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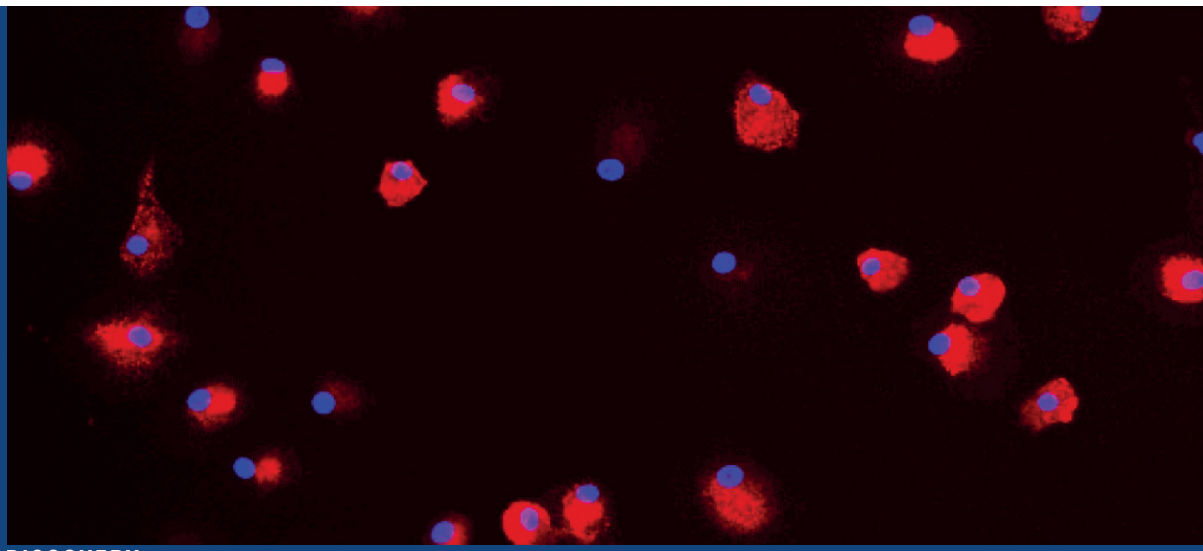
Summary

The multiple role of macrophages in immune responses provides numerous targets for drug therapies. We've developed an optimized, off-the-shelf panel of *in vitro* macrophage assays using human primary blood-derived cells from healthy donors to assess potential candidates to regulate the inflammatory process.



DISCOVERY

Off-the-Shelf Assay:

Complex Biology: *In Vitro* Immunological Assays
M2 Polarization

Click to learn more

M2 Polarization Assay – *Alternatively activated M2 macrophage polarization assay in primary human cells derived from healthy donor blood*

Monocytes and macrophages are critical effectors and regulators of the innate immune response. Through their ability to clear pathogens and elicit an immune response, these cells play a key role in protecting the host but also contribute to the pathogenesis of inflammatory and degenerative diseases.

Macrophages are remarkably plastic and can change their phenotype depending on the microenvironment. Classically activated macrophages (called M1) are pro-inflammatory cells, which promote Th1 responses and tumoricidal activity. In contrast, alternatively activated macrophages (called M2) have anti-inflammatory functions and elicit tissue repair responses, fibrosis, tumor growth and progression. Therefore, reprogramming of macrophages can be an interesting option for the treatment of diseases like cancer and autoimmune disorders. We have developed an optimized, off-the-shelf panel of *in vitro* macrophage polarization cell-based assays using human primary blood-derived cells to assess the translational potential of small molecules as novel therapies.

M2 macrophages are involved in parasite control, tissue remodeling, immune regulation, tumor promotion, and efficient phagocytic activity. Several studies demonstrate that M2 polarization occurs in primary human monocytes upon exposure to IL-4/IL-10 cytokine cocktail. Cells that have undergone M2 polarization increase secretion of CCL18 and gain *de novo* expression of the mannose receptor CD206.

A validated, robust IL-4/IL-10-induced M2 polarization assay has been developed using primary human blood cells that are derived from healthy donors, to evaluate therapeutic candidates. M2 polarization of monocytes is measured by CCL18 secretion via ELISA using high content imaging.

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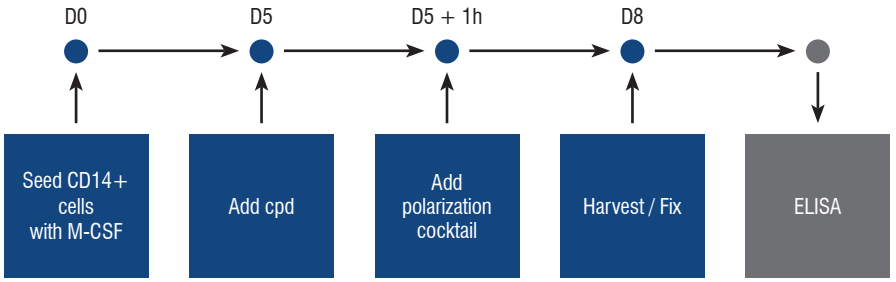
M2 Polarization Assay Principle

Blood-derived primary human CD14^{+} cells are seeded in presence of M-CSF to accommodate differentiation into so-called M(0) macrophages, then refreshed in preparation for addition of small molecule compounds and the IL-4/IL-10 cytokine cocktail to induce polarization into M2 macrophages. After 3 days, the culture supernatant is harvested and processed for CCL18 detection. In addition, the cells are fixed, stained for CD206 and DAPI, and imaged via HCA to assess the QC for M2 polarization.



M1 Polarization Assay Setup	
Cells	CD14^{+} cells from up to 2 healthy donors
Seeding density	5,000 cells/well in 384-well plates
Differentiation	M(0): M-CSF
Polarization	IL-4 / IL-10 cytokine cocktail
Assay controls	0.1% DMSO (negative control) and 1 μM tofacitinib (positive control)
Compounds	8-point concentration response curves (in biological duplicate)
Harvest / Fix	72 hours post polarization
Readout	ELISA: CCL18
QC	HCA: CD206 and DAPI

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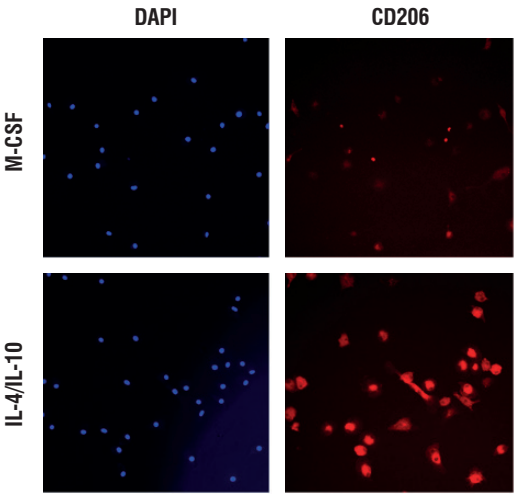
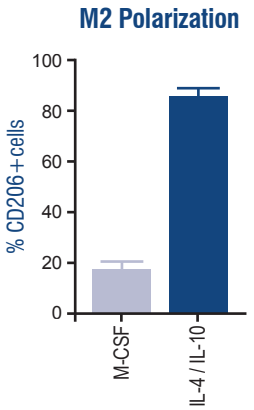
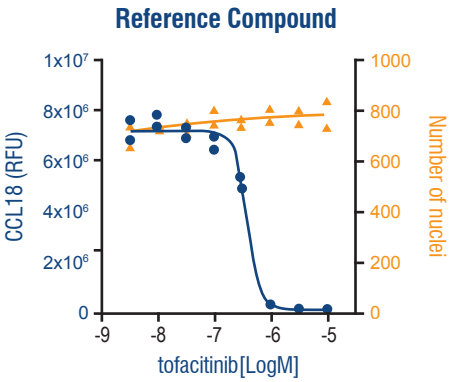
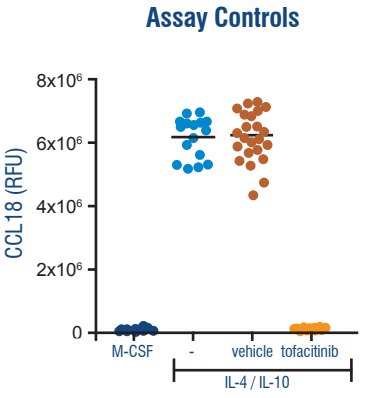
Assay Performance

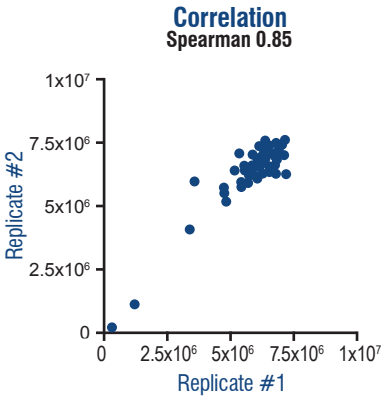
Representative images for IL-4/IL-10-mediated M2 polarization are shown below. In addition, representative concentration-response data shown for blood-derived M2 polarization.











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Summary

IL-4/IL-10 cytokine cocktail consistently induced polarization of M(0) macrophages into CD206 positive cells secreting CCL18, indicative of successful M2 polarization. IL-4/IL-10-mediated M2 polarization could be confined by treatment with the JAK1/3 inhibitor tofacitinib, showing full inhibition of CCL18 secretion regardless the presence of IL-4/IL-10. IC_{50} values were consistent among different donors (not shown) and strong Spearman rank correlation denotes consistency between biological replicates. Using this fibrosis assay, polarization of M2 macrophages can be monitored to evaluate therapeutic candidates in multiple diseases involving alternative activated macrophages, such as fibrosis, rheumatoid arthritis, inflammatory bowel disease, and cancer.

Therapeutic candidates can be evaluated in 8-point concentration response curves, using two different blood donors in biological duplicate for their effect on CCL18 secretion. In addition, potential cytotoxic side effects of the tested therapeutic candidate will be measured by monitoring the loss of DAPI-stained nuclei as a measure for cell death. Data package will contain QC results for M2 polarization (CD206 expression), signal-to-background ratios (M-CSF vs. IL-4/IL-10), assay windows (vehicle control vs. tofacitinib), reference compound (tofacitinib) data, and effect of the therapeutic candidate(s) on the CCL18 secretion. Results are issued within 6–8 weeks of compound receipt.

Assay Reference Code

M2 Polarization Assay – Alternatively activated M2 macrophage polarization assay in primary human cells derived from healthy donor blood.

OTS211-M2-polarization-blood-healthy

Complementary Fibrosis Assays

Fibroblast-to-Myofibroblast Transition (FMT) Assay

Epithelial-to-Mesenchymal Transition (EMT) Assay

M1 Polarization Assay

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