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

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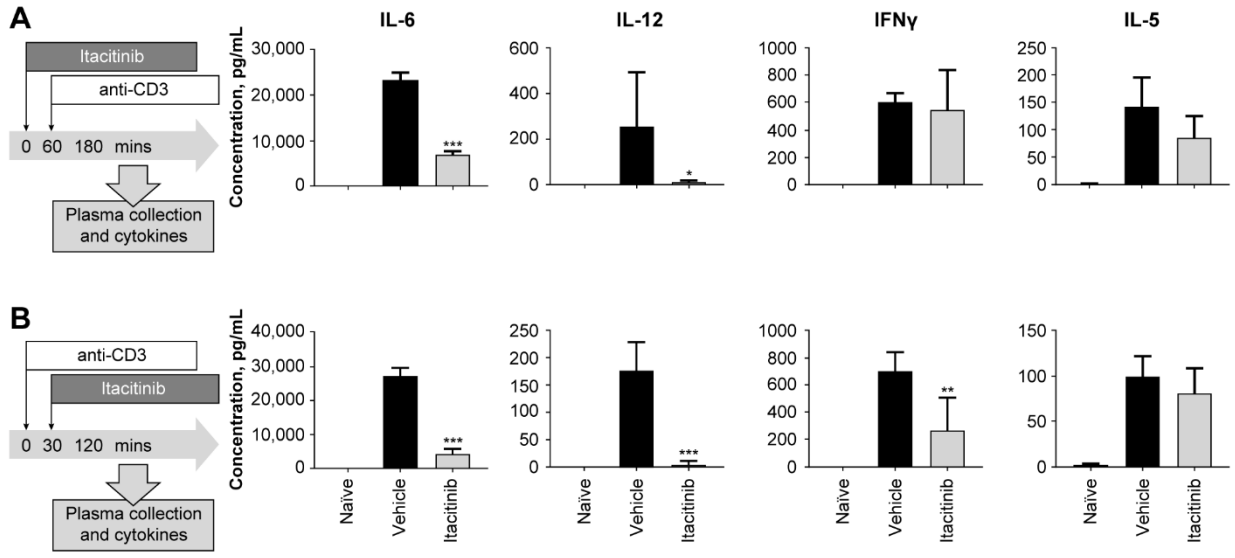
Supplementary Data

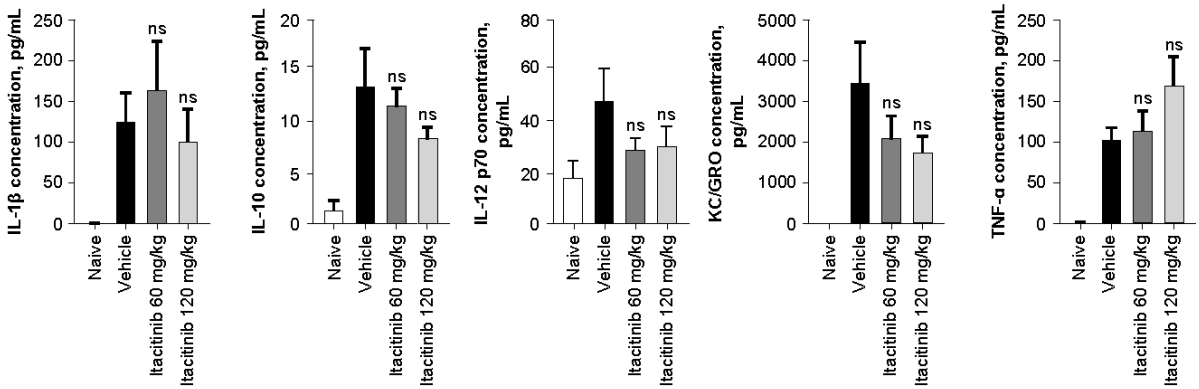
Supplementary Figure S1.

Itacitinib reduces cytokine levels in murine models of acute inflammation. (**A**) BALB/c mice were orally dosed with vehicle control, 60 or 120 mg/kg of itacitinib. One hour later animals were challenged with an anti-CD3 antibody, and 120 minutes later sacrificed and plasma collected (prophylactic). (**B**) BALB/c mice were challenged with an anti-CD3 antibody, 30 minutes later were orally dosed with vehicle control, 60 or 120 mg/kg of itacitinib, and 2 hours after anti-CD3 challenge sacrificed (therapeutic). N=5 animals per group. MSD analysis was performed to detect the levels of pro-inflammatory cytokines. Data represent mean + SEM, and P values were calculated by two-way ANOVA. * $p<0.05$, *** $p<0.001$. Data are representative of 2 independent experiments.



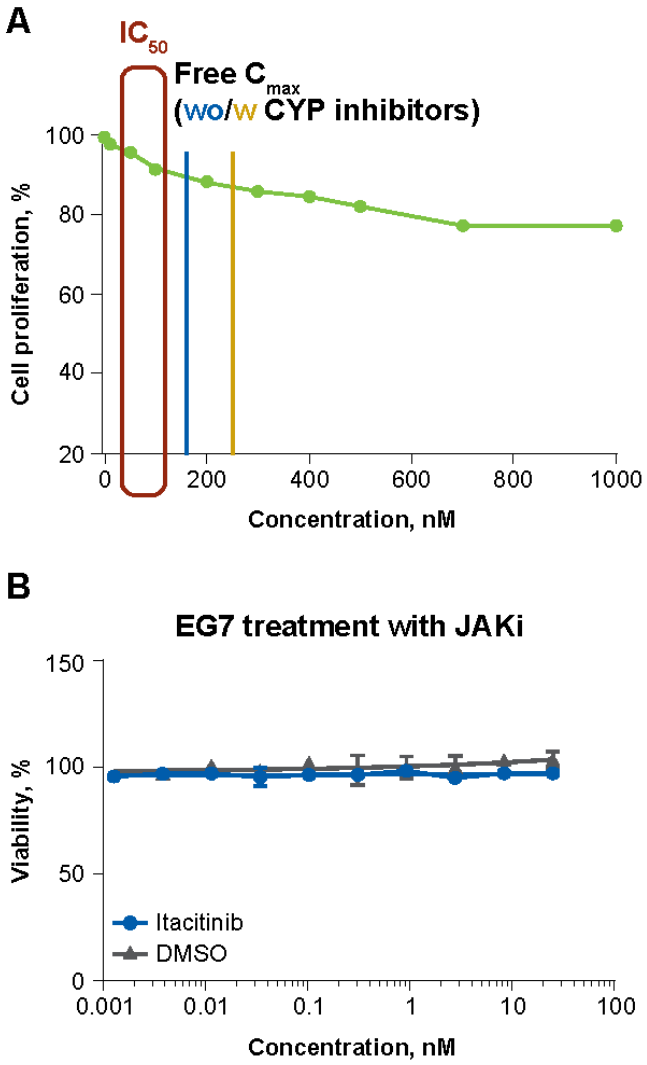
Supplementary Figure S2.

Itacitinib effect on cytokine production by macrophages. C57BL/6 mice were prophylactically orally dosed with vehicle, or 60 or 120 mg/kg of itacitinib b.i.d. for 3 days. Mice then received intraperitoneal injections of LPS (5 µg per animal). Two hours after injection, mice were euthanized and IL-1β, IL-10, IL-12p70, KC/GRO and TNF-α levels were measured in the peritoneal lavage. Data represent mean ± SEM, and P values were calculated by two-way ANOVA. n.s., not significant. Data are representative of two independent experiments.



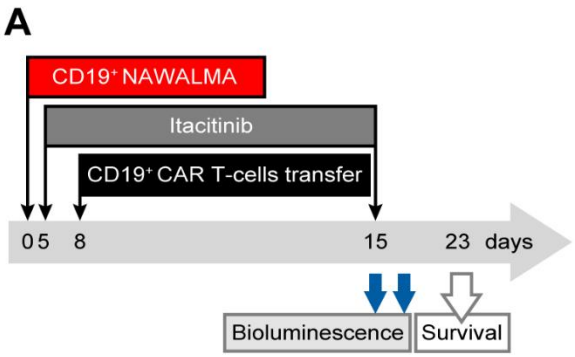
Supplementary Figure S3.

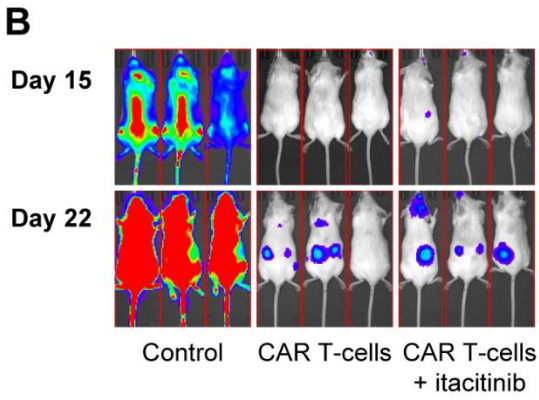
Clinically relevant concentrations of itacitinib does not affect OT-1 nor EG7 proliferation. (**A**) OT-1 T-cells were expanded with the SIINFEKL peptide in the presence of increasing concentrations of itacitinib, and their expansion was measured by flow cytometry. Itacitinib concentrations relevant to the cellular IC_{50} or maximum free concentrations induced a modest reduction on T-cell proliferation. (**B**) EG7 tumor cell line was expanded in the presence of increasing itacitinib concentrations.

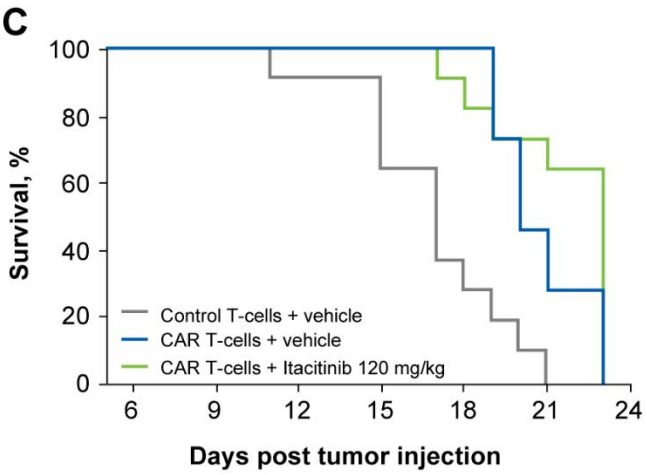


Supplementary Figure S4.

Itacitinib does not affect CD19-CAR T-cell antitumor activity *in vivo*. Immunodeficient NSG mice were inoculated with CD19^{+} human lymphoma NAMALWA-luciferase cell line. Starting five days later, animals received twice daily doses of itacitinib or PBS for 10 days. At day 8 post-tumor injection, corresponding animals received an adoptive transfer of CD19-CAR T-cells. N=12 animals per group. Data representative of 2 independent experiments. (**A**) Experiment scheme. (**B**) Bioluminescence was measured once a week in anesthetized mice. (**C**) Survival curve.







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