Title: Genomic determinants of acquired resistance to BRAF/MEK inhibitors in BRAF V600E mutant non-small cel lung cancer.

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Abstract

Background: BRAF mutations occur in approximately 4% of patients with non-small cell lung cancer (NSCLC), with half of those cases harboring the class I V600E variant. Combined BRAF and MEK inhibition (BRAFi+MEKi) has demonstrated meaningful antitumor activity and durable outcomes, yet resistance inevitably develops. BRAFi+MEKi therapy may drive the selection of specific tumor clones, promote the emergence of acquired mutations or copy-number alterations, and induce shifts in tumor immunophenotype. However, the full spectrum of resistance mechanisms to BRAFi+MEKi in NSCLC remains largely unknown.

Methods: We performed a global, multicenter, retrospective analysis of patients with metastatic BRAF V600E–mutant NSCLC treated with BRAFi±MEKi across multiple academic institutions, integrated with publicly available data. All included patients had paired pre- and post–targeted therapy next-generation sequencing (NGS) or a post-treatment biopsy documenting a defined, non-genomic mechanism of

resistance. NGS was accepted on either tissue or plasma samples; only oncogenic or likely oncogenic alterations per OncoKB and/or ClinVar were included.

Results: Among 26 patients with matched pre- and post–BRAFi/MEKi samples, median age was 65 years; 42.3% were women, 66.7% had a history of tobacco use, and 96.2% had adenocarcinoma at initial diagnosis. The median progression-free survival on BRAFi+MEKi was 8 months. Resistance mechanisms were heterogeneous and included both BRAF-independent and dependent activation of the RAS/RAF/ERK pathway and alterations leading to cell-cycle deregulation. Multiple concurrent resistance mechanisms were identified in 15% of cases. One patient developed small-cell lung cancer (SCLC) transformation. No clear resistance mechanism was identified in 7 (27%) patients (**Figure 1A**).

Among the 24 patients with complete NGS profiling, MET amplification (16%, N=4) and RAS mutations (KRAS G12V, N=1; KRAS Q61R, N=1; NRASQ61K, N=1; NRAS Q61R, N=1) emerged as the most frequent acquired alterations, followed by CDK4 amplification (N=2), and additional diverse events including an acquired BRAF kinase-domain duplication and FGFR4 amplification. Less well-characterized putative resistance events involving DNA repair genes such as CHEK2 were also observed (**Figure 1B**).

Clinically, one patient with acquired MET amplification showed loss of the MET-amplified clone on subsequent liquid biopsy after the addition of crizotinib to dabrafenib/trametinib, without grade≥3 adverse events (**Figure 1C**), highlighting a potentially targetable resistance mechanism with therapeutic implications.

Conclusions: These findings underscore the feasibility and clinical value of routine NGS at progression on BRAFi+MEKi, revealing multiple actionable or targetable mechanisms of resistance, including SCLC transformation, MET amplification, and emergent KRAS/NRAS alterations, that may inform personalized therapeutic strategies and guide post-BRAFi/MEKi treatment selection.

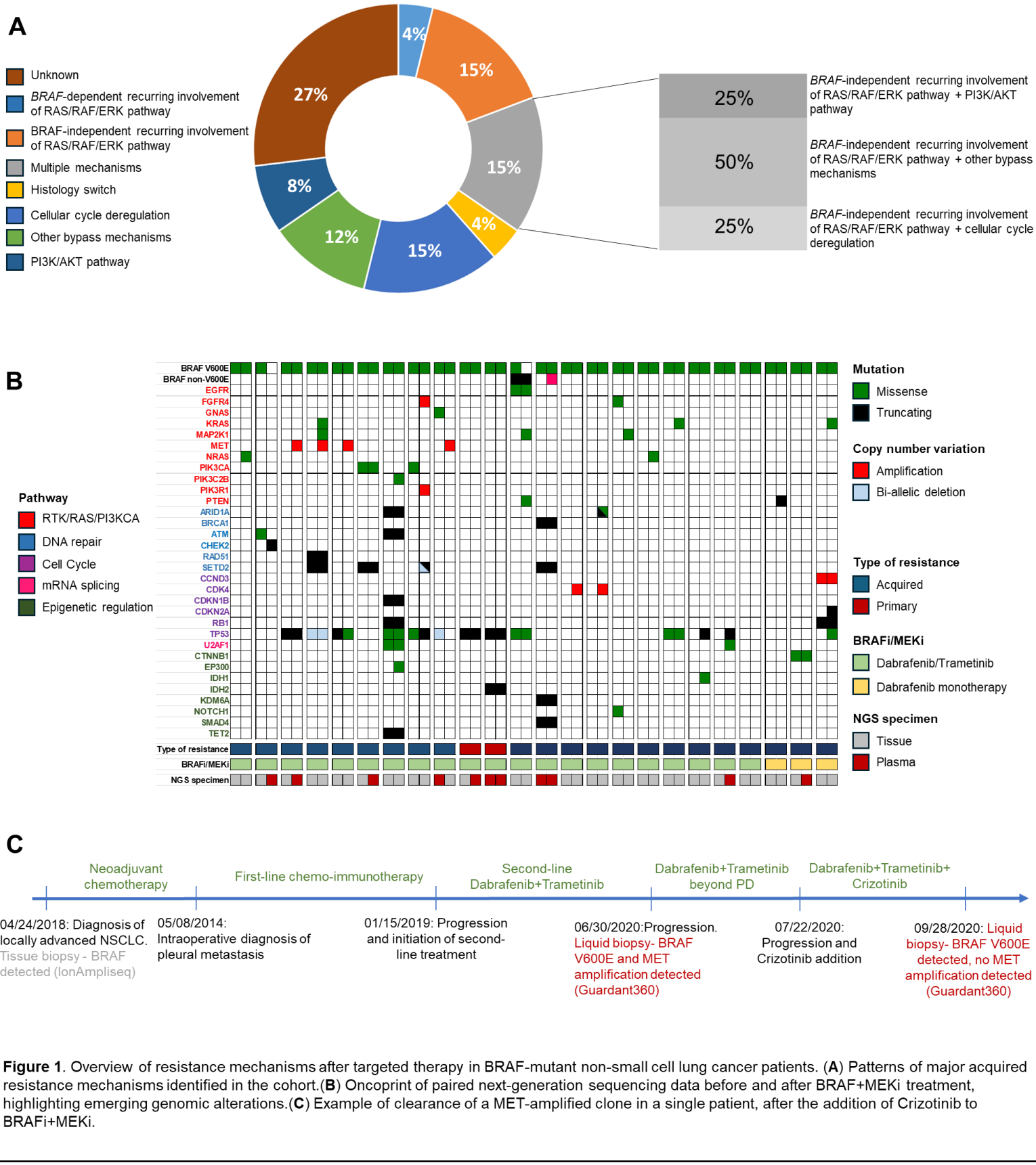

Figure 1. Overview of resistance mechanisms after targeted therapy in BRAF-mutant non-small cell lung cancer patients. (**A**) Patterns of major acquired resistance mechanisms identified in the cohort.(**B**) Oncoprint of paired next-generation sequencing data before and after BRAF+MEKi treatment, highlighting emerging genomic alterations.(**C**) Example of clearance of a MET-amplified clone in a single patient, after the addition of Crizotinib to BRAFi+MEKi.