

In Vivo Assessment of the Combination of the JAK1-Selective Inhibitor, Itacitinib, and First- and Second-Generation EGFR Inhibitors in Models of Non-Small Cell Lung Cancer

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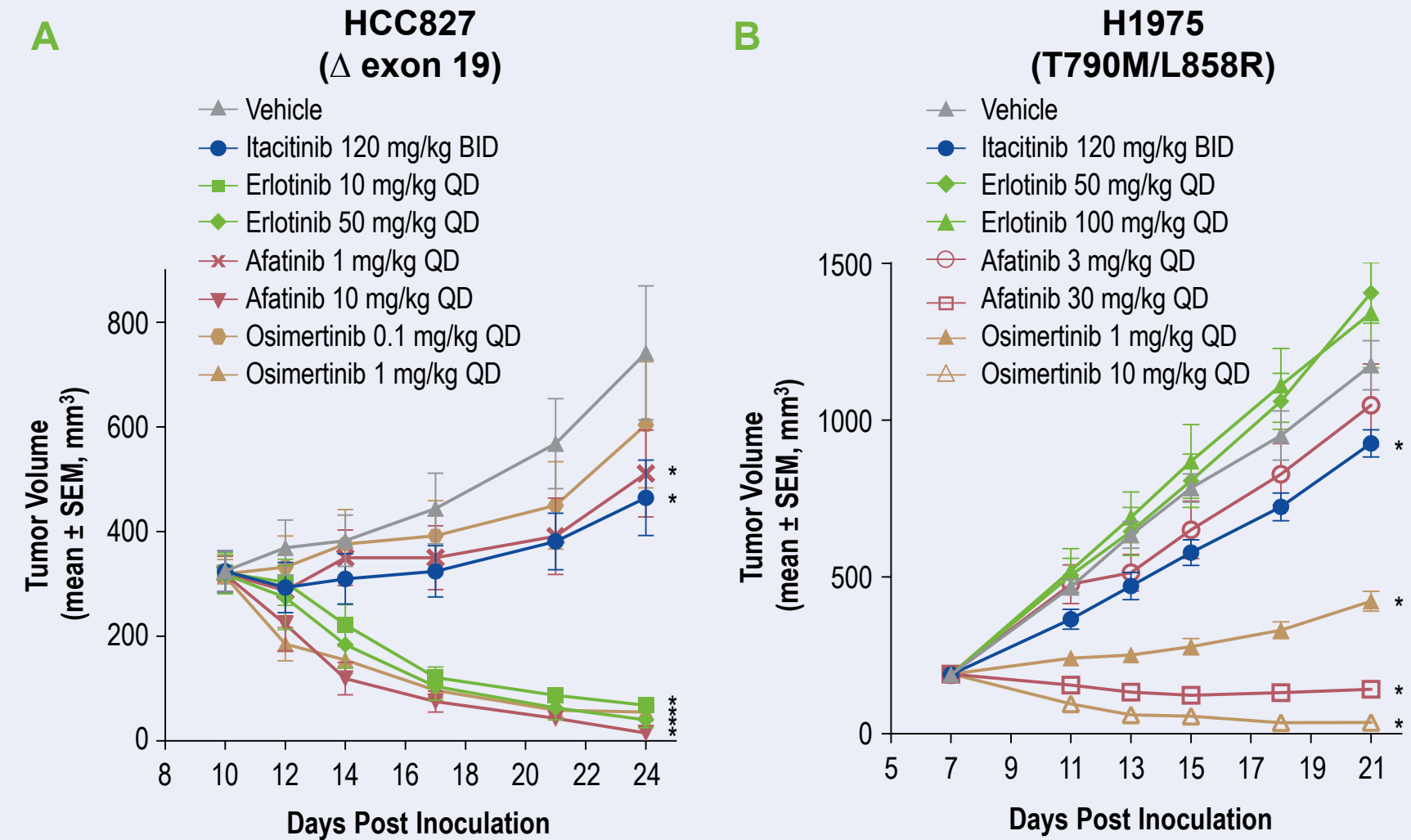
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Abstract

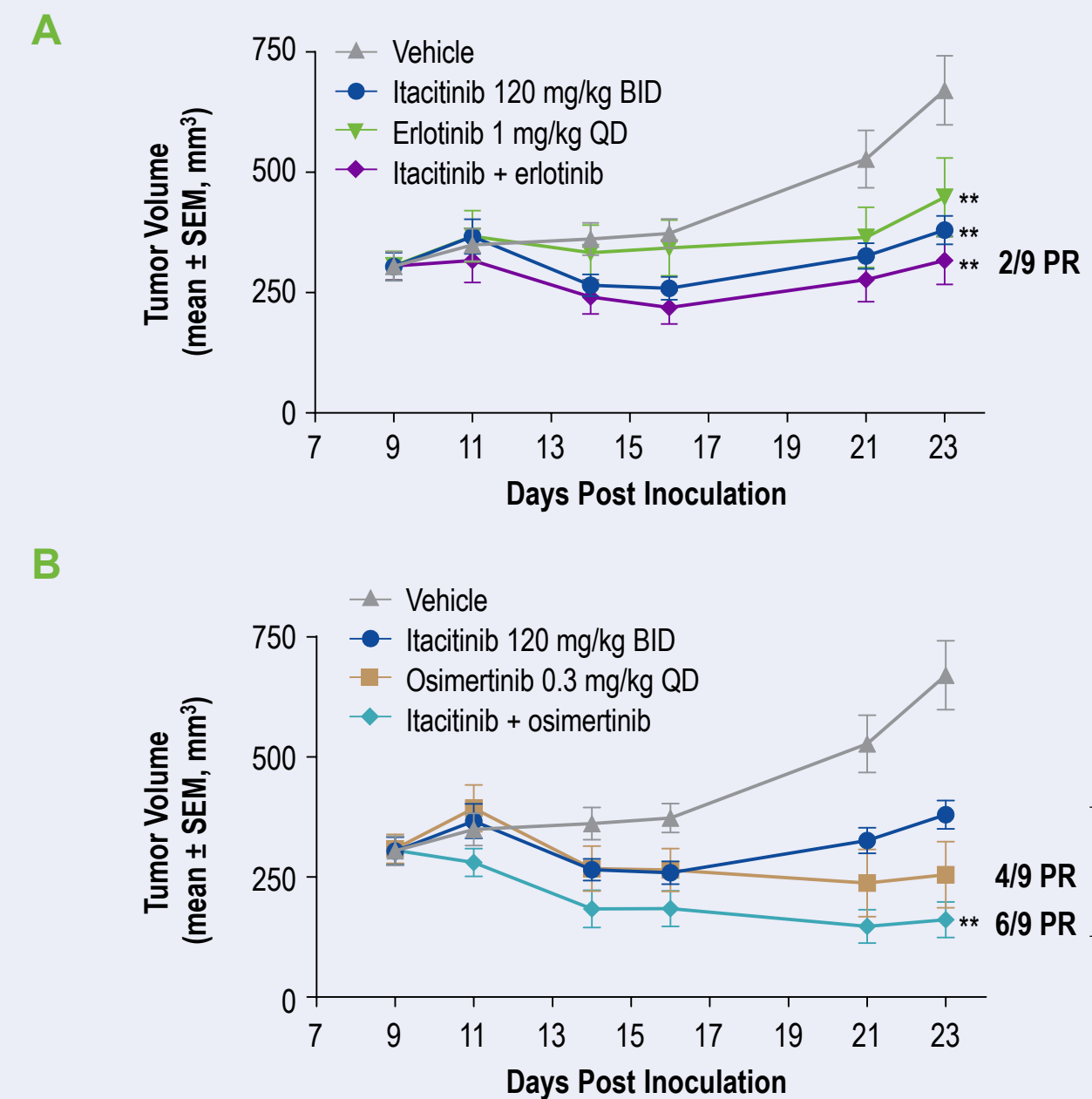
Non-small cell lung cancers (NSCLC) make up the majority of lung cancers, and are predominantly driven by aberrant kinase pathway signaling. Oncogenic mutations leading to activation of the epidermal growth factor receptor (EGFR) have been identified in a substantial fraction of NSCLC patients, leading to EGFR-targeted therapies such as erlotinib that have improved patient outcomes. However, inhibition of EGFR consistently leads to drug resistance through multiple pathways, creating a therapeutic need in NSCLC. One particular route to resistance of EGFR inhibitors is activation of pathways that can bypass the need for signaling through the EGFR, such as the JAK/STAT pathway. To explore the impact of JAK/STAT pathway modulation on EGFR inhibitor resistance, combination efficacy studies evaluating the JAK1-selective inhibitor itacitinib with either erlotinib or the EGFR T790M mutant inhibitor osimertinib were conducted in xenograft models of activated and erlotinib-resistant NSCLC. The HCC827 xenograft (EGFR-activating deletion in exon 19) model was very sensitive to both erlotinib and osimertinib, while the NCI-H1975 xenograft (EGFR T790M/L858R) model responded only to osimertinib. Itacitinib was efficacious in the HCC827 model, while only marginal tumor growth inhibition was observed with itacitinib in the NCI-H1975 model despite both models having detectable levels of pSTAT3. The combination of itacitinib with either erlotinib or osimertinib inhibited tumor growth to a greater degree than monotherapies in the HCC827 model. Despite marginal single-agent efficacy from itacitinib in the NCI-H1975 model, itacitinib enhanced the efficacy of osimertinib at several dose levels in this model. Importantly, itacitinib and erlotinib administration had synergistic efficacy in this erlotinib-resistant model, indicating that JAK1-specific signaling may be a critical bypass mechanism for resistance to EGFR inhibitors. Downstream of EGFR, both erlotinib and osimertinib inhibited different signaling pathways when combined with itacitinib in the NCI-H1975 model: STAT signaling was regulated by erlotinib, while the AKT/S6 and extracellular-regulated kinase (ERK) pathways were regulated by osimertinib. An analysis of possible upstream activators of signaling pathways relevant to NSCLC survival revealed that IL-6, monocyte chemoattractant protein-1 (MCP-1), and IL-8 levels were altered in H1975 tumors from mice treated with the combination of itacitinib and osimertinib, and to a lesser extent with the combination of itacitinib and erlotinib. These data demonstrate the potential utility of the JAK1-specific inhibitor itacitinib in EGFR-activated NSCLC, or for patients with EGFR mutations who are no longer responsive to a first-generation EGFR inhibitor such as erlotinib. The combination of itacitinib and osimertinib is currently in a phase 1/2 study (NCT02917993).

Single-Agent Activity of Itacitinib or EGFR Inhibitors in Xenograft Models of EGFR-Driven NSCLC



- A.** Female severe combined immunodeficient (SCID) mice were inoculated with 1×10^7 HCC827 cells in Matrigel® (Corning Life Sciences, Tewksbury, MA) (n = 8 per group). When tumors reached approximately 300 mm³, mice were dosed orally for 14 days. Statistical significance was determined by 2-way analysis of variance (ANOVA). * $P < 0.05$ versus vehicle.
- B.** Female SCID mice were inoculated with 1×10^7 H1975 cells in Matrigel (n = 10 per group). When tumors reached approximately 200 mm³, mice were dosed orally for 14 days. Statistical significance was determined by 2-way ANOVA. * $P < 0.05$ versus vehicle.

Itacitinib Enhances the Efficacy of EGFR Inhibitors in the Erlotinib-Sensitive HCC827 Model

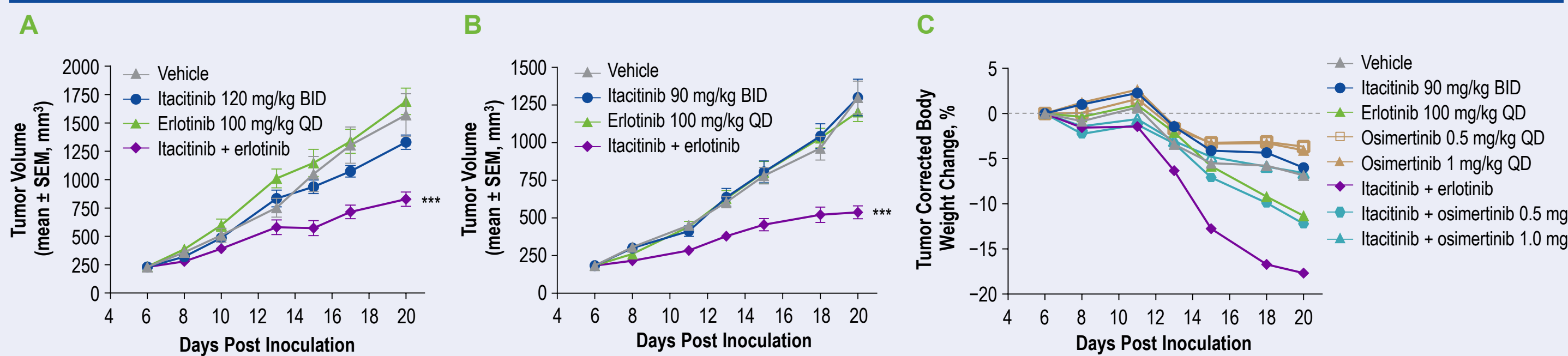


PR, partial response.

Female SCID mice were inoculated with 1×10^7 HCC827 cells in Matrigel (n = 9 per group). When tumors reached approximately 300 mm³, mice were dosed orally for 14 days. Statistical significance was determined by 2-way ANOVA.

- A.** Efficacy from combination of itacitinib (120 mg/kg BID) with erlotinib (1 mg/kg QD). ** $P < 0.01$ versus vehicle.
- B.** Efficacy from combination of itacitinib (120 mg/kg BID) with osimertinib (0.3 mg/kg QD). ** $P < 0.01$ combination versus itacitinib single agent; *** $P < 0.001$ versus vehicle.

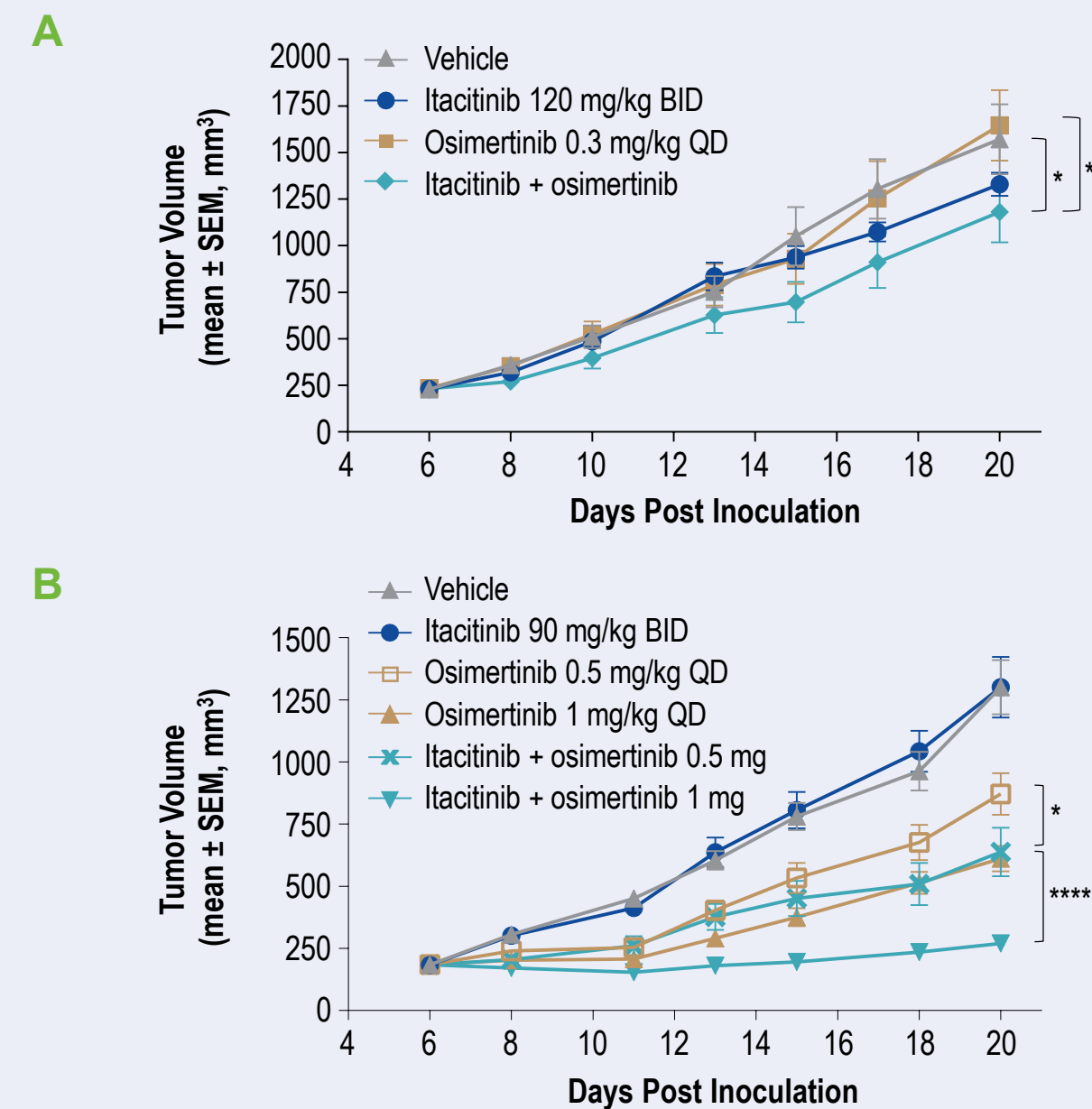
Itacitinib Overcomes Erlotinib Resistance in the H1975 Xenograft Model



Female SCID mice were inoculated with 1×10^7 H1975 cells in Matrigel. When tumors reached approximately 200 mm³, mice were dosed orally for 14 days. Statistical significance was determined by 2-way ANOVA.

- A.** Efficacy from combination of itacitinib (120 mg/kg BID) with erlotinib (100 mg/kg QD) (n = 8 per group). *** $P < 0.001$ versus each group.
- B.** Efficacy from combination of itacitinib (90 mg/kg BID) with erlotinib (100 mg/kg QD) (n = 10 per group). *** $P < 0.001$ versus each group.
- C.** Percentage change in body weight from baseline with itacitinib, erlotinib, osimertinib, and itacitinib combined with erlotinib or osimertinib (n = 8–10 per group).

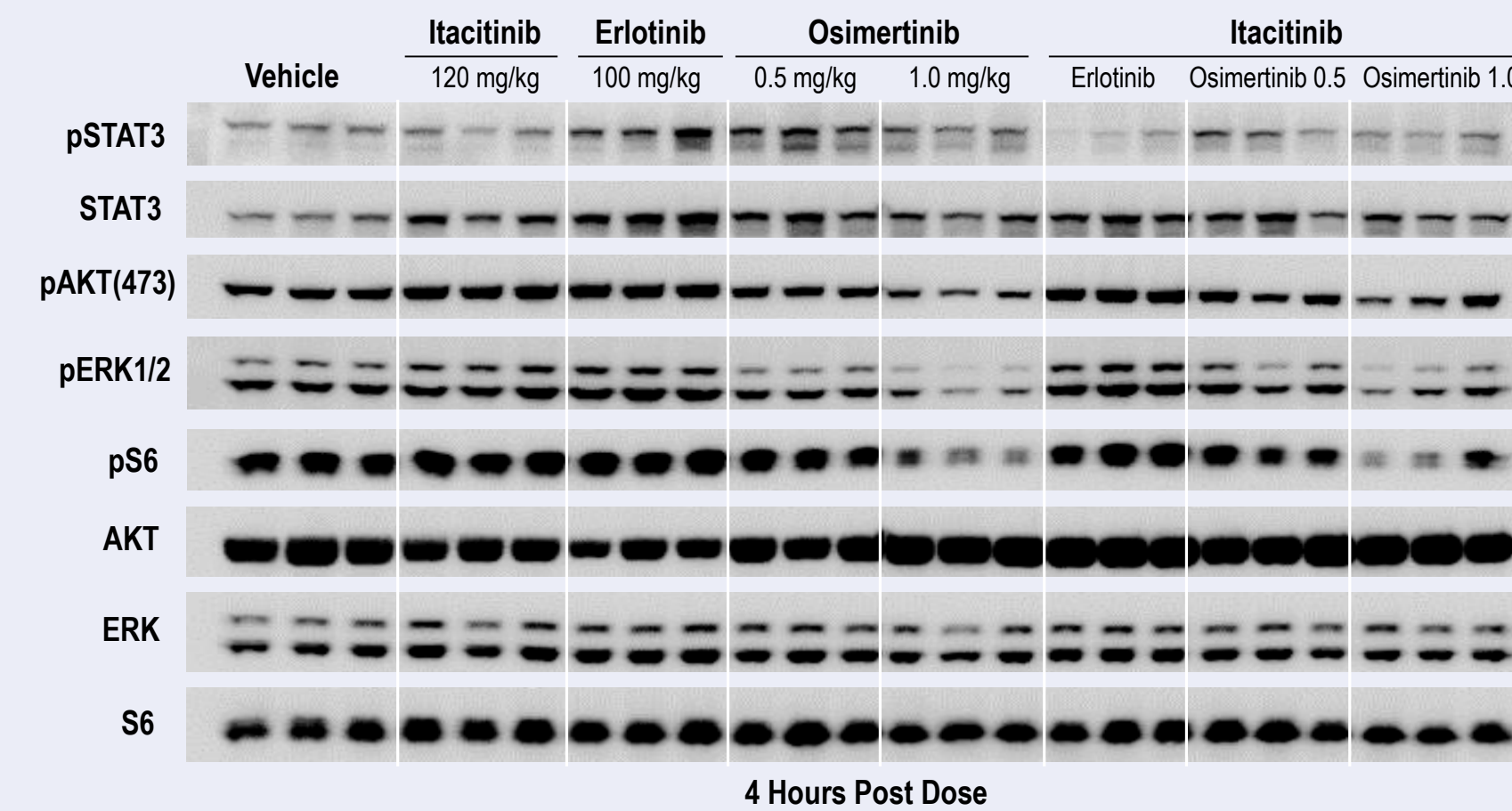
Itacitinib Enhances the Efficacy of Osimertinib in the Erlotinib-Resistant H1975 Model



Female SCID mice were inoculated with 1×10^7 H1975 cells in Matrigel (n = 8 per group). When tumors reached approximately 200 mm³, mice were dosed orally for 14 days. Statistical significance was determined by 2-way ANOVA.

- A.** Efficacy from combination of itacitinib (120 mg/kg BID) with osimertinib (0.3 mg/kg QD). * $P < 0.05$, ** $P < 0.01$.
- B.** Efficacy from combination of itacitinib (90 mg/kg BID) with osimertinib (0.5 and 1 mg/kg QD). * $P < 0.05$, **** $P < 0.0001$.

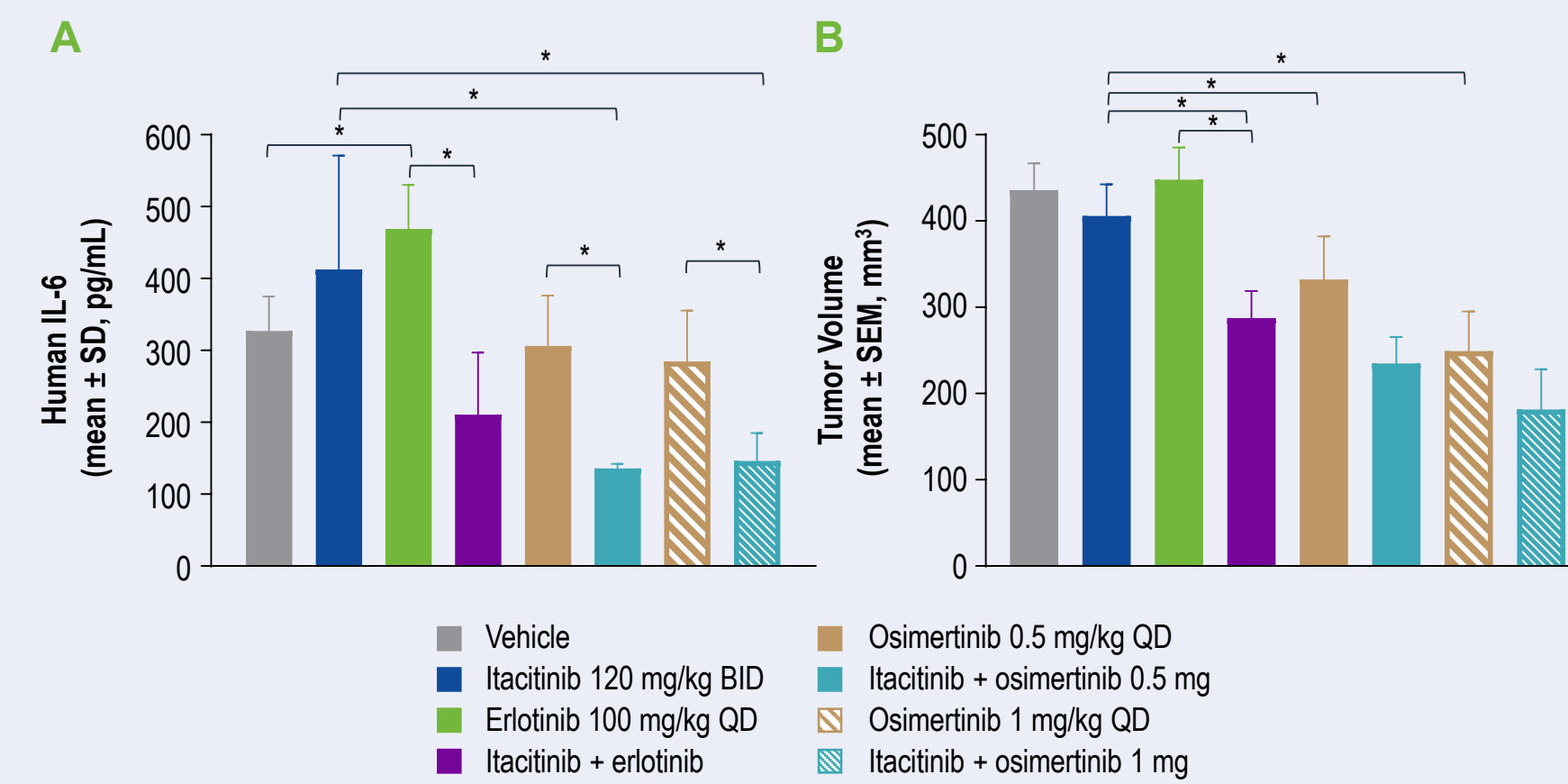
Pharmacodynamic Analysis Reveals Pathway Specificity for EGFR Inhibitors in the Erlotinib-Resistant H1975 Model



Female SCID mice were inoculated with 1×10^7 H1975 cells in Matrigel. When tumors reached approximately 200 mm³, mice were dosed orally once, and tumors were harvested after 4 hours for protein analysis. Western blots were performed for markers of JAK/STAT and EGFR pathway signaling.

- Erlotinib treatment inhibited signaling through the JAK/STAT pathway.
- Osimertinib treatment inhibited signaling through both S6 and ERK.

Reductions in Tumor IL-6 Levels Associated With Reductions in Tumor Growth in the Erlotinib-Resistant H1975 Model

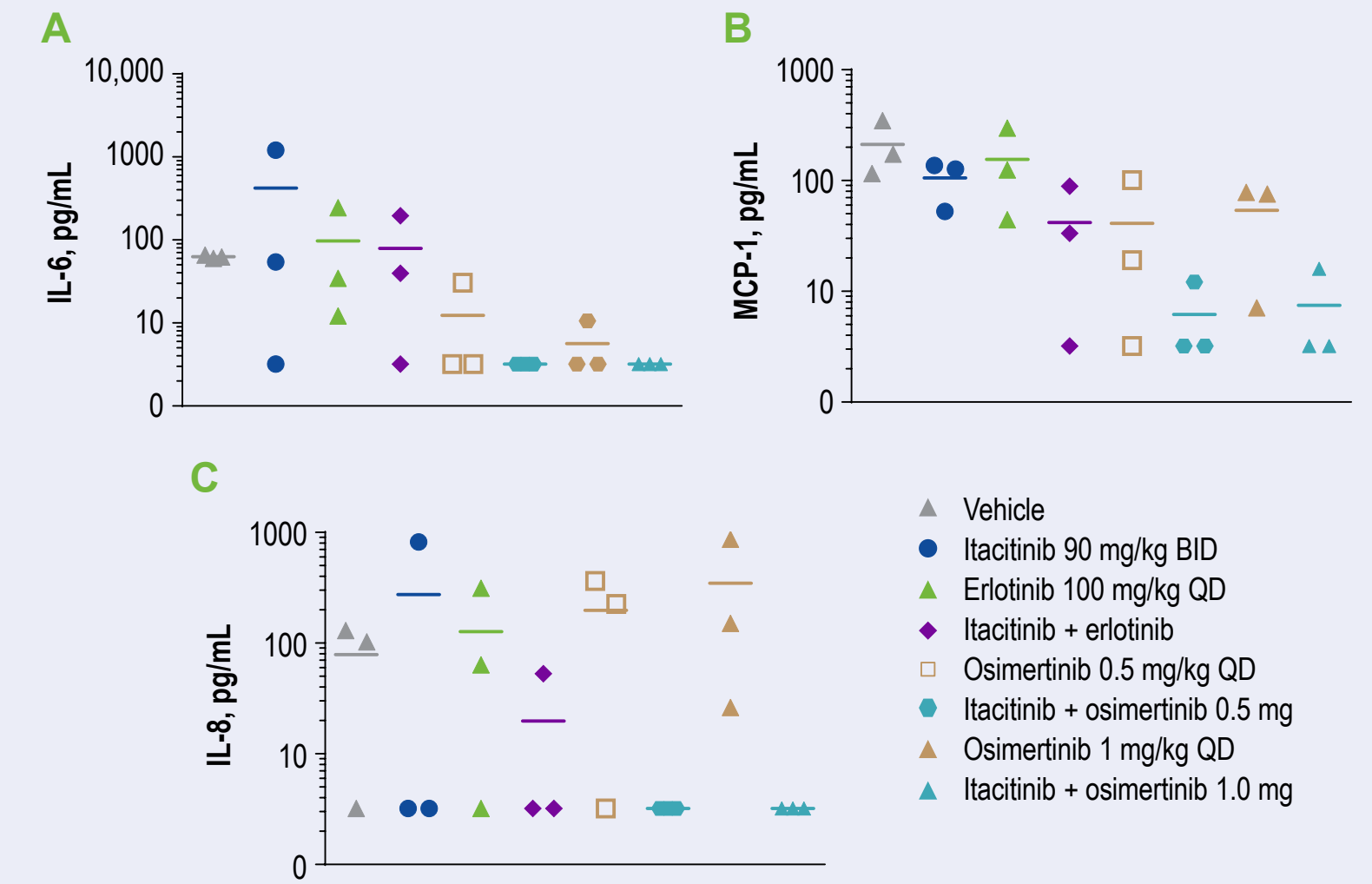


SD, standard deviation.

Female SCID mice were inoculated with 1×10^7 H1975 cells in Matrigel. When tumors reached approximately 200 mm³, mice were dosed orally for 5 days. Tumors were harvested for IL-6 enzyme-linked immunosorbent assay (ELISA; R&D Systems, Inc., Minneapolis, MN) analysis 4 hours post final dose.

- A.** IL-6 levels from H1975 tumor lysates were measured by ELISA. Statistical significance was determined by *t* test (n = 3 per group). * $P < 0.05$.
- B.** Efficacy on day 5 of itacitinib, erlotinib, osimertinib, and itacitinib (120 mg/kg BID) combined with erlotinib or osimertinib. Statistical significance was determined by 2-way ANOVA (n = 7–10 per group). * $P < 0.05$.

Combination of Itacitinib and Osimertinib Impacts Tumor Cytokine Levels in the Erlotinib-Resistant H1975 Model



A.–C. Female SCID mice were inoculated with 1×10^7 H1975 cells in Matrigel (n = 3 per group). When tumors reached approximately 200 mm³, mice were dosed orally for 14 days. Tumors were harvested 4 hours post final dose on day 14 for analysis of inflammatory cytokine levels using a Luminex cytokine panel. Combinations of itacitinib with osimertinib showed the strongest effects on suppression of IL-6, MCP-1, and IL-8 levels.

Conclusions

- Itacitinib was more efficacious in the erlotinib-sensitive HCC827 model than in the erlotinib-resistant H1975 model of NSCLC
- Itacitinib enhanced the efficacy of EGFR inhibitors in the HCC827 model
- Itacitinib overcomes erlotinib resistance in the H1975 model
- Combining itacitinib with erlotinib predominantly reduced pSTAT3 levels, whereas the combination of itacitinib with osimertinib resulted in reduced pERK and pS6 levels in the erlotinib-resistant H1975 model
- Despite the lack of efficacy with single-agent itacitinib in the erlotinib-resistant H1975 model, combination with osimertinib increased efficacy above predicted levels
- Combinations of itacitinib and osimertinib markedly reduced tumor production of several inflammatory cytokines, including IL-6 in the erlotinib-resistant H1975 model
- The combination of itacitinib and osimertinib is currently being evaluated in a phase 1/2 study (NCT02917993)

Disclosures

All authors: Employment and stock ownership – Incyte Corporation.

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