regulatory T cells in the bone marrow, thereby reducing leukemia burden.

Conclusions Our results suggest that eliminating STAT3 permits the TLR9-driven reprogramming of AML cells into APCs to unleash T cell-mediated responses against leukemia.

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PRE-CLINICAL DEVELOPMENT OF TNFR2 LIGAND-BLOCKING BI-1808 FOR CANCER IMMUNOTHERAPY


Background The pleiotropic TNF-alpha:TNFR axis plays a central role in the immune system. While the cellular expression of TNFR1 is broad, TNFR2 expression is mainly restricted to immune cells. The therapeutic potential of targeting TNFR2 for cancer treatment has been previously indicated and to gain further insight, we characterized a wide panel antibodies, generated from the n-CoDeR F.I.R.S.T target and antibody discovery platform. We identified parallel human and mouse TNFR2 specific, complete ligand (TNF-alpha) blocking antibodies and could show potent anti-tumor activity in several immune-competent models, both as single agent and in combination with anti-PD1 using a BI-1808 murine surrogate. The mechanism-of-action was shown to be FcγR dependent and likely mediated through a combination of intra-tumor T reg depletion, CD8+ T cell expansion and modulation of tumor-associated myeloid cells. These findings were confirmed using BI-1808 in a humanized mouse model.

Methods To address safety of the human lead-candidate BI-1808 two toxicological studies were performed in cynomolagus monkeys. The first study was a dose-range-finding study and the second a GLP study where three doses (2, 20 and 200 mg/kg) were given weekly for four consecutive weeks followed by a recovery period of eight weeks. In addition, cytokine release was further studied in T cell stimulation assays and in a humanized mouse model. Moreover, the BI-1808 murine surrogate was used to study the relationship between dose, receptor occupancy (RO) and efficacy in immune competent mouse cancer experimental models.

Results Four weekly administrations of BI-1808 to cynomolgous monkeys were well tolerated at all doses, with no associated clinical signs, and no histopathological changes. Non-adverse and reversible increases in neutrophil counts and decreases in T cells were observed at all dose levels. No drug-related adverse events were observed and consequently the NOAEL for BI-1808 was determined to be 200 mg/kg. Pharmacokinetic studies demonstrated an expected half-life of two weeks, covering the time it takes to generate a full adaptive Immune response.

Conclusions There is a clear association between RO and therapeutic effect and BI-1808 is well tolerated at doses associated with high and sustained RO. Collectively, these studies were used to determine the starting dose in upcoming phase I/II study in solid cancer aiming for first-patient in during December 2020.

Ethics Approval The study on cynomolgous monkeys was conducted by Citox/Charles River Laboratories in compliance with animal health regulations, in particular: Council Directive No. 2010/63/EU of 22 September 2010 and French decree No. 2013-118 of 01 February 2013 on the protection of animals used for scientific purposes. Studies in mice were approved by the Swedish Animal Experiment Ethics Board, ethical permit/ethical license numbers 5.2.18-17196/2018 and 5.8.18-03333/2020.

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SRF114 IS A FULLY HUMAN, CCR8 SELECTIVE IGG1 ANTIBODY THAT INDUCES DESTRUCTION OF TUMOR TREGS THROUGH ADCC

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1Surface Oncology, Inc., Cambridge, MA, USA; 2Vaccinex, Inc., Rochester, NY, USA

Background T regulatory cells (Tregs) are potent suppressors of immune activation in the periphery and tumor microenvironment (TME). Tumor-infiltrating Tregs have also been associated with resistance to cancer therapies. Loss of peripheral Tregs can lead to widespread autoimmunity and tissue destruction; therefore, specifically depleting tumor Tregs is an attractive therapeutic approach to locally activate the immune system. CCR8 expression is highly restricted to tumor Tregs across multiple cancer types, supporting the notion that CCR8 targeting may induce tumor-specific Treg depletion while sparing peripheral Tregs. Moreover, depletion of CCR8+ Tregs leads to significant tumor growth inhibition with correlative tumor Treg depletion in established CT-26 tumors. These data provide rationale for targeting CCR8 to deplete tumor Tregs. Here, we describe the development of SRF114, a fully human IgG1 anti-CCR8 antibody that induces tumor Treg destruction through antibody-dependent cellular cytotoxicity (ADCC).

Methods Virus panning against the N-terminal region of CCR8 and subsequent affinity maturation process led to discovery of SRF114, a fully human monoclonal antibody that is specific to CCR8. To evaluate SRF114 specificity, binding was profiled on recombinant CCR8 N-terminus, CCR8+ and CCR8- cell lines, and primary cell cultures. An extracellular protein target cell microarray was used to further validate specificity. SRF114 functional assays included the Promega CD16 (V/F variants) ADCC signaling assay, PBMC/293T-hCCR8+ cell co-culture experiments, and natural killer (NK)-activation assays targeting Raji-CCR8+ cell lines. To confirm tumor Treg binding and depletion, NK allogenic co-culture experiments were performed with SRF114 using isolated tumor infiltrating lymphocyte cultures from freshly resected tumors.

Results A tumor Treg-restricted pattern of CCR8 expression was confirmed using publicly available datasets and profiling of CCR8 expression on Tregs from fresh tumor tissues. SRF114 binds to CCR8-expressing 293T cells with pM affinity and not to parental cells. SRF114 does not bind any cell populations in PBMCs from healthy donors and has no other protein targets assessed by cell microarray. In dose-dependent ADCC assays, SRF114 induces cell killing with pM EC50 values, which is further enhanced by removing the fucose groups from the Fc-domain. Finally, SRF114 specifically binds to human tumor Tregs and induces killing of Tregs in NK co-culture experiments.

Conclusions The fully human anti-CCR8 antibody SRF114 specifically binds to and targets CCR8+ tumor Tregs for depletion, likely through ADCC. Through this mechanism, SRF114

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**INCREASED SERUM LEVELS OF EBI3 ARE ASSOCIATED WITH POOR OUTCOME IN HEPATOCELLULAR CARCINOMA PATIENTS AND SRF388, A FIRST-IN-CLASS IL-27 BLOCKING ANTIBODY, INHIBITS THE GROWTH OF MURINE LIVER TUMORS**

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Background IL-27 is a heterodimeric cytokine consisting of IL-27p28 and Epstein-Barr virus-induced gene 3 (EBI3) that binds the IL-27 receptor subunit alpha and glycoprotein 130. IL-27 is produced by activated macrophages and dendritic cells and limits the intensity and duration of immune responses in the tumor microenvironment by inducing the expression of immunoregulatory receptors (PD-L1, TIM3, LAG-3, TIGIT) and inhibiting production of proinflammatory cytokines (IFNγ). The IL-27 subunit EBI3 is elevated in plasma of human tumors. Blockade of IL-27 with SRF388, a first-in-class IL-27-blocking antibody that binds to IL-27p28, to reverse IL-27-induced inhibition of cytokine production in activated peripheral blood mononuclear cells from patients with hepatocellular carcinoma (HCC), the role of IL-27 was further explored in patient samples and a mouse model of HCC.

Methods Gene expression profiles from the Cancer Genome Atlas (TCGA) were analyzed to identify tumors with elevated IL-27 transcripts. Serum from patients with HCC was analyzed for levels of the IL-27 subunit EBI3. The ability of SRF388, a first-class IL-27-blocking antibody that binds to IL-27p28, to reverse IL-27-induced inhibition of cytokine production in human immune cell cultures from patients with HCC was assessed in vitro. Finally, the anti-tumor activity of SRF388 was assessed in an orthotopic murine model of HCC.

Results TCGA expression data revealed that IL-27p28 transcripts were elevated in tumors from patients with HCC relative to other indications. Serum levels of EBI3 were: 1) elevated in a subset of HCC patients; 2) inversely correlated with survival; 3) independent of serum alpha-fetoprotein levels; and 4) elevated in both hepatitis B/C virus positive and negative patients. Treatment with SRF388 stimulated increased cytokine production in activated peripheral blood mononuclear cells from patients with HCC that was further enhanced when combined with PD-1 blockade. Furthermore, SRF388 inhibited the growth of orthotopic Hepa-16 liver tumors. mRNA transcriptional profiling of treated tumors revealed that SRF388 profoundly altered the transcriptional landscape in this model. In particular, treatment with SRF388 inhibited expression of immunoregulatory receptors PD-L1 and TIGIT, repressed transcripts associated with TGF-β signaling, and altered myeloid and natural killer cell transcripts.

Conclusions These data indicate that elevated IL-27 subunit EBI3 is a hallmark of HCC and is associated with poor outcomes in these patients. Blockade of IL-27 with SRF388, currently being evaluated in a Phase 1 clinical trial in patients with advanced solid tumors (NCT04374877), may represent a promising therapy for patients with HCC where it can potentiate anti-tumor immune responses.

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**ANTIBIOTICS AND RESPONSE TO IMMUNOTHERAPY: REAL-WORLD EXPERIENCE**

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Background Immune checkpoint inhibitors (ICI) have altered the therapeutic paradigm of advanced non-small cell lung cancer (NSCLC) and have become an attractive treatment strategy in several malignancies. The identification of reliable predictors associated with resistance is essential to dictate new approaches to broaden responder groups. Growing evidence has shown that the gut microbiome is an important regulator of the systemic immune system and is involved in the response to ICI. The aim of the study was to evaluate the association between antibiotics use & ICI efficacy in advanced NSCLC.

Methods A retrospective, single-centre study of unselected patients with advanced NSCLC treated with ICI between June 2016 to May 2019. We included consecutive patients who received at least one dose of PD-1 inhibitors (Nivolumab or pembrolizumab) Clinico-pathologic characteristics and the status of any oral or intravenous antibiotic use were evaluated. Antibiotic use was defined as antibiotic treatment at any time between 4-weeks pre- and 4-weeks post the start of ICI (table 1). Progression-Free Survival (PFS) & Overall Survival (OS) were estimated with Kaplan-Meier method & compared between Abx groups. Cox proportional model was used for multivariate analyses.

Results After a median follow-up of 8.5 months [0.3–56.4], a significant improvement in PFS was observed in untreated group compared to Antibiotics treated group. 12.4 months (95%CI, 1.9–22.9) vs 4.1 months (95%CI, 2.6–5.6) (p < 0.001; figure 1). Similarly, OS among patients with no Antibiotics usage was significantly higher: 28.2 months (95%CI, not calculated) vs 12.5 months (95%CI, 10.8–14.2) (p < 0.001; figure 2).

**Abstract 728 Table 1**

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<tr>
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<tr>
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<td>Squamous cell carcinoma</td>
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http://dx.doi.org/10.1136/jitc-2020-SITC2020.0726

**Other**

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