

ALRN-0192519 is a potent and selective EGFR L858R mutant allosteric inhibitor that demonstrates anti-cancer activity in preclinical models as monotherapy or in combination with osimertinib

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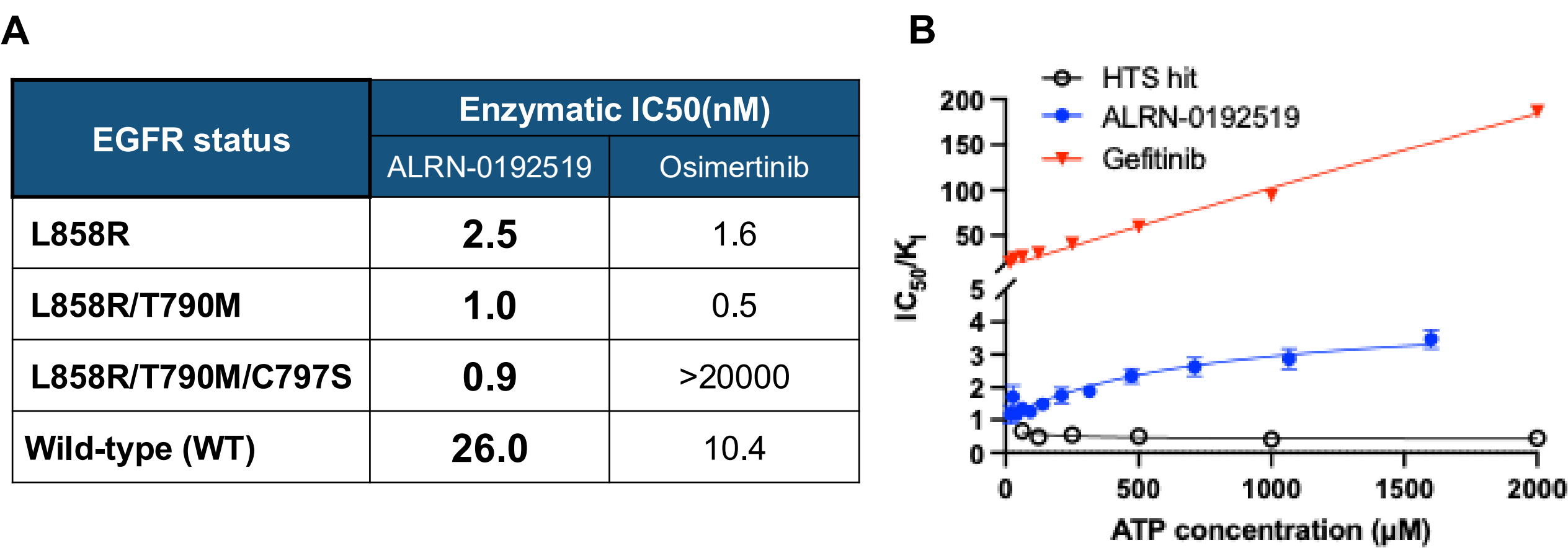
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Background

- Epidermal growth factor receptor (EGFR) therapy with small-molecule tyrosine kinase inhibitors (TKIs) is initially effective in patients with EGFR-mutant lung cancer, but resistance to the treatment emerges over time.
- The EGFR L858R-mutant lung cancer patient population has a significant unmet medical need, as third-generation EGFR TKIs are less effective in this group compared to those with exon 19del.
- EGFR L858R-mutant allosteric inhibitors bind to a distinct site from the ATP-binding site targeted by existing EGFR TKIs on the EGFR protein, providing a promising strategy to overcome resistance associated with EGFR mutations.
- Here, we report the preclinical characterization of ALRN-0192519 (ALRN-2519), a novel, orally bioavailable, mutant-selective allosteric EGFR L858R mutant inhibitor.

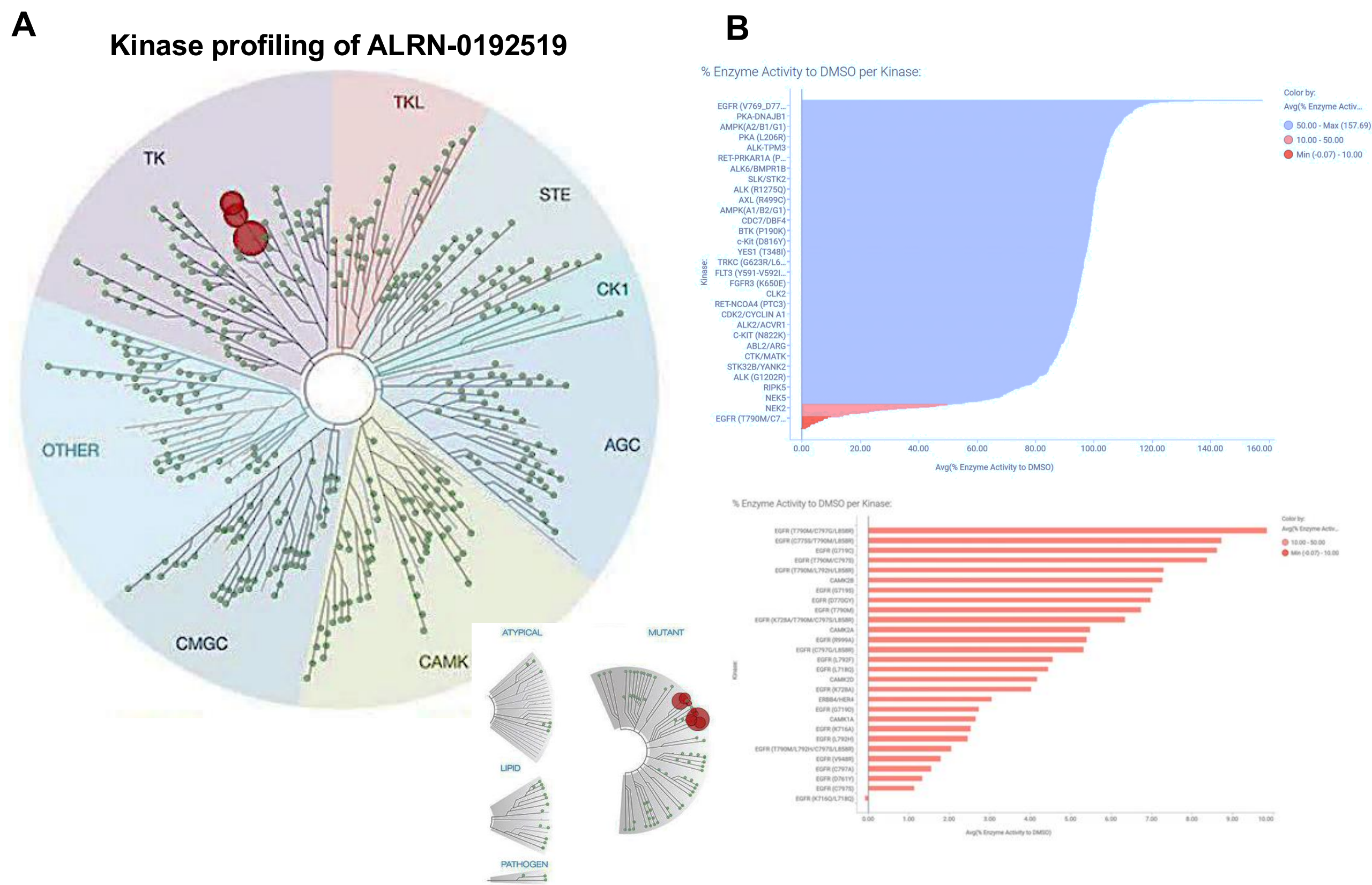
Results

Figure 1. ALRN-2519 is a potent allosteric inhibitor of EGFR L858R mutants



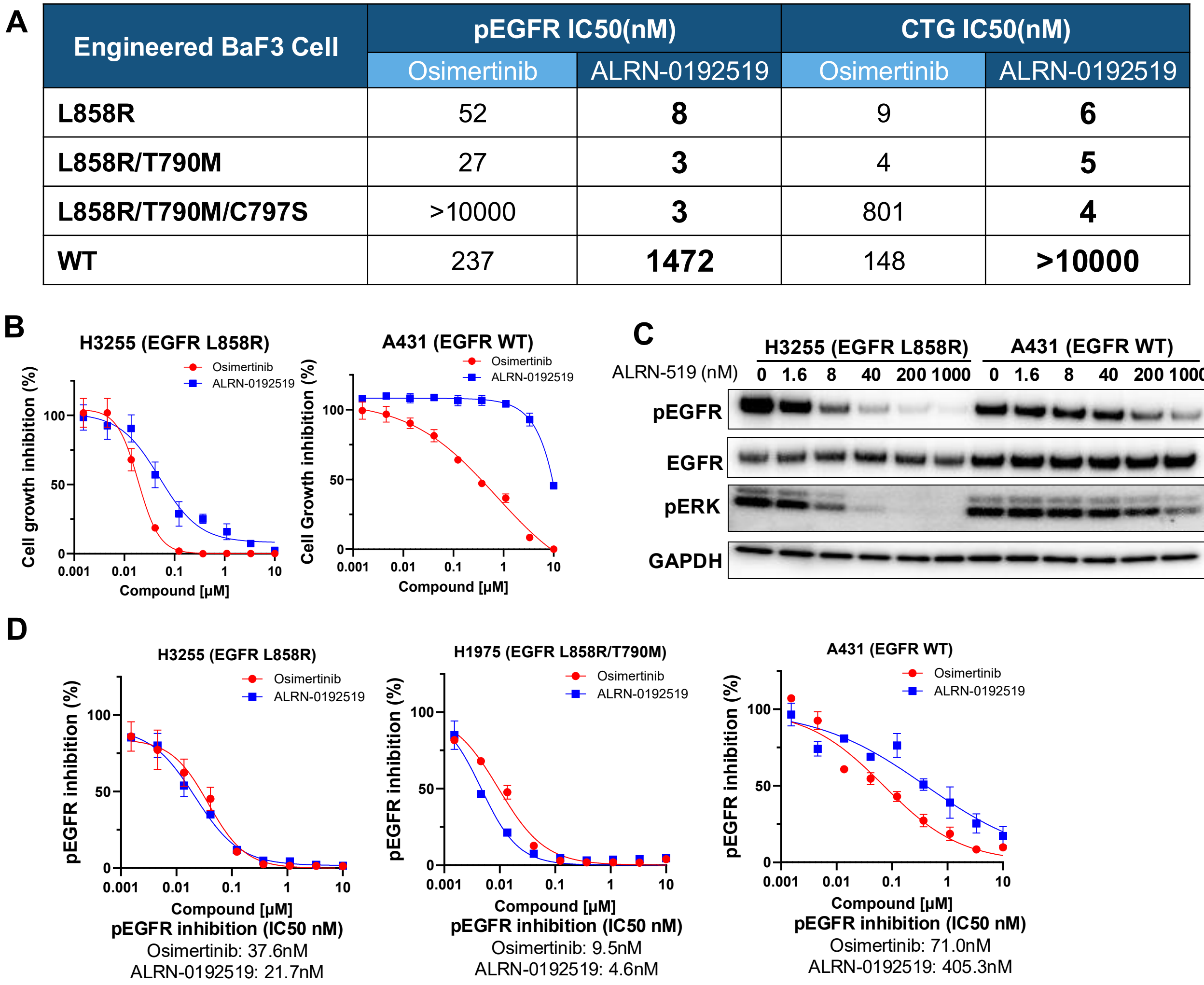
(A) Inhibition of EGFR L858R single, double and triple mutants by ALRN-2519 in enzymatic assays. Data represent results from n > 3 biological repeats. (B) Mechanism of inhibition by EGFR inhibitors. ALRN-2519 and its high-throughput screening hit demonstrated an allosteric inhibition mechanism, whereas gefitinib competed with ATP in the EGFR kinase containing the L858R mutation.

Figure 2. ALRN-2519 is highly selective against a broad panel of kinases



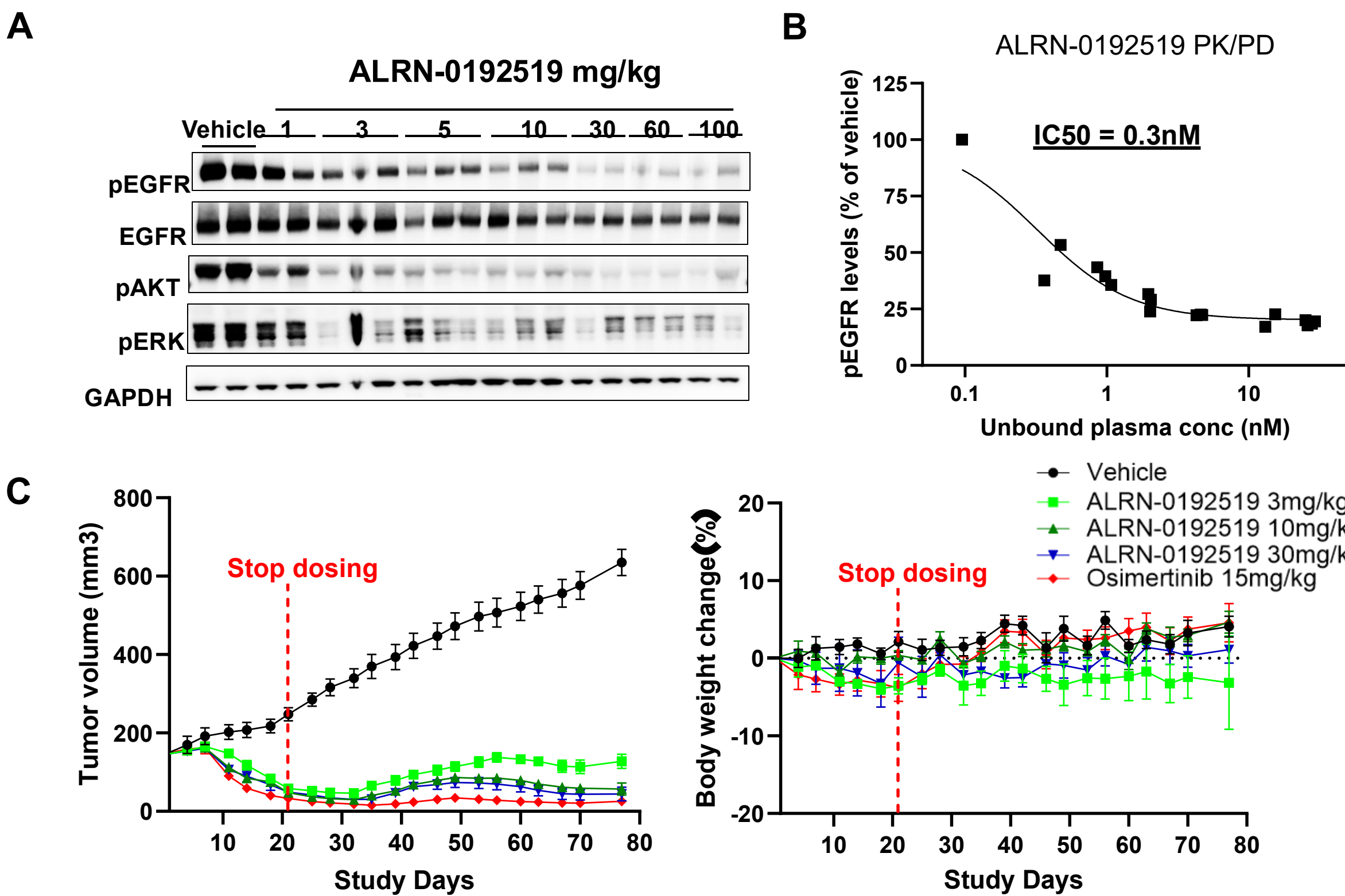
(A) Kinome profiling of ALRN-2519 at 10 μM using a binding assay (KINOMEScan, Eurofins). (B) Kinase activity profiling of ALRN-2519 at 10 μM across a panel of 757 kinases (Reaction Biology).

Figure 3. ALRN-2519 selectively inhibits mutant EGFR activity in cellular assays



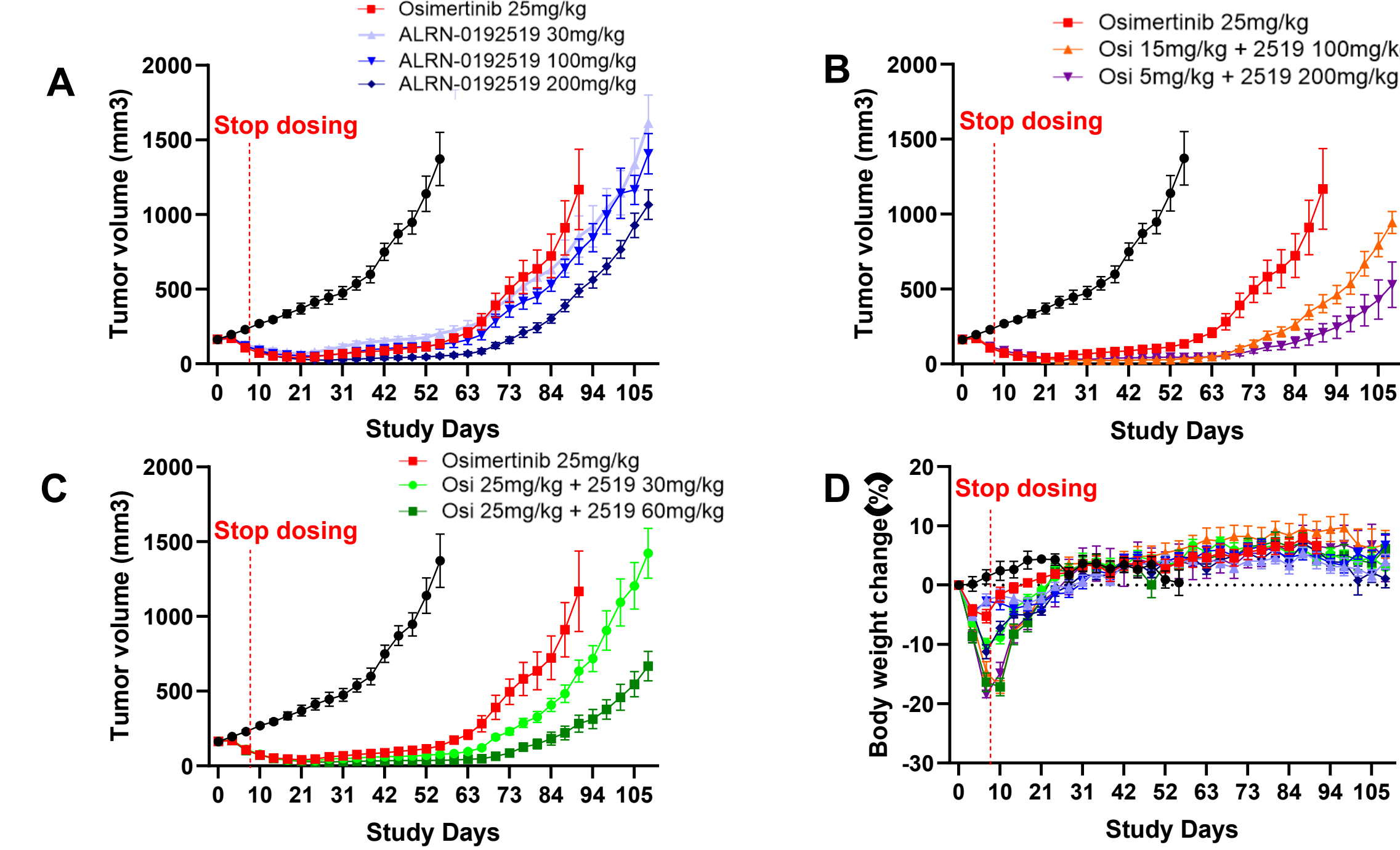
(A) Inhibition of cell proliferation and phospho-EGFR in engineered BaF3 cells. (B) Anti-proliferative activity of ALRN-2519 in patient-derived cell lines. (C) Phospho-EGFR inhibition in patient-derived cell lines assessed by Western blotting. (D) Phospho-EGFR inhibition in patient-derived cell lines assessed by AlphaLISA. All data represent results from n > 2 biological replicates.

Figure 4. ALRN-2519 effectively suppresses phospho-EGFR and tumor growth in the H3255 EGFR L858R CDX model



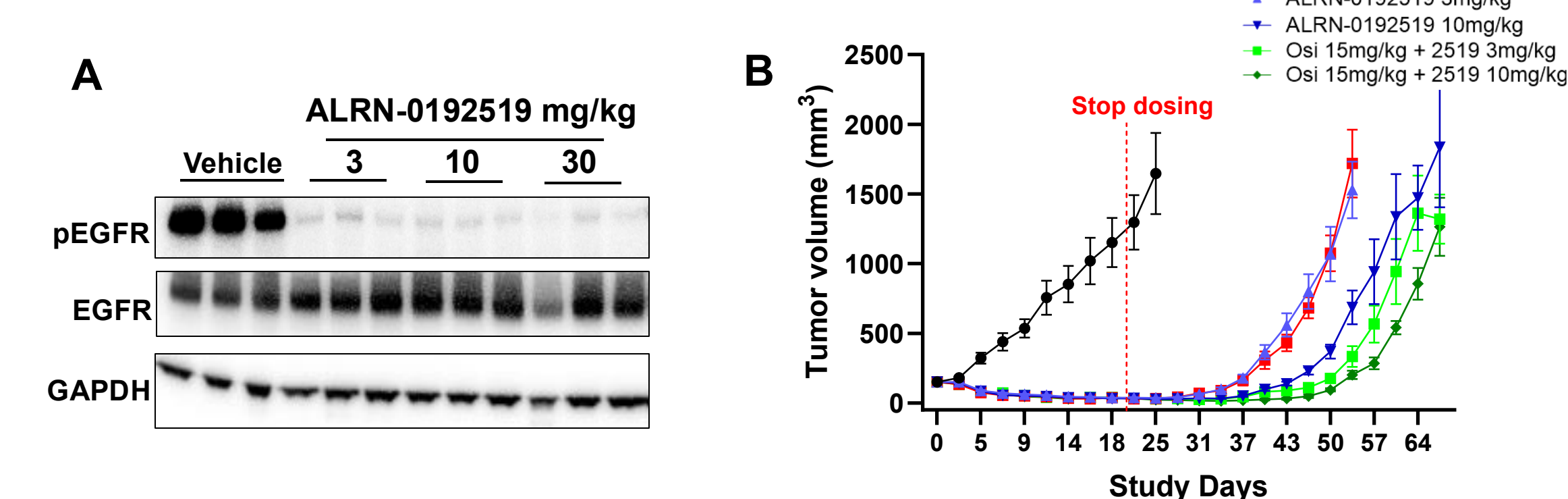
(A) EGFR pathway markers detected by Western blotting. H3255 tumors were collected 12 hours after a single dose of ALRN-2519. (B) Phospho-EGFR inhibition vs. unbound plasma concentration of ALRN-2519 in H3255 tumor-bearing mice (n=2 per dosing group). (C) Anti-tumor activity of ALRN-2519 in the H3255 CDX model and body weight changes.

Figure 5. ALRN-2519 delays tumor regrowth as monotherapy or in combination with osimertinib in the H3255 CDX model



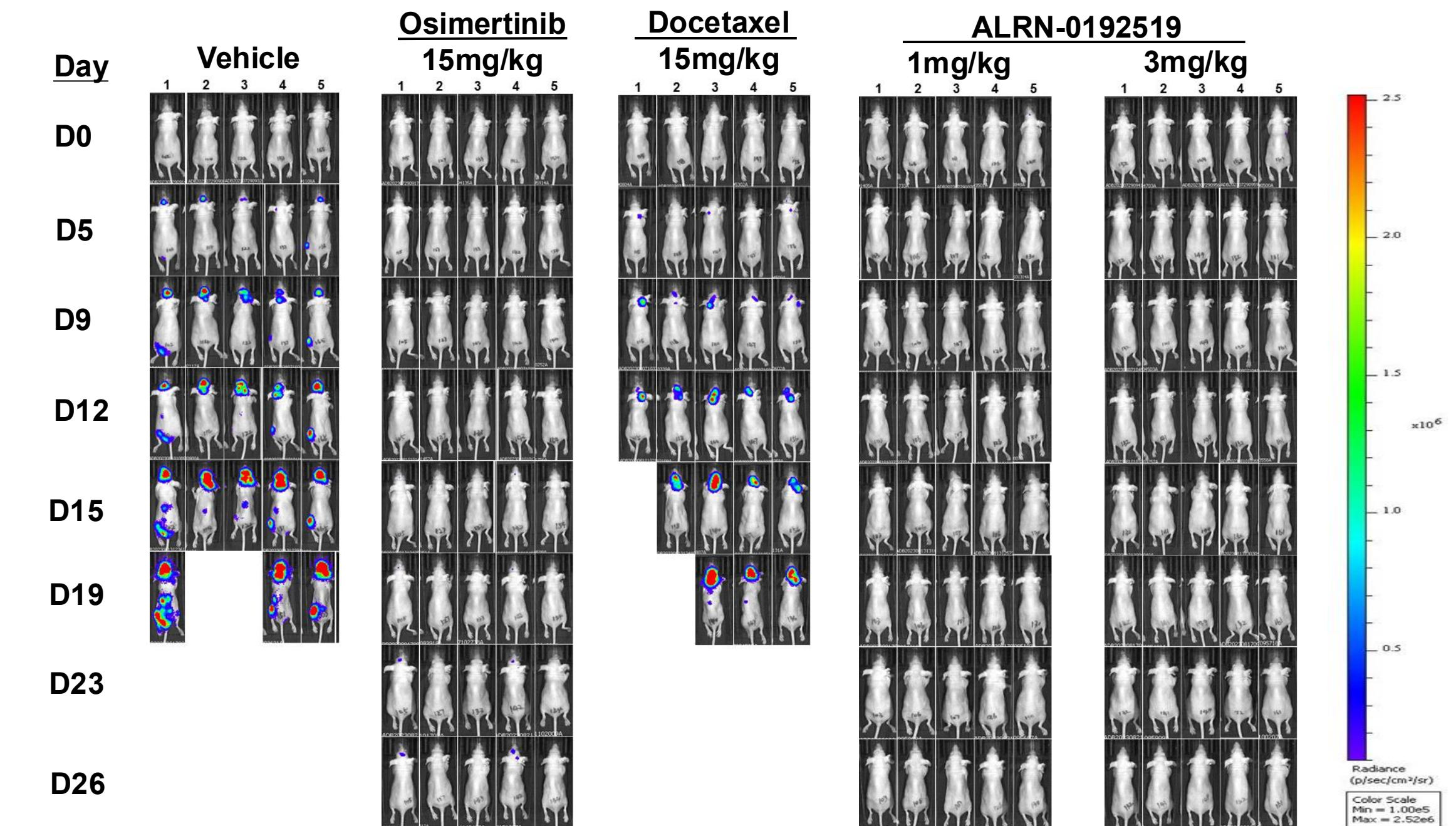
(A) Delayed tumor regrowth by ALRN-2519 as monotherapy. (B) and (C) Delayed tumor regrowth by ALRN-2519 in combination with osimertinib at 5, 15 and 25 mg/kg. (D) Body weight changes. Both compounds were dosed once daily via oral gavage (n = 8 mice per group). Data are presented as mean ± SEM.

Figure 6. ALRN-2519 suppresses tumor regrowth in the H1975 EGFR L858R/T790M mutant CDX model



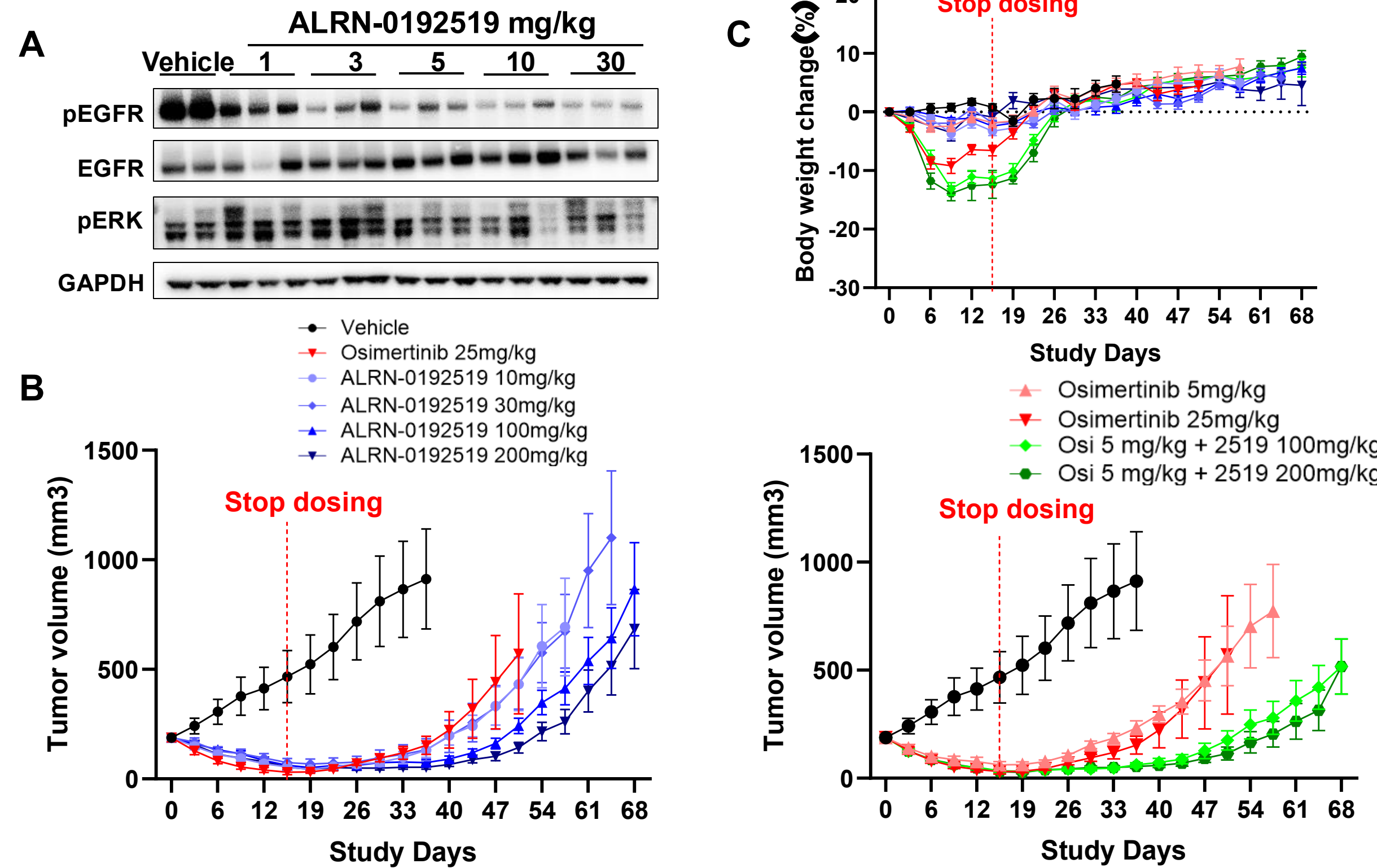
(A) Phospho-EGFR levels detected by Western blotting. Tumors were collected 12 hours after a single dose of ALRN-2519. (B) Anti-tumor activity in the H1975 xenograft model. Mice were treated with ALRN-2519 along or in combination with osimertinib via daily oral dosing.

Figure 7. ALRN-2519 inhibits tumor growth in the H1975 EGFR L858R/T790M brain metastases model



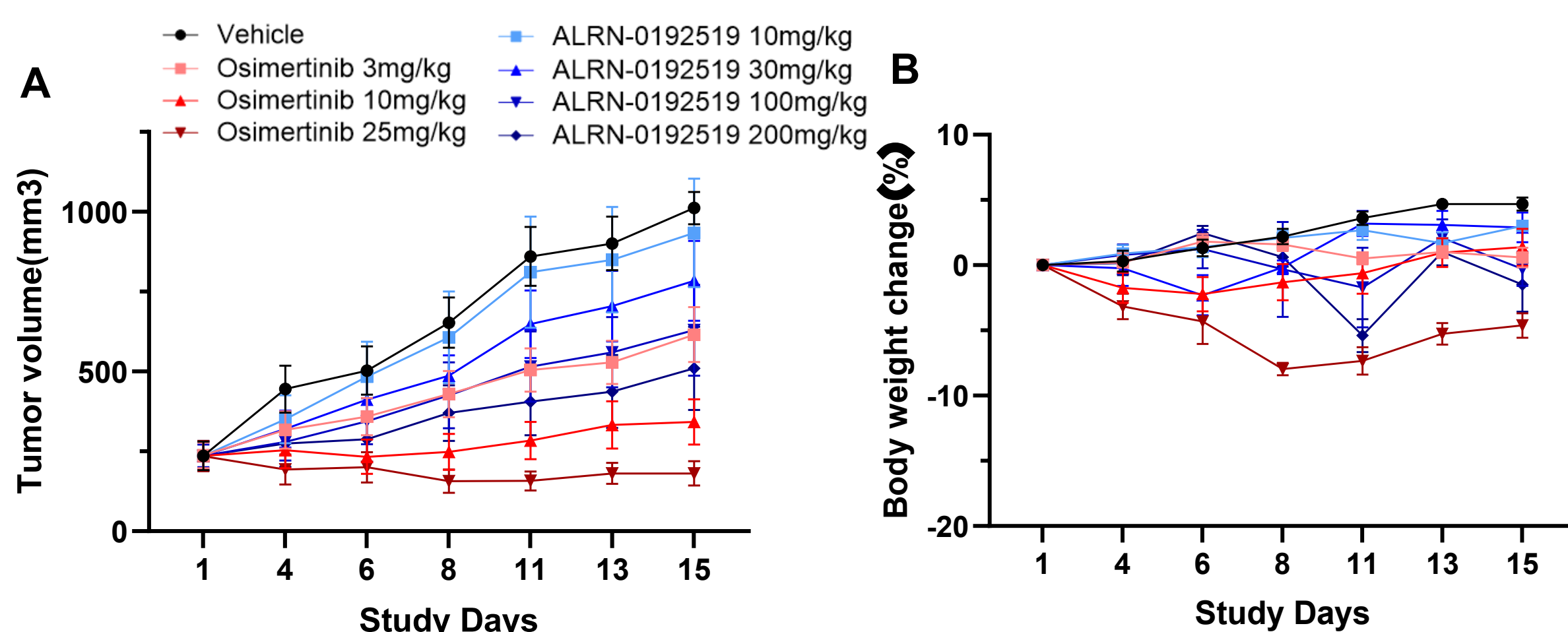
Anti-tumor activity in brain and body metastases of luciferized H1975 tumors. Mice were treated with ALRN-2519 and osimertinib via daily oral administration and docetaxel via weekly IP dosing.

Figure 8. ALRN-2519 effectively suppresses phospho-EGFR signaling and tumor regrowth in the EGFR L858R PDX model



(A) EGFR pathway markers detected by Western blotting. Tumor were collected 12 hours after a single dose of ALRN-2519. (B) Anti-tumor activity of ALRN-2519 as monotherapy or in combination with osimertinib. Both compounds were administered orally once daily for 15 days (n = 8 mice per group). Tumor regrowth was monitored until day 68. (C) Body weight changes. Data are presented as mean ± SEM.

Figure 9. ALRN-2519 exhibits minimal anti-tumor activity in the A431 EGFR WT CDX model



(A) Anti-tumor activity of osimertinib and ALRN-2519 in the A431 xenograft model. (B) Body weight changes. Both compounds were administered orally once daily for 14 days (n = 5 mice per group). Data are presented as mean ± SEM.

Summary

- ALRN-2519 is an allosteric inhibitor with sub-nanomolar IC₅₀ against EGFR L858R mutants in enzymatic assays and exhibits high selectivity against non-EGFR kinases.
- ALRN-2519 exhibits potent activity against EGFR-L858R-mutant cells while maintaining high selectivity against EGFR WT cells.
- ALRN-2519 induces tumor regression in multiple EGFR L858R-dependent CDX and PDX tumor models, including brain metastases, with daily oral dosing. In contrast, it shows minimal effects on tumor growth in EGFR WT xenograft models at equivalent doses.
- Combining ALRN-2519 with osimertinib prolongs tumor suppression compared to osimertinib alone, highlighting the potential benefits of combination therapy.
- These findings establish ALRN-2519 as a promising allosteric EGFR inhibitor for treating EGFR L858R-mutant lung cancers, either as monotherapy or in combination with osimertinib.