DUAL BLOCKADE OF THE PD-1 CHECKPOINT PATHWAY AND THE ADENOSINERGIC NEGATIVE FEEDBACK SIGNALING PATHWAY WITH A PD-1/CD73 Bispecific Antibody for Cancer Immune Therapy

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Background CD73 (ecto-5'-nucleotidase) is an ecto-nucleotidase that dephosphorylate AMP to form adenosine. Activation of adenosine signaling pathway in immune cells leads to the suppression of effector functions, down-regulate macrophage phagocytosis, inhibit pro-inflammatory cytokine release, as well as yield aberrantly differentiated dendritic cells producing pro-tumorigenic molecules.1 In the tumor microenvironment, adenosinergic negative feedback signaling facilitated immune suppression is considered an important mechanism for immune evasion of cancer cells.2, 3 Combination of CD73 and anti-PD-1 antibody has shown promising activity in suppressing tumor growth. Hence, we developed AK119, an anti-human CD73 monoclonal antibody, and AK123, a bi-specific antibody targeting both PD-1 and CD73 for immune therapy of cancer.

Methods AK119 is a humanized antibody against CD73 and AK123 is a tetrameric bi-specific antibody targeting PD-1 and CD73. Binding assays of AK119 and AK123 to antigens, and antigen expressing cells were performed by using ELISA, FACS and PACE assays. In-vitro assays to investigate the activity of AK119 and AK123 to inhibit CD73 enzymatic activity in modified CellTiter-Glo assay, to induce endocytosis of CD73, and to activate B cells were performed. Assay to evaluate AK123 activity on T cell activation were additionally performed. Moreover, the activities of AK119 and AK123 to mediate ADCC, CDC in CD73 expressing cells were also evaluated.

Results AK119 and AK123 could bind to its respective soluble or membrane antigens expressing on PBMCs, MDA-MB-231, and U87-MG cells with high affinity. Results from cell-based assays indicated that AK119 and AK123 effectively inhibited nucleotidase enzyme activity of CD73, mediated endocytosis of CD73, and induced B cell activation by upregulating CD69 and CD83 expression on B cells, and showed more robust CD73 blocking and B cell activation activities compared to leading clinical candidate targeting CD73. AK123 could also block PD-1/PD-L1 interaction and enhance T cell activation.

Conclusions In summary, AK119 and AK123 represent good preclinical biological properties, which support its further development as an anti-cancer immunotherapy or treating other diseases.

REFERENCES
Background The tumor-associated antigen 5T4 is expressed across a wide range of solid cancers. DuoBody-CD3x5T4 is a bispecific antibody (bsAb) that crosslinks CD3 on T cells with 5T4 on tumor cells, thereby inducing T-cell activation and T-cell mediated cytotoxicity in 5T4-expressing tumor cells. Here, we tested the capacity of DuoBody-CD3x5T4 to engage different T-cell subsets in vitro and investigated the mechanism of action (MoA) in vivo by combining preclinical efficacy studies with exploratory pharmacodynamic (PD) biomarker analyses.

Methods Immunohistochemistry was performed on patient-derived tumor tissue-microarrays using a commercial 5T4 monoclonal antibody (EPR5329). The capacity of DuoBody-CD3x5T4 to engage naïve and memory T-cell subsets was assessed in co-cultures of T cells and 5T4-positive tumor cells, using T-cell activation and T-cell mediated cytotoxicity as readouts. Anti-tumor activity in vivo as well as peripheral and intratumoral PD biomarkers were investigated in humanized mouse models bearing 5T4-expressing cell line-derived xenograft (CDX) or patient-derived xenograft (PDX) tumor models.

Results High prevalence of 5T4 expression (in >86% of biopsies) was observed in NSCLC, SCCHN, TNBC, bladder, esophageal, prostate and uterine cancer. In co-cultures of 5T4 + tumor cells and T cells in vitro, DuoBody-CD3x5T4 induced dose-dependent cytotoxicity, associated with T-cell activation, proliferation, and cytokine, perforin and granzyme production. Crosslinking of T cells with 5T4-expressing tumor cells was essential as no cytotoxicity was observed in CRISPR-production. Crosslinking of T cells with 5T4-expressing tumor cells was essential as no cytotoxicity was observed in CRISPR-Cas9-generated 5T4-knockout tumor cells or with control bsAbs targeting only CD3 or 5T4. Importantly, naïve and memory CD4+ or CD8+ T-cell subsets had equal capacity to mediate DuoBody-CD3x5T4-induced cytotoxicity, although naïve T-cell subsets showed slower kinetics. DuoBody-CD3x5T4 (0.5–20 mg/kg) demonstrated anti-tumor activity in 5T4+ breast and prostate cancer CDX and lung cancer PDX models in humanized mice. Treatment with DuoBody-CD3x5T4 was associated with intratumoral and peripheral T-cell activation as well as elevated cytokine levels, including IFNγ, IL-6 and IL-8, in peripheral blood.

Conclusions DuoBody-CD3x5T4 induced T-cell mediated cytotoxicity in 5T4-expressing tumor cells, associated with T-cell activation and cytokine production in vitro. DuoBody-CD3x5T4 efficiently engaged naïve and memory T cells within both CD4+ and CD8+ T-cell populations to induce T-cell mediated cytotoxicity in 5T4+ tumor cells. In humanized CDX and PDX mouse models, DuoBody-CD3x5T4 showed anti-tumor activity, in addition to PD biomarkers associated with T-cell activation in the tumor and periphery. Currently, DuoBody-CD3x5T4 is being investigated in a first-in-human clinical trial for the treatment of solid tumors (NCT04424641), in which exploratory biomarker analyses to study the clinical MoA and PD are included.

Ethics Approval All experimental animal studies were performed according to Codika BioSciences IACUC approved AUP CB2020-001.

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Abstracts

705 ANTI-TUMOR ACTIVITY OF CB-668, A POTENT, SELECTIVE AND ORALLY BIOAVAILABLE SMALL-MOLECULE INHIBITOR OF THE IMMUNO-SUPPRESSIVE ENZYME INTERLEUKIN 4 (IL-4)-INDUCED GENE 1 (IL4I1)

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Background Tumors evade destruction by the immune system through multiple mechanisms including altering metabolism in the tumor microenvironment. Metabolic control of immune responses occurs through depletion of essential nutrients or accumulation of toxic metabolites that impair immune cell function and promote tumor growth. The secreted enzyme interleukin 4 (IL-4)-induced gene 1 (IL4I1) is an L-phenylalanine oxidase that catabolizes phenylalanine and produces phenyl-pyruvate and hydrogen peroxide. IL4I1 regulates several aspects of adaptive immunity in mice, including inhibition of cytotoxic T cells through its production of hydrogen peroxide (reviewed in1). In human tumors, IL4I1 expression is significantly elevated relative to normal tissues and is notably high in ovarian tumors and B cell lymphomas. Motivated by the hypothesis that IL4I1 is an immuno-metabolic enzyme that suppresses anti-tumor immunity, we discovered CB-668, the first known small-molecule inhibitor of IL4I1.

Methods IL4I1 enzymatic activity was measured using an HRP-coupled enzyme assay. RNA in-situ hybridization was carried out on the RNAscope platform. Syngeneic mouse tumor models were used to evaluate the anti-tumor activity of CB-668. The level of phenyl-pyruvate in tumor homogenates was measured by LC/MS.

Results Our clinical candidate, CB-668 is a potent and selective non-competitive inhibitor of IL4I1 (IC50 = 15 nM). CB-668 has favorable in vitro ADME properties and showed low clearance and high oral bioavailability in rodents. Twice-daily oral administration of CB-668 was well-tolerated in mice and resulted in single-agent anti-tumor activity in the syngeneic mouse tumor models B16-F10, A20, and EG7. Oral CB-668 administration reduced the levels of phenyl-pyruvate in the tumor, consistent with inhibition of IL4I1 enzymatic activity. Anti-tumor activity of CB-668 was immune cell-mediated since efficacy was abrogated in CD8-depleted mice, and CB-668 treatment caused increased expression of pro-inflammatory immune genes in the tumor. Moreover, CB-668 had no direct anti-proliferative activity on tumor cells grown in vitro (IC50 > 50 μM). CB-668 also favorably combined with anti-PD-L1 therapy to reduce tumor growth in the B16-F10 tumor model.

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