Molecular characterization of 350 hepatocellular carcinomas identifies biomarker aberrations with potential novel therapeutic options

Celina Ang[1], John T. Miura[2], T. Clark Gamblin[2], Joanne Xiu[3], Sherri Z. Millis[3], Zoran Gatalica[3], Sandeep K. Reddy[3,4], Nelson S. Yee[5]

[1]Mt. Sinai, New York, NY, Hematology/Oncology Division [2]Medical College of Wisconsin, Milwaukee, WI; [3]Caris Life Sciences, Phoenix, AZ; [4]Harbor UCLA Medical Center, Los Alamitos, CA; [5]Penn State Hershey Cancer Institute, Hershey, PA.

Abstract #4086

Background: Effective treatment strategies for hepatocellular carcinoma (HCC) remain limited. Identification of additional therapies remains paramount as currently available agents have resulted in marginal improvements in overall survival or are not appropriate for this patient population.

Methods: 350 HCC samples were evaluated on a commercial platform, for both genetic and proteomic aberrations. Tests included Sanger or next generation sequencing (NGS), protein expression by immunohistochemistry (IHC) and gene amplification by in situ hybridization (ISH).

Results: TP53 was mutated in 34%, CTNNB1 in 20%, and BRCA2 in 18%; other gene mutation rates were < 5%.TP53-mutated tumors show significantly higher TOP2A (89% vs. 39%, p<0.0001), TS (70% vs. 32%, p=0.0067) and RRM1 expression (40% vs. 12%, p=0.017), implying high rates of proliferation and DNA synthesis. CTNNB1-mutated tumors showed significantly higher SPARC (67% vs. 21%, p=0.0013) and AR expression (53% vs. 22%, p=0.025). Changes in protein expression are shown.

% of samples with change, by IHC									
High expression levels						Low expression levels			
EGFR	TOPO1	PD-1	TOP2A	SPARC	cMET	RRM1	TS	PTEN	MGMT
83	52	60	38	36	25	82	80	72	31

Metastatic HCC (N=124) exhibited significantly higher PD-1 (79% vs. 50%, p=0.047) and TS expression (31% vs. 14%, p<0.0008) than non-metastatic (N=226).

Analysis of outcomes in a subset of patients treated based on biomarker-therapy associations is ongoing. In 1 patient an EGFR mutation (predictive of response to erlotinib in NSCLC) was identified, and the patient has begun treatment with erlotinib.

Conclusions: The molecular profile in HCC suggests potential targeted therapies, such as tyrosine kinase inhibitors, anti-PD1 agents, or PI3 kinase pathway inhibitors. Immuno-modulatory agents may be an option, particularly in metastatic HCC, based on levels of PD-1. Concurrent protein changes in CTNNB1-mutated tumors suggest potential benefit of combination therapies when targeting the WNT pathway. Review of responses to targeted therapies, such as is being tried with erlotinib in the patient with EGFR mutation may provide additional insight into efficacious therapies.

Patient Characteristics

Table 1. Patient demographics. Includes limited documented information of risk factors obtained on a very small subset of cases.

Median age: 61
Age range: 18-87
M:F ratio=2.5:1
Known mets: 36%
Known viral status: 3=HBV+, 8=HCV+
Known EtOH: 5

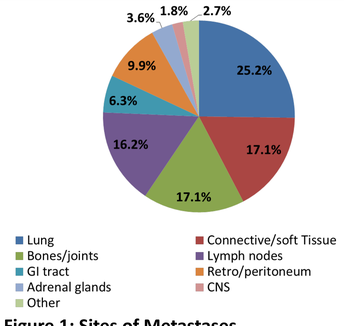
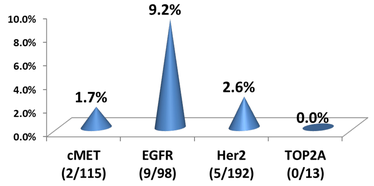


Figure 1: Sites of Metastases

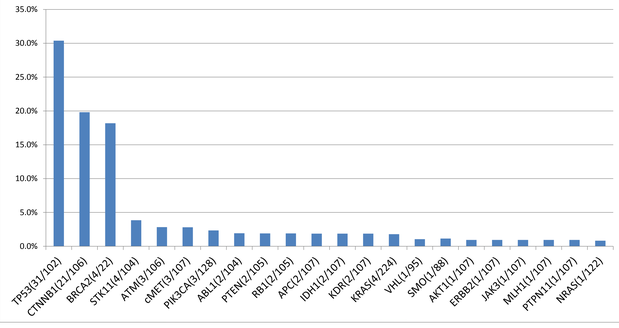
Results, ISH

Figure 2. Changes in gene copy number as measured by FISH or CISH were identified for cMET, EGFR, and HER2. No changes were seen for TOP2A.



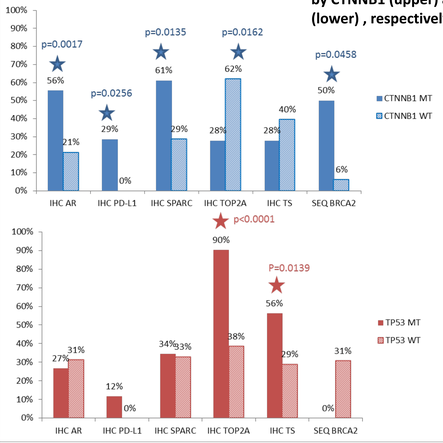
Results, Gene Mutations

Figure 4. Gene alterations. Mutations were found in 22 of 47 (47%) genes tested. Genes with no alterations identified included ALK, BRAF, CDH1, c-KIT, CSF1R, EGFR*, ERBB4, FBXW7, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, JAK2, MPL, NOTCH1, NPM1, PDGFRA, RET, SMAD4, SMARCB1and BRCA1. (* One additional tumor presented an EGFR activating mutation from an external lab.) 43% of cases tested had either a CTNNB1 or a TP53 gene alteration, including 6 cases with both a CTNNB1 and a TP53 gene alteration. No significant differences in gene mutations were found between primary and metastatic cases.



Results, Co-incidence

Figure 5. Biomarker features differentiated by CTNNB1 (upper) and TP53 mutations (lower) , respectively.



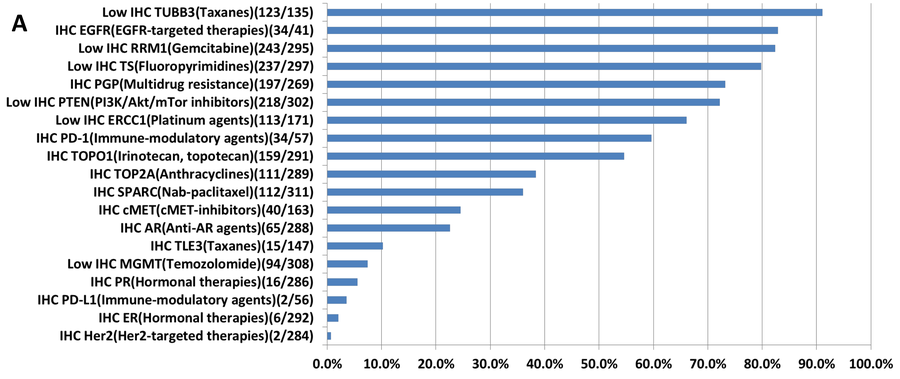
Stars indicate differences statistically significant by Fisher-Exact test.

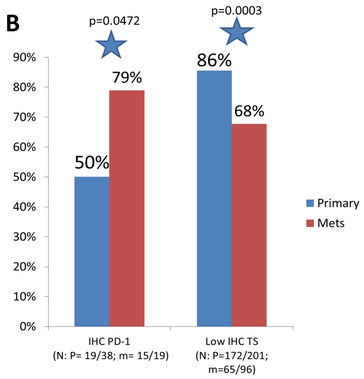
Significantly higher AR, PDL1, SPARC and BRCA2 suggest potential combinatorial strategies for treatment.

Higher expression of TOP2A and TS in TP53-MT cohort indicate higher cell proliferation and DNA synthesis activity.

Results, IHC

Figure 3A. Either overexpression, reported as percent positive of total cases tested, or loss, reported as percent negative. Therapeutic agents associated with the aberrations observed are listed in parenthesis. 2B: Comparison of protein expression, for those with significant differences between primary and metastatic cases (Stars indicate differences statistically significant by Fisher-Exact test.**).**





Conclusions

- These data suggest potential therapeutics, such as tyrosine kinase inhibitors, anti-PD-1 agents, or PI3 kinase pathway inhibitors. Although no evidence shows that cytotoxics are effective in patients with HCC, irinotecan, alkylating agents, fluoropyrimidines, anthracyclines, nab-paclitaxel, gemcitabine, or taxanes may be therapeutically relevant in a selected population.
- The unexpected high BRCA2 mutation rate observed highlights a population that may benefit from PARP inhibitors.
- The protein changes associated with CTNNB1-mutated tumors suggest potential benefit of targeting WNT pathway in combination with nab-paclitaxel, anti-androgens, anti-PD-1 agents and PARP inhibitors.
- Significantly higher PD-1+ tumor-infiltrating lymphocytes and TS expression in the metastases compared to primary HCC may suggest increased opportunity for immune checkpoint inhibitors in the metastases and higher likelihood of fluoropyrimidine agents to be effective in the primary tumors.
- Data presented herein and suggestions for therapeutic potential are limited by the lack of clinical outcomes.

References

1. Tornesello, M et al. Mutations in TP53, CTNNB1 and PIK3CA genes in hepatocellular carcinoma associated with hepatitis B and hepatitis C virus infections , *Genomics* 102, 2013: 74-83.
2. Li, L and Mao, M. Next generation sequencing reveals genetic landscape of hepatocellular carcinomas, *Cancer Letters* 340, 2013: 247–253.