**Development of a biologically selective treatment for lung adenocarcinoma**

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**Introduction.** Lung cancer remains the leading cause of cancer-related deaths in Australia, with a dismal 3% survival rate for patients diagnosed at stage IV—primarily due to treatment resistance. We have discovered that mRNA expression of the orphan G protein-coupled receptor bombesin 3 (BB3) is increased in lung adenocarcinoma (LUAC), a subtype of non-small cell lung cancer, and not found in healthy tissue. This presents a compelling drug opportunity.

**Aim.** To comprehensively characterise the pharmacology of BB3 to inform future LUAC drug development strategies.

**Methods.** BB3 expression in LUAC was assessed using bioinformatics and qPCR. LUAC cell survival was evaluated in response to current standard-of-care treatments with or without BB3 ligands, in cells expressing endogenous or exogenous BB3, with or without BB1 and BB2. BB3 signalling pathways were investigated using bioassays for canonical GPCR signalling in HEK293 cells overexpressing BB3. BB3 protein was subsequently purified from Expi293 cells in the presence of an antagonist for downstream applications.

**Results.** BB3 was expressed in 83% of LUAC cases—more frequently than any other LUAC marker (<44%)—but showed no correlation with patient survival or known LUAC drivers. BB3 agonism or antagonism did not influence LUAC cell viability or the efficacy of existing treatments. We found BB3 to be constitutively active, capable of engaging multiple GPCR signalling pathways, and likely to possess an allosteric binding site. Negative stain electron microscopy confirmed successful purification of monodisperse BB3 protein.

**Discussion.** BB3 exhibits exceptional tissue selectivity and is present in the majority of LUAC cases. Our finding that BB3 does not contribute to LUAC progression supports its use as a therapeutic conduit for cytotoxic delivery or antibody-mediated cell death. With our highly purified BB3 protein and detailed pharmacological characterisation of BB3 ligands, we are now positioned to develop targeted therapies via this receptor. Future work will focus on elucidating BB3 internalisation mechanisms, characterising orthosteric and allosteric binding sites through molecular pharmacology and structural biology, and computationally designing ligand-cytotoxic or ligand-radiochemical conjugates.