Molecular Heterogeneity in Gastric Cancer: Genomic Approaches and Clinical Impact

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Disclosure slide

• Nothing to Declare
Gastric Cancer: World’s 5th Most Common Cancer and 3rd Leading Cause of Cancer Death
Molecular and Clinical Heterogeneity in Gastric Cancer (GC)

Western Europe:
- Epirubicin + cisplatin + 5-FU (ECF)
- Modifications of ECF: EOX or ECX
- Fluoropyrimidine + platinum

Japan:
- S1 + cisplatin
- S1

USA:
- 5-FU + cisplatin + docetaxel
- 5-FU/leucovorin + oxaliplatin (FOLFOX)
- Cisplatin + irinotecan

Can Genomics Improve Gastric Cancer Patient Outcomes?

1) How many GC subtypes exist? What are their driver alterations and pathologic associations?

2) Which GC Subtypes are Clinically Relevant, for Patient Prognosis and Therapy Selection?

3) Are GCs from Asian and non-Asian localities the same?
There are ~3-4 Major GC Genomic Subtypes

A) Chromosomal Instability (CIN)

B) Microsatellite Instability (MSI)

C) Genome Stable (GS)

D) Epstein-Barr Virus (EBV)

USA TCGA (2014) Nature
GC Genomic Subtypes Show Distinct Molecular and Pathological Characteristics

Chromosomal Instability (CIN) (50%)
- Intestinal-type GCs
- \textit{TP53} mutations
- Focal somatic gene amplifications in RTK/RAS genes

Microsatellite Instability (MSI) (20%)
- Intestinal-type GC \textcolor{yellow}{\textit{ARID1A}, \textit{CIMP}}
- \textit{TGFBR2}, \textit{ACVR2A} mutations

Genome Stable (GSS) (20%)
- Diffuse-type GC
- \textcolor{green}{\textit{CDH1}, \textit{RHOA}\textsuperscript{**} mutations}

Epstein-Barr Virus (EBV) (10%)
- Global hypermethylation
- \textcolor{red}{\textit{ARID1A}, \textit{CIMP}}
- \textit{PDL-1/2} Gene Amplification\textsuperscript{**}

\begin{itemize}
  \item Matsusaka et al (2011) \textit{Cancer Res}
  \item Wang et al (2011) \textit{Nat Genetics}
  \item Zang et al (2012) \textit{Nat Genetics}
  \item Nagarajan et al (2012) \textit{Gen Biol.}
  \item Yoon et al (2013) \textit{Genome Res}
  \item USA TCGA (2014) \textit{Nature}
\end{itemize}
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Singapore Gastric Cancer Consortium
Translational Pipeline

Clinical Databases
“Gastronomica”
>250 Tumors
Demographics
Histopathology
Treatment
Survival Outcomes

Experimental Models
“GEMINI”
70 Cell Lines
High-Throughput
Screening
Synthetic Lethality
Cell Based Phenotypes

Preclinical Validation
Tumorgrafts
12 Gastric Lines
Patient Derived
Xenografts
Tumor Initiating
Cells

Patients
Clinical Trials
Phase I/II
Companion
Diagnostics

Genomics as a Bridging Technology
Patients with *Diffuse-Type* Genomic Signatures and/or Histology Exhibit Poor Prognosis

**Intestinal vs Diffuse**  
(Histopathology)

**G-INT vs G-DIF**  
(mRNA signature)

\[ P = 0.6 \]

\[ P = 0.004 \]
G-INT and G-DIF Cell Lines May Respond Differently to Distinct Platinum Agents

Cisplatin
P=0.03

Oxaliplatin
P=0.02

Tan et al (2011) *Gastroenterology*
Targeted Therapies in Gastric Cancer

ERBB2/HER2 Amplification

Van Cutsem, et al. ASCO 2009

The TOGA Trial

Bang et al (2011) Lancet

ERBB2 Positive (8-10%)
Exclusive RTK/RAS Amplifications in GC

ERBB2 Positive (8-10%)

Gastric Cancer

Deng et al (2012) *Gut*
Gastric CpG Island Methylator (CIMP) – A Targetable Epigenetic Phenotype?

DNA Methylation Clustering

Younger Patients (59 vs 65)

Undifferentiated Histology

PRC2 (Polycomb) Targets in Embryonic Stem Cells
Impact of DNA Demethylating Agents on Gastric CIMP Tumors

Decitabine (5-aza-2'deoxyctydine)
Inhibitor of DNA Methyltransferase

G-CIMP Lines

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Somatic Alterations (eg Amplifications) Between Asian and Non-Asian GCs Appear Similar

Singapore Cohort

TCGA Cohort (USA)
Phase III GC Clinical Trials Reveal an Association between Geography and Clinical Outcome

<table>
<thead>
<tr>
<th>Study</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVAGAST (Asian)</td>
<td>0.97 [0.75 - 1.25]</td>
</tr>
<tr>
<td>AVAGAST (Europe)</td>
<td>0.85 [0.63 - 1.14]</td>
</tr>
<tr>
<td>AVAGAST (America)</td>
<td>0.63 [0.43 - 0.94]</td>
</tr>
<tr>
<td>AVAGAST Overall</td>
<td>0.87 [0.73 - 1.03]</td>
</tr>
<tr>
<td>RAINBOW (Asia)</td>
<td>0.99 [0.73 - 1.34]</td>
</tr>
<tr>
<td>RAINBOW (Europe/ Americas/ Australia)</td>
<td>0.73 [0.59 - 0.91]</td>
</tr>
<tr>
<td>RAINBOW Overall</td>
<td>0.81 [0.68 - 0.96]</td>
</tr>
<tr>
<td>LOGIC (Asia)</td>
<td>0.68 [0.48 - 0.96]</td>
</tr>
<tr>
<td>LOGIC (N.America)</td>
<td>1.61 [0.53 - 4.83]</td>
</tr>
<tr>
<td>LOGIC (Rest of World)</td>
<td>1.04 [0.79 - 1.37]</td>
</tr>
<tr>
<td>LOGIC Overall</td>
<td>0.91 [0.73 - 1.12]</td>
</tr>
</tbody>
</table>

Hazard Ratio
Comparing Asian and Non-Asian GCs
Analysis of 1,600 Gastric Tumors

mRNA Profiles (>1000)

Stage 1

Stage 2

Validation analyses

Immunohistochemistry assessment in tissue array studies

IHC Profiles (>600)

9 expression profiling studies (n=1,016)

- 6 studies of Asian origin (n = 890)
- 3 studies of non-Asian origin (n = 126)

4 Affymetrix platform studies

- 2 studies of Asian origin (n = 207)
- 2 studies of non-Asian origin (n = 92)

5 non Affymetrix platform studies

- 4 studies of Asian origin (n = 683)
- 1 study of non-Asian origin (n = 34)

800 profile

Collaboration:

- Johann A. Gagnon-Bartsch
- Terry Speed, UC Berkeley

RUV algorithm: Nature Biotechnology (2014)
GC Expression Cohorts Recapitulate Well Known Geographic Differences in 5-yr Overall Survival

Non-Asian GCs are Enriched in T-cell Gene Signatures Relative to Asian GCs

Asian GCs are enriched in T-cell gene signatures relative to Non-Asian GCs. The differences are highlighted in the figures, where the normalized enrichment score (NES) and various gene sets are compared.
Immunohistochemistry Validation of T-cell Signatures in Non-Asian GCs
Adjusting for T-cell Signatures Impacts Geographic Differences in Overall Survival

<table>
<thead>
<tr>
<th>Variable used for adjustment</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geographic locality (Asian vs Non-Asian)</td>
<td>0.79</td>
<td>[0.67 - 0.93]</td>
<td>5 x 10^{-03}</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>0.61</td>
<td>[0.50 - 0.75]</td>
<td>1 x 10^{-06}</td>
</tr>
<tr>
<td>AJCC7 Staging</td>
<td>0.59</td>
<td>[0.50 - 0.71]</td>
<td>2 x 10^{-09}</td>
</tr>
<tr>
<td>Age</td>
<td>0.87</td>
<td>[0.74 - 1.02]</td>
<td>0.09</td>
</tr>
<tr>
<td>CD3</td>
<td>0.80</td>
<td>[0.63 - 1.02]</td>
<td>0.07</td>
</tr>
<tr>
<td>CD68</td>
<td>0.84</td>
<td>[0.66 - 1.06]</td>
<td>0.14</td>
</tr>
<tr>
<td>CD66b</td>
<td>0.87</td>
<td>[0.69 - 1.10]</td>
<td>0.25</td>
</tr>
<tr>
<td>CD8</td>
<td>1.17</td>
<td>[0.92 - 1.47]</td>
<td>0.20</td>
</tr>
<tr>
<td>CD45RO</td>
<td>0.86</td>
<td>[0.69 - 1.07]</td>
<td>0.17</td>
</tr>
<tr>
<td>FOXP3</td>
<td>0.94</td>
<td>[0.72 - 1.23]</td>
<td>0.67</td>
</tr>
</tbody>
</table>
Conclusions and Discussion

• Large-scale expression analysis reveals differences in the tumor microenvironment between Asian vs non-Asian GCs

• Non-Asian GCs appear enriched in T-cell pathways (eg CTLA-4) and immune cell infiltrates

• Tumor immunity differences do NOT seem to be due to differences in MSI or EBV frequency

• Adjusting for immune differences (esp CD68/CD3) impacts region-specific survival

• Tumor immunity differences may influence GC immunotherapy trials

Lin et al (in press) Gut
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