Molecular signature for management of hepatocellular carcinoma

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Outline

• Hepatocellular carcinoma (HCC)
• Clinical challenges
• Molecular signatures for
  ▪ Prognostic prediction
  ▪ HCC surveillance
  ▪ Molecular targeted therapy
  ▪ Chemoprevention
HCC: a common and lethal cancer

- Third leading cause of cancer death worldwide
  (750,000 new cases/year)
- Most rapidly increasing cancer death in the U.S.
  (Incidence tripled between 1975-2005)
- Incidence will increase and remain high next two decades
- Dismal prognosis
  (5-year survival <12% in US)

**Liver cirrhosis: driver of HCC**

**Healthy liver** → **Liver cirrhosis** → **HCC**

- 1-2% of population

**Risk factors**
- Hepatitis B: 350 million, 6% of population
- Hepatitis C: 170 million
- Alcohol
- Non-alcoholic fatty liver diseases (NAFLD)

Hepatitis C cirrhosis/HCC in the U.S.

- Major risk factor in industrialized countries: 50-60% in the U.S.
- Superseded HIV as cause of death by 2007.
- > 1 million “baby boomers” will develop cirrhosis and/or HCC by 2020.
- >$8.6 billion non-pharmacological cost by 2015.

Prognostic prediction

(AASLD, EASL practice guidelines)
Early-stage HCC

Advanced-stage HCC

HCC surveillance for cirrhosis

• Biannual abdominal ultrasound
• Prolong survival

<table>
<thead>
<tr>
<th>Findings</th>
<th>Screened group (pp × yr = 38 444)</th>
<th>Control group (pp × yr = 41 077)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC occurrence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>86</td>
<td>67</td>
</tr>
<tr>
<td>Early cancer</td>
<td>39</td>
<td>0</td>
</tr>
<tr>
<td>Total incidence (per 100 000)</td>
<td>223.7</td>
<td>163.1</td>
</tr>
<tr>
<td>Rate ratio (95% CI)</td>
<td>1.37 (0.99, 1.89)</td>
<td>Reference</td>
</tr>
<tr>
<td>Deaths from HCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deaths</td>
<td>32</td>
<td>54</td>
</tr>
<tr>
<td>Total mortality (per 100 000)</td>
<td>83.2</td>
<td>131.5</td>
</tr>
<tr>
<td>Rate ratio (95% CI)</td>
<td></td>
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</tbody>
</table>

• Only 17% are diagnosed through surveillance in the U.S.

Early-stage cirrhosis: burden in HCC surveillance

Prognostic biomarker for early cirrhosis needed!

Non-invasive fibrosis test

Cirrhosis: 1-2% of population

- Larger population
- Limited clinical prognostic indicators
Increasing early-stage HCC

- Larger population
- Limited clinical prognostic indicators

(Llovet, J Hepatol 2008, Ikeda, Liver Int 2011)
Molecular prognostic biomarkers

No clinical deployment

- Limited by biological hypothesis from previous studies
- Limited access to validation cohorts/samples
- Costly assay development
- Costly prospective evaluation

(D’Amico, J Hepatol 2006)
Genomic profiling

Hypothesis-free biomarker discovery from 10s $\rightarrow$ 10,000s variables

(Chen, Cell 2012)
Prognostic gene signatures in HCC?
Prognostic gene signature in HCC tumor

- Retrospective samples of convenience
- Too short clinical follow-up

Archived fixed tissue for genomic profiling

- Millions of specimens with decades of clinical follow-up in hospitals all across the world
- Analysis of previously completed prospective trials
- More reliable prognostic biomarkers
186-gene signature in cirrhotic liver predicts HCC prognosis

(Hoshida, NEJM 2008)
186-gene signature predicts HCC risk in HCV cirrhosis

(Hoshida, NEJM 2008, Hoshida, Gastro 2013)
Genome-based personalized HCC surveillance

Current recommendation

Cirrhosis no HCC → Ultrasound x2/yr

Signature-based schedule

Cirrhosis no HCC → Gene signature test →

- Poor (HCC: 5.8%/yr) → Ultrasound x4/yr
- Intermediate (2.2%) → Ultrasound x2/yr
- Good (1.5%) → Ultrasound x1/yr

Medical care cost ↓
Life expectancy ↑

EASL–EORTC Clinical Practice Guidelines: Management of hepatocellular carcinoma

European Association for the Study of the Liver*, European Organisation for Research and Treatment of Cancer

Requirements to be incorporated in practice guideline

(1) Randomized studies, cohort studies with training/validation sets (Study design).

(2) Independent prognostic association in multivariable analysis with clinical prognostic predictors.

(3) External validation by independent researchers.

(J Hepatol 2012)
## Molecular signatures in HCC

<table>
<thead>
<tr>
<th>Molecular signature</th>
<th>(1) Study design</th>
<th>(2) Multivariable analysis</th>
<th>(3) External validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>186-gene signature (prognosis)</td>
<td>✔</td>
<td>✔</td>
<td>Clinical assay development</td>
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<tr>
<td>G3/5-gene signature (prognosis)</td>
<td>✔</td>
<td>✔</td>
<td>Clinical assay development</td>
</tr>
<tr>
<td>miR-26a (prognosis, interferon response)</td>
<td>✔</td>
<td>✔</td>
<td>Clinical assay development</td>
</tr>
<tr>
<td>EpCAM signature (prognosis)</td>
<td>✔</td>
<td>✔</td>
<td></td>
</tr>
</tbody>
</table>

### Molecular prognostic biomarkers

<table>
<thead>
<tr>
<th>Variables significant among the first 5 in 2–10 studies divided by the total studies in which the variable was tested (%)</th>
<th>Variables significant among the first 5 in only one study divided by the total studies in which the variable was tested (%)</th>
<th>Variables significant among the first 5 and tested in only one study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varices</td>
<td>9/36 (25%)</td>
<td>Factor V</td>
</tr>
<tr>
<td>Gender</td>
<td>9/68 (13%)</td>
<td>Midarm circumference</td>
</tr>
<tr>
<td>UGI hemorrhage</td>
<td>8/32 (25%)</td>
<td>PIVKA-II</td>
</tr>
<tr>
<td>Platelets</td>
<td>7/33 (21%)</td>
<td>Portal vein tumoral thrombosis</td>
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<tr>
<td>MELD</td>
<td></td>
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<tr>
<td>HVPG</td>
<td></td>
<td></td>
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<tr>
<td>HCC</td>
<td></td>
<td></td>
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<tr>
<td>BUN/Azotemia</td>
<td></td>
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<tr>
<td>γ-globulins</td>
<td></td>
<td></td>
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<tr>
<td>Creatinine</td>
<td></td>
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<tr>
<td>Alkaline phosphatase</td>
<td></td>
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<tr>
<td>Aminopyrine</td>
<td></td>
<td></td>
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<tr>
<td>Pseudoephedrine</td>
<td></td>
<td></td>
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<tr>
<td>Sodium/hypertension</td>
<td></td>
<td></td>
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<tr>
<td>AST</td>
<td></td>
<td></td>
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<tr>
<td>Factor VII</td>
<td></td>
<td></td>
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<tr>
<td>ICG clearance</td>
<td></td>
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<tr>
<td>Nutrition</td>
<td></td>
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<tr>
<td>Blood pressure</td>
<td></td>
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<tr>
<td>Pre-kallikrein</td>
<td></td>
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<tr>
<td>TNF</td>
<td></td>
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<tr>
<td>Hyaluronic acid</td>
<td></td>
<td></td>
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<tr>
<td>Liver iron</td>
<td></td>
<td></td>
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<tr>
<td>Decompensation T4</td>
<td></td>
<td></td>
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<tr>
<td>Bile acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN treatment</td>
<td>2/6 (33%)</td>
<td></td>
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<tr>
<td>SBP/infection</td>
<td>2/7 (29%)</td>
<td></td>
</tr>
<tr>
<td>Galactose elimination</td>
<td>2/8 (25%)</td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>2/11 (18%)</td>
<td></td>
</tr>
<tr>
<td>Vascular spiders</td>
<td>2/12 (17%)</td>
<td></td>
</tr>
<tr>
<td>Alcohol abstinence</td>
<td>2/12 (17%)</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>2/13 (15%)</td>
<td></td>
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<tr>
<td>Spleen size</td>
<td>2/17 (12%)</td>
<td></td>
</tr>
</tbody>
</table>

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**No clinical deployment**

- Limited by biological hypothesis from previous studies
- Limited access to validation cohorts/samples
- Costly assay development
- Costly prospective evaluation

(D’Amico, J Hepatol 2006)
Genomic profiles for prognostic biomarker assessment

Butyryl-cholinesterase

Osteopontin

Early-stage HCV cirrhosis (n=216)
Web-app of prognostic biomarker assessment
Molecular signatures for HCC therapy?
Molecular targeted therapies in HCC

**Sorafenib** (BRAF, VEGFR, PDGFR inhibitor)
First-line

Still limited survival benefit (only 3 months)

(Llovet, NEJM 2008)
# Phase 3 trials in HCC for regulatory approval

<table>
<thead>
<tr>
<th>Indication</th>
<th>ID</th>
<th>Acronym</th>
<th>Active arm</th>
<th>Control arm</th>
<th>Primary outcome</th>
<th>Child–Pugh</th>
<th>Target population</th>
</tr>
</thead>
<tbody>
<tr>
<td>First line</td>
<td>NCT00901901</td>
<td>SEARCH</td>
<td>Erlotinib + sorafenib</td>
<td>Sorafenib</td>
<td>Overall survival</td>
<td>A</td>
<td>Advanced liver cancer</td>
</tr>
<tr>
<td></td>
<td>NCT00858871</td>
<td>BRISK-FL</td>
<td>Brivanib</td>
<td>Sorafenib</td>
<td>Overall survival</td>
<td>A</td>
<td>Advanced HCC(^a)</td>
</tr>
<tr>
<td></td>
<td>NCT01009593</td>
<td>—</td>
<td>Linifanib</td>
<td>Sorafenib</td>
<td>Overall survival</td>
<td>A</td>
<td>Unresectable or metastatic HCC</td>
</tr>
<tr>
<td>Second line</td>
<td>NCT01035229</td>
<td>EVOLVE-1</td>
<td>Everolimus</td>
<td>Placebo</td>
<td>Overall survival</td>
<td>A</td>
<td>Advanced liver cancer</td>
</tr>
<tr>
<td></td>
<td>NCT00825955</td>
<td>BRISK</td>
<td>Brivanib</td>
<td>Placebo</td>
<td>Overall survival</td>
<td>A(^b)</td>
<td>Advanced HCC(^a)</td>
</tr>
<tr>
<td></td>
<td>NCT01108705</td>
<td>BRISK-APS</td>
<td>Brivanib</td>
<td>Placebo</td>
<td>Overall survival</td>
<td>A(^b)</td>
<td>Advanced HCC(^a) Asian ethnicity</td>
</tr>
<tr>
<td>Prevention of recurrence after resection or ablation</td>
<td>NCT00692770</td>
<td>STORM</td>
<td>Sorafenib</td>
<td>Placebo</td>
<td>Recurrence-free survival</td>
<td>A(^b)</td>
<td>BCLC 0/A treated with resection or ablation</td>
</tr>
<tr>
<td>Prevention of recurrence after transplantation</td>
<td>NCT00554125</td>
<td>—</td>
<td>Rapamycin</td>
<td>FK506</td>
<td>Disease-free survival</td>
<td>A-B-C</td>
<td>HCC exceeding Milan Criteria</td>
</tr>
<tr>
<td></td>
<td>NCT00355862</td>
<td>SILVER</td>
<td>Rapamycin</td>
<td>mTOR-free–based immunosuppression</td>
<td>Recurrence-free survival</td>
<td>A-B-C</td>
<td>HCC within or exceeding Milan Criteria</td>
</tr>
</tbody>
</table>

(Villanueva, Gastro 2011)
Failing “all-comer” approaches in HCC

Brivanib
(VEGF, FGF inhibitor)
Second-line

Sunitinib
(VEGFR, PDGFR, KIT inhibitor)
First line

(Llovet, JCO 2013, Cheng, JCO 2013)
Responders to molecular target drug

Crizotinib (ALK inhibitor) kills ALK-positive lung cancer

Molecular biomarker-informed personalized treatment

Biomarkers of sorafenib response?

- No established predictive biomarker
- Limited opportunity: no biopsy tissue

(Abou Alfa, JCO 2006, Llovet, CCR 2012)
Predictive biomarker in HCC?

Tivantinib (MET inhibitor)
Second-line, phase 2 trial

All comers

MET-high tumors

p=0.63

p=0.01

Phase 3 trial with biomarker enrichment on-going

(Santoro, Lancet Oncol 2013)
Molecular classification of HCC

- HCV, alcohol
  Early ~ intermediate

- HBV
  Intermediate ~ advanced

- HCV
  Early ~ intermediate

- HCV, alcohol
  Early ~ intermediate

- HBV
  Intermediate ~ advanced

- HCV
  Early ~ intermediate
Molecular classification of HCC

Subclass

- S1
- S2
- S3

(Hoshida, Cancer Res 2009)
## Molecular classification of HCC

### Aggressive HCC
- **S1**
  - TGF-β, WNT activation
  - E2F1 activation, TP53 inactivation
  - Met-related genes ↑
  - Cellular proliferation, KRT19 (+)
  - Progenitor cell-like
- **S2**
  - MYC, AKT activation
  - IFN-related genes ↓
  - AFP, IGF2 ↑
  - EPCAM, GPC3 ↑
  - Hepatoblastoma-like
  - Vascular invasion
- **S3**
  - Normal hepatocyte-like
  - CTNNB1 mutations
  - More differentiated
  - Smaller tumor
  - Better prognosis

### Less-Aggressive HCC
- Less differentiated
- Larger tumor
- Poorer prognosis in advanced HCC

(Hoshida, Semin Liver Dis 2010)
Molecular signature for HCC chemoprevention?
“When we think of cardiovascular or infectious diseases, we first consider ways to prevent them rather than drugs to cure their most advanced forms.

But when we think about eradicating cancer, we generally think about curing advanced cases.”

(Volgelstein, et al. Science 2013)
Treatment of advanced cancer

- **Intrinsic/acquired resistance**

- **Clonal heterogeneity/evolution**

“Cancer deaths can be reduced by more than 75% in the coming decades only when greater efforts are made toward early detection and prevention.”

(Volgelnstein, et al. Science 2013)
Cancer prevention trial

Selenium + Vit E for Prostate cancer prevention

N=35,533
Follow-up: >7 years

High-risk population?

P=0.52

(Lippman, JAMA 2009)
HCC: best target of prevention

- Distinct high-risk population
  Liver cirrhosis caused by
  - Hepatitis virus B/C
  - Alcohol
  - Non-alcoholic fatty liver diseases (NAFLD)

- High incidence in high-risk population
  3-7%/year

HCC prevention trial needs
- Smaller sample size
- Shorter follow-up time
Chemoprevention trial enrichment by gene signature

High risk of HCC

Test Positive
- New RX
- Control

Test Negative
- Off Study

Evaluate Diagnostic Test

HCC chemoprevention drugs

(Simon, CCR 2008, Hoshida, CCDT 2012)
Chemoprevention target of “poor prognosis”

Epidermal growth factor (EGF)

(Fuchs, Hepatol 2013)
Inhibitor of “poor-prognosis” target reversed gene signature, reduced fibrosis, and prevented HCC

Poor-prognosis signature

\[ q = 0.97 \quad 0.002 \]

Planning phase 1 trial

(Fuchs, Hepatol 2013)
Biopsy is critical especially during development of therapeutic strategy

(Garraway, JCO 2013)
“Randomized studies testing molecular targeted therapies should optimally include biomarker analysis (tissue and/or serum samples) to enable the identification of molecular markers of response and for pharmacokinetic purposes, as reported in other cancers.”

(J Hepatol 2012)
Summary

Molecular signatures

- Refine prognostic prediction
- Refine HCC surveillance
- Guide molecular targeted therapies
- Guide molecular targeted chemoprevention
Thank you!