Comprehensive multiplatform biomarker analysis of 313 hepatocellular carcinomas identifies potential novel therapeutic options

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Disclosures

- Support for this study was provided by Caris Life Sciences

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- **John T Miura**: none
- **Ruth He**: none
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- **Nelson Yee**: none

Therapy for Advanced Hepatocellular Carcinoma

- Sorafenib is the only active agent
- Multiple negative trials
- No predictive biomarkers
- Rationale for molecular profiling
  - Identify therapeutic targets
  - Predict therapeutic response
  - Risk stratification and prognosis

Negative phase III trials in HCC	
Agent	***Trial/Setting***
Sorafenib	STORM – vs placebo, adjuvant
Sunitinib	vs sorafenib, 1st line
Erlotinib + sorafenib	SEARCH – vs sorafenib, 1st line
Linifanib	vs sorafenib, 1st line
Brivanib	BRISK-FL – vs sorafenib, 1st line
	BRISK-PS – vs placebo, 2nd line
Everolimus	EVOLVE – vs placebo, 2nd line
Ramucirumab	REACH – vs placebo, 2nd line

Study Design and Objective

- **Design:** Retrospective review of HCC molecular profiling data generated by multiplatform analysis

- **Objective:** To describe molecular patterns and associations, and potential novel therapeutic targets in HCC

Methods

- Formalin-fixed paraffin-embedded samples
- Multiplatform profiling at CLIA certified lab
- Specimens reviewed by board-certified pathologists
- Immunohistochemistry (IHC)
  - 21 protein panel
  - Slides stained using automated system (Ventana Medical Systems, Tucson, AZ) as per manufacturer's protocol with proprietary reagents
  - IHC stained slides scored by pathologists
  - Tumor staining scored for all markers except PD-1 which was scored in tumor infiltrating lymphocytes
  - Standard thresholds specific to each antibody

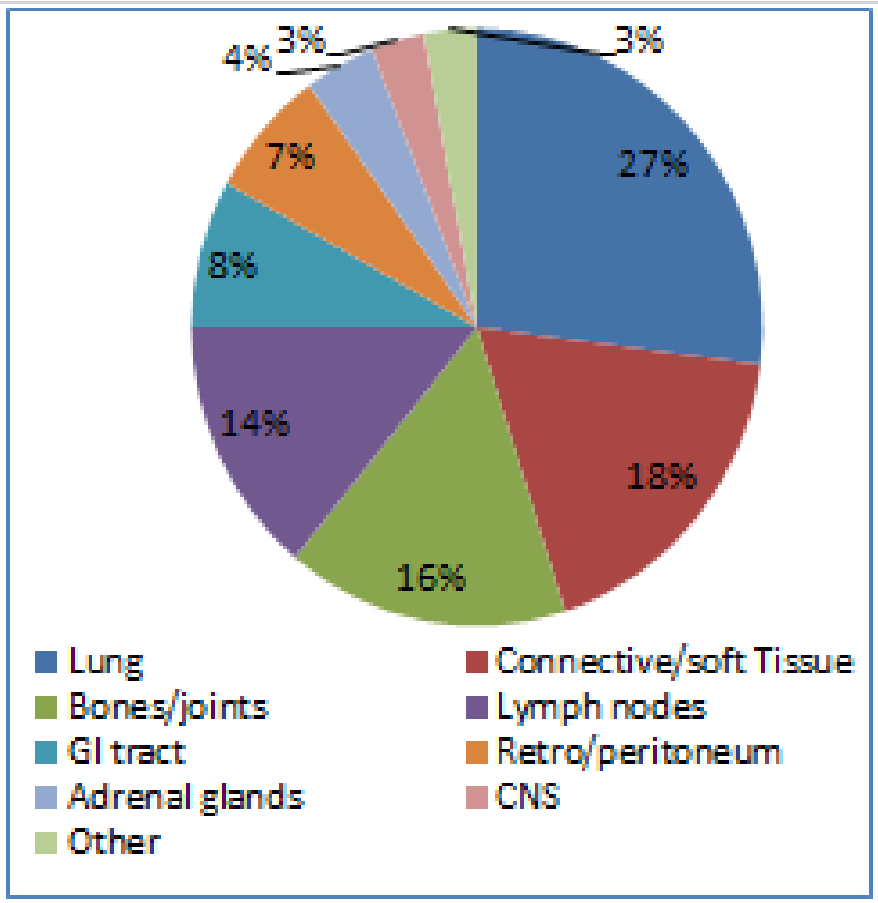
Methods (cont.)

- Fluorescence/chromogenic *in situ* hybridization (FISH/CISH)
  - Detect gene amplifications
  - Standard scoring systems applied
- DNA sequencing (Next generation sequencing or Sanger sequencing)
  - 47 genes
  - NGS using Illumina MiSeq platform (Illumina TruSeq Amplicon Cancer Hotspot panel)
  - Sequencing plots read by board-certified geneticists

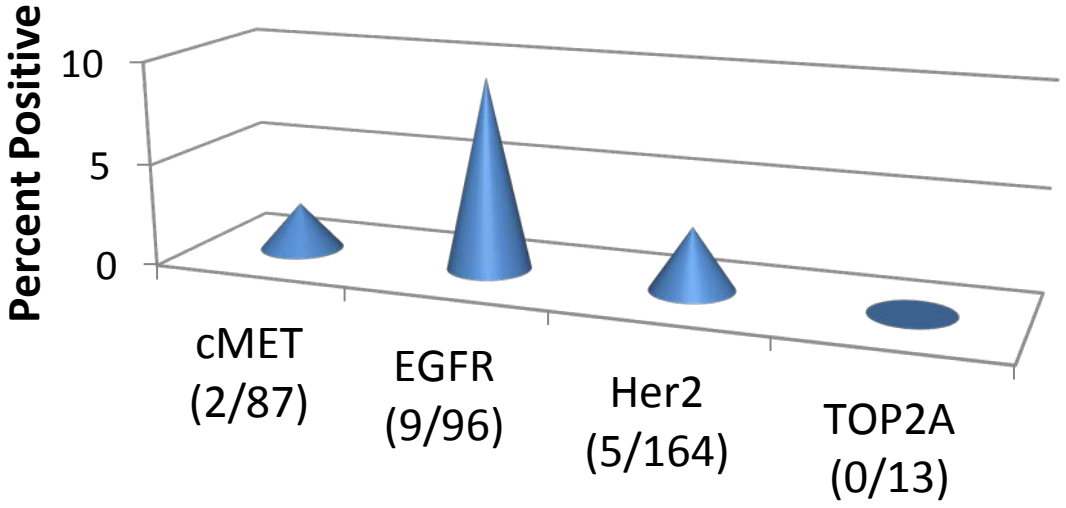
Results

Specimens

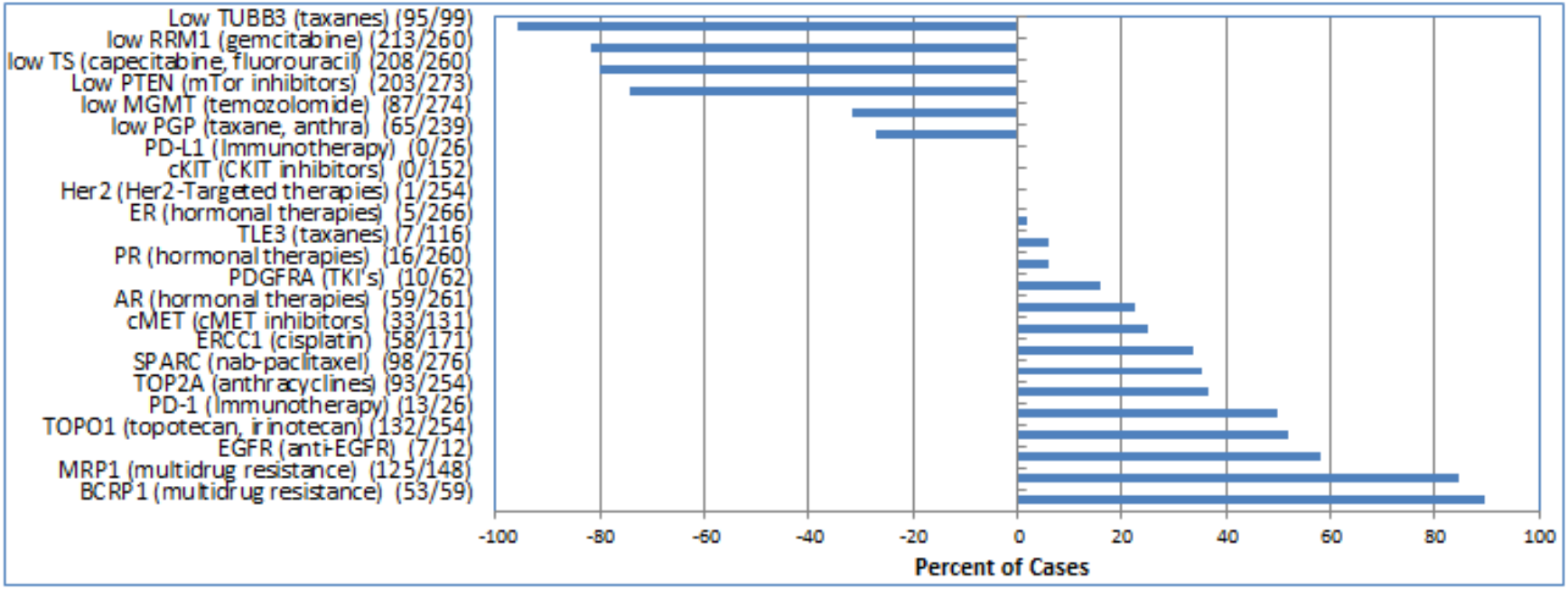
- 313 individual patient specimens
- Heterogeneous
- Median age 61 years (18-87 years)
- Male:female ratio = 2.7:1
- Metastases in 36% of subjects



Changes in gene copy number by FISH or CISH



Protein expression by IHC

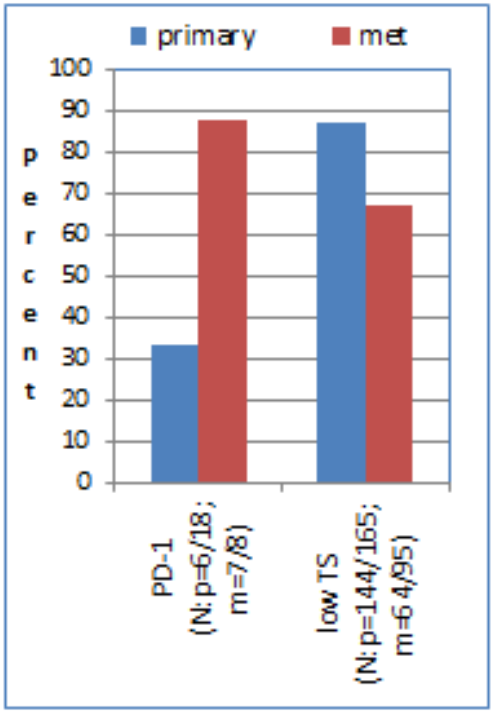


Percentage of samples with change in protein expression, by IHC									
High expression levels						Low expression levels			
EGFR	TOPO1	PD-1	TOP2A	SPARC	cMET	RRM1	TS	PTEN	MGMT
58	52	52	36	35	25	82	80	66	32

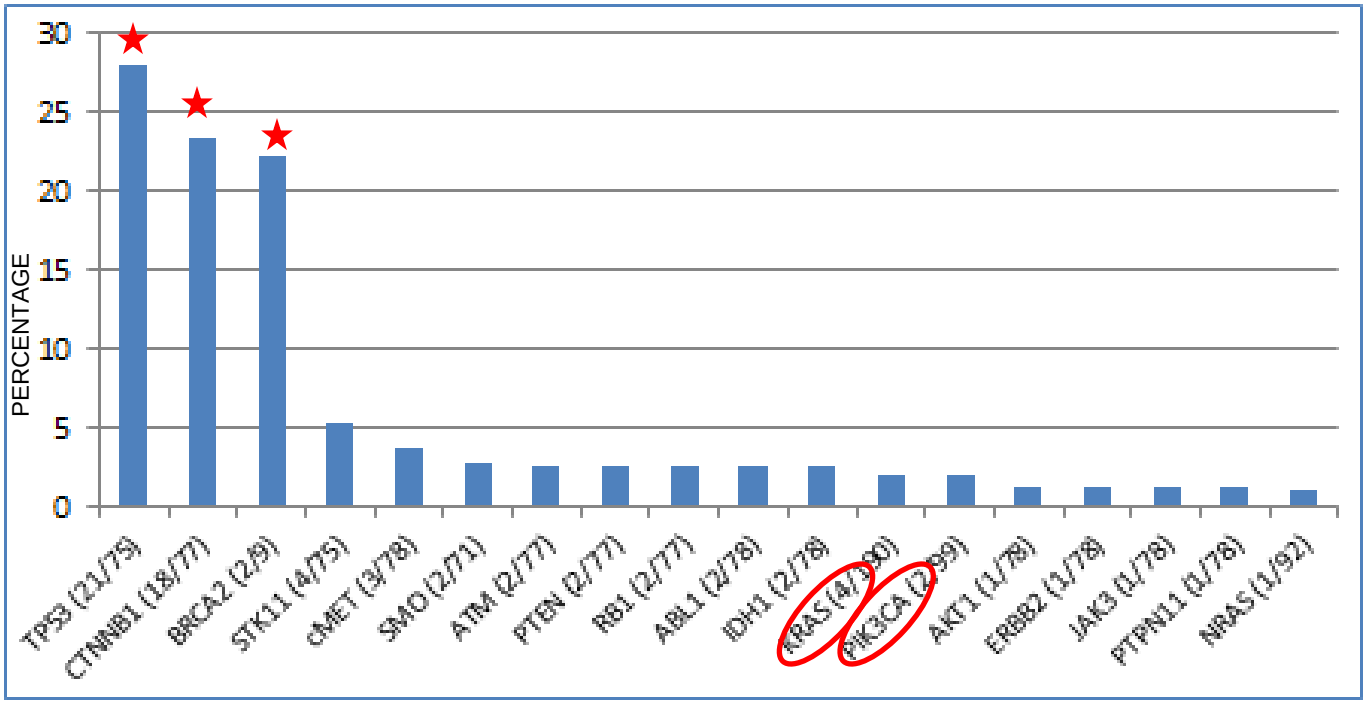
Protein expression in primary and metastatic tumors

- Significantly higher numbers of PD-1+ tumor infiltrating lymphocytes in metastatic lesions ($p = 0.0128$)

- Low TS (thymidylate synthase) significantly more frequent in primary tumor ($p = 0.0004$)
  - Low TS associated with sensitivity to fluoropyrimidines

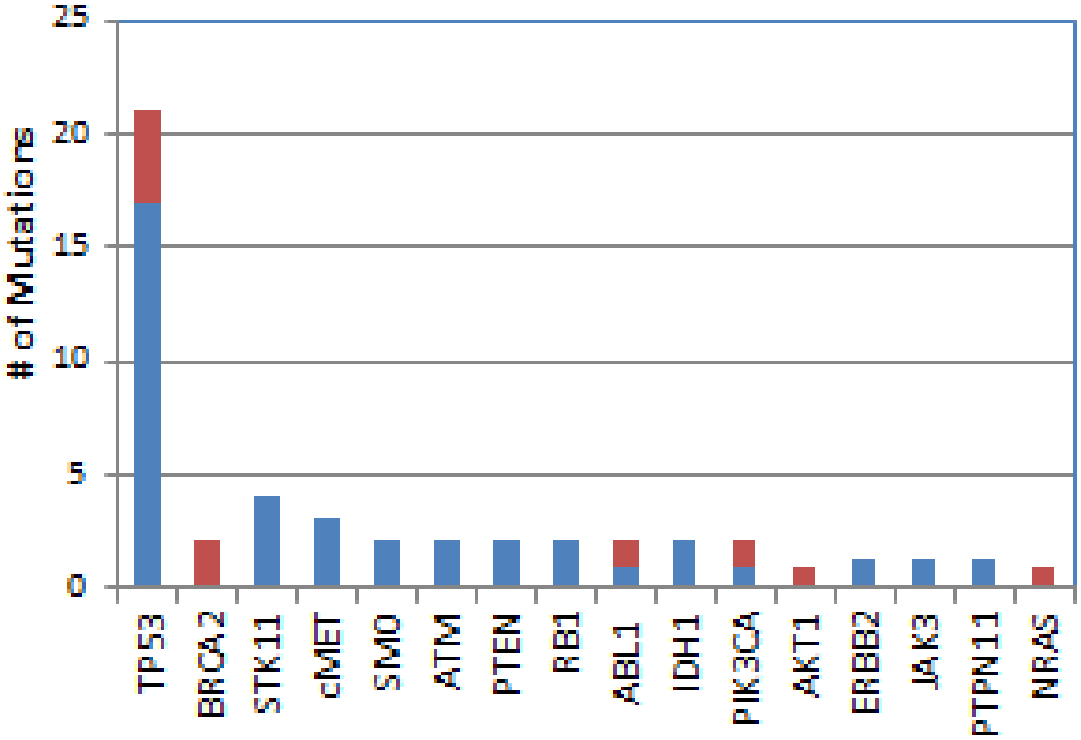


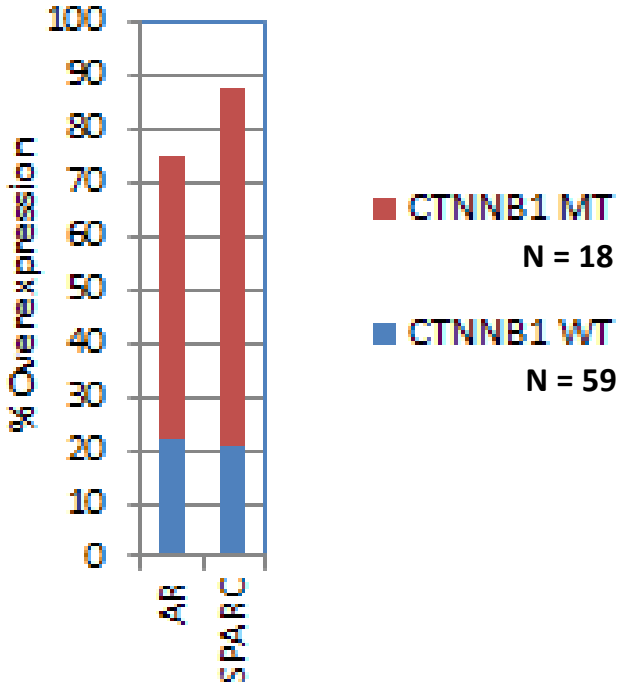
Incidence of gene mutations



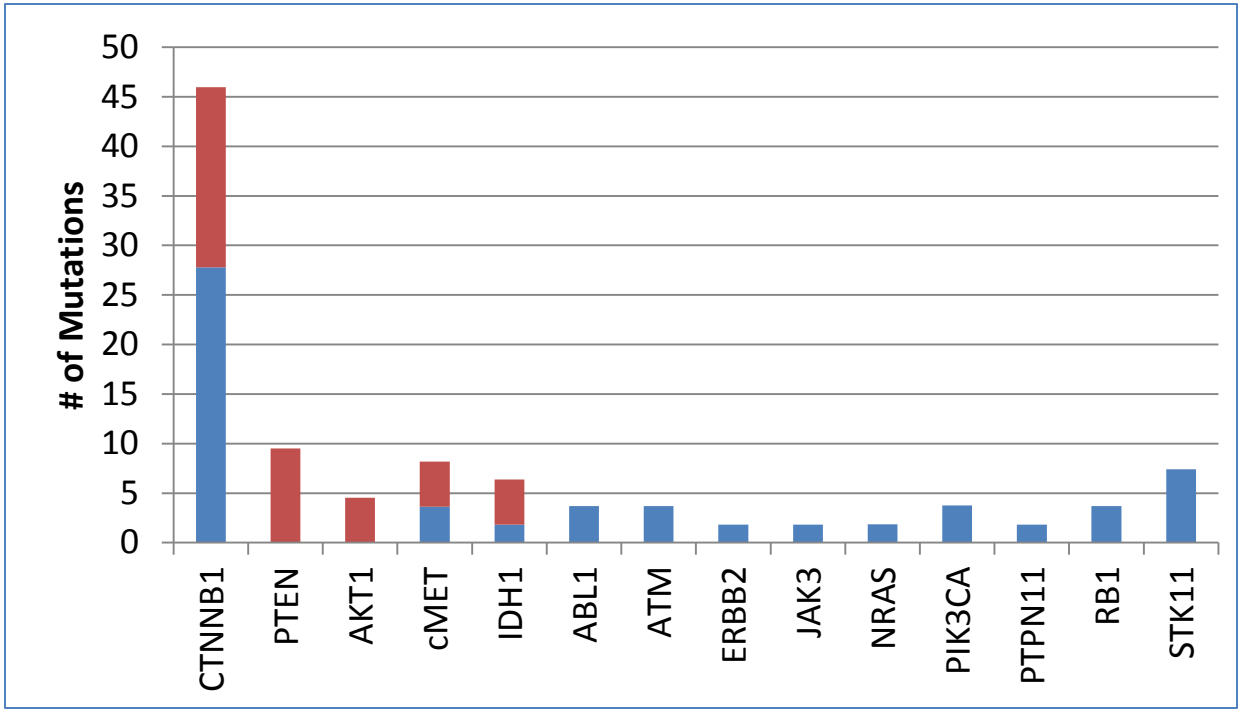
Genes without mutations		
ALK	FGFR1	MLH1
APC	FGFR2	MPL
BRAF	FLT3	Notch1
CDH1	GNA11	NPM1
c-Kit	GNAQ	PDGFRA
CSF1R	GNAS	RET
EGFR	HNF1A	SMAD4
ERBB4	HRAS	SMARCB1
FBXW7	JAK2	VHL
VEGFR2		BRCA1

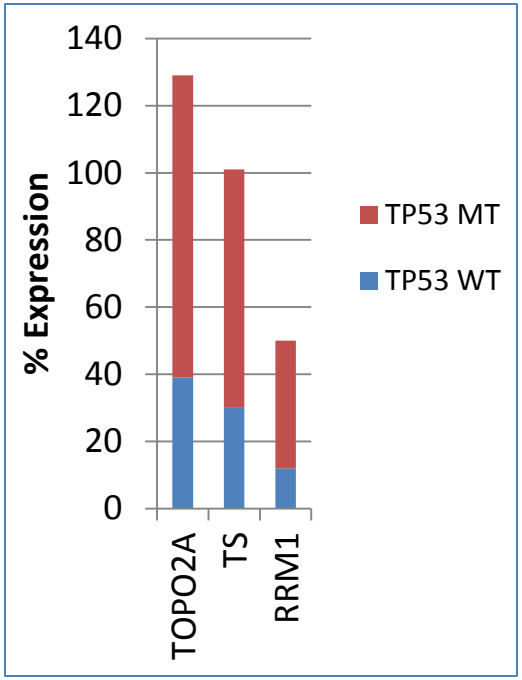
Co-incidence of gene alterations and changes in protein expression by CTNNB1 mutation status





Co-incidence of gene alterations and changes in protein expression by TP53 mutation status





Actionable Targets

- EGFR
- PI3K/Akt/mTOR
- PD-1
- Wnt

- C-Met
- BRCA2

SEARCH (sorafenib + erlotinib)

Conclusions

- Novel therapeutic targets and potential combinations identified
- Findings supported by existing literature
- Needs clinical validation

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