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Pharmacodynamic Biomarkers Demonstrate T-Cell Activation in Patients Treated With the Oral PD-L1 Inhibitor INCB086550 in a Phase 1 Clinical Trial

Sarina A. Piha-Paul,[1]* Tara C. Mitchell,[2] Solmaz Sahebjam,[3] Janice Mehnert,[4] Thomas Karasic,[2] Kevin O'Hayer,[5] Ryan Geschwindt,[5] Susan Spitz,[5] Hao Liu,[5] Johanna Bendell[6]

[1]University of Texas MD Anderson Cancer Center, Houston, TX; [2]University of Pennsylvania, Philadelphia, PA; [3]Moffitt Cancer Center, Tampa, FL; [4]New York University Langone Health's Perlmutter Cancer Center, New York, NY; [5]Incyte Corporation, Wilmington, DE; [6]Tennessee Oncology, Nashville, TN

*Presenting author

Background

- Programmed cell death protein 1 (PD-1) binds to programmed cell death ligand 1 (PD-L1; also known as CD274), which is highly upregulated on many types of tumor cells as well as immune cells. Interaction of PD-1 with PD-L1 delivers a signal that inhibits interleukin (IL)-2 and interferon-γ (IFNγ) production, as well as cell proliferation induced upon T-cell receptor activation[1-3]
  - Thus, the pharmacological blockade of the PD-1:PD-L1 interaction may affect these pathways
- Treatment with monoclonal antibody drugs has been associated with enhancement of the antitumor effector functions of T cells as evidenced by increased peripheral T-cell proliferation and infiltration of T cells in tumors, together with increased expression of key IFNγ pathway genes and proteins in plasma
- The reported observed changes in the periphery include increases in C-X-C motif chemokine ligand (CXCL) 9, CXCL10, IL-18, IFNγ, and granzyme serine proteases, as well as increases in circulating $CD8^+HLA\text{-}DR^+Ki\text{-}67^+$ T cells[4,5]
- RNA sequencing (RNASeq) has demonstrated that the blockade of PD-L1 with a monoclonal antibody drug in the breast cancer cell line MDA-MB-231 downregulates genes related to epithelial-mesenchymal transition (EMT), cell migration/invasion and metastasis, cell proliferation/growth, and anti-apoptosis[6]
- In the INCB86550-102 study, we assessed markers that have previously demonstrated modulation with antibody treatment, such as soluble target, cytokines, and T-cell proliferation, and evaluated a panel of 1104 proteins and performed RNASeq to identify additional markers that may be unique to an oral anti–PD-L1 inhibitor

Objective

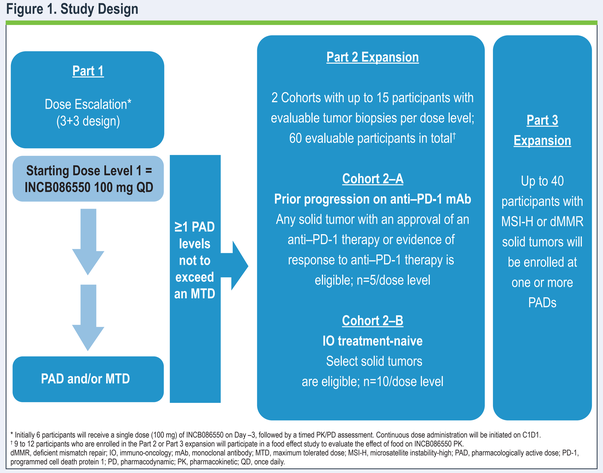
- To describe peripheral pharmacodynamics activity following treatment with INCB086550

Methods

Study Design and Patients

- Men and women ≥18 years of age with advanced solid tumors who have received prior standard therapy, which may include immune checkpoint therapy, were enrolled
- The study design is shown in **Figure 1**

Figure 1. Study Design



* Initially 6 participants will receive a single dose (100 mg) of INCB086550 on Day –3, followed by a timed PK/PD assessment. Continuous dose administration will be initiated on C1D1.
† 9 to 12 participants who are enrolled in the Part 2 or Part 3 expansion will participate in a food effect study to evaluate the effect of food on INCB086550 PK.
dMMR, deficient mismatch repair; IO, immuno-oncology; mAb, monoclonal antibody; MTD, maximum tolerated dose; MSI-H, microsatellite instability-high; PAD, pharmacologically active dose; PD-1, programmed cell death protein 1; PD, pharmacodynamic; PK, pharmacokinetic; QD, once daily.

- Cancer types and dose in the current INCB86550-102 data set are shown in **Table 1**

Table 1. Cancer Types and INCB086550 Doses in the INCB86550-102 Data Set

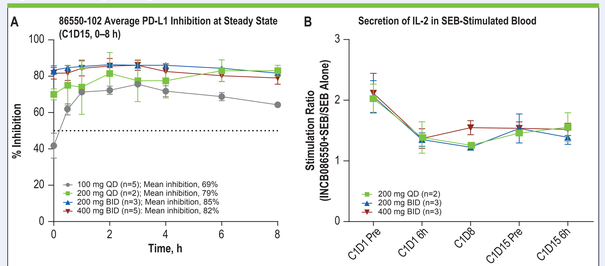
Cancer Type	Dose
High-grade serous fallopian	100 mg QD
High-grade epithelial ovarian	100 mg QD
Dedifferentiated liposarcoma	100 mg QD
Undifferentiated pleomorphic sarcoma	100 mg QD
Acinic cell carcinoma of the parotid gland	100 mg QD
Prostate adenocarcinoma	100 mg QD
Endometrial leiomyosarcoma	200 mg QD
Cholangiocarcinoma	200 mg QD
Adrenocortical carcinoma	200 mg QD
Renal cell adenocarcinoma (post PD-1 treatment)	200 mg BID
Clear cell endometrial carcinoma	200 mg BID
Pancreatic ductal adenocarcinoma	200 mg BID
Basal cell carcinoma	400 mg BID
Adrenocortical carcinoma	400 mg BID
Cholangiocarcinoma (n=2)	400 mg BID
Prostate adenocarcinoma	400 mg BID
CRC (MSI-H; post CTLA4/PD-1 treatment)	400 mg BID
Squamous cell anal carcinoma	400 mg BID

BID, twice daily; CRC, colorectal cancer; CTLA4, cytotoxic T-lymphocyte antigen 4; MSI-H, microsatellite instability-high; PD-1, programmed cell death protein 1; QD, once daily.

Results

- The ex vivo stimulation of whole blood from patients showed a dose-related decrease of free PD-L1 receptors and increases in IL-2 secretion **(Figure 2)**

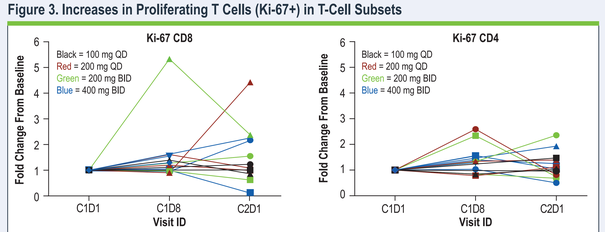
Figure 2. Binding of Drug to PD-L1 and Secretion of IL-2 With Ex Vivo Restimulation



The binding activity to PD-L1 on human blood monocytes was monitored in whole blood stimulated with exogenous IFNγ followed by the analysis of expression of PD-L1 by flow cytometry. The average inhibition over the dosing interval (0–8 h) at steady state (C1D15) in Cohort 1 (100 mg QD), Cohort 2 (200 mg QD), Cohort 3 (200 mg BID), and Cohort 4 (400 mg BID) was 69%, 79%, 85%, and 82%, respectively (**A**). Since the percent inhibition for the 100-mg QD dose group at time 0 was below 50%, suggesting lower exposure, the 100-mg dose group is excluded from the presentation of the targeted analysis of peripheral blood protein markers. A functional assay to determine if INCB086550 enhances T-cell effector function by examining its effects on cytokines secreted from SEB-stimulated blood was utilized to evaluate the effect of drug in study INCB86550-102. Maximum secretion of IL-2 was demonstrated by 6 hours on-treatment in all cohorts (**B**). BID, twice daily; IL, interleukin; IFNγ, interferon-γ; PD-L1, programmed cell death ligand 1; QD, once daily; SEB, staphylococcal enterotoxin B.

- Increases in the proliferation of circulating T cells, as measured by Ki-67, were observed post treatment **(Figure 3)**

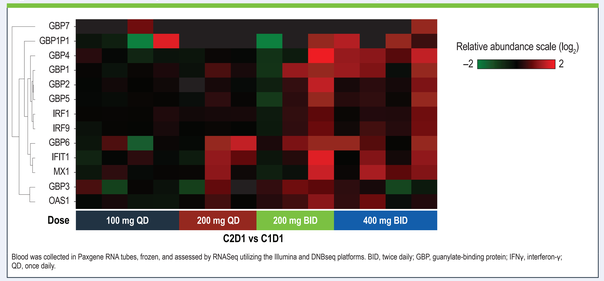
Figure 3. Increases in Proliferating T Cells (Ki-67+) in T-Cell Subsets



Whole blood was collected at various time points following treatment with INCB086550 and shipped overnight for analysis. Staining for T-cell subsets and the marker of proliferation, Ki-67, was performed. Increases in the fold change in the frequency of proliferating cells (Ki-67+ cells) in different T-cell subsets, $CD3^+/CD8^+$, $CD3^+/CD4^+$ (shown), is observed in all cohorts except the lowest (100 mg). Increases were also observed in the total $CD3^+$ population (not shown). BID, twice daily; QD, once daily.

- Increases in multiple IFN genes were observed in a dose-dependent manner by RNASeq **(Figure 4)**

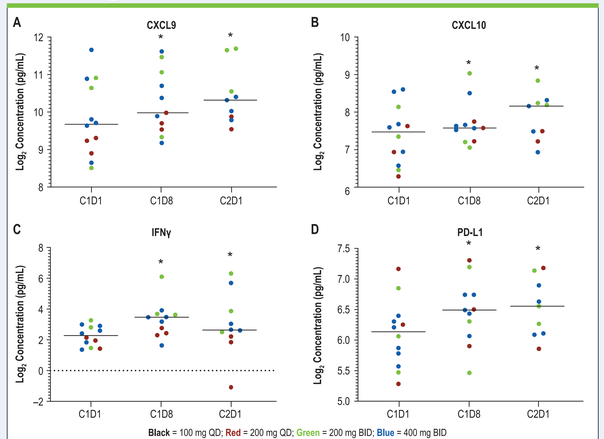
Figure 4. Elevation of IFNγ and GBP Gene in On-Treatment Samples



Blood was collected in Paxgene RNA tubes, frozen, and assessed by RNASeq utilizing the Illumina and DNBseq platforms. BID, twice daily; GBP, guanylate-binding protein; IFNγ, interferon-γ; QD, once daily.

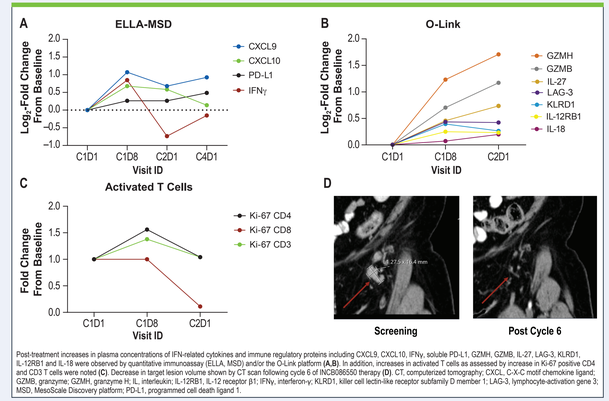
- Plasma concentrations of the IFN-related cytokines and immune regulatory proteins increased following INCB086550 treatment **(Figure 5)**

Figure 5. Plasma Concentrations of the Interferon-Related Cytokines and Immune Regulatory Proteins Following INCB086550 Treatment



A targeted panel of interferon-related cytokines and immune regulatory proteins were assessed in plasma by immunoassays (ELLA and MesoScale Discovery platforms). The mean concentrations of CXCL9 and CXCL10 increased by 1.8- and 1.4-fold, respectively, from baseline to C2D1 (**A**,**B**), and a 3.8-fold increase in IFNγ was also observed on-treatment at C2D1 (**C**). A 1.4-fold increase in the plasma concentration of soluble target (PD-L1) was observed (**D**). In addition, the plasma samples were assessed on a panel of 1104 analytes utilizing the O-Link Proximity Extension Assay platform. On-treatment increases in CXCL9, CXCL10, IFNγ, and PD-L1 were confirmed by the O-Link platform. The O-Link platform identified increases in proteins not included in the targeted assessment, including IL-18, IL-27, granzyme B, and granzyme H. The heat map of 39 analytes in samples from INCB86550-102 demonstrates similarities of upregulation with antibody drugs in this class (**E**). BID, twice daily; CXCL, C-X-C motif chemokine ligand; IFNγ, interferon-γ; PD-L1, programmed cell death ligand 1; QD, once daily.
* $P<0.05$.

Figure 6. Immune Activation and Clinical Activity Observed in a 54-Year-Old Patient Previously Treated With Chemoradiotherapy and Immunotherapy Naive



Post-treatment increases in plasma concentrations of IFN-related cytokines and immune regulatory proteins including CXCL9, CXCL10, IFNγ, soluble PD-L1, GZMH, GZMB, IL-27, LAG-3, KLRD1, IL-12RB1 and IL-18 were observed by quantitative immunoassay (ELLA, MSD) and/or the O-Link platform (**A**,**B**). In addition, increases in activated T cells as assessed by increase in Ki-67 positive CD4 and CD3 T cells were noted (**C**). Decrease in target lesion volume shown by CT scan following cycle 6 of INCB086550 therapy (**D**). CT, computerized tomography; CXCL, C-X-C motif chemokine ligand; GZMB, granzyme; GZMH, granzyme H; IL, interleukin; IL-12RB1, IL-12 receptor β1; IFNγ, interferon-γ; KLRD1, killer cell lectin-like receptor subfamily D member 1; LAG-3, lymphocyte-activation gene 3; MSD, MesoScale Discovery platform; PD-L1, programmed cell death ligand 1.

Conclusions

- **Ex vivo pharmacologic effects of drug exposure were demonstrated by 2 methods: measurement of unoccupied PD-L1 receptors and IL-2 secretion after restimulation of blood cells**
- **Changes in the peripheral blood were observed that are consistent with the mechanism of action of the drug**
  - **CD4 and CD8 T-cell activation as measured by Ki-67 was observed by flow cytometry**
  - **Multiple changes in proteins were observed in plasma from patients who received INCB086550**
    - **IFNγ-related proteins, CXCL9, CXCL10, IFNγ, and IL-18 in the periphery were increased, indicating T-cell activation**
    - **Increases in target, PD-L1, and other immune regulatory protein markers, including LAG-3, granzyme B, and granzyme H, were detected**
  - **Increases in IFN-related genes were demonstrated by RNASeq**
- **The peripheral immune alterations observed with the administration of the oral PD-L1 inhibitor INCB086550 are consistent with those observed for PD-1 and PD-L1 monoclonal antibodies**
- **Taken together, these results demonstrate that INCB086550 is biologically active and leads to an increase in circulating T-cell activation**

Disclosures

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