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Discovery of INCB098377: A Potent Inhibitor of Phosphoinositide 3-Kinase Gamma (PI3Kγ)

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Abstract

Immune checkpoint blockade has shown impressive efficacy in patients with inflamed tumors, although minimal activity has been observed in tumors lacking T cells. Myeloid cells are one of the most abundant cell types in both inflamed and noninflamed tumors, and may contribute to immune checkpoint blockade resistance. The plasticity of macrophages enables them to directly and indirectly modulate T cell responses and directly kill tumor cells via phagocytosis. This suggests that targeting myeloid cells could be an effective therapeutic approach. Class I phosphatidylinositol-3 kinases (PI3Ks) are a family of dual specificity lipid and protein kinases. Unlike other class I PI3Ks, PI3Kγ is predominantly expressed in myeloid cells. PI3Kγ has been shown to be a key mediator that drives the immunosuppressive macrophage program by stimulating AKT/mTOR signaling and promote C/EBPβ expression while inhibiting NF-κB activity (Kaneda MM. *Nature.* 2016;17:437-442). Here, we present the discovery and characterization of INCB098377, a potent and selective PI3Kγ inhibitor. Specific inhibition of PI3Kγ with INCB098377 may induce antitumor activity by reshaping the tumor immune microenvironment.

In cell-based assays, INCB098377 has a IC_{50} of 1.4 nM and is greater than 100-fold selective over other PI3K isoforms. It also shows a favorable PK profile in several animal species. Treatment of M2 polarized macrophages with INCB098377 resulted in changes towards a more pro-inflammatory phenotype. CD163 and CD206 were decreased, whereas HLA-DR and co-stimulatory CD80/86 molecules were increased. MHC-I expression was unchanged, suggesting a role for these macrophages in MHC-II–mediated antigen presentation. Furthermore, INCB098377 treatment reduced macrophage-mediated immunosuppression and restored T cell proliferation in M2 polarized macrophages cocultured with allogeneic human T cells.

In vivo, significant tumor growth inhibition was observed with once-daily dosing of 10 mg/kg INCB098377 in both syngeneic and humanized mouse tumor models without toxicity. Moreover, efficacy was observed in inflamed and noninflamed tumor models. Consistent with the proposed mechanism of action, INCB098377 inhibited phosphoAKT (pAKT) levels in vivo and in human peripheral blood mononuclear cells. Treatment with INCB098377 induced pro-inflammatory responses without macrophage depletion, which suggests that robust tumor microenvironment (TME) changes are responsible for observed antitumor efficacy. In addition, INCB098377 inhibited neutrophil migration in the Carrageenan-induced paw inflammation model.

INCB098377, a potent and selective inhibitor of PI3Kγ, shows effective antitumor activity in a variety of mouse and humanized cancer models through the inhibition of immunosuppressive cells trafficking into the tumor, modulation of myeloid cell function, and enhancement of T cell proliferation.

INCB098377 Is a Potent PI3Kγ Inhibitor With Favorable PK Properties

Compounds Synthesized (N=7547)

Caco2 Permeability	Human Int Cl	Protein Binding	Whole Blood Potency
n=2004	n=2404	n=1350	n=1926
>1×10^{-6} cm/s	<1 L/h/kg	>1% free	<1 μM/<200 nM
1359 (68%)	1300 (54%)	174 (13%)	1542 (80%)/ 589 (31%)

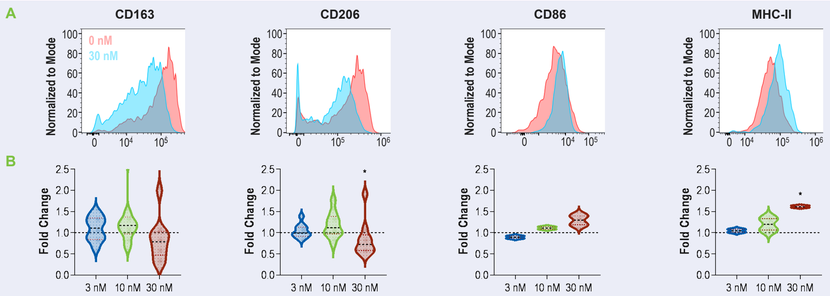
Int Cl, intrinsic clearance.

- 589 compounds selected based on potency
- 199 compounds selected based on favorable in vitro ADME
- 134 compounds selected based on rat PK
- 41 compounds selected based on cyno PK
- 10 lead candidates were characterized in vivo determining their PK/PD, efficacy, and toxicity

Parameter	INCB098377
Potency	
Enzyme PI3Kγ IC_{50}, nM	3.7
Cell PI3Kα IC_{50}, nM	126
Cell PI3Kβ IC_{50}, nM	1430
Cell PI3Kδ IC_{50}, nM	100 (71X)
Cell PI3Kγ IC_{50}, nM	1.4
Monocyte WB PI3Kγ IC_{50}, nM	43
Neutrophil WB PI3Kγ IC_{50}, nM	90
In Vitro ADME	
Caco, Pm × 10^{-6} cm/s	6.7
Int Cl, L/h/kg	0.5
Human PB, % Free	10
Preclinical PK/PD	
Rat % HBF	48
Rat $AUC_{0\text{-}24}$ at 3 mg/kg, μM•h	4.32
Cyno % HBF	7
Cyno $AUC_{0\text{-}24}$ at 1.5 mg/kg, μM•h	16.4
Dog % HBF	4
Dog $AUC_{0\text{-}24}$ at 1.5 mg/kg, μM•h	27.6

ADME, absorption, distribution, metabolism, and excretion; $AUC_{0\text{-}24}$, area under the concentration-time curve from 0-24 hours post dose; HBF, hepatic blood flow; IC_{50}, half maximal inhibitory concentration; Int Cl, intrinsic clearance; PB, peripheral blood; PD, pharmacodynamics; PI3K, phosphatidylinositol-3 kinase; PK, pharmacokinetics; WB, whole blood.

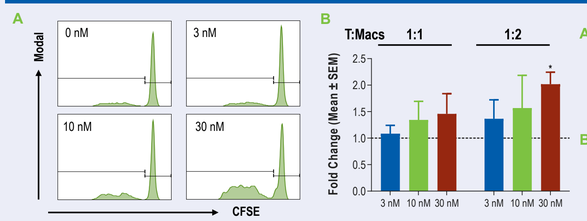
INCB098377 Promotes Pro-inflammatory Phenotype in Human Macrophages



A. Human blood CD14$^+$ monocytes were differentiated for 6 days with M2 polarization kit (R&D Systems, Inc., Minneapolis, MN) followed by 10 ng/mL IL-10 for 24 hours into immunosuppressive M2 macrophages in the presence of increasing concentrations of INCB098377 for 7 days. Macrophage markers were assessed by flow cytometry

B. Quantification of macrophage polarization shows a decrease in CD163 and CD206 and an increase in MHC-II and the costimulatory molecule CD86 expression, suggesting macrophage change from M2 towards pro-inflammatory antigen presenting cell phenotype. **P*<0.05

INCB098377 Restores T Cell Proliferation in the Presence of M2 Polarized Macrophages

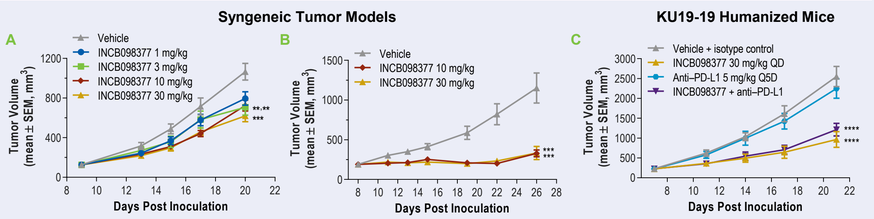


A. Representative flow cytometry histograms of T cell suppression assay. Human CD4$^+$ T cells were labeled with CFSE and cocultured for 5 days with M2 polarized macrophages pretreated with INCB098377

B. Assay quantification shows INCB098377 dose-dependent increase in proliferation of CD4$^+$ T cells (3 normal donors) as determined by CFSE dilution. **P*<0.05

CFSE, carboxyfluorescein succinimidyl ester; SEM, standard error of the mean; T:Macs, T cell to macrophages ratio.

INCB098377 Induces Significant Tumor Growth Inhibition in Inflamed and Noninflamed Tumor Models



***P*<0.01; ****P*<0.001; *****P*<0.0001.
PD-L1, programmed death ligand 1; Q5D, every 5 days; QD, every day; SEM, standard error of the mean.

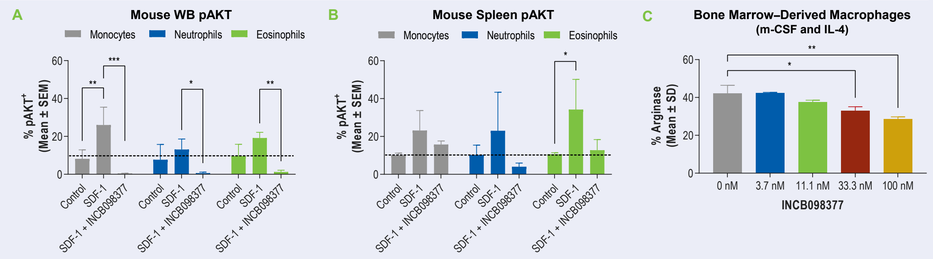
A. INCB098377 dose-response study in noninflamed LLC mouse model shows significant tumor growth inhibition (TGI) at 30, 10, and 3 mg/kg doses (42% TGI at 30 mg/kg)

B. INCB098377 treatment in inflamed mouse model MC38 shows efficacy at both 30 and 10 mg/kg (71% TGI)

C. INCB098377 significantly inhibits growth of noninflamed KU19-19 bladder tumors either alone (62% TGI) or in combination with anti–PD-L1 (52% TGI)

Significant TGI was observed in additional mouse models (A20, B16F10, CT26, EMT6, RM-1, Pan02) treated with INCB098377 alone or in combination

PD Assays In Vitro: Dose-Dependent Inhibition of pAKT and Arginase by INCB098377

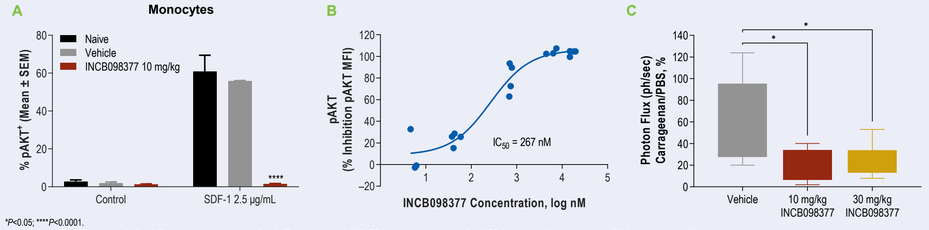


*P<0.05; **P<0.01; ***P<0.001.
SD, standard deviation; SDF-1, stromal cell–derived factor-1; SEM, standard error of the mean.

A&B. Whole blood (WB) and mouse spleen cells were pretreated with 2 μM INCB098377 for 30 minutes, then stimulated with 2.5 μg/mL SDF-1 for 3 minutes. Percentages of pAKT were measured in cell subsets by flow cytometry. INCB098377 inhibited AKT phosphorylation in all subsets tested

C. In vitro bone marrow–derived mouse macrophages were treated with INCB098377. Percentage of arginase-positive cells was measured by flow cytometry. INCB098377 inhibited arginase expression in a dose-dependent manner, with 32% inhibition at 100 nM, suggesting functional effect of the drug on macrophage immunosuppressive activity

PD Assays In Vivo: Dose-Dependent Inhibition of pAKT and Neutrophil Migration by INCB098377



*P<0.05; ****P<0.0001.
IC_{50}, half maximal inhibitory concentration; MFI, mean fluorescence intensity; pAKT, phosphorylated AKT; PBS, phosphate-buffered saline; SDF-1, stromal cell–derived factor-1; SEM, standard error of the mean.

A. Mice were dosed with INCB098377. Whole blood was collected 1 hour later and stimulated with 2.5 μg/mL SDF-1 for 3 minutes. Percentage of pAKT was measured in monocytes by flow cytometry

B. PK/PD relationship of INCB098377

C. Inhibition of neutrophil migration by INCB098377 in the Carrageenan-induced paw edema model

Conclusions

- **INCB098377 is a potent and specific PI3Kγ inhibitor with favorable PK properties in multiple species**
- **INCB098377 induces significant tumor growth inhibition in inflamed and noninflamed syngeneic as well as humanized mouse models**
- **In vitro and in vivo treatment with INCB098377 results in a dose-dependent PI3Kγ pathway inhibition measured by pAKT, arginase expression, and neutrophil migration**
- **INCB098377 remodels TME via direct and indirect effects on myeloid cells and T cells, respectively, suggesting the ability to synergize with immuno-oncology therapies**

Disclosures

Alvarez Arias, Douglass, Truong, Wang, He Wang, Yang, Hansbury, Collins, Stubbs, Stevens, Maddage, Douty, Covington, Leffet, Yue, Kim, Hess: Employment and stock ownership – *Incyte Corporation.* **O'Connor, Bowman, Hall, Combs, Shin, Koblish:** Former employment and stock ownership – *Incyte Corporation.*

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