TT-816, A NOVEL SMALL MOLECULE IMMUNE CHECKPOINT INHIBITOR TARGETING CANNABINOID CB2 RECEPTOR, STIMULATES INNATE AND ADAPTIVE IMMUNITY FOR CANCER THERAPY

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**Background** The endocannabinoid system is widely expressed in the human body, including the innate and adaptive immune system, where endocannabinoids, Δ9-tetrahydrocannabinol and synthetic ligands regulate immune response. The effects of endocannabinoids on immune regulation are primarily mediated by G-protein coupled cannabinoid CB2 receptors (CB2R) via several mechanisms, including development, migration, proliferation and effector functions. The upregulated expression of CB2R and elevated levels of endocannabinoids have been observed in a variety of tumor microenvironments and are associated with the aggressiveness of cancer.

**Methods** Membranes prepared from CHO-K1 cells stably expressing human CB2R were used for receptor binding assays in the presence of TT-816 and [3H]CP-55,940, and for GTPγS binding assay in the presence of TT-816, 10 μM GDP and 0.3 nM [35S]GTPγS. cAMP assay was performed by incubating the CHO-K-1 cells for 30 min with TT-816, 25 μM forskolin and 12 nM CP-55,940, or with TT-816 and 5 μM forskolin. NK cell function was determined by co-culturing TT-816 pretreated NK cells with K562 cancer cells for 24 hours. The mixed lymphocyte reaction assay was conducted by co-culturing human CD4+ T cells with monocyte-derived dendritic cells. Cell viability was measured by FACS and IFN-γ by MSD.

**Results** TT-816 is a competitive and selective CB2R antagonist. It bound to human CB2R with an IC50 26.2 nM, showing greater than 380-fold selectivity over cannabinoid CB1 receptors. The ability of TT-816 to inhibit the constitutive activity of CB2R was characterized in both GTPγS and cAMP assays. TT-816 concentration-dependently inhibited the basal GTPγS binding response, antagonized CB2R agonist-mediated cAMP production and enhanced the forskolin response on basal cAMP level. Consistent with its inhibition of CB2R function, TT-816 inhibited the growth of human breast, colorectal and lung cancer cells. In addition, TT-816 concentration-dependently enhanced the functions of NK cells, dendritic cells and T cells. It increased NK cell killing of the human cancer cells and IFN-γ production, significantly stimulated the expression of CD86, HLA-DR, IL-12 and TNF-α in monocyte-derived dendritic cells, and enhanced CD4+ T cell proliferation and IFN-γ production in a mixed lymphocyte reaction assay.

**Conclusions** TT-816 is a novel, oral small molecule immune checkpoint inhibitor that selectively blocks CB2R on cancer cells and immune cells. Preclinical data have demonstrated that it stimulates antitumor innate and adaptive immune response and inhibits cancer cell proliferation. TT-816 is currently undergoing phase 1 clinical trials for the treatment of a broad range of solid tumors.