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A Human Bispecific Antibody Targeting LAG-3 and PD-1 (INCA32459) Potently Activates Exhausted T Cells

Shaun Stewart,[1] Floris Fransen,[2] Shane Harvey,[1] Franziska Mortensen,[2] Anita Stam,[2] Rahel Awdew,[1] Arpita Mondal,[1] Christina Stevens,[1] Eric Rovers,[2] Steef Engels,[2] Melissa Rentrop-Boeijen,[2] Linda J. A. Hendriks,[2] Brenda van Dieren,[2] Rebecca Buonpane,[1] Therese Visser,[2] Pepijn Schellen,[2] Ashwini Kulkarni,[1] Jonathan Rios-Doria,[1] Jing Zhou,[1] Paul Tacken,[2] Lu Lu,[1] Vanessa Zondag-van der Zande,[2] Cheng-Yen Huang,[1] Renate den Blanken-Smit,[2] John de Kruif,[2] Rinse Klooster,[2] Simon Plyte,[2] Horacio Nastri,[1] Patrick Mayes[1]

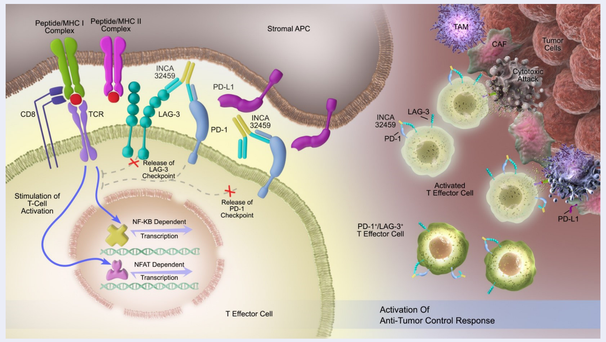
[1]Incyte Corporation, Wilmington, DE, USA; [2]Merus N.V., Utrecht, The Netherlands

Abstract

Exhausted T cells are characterized by the expression of negative immune regulatory receptors, including programmed death protein-1 (PD-1) and lymphocyte activation gene 3 (LAG-3), which inhibit the proliferation and function of T cells and limit antitumor immunity. We describe the generation and characterization of INCA32459, a human immunoglobulin G1 (IgG1) Fc-silenced bispecific antibody that simultaneously binds to PD-1 and LAG-3 and reverts their inhibitory function. INCA32459 was generated using the Merus common light chain Biclonics® platform. LAG-3 and PD-1 Fab panels were generated through immunization of Merus MeMo® mice, and large panels of Biclonics® libraries were screened before optimizing lead candidate molecules. INCA32459 binds with high affinity to both human (K_D=0.39 nM) and cynomolgus monkey (K_D=0.44 nM) PD-1, and human (K_D=1.15 nM) and cynomolgus monkey (K_D=0.20 nM) LAG-3, as measured by surface plasmon resonance. The monovalent PD-1 arm of INCA32459 blocks PD-1 with similar potency as a bivalent PD-1 antibody (nivolumab analog) in a PD-1/PD-L1 reporter assay. In a loss-of-function reporter assay where luciferase expression increases upon blockade of both LAG-3 and PD-1, INCA32459 significantly induced luciferase expression to a greater extent than either PD-1 (nivolumab analog) or LAG-3 (relatlimab analog) single-agent antibody controls, and greater than PD-1 (nivolumab) and LAG-3 (relatlimab) analog antibodies combined. In 3 human primary immune cell assays, a T-cell exhaustion model using staphylococcal enterotoxin B (SEB)–stimulated peripheral blood mononuclear cells (PBMCs), exhausted mixed lymphocyte reaction (MLR) assays, and an antigen recall assay using CEFT major histocompatibility complex class II (MHCII) peptide pool-stimulated PBMCs, INCA32459 treatment resulted in higher interleukin-2 (IL-2) and interferon (IFN)-γ induction, respectively, compared with PD-1 (nivolumab analog) and LAG-3 (relatlimab analog) antibodies combined. In a human MDA-MB-231 breast tumor model in CD34+ humanized NSG mice, INCA32459 treatment decreased tumor growth compared with a combination of PD-1 (pembrolizumab) and LAG-3 (relatlimab analog) antibodies. Pharmacodynamic analysis in mice demonstrated a dose-dependent increase in receptor occupancy at 1 and 10 mg/kg. Pharmacokinetic characterization of INCA32459 in cynomolgus monkeys after a single intravenous (IV) infusion at 3 and 30 mg/kg demonstrated an average clearance, steady-state volume of distribution, and mean residence time of 0.515 mL/h/kg, 74.1 mL/kg, and 144 h, respectively. We have developed INCA32459, a potent dual inhibitor of PD-1 and LAG-3 in preclinical models, which induces activation of exhausted T cells to a greater extent than a combination of bivalent monospecific antibodies targeting PD-1 (nivolumab analog) and LAG-3 (relatlimab analog). These data support the clinical evaluation of INCA32459, and a phase 1 study in patients with cancer is underway.

Introduction

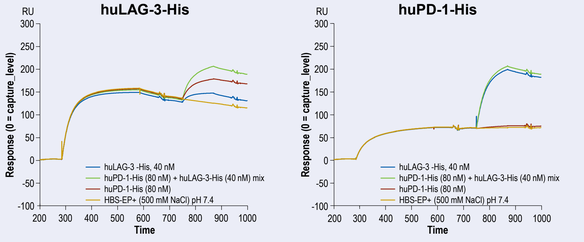
- Exhausted T cells are characterized by the expression of negative immune regulatory receptors, including PD-1 and LAG-3, which inhibit the proliferation and function of T cells and limit antitumor immunity[1-4]
- The blocking of LAG-3 and PD-1 binding to their ligands MHCII and PD-L1 releases the suppressive effect of checkpoint inhibition and enables activation of effector T cells[4]
- INCA32459 is a highly selective human IgG1 Fc-silenced bispecific antibody that simultaneously binds to PD-1 and LAG-3 and reverts their inhibitory function



APC, antigen-presenting cell; CAF, cancer-associated fibroblast; NFAT, nuclear factor of activated T cell; NF-kB, nuclear factor kappa B; TAM, tumor-associated macrophage; TCR, T-cell receptor.

INCA32459 Simultaneously Binds Both PD-1 and LAG-3 With High Affinities

- INCA32459 binds to human PD-1 with 3-fold greater affinity than LAG-3

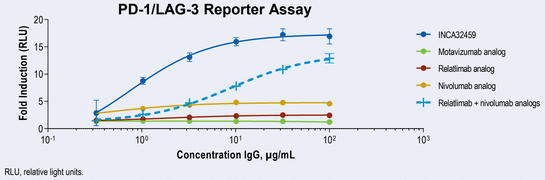


Bispecific antibodies at 20 nM were captured by anti-huFc immobilized on a CM5 sensor chip. Then, one of the antigens was injected at a saturating concentration (80 nM for huPD-1-His and 40 nM for huLAG-3-His) for 300 seconds, to occupy all antigen-binding sites. The second antigen was injected sequentially at the same concentration used in injection 1, either alone or in combination with the first antigen (to ensure that all binding sites remained occupied)

	INCA32459	
	PD-1 K_D	LAG-3 K_D
Human	0.39	1.15
Cynomolgus	0.44	0.20

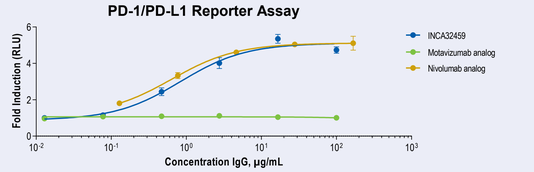
INCA32459 Is More Potent Than Nivolumab and Relatlimab Analogs in Reporter Assays

- INCA32459 is 10-fold more potent than an equimolar mixture of nivolumab and relatlimab analogs



RLU, relative light units.
PD-L1+ Raji cells (antigen-presenting cells) and Jurkat PD-1+/LAG-3+ effector cells were incubated for 6 hours in the presence of staphylococcal enterotoxin D (SED) and antibody titrations starting at 100 µg/mL, except nivolumab analog + relatlimab analog (50 µg/mL + 50 µg/mL). Luciferase reporter gene activity was determined by measuring luminescence, and fold induction induced by each antibody was calculated relative to wells containing no antibody (no IgG)

- Monovalent α-PD-1 arm of INCA32459 blocks PD-1 engagement to PD-L1 with similar potency as bivalent nivolumab analog

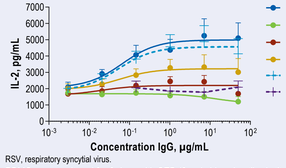


Jurkat NFAT-RE reporter T cells expressing PD-1 and CHO-K1 cells expressing PD-L1 were incubated for 6 hours in the presence of antibody titrations as indicated. Luciferase reporter gene activity was determined by measuring luminescence, and fold induction induced by each antibody was calculated relative to wells containing no antibody (no IgG)

INCA32459 Increases T-Cell Activation in Primary Immune Cell Assays

SEB Assay

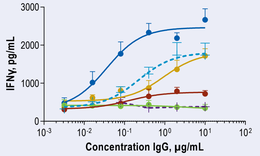
- INCA32459 generates equal IL-2 production in a PBMC SEB stimulation assay to an equimolar mixture of nivolumab and relatlimab analogs



RSV, respiratory syncytial virus.
PBMCs were cultured for 3 days with SEB (2 µg/mL) and antibody titrations starting at 50 µg/mL, except nivolumab analog + relatlimab analog (25 µg/mL + 25 µg/mL). Levels of IL-2 in the culture supernatant were measured by Luminex

Antigen Recall Assay

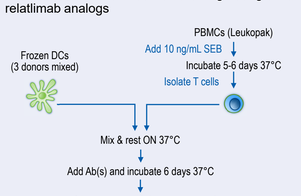
- INCA32459 is 20-fold more potent in IFNγ production and induces greater IFNγ secretion than an equimolar mixture of nivolumab and relatlimab analogs



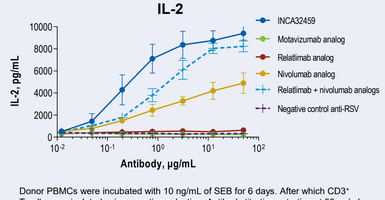
Donor PBMCs were incubated for 6 days with 1 µg/mL CEFT MHCII peptide pool and antibody titrations starting at 10 µg/mL, except nivolumab analog + relatlimab analog (5 µg/mL + 5 µg/mL). IFNγ levels were measured by Luminex

Exhausted MLR

- INCA32459 is more effective at reinvigorating exhausted T cells than an equimolar mixture of nivolumab and relatlimab analogs



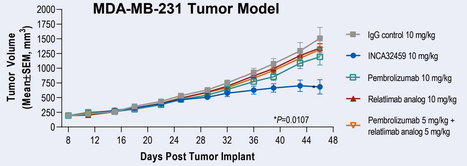
DC, dendritic cell; MSD, MesoScale Discovery assay.



Donor PBMCs were incubated with 10 ng/mL of SEB for 6 days. After which CD3+ T cells were isolated using negative selection. Antibody titrations starting at 50 µg/mL, except nivolumab and relatlimab analogs (25 µg/mL + 25 µg/mL). IL-2 levels were measured by MSD

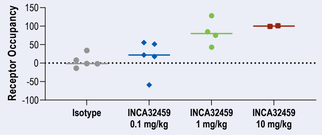
Antitumor Activity and Receptor Occupancy of INCA32459 in Humanized MDA-MB-231 Mouse Model

- INCA32459 enhances the antitumor activity of human CD34+ cells engrafted in NSG mice with MDA-MB-231 tumors
- The efficacy of INCA32459 in the MDA-MB-231 model was statistically significant compared with IgG1 control by 2-way analysis of variance



Receptor Occupancy of CD8+ T Cells in Humanized MDA-MB-231 Mouse Model

- There is a dose-dependent titration of receptor occupancy in CD8+ T cells among mice dosed with INCA32459



Pharmacokinetic Analysis of INCA32459 in Cynomolgus Monkeys

- After a single-dose IV infusion at 3 and 30 mg/kg in monkeys, INCA32459 exhibited pharmacokinetic profiles typical of an antibody

Dose	3 mg/kg	30 mg/kg*
C_{max}, µg/mL	68.3	657
t_{max} after SOI, h	0.5	0.5
$AUC_{0\text{-}t}$, h•µg/mL (time for calculation)	5360 (0 to 336.5 h)	59,800 (0 to 240.5-336.5 h)
$AUC_{inf,pred}$, h•µg/mL	5820	NR
$t_{½,term}$, h	108	NR
$MRT_{inf,pred}$, h	144	NR
CL_{pred}, mL/h/kg	0.515	NR
$V_{ss,pred}$, mL/kg	74.1	NR

All data presented as average.
*The concentration data at or after those time points that showed a sharp decrease were not included in the calculation of pharmacokinetic parameters.
$AUC_{0\text{-}t}$, area under the concentration time curve from time 0 to time t; $AUC_{inf,pred}$, AUC from time of dosing extrapolated to infinity, based on the last predicted concentration; CL_{pred}, predicted clearance; C_{max}, maximum plasma drug concentration; MRT, mean residence time; NR, not reported due to the extrapolation of the AUC_{inf} representing >20% of the total area; $t_{½,term}$, terminal half-life; t_{max}, time to maximum plasma drug concentration, $V_{ss,pred}$, steady-state volume of distribution.

Conclusions

- **INCA32459 is a potent dual inhibitor of PD-1 and LAG-3**
- **INCA32459 induced greater reporter cell activation, IL-2 and IFNγ production than nivolumab and relatlimab analogs in SEB stimulation and memory recall assays, respectively**
- **INCA32459 induces activation and reinvigoration of exhausted T cells in an exhausted MLR model**
- **INCA32459 demonstrates antitumor activity in MDA-MB-231 humanized mouse models**
- **In cynomolgus monkeys, INCA32459 exhibited pharmacokinetic profiles typical of an antibody**
- **The unique ability to simultaneously inhibit both PD-1 and LAG-3 leads to more potent T-cell reinvigoration than a dual therapy of PD-1 and LAG-3 monoclonal antibodies**

Disclosures

Stewart, Harvey, Awdew, Mondal, Stevens, Buonpane, Kulkarni, Rios-Doria, Zhou, Lu, Huang, Nastri, Mayes: Employment and stock ownership – *Incyte Corporation*. **Fransen, Mortensen, Stam, Rovers, Engels, Rentrop-Boeijen, Hendriks, van Dieren, Visser, Schellen, Tacken, Zondag-van der Zande, den Blanken-Smit, de Kruif, Klooster, Plyte:** Employment and stock ownership – *Merus N.V.*

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