INHIBITION OF ACID SENSING BY GPR65 NORMALISES GENE EXPRESSION IN MACROPHAGES, INCREASES IMMUNE CELL INFILTRATION IN TUMORS, AND RESTRAINS SUBCUTANEOUS MC38 GROWTH IN MICE

Alastair Corbin, Stuart Hughes, Mussa Quareshy, Tobias Bopp, Barbara Cipriani, David Miller, Alan Naylor, Gavin Milne, Darryl Turner, Barbara Young, Anastasia Nika, Preeti Singh, Rupert Satchell, Sourav Sarkar, Gavin Knox, Toszka Bohn, Tom McCarthy, Pathios Therapeutics Ltd., Oxford, UK; Sygnature Discovery Ltd., Nottingham, UK; University Medical Centre Mainz, Mainz, Germany; Malvern Panalytical, Edinburgh, UK

Background High frequencies of Tumor Associated Macrophages (TAMs) are related to poor patient prognosis. The Tumor Microenvironment (TME) is characterised by resource scarcity, toxic metabolic by-products, and low pH, together creating an immunosuppressive environment which polarises TAMs towards a pro-tumorigenic state.

Methods We identified the proton-sensing G-Protein-Coupled Receptor 65 (GPR65) as a key determinant of low-pH-induced immunosuppression in human cancers, specifically via modulating TAM phenotype in response to the acidic TME. The importance of GPR65 in human cancers is highlighted by three key findings: (1) cancer patients homozygous for the hypomorphic I231L variant exhibit a pronounced survival benefit, (2) GPR65 and downstream pathway genes are highly expressed in innate immune cells from all human solid tumors when assessed by single cell RNA sequencing, and (3) low pH treatment of macrophages in vitro leads to a marked suppression of inflammatory genes and an upregulation of a tissue repair signature.

Results We have identified potent and selective small-molecule antagonists of human GPR65 that inhibit the low pH-induced accumulation of cAMP in recombinant cell systems and primary human macrophages with single-digit nanomolar potencies. These compounds dose-dependently prevent the low pH-driven suppression of inflammatory cytokine and chemokine genes and counteract the upregulation of pro-tumorigenic and tissue repair genes in both human and mouse macrophages.

Oral administration of our exemplar compound PTT-3213 in subcutaneous MC38 tumor-bearing mice caused gene expression changes consistent with those observed in primary macrophages in vitro, indicative of a dramatic impact on the TME. Weekly dosing of PTT-3213 significantly reduced MC38 Tumor Volume (TV) compared to vehicle (46%). This monotherapy activity was comparable to bi-weekly administration of anti-PD1, whilst combination of PTT-3213 and anti-PD-1 led to a more pronounced curtailment of TV vs vehicle-treated animals (61%). In accordance with the increased expression of chemokine genes, PTT-3213 monotherapy in MC38-bearing mice markedly elevated the frequency of tumor-infiltrating NK cells (up to 22-fold). There was also an increase in the CD8+/CD4+ T cell ratio which attained statistical significance in combination with anti-PD-1.

Conclusions Taken together, we have identified GPR65 as a key innate immune checkpoint and therapeutic target in solid tumors and propose that macrophage conditioning via GPR65 inhibition may provide an efficacious strategy to counteract the immunosuppressive action of the acidic TME on TAMs in patients.

Ethics Approval Protocols or procedures involving the care and use of animals in studies in China were reviewed and approved by the Institutional Animal Care and Use Committee of Crown Bioscience. During studies, the care and use of animals was conducted in accordance with the regulation of the Association for Assessment and Accreditation of Laboratory Animal Care.

Studies involving the welfare and use of animals within the UK complied with the UK Animals Scientific Procedures Act 1986 (ASPA) in line with Directive 2010/63/EU of the European Parliament and Council of 22/September/2010 on the protection of animals used for scientific purposes and UK Home Office guidance on the implementation of the Act and applicable codes of practice for the care and housing of laboratory animals.