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MAPK Pathway Mutations Emerge in Mutant-IDH1 Inhibitor–Resistant Cholangiocarcinoma and Attenuate the Interferon Response

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Background

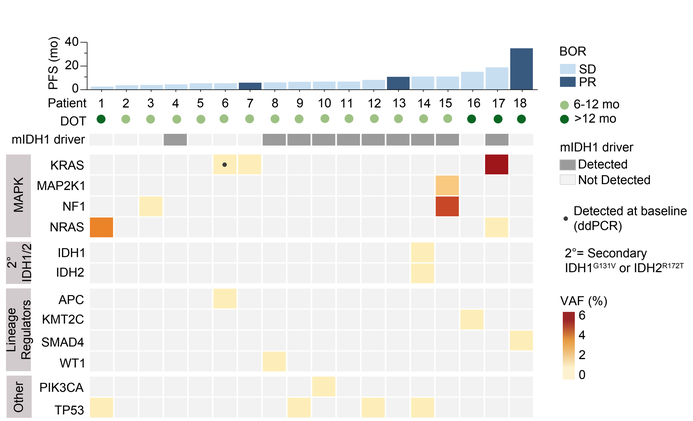
- Ivosidenib (IVO) is a first-in-class oral inhibitor of the isocitrate dehydrogenase 1 (IDH1) mutant enzyme.
- In the Phase III ClarIDHy study, conducted in previously treated patients with IDH1-mutated cholangiocarcinoma (CCA), IVO treatment led to improved progression-free survival (PFS) and overall survival (OS) compared with placebo (median PFS 2.7 versus 1.4 months; median OS 10.3 versus 5.1 months when adjusted for the 70% crossover rate).1.2
- Here, we studied the dynamics of mutations emerging after 6 months of IVO treatment using circulating tumor DNA (ctDNA) analyses.
- Alterations detected at end-of-treatment (EOT), but not at screening, were further studied in preclinical models for their potential functional roles in IVO resistance.

Methods

- Plasma samples from 81 patients were analyzed by PGDx elioTM plasma complete, a next generation sequencing (NGS)-based 521 gene panel.3
- Clinical parameters (PFS, treatment duration, and tumor burden [evaluated by RECIST v1.1]) were correlated with ctDNA abundance and presence of specific co-mutations at baseline..
- Evaluation of matched baseline and EOT samples from 18 patients treated with IVO for ≥ 6 months revealed the emergence of mutations in mitogen-activated protein kinase (MAPK) pathway genes (e.g., in KRAS, NRAS, and NF1) and in IDH1/IDH2.
- Digital droplet polymerase chain reaction (ddPCR) analysis conducted on longitudinal plasma samples validated the emergence of these mutations and established their allelic frequency relative to clonal (truncal) mutations.
- IVO promotes innate immune signaling (type I interferon [IFN] pathway) and restores responsiveness of tumor cells to exogenous IFN-γ as part of its mechanism of action. Hence, we tested the impact of neurofibromin 1 (NF1) deletion on induction of IFN pathway target genes after IVO ± IFN-γ treatment4 in the IDH1 mutant CCA cell line, SNU1079.
- Roles of IDH1 second-site mutation and IDH2 mutant alleles in resistance was tested in an IDH1 mutant CCA mouse allograft model.5

Results

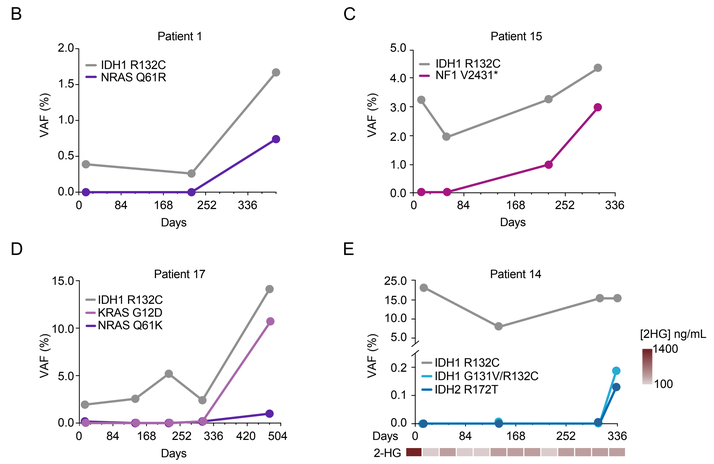
Figure 1. ctDNA analysis reveals emergent MAPK and IDH1/2 mutations during ivosidenib resistance in ICC.



A. Heatmap showing VAF of alterations detected by NGS in EOT ctDNA samples but not in baseline samples from patients treated with ivosidenib (IVO) for >6 months. All patients had confirmed IDH1 driver mutations in tumor tissue. Patients with IDH1 mutations detected in baseline ctDNA are indicated. 2° IDH1/IDH2 denotes secondary IDH1G131V and IDH2R172T mutations that emerged in patient 14. For patient 6, the asterisk indicates a KRAS mutation classified as emergent by NGS but subsequently detected at baseline by ddPCR.

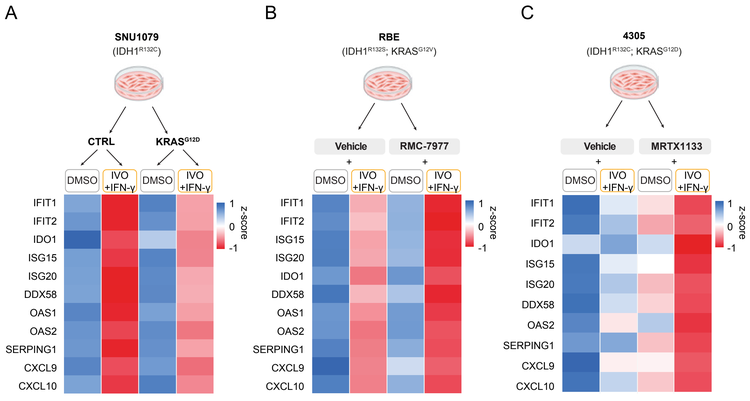
Results

Figure 1. ctDNA analysis reveals emergent MAPK and IDH1/2 mutations during ivosidenib resistance in ICC. (continued)



B-E. ddPCR analysis of serial ctDNA samples for select candidate resistance mutations identified by NGS (from panel A). Patient 1 had radiographic progression at 85 days but continued to receive clinical benefit and was treated for 517 days. For the other patients, progression occurred at or after the final time point depicted (patient 15, 33 days; patient 17, 96 days; patient 14, 2 days). In E, the heat map below the graph shows 2HG levels at the beginning of each 28-day cycle of treatment.

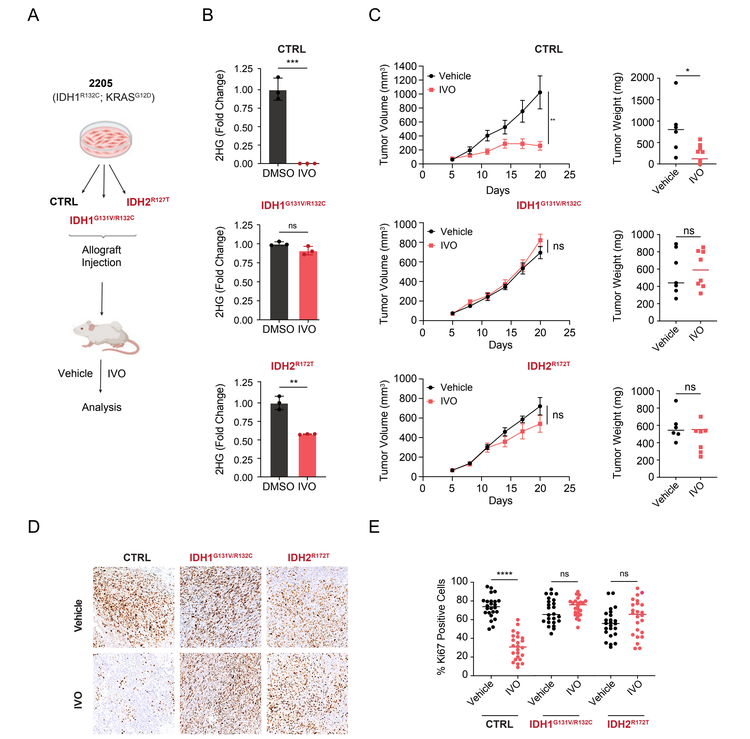
Figure 2. KRAS activation blunts response to ivosidenib plus IFN-γ in mIDH1 ICC cells.



A. SNU1079 cells engineered with doxycycline (DOX)-inducible KRASG12D or empty vector were cultured in DOX-containing media (50 ng/mL) and treated with vehicle or 1 uM ivosidenib + 10 ng/mL IFN-γ (see Methods) and analyzed by qRT-PCR. Targets were selected based on prior analysis of ivosidenib+IFN-γ-treated parental SNU1079 cells. **B, C.** RBE cells treated with 100 nM RMC-7977 or vehicle (**B**) and 4305 cells were treated with 100 nM MRTX1133 or vehicle (**C**) were co-treated with DMSO or ivosidenib+IFN-γ and analyzed by qRT-PCR.

Results

Figure 3. Clinically observed acquired IDH1/2 mutations drive ivosidenib resistance in a mouse model of mIDH1 ICC



A. Schematic of study. **B.** Measurement of 2HG levels in 2205 derivatives treated with 1 mM ivosidenib or vehicle *in vitro* for 24 hrs. **C, D.** Subcutaneous 2205 allografts were treated with 150 mg/kg ivosidenib or vehicle twice daily once tumors reached ~75 mm^3. **C.** Serial tumor volume measurements (left) and final tumor weigh (right). **D, E.** IHC staining for Ki-67 in end of study samples collected 6 hrs after the last treatment.

Conclusion

MAPK pathway alterations represent a recurrent mechanism of resistance to mIDH1 inhibition in ICC, while emergent IDH1/IDH2 mutations appear infrequent. Functional data suggest that MAPK-mediated resistance may involve impaired IFN signaling. These results support MAPK-directed combination strategies and highlight the utility of ctDNA profiling to identify predictive and resistance biomarkers in mIDH1-driven ICC.

References

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