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 granzyme 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 leukaemia 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 signalling receptor which recognise 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). In 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


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Abstracts

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

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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 synthesize 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 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.
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**P01.09 CRD3874-SI: A NOVEL ALLOSTERIC STING AGONIST WITH HIGH SYSTEMIC TOLERABILITY**

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**Background**

STING activation by natural and targeted competitive ligands leads to the generation of Type I interferons as well as pyroptosis and autophagy of the cells expressing STING caused by runaway inflammation. This overt inflammation is due to STING’s newly discovered proton channel activity. STING agonists tested in the clinic thus far have shown limited systemic tolerability and also a tendency to kill the very immune cells that they are designed to activate. CRD3874 is a novel allosteric small-molecule human STING agonist with a unique binding mode that activates canonical STING pathway while simultaneously blocking STING’s proton transport activity that is associated with pyroptosis and autophagy.

**Materials and Methods**

Allosteric binding was established using radioligand binding assays. Anti-cancer activity was established in human STING KI C57/BL6 mice (genOWay, France). Inflammamson and autophagy markers were studied in human and mouse cells. Safety studies were evaluated in cynomolgus monkeys.

**Results**

As opposed to competitive STING agonists, CRD3874 potentiates the binding of radiolabeled cGAMP to STING. CRD3874, like other STING agonists, led to dose dependent increase in TypeI interferons and other pro-inflammatory cytokines from immune cells. In contrast to other STING agonists, activation of STING by CRD3874 did not lead to induction of autophagy or Inflammamson markers in cells or mice. Intravenous administration of CRD3874-SI in human STING knock-in mice caused potent anti-tumor effect and survival benefit in multiple murine tumor models. Intravenous infusion of the compound was well tolerated at high doses in the primate GLP study and caused exposure dependent increases in cytokines. This profile of retaining the high efficacy of a STING agonist while demonstrating systemic safety is unique to CRD3874-SI.

**Conclusions**

CRD3874-SI is a systemically administered novel allosteric STING agonist with promising single agent activity and an excellent IV safety profile. An investigator sponsored FIH phase 1 trial with CRD3874-SI has been initiated at Memorial Sloan Kettering, NY, in sarcoma, MCC patients under the supervision of Dr. Ciara Kelly (NCT06021626).

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**P01.10 REAL-TIME INTERACTION CYTOMETRY REVEALS DIFFERENT BINDING KINETICS OF ANTIBODIES TARGETING MEMBRANE PROTEINS ON FIXED VERSUS LIVING CELLS**

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**Background**

The field of therapeutic antibody development has witnessed significant growth since the introduction of the first therapeutic monoclonal anti-CD3 antibody, Muronomab, in 1986 [1]. Numerous investigations indicate that the dissociation rate of antibodies plays a predictive role in their clinical effectiveness [2, 3]. Therefore, it is imperative to conduct kinetic rate analyses to enhance the efficacy and safety of these therapeutic agents. Transmembrane proteins such as PD-(L)1, CD3 or HER2 represent the most common targets. Their binding kinetics are influenced by neighbouring coreceptors as well as by their density and mobility within the membrane. Preserving these target molecules within their native cell membrane...