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

Milind M. Javle[1], Karthikeyan Murugesan[2], Rachna T. Shroff[1], Mitesh J. Borad[3], Reham A. Wahab[1], Russell Madison[2], Alexa B. Schrock[2], Jon Chung[2], Lipika Goyal[4], Garrett M. Frampton[2], Robin K. Kelley[5], Vincent A. Miller[2], Jeffrey S. Ross[2,6], Tanios S. Bekaii Saab[7], Siraj M. Ali[2], Lee A. Albacker[2]
University of Texas MD Anderson Cancer Center, Houston, TX[1], Foundation Medicine Inc., Cambridge, MA[2], Mayo Clinic Cancer Center, Scottsdale, AZ[3], Massachusetts General Hospital, Boston, MA[4], University of California San Francisco, Helen Diller Family Comprehensive Cancer Center, San Francisco, CA[5], SUNY Upstate Medical University, Syracuse, NY[6], Mayo Clinic, Phoenix, AZ[7]

ABSTRACT

Background: The management of CCA has evolved as targeted and immune checkpoint inhibitor (ICPI) 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 on 0.8-1.1 Mb of the coding genome to identify GAs in exons and select introns in up to 404 genes, TMB, microsatellite instability status (MSI) and % monoallelic genome (gLOH). *PDL1* expression was determined by IHC (Dako 22C3).
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 (3.5%), and lung (2%). MSI high was rare (1%), 150 and 57 cases had TMB > 10 and > 20 mutations/mb respectively. Of the latter, 54% (31/57) were MSI-H. *PDL1* amplification (AMP) was present in 0.25%. Of 490 CCA tested, 43 (9%) were positive for *PDL1* expression. Of the 61% of cases tested for gLOH, 21% cases (551/2665) have a gLOH > 16%, only 1 case had both TMB > 20 and gLOH > 16%. GAs were most common in *TP53* (32%), *CDKN2A* (30%), *KRAS* (20%) and *ARID1A* (18%). Potentially targetable GAs include *FGFR2* (11%, 85% fusions), *BRAF* (5%, 52% V600E), *ERBB2* (5.7%, 75% AMP), *MET* (2%, 92% AMP), *EGFR* (2%, 26% short variants (SV)) and rarer (< 0.5%) *FGFR3*, *RET*, *FGFR1*, *ALK*, and *ROS1* fusions. The *FGFR2* fusions had 144 unique 3' partner genes including *BICC1* (26%), *CCDC6* (3.2%), *AHCYL1* (2.4%) and *KIAA1217* (2.4%). *FGFR2* fusions occurred in a mutually exclusive fashion from high gLOH (p < 5e-05), but not high TMB. GA in *IDH1* (15%) were mutually exclusive of *FGFR2* fusions (p < 5.5e-16), but cooccurred with *PBRM1* GA (22%, p < 7.5e-21), *ARID1A* (26%, p < 1.5e-09). *IDH1* GA had gLOH similar to the overall CCA population but were enriched for low TMB (p < 2.5e-04).
Conclusions: Nearly 20% of CCA cases 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 *PDL1* AMP may benefit from PARP inhibitors and ICPI respectively. Independently, comutation of *IDH1* and *PBRM1/ARID1A* defines a class of CCA that warrants further investigation for sensitivity to PARP 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 (Frampton GM, Nat Biotechnol, 2013, PMID: 24142049) 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 select introns in up to 404 genes, TMB, microsatellite instability status (MSI) and % monoallelic genome (gLOH). CGP was performed in a CLIA certified, NY State, CAP accredited laboratory (Foundation Medicine, Inc, Cambridge MA). PDL1 expression was determined by IHC (Dako 22C3).
- TMB was calculated as the number of non-driver somatic coding mutations per megabase of genome sequenced (Chalmers et al. 2017, PMID: 28420421). TMB levels were divided into two groups: low (<= 20 mutations/mb) and high (>20 mutations/mb).
- High genome-wide loss-of-heterozygosity (gLOH) was classified as >16% LOH, as was used in the ARIEL3 PARP inhibitor trial in ovarian cancer (Coleman et al, Lancet, 2017, PMID: 28916367)

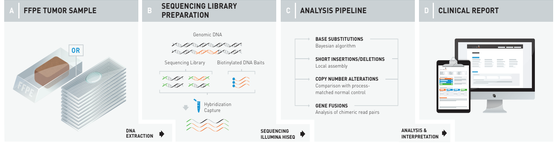


Figure 1. Hybrid capture based Comprehensive Genomic Profiling

RESULTS

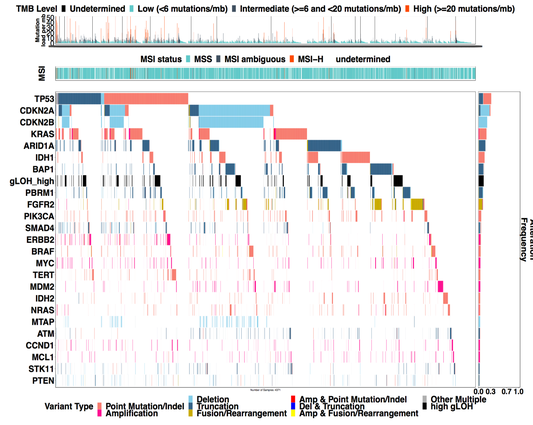


Figure 2. Oncoprint: Prevalence of the top 25 GAs, TMB, MSI and gLOH across the CCA cohort

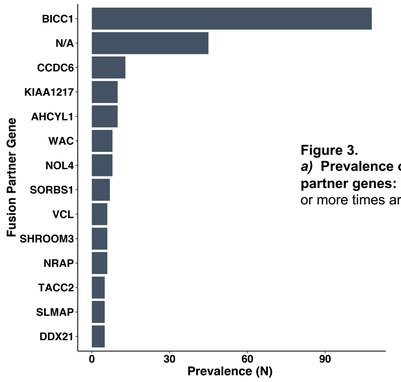
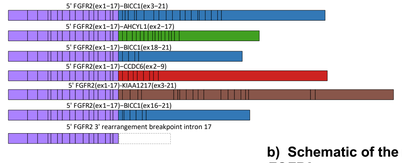


Figure 3.
***a)* Prevalence of *FGFR2* fusion partner genes:** Only fusions seen 5 or more times are shown in this plot.



b) Schematic of the most common *FGFR2* rearrangements

RESULTS

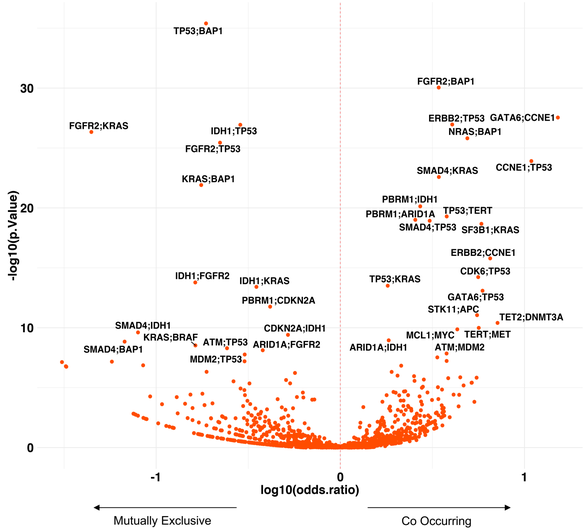


Figure 4. Co-Occurrence and Mutual Exclusivity of GAs: Co-Occurrence and Mutual Exclusivity was calculated using the Fischer's exact test and the Fischer's p-values were adjusted for multiple comparisons by the Benjamini-Hochberg method. Only genes with a prevalence ≥1.5% are shown in this plot and only gene pairs with an FDR < 0.001 have been labelled. Not shown - *KRAS;NRAS* and *IDH1;IDH2* are mutually exclusive (FDR < 0.001). *MDM2;FRS2*, *CDK6;MET*, all permutations in [*CDKN2A*, *CDKN2B*, *MTAP*] and [*FGF3, FGF4, FGF19, CCND1*] 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 *FGR2* fusions (p < 1.5e-27).
- *KRAS* seen at 20% is mutually exclusive from *FGFR2* fusions (p < 2e-24), *IDH1/2* short variants (p < 4e-21) and targetable kinases (p < 8.5e-11). *KRAS* is also not significantly associated with high TMB (Odds Ratio = 1.5, p-value = 0.24).
- Amongst 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%), *PTEN* (3.2%), *BRCA2* (2.24%), *CHEK2* (1.65%), *BRCA1* (1%) and other genes at < 1%.
- *ATM*, *PTEN*, *BRCA2*, *BRCA1*, *PALB2*, *BARD1*, *FANCG* were also associated with high gLOH (p < 0.05, Fig 5)

RESULTS

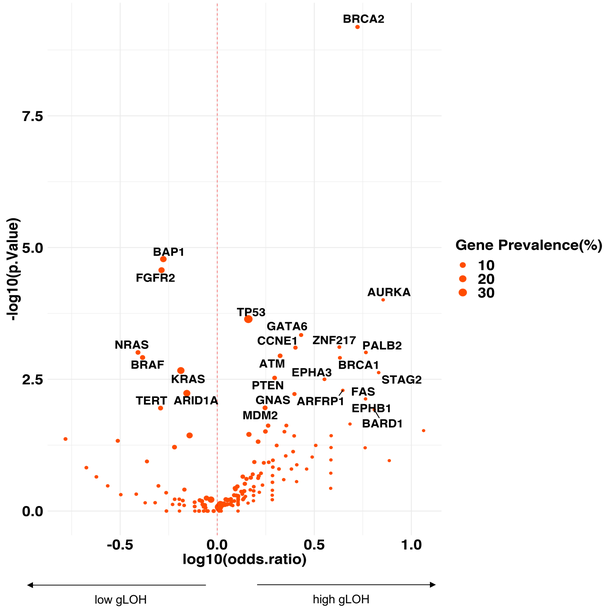


Figure 5. Association of GAs with gLOH: Enrichment of GAs between high gLOH and low gLOH was calculated using the Fischer's exact test. Only gene:gLOH associations with a p-value < 0.01 have been labelled. Not shown - *MLH1* is associated with low gLOH (p-value < 0.01)

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.
- PARP (poly ADP ribose polymerase) inhibitors could play an important role in the management of an additional 10% of cholangiocarcinomas with high gLOH.
- Activating *FGFR2* fusions were seen with a multitude of 3' partner genes emphasizing the importance of partner agnostic detection of these GAs.
- Comutation of *IDH1* and *PBRM1/ARID1A* defines an additional class of CCA that warrants further investigation for sensitivity to PARP inhibitors.