Novel assay technology

LBA007 Discovery and characterization of oncogenic KRAS: RAF1 conformational modulators with *in vitro* and *in vivo* MAPK inhibition Elizabeth Donohue Vo[1], Juan Luengo[2], Hong Lin[2], Jerry Chen[2], Ben Reid[1], Brooke McDonough[1], Norman Fultang[2], Jillian Silva[1], Cameron Pitt[1]. [1]Quanta Therapeutics, Inc., South San Francisco, CA, [2]Quanta Therapeutics, Inc., Malvern, PA.

Oncogenic mutations in the RAS family are the most frequently occurring among human cancers. The recent development of KRAS-targeted covalent inhibitors display efficacy in KRAS-mutant tumors; however, this approach is limited to $KRAS^{G12C}$ mutant cancers. Here, we describe a novel drug discovery program to target the full-scope of mutant RAS-driven cancers through allosteric inhibition of the oncogenic KRAS-RAF1 signaling complex. To identify novel allosteric inhibitors, we developed a second harmonic generation (SHG) assay to detect conformational changes in the KRAS-RAF1 membrane-bound complex. Fully processed farnesylated and methylated KRAS4b-G12D protein ($KRAS^{G12D}$-FMe) was complexed with the RAF1 N-terminal RAS-binding domain (RBD) and cysteine rich domain (CRD) and immobilized on a phosphatidylserine-enriched bilayer. The RAF1RBD-CRD protein was rendered SHG-active by chemical conjugation and tethered to the bilayer by $KRAS^{G12D}$-FMe, thus replicating the physiological complex orientation. Ligands that disrupted or altered the orientation of the complex relative to the surface were identified from a curated diversity library of 60,000 chemical compounds based on % SHG signal change relative to baseline. Validated hit compounds were selected for SAR characterization and development wherein newly synthesized molecules were assayed by SHG and inhibition of cellular ERK phosphorylation by HTRF. Quanta (QTX) molecules were confirmed to rapidly inhibit ERK phosphorylation across multiple RAS-mutant cell lines by Western blot. Biochemical cellular target engagement studies reveal inhibition of RAF1:BRAF dimerization downstream of allosteric modulation of the KRAS:RAF1 signaling complex. Mass spectrometry with a photoactive derivative identified modified residues clustered along the KRAS:RAF1 interface, thus providing structural evidence for this unique mechanism of action. In addition to MAPK inhibition, we observe inhibition of cellular proliferation across mutant RAS cell lines. Cellular growth assays in the presence of QTX molecules combined with the SHP-2 inhibitor, RMC4550, displayed synergistic growth inhibition, providing a rationale combination therapy. Moreover, RAS-mutant cell-line derived xenograft tumors treated with QTX inhibitors elicited significant tumor growth inhibition and dose-dependent reductions in the pharmacodynamic MAPK targets, P-ERK and DUSP6, which is consistent with anti-tumor activity. This research illustrates a promising approach towards the development of mutant RAS-targeted inhibitors with a unique mechanism of action and target engagement.