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Prophylactic Itacitinib (INCB039110) for the Prevention of Cytokine Release Syndrome Induced by Chimeric Antigen Receptor T-Cell (CAR T-Cell) Therapy

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Eduardo Huarte,[1] Roddy S. O'Connor,[2] Melissa Parker,[1] Taisheng Huang,[1] Michael C. Milone,[2] Paul Smith[1]

[1]Incyte Research Institute, Wilmington, DE; [2]Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

Abstract

Background: T-cells engineered to express a chimeric antigen receptor (CAR T-cells) are a promising cancer immunotherapy. Such targeted therapies have shown long-term relapse survival in patients with B-cell leukemia and lymphoma. However, cytokine release syndrome (CRS) represents a serious, potentially life-threatening, side effect often associated with CAR T-cell therapy. The Janus kinase (JAK) kinase family is pivotal for the downstream signaling of inflammatory cytokines, including interleukins (ILs), interferons (IFNs), and multiple growth factors. CRS manifests as a rapid (hyper)immune reaction driven by excessive inflammatory cytokine release, including IFN-gamma and IL-6. Itacitinib is a potent, selective JAK1 inhibitor, which is being clinically evaluated in several inflammatory diseases.

Aim: To evaluate in vitro and in vivo the potential of itacitinib to modulate CRS without impairing CAR T-cell antitumor activity.

Materials and Methods: In vitro proliferation and cytotoxic activity of T-cells and CAR T-cells were measured in the presence of increasing concentrations of itacitinib or tocilizumab (anti–IL-6R). To evaluate itacitinib effects in vivo, we conducted experiments involving adoptive transfer of human CD19-CAR T-cells in immunodeficient animals (NOD-*scid IL2rγ*^{null} [NSG]) bearing CD19-expressing NAMALWA human lymphoma cells. The effect of itacitinib on cytokine production was studied on CD19-CAR T-cells expanded in the presence of itacitinib or tocilizumab. Finally, to study whether itacitinib was able to reduce CRS symptoms in an in vivo setting, naive mice were stimulated with Concanavalin-A (ConA), a potent T-cell mitogen capable of inducing broad inflammatory cytokine releases and proliferation.

Results: In vitro, itacitinib at IC_{50} relevant concentrations did not significantly inhibit proliferation or antitumor killing capacity of human CAR T-cells. Itacitinib and tocilizumab (anti–IL-6R) demonstrated a similar effect on CAR T-cell cytotoxic activity profile. In vivo, CD19-CAR T-cells adoptively transferred into CD19$^+$ tumor–bearing immunodeficient animals were unaffected by oral itacitinib treatment. In an in vitro model, itacitinib was more effective than tocilizumab in reducing CRS-related cytokines produced by CD19-CAR T-cells. Furthermore, in an in vivo immune hyperactivity (ConA) model, itacitinib reduced serum levels of CRS-related cytokines in a dose-dependent manner.

Conclusion: Itacitinib at IC_{50} and clinically relevant concentrations did not adversely impair the in vitro or in vivo antitumor activity of CAR T-cells. Using CAR T- and T-cell in vitro and in vivo systems, we demonstrate that itacitinib significantly reduces CRS-associated cytokines in a dose-dependent manner. Together, the data suggest that itacitinib may have potential as a prophylactic agent for the prevention of CAR T-cell–induced CRS.

Itacitinib Is a JAK1 Selective Inhibitor

Enzyme	IC_{50} Mean ± SD, nM	Number of Experiments	Fold Selectivity for JAK1
JAK1	3.2 ± 2.2	70	—
JAK2	71.6 ± 32.0	68	22
JAK3	>2000	13	>600
TYK2	818 ± 272	13	256

SD, standard deviation; TYK, tyrosine kinase.

- Comparison of enzyme inhibitory activities of itacitinib against different members of the human JAK family reveals itacitinib to be a potent inhibitor of JAK1, with 22- to >600-fold selectivity for JAK1 vs other JAK family members[1]

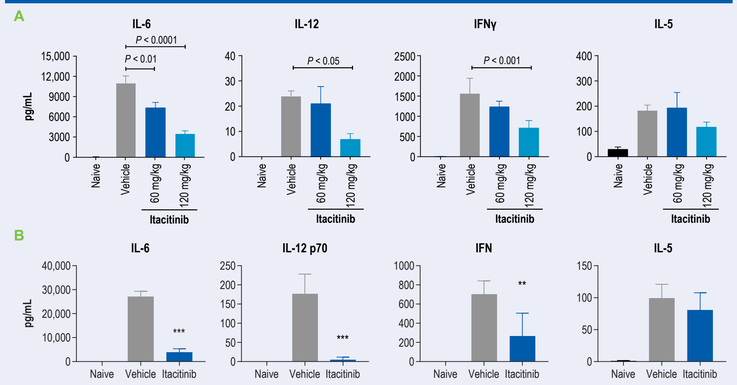
Itacitinib Pharmacokinetics

Parameter	Dose, mg/kg	
	60	120
C_{max}, nM	2093	5310
T_{max}, h	0.5	0.5
AUC, nM•h	3349	7764
$t_{1/2}$, h	2.1	1.0
WB JAK1 IC_{50} (141 nM) coverage, h	4	12

AUC, area under plasma concentration-time curve; C_{max}, maximum plasma drug concentration; IC_{50}, half maximal inhibitory concentration; $t_{1/2}$, terminal half-life; T_{max}, time to maximum plasma drug concentration; WB, whole blood.

- Current estimate of whole blood IC_{50} (141 nM) coverage in human is ≈6.5–10 hours at 200 mg. Mouse doses were selected to mimic predicted human coverage[2]

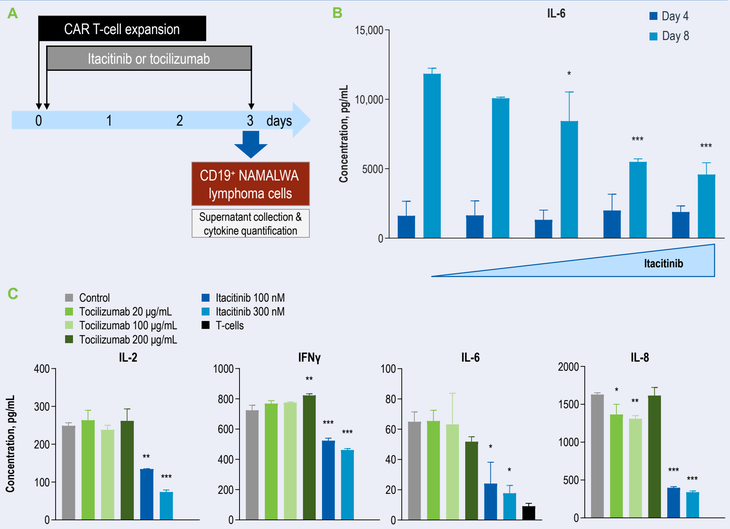
Itacitinib Did Not Impair CAR T-Cell Proliferation or Effector Function



A. BALB/c animals were orally dosed with vehicle control or 60 or 120 mg/kg of itacitinib. One hour later, animals were challenged with ConA, and 60 minutes later sacrificed and plasma collected. N = 5 animals per group. Cytokine analyses were performed to detect the levels of proinflammatory cytokines. Data represent mean ± SEM, and *P* values calculated by 2-way ANOVA. Data are representative of 4 independent experiments

B. Animals were immunized intravenously with 100 µg of anti-CD3 antibody and 30 minutes later orally treated with vehicle or itacitinib (120 mg/kg). Plasma was collected 2 hours after treatment and cytokines measured. $^{**}P < 0.01$ vs vehicle, $^{***}P < 0.001$ vs vehicle

Itacitinib Reduced Cytokine Production by Macrophages and CAR T-Cells

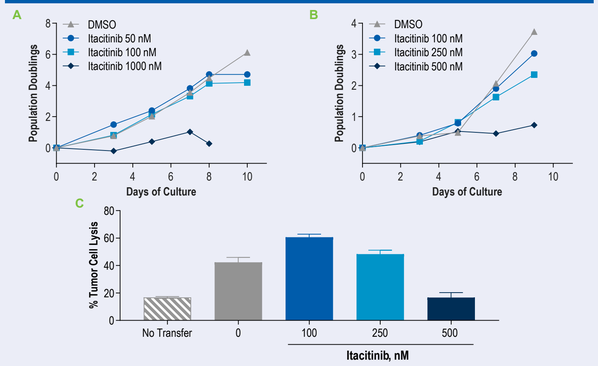


A. Schematic timeline of the experiment

B. Bone marrow–derived macrophages were expanded with granulocyte-colony stimulating factor and stimulated at day 7 with lipopolysaccharide (10 µg/mL) and increasing concentrations of itacitinib. IL-6 levels at day 4 (dark blue columns) and day 8 (blue columns) were measured using a Meso Scale Discovery biomarker assay (Meso Scale Diagnostics, Rockville, MD). Data represent mean ± SEM, and *P* values calculated by 2-way ANOVA. $^{*}P < 0.05$, $^{***}P < 0.001$ against control without itacitinib.

C. CD19-CAR T-cells were expanded with CD3/CD28 beads in the presence of itacitinib or tocilizumab. Three days later, they were co-cultured with CD19$^+$ lymphoma cells (E:T ratio = 2.5:1) for 6 hours and cytokines measured in the supernatant. Data represent mean ± SEM, and *P* values calculated by 2-way ANOVA. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ against control

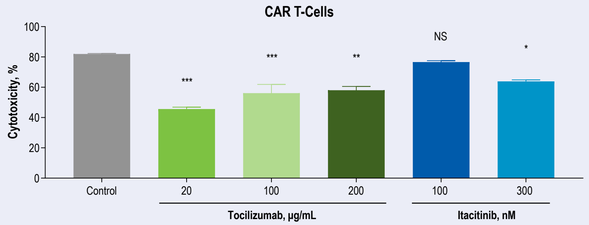
Itacitinib Did Not Impair CAR T-Cell Proliferation or Effector Function



A & B. Human peripheral blood mononuclear cells or GD2-CAR T-cells, respectively, were expanded with anti-CD3/CD28 beads in the presence of increasing itacitinib concentrations and proliferation was quantified by flow cytometry

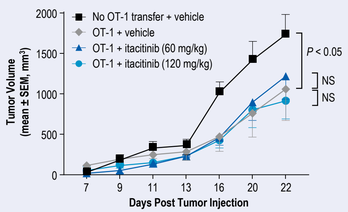
C. GD2-CAR T-cells were expanded with increasing itacitinib concentrations and then co-cultured with luciferase-expressing SY5Y neuroblastoma cells (GD2$^+$). Lytic activity was determined by luciferase activity

CAR T-Cell Tumor Killing Was Similar Between Itacitinib and Tocilizumab



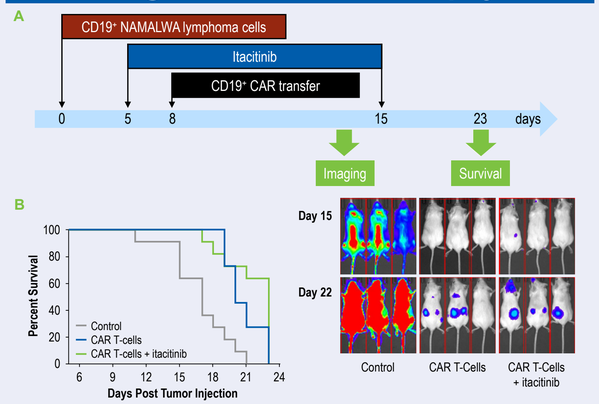
- CD19-CAR T-cells were expanded with either itacitinib or tocilizumab at doses relevant to clinical concentrations. Three days later, they were co-cultured with luciferin-expressing CD19$^+$ lymphoma cells and luminescence recorded. $^{*}P < 0.05$ vs control, $^{**}P < 0.01$ vs control, $^{***}P < 0.001$ vs control. NS, not significant

Itacitinib Did Not Impair OT-1 Cell Antitumor Activity



- C57BL/6 animals were subcutaneously inoculated with ovalbumin-expressing EG-7 tumor cell line. Starting at day 5, corresponding groups received oral doses of itacitinib or vehicle for 2 weeks, and at day 8 an adoptive transfer of naive OT-1 CD8 T-cells. Tumor growth was monitored and compared between groups. N = 10 animals per group. Data represent mean ± SEM, and *P* values calculated by 2-way ANOVA. Data are representative of 2 independent experiments. NS, not significant

In Vivo CAR T-Cell Antitumor Activity Was Unchanged With Itacitinib Prophylaxis



C

Comparison	*P* Value
Control T-cells vs CAR T-cells + vehicle	0.0014
Control T-cells vs CAR T-cells + itacitinib	0.0003
CAR T-cells + vehicle vs CAR T-cells + itacitinib	0.1860

- Immunodeficient mice were inoculated with CD19$^+$ human lymphoma NAMALWA-luciferase cell line and treatment initiated 5 days later
  - **A.** Schematic timeline of the experiment
  - **B.** Animal received twice-daily doses of itacitinib or vehicle for 10 days. At day 8, animals received an adoptive transfer of either control T-cells or CD19-CAR T-cells. N = 12 animals per group. Left panel represents a Kaplan-Meier curve for mice survival. In vivo bioluminescence imaging of representative animals challenged with luciferase-expressing NAMALWA lymphoma cells and different treatments over time (right panel). Data representative of 2 independent experiments
  - **C.** Statistical analysis

Conclusions

- **Itacitinib potently inhibits JAK1 (IC_{50} = 3.2 nM), with 22- to >600-fold selectivity for the other JAK family members, JAK2, JAK3, and TYK2**
- **Itacitinib 120 mg/kg dosing in mice closely mimics estimated JAK1 coverage in human (~6.5–10 hours at 200 mg)**
- **In 2 in vivo models of nonspecific T-cell activation (ConA and anti-CD3), itacitinib reduced serum levels of CRS-related cytokines in a dose-dependent manner**
- **In an in vitro model, itacitinib, but not tocilizumab, significantly reduced the level of CRS-related cytokines produced by CD19-CAR T-cells**
- ***Of note, in vitro assays demonstrate that itacitinib does not significantly affect proliferation or antitumor activity of human CAR T-cells at clinically relevant concentrations***
- **Itacitinib did not adversely impact human CD19-CAR T-cells in a preclinical CD19$^+$ tumor bearing model in vivo**
- **A phase 2 clinical trial of itacitinib for the prevention of CRS induced by CAR T-cell therapy was recently initiated (NCT04071366)**

Disclosures

Huarte, Parker, Huang, Smith: Employment and stock ownership – *Incyte Corporation*. **Milone:** Consultant – *Novartis*; cofounder and co-chair – *Cabaletta Bio*. **O'Connor:** nothing to disclose.

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1. Incyte Corporation, data on file (March 15, 2019).
2. Juvekar A, et al. *Blood*. 2018;132(Suppl 1). Abstract 4523.

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