than resveratrol in inhibiting chemotaxis of HL-60 cells and blocking cell cycle of THP-1 and HL-60 cells at G1/S transition. In addition, NBT-167, but not resveratrol, could increase IL-2 production and T cell proliferation stimulated with anti-CD3 and anti-CD28 and synergize with anti-PD-1 antibody to increase IL-2 and IFN- γ production in co-culture of allo-type T cells and dendritic cells (MLR).

Conclusions Our data showed that NBT-167, a dimer of resveratrol, had anticancer and immunomodulatory activities such as modulation of expression of cytokines in immune cells and induction of cancer cell-killing activities of NK and gamma delta T cells. Generally, NBT-167 appeared to have higher activities than resveratrol in modulating immune cells and inhibiting cancer cells. NBT-167 could be a promising cancer immunotherapeutic agent targeting both cancer cells and immune cells.

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601 DEVELOPMENT OF IMPROVED SMALL MOLECULE STING AGONISTS SUITABLE FOR SYSTEMIC ADMINISTRATION

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Background Stimulator of Interferon Genes (STING) is a major player in the activation of robust innate immune response leading to initiation and enhancement of tumor-specific adaptive immunity. Several clinical and pre-clinical programs have shown that activation of the STING pathway triggers immune-mediated antitumor response. Although vast majority of programs focus on development of analogues of the endogenous STING ligands, their chemical nature and stability often limit their use to local administration. Herein, we present recent results from the development of our selective non-nucleotide, non-macrocyclic, small molecule direct STING agonists, suitable for systemic administration, characterized by improved activity in human immune cells.

Methods Binding to recombinant STING protein was examined using FTS, MST, FP and crystallography studies. Phenotypic screen was performed in THP-1 Dual reporter cells. Mouse bone marrow-derived dendritic cells (BMDC) were obtained from C57BL/6 mice and differentiated with mIL-4 and mGM-CSF. STING agonists were administered into BALB/c mice and cytokine release was measured in plasma. Additionally, mice were inoculated with CT26 murine colon carcinoma or EMT6 murine breast carcinoma cells and the compound was administered, followed by the regular tumor growth and body weight monitoring.

Results Ryvu's small-molecule agonists demonstrate strong binding affinity to recombinant STING proteins across all tested species. The compounds bind to all human STING protein variants and trigger pro-inflammatory cytokine release from human immune cells regardless of the STING haplotype. Moreover, new generation of developed agonists show significantly improved binding to human protein as well as in vitro activity on human cells. Systemic, intravenous in vivo administration leads to a dose-dependent upregulation of STING-

dependent pro-inflammatory cytokines, which results in a dose-dependent antitumor efficacy observed in CT26 and EMT6 mouse cancer models, leading to complete tumor remissions in all treated animals. Furthermore, observed efficacy is accompanied by development of a lasting immunological response demonstrated by lack of tumor engraftment or a delayed tumor growth in cured animals challenged with repeated inoculation of cancer cells.

Conclusions New generation Ryvu's STING agonists are strong and selective activators of STING-dependent signaling in both mouse and human immune cells promoting anti-tumor immunity. Treatment with Ryvu's small-molecule STING agonists leads to engagement of the immune system which results in a complete tumor remission and development of immunological memory of the cancer antigens. The compounds show good selectivity and ADME properties enabling development for systemic administration. In addition developed compounds maintain small functional handles amenable to linker attachment making the series suitable for versatile development as single agents, for combinations with immunotherapies or as targeted agents.

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602 STING AGONIST-BASED TREATMENT PROMOTES VASCULAR NORMALIZATION AND TERTIARY LYMPHOID STRUCTURE FORMATION IN THE THERAPEUTIC MELANOMA MICROENVIRONMENT

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Background The degree of immune infiltration in tumors, especially CD8⁺ T cells, greatly impacts patient disease course and response to interventional immunotherapy. Hence, enhancement of TIL prevalence is a preferred clinical endpoint, one that may be achieved via administration of agents that normalize the tumor vasculature (VN) leading to improved immune cell recruitment and/or that induce the development of local tertiary lymphoid structures (TLS) within the tumor microenvironment (TME).

Methods Low-dose STING agonist ADU S-100 (5 μ g/mouse) was delivered intratumorally to established s.c. B16.F10 melanomas on days 10, 14 and 17 post-tumor inoculation under an IACUC-approved protocol. Treated and control, untreated tumors were isolated at various time points to assess transcriptional changes associated with VN and TLS formation via qPCR, with corollary immune cell composition changes determined using flow cytometry and immunofluorescence microscopy. In vitro assays were performed on CD11c⁺ BMDCs treated with 2.5 μ g/mL ADU S-100 (vs PBS control) and associated transcriptional changes analyzed via qPCR or profiled using DNA microarrays. For TCR β -CDR3 analyses, CDR3 was sequenced from gDNA isolated from enzymatically digested tumors and splenocytes.

Results We report that activation of STING within the TME leads to slowed melanoma growth in association with increased production of angiostatic factors including Tnfsf15 (Vegf), Cxcl10 and Angpt1, and TLS inducing factors including Ccl19, Ccl21, Lta, Ltb and Tnfsf14 (Light). Therapeutic responses from intratumoral STING activation were characterized by increased vascular normalization (VN), enhanced tumor infiltration by CD8⁺ T cells and CD11c⁺ DCs and local TLS neo-genesis, all of which were dependent on host

expression of STING. Consistent with a central role for DC in TLS formation, ex vivo ADU S-100-activated mCD11c+ DCs also exhibited upregulated expression of TLS promoting factors including lymphotoxin- α (LTA), IL-36, inflammatory chemokines and type I interferons. TLS formation was associated with the development of a therapeutic TIL TCR repertoire enriched in T cell clonotypes uniquely detected within the tumor but not the peripheral circulation in support or local T cell cross-priming within the TME.

Conclusions Our data support the premise that i.t. delivery of STING agonist promotes a pro-inflammatory TME in support of VN and TLS formation, leading to the local expansion of unique TIL repertoire in association with superior anti-melanoma efficacy.

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COVALENT ATTACHMENT OF A TLR7/8 AGONIST TO TUMOR-TARGETING ANTIBODIES DRIVES POTENT ANTI-TUMOR EFFICACY BY SYNERGISTICALLY ACTIVATING FcGR- AND TLR- SIGNALING AND ENABLES SAFE SYSTEMIC ADMINISTRATION

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Background Immune stimulating antibody conjugates (ISACs) covalently attach TLR7/8 immune stimulants to tumor-targeting antibodies. ISACs can be delivered systemically and act locally in the tumor microenvironment by requiring the following biological steps to elicit immune activation: 1) tumor antigen recognition, 2) Fc receptor mediated phagocytosis by myeloid antigen presenting cells (APCs), and 3) activation of endosomal TLR7 and TLR8. Here, we demonstrate that covalent attachment of our TLR7/8 agonist to tumor-targeting antibodies not only enables the resulting ISACs to be safely administered systemically in preclinical models, but also unexpectedly promotes synergy between the FcGR and TLR pathways that results in amplified anti-tumor immunity in mice and robust immune activation in human leukocytes as compared to the co-administration of the components.

Methods ISAC activity and mechanistic studies were analyzed via flow cytometry, ELISA and CyTOF following in vitro coculture of human leukocytes with tumor cell lines. In vivo efficacy of HER2-targeting ISACs following systemic administration was assessed in a trastuzumab-resistant HER2+ human tumor xenograft model. Safety and tolerability were assessed in tumor-bearing mice and healthy non-human primates (NHP).

Results While co-administration of intratumoral TLR7/8 agonist and intraperitoneal trastuzumab failed to control tumor growth, systemic administration of the same TLR7/8 agonist and trastuzumab in our ISAC format was efficacious and induced complete tumor regression in an Fc- and TLR-dependent manner. Analysis of primary human leukocytes stimulated with ISACs in tumor co-culture assays indicated that ISACs elicit amplified and sustained phosphorylation of Fc and TLR signaling pathways, such as pERK1/2 and pIRF-7, as compared to the unconjugated mixture of the same TLR7/8 agonist and tumor targeted antibody. ISAC stimulation was

largely restricted to antigen presenting cells such as dendritic cells and plasmacytoid dendritic cells that express the relevant Fc receptors and TLR7 and/or TLR8. Modifications to the ISAC that reduce FcGR engagement (N297A/Q) or render the agonist inactive halted ISAC-mediated activation and in vivo anti-tumor efficacy. Lastly, our HER2-targeting ISACs were well-tolerated when delivered systemically in mice and NHPs.

Conclusions Our ISACs enable potent TLR agonists to be safely administered systemically in preclinical models. ISACs provide distinct and unexpected advantages over unconjugated TLR agonists, notably by driving synergy between FcGR and TLR pathways, leading to robust myeloid activation and anti-tumor efficacy. These data support the evaluation of BDC-1001, a HER2-targeted ISAC in the ongoing Phase 1/2 trial (NCT04278144).

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INTRAVENOUS CMP-001, A CPG-A TOLL-LIKE RECEPTOR 9 (TLR9) AGONIST DELIVERED VIA A VIRUS-LIKE PARTICLE, CAUSES TUMOR REGRESSION IN SYNGENEIC HEPA1-6 MOUSE MODELS OF HEPATOCELLULAR CARCINOMA

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Background Therapeutic options are limited for patients with liver metastases and hepatocellular carcinoma (HCC). Intratumoral and subcutaneous injections of CMP-001, a CpG-A TLR9 agonist packaged within a virus-like particle, have shown evidence of antitumor activity in patients with melanoma refractory to PD-1 blockade. In mice, CMP-001 intravenous distributes primarily to the liver, while CMP-001 subcutaneous is found mostly in local tissues and draining lymph nodes. The antitumor activity of CMP-001 intravenous and subcutaneous were compared with PD-1 blockade or sorafenib in two Hepa1-6 orthotopic mouse models of HCC.

Methods Groups of 10–15 C57BL/6J mice were orthotopically implanted with syngeneic murine hepatoma cells using two different models. Model 1 used 1.5×10^6 Hepa1-6 cells injected into the spleen following a partial hepatectomy; Model 2 used 1×10^6 Hepa1-6-Luc cells injected into the upper left lobe of intact liver. Treatment was initiated 3–7 days later with either CMP-001 intravenous or subcutaneous Q4-5Dx3-4 doses, PD-1 blocking antibody intraperitoneal Q3-4Dx2 (Bio X Cell clone RPM1-14), or sorafenib QD oral. Antitumor activity was assessed by tumor imaging, liver weight, and/or survival.

Results CMP-001 was compared with PD-1 blocking antibody therapy in Model 1, the more aggressive model. All animals were sacrificed at day 15 due to institutional welfare requirements. Tumor growth inhibition (TGI) was assessed by comparison of liver weight to body weight ratios, which relative to untreated control mice showed that CMP-001 intravenous achieved 85% mean TGI compared with 63% mean TGI for CMP-001 subcutaneous and 15% mean TGI for PD-1 blocking antibody intraperitoneal (table 1). CMP-001 intravenous was compared to sorafenib oral in Model 2, which utilized an engineered Hepa1-6 cell line that expresses luciferase to enable noninvasive monitoring of liver tumor growth. CMP-001 intravenous was active, with a 67% mean TGI, and survival that was comparable to sorafenib (table 2; figure 1).