Profiling of cholangiocarcinomas (CCA) to identify genomic alterations, tumor mutational burden (TMB) and genomic loss of heterozygosity (gLOH)

Background: The management of CCA has evolved as targeted and immune checkpoint inhibitors (ICI) therapies have emerged. We used comprehensive genomic profiling (CGP) to characterize the genomic alterations (GA) that have potential to personalize therapy for CCA.

Methods: 4371 CCA underwent hybrid capture based CGP (Foundation GM, Nat Biotechnol., 2013, PMID: 24143048) on 0.8-1.1 Mb of the coding genome to identify GAs in exons and selected introns in up to 404 genes, TMB, microsatellite instability status (MSI) and microsatellite genomic loss of heterozygosity (gLOH). PD1 expression was determined by IHC (Dako, 2023).

Results: 51% of CCA were female, with a median age of 62 years (range 16-89). The most common biopsy sites were liver (75%), lymph node (4%), bile duct (5%) and lung (2%). MSI high was rare (1%), 1G and 2G cases had TMB >10 and >20 mutations/megabase, respectively. Of the 54 (15%) MSI-H, PD-L1 amplification (AMP) was present in 20%, 25% of 460 CCA tested, 43 (12%) were positive for PD1 expression. Of the 61% gLOH tested for CCA, 21% (55/2658) have a gLOH >10; only 1% had both TMB >20 and gLOH >16%. GAs were most common in TP53 (32%), CCND1 (30%), ARID1A (18%). Potentially targetable GAs include FGFR2 (11%, 85% fusions), BRAF (5%, 52% V600E), ERBB2 (9.7%, 72% AMP), MET (2%; 53% AMP), EGFR (2%; 21% short variants (SV)) and rarer (<0.5%) FGFR3, RET, RET/PTK4, ALK, and ROS1 fusions. The FGFR2 fusions had 144 unique 3 partner genes including BCCl (26%), CDCA8 (23%), AHCY1 (2.4%) and KIAA1271 (2.4%). FGFR2 fusions occurred in a mutually exclusive fashion from high gLOH (p >0.05), but not high TMB. GA in high TMB (19%) were mutually exclusive of FGFR2 fusions (p = 5.1e-16), but coincurred with PD-L1 (GA 22%, p < 7.5e-21), ARID1A (24%, p < 1.5e-09). PD1 GA had similar to the overall CCA population but were enriched for low TMB (p = 2.5e-04).

Conclusions: CCA harbor potentially targetable kinase GA, half of which were FGFR2 fusions. Independently, an additional 10% of cases with high gLOH and 1% of cases with high TMB, MSI and/or PD-L1 AMP may benefit from PPAR inhibitors and ICI respectively. Independently, combination of IDH1 and PBRM1/ARID1A defines a class of CCA that warrants further investigation for sensitivity to PPAR inhibitors and may serve as a paradigm for other tumors (i.e. gliomas) with a similar cooccurrence landscape.

Materials and Methods

- 4371 CCA underwent hybrid capture based CGP (Foundation GM, Nat Biotechnol., 2013, PMID: 24143048) on 0.8-1.1 Mb of the coding genome to identify GAs (base substitutions, small indels, copy number alterations and rearrangements) in exons and selected introns in up to 404 genes. TMB, microsatellite instability status (MSI) and microsatellite genomic loss of heterozygosity (gLOH). CGP was performed in a CLIA certified, NY State, CAP accredited laboratory (Foundation Medicine, Inc, Cambridge MA). PD1 expression was determined by IHC (Dako, 2023).

- TMB was calculated as the number of non-driver somatic coding mutations per megabase of genome sequenced (Cherem et al. 2017, PMID: 28404211). TMB levels were divided into two groups: low (0-20 mutations/megabase) and high (>20 mutations/megabase).

- High genome-wide loss of heterozygosity (gLOH) was classified as >16% LOH, as used in the ARIEL3 PARP inhibitor trial in ovarian cancer (Coleman et al., Lancet, 2017, PMID 28618360).

- The most common GAs included FGFR2 fusions, seen in 5% or more cases are shown in this plot.

- b) Schematic of the most common FGFR2 rearrangements

- Co-Disclosure and Mutual Exclusivity of GAs: Co-Disclosure and Mutual Exclusivity was calculated using the Fisher’s exact test and the FDR p-values were adjusted for multiple comparisons by the Benjamin-Hochberg method. Only GAs with a p-value < 0.001 and an FDR <0.05 were considered statistically significant. Not shown - KRAS/BRCA and IDH1/SHJ are mutually exclusive (FDR = 0.005). FGFR2/FGFR3, CNOT12/MAPK, and mutations in DCC, Tubb8, MPRF, and FGFR4, FGFR5, FGFR6, CNOT12 co-occur (FDR < 0.001).

- Targetable kinases (defined as EGFR short variants, ERBB2 amplifications and short variants, MET amplifications, BRAF V600E alterations, RET, ROS1 and ALK fusions) were mutually exclusive from FGFR2 fusions (p < 1.5e-08) and IDH1/2 short variants (p = 6e-12).

- FGFR2 fusions were mutually exclusive from IDH1/2 short variants (p = 2e-20) while BAP1 frequently co occurred with FGFR2 fusions (p < 1.5e-27).

- KRAS seen at 20% is mutually exclusive from FGFR2 fusions (p < 2e-24). (FGFR2 non-driver variants 131 fusions were seen in this group and targetable kinases (p=5.1e-15). KRAS is also not significantly associated with high TMB (Odds Ratio 1.5, p-value = 0.24).

- Among the FGFR2 fusion partners, 131 fusion partners were seen less than 5 times.

- GAs in the Homologous Recombination Repair pathway genes were most common in ATM (3.7%), PTEF1 (3.2%), BRCA2 (2.24%), CHEK2 (1.65%), BRCA1 (1%) and other GAs at < 1%.

- ATM, PTEF, BRCA2, BRCA1, RAD51, FANC5 were also associated with high gLOH (p < 0.05, Fig 5).

Conclusions

- We estimate that 35% of cholangiocarcinomas have potentially actionable GAs that may benefit from targeted therapy with IDH or kinase inhibitors.

- Around 1% of cholangiocarcinomas could possibly benefit from immune checkpoint inhibitors.

- Patients with FGFR (gene fusions) inhibitors could play an important role in the management of an additional 10% of cholangiocarcinomas with high gLOH.

- Achieving FGFR2 fusions were seen in a multitude of 3 partner genes emphasizing the importance of partner agnostic detection of these GAs.

- Co-occurrence of IDH1 and PBRM1/ARID1A defines an additional class of CCA that warrants further investigation for sensitivity to PARP inhibitors.

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