# MICROBIAL AND IMMUNE METABOLIC MODULATION

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Our lab is interested in the interplay between microbes, epithelium and the immune system in the intestine. Bacteria have been used experimentally as cancer therapeutic agents since the work of William Coley in the early 1900's, yet only one bacterial cancer therapy (BCT) is in clinical use; BCG therapy for superficial bladder cancer. We are working toward understanding and improving BCTs by investigating mechanisms of bacterial adaptation to the tumour, direct effects of bacteria on tumour growth and effects on immune activation and responses. More broadly, we are interested in host-microbe interplay within the intestine (Figure 1).

The immune system protects us from infectious agents such as bacteria, viruses and fungi, as well as from malignant growth of our own tissues. In the intestine, we have both positive (commensals) and negative (pathogenic) interactions with bacteria. We are co-inhabited with trillions of microbes which, for the most part, do not elicit immune responses and exist in a symbiotic relationship with the host; and some bacteria even performing essential functions. The intestinal epithelium harbours innate sensors and is able to recognise and respond to pathogenic insults and help shape innate and adaptive immune responses. Intriguingly, many of these innate pathways can also act to suppress, or promote, tumourigenesis. This is where our intrigue lies; what microbial cues could we utilise to impair tumour growth and improve anti-tumour immunity? We use attenuated Salmonella typhimurium (STm) which selectively home tumours and efficiently reduce tumour growth. In particular, we study effects of STm on colorectal cancer (CRC) using both mouse models of CRC and tumour organoids (mouse and patient-derived). We aim to uncover mechanisms that both drive effective therapeutic responses as well as less-desirable side-effects, in an effort to best engineer STm therapy.

This year our lab has grown as we continue to settle into the CRUK Scotland Institute. Declan McLelland joined the group in April as a Scientific officer, Ross McInnes joined in May as a postdoctoral scientist, Taiitsu Masunaga in July as an MSci student and Sofia Sandalli joins us at the start of 2025 as a post-doctoral scientist.


**Figure 1.** Word cloud generated from publication titles highlighting the laboratory's interests.

## Metabolic suppression of tumours at the cost of T cell immunity

In previous work we demonstrated that attenuated Salmonella therapy altered the tumour metabolic landscape, with large reductions in a range of metabolites including sugars, TCA cycle intermediates and amino acids or their precursors (Mackie *et al.*, 2021, *JCI Insight*). From this we surmised that part of the mechanism of Salmonella therapy was metabolic competition – essentially the microbes could outcompete tumour cells for essential fuel sources and thereby limit tumour growth. Over the past few years one of the lab's research focuses has been to understand the role T cells play in BCT. Previous research had shown that T cells are not required for effective STm therapy, yet the mechanisms behind this have not been addressed. Further, there is effort to develop BCTs alongside checkpoint blockade therapies, thus it is important we resolve how T cells function during BCT. We indeed found that T cells from STm-treated tumours were dysfunctional; they could not sustain their activation and showed poor cytokine production and failed proliferation. This was not associated with defects in TCR signalling, but instead potent inhibition of glycolysis; upregulation of which is essential for T cell proliferation and gain of effector function. T cell metabolic dysfunction was due solely to asparagine depletion by bacteria, leading to depleted c-Myc protein; reversal of this depletion restored T cell function (Figure 2). Critically, STm-mediated c-Myc suppression was also detected in the tumour itself, which dampened tumour stemness and survival, highlighting an important 'double-edged sword' for STm BCT in which tumour control by bacteria comes at the detriment of adaptive immunity. These findings provide a strong rationale for addressing a previously unknown cardinal defect in Salmonella-based cancer therapies to yield more successful clinical outcomes. This work is now published in EMBO Molecular Medicine (https://doi.org/10.1038/s44321-024-00159-2). Future work will now aim to further dissect the efficacy of the asparaginase-deficient STm when in combination with immune checkpoint blockade therapies, or the asparaginase-sufficient strain with different timing regimens to improve tumour suppression.

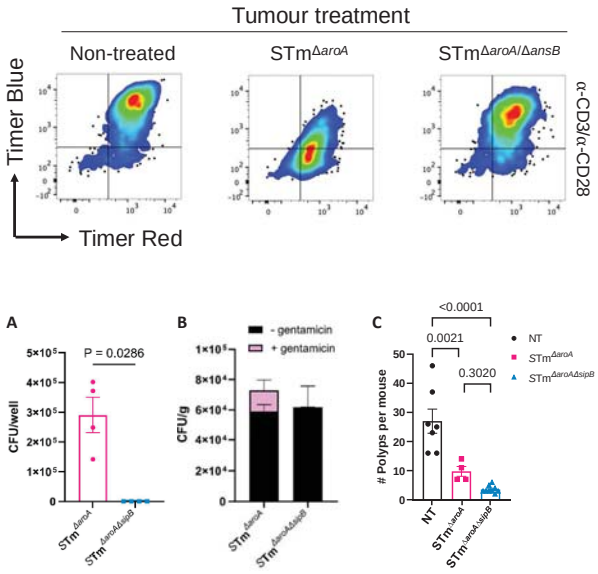


**Figure 2.** CD4+ T cells cultured in tumour conditioned medium from tumour organoids given the indicated Salmonella strains. Timer blue and red expression indicates persistent T cell receptor activation. Timer$^{red+}$Timer$^{blue-}$ indicates terminated T cell receptor signalling. aroA=aromatase deficient STm; ansB=asparaginase.

**Figure 3.** A) Tumour organoids were infected with ΔaroA or ΔaroaΔsipB STm and CFU analysed. Loss of sipB ameliorates STm invasion. B) *Apc*$^{min/+}$ mice were infected with each STm strain and CFU in polyp tissue assessed. Gentamycin added to quantify STm residing intracellularly, the remaining are extracellular. C) *Apc*$^{min/+}$ mice were treated with indicated STm strains or control for 8 weeks, from 8 weeks of age. Polyp burden shows reduction in polyp number in STm treated mice.

## Intra versus extracellular bacterial targeting

We had observed preferential invasion of Lgr5+ stem cells within the tumour and noted that in fact only a small percent of STm are intracellular; the vast majority reside in the extracellular spaces. Our questions were: why does STm preferentially invade Lgr5+ stem cells? And is intracellular invasion important or necessary for STm therapeutic effect? Using patient-derived colorectal cancer organoids we found that, like mouse-derived organoids, STm preferentially invade proliferating cells, and blocking cellular proliferation prevents STm invasion. We found this was due to part of the type III secretion system apparatus, and particularly SipB, which mediates tight binding to the host cell by interaction with membrane cholesterol. We have used invasion deficient STm to start to investigate the necessity of intracellular invasion for therapeutic effect of STm therapy (Figure 3). Invasion-deficient STm may represent a safer therapeutic avenue, decreasing the (very low) risk of bacterial dissemination in immune compromised cancer patients. However, some STm therapy strategies are focussed on using STm as a vehicle to deliver intracellular cargo – thus it is important to further understand how, how many and which cells are actually targeted intracellularly, and how much bearing that has on therapeutic success.

## Epithelial innate sensors

Another avenue of interest for our lab are intracellular innate sensors that are expressed by epithelial cells, particularly how they contribute to, or control, tumorigenic growth. Previously, we have shown that a family of proteins called NLR apoptosis inhibitory proteins (NAIPs) suppress epithelial tumorigenesis in a cell-intrinsic manner. NAIPs belong to a family of inflammasome-forming proteins, and other groups have also identified tumour suppressive roles for other family members, suggesting a kind of innate sensing and checkpoint in epithelial transformation. Recently, we have been asking what effect loss of epithelial NAIPs, which we observed during tumorigenesis, might have on the intra-epithelial / tumoral immune response, particularly on intraepithelial lymphocyte populations. We have found some alterations in gamma delta T cells, which we aim to follow up in collaboration with the Coffelt group.

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