ImmunoTools *special* Award 2014

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Investigating the role of the immune system in imatinib-treated dermatofibrosarcoma protuberans patients

Background and Rationale

Dermatofibrosarcoma Protuberans (DFSP) is a rare mesenchimal soft tissue sarcoma arising in the dermis. More than 90% of DFSP carry a translocation resulting in the COL1A1/PDGFB fusion gene. This gene results in a fusion-protein that is processed to mature PDGF-BB and thereby responsible for PDGFRβ activation allowing DFSP to grow, through an autocrine-paracrine loop. Surgery is the standard of care for DFSP, but there is a high risk of local recurrence. Moreover, DFSP can develop distant metastases. A metastatic behavior is mainly associated with the occurrence in approximately 15% cases of a fibrosarcomatous (FS) transformation, characterized by a high-grade sarcoma component (DFSP-FS). Conventional chemotherapy has proven to be ineffective and based on its molecular phenotype imatinib mesilate (IM) has been currently approved for the treatment of patients with unresectable, recurrent, or metastatic DFSP. Importantly, it has been shown that DFSP-FS maintained the translocation, thus confirming their sensitivity to the drug. Clinical studies underlined that IM is a potentially effective therapy in the management of these patients. However, responses are often short-lasting and there are no effective options after progression. Mechanisms responsible for a sustained response as well as for the acquisition of resistance to IM have not been clarified yet. Numerous data suggest that the success of treatments for cancer might depend on re-establish anti-tumor T cell responses, and the clearence of tumor cells by phagocytic cells of the innate immune system might represent a crucial step in this scenario. So far, no studies have underlined the effect of IM treatment in shaping the immunogenicity of this tumor. This might be critical in developing novel approaches for achieving long-term disease control. A set of primary cultures of classic as well as

DFSP-derived FS, have been already established and characterized (presence of the translocation, PDGFRβ phosphorilation, staining for the tumor-positive marker CD34).

Aims and study design

1. Analyze the effect of IM treatment on tumor cells expression of pro-phagocytic ("eat me") and anti-phagocytic ("don't eat me") signals required for recognition by neighboring antigen-presenting cells (APC).

Tumor cells will be treated with IM and the level of expression of several "eat me" signals, such as, phosphatidylserine exposure (Annexin V), calreticulin (CRT), will be analyzed by flow cytometry and compared to that of untreated tumors. In parallel, the expression of a well-known anti-phagocytic molecule (CD47) will be evaluated. In association to IM the ability of inflammatory factors (IFNγ, M-CSF, GM-CSF, TNFα, IL-1β) to modulate these molecules will be also tested.

2. Evaluate the ability of untreated as opposed to IM-treated tumor to:
    - polarize the tumor microenvironment (i.e. inhibition of monocyte-derived dendritic cells (mo-DC) maturation, M2 macrophage skewing)

Monocytes or human-monocyte derived macrophages (HMDM) (M-CSF) will be treated with tumor-derived supernatant and assessed for their functional polarization towards a cancer-supporting M2, or a more anti-tumor M1 macrophage phenotype (IL-10 vs IL-12 production). Moreover, mo-DC (IL-4 and GM-CSF) differentiation (CD14, CD1a) and maturation following LPS (CD80, CD86, HLADR, CD83) will be assessed. In parallel rhPDGF-BB will be use to condition the above mentioned innate immune cells and assess its *in vitro* polarizing capability (i.e. M1 vs M2, inhibition of DC maturation)

    - attract and be engulfed by macrophages and the subsequent ability of these immune cells to activate/inhibit $CD4^+$ and $CD8^+$ T cells

The collected supernatants will be used to set up migration assay with monocytes. Moreover, an *in vitro* phagocytosis assay will be conducted with HMDM and CFSE labeled IM-treated and untreated tumor. Macrophages isolated from the co-culture will be analyzed (CD40 expression, IL-12 production) and tested in a mixed lymphocyte reaction for their ability to activate T cells (CD38, CD69, CFSE) and prime a Th1 response (IFNγ, Tbet).

These *in vitro* data will be further support by *ex vivo* phenotype and functional analysis of immune cells ($CD45^+CD34^-$) isolated from tumor specimens surgically removed prior and after drug treatment as well as by immunohistochemical characterization on a retrospectically collected series of pre-post DFSP and DFSP-derived FS displaying an IM-sensitive or -resistant phenotype. ImmunoTools will greatly assist in providing a broad array of reagents necessary for enabling this translational proposal.

ImmunoTools *special* AWARD for **Marcella Tazzari** includes 25 reagents

FITC - conjugated anti-human CD1, CD45, CD47, CD86, HLA-DR,

PE - conjugated anti-human CD34, CD38, CD80, isotype control IgG1,

APC - conjugated anti-human CD14, CD40, CD69, isotype control IgG1, Annexin V,

human IL-12p40 total ELISA-set for 96 wells (3 reagents),

recombinant human cytokines: rh GM-CSF, rh IFN-gamma, rh IL-1beta /IL-1F2, rh IL-4, rh MCP1 / CCL2, rh M-CSF, rh PDGF-BB, rh TNFα

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