Development of IMGS-001, a novel anti–PD-L1/PD-L2 dual-specific, multifunctional antibody, to treat immune-excluded tumors

C Gagliardi,[1] A Salameh,[1] P Blezinger,[1] M Hemberger,[1] C Schweizer,[1] J Barlow,[1] M Curran,[2] F Pericle[1]

[1]ImmunoGenesis, Inc, Houston, TX, USA; [2]MD Anderson Cancer Center, Houston, TX, USA.

Background

Interruption of the programmed cell death-1 (PD-1) inhibitory pathway by binding PD-1 or its ligand PD-L1 is an effective treatment for various cancers, although resistance is common. PD-1 has a second ligand, PD-L2, that can be expressed by a variety of immunosuppressive stromal cells, endothelial cells, and tumor cells. IMGS-001 is a dual-specific monoclonal antibody designed to bind PD-L1 and PD-L2 and block their engagement with PD-1 (Figure 1). The Fc region is engineered to induce robust cell-mediated cytotoxicity, enabling depletion of PD-L1$^+$ and PD-L2$^+$ immunosuppressive cells throughout the tumor microenvironment. Here we describe the development of IMGS-001, including potency, specificity, cytokine release potential, pharmacokinetics (PK), and repeat-dose toxicity.

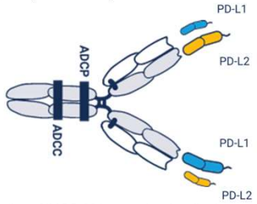

Figure 1. Graphical representation of IMGS-001 monoclonal antibody.

One important reason for failure of anti–PD(L)-1 therapy is the dominant T-cell suppression resulting from non-checkpoint mechanisms of immune suppression. Immune suppression via cytokines (TGF-β, IL-35), nutrient-depleting enzymes (arginase, indoleamine 2,3 dioxygenase), and suppressive metabolites (adenosine, kynurenines), as well as triggering of Fas-induced apoptosis by stromal cells (cancer-associated fibroblasts, endothelial cells), acts to deplete and disable T cells independently of PD-1 inhibition. Thus, to expand the percentage of patients that benefit from immunotherapy, drugs are needed that can diminish multimodal immune suppression in immune-excluded tumors to the extent that T cells can accumulate and expand sufficiently to benefit from PD-1 pathway blockade. IMGS-001 is being developed to address this gap in the existing arsenal of clinical immunotherapies.

Methods

Affinities were measured with the Octet system. Reporter cell assays (Promega) assessed PD-1 pathway blockade, antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent cellular phagocytosis (ADCP). Specificity was evaluated by Retrogenix microarray technology. Potential for spontaneous cytokine release was measured by coculturing with healthy donor peripheral blood mononuclear cells (PBMCs). PK studies were performed in mice and in cynomolgus monkeys. In a GLP toxicity study, IMGS-001 was dosed weekly over 4 weeks at 10, 50, or 100 mg/kg with a 4-week recovery period.

Results

In Vitro Characterization

IMGS-001 binds with high affinity to PD-L1 and PD-L2.

IMGS-001 binds with low nanomolar affinity to human PD-L1 and PD-L2 (Figure 2). It has higher-affinity binding to PD-L2, reflecting the natural characteristic of PD-1 in humans.

IMGS-001 binds cynomolgus (0.468 nM, 0.724 nM) PD-L1 and PD-L2. It binds mouse antigens, with lower affinity to mouse PD-L1. Mice were used for preclinical efficacy studies and cynomolgus monkeys for toxicology because of the high homology to human proteins.

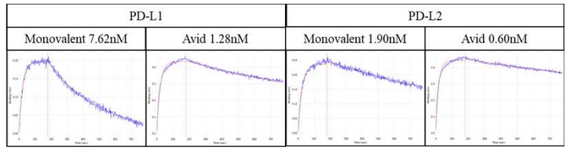

Figure 2. Octet images of IMGS-001 binding human PD-L1 and PD-L2 in monovalent and avid presentations. IgG on AHC sensor, 100 nM human PD-L1-His, PD-L1-Fc, PD-L2-His, or PD-L2-Fc (L-R) in solution.

IMGS-001 blocks the entire PD-1 inhibitory pathway.

IMGS-001 interrupts the ability of PD-1 to bind its ligands and inhibits the pathway in the presence of PD-L1$^+$ cells, PD-L2$^+$ cells, or a mixture of cells expressing both ligands (Figure 3).

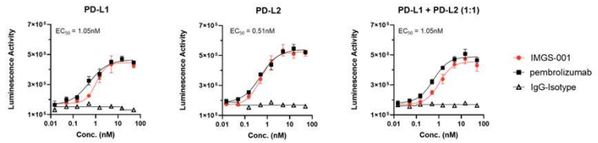

Figure 3. The concentration of IMGS-001, pembrolizumab, or IgG isotype is displayed on the x-axis, and luminescence activity is displayed on the y-axis. Mean ± SEM (n = 3) is displayed for each concentration of each antibody. A dose range of 0.015 to 50.000 nM was tested against PD-L1$^+$ cells (left), PD-L2$^+$ cells (center), and a 50:50 mixture of PD-L1$^+$ and PD-L2$^+$ cells (right).

IMGS-001 has potent ADCC and ADCP activity in vitro.

The Fc region is bound by FcγRIIIa and FcγRIIa receptors when IMGS-001 engages PD-L1 and/or PD-L2 on target cells, indicating its ability to mediate potent ADCC and ADCP (Figure 4).

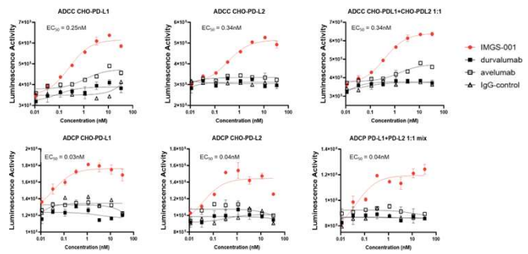

Figure 4. The concentration of IMGS-001, durvalumab, avelumab, or IgG isotype is displayed on the x-axis, and luminescence activity is displayed on the y-axis. Mean ± SEM (n = 2) is displayed for each concentration of each antibody. A dose range of 0.011 to 33.333 nM was tested against PD-L1$^+$ cells (left), PD-L2$^+$ cells (center), and a 50:50 mixture of PD-L1$^+$ and PD-L2$^+$ cells (right) in reporter cell assays testing ADCC (top) and ADCP (bottom).

Specificity

IMGS-001 does not have evidence of potentially toxic off-target binding.

Tested against 5861 human plasma membrane proteins and cell surface–tethered secreted proteins in addition to 371 human heterodimers expressed in HEK293 cells, IMGS-001 showed strong specific interactions with PD-L1 and PD-L2, as well as potential weak off-target interactions with CST1 and CST4 (Figure 5; in green). These are secreted proteins found in saliva and therefore do not present a risk of adverse effects.

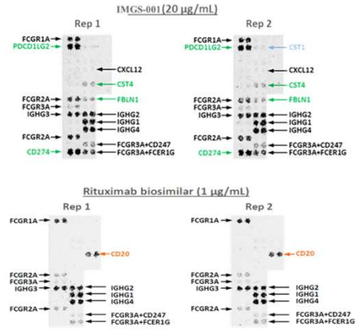

Figure 5. Images of fixed-cell microarrays for IMGS-001 (top) and a control antibody (bottom). Specific hits are in green. Nonspecific hits are in black. The positive control is in orange.

FBLN was detected as a potential target in the fixed cells screen but not when IMGS-001 was tested against live cells. Proteins noted in black were also detected for at least one of the control antibodies and classified as nonspecific. These include Fc gamma receptors and proteins bound directly by the detection antibody.

Pharmacokinetics

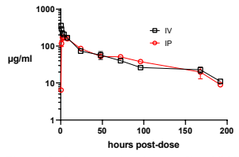

Figure 6. Concentration-time profile of IMGS-001 dosed at 10 mg/kg IP and IV in mice.

A mouse study characterized the PK of IMGS-001 after IP and IV administration at the efficacious dose (10 mg/kg, Figure 6). As would be expected, the C_{max} for the IV group was higher than that for the IP group. The elimination half-life, clearance, and volume of distribution were similar for both routes of administration.

Weekly IV infusions of IMGS-001 at 10, 50, or 100 mg/kg in cynomolgus monkeys resulted in approximately dose-proportional increases in mean C_{max} and AUC with increasing dose (Figure 7). Systemic exposure was independent of sex. In all dose groups, some animals developed antidrug antibodies.

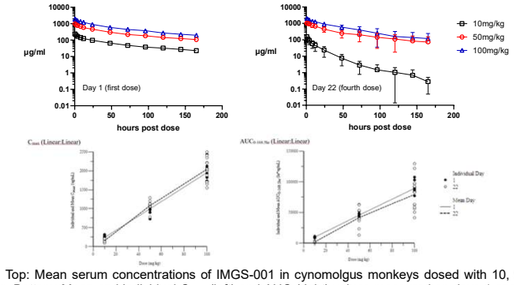

Figure 7. Top: Mean serum concentrations of IMGS-001 in cynomolgus monkeys dosed with 10, 50, or 100 mg/kg. Bottom: Mean and individual C_{max} (left) and AUC (right) values measured on days 1 and 22 in cynomolgus monkeys dosed with 10, 50, or 100 mg/kg IMGS-001.

Cytokine Release

IMGS-001 was tested for its effects on cytokine/chemokine release from human PBMCs (8 donors) under wet bound and soluble conditions and at concentrations up to 250 µg/mL IFNγ, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-17A, MIP-1α, and TNFα were measured (Figure 8).

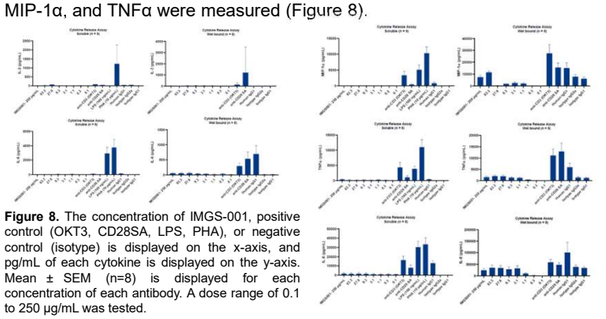

Figure 8. The concentration of IMGS-001, positive control (OKT3, CD28SA, LPS, PHA), or negative control (isotype) is displayed on the x-axis, and pg/mL of each cytokine is displayed on the y-axis. Mean ± SEM (n=8) is displayed for each concentration of each antibody. A dose range of 0.1 to 250 µg/mL was tested.

Toxicology

In a repeat-dose toxicity study of 4 weeks in cynomolgus monkeys, there were no mortalities and no IMGS-001–related changes in body weights, qualitative food consumption, ophthalmology, electrocardiology, neurological parameters, blood pressure, heart rate, urinalysis parameters, organ weights or weight ratios, or macroscopic examinations after weekly 30-minute IV infusions of 10, 50, or 100 mg/kg.

IMGS-001–related effects at ≥10 mg/kg:

- Dose-dependent decrease in CD16+ cells in peripheral blood
- Dose-independent increase in plasma IL-1RA concentrations
- Slight to moderate emesis during infusion (n=3)

IMGS-001–related effects at ≥50 mg/kg:

- Mild to marked decrease in neutrophils (n=3) and/or platelets (n=4)
- Mild to marked increase in monocytes (n=2)
- Dose-dependent minimal to mild increase in red cell distribution width
- Minimal to mild decrease in albumin and minimal to mild increase in globulins
- Minimal to mild mononuclear cell and/or mixed infiltrations, often with perivascular/vascular distribution, within various tissue

At 100 mg/kg, 1 of 12 animals presented with clinical symptoms, including dehydration, labored breathing, increased respiration, and high body temperature. Pathology revealed moderate mixed-cell inflammation in the lungs, mild thrombus within the kidney, and mild mixed-cell inflammation in the spleen. These findings set the highest nonseverely toxic dose (HNSTD) at 50 mg/kg.

Conclusions

These data indicate that IMGS-001 binds to PD-L1 and PD-L2 and functions per its design. It shows no biologically relevant off-target effects and has a viable PK profile for human administration. A 4-week repeat dose toxicity study showed some immune-related effects, with an HNSTD of 50 mg/kg.

The IMGS-001 mechanisms of elimination of immunosuppressive cells with PD-1 pathway blockade could benefit patients that are resistant to existing PD-(L)1 drugs by restoring immune-driven anti-tumor activity. IMGS-001 is poised to enter clinical trial in immune-excluded tumors by the end of 2022.

This research is supported in part through a grant award from the Cancer Prevention & Research Institute of Texas (“CPRIT”).