CDK4/6 INHIBITION WITH LEROCICLIB (G1T38) DELAYS ACQUIRED RESISTANCE TO TARGETED THERAPIES IN PRECLINICAL MODELS OF NON-SMALL CELL LUNG CANCER

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BACKGROUND

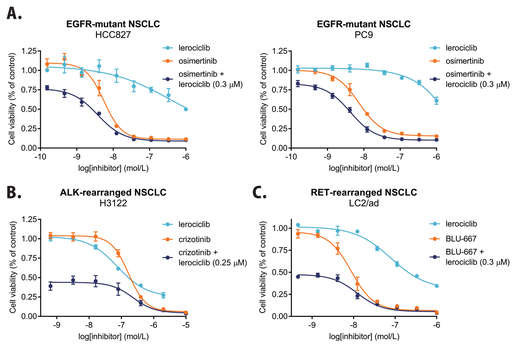
- Tyrosine kinase inhibitors (TKIs) targeting oncogenic drivers such as EGFR, ALK, or RET have substantially improved anti-tumor efficacy in patients with non-small cell lung cancer (NSCLC). Despite the clinical benefit offered by these agents, tumor responses to TKIs are still limited in magnitude and duration. Efforts to address these challenges have focused on combination therapy approaches to enhance efficacy and delay the development of resistance.
- The recent FDA approvals of palbociclib, ribociclib, and abemaciclib in breast cancer validate cyclin-dependent kinases 4 and 6 (CDK4/6) as key therapeutic targets and provide strong rationale to investigate CDK4/6 inhibitors in other tumor types, including NSCLC. Moreover, compared to currently-approved targeted therapies in NSCLC, CDK4/6 inhibitors have a complementary mechanism of action – making them attractive partners for combination therapy.
- Lerociclib (G1T38) is an oral, potent, and selective small-molecule CDK4/6 inhibitor in clinical development (NCT02983071; NCT03455829). Preclinical and early clinical data have demonstrated that lerociclib is differentiated based on its favorable safety/tolerability profile and ability to be dosed continuously with less dose-limiting neutropenia.
- Here, we evaluate whether combination therapy with lerociclib can enhance TKI efficacy and thwart resistance in various NSCLC subtypes.

OBJECTIVES

- Assess the ability of lerociclib to enhance anti-tumor efficacy of TKIs in preclinical models of NSCLC driven by mutations in EGFR, ALK, or RET.
- Determine whether lerociclib can delay or overcome acquired resistance to TKIs in NSCLC models with various oncogenic alterations.
- Identify the nature of resistance mechanisms that can be addressed with lerociclib + TKI combination treatment.

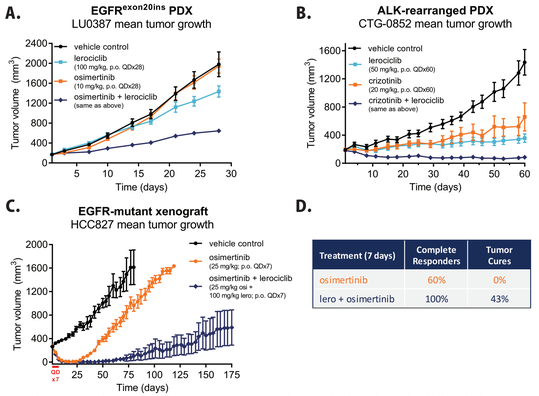
RESULTS

FIGURE 1. LEROCICLIB ENHANCES EFFICACY OF TARGETED THERAPIES IN NSCLC CELL LINES WITH VARIOUS ONCOGENIC ALTERATIONS



The ability of lerociclib to enhance efficacy of TKIs in NSCLC cells harboring **(A)** EGFR exon 19 E746–A750 deletion mutations (HCC827 and PC9), **(B)** EML4-ALK fusion (H3122), or **(C)** CCDC6-RET fusion (LC2/ad) was evaluated using the CellTiter-Glo proliferation assay. Cells were seeded in 96-well plates and incubated with DMSO or increasing concentrations of lerociclib, TKI, or TKI + lerociclib (at the indicated concentration) for 4-6 days. Data are plotted as mean ± SD for n = 3 technical replicates.

FIGURE 2. LEROCICLIB ENHANCES EFFICACY OF TARGETED THERAPIES IN NSCLC XENOGRAFT MODELS

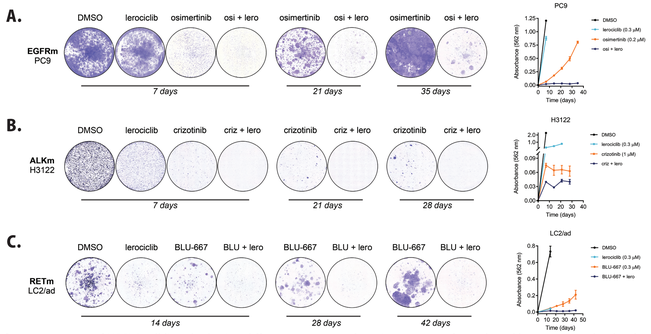


D.

Treatment (7 days)	Complete Responders	Tumor Cures
osimertinib	60%	0%
lero + osimertinib	100%	43%

(A) BALB/c nude mice (n = 10 per group) bearing EGFR exon 20 insertion mutation (EGFR H773_V774insNPH) PDX tumors were treated orally once daily with vehicle, lerociclib, osimertinib, or osimertinib + lerociclib for 28 days at the indicated doses. Study performed at CrownBio. **(B)** Athymic nude mice (n = 10 per group) bearing EML4-ALK fusion PDX tumors were treated orally once daily with vehicle, lerociclib, crizotinib, or crizotinib + lerociclib for 60 days at the indicated doses. Study performed at Champions Oncology. **(C)** HCC827 cells harboring the EGFR exon 19 E746–A750 deletion were implanted subcutaneously in SCID Beige mice (n = 10 per treatment group). Mice were treated orally once daily for 7 days with vehicle, osimertinib, or osimertinib + lerociclib at the indicated doses. Study performed at Charles River. (D) Percentage of complete responses (3 consecutive tumor measurements below 13.5 mm^3) and tumor cures (complete response that persists until end of study) for the experiment shown in (C). All data plotted as mean tumor growth ± SEM.

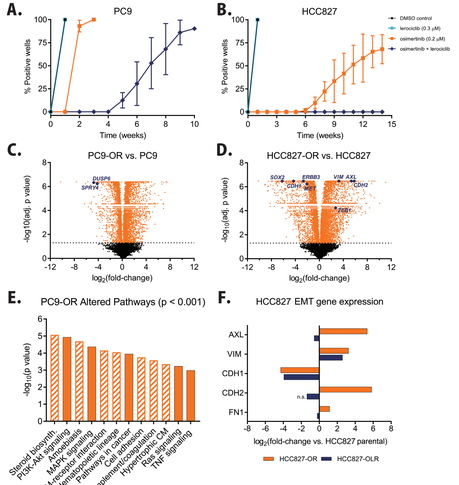
FIGURE 3. LEROCICLIB DELAYS ACQUIRED RESISTANCE TO TARGETED THERAPIES IN NSCLC CELL LINES WITH VARIOUS ONCOGENIC ALTERATIONS



Colony formation assays of NSCLC cell lines harboring different oncogenic alterations were performed weekly to monitor the development of TKI resistance at TKI concentrations >10-fold above the cellular IC$_{50}$ for proliferation. **(A)** EGFR exon 19 deletion PC9 cells, **(B)** EML4-ALK fusion H3122 cells, or **(C)** CCDC6-RET fusion LC2/ad cells were treated with DMSO, lerociclib (0.3 μM), relevant TKI (osimertinib = 0.2 μM, crizotinib = 1 μM, BLU-667 = 0.3 μM), or lerociclib + TKI. Culture media and inhibitors were refreshed weekly, and cells were fixed and stained with crystal violet. Representative images (n = 3 biological replicates) from chosen timepoints are shown on the left, and absorbance quantification of staining is shown on the right, graphed as mean ± SD. H3122 experiments were performed in collaboration with MI Bioresearch, and similar results were seen with alectinib ± lerociclib.

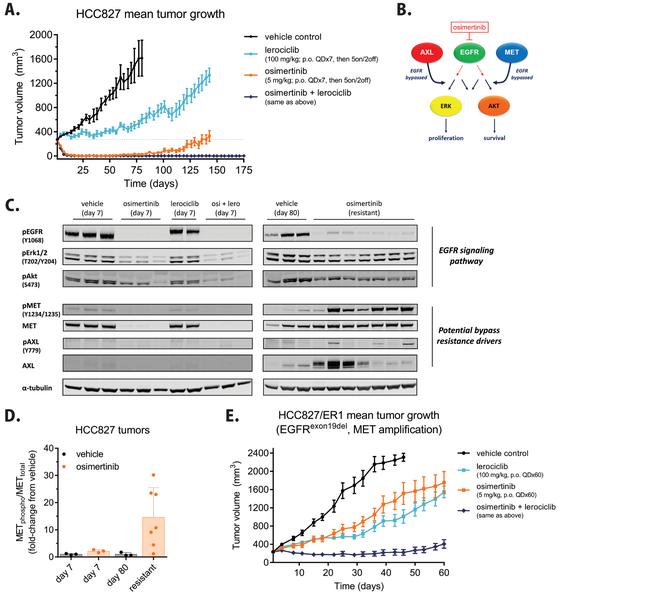
RESULTS

FIGURE 4. LEROCICLIB DELAYS ACQUIRED RESISTANCE IN EGFR-MUTANT NSCLC CELL LINES



To measure the development of acquired resistance and analyze gene expression of resistant cell pools, EGFR-mutant **(A)** PC9 or **(B)** HCC827 NSCLC cells were seeded at low density in the central 24 wells of 48-well plates. Each well in a respective plate was treated with either DMSO, lerociclib (0.3 μM), osimertinib (0.2 μM; >20-fold above the IC$_{50}$ for cellular proliferation), or lerociclib + osimertinib, giving 24 treatment replicates for each plate. Culture media and inhibitors were refreshed weekly, and cell confluence was measured. The percentage of positive wells (> 50% confluence) in each plate were plotted as mean ± SD (n = 3 biological replicates, 1 plate each). Resulting resistant cell pools are denoted with the suffix "-OR" (osimertinib resistant) or "-OLR" (osimertinib + lerociclib resistant). **(C-F)** RNA-Seq of resistant PC9 and HCC827 cell pools identified potential osimertinib resistance mechanisms. Volcano plots of **(C)** PC9-OR and (D) HCC827-OR cells compared to their respective parental control cells reveal differentially expressed genes. The dotted line at p = 0.05 separates significant (orange) and non-significant (black) genes, and potential osimertinib resistance drivers are labeled in dark blue. **(E)** Pathways significantly altered (p < 0.001) in PC9-OR cells compared to parental PC9 cells; pathways previously shown to drive resistance to EGFR TKIs are filled in solid orange. **(F)** Fold-change of epithelial-mesenchymal transition (EMT) related genes AXL (AXL receptor tyrosine kinase), VIM (vimentin), CDH1 (E-cadherin), CDH2 (N-cadherin), and FN1 (fibronectin). Changes in EMT-related gene expression are shown for HCC827-OR or HCC827-OLR cells compared to parental HCC827 cells. All gene expression changes are statistically significant (adj. p < 0.05), with the exception of CDH2 in HCC827-OLR cells.

FIGURE 5. LEROCICLIB PREVENTS AND OVERCOMES BYPASS RESISTANCE TO OSIMERTINIB IN XENOGRAFT MODELS OF EGFR-MUTANT NSCLC



(A) SCID Beige mice (n = 10 per group) bearing HCC827 EGFR-mutant NSCLC tumors were treated orally with vehicle, lerociclib, osimertinib, or osimertinib + lerociclib at the indicated doses once daily for 7 days, followed by a 5on/2off dosing schedule. In the osimertinib monotherapy group, 8/10 mice developed resistant tumors, whereas 0/10 mice developed resistant tumors in the osimertinib + lerociclib combination group. The gray dotted line represents mean initial tumor volume. Study performed at Charles River. **(B)** Simplified cartoon illustrating how bypass resistance to osimertinib can arise through reactivation of signaling pathways downstream of EGFR, while EGFR kinase activity remains effectively inhibited. **(C)** Western blots of HCC827 tumors from early (day 7) and late (day 80 or at resistance) timepoints. No significant group-wide changes were seen in Ras, Cyclin D1, Vimentin, or E-cadherin protein levels between vehicle and osimertinib-resistant tumors. α-tubulin was used as a loading control. **(D)** Quantification of phosphorylated and total MET protein on western blots, expressed as the ratio of phosphorylated/total MET protein. **(E)** BALB/c nude mice (n = 10 per group) bearing HCC827/ER1 EGFR-mutant, MET-amplified xenograft tumors were treated orally once daily with vehicle, lerociclib, osimertinib, or osimertinib + lerociclib for 60 days at the indicated doses. Study performed at CrownBio. All data plotted as mean tumor growth ± SEM (A, E) or SD (D).

SUMMARY

- CDK4/6 inhibition with lerociclib enhances anti-tumor efficacy of targeted therapies in preclinical NSCLC models harboring a variety of oncogenic alterations, including EGFR, ALK, and RET.
- Seven days of combination treatment with lerociclib + osimertinib resulted in 100% complete responses and 43% tumor cures after 175 days in an EGFR-mutant NSCLC xenograft model, compared to 60% complete responses and 0% tumor cures in the corresponding osimertinib monotherapy group.
- Lerociclib significantly delays acquired resistance to new-generation targeted therapies in NSCLC cell lines, suggesting combination therapy with lerociclib may effectively delay resistance regardless of the specific mechanism.
- RNA-Seq analysis reveals potential osimertinib resistance mechanisms in EGFR-mutant cell lines, and suggests combination treatment with lerociclib + osimertinib may delay or prevent resistance arising from epithelial-mesenchymal transition.
- In EGFR-mutant xenograft tumors, lerociclib prevents osimertinib resistance putatively acquired through bypass activation of MET and/or AXL signaling. In EGFR-mutant xenograft tumors with existing resistance to osimertinib caused by MET amplification, combination treatment with lerociclib plus osimertinib overcomes resistance.
- A phase 1b/2 clinical trial evaluating lerociclib + osimertinib in EGFR-mutant NSCLC is ongoing (NCT03455829).