Validation of Biomarkers for Hepatocellular Carcinoma

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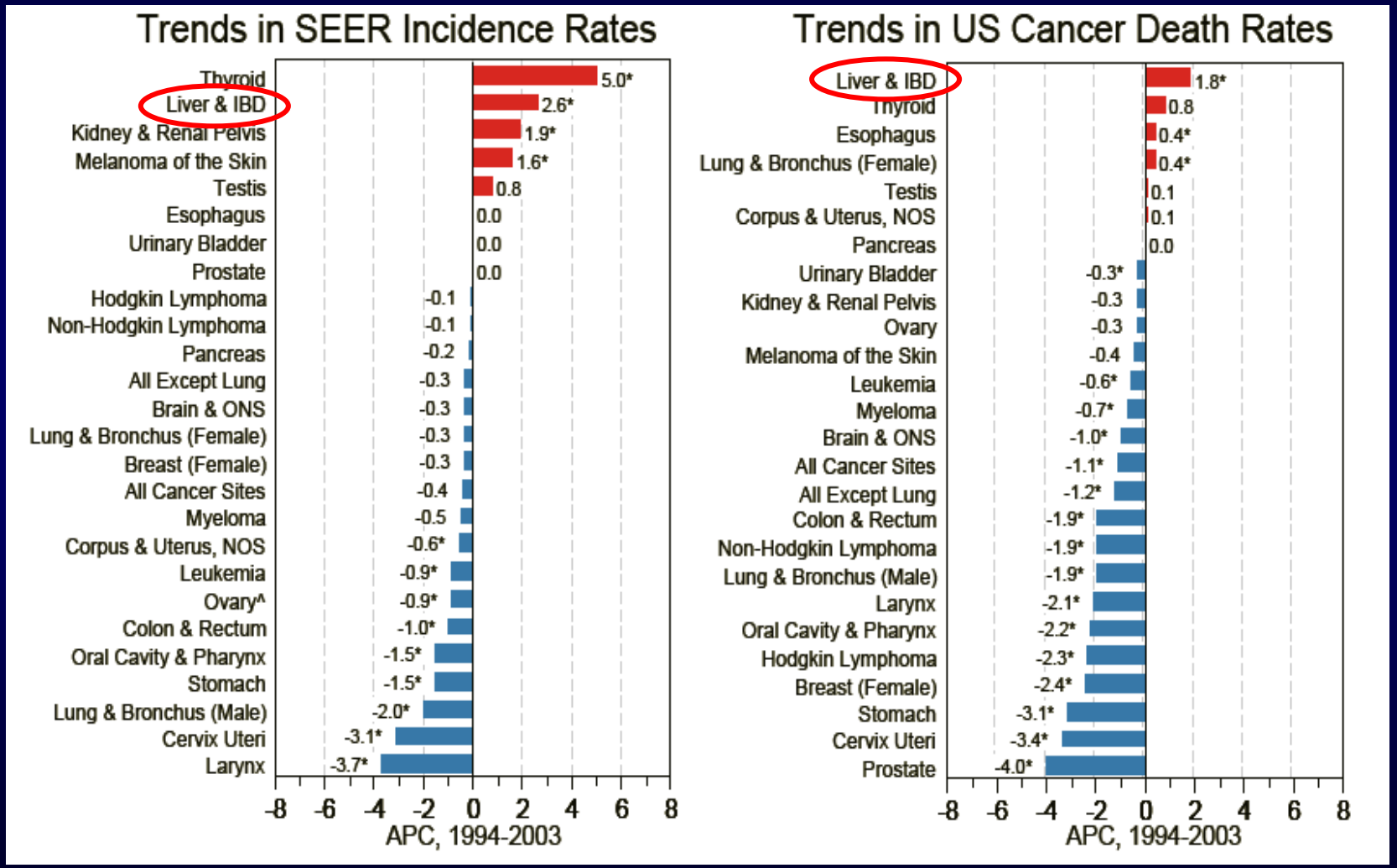
Overview

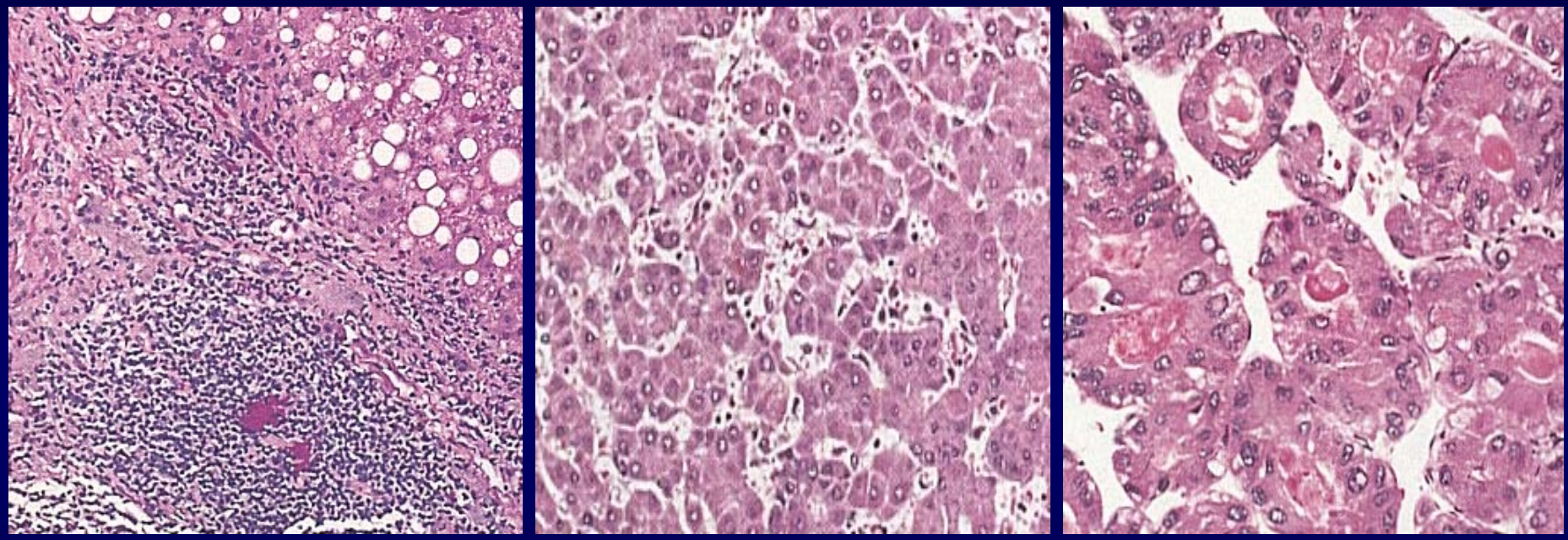
- Epidemiology of Hepatocellular Carcinoma (HCC)
- Current Markers for HCC
- Validation of Biomarkers for HCC
  - Des-gamma carboxyprothrombin (DCP)
  - Others

Trends in Incidence and Death Rates 1994-2003



www.seer.cancer.gov

HCC Progression



Chronic Liver Disease → **Cirrhosis** → **HCC**

Nat Genet. 2002;31:339-46.

Incidence of Hepatocellular Carcinoma in HCV and HBV

Hepatitis C

Clinical setting	Geographic area	No. studies	References[a,b]	No. patients	Mean follow-up (y)	HCC incidence[c]	95% Confidence interval
Chronic hepatitis[d]	Europe	1	30	329	4.2	0	—
	Japan	6	31–36	1451	6.2	1.8	1.50–2.05
	Taiwan	1	37[e,f]	553	9.2	0.3	0.16–0.46
Compensated cirrhosis[g]	Europe and United States	13	5, 8, 30, 38–47	1284	4.5	3.7	3.20–4.17
	Japan	7	32, 34, 35, 48–51	626	5.8	7.1	6.19–7.96

Hepatitis B

Clinical setting	Geographic area	No. studies	References[a,b]	No. patients	Mean follow-up (y)	HCC incidence[c]	95% Confidence interval
Asymptomatic carrier	North America	2[d]	74, 75	1804	16	0.1	0.07–0.14
	Taiwan and China	4[e]	37, 76–78	18,869	8	0.7	0.61–0.70
	Japan	1[f]	79	513	7.3	0.2	0.08–0.39
Inactive carrier[g]	Europe	3	80–82	410	16	0.02	0–0.04
	Taiwan	1	83	189	8	0.2	0–0.42
Chronic hepatitis[h]	Europe	6	84–89	471	5.9	0.1	0–0.27
	Taiwan	2	90–91	461	4.0	1.0	0.36–1.56
	Japan	2	31, 92	737	5.1	0.8	0.46–1.06
Compensated cirrhosis[i]	Europe	6	8, 38, 40, 41, 85, 89	401	5.8	2.2	1.62–2.80
	Taiwan and Singapore	3	76, 93, 94	278	4.3	3.2	1.94–4.55
	Japan	2	48, 95	306	5.8	4.3	3.40–5.25

Fattovich G, et al. Gastroenterology 2004;127:S35

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Screening for HCC: AASLD Recommendations

- Surveillance should be performed with ultrasonography (level II)
- AFP alone should not be used for screening unless it is with ultrasound (level II)
- Screening should occur every 6-12 months (level II)
- Need for better screening tests

Bruix J, et al. Hepatology 2005;42:1208

Alpha-fetoprotein Cross-Sectional Studies

Author	Cutoff	No. of HCC	Sensitivity %	Specificity %
Peng	20	205	65	88
Trevisani	16#	170	60	90
Cedrone	100#	74	25	95
Soresi	30#	197	65	89
Lee	200	54	53	79
Nguyen	20	163	63	79

determined by ROC curves

Marrero JA. Clin Liver Dis 2005;9:235.

Alpha-fetoprotein Prospective Cohort Studies

Author	No. of cirrhotics	No. of HCC	PPV %	NPV %	Sensitivity %	Specificity %
Pateron	118	14	33	-	50	86
Oka	260	55	32	82	39	76
Bolondi	313	61	46	85	41	82
Tong	602	31	12	99	41	95
Chalasani	285	27	30	-	63	87

Marrero JA. Clin Liver Dis 2005;9:235.

Ultrasound in HCC in Cohort Studies

Author	Year	Sens%	Spec%	Likelihood Ratio Pos	Likelihood Ratio Neg
Okazaki	84	86	99	66	0.14
Maringhni	84	92	86	6.5	0.09
Kobayashi	85	75	98	32.6	0.26
Tanaka	86	47	100	589	0.41
Dodd	92	43	98	21.5	0.58
Saada	97	33	100	333	0.67
Chalasani	99	59	92	8.4	0.45
Rode	01	46	95	9.2	0.57
Bennett	01	30	97	7.4	0.72
Teefey	03	89	73	3.3	0.15
Libbrecht	03	40	100	400	0.6
Pooled Estimates (95%CI)		60.5 (44-76)	96.9 (95-98)	17.7 (8.5-36.9)	0.5 (0.4-0.6)

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Phases of Biomarker Development for Early Detection of Cancer

Phase 1: Preclinical exploratory studies

Phase 2: Clinical Assay Development for Clinical Disease

Phase 3: Retrospective Longitudinal Study

Phase 4: Prospective Screening Studies

Phase 5: Cancer Control Studies

Pepe MS, et al. J NCI 2001;93:1054

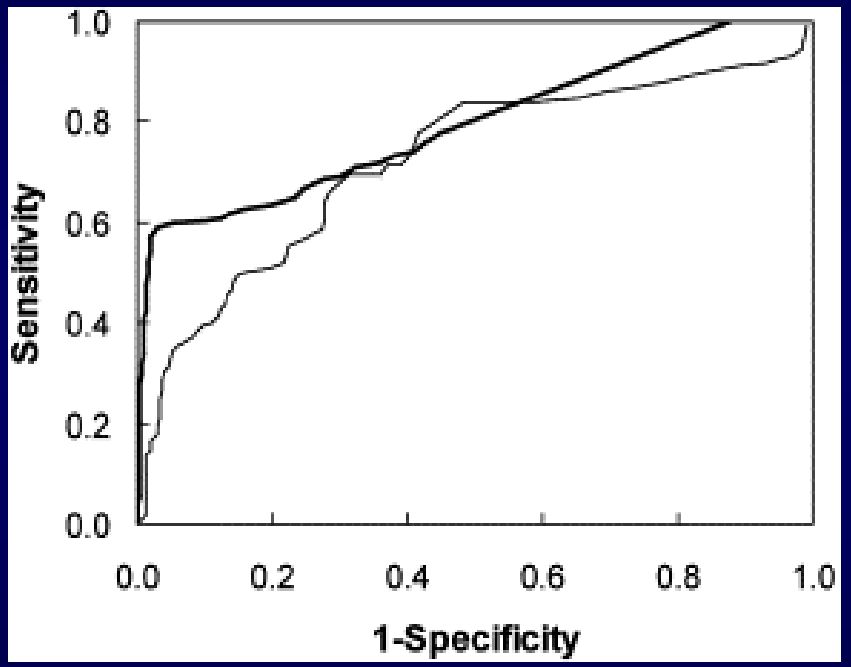
Des-gamma carboxyprothrombin (DCP) in HCC

- Increased prothrombin precursor
- The activity of the γ-glutamyl-carboxylase has been shown to be decreased in HCC
- The increase production of precursor is named des-gamma carboxy prothrombin

Tsai et al Hepatology 1990;11:481

Lectin-bound Alpha-fetoprotein AFP-L3

- The sugar chain structures of AFP obtained from patients with LC and HCC have different affinities for lectins
- One subspecies, *Lens culinaris* agglutinin (LCA)-reactive AFP (AFP-L3) is more specific to HCC



Yamagata et al. Clin Chim Acta. 2003;327:59

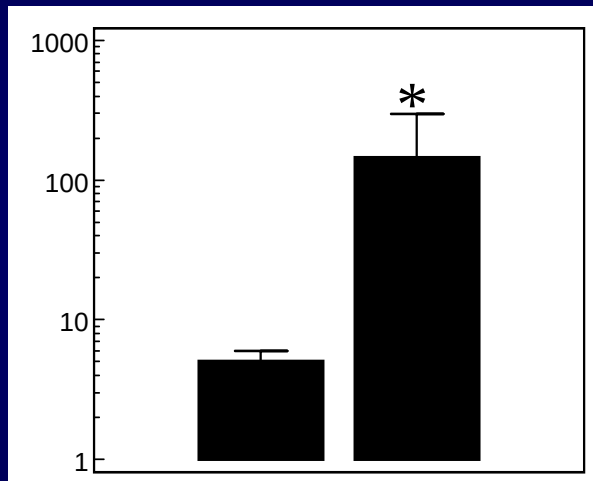
Phase I validation study of DCP in HCC

- 4 Groups
  - *Group 1*: normal volunteers: 60
  - *Group 2*: non-cirrhotic chronic hepatitis: 61
  - *Group 3*: cirrhosis: 63
  - *Group 4*: HCC: 65
- Blood was obtained and centrifuged before treatment at the time of diagnosis (after sitting in 32°F for 12 hrs), aliquoted and stored at -80°C
  - Each sample barcoded to link to clinical data
- DCP performed by sandwich ELISA in duplicates in an external lab and measured blindly
- AFP-L3 was performed by Wako diagnostics in a blind fashion
- Assay intra- and interassay variability ~ 10%

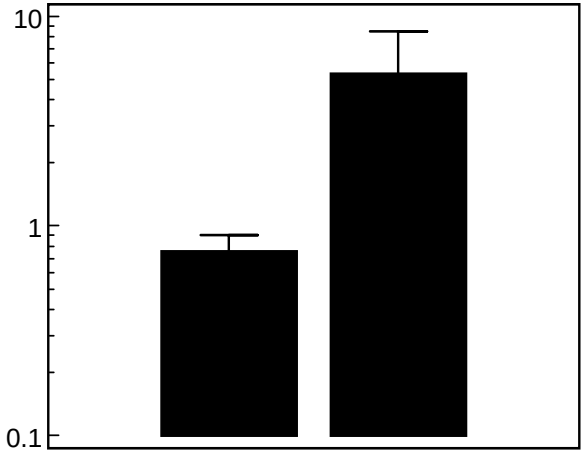
Marrero, Hepatology 2003

AFP, AFP-L3 and DCP

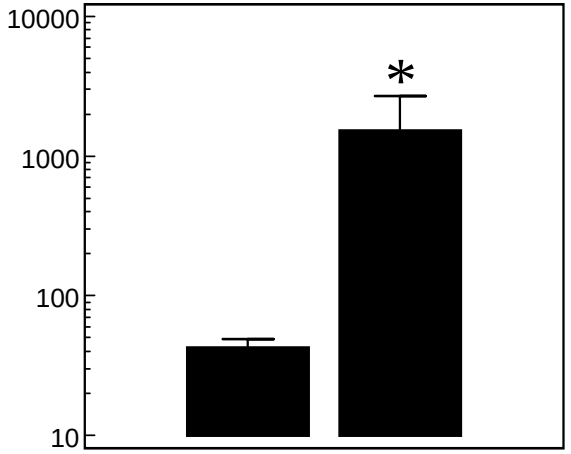
AFP



AFP-L3%

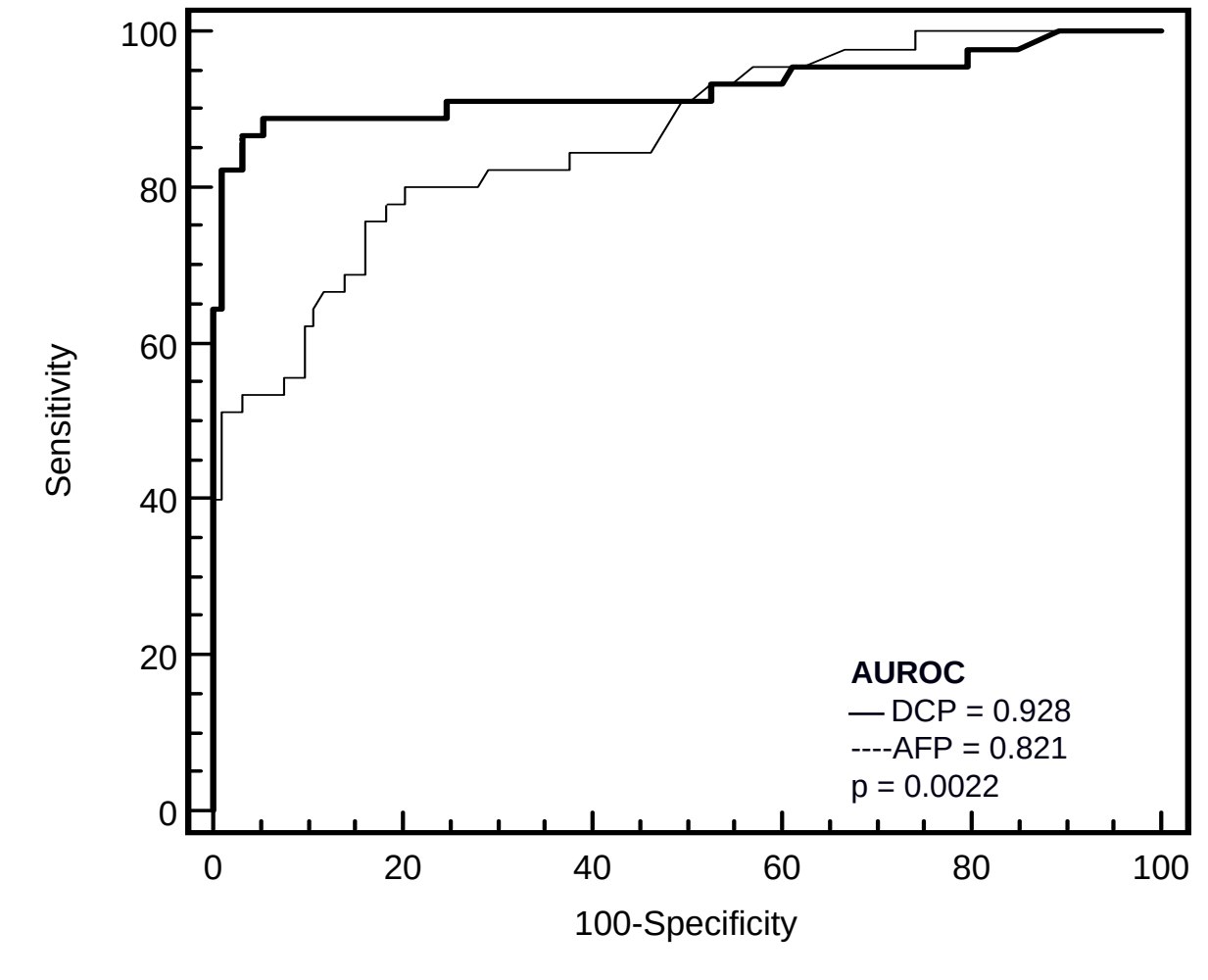


DCP



Control = cirrhosis without HCC (n=159)
Case = HCC with underlying cirrhosis (n=84)

DCP Differentiates Cirrhosis from HCC



* Cutoff Values

DCP = 150 mAU/ml
- Sens: 89%
- Spec: 96%
- PPV: 91%
- NPV: 88%

AFP = 13 ng/ml
- Sens: 62%
- Spec: 76%
- PPV: 78%
- NPV: 71%

Marrero et al; Hepatology 2003;37:490

Performance Characteristics of Markers-Early Stage (n=52)

Marker	AUROC	Sens	Spec	+LR	-LR
AFP 14 ng/mL	0.81	64	88	8.5	0.38
AFP-L3 3%	0.71	50	88	4.5	0.56
DCP 150 mAU/ml	0.93	92	93	13.9	0.08
Comb	0.94	90	91	10.7	0.11

DCP Validation Study Design Phase 2

- Aims
  - To determine the sensitivity and specificity of des-gamma carboxyprothrombin (DCP) for the diagnosis of early hepatocellular carcinoma (HCC).
  - compare performance characteristics of DCP and Alpha-fetoprotein (AFP) in the diagnosis of early HCC.
  - To determine whether demographic or etiology of underlying liver disease alter the expression of DCP or AFP.

- Case-control study
  - cases: modified TNM stage I and II HCC (eligible for liver transplant), prior to any cancer therapy
  - controls: cirrhosis without tumor

Participating Centers

- University of Michigan
- Mount Sinai University Hospital
- University of Pennsylvania
- Mayo Clinic, Rochester
- Mayo Clinic, Jacksonville
- Saint Louis University
- Stanford University

Sample size

AFP

True Positive Fraction = 0.57 (0.65 for power analysis)
False Positive Fraction = 0.22 (0.20 for power analysis)

DCP

True Positive Fraction = 0.85
False Positive Fraction = 0.10

- **Power 90% and 92% for TPF and FPF**
- **We will need to recruit 190 early HCC cases and 410 cirrhosis controls.**

Recruitment

- Patients are identified at the time of the clinic visit, consented prior to the physician seeing the patient
- VSIMS has a tool to determine if a potential control matches to a case (gender, age ± 10 y, etiology of liver disease –viral vs nonviral liver disease)
- Cases
  - Histology or 2 imaging tests showing characteristics of HCC (> 2 cm)
  - Most sites have a centralized area for the care of patients with primary liver tumors
    - Multidisciplinary liver tumor clinic
- Controls
  - Histology or evidence of portal hypertension (splenomegaly, low platelets, presence of esophageal varices)
  - Liver clinics because controls have chronic liver disease
- Patients are confirmed if they meet the eligibility criteria and if serum was obtained
- Data is collected regarding demographics, family history, social history, medical history, detail data about their liver disease, obesity

Samples

- Serum collection
  - Phlebotomy, blood sits for 30 minutes and then 12 hrs in 32°C, followed by centrifuge and aliquoted in 500 µL, then stored at -80°C. Sites ship to central facility monthly located at the University of Michigan.
  - Each aliquot is barcoded for identification purpose and link to clinical data
  - Data systems allow for sample tracking (VSIMS)
  - An aliquot (500 µL) from each patient will be shipped to UCLA for assay, aliquot identified by DMCC
  - UCLA then send raw results to DMCC for analysis

- Assays
  - DCP will be performed as sandwich ELISAs' in a blind fashion at UCLA in duplicates
  - AFP and AFP-L3 will be performed singly UCLA (already FDA approved)
  - 10% of all samples will undergo QC
    - DCP at UMich
    - AFP and AFP-L3% at Wako
  - Additional samples will be collected and stored at NCI Frederick once the study concludes

Data Management

- DMCC (FHCRC) will perform data coordination and management
- Database utilized is the EDRN Validation Study Information Management System (VSIMS)
- Data Quality Monitoring Board in place

Next Step

- Phase 2 study will be the largest involving early stage tumors. Important to determine:
  - Appropriate cutoffs for the biomarkers
  - Determine performance characteristics in early stage HCC
  - To better select the population for future studies
- A phase 3 study: a cohort of patients with cirrhosis to determine efficacy of biomarker to detect preclinical HCC
  - 2 ongoing studies

AFP-L3

- FDA approved for the determination of risk of HCC among patients with cirrhosis
- Approval based on a multicenter US study (as well as data from Japan)
  - Prospective study of patients with cirrhosis 332 of which 34 had HCC
    - Each site had its own definition of HCC
    - Could not go back to when HCC first diagnosed
  - Serum obtained locally and AFP-L3 done centrally at Wako
  - Unclear if they studied other important risk factors for the development of HCC such as liver function, alcohol, tobacco exposures, obesity

Conclusion

- For the validation of biomarkers we have developed a reference set for discovery, followed by a large phase 2 study powered for novel biomarker(s) to “beat” AFP (standard), and then a phase 3 study for the ability of the biomarker to detect preclinical disease
- It is important to test the quality of the assay as well as the design evaluating the clinical indication
- A DMCC is critical in the validation process