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**P01.07 INTRACELLULAR AND EXTRACELLULAR BIOCHEMICAL ACTIVITIES OF V-DOMAIN IG-CONTAINING SUPPRESSOR OF T CELL ACTIVATION OR VISTA**

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**Background** V-domain Ig suppressor of T cell activation (VISTA) is the immune checkpoint protein which could display both receptor and ligand properties. It is also engaged in intracellular biochemical networks, where its role remains to be identified. We have recently reported [1] that galectin-9 could act as a ligand for VISTA, when it is located on the T cell surface where it displays receptor properties. This suppresses granzyne B-dependent cytotoxic activities of T cells [1]. However, VISTA could also display T cell suppressive activity when acting as a ligand derived from cancer cells. But, its receptor and downstream biochemical activities are yet to be discovered. The aim of this work is to investigate T cell suppressive activities of VISTA as a ligand and its contribution to intracellular biochemical networks.

**Materials and Methods** We used human cancer and non-cancerous cell lines including LN-18 glioblastoma cells, BEAS-2B bronchial epithelial cells, THP-1 human acute myeloid leukemia monocytes, Jurkat T cells, TALL-104 cytotoxic T cells and primary human CD3-positive T cells. Western blot analysis, ELISA set-ups, flow cytometry, on-cell Western analysis, fluorescent microscopy, quantitative RT-PCR, a wide range of biochemical assays and synchrotron radiation circular dichroism spectroscopy were used to conduct the studies.

**Results** In this work we identified the T-cell associated signaling receptor which recognises VISTA as a ligand. We found that VISTA downregulates PI-3-kinase/Akt [2, 3] and suppresses IL-2 production by T helpers. This effect is taking place due to VISTA-dependent activation of Src homology 2 (SH2) domain containing non-transmembrane protein tyrosine phosphatase (SHP2). By cytotoxic T cells (CTCs), VISTA-dependent impact on these signalling events leads to downregulation of BCL-XL family proteins thus preventing anti-apoptotic activities and allowing CTCs to die when they suffer from leakage of granzyme B from the granules inside them. This process of granzyme B leakage in CTCs can be induced by other components of immune evasion machinery operated by cancer cells (e.g. galectin-9 [1, 2]). We found that on the intracellular level VISTA can be involved in activation of AMP-dependent kinase (AMPK) and thus in control of mTOR activity in partnership with transforming growth factor-β-activated kinase 1 and galectin-9.

**Conclusions** Our results suggest that malignant tumours escape immune surveillance by operating complex biochemical machinery, where immune evasion pathways are cross-linked with each other forming complementary network. The immune checkpoint protein VISTA is a multifunctional component of cancer immune evasion machinery and T cell suppression displaying both ligand and receptor properties on the extracellular/inter-cellular levels. In addition, VISTA can be involved in intracellular signalling networks.

**REFERENCES**

**P01.08 DISCOVERY OF CRD1601, A POTENT AND SELECTIVE HPK1 INHIBITOR WITH ROBUST IN VIVO ANTI-CANCER ACTIVITY**


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**Background** Despite the promise shown by the CPIs targeting the PD-1 or CTLA-4 axes, recent estimates of the percentage of responders to these CPI across different cancers has been modest. One of the causes for non-response is impaired capacity of invigoration of exhausted T cells. Haematopoietic Progenitor Kinase (HPK-1, MAP4K1), a member of the STE20 family of serine/threonine kinases, is expressed predominantly in haematopoietic cells. HPK-1 acts as a negative regulator of T-cell and B-cell receptor activation and triggers the protosomal degradation and disrupts signalosome complexes downstream of TCR and BCR. Promising initial clinical results with novel HPK1 inhibitor have established HPK1 inhibition an attractive novel IO strategy that could combine with existing chemotherapies and immunotherapies.

**Materials and Methods** Pharmacophore based approach was used to design and synthesise novel small molecule HPK-1 inhibitors using in vitro enzymatic and primary cell based phenotypic cellular screens. CRD1601, the clinical candidate was selected based on its ability to enhance human T-cell proliferation through the induction of IL2, IFNγ and TNFα. Synergistic tumor models were used to assess anti-tumor activity.

**Results** CRD1601 is a potent single digit nanomolar HPK1 inhibitor as determined in enzymatic assay. It potently inhibits the phosphorylation of cellular SLP76 and leads to T cell activation and proliferation. Further, CRD1601 reversed the immunosuppressive effect of PGE2 and NECA on T cells. A single dose of CRD1601 in mice treated with an anti-CD3 antibody caused systemic enhancement of pro-inflammatory cytokines such as IL2, IFNγ and reduced pSLP76 levels in the spleen. CRD1601 demonstrated significant single agent activity in multiple murine tumor models and also synergizes with chemotherapy and immunotherapy.

**Conclusions** HPK-1 inhibition is a promising therapeutic modality that could augment the effects of existing anti-cancer treatments. CRD1601 is a potent and selective HPK-1 inhibitor with favorable drug like properties which shows promising in vivo activity in multiple tumor models as single agent and in combination with existing therapies.