Targeting tumor-associated macrophages to enhance anti-tumor immunity with the Dectin-2 agonistic antibody BDC-3042

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ABSTRACT 2964

INTRODUCTION

Tumor-associated macrophages (TAMs) are the largest immune cell population in many cancers and play a key role in establishing the immunosuppressive tumor microenvironment (TME) that enables tumor progression. However, TAMs are phenotypically plastic and have the potential to be reprogrammed into immunostimulatory cells that enhance innate and adaptive anti-tumor immunity. To this end, we developed BDC-3042, an agonistic antibody targeting an immune-activating receptor expressed on TAMs known as Dectin-2 (CLEC6A). Dectin-2 is a C-type lectin receptor (CLR) known best for its role in pathogen recognition and induction of protective immune responses against fungi and other microbes. We previously demonstrated that Dectin-2 agonism with natural ligands stimulates pro-inflammatory cytokine secretion and antigen presentation by TAMs, resulting in robust CD8+ T cell-mediated anti-tumor immunity in syngeneic mouse models. Here we present our preclinical studies demonstrating the therapeutic potential of the Dectin-2 agonistic antibody, BDC-3042, as a novel TAM-directed immunotherapy for diverse human cancers.

Dectin-2 agonism activates TAMs and elicits anti-tumor immune response

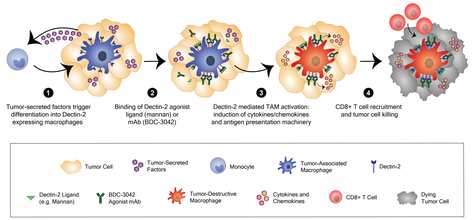


Figure 1: Schematic of proposed mechanism of action driving Dectin-2-mediated anti-tumor activity.

RESULTS

Dectin-2 gene expression is elevated across tumor types

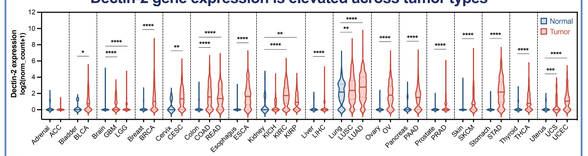


Figure 2: Dectin-2 gene expression is elevated in tumors but low in most normal tissues. Dectin-2/CLEC6A mRNA expression in human tissue samples from the TCGA (tumor) and GTEX (normal) datasets (accessed Oct. 2019). TCGA study abbreviations are shown for the tumor subtypes. TCGA and GTEX data were processed using a uniform bioinformatic pipeline and obtained from UCSC Xena (xena.ucsc.edu). Median and interquartile range are shown on the violin plots. Statistics were calculated by Mann-Whitney U test; *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001.

BDC-3042 binds strongly to anti-inflammatory M-CSF macrophages relative to circulating immune cells

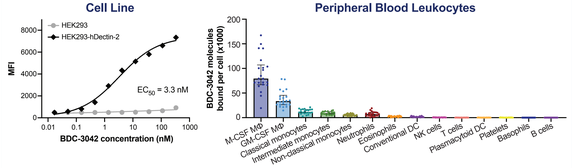


Figure 3: BDC-3042 binds strongly to Dectin-2-expressing macrophages with minimal binding to circulating immune cells. (A, B) Binding of fluorescently labeled BDC-3042 to the indicated cell types was measured by flow cytometry. (A) BDC-3042 binds to HEK293 cells expressing human Dectin-2 with single-digit nM EC_{50}s. MFI refers to median fluorescence intensity. (B) Binding of BDC-3042 (10 µg/mL, 67 nM) to monocyte-derived macrophages (MΦ) generated with M-CSF or GM-CSF, and to immune cell subsets in peripheral blood from healthy donors (n=22). The number of BDC-3042 molecules bound per cell was determined by bead-based quantification. Data are shown as median with interquartile range.

BDC-3042 elicits strong pro-inflammatory responses from macrophages in an FcγR-dependent manner

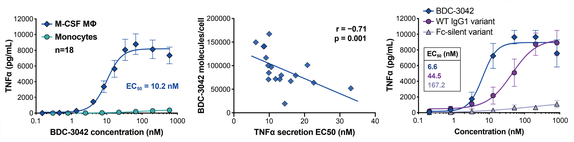


Figure 4: BDC-3042 robustly activates macrophages but not monocytes in an Fcγ receptor-dependent manner. (A) Human monocytes and monocyte-derived M-CSF MΦ from healthy donors (n=18) were stimulated overnight with BDC-3042 and TNFα secretion was measured using an MSD immunoassay kit. (B) Correlation between M-CSF MΦ activation, assessed by TNFα secretion EC_{50}, and BDC-3042 binding, expressed as BDC-3042 molecules bound per cell (n=18). Spearman correlation coefficient and p-value are shown. (C) GM-CSF MΦ were stimulated with BDC-3042 (Fc-enhanced IgG1) or Fc variants of BDC-3042 with wild-type or attenuated Fc domains (n=4). TNFα secretion was measured by ELISA.

BDC-3042 binding to macrophages is enhanced following polarization with selected TME-associated cytokines

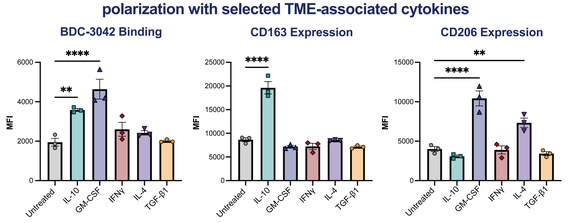


Figure 5: BDC-3042 binding to macrophages is enhanced following polarization with selected cytokines found in the tumor microenvironment. Human monocyte-derived MΦ generated with M-CSF were further polarized with the indicated cytokines for 24 hr (n=3 donors). Binding of BDC-3042 and expression of the M2 markers CD163 and CD206 were assessed by flow cytometry. Data are shown as mean with SEM. Statistics were calculated by one-way ANOVA comparing the treated groups to the untreated group. **, p<0.01; ****, p<0.0001

BDC-3042 preferentially binds to TAMs and stimulates pro-inflammatory responses from human tumor samples

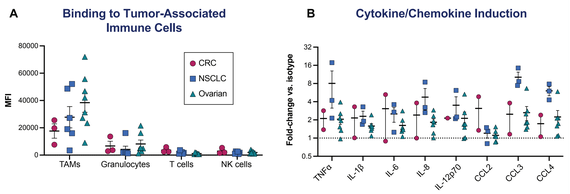
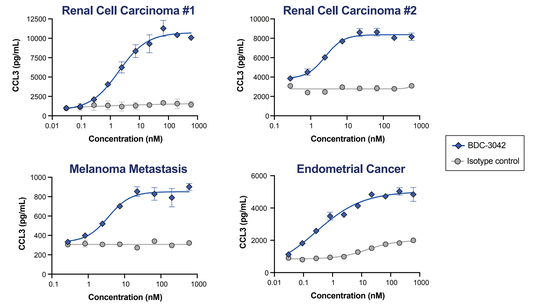


Figure 6: BDC-3042 preferentially binds to TAMs and stimulates pro-inflammatory responses from human tumor samples. (A) BDC-3042 binding to immune cell subsets from cryopreserved dissociated tumor samples from colorectal cancer (CRC, n=3), non-small cell lung cancer (NSCLC, n=6), and ovarian cancer (n=7) patients was assessed by flow cytometry. MFI indicates median fluorescence intensity. (B) A subset of the samples were stimulated overnight with BDC-3042 or an isotype control mAb (both at 67 nM). Cytokine/chemokine secretion was measured using MSD kits.

BDC-3042 elicits dose-dependent responses from diverse human tumor samples



Tumor Sample	EC_{50} (nM)			
	TFNα	IL-1β	CCL3	CCL4
Renal Cell Carcinoma #1	2.48	0.63	1.87	1.89
Renal Cell Carcinoma #2	5.55	2.80	1.88	1.60
Melanoma Metastasis	4.61	2.05	2.65	2.04
Endometrial Cancer	2.99	NC	0.25	0.99
Median EC_{50} (nM) for All Samples	3.80	2.05	1.88	1.75

Figure 7: BDC-3042 elicits dose-dependent responses from diverse human tumor samples. Freshly resected primary tumor samples (Renal Cell Carcinoma #1 & #2, Endometrial Cancer) and lymph node metastasis (Melanoma Metastasis) were processed into single-cell suspensions and then stimulated overnight with a dose titration of BDC-3042 or isotype control mAb. Cytokine/chemokine secretion was measured using MSD kits. Dose-response curves for CCL3 are shown. The table displays EC_{50}s for induction of CCL3 and other key cytokines and chemokines. NC, not calculable.

Humanized mice for in vivo assessment of BDC-3042 anti-tumor activity

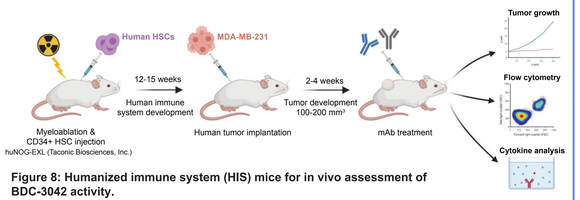


Figure 8: Humanized immune system (HIS) mice for in vivo assessment of BDC-3042 activity.

Kenkel JA, et al. AACR Poster (2022).
Acknowledgments: The HIS mouse work described was performed in collaboration with Taconic Biosciences, Inc.

BDC-3042 binds to TAMs and stimulates pro-inflammatory responses from humanized mouse tumors ex vivo

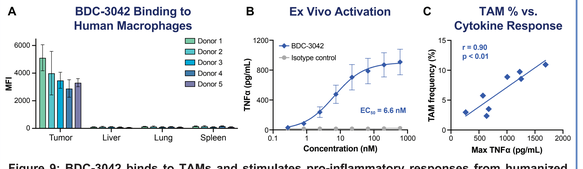


Figure 9: BDC-3042 binds to TAMs and stimulates pro-inflammatory responses from humanized mouse tumors ex vivo. (A) BDC-3042 binding to human macrophages recovered from the indicated tissues from MDA-MB-231 tumor-bearing huNOG-EXL mice generated using five unique HSC donors (n=4-5 mice/donor). (B) Dissociated MDA-MB-231 tumor samples from huNOG-EXL mice were incubated overnight with BDC-3042 or isotype control mAb, and human TNFα secretion was measured by ELISA (n=8). (C) Correlation between the frequency of human TAMs in the dissociated tumor samples and their peak TNFα secretion values. Pearson correlation coefficient and p-value are shown.

BDC-3042 elicits infiltration of lymphocytes and production of pro-inflammatory cytokines and chemokines in the TME

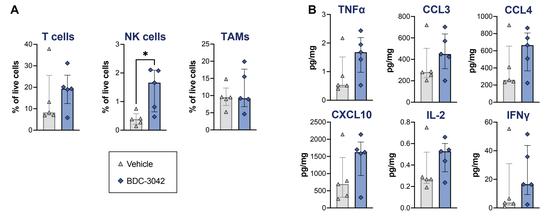


Figure 10: BDC-3042 elicits infiltration of lymphocytes and production of pro-inflammatory cytokines and chemokines in the tumor microenvironment. (A, B) MDA-MB-231-bearing huNOG-EXL mice from a single donor were treated with vehicle or BDC-3042 (0.3 mg/kg) administered intraperitoneally on Days 0, 3, and 7 (n=5/group). Tumors were collected 24 hours after the last dose for assessment of human immune cell infiltration and cytokine/chemokine production. (A) Frequencies of the indicated cell subsets expressed as percent of total live cells. (B) Levels of the indicated cytokines and chemokines in tumor lysates measured using MSD kits. Data are shown as median with interquartile range. *, p<0.05 by Student's t-test.

Anti-PD-1 therapy increases Dectin-2 expression in human tumors and improves anti-tumor activity of BDC-3042 in humanized mice

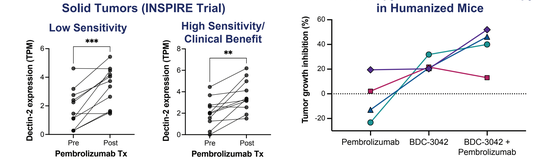


Figure 11: Anti-PD-1 therapy increases Dectin-2 gene expression in human tumors and generally improves anti-tumor activity of BDC-3042 in humanized mice. (A) Dectin-2 gene expression in tumor samples obtained from patients with mixed solid tumors before and after 2-3 cycles of pembrolizumab treatment (data obtained from Cindy Yang et al., Nat Commun 2021). Patients were stratified into subgroups showing "Low Sensitivity" (LS) or "High Sensitivity/Clinical Benefit" (HS/CB) in response to pembrolizumab according to changes in circulating tumor DNA (ΔctDNA) and target lesion measurement (Δ TM) as well as clinical response (described in Cindy Yang et al.) (n=11 per subgroup). LS: ΔctDNA and Δ TM positive; 10/11 PD, 1/11 SD. HS/CB: ΔctDNA negative and/or ΔTM negative; 3/11 SD, 6/11 PR, 2/11 CR. Statistics were calculated by paired t-tests. **, p<0.01; ***, p<0.001. (B) MDA-MB-231-bearing huNOG-EXL mice from 4 HSC donor cohorts were treated Q7D x 5 with the indicated test article via intraperitoneal administration (BDC-3042: 0.5 mg/kg; pembrolizumab: 5 mg/kg). Tumor growth inhibition relative to the isotype control was calculated on day 35. The connected lines represent data for each HSC donor cohort.

CONCLUSIONS

- Dectin-2 is a novel immuno-oncology target expressed by tumor-associated macrophages (TAMs) across a range of solid tumor types
- BDC-3042 is an agonistic antibody targeting Dectin-2 that is designed to reprogram immunosuppressive TAMs into immunostimulatory cells that drive anti-tumor immunity
- BDC-3042 selectively binds to Dectin-2-expressing macrophages and induces an array of pro-inflammatory cytokines, chemokines, and antigen presentation molecules
- BDC-3042 exhibits minimal binding to and activation of peripheral leukocytes
- BDC-3042 repolarizes TAMs toward an immunostimulatory phenotype and mediates anti-tumor activity in tumor-bearing humanized mice
- Preclinical data support clinical evaluation of BDC-3042, with initiation of a Phase I clinical trial planned for 2023