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Conclusions In this study avß8 exhibited dramatic TGF-ß inhibitory activity compared with a wide range of inhibitors in development. Because integrin avß8 may direct TGF-ß signaling from within its latent complex, this may offer an advantage for target specificity and avoid the challenges faced by non-specific TGF-ß inhibitors. These findings characterize avß8 as a novel and potent immunotherapy drug for further clinical investigation.

Trial Registration NA

Ethics Approval NA

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723 LYMPH NODE-TARGETED AMP-VACCINE ENABLES TUMOR-DIRECTED MKRAS-SPECIFIC IMMUNE RESPONSES WITH POTENT POLYFUNCTIONAL AND CYTOLYTIC ACTIVITY

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Background Subunit vaccines targeting tumor antigens have shown limited capacity for expanding cytotoxic T-cells against tumors in the clinic. Especially in the case of KRAS-driven tumors, responses elicited by conventional vaccines have been exceedingly weak. For molecular immunogens including peptides and oligonucleotides, inefficient delivery to immune cells residing in the lymphatics is a significant challenge limiting their ability to induce cancer-directed immune responses of sufficient strength and functionality to impact tumors. Improving the targeting of immunogens to lymph nodes (LN), where resident immune cells potently orchestrate immunity, can substantially amplify their ability to induce effective tumor-directed immunity. Here, we demonstrate such an approach for significantly enhancing mKRAS-directed T-cell responses by precisely targeting antigens and adjuvants directly to the draining LN through a simple one-step conjugation to albumin-binding lipids. These amphiphilic conjugates ('Amphiphiles', or AMP) then 'hitch-hike' on albumin into the LNs where they elicit strong immune responses. LN accumulation of structurally optimized amphiphiles in mice is greatly improved over soluble equivalents.

Methods C57BL/6J mice received two or more doses of benchmark or amphiphile-modified vaccines, comprised of mKRAS peptide and CpG adjuvant, subcutaneously injected into the tail base in two-week intervals. Immunological readouts were performed 7 days post dosing. For ELISpot analysis of IFNγ and Granzyme B production and flowcytometric bead array analysis of Th1/2 cytokines, splenocytes were harvested and re-stimulated with antigen overnight. In vivo, cytolytic capabilities of antigen-specific T-cells were evaluated by pulsing CFSE-stained splenocytes from naïve mice with mKRAS antigen and injecting these cells intravenously into immunized mice. Recovery of CFSE-labeled target cells from immunized mice was performed 24h later and analyzed flowcytometrically.

Results We show robust immune responses that yield strong activation against all common mutations in the mKRAS protein compared to low or undetectable responses generated by soluble or benchmark treatments. Further, this response is composed of CD4+ as well as CD8+ T-cells resulting in the production of high levels of TH1-associated cytokines upon re-stimulation with mKRAS-specific peptides in vitro. In vivo, robust cytolytic function towards mKRAS-presenting targets can be measured in T-cells.

Conclusions By targeting immunogens directly and precisely to the LNs, the Amphiphile platform can significantly amplify the potency of subunit vaccines. In the case of mKRAS, substantially improved cytolytic immune responses represent a promising therapeutic strategy for targeting mKRAS-driven tumor growth and survival in a large fraction of human tumors. Furthermore, this platform technology is simple, rapid and scalable for broad clinical application.

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724 STAT3 INHIBITION IN ACUTE MYELOID LEUKEMIA CELLS ALLOWS FOR TLR9-DRIVEN DIFFERENTIATION TO IMMUNOGENIC MONOCYTIC CELLS AND INDUCTION OF T-CELL MEDIATED IMMUNE RESPONSES

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Background Signal transducer and activator of transcription factor 3 (STAT3) is commonly activated in acute myeloid leukemia (AML) and known for supporting cancer cell proliferation and survival. Recently, we demonstrated that STAT3 also plays a critical role ensuring AML immune evasion. Intravenous injections of bi-functional decoy oligodeoxyribonucleotides (CpG-STAT3dODN) blocked STAT3 activity and induced TLR9 signaling in Cbfb/MYH11/Mpl (CMM) AML cells, thereby resulting in immunogenic effects and T cell-mediated immune responses and leukemia regression.

Methods To understand the molecular mechanisms of the CpG-STAT3 decoy-induced AML differentiation and immunogenicity, we performed global gene expression analysis on the in vivo treated AML cells using oligonucleotide strategy as well as an inducible STAT3 gene silencing.

Results Transcriptional profiling revealed the upregulation of myeloid cell differentiation related genes, such as Irf8, Cebpa, and Gadd45A with reduction of oncogenic Runx1 and Run1t1 in CMM leukemic cells after CpG-STAT3dODN but not after control treatments. CpG-STAT3dODN treatment also upregulated set of antigen-presentation related genes, such as CIIta, Il12a, and Ifng in CMM AML cells. Importantly, the induction of Irf8 and Cebpa, with the concomitant suppression of Runx1 were found specifically in the subset of differentiated CD11b+ CMM cells but not in the bulk CD11b– leukemic cells. These effects were likely related to epigenetic reprogramming of AML cells as indicated by treatment-induced changes in the expression and protein levels of STAT3 regulated DNA methyltransferases, DNMT1 and DNMT3a/b. Furthermore, our initial studies suggest that STAT3 inhibition/TLR9 activation leads to immunogenic effects also in a xenotransplanted model of human FLT3-ITD MV4-11 leukemia in humanized mice. CpG-STAT3dODN alone or together with clinically-relevant demethylating agent (Decitabine) triggered differentiation of MV4-11 cells into CD11b+HLA-DR+CD86+ antigen-presenting cells (APCs) and increased ratio of CD8+ to

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

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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 FcgR 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 cynomolgus 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 cynomolgus 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 at receptor saturation. There were no indications of cytokine release in any of the systems tested. Finally, we could show that to achieve max therapeutic effect, sustained RO was needed for approximately 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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726 SRF114 IS A FULLY HUMAN, CCR8 SELECTIVE IGG1 ANTIBODY THAT INDUCES DESTRUCTION OF TUMOR TREGS THROUGH ADCC

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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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