ImmunoTools *special* Award 2018

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Investigating the applicability of novel cancer immunotherapies in upper gastrointestinal cancers and the effect of concurrent combination chemotherapy

Oesophageal cancer (OC) is the 8th most common cancer worldwide and the 6th most common cause of cancer deaths. It is an aggressive and deadly disease with a high propensity to metastasise. The five year survival rate is currently <20%. There are two main subtypes of OC; squamous cell carcinoma (SCC) and adenocarcinoma (OAC). The incidence of SCC is stabilising but the incidence of OAC has increased by 50% in the last two decades and continues to rise, with expected increases of 130% forecast for 2040. Therefore, there is an urgent unmet clinical need to identify new treatments options for OAC patients.

In recent years use of immunotherapeutics in cancer therapy has sparked huge excitement and has been described as a paradigm-shift for the treatment of several cancer-types and life-changing for patients. Targeting immune checkpoints (ICs) using immune checkpoint inhibitors (ICIs) to keep tumour killing T-cells active, has proved among the most beneficial approaches in the clinic. Currently no ICI has been approved for use in OAC patients but clinical trials are ongoing. One of the biggest clinical challenges is how to effectively combine ICIs with current standards of care such as chemotherapy. As focus is now shifting to identify the best scheduling regimens for combination chemotherapy with immunotherapy, no study has focused on the direct effect of individual chemotherapy agents on IC expression in OAC. Preliminary data from our group demonstrates that combination chemoradiotherapy significantly decreases the expression of PD-1 in OAC patients and *in vitro* experiments show that a range of chemotherapies including 5-fluorouracil, cisplatin, epirubicin and capecitabine, substantially alter the expression levels of a range of ICs and their ligands on the surface of OAC cells as determined by flow cytometry.

Therefore, this novel study examines the expression of a range of newly identified ICs in OAC in order to identify potential ICs that could be targeted in OAC patients and

importantly determine how clinically relevant chemotherapies affect their expression. A large range of ICs and their ligands will be examined including LAG-3, PD-1, TIM-3, VISTA, CD160, A2aR, PD-L1, PD-L2, CTLA-4 and the following ICs which are supplied by ImmunoTools will also be examined: CD47 and CEACAM-1.

Additionally, it is well known that IFN-γ and TNF-α (supplied by ImmunoTools) upregulates PD-L1 on the surface of cancer cells and immune cells. Therefore, I will also investigate the effect of IFN-γ on PD-L1 expression in combination with chemotherapies, hypoxia, acidosis and nutrient deprivation to simulate the tumour microenvironment which is characterised by low oxygen, low nutrient and acidic conditions. The expression of all the other immune checkpoints will also be investigated under these conditions.

As mentioned above several clinical trials are now ongoing combining chemotherapy with ICIs to treat OAC patients. However, there is little known about the effect of chemotherapy on the activation status of T cells. For example, certain chemotherapies could increase the expression of activation markers on the surface of T cells, therefore, these chemotherapies would be ideal to combine with immunotherapies and could potentially synergise with ICIs improving treatment responses. Paradoxically, certain chemotherapies could decrease the expression of activation markers on T cell surfaces, therefore combining these chemotherapies with immunotherapies would decrease treatment responses. Therefore, it is essential to determine the direct effect of individual clinically relevant chemotherapies on the activation status of T cells. T cells will be treated for 24, 48 and 72h with a range of chemotherapy concentrations for 6 drugs (cisplatin, oxaliplatin, 5-fluorouracil, docetaxel, capecitabine and epirubicin) and the expression of activation markers including CD45RO, CD45RA, CD69, CD62L, CD27 and CD56 on the surface of T cells (all supplied by ImmunoTools) will be examined using flow cytometric analysis. T cells will also be stained with CD3, CD4 and CD8 (supplied by ImmunoTools) to elucidate the effect of chemotherapies on the activation status of both CD4-positive and CD8-positive T cells.

This research has incremental value in providing key insights into identifying the most appropriate ICIs to combine with specific chemotherapies for the treatment of OAC patients and potentially many other cancer types. There are three specific aims to my project, listed below.

ImmunoTools *special* AWARD for **Maria Davern** includes 25 reagents

FITC - conjugated anti-human CD27, CD45RA, CD47, CD56, CD62L, CD69, CEACAM-1

PE - conjugated anti-human CD27, CD45RA, CD45RO, CD56, CD62L, CD69, CEACAM-1-PE

PerCP - conjugated anti-human CD4, CD45RA

APC - conjugated anti-human CD3, CD8, CD27, CD45RA, CD62L, CD69, CEACAM-1

recombinant human cytokines: IFNγ, TNF-α DETAILS more AWARDS