822 OVERCOMING THE SUPPRESSIVE TUMOR MICROENVIRONMENT WITH A LIVE BACTERIAL IMMUNOTHERAPY

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Background Tumor-infiltrating myeloid cells supress anti-tumor immunity within the tumor microenvironment (TME) through direct and indirect inhibitory mechanisms. However, inherent myeloid plasticity offers the opportunity for treatments to reprogramme these cells. One class of agents with potential to reprogramme myeloid cells are bacteria and their products, which act locally on suppressive cell populations, but also induce long-lasting systemic reprogramming of myeloid cells, a process termed trained immunity. Prokarium is developing a live attenuated *Salmonella enterica* serovar Typhi strain (ZH9) to be the next cancer immunotherapy and sought to establish whether *Salmonella* reprograms myeloid cells and enhances anti-tumor immune function.

Methods The effects of *Salmonella* on established suppressive myeloid cell phenotypes was assessed by flow cytometry staining, cytokine release and T cell suppression assays using M2 polarised human macrophages. The effects of *Salmonella* on unpolarised myeloid cells was investigated after oral *Salmonella* infection of healthy mice by phenotyping splenic myeloid cells using flow cytometry and assessing their cytokine production after *ex vivo* re-stimulation. Anti-tumor activity of *Salmonella* treatment was measured in syngeneic subcutaneous colon (MC38) and experimental metastasis (4T1) models. Finally, potential synergy with established therapies was assessed utilising a co-culture system of human monocyte-derived M2 macrophages treated with *Salmonella* and autologous T cells, with or without checkpoint inhibitor antibodies.

Results *Salmonella* treatment repolarized human M2 macrophages towards an anti-tumor phenotype, upregulating costimulatory molecules, increasing secretion of pro-inflammatory cytokines and relieving suppression of co-cultured T cells. Oral *Salmonella* treatment of mice induced long-term phenotypic and functional myeloid changes, including upregulation of costimulatory and MHC molecules on systemic dendritic cells, monocytes and macrophages, and increased responsiveness of CD11c+ splenocytes to secondary stimuli, suggesting *Salmonella* also affects unpolarised myeloid cells. Oral treatment with *Salmonella* as a monotherapy was able to suppress tumour growth in subcutaneous and experimental metastasis models, indicating this *Salmonella*-induced myeloid phenotype may translate to changes in the myeloid compartment of the TME. Finally, *Salmonella* complemented other cancer therapies both *in vitro* and *in vivo*. *In vitro, Salmonella*-trained human monocytes overcame the suppressive phenotype induced by subsequent culture in M2-polarizing conditions to synergize with checkpoint inhibitors in driving T-cell proliferation. *In vivo*, oral *Salmonella* treatment synergized with anti-PD-L1 in supressing growth of subcutaneously implanted MC38 tumors.

Conclusions *Salmonella* immunotherapy can both reverse established suppressive myeloid phenotypes and systemically prime myeloid cells, likely rendering them resistant to immunosuppression in the TME and ultimately leading to improved efficacy of existing cancer immunotherapies.

Ethics Approval All animal studies were conducted under authority of United Kingdom Animals (Scientific Procedures) Act 1986 project license number PP8366809.