various immune suppression mechanisms in tumor microenvironment, iTeos Therapeutics has developed a target discovery and drug repurposing platform based on phenotypic screening assays. We established a co-culture assay combining human tumor immune suppressive cells and T cells. This assay is favored to the screening of immunosuppressors, small-molecule or DMSO libraries. Multi-parametric readouts are combined to assess both T cell activation and proliferation, through high-content imaging of T cell coiler formation concomitant with detection of IFNγ secretion, and tumor cell death, assessed using a cyclometry assay. The readout format of the assay allows medium-throughput by keeping up to 3000 wells screened in 24 hours to identify ESCs, potency ranking and target deconvolution.

We report here a proof-of-concept study in which we evaluated the ability to detect metabolic immune-oncology targets at 48h in a panel of cancer immune suppressive cell lines. 4A9 expresses indoleamine-2,3-dioxygenase (IDO1), an enzyme overexpressed in cancer that mediates local tumor suppression through depletion of the essential amino acid tryptophan. The assay conditions were validated with an IDO1 inhibitor as positive control and subsequently assessed for automation. A commercially available small molecule library of 192 compounds, with a high percentage of structurally active drugs was screened. The library was tested at two different concentrations (1µM and 3µM), with two independent T cell donors and spiked with DMSO as inhibitor as control. Combined analysis of T cell activity and tumor killing led to the identification of hit compounds in a study of multiple potential immune suppressive pathways, including metabolism, epigenetics, autophagy, TGFβ, LDH, citrate and THF-KF activity.

READOUT VALIDATION

HIT IDENTIFICATION

Proof of concept confirmation run. Positive compounds resulting from the proof of concept screen were tested for their ability to inhibit Expression of TGFβ ETA/ETB cells. Sample means ± SEM. T-test for different doses. Shown are representative images of both screen and confirmation panel. Shown are representative images of both screen and confirmation panel.

CONCLUSIONS

- We designed an affordable, automated assay system to identify novel immuno-oncology targets.
- The assay has been validated by performing a medium-throughput screen using immunosuppressive 4A9 and CD8+ T cells.
- Our screen successfully detected 4D4 inhibitors present in the Ebiolife Library.
- Additionally, we identified hits representing various signaling pathways for their ability to revert immune suppression.