overcome the checkpoint resistance seen in contemporary treatments involving PD-1.

**Ethics Approval** All mouse studies described in this work were carried out in accordance with the principles of the Guide for the Care and Use of Animals and were approved by the Institutional Animal Care and Use Committee of Dartmouth College, NH, USA (protocol 2012).

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**DISCOVERY OF CLINICAL CANDIDATE IK-175, A SELECTIVE ORALLY ACTIVE AHR ANTAGONIST**

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**Background** Aryl Hydrocarbon Receptor (AHR) is a transcription factor that regulates the activity of multiple innate and adaptive immune cells subsequent to binding to a diverse set of endogenous and exogenous ligands. One such endogenous AHR ligand is kynurenine, generated from the precursor tryptophan by indoleamine-pyrrole 2,3-dioxygenase 1 (IDO1) and tryptophan 2,3-dioxygenase 2 (TDO2). Binding of kynurenine to AHR leads to a net immunosuppressive tumor microenvironment. In addition, increased levels of serum kynurenine are associated with resistance to checkpoint inhibitors. Given that kynurenine can be generated by both IDO1 and TDO2 and that AHR is activated by multiple other endogenous ligands, AHR inhibition provides a novel and ideal approach to overcome immunosuppression in a broad range of cancers.

**Methods** We sought to identify an orally active AHR antagonist as an immunomodulatory agent for the treatment of solid tumors. Lead optimization efforts identified IK-175 as an AHR antagonist with a favorable ADME and pharmacokinetic profile in preclinical species.

**Results** IK-175 inhibits AHR activity in rodent and human cancer cell lines as well as human and nonhuman primate primary immune cells, with concentration dependent effects on AHR target gene expression and cytokine release. IK-175 is inactive in a broad panel of kinases, receptors, and transporters. Orally administered IK-175 dose-dependently blocks ligand-stimulated-AHR activation of Cyp1a1 transcription in liver and spleen, demonstrating on-target in vivo activity in mice. IK-175 alone and in combination with an anti-PD-1 antibody demonstrates significant antitumor activity in syngeneic mouse models of colorectal cancer (CT26.WT) and melanoma (B16-IDO1). In addition, IK-175 in combination with liposomal doxorubicin demonstrates antitumor activity in syngeneic mouse models of colorectal cancer (CT26.WT and MC38).

**Conclusions** These studies provide rationale for targeting AHR in cancer patients. Ikena will evaluate the anti-tumor activity of IK-175 as a single agent in cancers with activated AHR and in combination with other therapies. Overall, our data demonstrates that IK-175 is a selective orally active AHR antagonist that inhibits tumor growth and reverses immune suppression in mouse tumors models. IK-175 is currently being evaluated in a Phase 1 clinical trial in patients with advanced solid tumors and urothelial carcinoma (Clinicaltrials.gov NCT04200963).

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**NEOADJUVANT CYCLIC DINUCLEOTIDES COMBINED WITH INTERLEUKIN-2 AND ANTI-PD-1 ANTI-BODY LIMIT LUNG METASTASIS OF ORTHOTOPIC BREAST TUMORS THROUGH PROLONGED NK CELL ACTIVATION**

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**Background** Cyclic dinucleotides (CDN) – agonists of stimulator of interferon genes – can initiate potent anti-tumor immunity by activating antigen presenting cells which prime CD8+ T cells. Recent studies have also highlighted CDN activation of NK cells via IL-15 in T cell-resistant tumors. Thus far, limited analysis has been made of the impact of CDN-based therapies on cancer metastasis. We employed a surgical resection model of metastatic mammary carcinoma to examine the effects of surgery – a predominant breast cancer intervention – and lung metastasis on neoadjuvant therapy with CDNs combined with other clinically-relevant immunotherapies including IL-2 and anti-PD-1.

**Methods** 4T1-luciferase cells were inoculated in the mammary fat pad, palpable tumors were treated with immunotherapy starting eight days later, any remaining primary tumor was surgically resected on day 17, and metastases were monitored by luciferase imaging. Combinations of intratumoral bisphosphonatoate 2’3’-c-di-AMP (CDN), intraperitoneal (i.p.) albumin-IL2 fusion protein (Alb-IL2), and i.p. anti-PD-1 were tested in this model by measuring primary tumor growth and monitoring overall survival. CD8+ T cells, CD4+ T cells, or NK cells were depleted using anti-CD8 (2.43), anti-CD4 (GK1.5), and anti-asialo-GM1 antibodies, respectively, administered i.p. every 3 days beginning one day prior to treatment initiation. Immunophenotyping of primary tumors and lungs was conducted at several timepoints after starting therapy.

**Results** In mice bearing orthotopic 4T1-luciferase tumors, administration of three doses of CDN resulted in no cures in the absence of surgical resection. When administered prior to surgical resection CDN monotherapy yields a 20% cure rate and enhanced median overall survival compared to untreated mice (median survival 44.5 days vs 38 days, p=0.0026). Combination of CDN with Alb-IL2 and anti-PD-1 substantially improved survival, with 60% of mice surviving long-term. Through cellular depletions we determined that neither CD8+ nor CD4+ T cells were required for efficacy in this neoadjuvant therapy model, while NK cell depletion decreased survival rate by approximately 50%. Lung immunophenotyping of CDN/Alb-IL2/anti-PD-1-treated mice revealed a near doubling of the absolute NK cell count compared to untreated controls. More strikingly, lung infiltrating NK cells in the CDN/Alb-IL2/anti-PD-1 cohort exhibited prolonged granzyme B production compared to CDN monotherapy (6.24x higher after 6 days) and Alb-IL2 monotherapy (25x higher after 6 days) cohorts.

**Conclusions** Our findings suggest that combining intratumoral CDN with systemic Alb-IL2 and anti-PD-1 can delay the growth of primary breast tumors and limit metastatic outgrowth in the lungs. Efficacy is attributed to sustained cytotoxicity of NK cells.

**Ethics Approval** All mouse experiments were approved by MIT’s Committee on Animal Care, protocol #0720-070-23.

**REFERENCES**

COMBINATION OF RHIL-7-HyFC AND ANTI-PD-L1xCD3-bispecific antibody enhances antitumor response in mice

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Background rhIL-7-hyFc is a hybrid Fc-fused recombinant human interleukin-7 (NT-I7; efineptakin-alfa) with enhanced bioactivity. In a previous study, we found that a systemic administration of rhIL-7-hyFc induced antitumor effect by increasing CD8+ T cells in the tumor microenvironment. rhIL-7-hyFc monotherapy increased not only PD-1+ tumor-reactive but also intratumoral PD-1 bystander CD8+ T cells. Therefore, we hypothesized that the activation of PD-1 bystander T cells in tumors would enhance the antitumor activity of rhIL-7-hyFc. Here we evaluated the antitumor effect of combination therapy with rhIL-7-hyFc and a bispecific antibody (bsAb), anti-PD-L1xCD3, targeting both a tumor-associated antigen (PD-L1) and a T-cell stimulatory antigen (CD3ε).

Methods In vitro cell culture. For analysis of T cell activation and cytotoxicity, splenocytes were isolated from PD-L1 knockout (KO) mice and co-cultured with either wild type (MC-38WT) and PD-L1-depleted (MC-38SPD-L1) tumor cells in the presence of bsAb for 48 hours. In vivo treatment. Tumor-bearing mice were treated subcutaneously (s.c.) with 1.25 mg/kg of rhIL-7-hyFc. An indicated dose of bsAb was daily treated intravenously (i.v.) or intratumoral (i.t.) route starting from 3 days after the rhIL-7-hyFc treatment for a total 5 times.

Preparation of tumor-infiltrating cells. Tumor tissues were harvested after 7 days of rhIL-7-hyFc treatment. Single-cell suspensions were prepared through mechanical separation followed by caspase D and DNAse I treatment.

Results Anti-PD-L1xCD3 bsAb induced the PD-L1-specific activation and cytotoxicity of CD8+ T cells in vitro (figure 1). rhIL-7-hyFc combined with a systemic administration of bsAb enhanced antitumor responses, although loss of body-weight was shown with high-dose bsAb combination (figure 2) The combination of rhIL-7-hyFc with a systemic administration of bsAb increased not only the frequency of CD8+ T cells in tumors but also the PD-1 bystander CD8+ T cells with enhanced expression of a Granzyme B (figure 3). Intratumoral administration of high-dose bsAb enhanced antitumor response of rhIL-7-hyFc without body-weight loss (figure 4).

Abstract 450 Figure 1 MC-38WT and MC-38SPD-L1 tumor cells were cultured in vitro. (a) PD-L1 expression levels on each cell line. (b) Splenocytes isolated from PD-L1 KO mice were co-cultured with indicated tumor cells (E:T = 20:1) in the presence of bsAb. Expression levels of activation markers, such as CD69 and CD25, on the CD8+ T cells were analyzed by flow cytometry. (c) Cytotoxicity against tumors was analyzed in the presence of bsAb. Cytotoxicity was calculated using the formula: (1 - live target cells/sample/live target cells/control) × 100

Abstract 450 Figure 2 (a-b) Mice bearing MC-38 tumors were treated with different doses of bsAb (i.v.) as indicated in (a) (n = 5 per group). (b) Shown are mean tumor growth curves (left) and body-weight changes (right). (c-d) Mice bearing MC-38 tumors were treated either 1.25 mg/kg of rhIL-7-hyFc (s.c.), indicated doses of bsAb (i.v.), or combination of each therapy as indicated in (c). In the case of combination therapy with 1 ug bsAb, mice were treated only for the first 3 doses of bsAb because of body-weight loss (n = 5–7 per group). (d) Shown are mean tumor growth curves (left) and body-weight changes (right). Arrows indicate the dosing of bsAb. Data are represented as mean ± SEM. Statistical significance was analyzed by two-way ANOVA with bonferroni’s multiple comparisons for (b and d).

Abstract 450 Figure 3 (a) Experimental scheme for the analysis of tumor-infiltrating T cells (n = 4 per group). (b) Frequencies of CD8+, CD4+Foxp3+ T helper (Th), and CD4+Foxp3- regulatory (Treg) cells among CD4+ T cells. (c) Frequencies of CD4+Foxp3- Treg cells among CD4+ T cells. (d) The ratio of CD8+ T cells to Treg cells. (e) Frequencies of Granzyme B (GzmB) expressing cells among PD-1- or PD-1+ CD8+ T cells. Data are represented as mean ± SD. Statistical significance was analyzed by one-way ANOVA with bonferroni’s multiple comparisons. *P<0.05; **P<0.01; ***P<0.001