CT085

Pharmacodynamic Correlates in a Phase 1 Study of INCMGA00012, a PD-1 Antagonistic Monoclonal Antibody

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Abstract

Background: The role of the programmed cell death (PD)-1/programmed cell death ligand (PD-L)1 axis in limiting T cell activity has been well established. Effective blockade of this pathway has been demonstrated to lead to an increase in T cell activity and to yield clinical activity in cancer patients. INCMGA00012 is a humanized IgG4 monoclonal antibody that binds to human PD-1 and blocks its interaction with PD-L1/PD-L2. This therapeutic antibody has demonstrated acceptable tolerability with evidence of clinical activity in a phase 1 study in patients with solid tumors (NCT03059823). Pharmacodynamic markers demonstrating biological activity of INCMGA00012 were assessed in samples collected during the study.

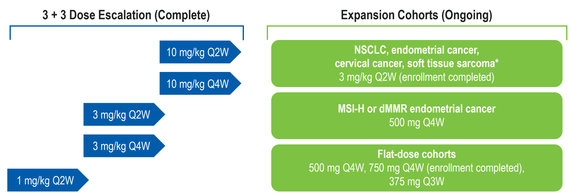
Methods: Blood samples were collected at baseline and at various time points following treatment from patients receiving doses of either 3 mg/kg (Q2W) in the tumor-specific expansion cohort or a flat-dose of 500 or 750 mg (Q4W) in the tumor-agnostic expansion cohort. Receptor occupancy was measured on circulating T cells by flow cytometry and serum cytokine levels were measured using either a proximity extension assay or immunoassays. In addition, circulating immune cell phenotyping was analyzed using flow cytometry and peripheral blood mononuclear cell (PBMC) functionality evaluated using ex vivo restimulation.

Results: All tested doses of INCMGA00012 evaluated demonstrated a full saturation of the PD-1 receptor at trough on circulating CD4 and CD8 T cells. In all tested cohorts, an increase in serum CXCL9 and CXCL10 was observed following infusion with INCMGA00012. Moreover, an increase in the proliferation of circulating T cells was observed on treatment compared to baseline. Concurrently, following ex vivo restimulation, T cells isolated from PBMC demonstrated an increased capacity to secrete cytokines.

Conclusion: These results provide evidence that INCMGA00012 is biologically active and leads to an increase in IFNγ-related protein levels (ie, CXCL9 and CXCL10) and in T cell proliferation. The clinical activity of INCMGA00012 is undergoing further evaluation in several indications, including endometrial cancer, Merkel cell carcinoma, and squamous carcinoma of the anal canal. Several combination studies have also been initiated.

Methods

Overall Study Design



* The following histologies of soft tissue sarcoma are allowed: undifferentiated pleomorphic sarcoma (including malignant fibrous histiocytoma), de-differentiated or poorly differentiated liposarcoma, synovial sarcoma, or rhabdomyosarcoma.
dMMR, deficient mismatch repair; MSI-H, microsatellite instability-high; NSCLC, non-small cell lung cancer; Q2W, every 2 weeks; Q3W, every 3 weeks; Q4W, every 4 weeks.

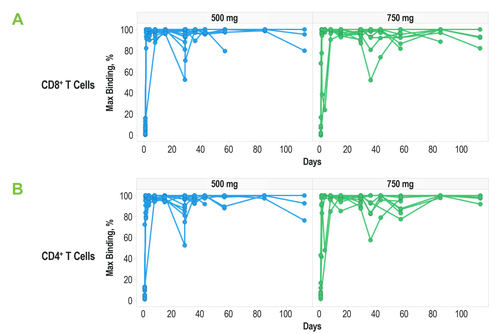
Eligibility Criteria (Cohort Expansion Phase)

- Tumor-specific cohorts: patients with unresectable, locally advanced, or metastatic NSCLC, endometrial cancer, cervical cancer, and soft tissue sarcoma who have progressed during or following 1–5 prior treatments
- Flat-dose cohorts: patients with carcinoma of any tumor histology who have progressed during or following 1–5 prior treatments consistent with the standard of care for respective tumor types

Results

Receptor Occupancy for the Q4W Flat-Dosing Cohort

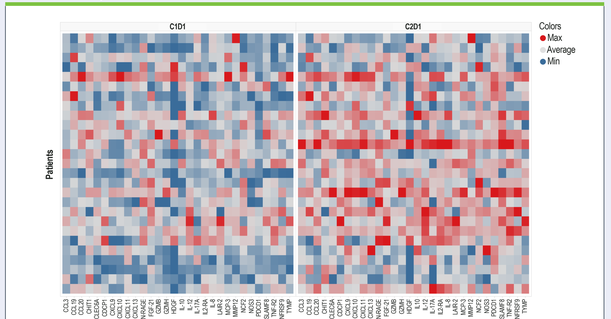
PD-1 Receptor on Circulating T Cells Is Fully Saturated by INCMGA00012



INCMGA00012 receptor occupancy on CD8+ (A) and CD4+ (B) T cells from participants dosed with INCMGA00012 was measured using flow cytometry technology evaluating the percentage of maximal binding over time (as measured by anti-IgG4 to detect INCMGA00012). Receptor occupancy analysis from 30 participants receiving flat doses of INCMGA00012 (500 mg Q4W [n = 15] and 750 mg Q4W [n = 15]) demonstrated full occupancy in both dosing cohorts. Specifically, average receptor occupancy for both CD8+ and CD4+ T cells was above 90% at all time points assessed.

Plasma Protein Upregulated on Treatment

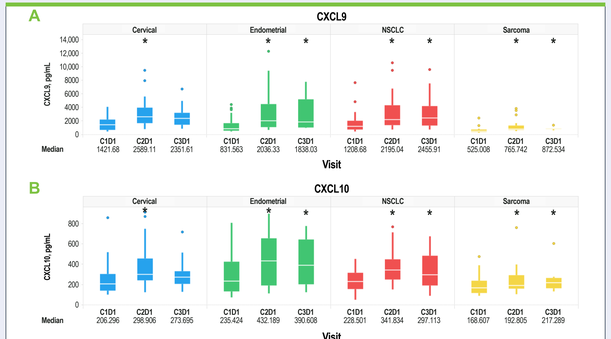
Patients Receiving Infusion of INCMGA00012 Present an Increase in Several Serum Proteins Including Various Chemokines and Cytokines



Serum samples collected pretreatment (C1D1 – left panel) and on treatment (C2D1 – right panel) in patients enrolled in the 500 mg and 750 mg flat-dose cohort (Q4W) were analyzed using Olink proximity extension assay technology (11 panels). Fold change in plasma protein levels was calculated by comparing C2D1 (day 29) levels to C1D1 levels (day 1). Only proteins with a fold change >1.25 are depicted in this figure.

CXCL9 and CXCL10 Upregulation in Tumor-Specific Cohorts

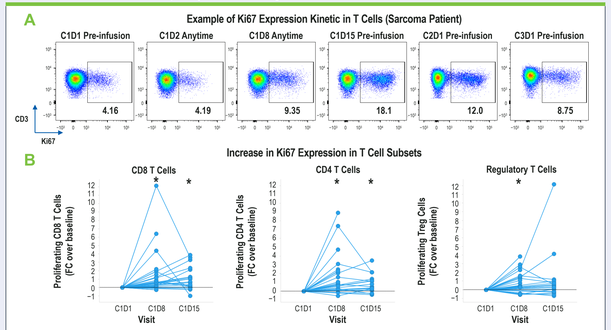
CXCL9 and CXCL10 Levels in Serum Are Increased in Patients Dosed With INCMGA00012



Level of CXCL9 (A) and CXCL10 (B) were measured by immunoassays in serum samples collected at baseline (C1D1 pretreatment) and on treatment (C2D1: day 29 and C3D1: day 57) in patients enrolled in the tumor-specific cohorts. The table below the box plot summarizes the median value at each visit (1 indication per panel as indicated) (* $P < 0.05$ by Wilcoxon matched pairs test – each time point was compared with C1D1).

Kinetics of Ki67 Increase in T Cells

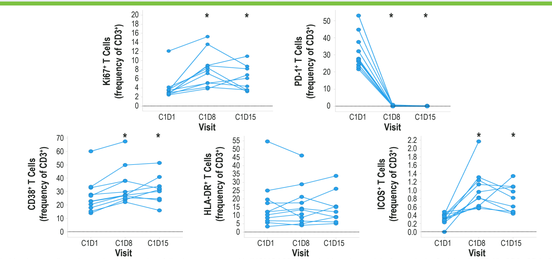
INCMGA00012 Increases the Frequency of Proliferating T Cell Subsets in the Blood of Treated Patients



Whole blood was collected at various time points following treatment with INCMGA00012 and shipped overnight for analysis. Staining for CD45, CD3, CD4, CD8, CD25, FoxP3, and Ki67 was performed. Cells were first gated on single cells, then on CD45+ cells (leukocytes), and then on CD3+ cells (T cells). A. Dot plots depicting the frequency of Ki67+ T cells (CD45+/CD3+ cells) at various times in a patient with sarcoma receiving 3 mg/kg of INCMGA00012 Q2W. B. Fold change in the frequency of proliferating cells (Ki67+ cells) in different T cell subsets (left panel: CD3+/CD8+; middle panel: CD3+/CD4+; right panel: CD3+/CD4+/CD25+/ FoxP3+). This analysis includes 28 patients enrolled in the tumor-specific cohorts (* $P < 0.05$ by Wilcoxon matched pairs test – each time point was compared with C1D1).

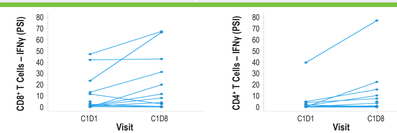
Circulating T Cells Present a More Activated Phenotype Early on Treatment

INCMGA00012 Increases the Frequency of T Cells Expressing Activation Markers in the Blood of Treated Patients



Whole blood was collected at various time points following treatment with INCMGA00012 and shipped overnight for analysis. Staining for CD45, CD3, CD4, CD8, PD-1, CD38, HLA-DR, ICOS, and Ki67 was performed. Cells were first gated on single cells, then on CD45+ cells (leukocytes), and then on CD3+ cells (T cells). Frequency of each marker at C1D1, C1D8, and C1D15 is depicted for 11 patients enrolled in the 375 mg (Q3W) cohort (* $P < 0.05$ by Wilcoxon matched pairs test – each time point was compared with C1D1).

Ex Vivo Restimulated CD8+ and CD4+ T Cells Secrete Higher Level of IFNγ Post INCMGA00012 Treatment



CD8+ and CD4+ T cells were isolated from frozen PBMCs collected from patients enrolled in the 500 mg and 750 mg flat-dosing cohorts. T cell subsets were restimulated overnight using anti-CD3 and anti-CD28 antibodies. The next day, cells were harvested and cytokine secretion by single cells analyzed using the Isoplexis Isolight system (Branford, CT). PSI, Polyfunctional Strength Index.

Discussion and Conclusions

- **INCMGA00012 administered at 375 mg (Q3W), 500 mg (Q4W), and 750 mg (Q4W) fully occupy PD-1 receptors on circulating CD4+ and CD8+ T cell subsets**
- **An array of serum protein, including cytokines and chemokines, was found to be increased in patients dosed with INCMGA00012. The IFNγ-inducible chemokines, CXCL9 and CXCL10, were among the highest upregulated serum proteins identified as induced by INCMGA00012**
- **An increase in the frequency of proliferating T cells and activated T cells was observed in patients receiving INCMGA00012. This effect seemed to peak 8 days following the first infusion. In addition, ex vivo restimulation suggests that activated T cells secrete larger amounts of IFNγ per cell**
- **Increases in circulating serum chemokines and frequency of proliferating T cells were observed irrespective of tumor indication, and similar to previously reported data with other PD-(L)1 agents**
- **Taken together, these results demonstrate that INCMGA00012 at all regimens/doses tested is biologically active and leads to an increase in circulating T cell activation. The clinical activity is undergoing further evaluation in monotherapy and combination therapy studies**

Disclosures

Thomas Condamine, Sherry Owens, Pat Feldman, and **Robert Newton:** Employment and stock ownership – *Incyte Corporation.*
Ross La Motte-Mohs, John Muth, Bradley Sumrow, and **Paul Moore:** Employment and stock ownership – *MacroGenics Inc.*

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