Characterisation of myeloid derived suppressor cells generated *in vitro* from human peripheral blood mononuclear cells

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Myeloid derived suppressor cells (MDSCs) are a population of pathologically activated, immature myeloid cells with potent immunosuppressive capabilities. As a key mediator of immune suppression, in the setting of many tumours, the infiltration of MDSCs may be a significant obstacle in achieving effective tumour immunotherapy, which is a viable and rapidly advancing strategy for cancer treatment. It is well established that depletion of MDSCs improves T cell immune responses, whilst delaying tumour progression and prolonging survival, in both cancer patients and animal models. As a result, increasing attention has been focused on understanding the mechanisms underlying MDSC induction.

Using an *in vitro* approach, we have generated $CD33^{+}$ HLA-DR^{low} MDSCs from healthy donor PBMCs via cytokine induction using recombinant human GM-CSF, in combination with either IL-6 or VEGF, over a period of 6-8 days. The clinically relevant A375 melanoma cell line, known to induce formation of suppressive MDSCs, was found to secrete higher levels of both IL-6 and VEGF, via MSD and ELISA, underscoring the selection of these factors for MDSC induction.

Co-culture of the *in vitro* generated MDSCs with autologous T cells, at a ratio of 2:1 T cells: MDSC, demonstrated significant inhibition of T cell proliferation in response to CD3/CD28 stimulation of T cells using an ImmunoCult reagent, during a 4-day co-culture period (one-way ANOVA, $p<0.01$). This suppressive effect on T cell proliferation, which was accompanied by reduced T cell secretion of IFN-γ in response to CD3/CD28 activation, indicated acquisition of an immunosuppressive phenotype by the in vitro generated MDSCs; characteristic of this myeloid cell population.

Overall, suppressive $CD33^{+}$ cells generated from human peripheral blood PBMCs via GM-CSF and IL-6 or GM-CSF and VEGF stimulation were consistent with human MDSCs. The next phase of this study will be to induce MDSCs using physiologically relevant A375 conditioned media, to more closely mimic the conditions experienced by MDSC precursors in the tumour microenvironment. Further study of this cell population could enable the development of novel therapeutic reagents for cancer immunotherapy.