expression of STING. Consistent with a central role for DCs in TLS formation, ex vivo ADU S-100-activated mCD11c+ DCs also exhibited upregulated expression of TLS promoting factors including lymphotixin-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.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0602

603

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

1Shelley Ackerman, 1Felix Hartmann, 1Cecelia Pearson, 1Joseph Gonzalez, 1Po Yi Ho, 2Samuel Kimmy, 1Andrew Luo, 2Benjamin Ackerman, 1Arthur Lee, 1Richard Laura, 2Jason Paik, 1Karla Henning, 1David Jackson, 1Steven Chapin, 1Bruce Devens, 1David Dornan, 2Sean Bendall, 2Edgar Engleman, 1Michael Alonso. 1Bolt Biotherapeutics, Redwood City, CA, USA; 2Stanford University, Stanford, CA, USA; 3Johns Hopkins University, Baltimore, MD, USA

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 FcγR 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 trastuzumab was ineffective, ISACs were efficacious and induced complete tumor regression in an Fc- and TLR-independent manner. Assessment 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 FcγR 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).

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0603

604

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

1Aaron Morris, 1Evan Walters, 2Bassel Akache, 2Michael McCuskie, 1Arthur Krieg*. 1Bolt Biotherapeutics, Pembroke Pines, FL, USA; 2Checkmate Pharmaceuticals, Cambridge, MA, USA; 3National Research Council Canada, Ottawa, Canada

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 15 C57BL/6J mice were orthotopically implanted with syngeneic murine hepatoma cells using two different models. Model 1 used 1.5 x 106 Hepa1-6 cells injected into the spleen following a partial hepatectomy; Model 2 used 1 x 106 Hepa1-6-Luc cells injected into the upper left lobe of intact liver. Treatment was initiated 3 days later with either CMP-001 intravenous or subcutaneous Q4-5D x3-4 doses, PD-1 blocking antibody intraperitoneal Q3-4Dx2 (Bio X Cell clone RPMI1-14), 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 CPG-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).

Conclusions In orthotopic mouse models of HCC, the antitumor activity of CMP-001 intravenous was greater than PD-1 blockade and comparable to sorafenib. CMP-001 intravenous was more active than CMP-001 subcutaneous in this model, which we hypothesize is due to increased liver exposure with intravenous infusion. Antitumor activity of CMP-001 monotherapy may be increased by combining it with standard of care or other therapies, as observed relative to historical benchmarks in ongoing CMP-001 clinical trials in patients with melanoma. CMP-001 intravenous may be a promising treatment option for patients with primary or metastatic liver cancers.

Acknowledgements This work was supported by Checkmate Pharmaceuticals. Studies were performed at Oncodesign Biotechnology (Dijon, France) and Crown Bioscience UK Ltd (Osgathorpe, UK) and National Research Council Canada (Ottawa, Ontario, Canada) and funded by Checkmate Pharmaceuticals.

Ethics Approval At Oncodesign Biotechnology, animal housing and experimental procedures were conducted according to French and European Regulations and the National Research Council Guide for the Care and Use of Laboratory Animals. The animal facility is authorized by the French authorities (Dijon: Agreement B21231011EA). The study and all animal procedures were approved by the Institutional Animal Care and Use Committee of Oncodesign (Oncomet) approved by French authorities (CNREEA agreement number 91). At Crown Bioscience, animal care and experimental procedures were compliant with the UK Animals Scientific Procedures Act 1986 (ASPA) in line with Directive 2010/63/EU of the European Parliament and the Council of 22 September 2010 on the protection of animals used for scientific purposes. At National Research Council Canada, animals were maintained in accordance with the guidelines of the Canadian Council on Animal Care, and all experimental procedures were performed in accordance with regulations and guidelines reviewed and approved by the NRC Human Health Therapeutics Ottawa Animal Care Committee.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0604