# ImmunoTools *special* Award 2019

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## Unravelling the immunomodulatory properties of mutant KRAS in MSI colorectal carcinogenesis

### Background

Tumor cells have the capacity to escape immune surveillance and to induce a protumorigenic immune microenvironment, taking advantage to inappropriately grow, invade and ultimately disseminate to distant organs. KRAS mutations are present in about 40% of colorectal cancer cases (CRC) and confer to the tumour a greater potential for malignancy. Few evidences also highlight an association between oncogenic KRAS signalling and the type of immune response present within the tumour tissue. Our preliminary results evidenced a dependence on KRAS activation for IFN-γ-induced PD-L1 expression in an Microsatellite instability (MSI) context and for CD47 expression in some CRC cell lines. These results award oncogenic KRAS signalling a novel role as a modulator of the immune escape mechanisms, which may impact not only cancer progression but also the response to immunotherapy strategies.

### Project

We propose to characterize the influence of mutant KRAS signalling in the regulation of the immune response in the context of MSI CRC, and to address the role of mutant KRAS in the regulation of immune cell infiltration in primary tumor lesions.

To address our objectives, we will perform *in vitro* and *in vivo* experiments:

- ***in vitro:*** we will evaluate the biological effect of PD-L1 upregulation in KRAS-mutant MSI CRC using a co-culture system composed of KRAS silenced and non-silenced MSI CRC cells (stimulated or not with IFN-γ, ImmunoTools) and lymphocytes previously activated with CD3/CD28 beads and labelled with CellTrace Violet Cell.

After co-cultured, KRAS downregulation will be validated by western-blot and both cancer cells and lymphocytes will be profiled by flow cytometry to evaluate the expression of PD-L1 in cancer cells, and the expression of PD-1, activation markers (CD69, CD25, HLA-DR, ImmunoTools) and proliferation rate in CD4+ and CD8+ Tcell populations (ImmunoTools). By doing so, we expect to functionally validate the role of KRAS in downregulating Tcell activation through the induction of PD-L1.

The effect of KRAS-induced CD47 upregulation in downregulating the phagocytic activity of macrophages will also be evaluated through a co-culture system. Accordingly, monocytes will be isolated from healthy blood donors' buffy coats, and differentiated into macrophages. Afterwards, KRAS silenced and non-silenced CRC cells will be labelled with CFSE and co-cultured with the differentiated macrophages to allow phagocytosis. Phagocytosis will be calculated as the % of CD14+ cells (ImmunoTools) labelled with CSFE.

- ***in vivo:*** The role of oncogenic KRAS in controlling the response of cancer cells to both innate and adaptive immune mechanisms will be further evaluated using in vivo models. We are currently establishing a *Msh2*$^{-/-}$; *Kras*$^{LSL-G12D/+}$ *Villin Cre* mouse model to obtain MSI KRAS mutant CRCs (*Cre* expression under the *Villin* promoter will dictate *Msh2* knockdown and *Kras*$^{G12D}$ expression specifically in the small and large intestine). Tumour load and histology will be compared in tumours from mice from both genotypes (*Msh2*$^{-/-}$; *Kras*$^{LSL-G12D/+}$; *Villin Cre* and *Msh2*$^{-/-}$; *Villin Cre*); additionally, the expression of PD-1 and PD-L1, as well the characterization of the lymphocyte infiltrate (CD3, CD4, CD8 and FoxP3) and macrophage populations (F4/80 and CD86) will be also determined by immunohistochemistry. Splenic myeloid and lymphoid populations will also be characterized in terms of populations present and Tcell activation status.

We expect to validate a role for KRAS activation in regulating PD-L1 expression thus affecting the anti-tumoral immune response at the TME and the spleen, as well as to determine its repercussions on tumour development and progression.

**ImmunoTools** *special* AWARD for **Sérgia Velho** includes 25 reagents:

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| FITC - conjugated anti-human | CD4, CD8, CD25, CD69, HLA-DR, CD47 |
| PE - conjugated anti-human | CD3, CD4, CD8, CD69 |
| PerCP - conjugated anti-human | CD3, CD4, CD25, CD69, HLA-DR |
| APC - conjugated anti-human | CD3, CD4, CD8, CD25 |
| recombinant human cytokines | rh IFNgamma |
| FITC - conjugated anti-mouse | CD3e, CD11b |
| PE - conjugated anti-mouse | CD4 |
| APC - conjugated anti-mouse | CD8a, CD25 |

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