ImmunoTools *special* Award 2023

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Elucidating the tumourigenic effects of fractalkine and its therapeutic utility in oesophageal adenocarcinoma

Oesophageal Adenocarcinoma (OAC) affects more than 400 people in Ireland every year and 600,000 worldwide. OAC is regarded as an inflammation-driven cancer and unfortunately, it remains a poor prognosis cancer with a 5-year survival rate of less than 25%, which is largely due to only 30% of patients currently responding to surgery, chemotherapy, and radiotherapy. Current standard-of-care (SOC) treatment for OAC is surgical resection and chemoradiotherapy. In addition, two immunotherapies (IT) have been approved for the treatment of OAC. However, current efficacy rates for these ICIs stands between 10 to 15% for OAC therefore there is an urgent need for novel therapies and treatment combinations for this poor prognosis malignancy.

Obesity is a burgeoning global health problem with the World Health Organisation (WHO) reporting that its prevalence has tripled worldwide since 1975. Robust epidemiological evidence has established links between obesity and the incidence of cancers, notably oesophageal cancer. Our group has shown that visceral obesity is characterised by immune dysfunction in OAC and that the largest depot of visceral adipose tissue (VAT) in humans known as the omentum is the source of many driving factors in this dysregulation. In light of this, targeting key drivers of obesity-associated tumorigenesis, inflammation, and immune dysfunction in OAC is a desirable therapeutic approach.

It is well established that cancer patients with the lowest frequencies of NK cells have the lowest survival rates. Moreover, our group has shown that the most obese OAC patients have the lowest numbers of intratumoural NK cells, contributing to ‘cold’ or ‘immune-excluded’ tumours in this poor prognosis malignancy. Since OAC patients have impaired anti-tumour immunity, we propose that they would benefit from immunotherapies that restore NK cell anti-tumour functions.

Our group has previously reported that the pro-inflammatory chemokine fractalkine plays a role in the disruption of anti-tumour immunity in OAC via its recruitment of NK cells to the visceral

adipose tissue (VAT). Furthermore, we have reported that antagonism and modulation of the fractalkine receptor CX3CR1 can prevent NK cell migration towards VAT and holds therapeutic potential to re-invigorate their infiltration and killing of OAC tumours. Other groups have reported the direct pro-tumourigenic effects of fractalkine in cancer of the stomach, lung, pancreatic and prostate. Therefore, this study aims to elucidate the direct role of fractalkine in OAC tumour progression and its potential as a therapeutic target. Moreover, this study will assess the utility of a highly cytotoxic and fractalkine-resistant tumour-homing NK cell-based immunotherapy to bypass the VAT and infiltrate OAC tumours.

Due to their robust anti-tumour activity and safety profile, NK-92 cells are the NK cell line that have been the most explored in clinical trials for various cancer types. Therefore the NK-92 cell line is of interest to our project, but also the cell line KHYG-1 as it has previously been reported by other groups to be CX3CR1-. In order to support their growth and survival, these cell lines have to be stimulated with rh cytokine IL-2, IL-12 and IL-15. We will observe NK cell markers on these cell lines such as FITC-CD56, PerCp-CD3, PE-CD27 and APC-CD11b but also activation marker PE-CD69, APC-CD54 and CD62L. To assess NK cell migration towards different chemotactic gradients, we will use rh MIP-1alpha, rh MIP-3alpha and rh RANTES in our chemotaxis experiments.

Furthermore, to evaluate the role of fractalkine in OAC tumour progression, OAC cell lines will be treated with fractalkine +/- rh TNF-alpha/rh IFN-y and cell viability, cell proliferation and cell invasion will be assessed.

Ultimately, our research aims to confirm the utility of two novel immunotherapies to promote OAC tumour eradication and improve OAC patient survival.