**Characterising BB3: a novel therapeutic target in lung adenocarcinoma**

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**Introduction.** Highly innovative strategies are urgently needed to target lung cancer, one of the biggest killers of Australians. Early evidence suggests that the orphan G protein-coupled receptor, bombesin 3 (BB3), may be overexpressed in lung cancer and absent in healthy tissue. We seek to improve outcomes for lung adenocarcinoma (LUAC), a subtype of non-small cell lung cancer, by exploiting the biological selectivity of BB3.

**Aims**. To deconvolute the expression, activation and signal transduction of the poorly defined receptor, and then determine any possible interactions between BB3 ligands and standard therapeutics.

**Methods**. To determine the extent of BB3’s biological selectivity, gene expression data was mined from RNA-sequencing databases. To validate the pharmacological properties of BB3, the following assays were used: reporter gene assays to investigate Gαq/11, Gα12/13, and Gαs signalling; NanoBiT assay for β-arrestin recruitment; and BRET1-based biosensors for ERK1/2 phosphorylation and cAMP accumulation. To assess the impact of BB3 activity in cancer, a panel of lung cancer cell lines was assessed for BB3 expression via qPCR, and the effect of agonist stimulation was measured using the CellTiter-Glo cell viability assay.

**Results.** BB3 mRNA was found exclusively in LUAC, and not in healthy human tissue. BB3 expression in LUAC was more prevalent (83%) than any other LUAC marker (<44%). BB3 signals in the absence of ligand via the Gαq/11, Gα12/13, and Gαs pathways (Emax = 439.8, 174.2, 118.7 RLU respectively), and agonism increases Ca2+ (MK-5046 pEC50 = 8.75, BAG-1 pEC50 = 9.63). Cell lines expressing BB3 were susceptible to traditional cancer therapies, but targeted BB3 ligands had no effect on cell viability.

**Discussion.** BB3 appears to be a cell-surface biomarker of LUAC, making it an ideal candidate for selective targeting in a disease with high treatment resistance. By characterising BB3’s pharmacology and demonstrating its selective expression in LUAC, we provide a strong foundation for the future development of BB3-targeted therapies.