

immundominant SARS-CoV-2-specific CD8 T cell response revealed a regulated activation program that maintains CD8 T cell survival while halting their effector function and migratory capacity.

**Conclusions** The ORF1ab, that was found to be the source of an immunodominant SARS-Cov-2-specific CD8 T cell epitope, is not included in the majority of vaccine candidates in development, which may influence their clinical activity. Furthermore, these data may be a cautious indication that SARS-CoV-2 specific CD8 T cells – unlike CD4 T cells – are less likely to contribute to the immunopathology observed in severely and critically ill COVID-19 patients.

**Ethics Approval** The samples from both COVID-19 patients were collected in accordance with the Declaration of Helsinki after approval by the institutional review boards.

**Consent** Each participant signed informed consent.

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## Tumor and stromal cell biology

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### THE MESENCHYMAL STROMAL COMPARTMENT IN COLORECTAL CANCER GREATLY ALTERS THE INNATE TUMOUR IMMUNE MICROENVIRONMENT BOTH IN 2D AND 3D CULTURE SYSTEMS

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**Background** Colorectal cancer is the fourth most common occurring cancer and despite new treatment options it remains the third leading cause of cancer related deaths worldwide.<sup>1</sup> Of the four Consensus Molecular Subtypes (CMS), the mesenchymal stromal rich CMS4 tumours are shown to have the worst disease free progression survival. However the role mesenchymal stromal cells (MSC) play in the tumour immune microenvironment has yet to be fully elucidated. Understanding the complex communication in this stromal cell rich multicellular environment is challenging but may reveal novel targets for the treatment of colorectal cancer patients.

**Methods** Tumour cell secretome (TCS) was generated from colon cancer cells with/without the addition of TNF- $\alpha$ , an inflammatory stimulus using both human and mouse cell lines. MSCs were then conditioned with the TCS and inflammatory TCS and changes in surface and secreted immunomodulatory molecules were assessed using RNA-seq, flow cytometry and ELISA analysis. Macrophage antigen processing and migration following co-culture with the TCS conditioned MSCs was observed using DQ-ova and transwell experiments. A Gelatin Methacryloyl hydrogel, 3D triple culture systems was established to study the role of MSC in the colon tumour immune microenvironment. HCT116 colon cancer cell line with THP1 monocytic cell line and primary bone marrow derived MSCs were embedded in the hydrogel and incubated for 10days, changing the media on Day 8 with/without the addition of TNF- $\alpha$ . Cell proliferation viability and protein secretion were assessed from the 3D CRC system.

**Results** Bioplex analysis revealed secretion of potent chemokines and cytokines from the cancer cells. This inflammatory TSC resulted in increased expression of cell surface MSC immunomodulatory markers PD-L1 and CD47 and a variety of secreted molecules. These conditioned MSCs reduced macrophage-mediated antigen processing and increase monocyte migration. A triple culture 3D model of CRC was successfully developed, and while the addition of MSC to the system did not alter spheroid size they increased the release of potent chemokines(CCL2, CXCL12), cytokines (IL-6, IL8) and growth factors (GM-CSF) from the culture system.

**Conclusions** The inflammatory tumour cell secretome can alter MSC surface expression and secretion of a variety of immunomodulatory makers. These tumour conditioned MSCs can alter innate immune cell antigen processing and migration. When MSCs are combined in 3D with monocytes and colon cancer cells the MSC significantly alter the secretion of immune modulating and tumour promoting factors from the culture system. Targeting MSC immune suppression in the colon tumour microenvironment could be a novel therapeutic target.

**Ethics Approval** Human MSC (hMSC) were isolated from the bone marrow of three healthy volunteers at Galway University Hospital under an ethically approved protocol (NUIG Research Ethics Committee, Ref: 08/May/14) according to a standardized procedure.

#### REFERENCE

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### SUGAR HIGH: DOES THE SIALIC ACID PROFILE OF CANCER-ASSOCIATED FIBROBLASTS INDUCE A MORE TUMOUR-PERMISSIVE MICROENVIRONMENT?

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**Background** Immunosuppressive tumour microenvironments (TME) inhibit the effectiveness of cancer immunotherapies. Sialic acids, which exist as terminal sugars of glyco-conjugates, are highly expressed on cancer cells and are involved in various pathological processes including increased immune evasion, tumour invasiveness and tumour cell metastasis.<sup>1</sup> Siglecs (Sialic acid-binding immunoglobulin-type lectins) are expressed on immune cell surfaces and bind sialic acid. Siglec binding to hypersialylated tumour glycans blocks immune cell activation to promote immunosuppression.<sup>1</sup> <sup>2</sup>Intestinal stromal cells (iSCs), precursors to cancer-associated fibroblasts (CAFs), are a key component of the TME and play a vital role in tumour progression by enhancing a tumour-promoting microenvironment. The aim of this study was therefore to investigate if iSC/CAF sialylation contributes to enhanced immunosuppression in the TME.

**Methods** iSCs were isolated from colorectal cancer patient biopsies and cultured ex vivo. Informed consent was obtained from all patients prior to sampling. Tumour-derived iSCs were termed CAFs while control iSCs, isolated from tumour-adjacent non-cancerous tissue, were termed normal-associated