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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603 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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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

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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 x 10^6 Hepa1-6 cells injected into the spleen following a partial hepatectomy; Model 2 used 1 x 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).

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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.

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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.

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Abstract 604 Table 1

Terminal Liver/Body Weight % All Mice

Study Group #	Group Description	Mean LW/BW%	SEM	Delta From No Tumor Baseline	% Growth	% Growth Inhibition
1	Vehicle	20.71	2.96	15.48	100	0
2	PD-1	18.36	2.19	13.13	85	15
3	IV CMP-001	7.57	0.84	2.34	15	85
4	SC CMP-001	10.96	1.83	5.73	37	63
5	No tumor	5.23	0.14	0	0	NA

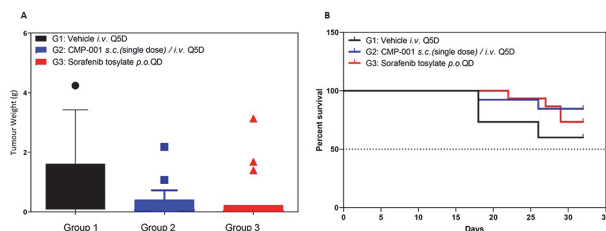
IV, intravenous; LW/BW, liver to body weight; NA, not applicable; PD-1, programmed cell death protein 1; SC, subcutaneous; SEM, standard error of the mean.

Abstract 604 Table 2

Terminal Tumor Weight All Mice

Study Group #	Group Description	Mean Tumor Weight (g)	SEM	Delta From No Tumor Baseline	% Growth	% Growth Inhibition
1	Vehicle	1.011	0.33	1.011	100	0
2	IV CMP-001	0.335	0.18	0.335	33	67
3	Sorafenib	0.45	0.24	0.45	45	55

IV, intravenous; LW/BW, liver to body weight; NA, not applicable; PD-1, programmed cell death protein 1; SC, subcutaneous; SEM, standard error of the mean.



Abstract 604 Figure 1

605 SYSTEMICALLY ADMINISTERED HER2-TARGETED ISACS PROVOKE A RAPID, LOCAL RESPONSE THAT ENGAGES THE INNATE AND ADAPTIVE ARMS OF THE IMMUNE SYSTEM TO ERADICATE TUMORS IN PRECLINICAL MODELS

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Background Immune-stimulating antibody conjugates (ISACs) covalently attach immune stimulants to tumor-targeting antibodies such as trastuzumab. We have shown that HER2-targeted TLR7/8 ISACs elicit robust myeloid activation and tumor eradication in a TLR- and Fc-dependent manner in trastuzumab-resistant and HER2-low models. Upon treatment with ISACs, T cell-mediated immunological memory extends to tumor antigens beyond HER2.[1] Here we describe the ISAC mechanism of action in vivo that leads to eradication of tumors in mice.

Methods Established syngeneic rHER2- or xenograft HER2-expressing tumors treated with anti-HER2 ISACs or appropriate controls were assessed for gene expression by NanoString Pan-Cancer Immune Profiling panel comprising 750 genes related to tumor immune biology. Tumor cytokines were measured using MesoScale Discovery (MSD) technology, and immune cell infiltrates were assessed by immunohistochemistry (IHC). Anti-tumor efficacy was assessed after depletion of CD8+ T cells and phagocytes.

Results Within 24 hours of administration, HER2-directed ISACs induced robust, target-dependent activation of the immune system. In a syngeneic tumor model, 34% of the measurable genes were significantly upregulated after treatment with the rHER2-targeted ISAC vs 0.1% with the non-binding ISAC control. Similarly, 13% vs 0% of genes were upregulated in a xenograft model after HER2-targeted vs control ISAC treatment. In both models anti-HER2 ISAC treatment led to activation of pathways indicative of TLR7/8 agonism (e.g. IRF-7; type 1 interferons), and FcgR engagement (e.g. NF-kappaB associated genes). Cytokines and chemokines, including myeloid chemokines CCL2/3/4 and T cell chemokines CXCL9/10/11 were specifically upregulated in the tumors at the gene and protein level, indicating robust activation of myeloid cells following anti-HER2 ISAC treatment. Furthermore, in syngeneic tumors T cell activation markers (e.g. Granzyme B; IFN-gamma) were induced within 24 hours post treatment with an

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