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A Bispecific Fc-Silenced IgG1 Antibody (MCLA-145) Requires PD-L1 Binding to Activate CD137

Presented at the
AACR Annual Meeting 2019
Atlanta, GA, USA • March 29–April 3, 2019

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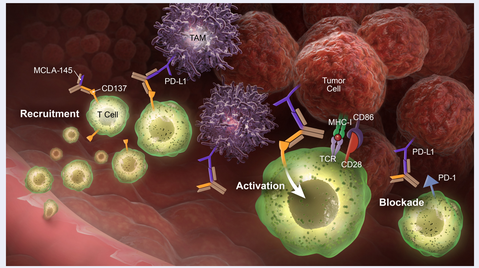
Abstract

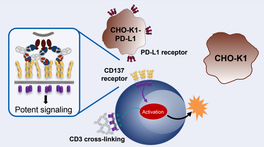
CD137 (4-1BB) is a transmembrane costimulatory receptor on T and NK cells that enhances adaptive immune responses and is a critical mediator of antitumor immunity. The development of CD137-targeted agents for cancer therapy has been hampered by on-target off-tumor toxicity in the case of agonist monospecific, bivalent mAbs or limited antitumor activity in the case of crosslinking mAbs. Here we have developed an Fc-silenced bispecific IgG1 antibody to CD137 and PD-L1 with monovalent binding specificity to each target. MCLA-145 drives transactivation of CD137 in the vicinity of cells expressing PD-L1, such as in the immunosuppressive tumor microenvironment. The degree of CD137 agonistic activity in T cells correlated with the expression level of PD-L1 on neighboring cells, as demonstrated in transactivation assays whereby reporter T cells were co-cultured with cells expressing different levels of PD-L1. PD-L1 expression as low as 6000 receptors per cell was sufficient to activate CD137 in neighboring T cells. In contrast, MCLA-145 blocked PD-1 signaling without requirement for CD137 binding in a PD-1/PD-L1 reporter assay. CD137 signaling was induced by MCLA-145 in multiple primary human immune cell assays including the mixed lymphocyte reaction, human PBMC, and whole blood SEB stimulation assays. MCLA-145 reversed T cell suppression mediated by M2 macrophages or Tregs, in vitro. In addition, MCLA-145 enhanced Ag-specific expansion and differentiation of human naive CD8⁺ T cells in vitro. In vivo, MCLA-145 treatment resulted in significant tumor immune activation and antitumor responses in 2 separate humanized mouse tumor models. In one model, human T cells expressing NY-ESO–specific TCR were adoptively transferred to mice bearing A549 tumors, which expressed NY-ESO antigen and human PD-L1. MCLA-145 treatment at 5 mg/kg resulted in 54% tumor growth inhibition (TGI) as compared to T cell only–treated mice. In the tumors of MCLA-145–treated mice, the percentage of NY-ESO–specific CD8⁺ T cells were significantly increased compared with controls. In a second model, mice engrafted with human CD34⁺ cells were implanted with the breast tumor cell line MDA-MB-231. MCLA-145 at 0.5 mg/kg and 5 mg/kg induced significant TGI (55% and 57%, respectively) as compared to vehicle control or Fc-silenced huIgG1 controls. Additionally, 2 out of 9 animals in the 5 mg/kg MCLA-145–treated group had complete tumor regression. MCLA-145 increased the number of infiltrating CD8⁺ T cells, as well as the percentage of central memory CD8⁺ T cells. The cured animals were then re-challenged with MDA-MB-231 tumor cells, and none of previously cured mice were rejected as compared to no growth inhibition in treatment-naive CD34⁺ NSG mice. In conclusion, these data support the clinical evaluation of MCLA-145 as a novel, PD-L1–dependent CD137 agonist immune therapy.

Introduction

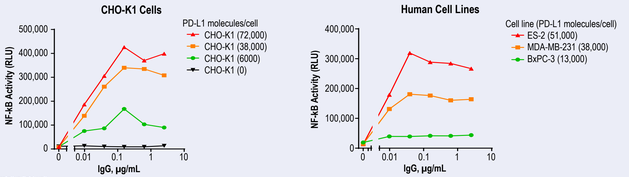
- CD137 (4-1BB) is a transmembrane costimulatory receptor on T and NK cells that enhances adaptive immune responses and is a critical mediator of antitumor immunity
- The development of CD137-targeted agents for cancer therapy has been hampered by on-target off-tumor toxicity
- CD137 signaling requires receptor clustering by the trimeric CD137 ligand, agonistic monoclonal antibodies (mAbs), or indirectly via cross-linking of CD137-binding antibodies by Fcγ receptors on neighboring cells
- PD-L1 expression is frequently observed on tumor cells, and mAb-based PD-L1 inhibitors have demonstrated durable tumor remission in patients with diverse advanced cancers in the clinic
- MCLA-145 is a Biclonics® T cell agonist that binds with high affinity and specificity to human PD-L1 and CD137



MCLA-145 Activity Correlates With PD-L1 Expression Levels



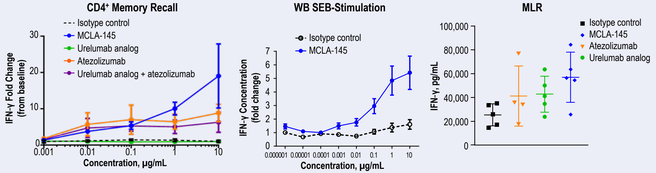
- CHO cell lines stably expressing various levels of human PD-L1 and human cell lines endogenously expressing PD-L1 were co-cultured with CD137 Jurkat NF-kB/luc reporter cells
- MCLA-145–mediated CD137 reporter cell signaling intensity correlated with PD-L1 expression levels on neighboring cells

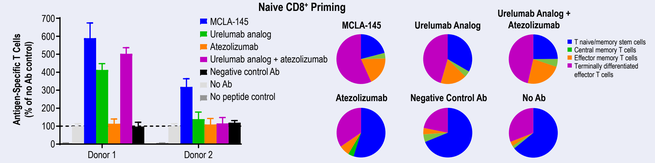


RLU, relative light unit.

MCLA-145 Increases T Cell Activation in Primary Immune Assays

- MCLA-145 induces IFN-γ production in human primary CD4⁺ memory T cells restimulated with CEFT peptide pools, human whole blood (WB) stimulated with staphylococcal enterotoxin B antigen (SEB), and CD4⁺ T cells activated by culture with allogenic human dendritic cells (mixed lymphocyte reaction [MLR])
- MCLA-145 induces the number of antigen-specific T cells and enhances differentiation of naive T cells into effector T cells in an in vitro priming assay with peptide-pulsed human dendritic cells and naive CD8⁺ T cells

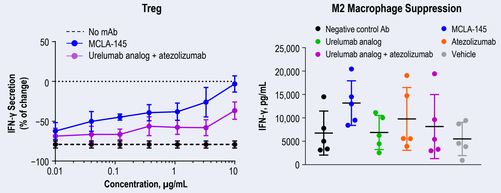




- Human-naive CD8⁺ T cells were primed with Melan-A peptide-pulsed dendritic cells. Dextramer, CD45RA, and CCR7 FACS staining was used to determine antigen-specific CD8⁺ T cell numbers (bar graph) and differentiation status (pie chart)
- MCLA-145 increases antigen-specific CD8⁺ T cell numbers and differentiation

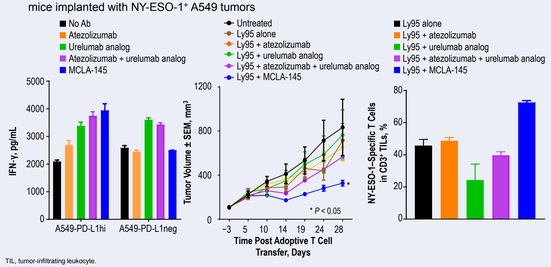
MCLA-145 Reverses M2 Macrophage and Treg Suppression

- MCLA-145 induces IFN-γ production by anti-CD3/CD28 activated human T cells when cultured with human regulatory T cells in vitro
- MCLA-145 induces IFN-γ production by anti-CD3/CD28 activated human T cells when cultured with M2-polarized macrophages in vitro



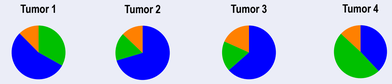
Ly95-NY-ESO Adoptive T Cell Transfer Model

- MCLA-145 induces IFN-γ production in NY-ESO-1–specific T cells (Ly95 cells) co-cultured with NY-ESO-1⁺ A549 cells for 72 hours
- MCLA-145 enhances the antitumor activity and tumor infiltration of NY-ESO-1–specific T cells in NSG mice implanted with NY-ESO-1⁺ A549 tumors



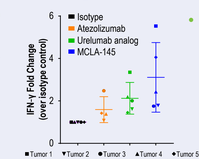
TIL, tumor-infiltrating leukocyte.

Activity of MCLA-145 in Ex Vivo Human Primary Tumor Samples



	Total Cell Number		
	CD8⁺	Treg⁺	Ratio
Tumor 1	6091	1394	4.37
Tumor 2	24,410	18,864	1.29
Tumor 3	13,189	13,250	1.00
Tumor 4	5931	1590	3.73
Tumor 5	1026	202	5.08

	CD137, %			PD-L1, %		
	CD4⁺	CD8⁺	Treg⁺	CD45⁺	CD45⁻	IFN-γ Fold Change Over Control
Tumor 1	0.86	1.99	49.2	1.52	0.8	5.5
Tumor 2	0.3	0.77	10.3	0.19	0.39	1.8
Tumor 3	2.21	23.1	51.3	1.78	1.89	1.8
Tumor 4	0.3	5.56	42.9	2.13	0.46	2.4
Tumor 5	2.16	1.17	43.6	3.01	1.01	4.1

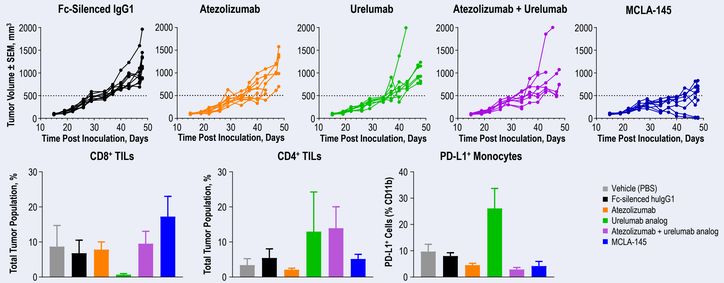


Data for atezolizumab treatment are missing for Tumor 1 due to technical issues

- Surgically resected endometrial tumors were dissociated into single cell suspension and analyzed for immune infiltration as well as surface marker expression by flow cytometry
- Cells were placed into 96-well round bottom plates and incubated with indicated antibodies in the presence of soluble anti-CD3 antibody (Clone Hit3a) for 6 days. IFN-γ levels in the supernatant were measured by Meso Scale Discovery electrochemiluminescence assay

Antitumor Activity of MCLA-145 in Humanized MDA-MB-231 Model

- MCLA-145 enhances the antitumor activity of human CD34⁺ cells engrafted in NSG mice with MDA-MB-231 tumors. MCLA-145, atezolizumab, and urelumab analog were given intraperitoneally at the dose of 5 mg/kg once every 5 days for a period of 31 days
- Human CD34⁺ engrafted NSG mice bearing MDA-MB-231 tumors had increased total CD8⁺ tumor infiltrating leukocytes in response to MCLA-145 treatment as measured by flow cytometry. No significant changes were observed for CD4⁺ or PD-L1⁺ cells



Conclusions

- **MCLA-145 is an Fc-silenced Biclonics® that engages human CD137 and PD-L1**
- **MCLA-145 induces CD137 signaling provided PD-L1 is present in its environment**
- **MCLA-145 induces cytokine production from T cells in ex vivo primary human tumors cells**
- **MCLA-145 demonstrates antitumor activity in humanized mouse tumor models**
- **The unique binding properties of MCLA-145 may result in an increased therapeutic window by specifically activating CD137-expressing cells in the tumor niche where PD-L1 is expressed, while simultaneously blocking inhibitory input from the PD-1/PD-L1 axis**

Disclosures

Patrick Mayes, Steve Wang, Thomas Condamine, Ashwini Kulkarni, Yao-bin Liu, Arpita Mondal, Leslie Hall, Peggy Scherle, Gregory Hollis, and **Reid Huber:** Employment and stock ownership – *Incyte Corporation.* **Paul Tacken, Pieter Fokko van Loo, Hans van der Maaden, Eric Rovers, Steef Engels, Floris Fransen, Mark Throsby,** and **Cecile Geuijen:** Employment and stock ownership – *Merus NV.* **Edmund Moon** and **Steven Albelda:** Research funding – *Incyte Corporation.* **Soyeon Kim, Marina Martinez,** and **Shaun O'Brien:** Nothing to disclose.

Acknowledgments

This study was sponsored by Merus NV (Utrecht, The Netherlands) and Incyte Corporation (Wilmington, DE). Editorial, graphics, and printing support was provided by Envision Pharma Group, Inc. (Philadelphia, PA), funded by Incyte Corporation.

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