

Conclusions These data support an immune-mediated anti-tumor effect of IL411 inhibition by CB-668, and suggest inhibition of IL411 represents a novel strategy for cancer immunotherapy.

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BT7480, A FULLY SYNTHETIC TUMOR-TARGETED IMMUNE CELL AGONIST (TICA™) INDUCES TUMOR LOCALIZED CD137 AGONISM AND MODULATION OF TUMOR IMMUNE MICROENVIRONMENT

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Background After disappointing first clinical experiences with agonistic anti-CD137 (4-1BB) antibodies, a new generation of both systemic and targeted CD137 agonists is entering clinical development.^{1–3} These strategies rely on biologic agents with suboptimal properties for CD137 agonism due to their relatively large sizes and long circulating half-lives. These properties may limit their tissue penetration and cause sustained agonism resulting in overstimulation and activation-induced cell death of lymphocytes due to continuous exposure. Fully synthetic constrained bicyclic peptides (Bicycles™) with antibody-like affinities and target selectivity are uniquely suited to circumvent the above barriers to optimal targeted CD137 agonistic therapeutics. BT7480 is a tumor-targeted immune cell agonist (TICA) designed to deliver a highly potent CD137 agonist to Nectin-4 overexpressing tumor tissue with a flexible dosing schedule maximizing anti-tumor activity while circumventing the need for continuous systemic exposure.

Methods BT7480 functional activity in vitro was analyzed by measuring IL-2 and IFN gamma production from primary human PBMC/tumor cell co-cultures. BT7480 in vivo activity was determined in huCD137-syngeneic tumor models using tumor immune cell and transcriptional profiling by FACS, IHC, and Nanostring as well as tumor growth kinetics as read-outs.

Results BT7480 binds potently and simultaneously to Nectin-4 and CD137 as assessed biochemically and caused Nectin-4-dependent CD137 agonism in primary human PBMC co-cultured with tumor cells. Treatment of Nectin-4 expressing tumors in immunocompetent mice with BT7480 leads to profound reprogramming of the tumor immune microenvironment including increased T cell infiltration and upregulation of a cytotoxic cell gene signature. BT7480 treatment induces complete tumor regressions and subsequent resistance to tumor rechallenge. TICA-dependent anti-tumor activity and established immunologic memory are dependent on cytotoxic T cells. Importantly, BT7480 in vivo activity is not dependent on continuous plasma exposure since once weekly dosing of BT7480 provides a maximum anti-tumor activity despite minimal BT7480 plasma exposure after day 2. BT7480 demonstrates linear pharmacokinetics in non-human primates and appears well tolerated at exposures in excess of the predicted efficacious exposure in humans.

Conclusions BT7480 is a highly potent Nectin-4 expression dependent CD137 agonist with optimal target binding, pharmacologic, and pharmacokinetic properties that enable intermittent dosing for curative effect through modulation of tumor immune microenvironment in syngeneic mouse tumor models. BT7480 is currently being evaluated in IND-enabling safety studies.

Ethics Approval The care and use of animals were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of WuXi AppTec and conducted in accordance with the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

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DISCOVERY OF A NOVEL EP2 AND EP4 DUAL ANTAGONIST

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Background Prostaglandin E2 (PGE2) is one of the most abundant prostaglandins, with crucial roles in normal and pathologic physiology. Especially, PGE2 levels are abnormally elevated in many cancers, and high levels of PGE2 are known to be pro-tumorigenic, likely due to the immune suppressive effect in the tumor microenvironment.^{1–4} There are four types of PGE2 receptors; EP1, EP2, EP3 and EP4. Among them, EP2 and EP4 activate adenylate cyclase and increase cAMP levels, which induce the cAMP-dependent protein kinase (PKA) signaling pathway. EP2 and EP4 are expressed in various immune cells (e.g. macrophages, dendritic cells, NK cells and CTLs), and genetic and pharmacological inhibition of EP2 and EP4 increases immune activity and suppresses tumor growth.

Methods To evaluate the binding affinity against EP2 and EP4, a radioligand binding assay was conducted using EP2 or EP4 transfected HEK293 cells. Cell membrane homogenates were incubated with [³H]PGE2 in the absence or presence of the test compounds. Following incubation, the samples were filtered rapidly under vacuum through glass fiber filters and rinsed several times with cold Tris-HCl. The filters were dried then counted for radioactivity in a scintillation counter using a scintillation cocktail. The results were expressed as a percent inhibition of the control radioligand specific binding. The antagonistic activity against EP2 and EP4 was assessed via LANCE Ultra cAMP assay (PerkinElmer). HEK293 cells overexpressing EP2 or EP4 were seeded into the plate and treated by PGE2 and compounds. After 30 minutes of incubation, cAMP levels were measured by FRET signal using Varioskan plate reader, following the manufacturer's protocol. Anti-tumor activity of KT-00113 was evaluated using LLC1 syngeneic model. When tumor volume reached approximately 100 mm³, mice were treated PO, QD. Tumor size was measured twice every week.