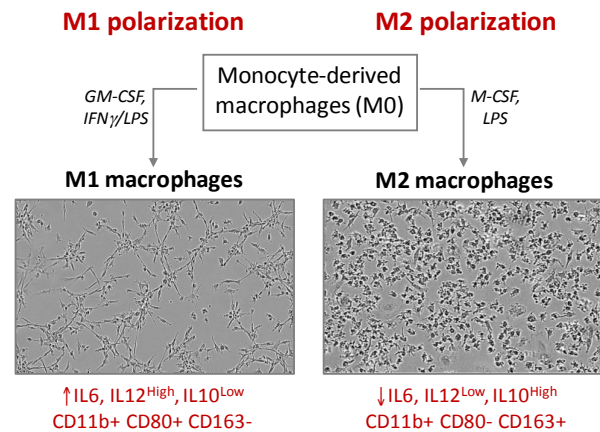


# M2 MACROPHAGE SUPPRESSION ASSAY

Macrophages - the most abundant antigen-presenting cells in the periphery - represent by themselves a wide group of functionally-different subpopulations involved in several immunological functions, including inflammatory responses and tissue homeostasis maintain & repair. Unlike M1 macrophages which promote a pro-inflammatory response, alternatively activated M2 macrophages, with diametrically opposed functions, trigger inflammation resolution and suppress T cell activation via several mechanisms including immune checkpoint engagement (e.g. PDL1), release of anti-inflammatory cytokines such as IL10 and TGF $\beta$ , and metabolic activities which promote essential amino acid depletion (e.g. arginine) - which characteristics that they share with the so-called tumor-associated macrophages (TAMs).

Our newly validated M2 suppression assay based on **i)** the co-culture of autologous monocyte-derived M2 macrophages and activated CD4+ T cells (or PBMCs) and on **ii)** the quantitation of IFN $\gamma$  levels as surrogate of T cell activation, is specifically designed to assess new immunotherapeutics for their modulatory activity on the phenotype and function of M2 macrophages. Candidate compounds can thus be evaluated as single agents or in combinatorial treatments, for their potential to repolarize / switch M2 macrophages and to antagonize M2-mediated T cell suppression.

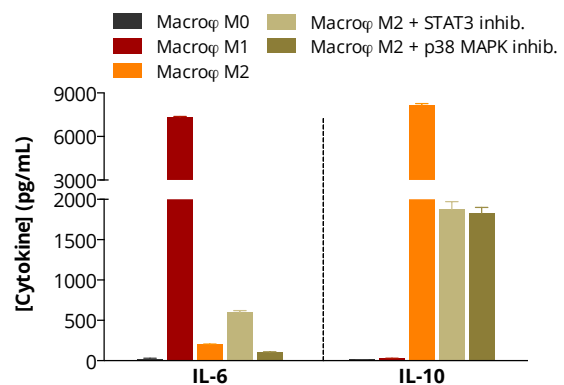
## Schematic diagram of differentiation & activation of functional phenotypes of M1 and M2 effector macrophages



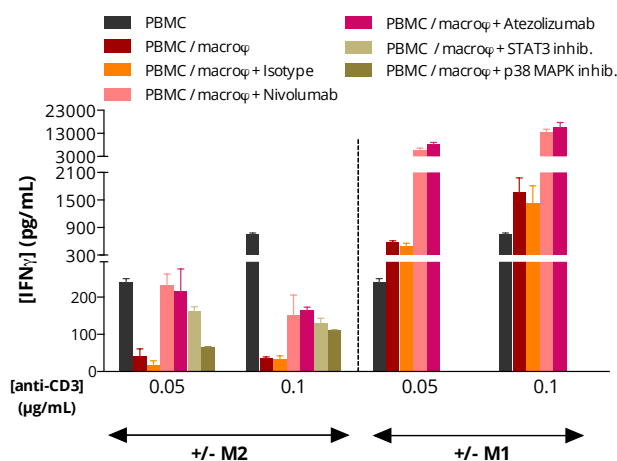
## M2 MACROPHAGES: A GOOD CANDIDATE TO IMMUNOTHERAPIES MODULATING T CELL RESPONSE SUPPRESSION

### ◆ Cytokinetic profile of M2 macrophages is reversed by immunosuppression-mediating pathways inhibitors

While M2 macrophages are known to display a IL6<sup>Low</sup> IL10<sup>High</sup> profile, this latter is involved in T cell response suppression and is underlied by signaling pathways including JAK/STAT and SMAD/p38 MAPK. Indeed, additions of a STAT3 inhibitor (niclosamide) or p38 MAPK inhibitor (LY2228820) during the M2 polarization process are shown here to be capable of repolarizing/switching M2 phenotype by reverting, at least partially, the IL6<sup>Low</sup> IL10<sup>High</sup> profile.



### ◆ M2 macrophage-mediated T cell suppression is antagonized by immune checkpoint inhibitors and involved molecular pathway modulators



Unlike M1 macrophages, which boost T cell activation thereby inducing an increase in IFN $\gamma$  production in anti-CD3-activated PBMC/M1 co-culture, M2 macrophages exhibit a strong suppression of T cell activity shown through IFN $\gamma$  level decrease. M2 suppression function is known to be exerted via multiple mechanisms including PD1/PDL1 axis engagement, among other ways. Interestingly, treatments of anti-CD3-activated PBMC/M2 co-cultures with either nivolumab or atezolizumab result, at least partially, in a reversal of the T cell activation suppression compared to untreated PBMC/M2 co-cultures, and to M1-mediated induced T cell activation. Likewise, nivolumab and atezolizumab also induce an optimized response of M1-mediated T cell activation. Very interestingly, repolarization/switch of M2 macrophage phenotype, through e.g. STAT3 or p38 MAPK inhibitors, prior to their co-cultivation with anti-CD3-activated PBMC, is also shown here to relieve the immunosuppressive M2 function on T cell response, an effect which occurs subsequently to the reversal of the M2 cytokinetic profile.