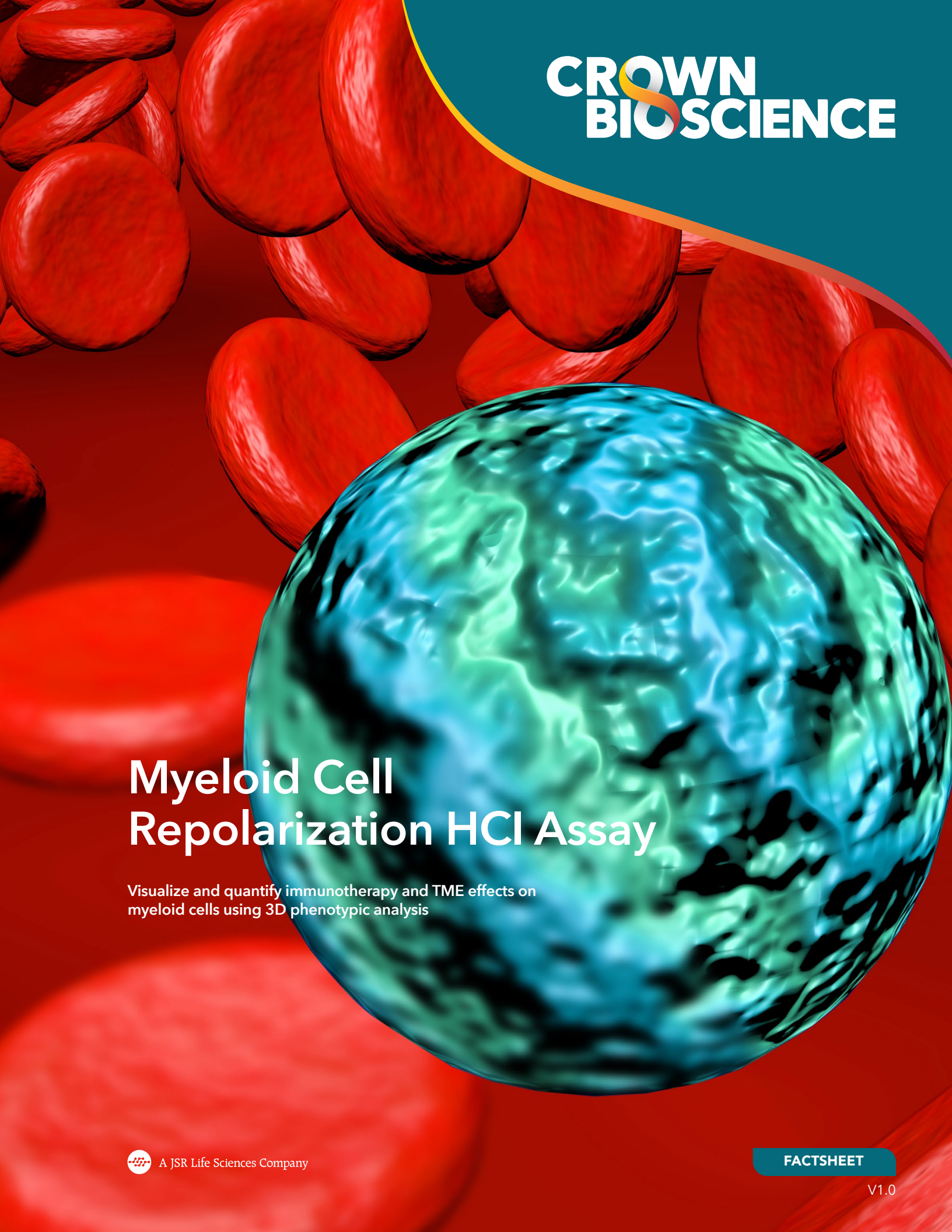


CROWN BIOSCIENCE

Myeloid Cell Repolarization HCI Assay

Visualize and quantify immunotherapy and TME effects on myeloid cells using 3D phenotypic analysis

A JSR Life Sciences Company

FACTSHEET

V1.0

The myeloid cell compartment plays an important role in anti-tumor immune responses and represents a heterogeneous population with both cancer-promoting and cancer-restraining actions. 3D image-based co-cultures of tumor and immune cells allow ecacy statement of immune modulators, that aim to enhance immune cell inltration and tumoroid killing. Incorporation of dierent myeloid cell populations results in a better representation of the human immune system in the tumor microenvironment (TME) within our assays.

CD14+ monocytes derived from healthy PBMCs cultured in 3D are polarized into different subsets of myeloid cells with cytokine cocktails. After polarization of the myeloid subsets, the nuclei are stained with DAPI (blue) and the actin cytoskeleton is stained with Rhodamine (red), as shown in the enhanced fluorescent images, with a maximum projection of the full 3D image stack (left panel). Subsequently, the images are segmented to extract each individual myeloid cell for further phenotypic analysis.

Myeloid Cell Identification Based on Phenotypic Profile

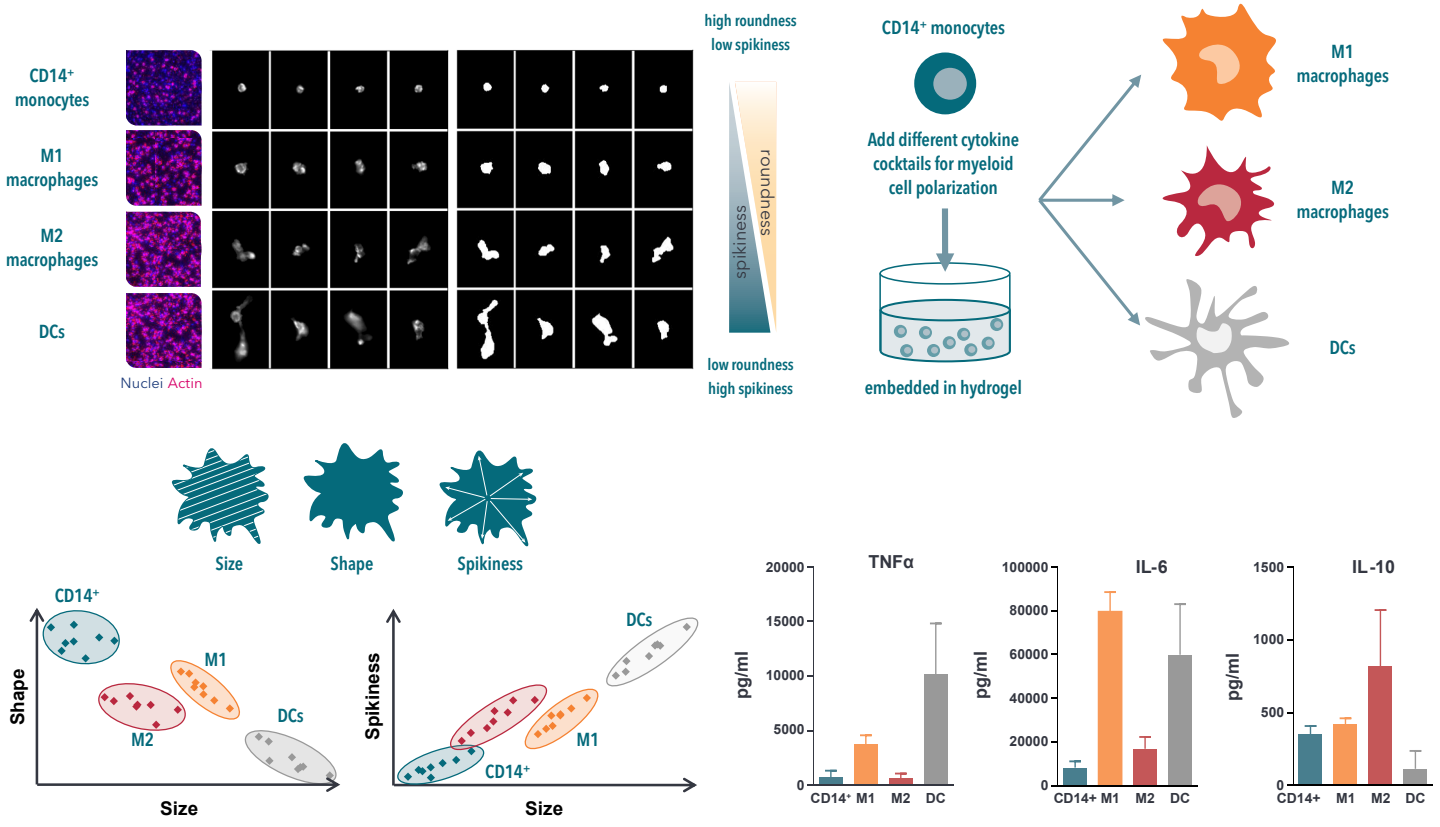
Image analysis with in-house developed software identifies the most distinguishing phenotypic characteristics for each population. Subsequently, a classifier is trained to understand selected features to create phenotypic profiles for each cell population. Examples of selected features are size, shape, and spikiness of the cells as shown below.

Key Advantages:

- Visualize and quantify effects of immunotherapies and the TME on myeloid cells using 3D phenotypic analysis
- Analyze the functional reprogramming of the suppressive tumor-associated population towards an M1 phenotype induced by drug candidates
- Understand drug effects in a suppressive TME in both mono-cultures of myeloid cells and co-cultures with tumor and/or T cells through functional readouts including tumoroid volumes and immune cell infiltration

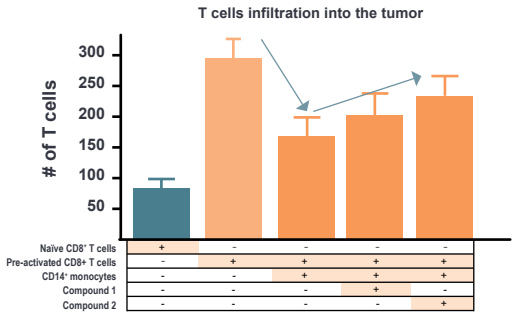
Specific Cytokine Secretion Profile

The phenotypic profiles of the different subset of myeloid cells are confirmed with functional read-out.



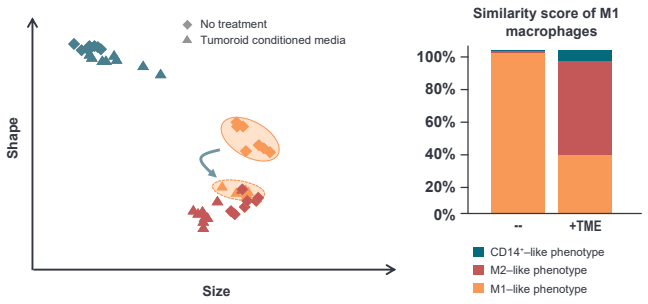
Suppression of T Cell Infiltration by Myeloid Cells

Immunosuppressive macrophages and monocytes are used to generate a suppressive TME to study the effect on T cells. Naïve or pre-activated CD8+ T cells were co-cultured with tumoroids in the presence of monocytes. Pre-activated T cells' capacity to infiltrate tumoroids is impaired when cocultured with immunosuppressive monocytes, which is partially restored with treatment.



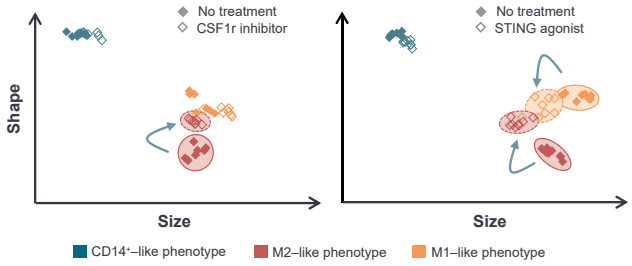
Myeloid Cell Repolarization by the TME

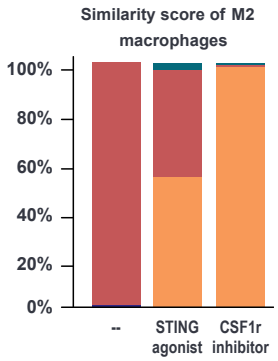
The TME can skew the phenotypic profile of myeloid cells. Both M1 and M2 macrophages demonstrated a change in their phenotypic profile after coculture with tumor conditioned media coming from cancer cell line-derived tumoroids. A switch of M1 macrophages towards M2 macrophages was observed which was also reflected by a higher M2 similarity score.



Repolarization of Myeloid Cells with Compounds

Phenotypic profiles can be used to analyze repolarization of myeloidcells upon treatment with CSF1r inhibitor and STING agonist. The analysis shows that M2 macrophages change their profile upon treatment and they demonstrate characteristics that overlap with M1 macrophages, which is confirmed with a higher similarity score to M1 macrophages. Image analysis offers the possibility to visualize and quantify the phenotypic shift of myeloid cells induced by repolarization agents.

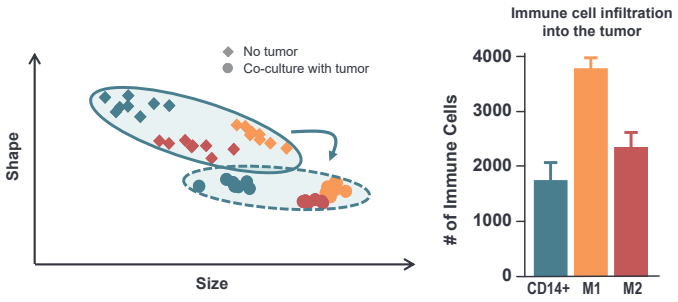


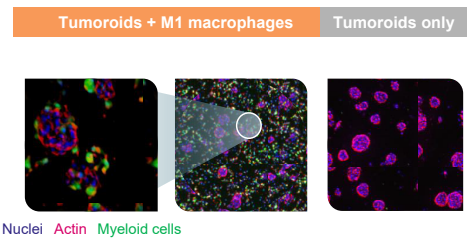


Co-Culture of Myeloid Cells with Tumoroids

Co-culture of tumoroids with the myeloid subsets leads to a change in their phenotypic profile. CD14+ monocytes show characteristics associated with macrophage differentiation, while M1 and M2 macrophages start to overlap their phenotypic profiles. M1 macrophages are the most efficient in infiltrating tumoroids and they are located in closest proximity to the tumoroids.

Image analysis offers insight into the cross-talk between tumor cells and myeloid cells, and to measure their phenotypic changes induced by drug candidates.





Get in touch

Sales
US: +1 858 622 2900
UK: +44 870 166 6234
busdev@crownbio.com
www.crownbio.com

Science
consultation@crownbio.com