Comprehensive Molecular Characterization of Gastric Cancer:

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Dana-Farber Cancer Institute

On Behalf of the STAD Working Group
Co-Chairs: Peter Laird & Ilya Shmulevich

TCGA Symposium
May 13, 2014
Gastric Cancer: ~723,000 deaths annually

**Most Common Causes of Cancer Death**

<table>
<thead>
<tr>
<th>Cause</th>
<th>Deaths per year (thousands)</th>
<th>Total: 8.2 million</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>1,590</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>746</td>
<td>2</td>
</tr>
<tr>
<td>Stomach</td>
<td>723</td>
<td>3</td>
</tr>
<tr>
<td>Bowel (inc. anus)</td>
<td>694</td>
<td>4</td>
</tr>
<tr>
<td>Breast</td>
<td>522</td>
<td>5</td>
</tr>
<tr>
<td>Oesophagus</td>
<td>400</td>
<td>6</td>
</tr>
<tr>
<td>Pancreas</td>
<td>330</td>
<td>7</td>
</tr>
<tr>
<td>Prostate</td>
<td>307</td>
<td>8</td>
</tr>
<tr>
<td>Cervix</td>
<td>266</td>
<td>9</td>
</tr>
<tr>
<td>Leukaemia</td>
<td>265</td>
<td>10</td>
</tr>
<tr>
<td>Lip, oral cavity</td>
<td>145</td>
<td>15</td>
</tr>
<tr>
<td>Kaposi sarcoma</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td>Other cancers</td>
<td>2,186 (27%)</td>
<td></td>
</tr>
</tbody>
</table>

*International Agency for Research on Cancer*
### Histopathology: Lauren’s classification

<table>
<thead>
<tr>
<th></th>
<th>Intestinal</th>
<th>Diffuse</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphology</strong></td>
<td><em>Glandular</em> structure</td>
<td><em>Poorly cohesive</em>, or dispersed single cells</td>
</tr>
<tr>
<td>Frequent sites of metastasis</td>
<td>Liver</td>
<td>Ovary, Peritoneum</td>
</tr>
<tr>
<td>Other remarks</td>
<td>Associated with atrophic gastritis, intestinal metaplasia</td>
<td>Familial variant involving <em>CDH1</em> germline mutation</td>
</tr>
</tbody>
</table>
Gastric Adenocarcinoma: What Disease are We Trying to Study?

- Histologic
  - Intestinal vs Diffuse

- Anatomic
  - GEJ vs body vs pylorus

- Geographic
  - East vs West

- Molecular
  - MSI vs MSS, ERBB2+.....

But when it comes time to clinical care and clinical trials, all of this gets ignored.

“We did a trial of xxxx in patients with stomach cancer....”
Goals for the Gastric TCGA

• To better classify tumors
  – And to use a schema that can be applied in a more ‘real world’ setting
• To identify key pathways in distinct tumor types
• To identify targets/biomarkers for distinct tumors, tumor types
Comprehensive Molecular Characterization of Gastric Cancer – Data Types

**Clinical Data**
- 295 Cases

**Copy Number Variation**
- 293 Cases

**Whole Exome Seq**
- 289 Cases

**RNA-seq**
- 265 Cases

**DNA Methylation Arrays**
- 295 Cases
Developing Classification

• Use Agnostic Molecular Classification
  – Cluster of Cluster Assignments
  – iCluster
• Identify key identifying features of molecular clusters of tumors
• Use identifying features to categorize tumors using a decision tree
  – Analysis based upon assignments from decision tree rather than based upon initial clustering
Cluster of Cluster Assignments...

MSI  Diffuse  EBV  Aneuploid

Vesteinn Thorsson
Genome-Guided Classification Correlations
Example of Value of TCGA Integrated Analysis: Features of EBV-Positive GC
Distinct CIMP Profiles Differentiate EBV+ and MSI+ Gastric Cancer

Toshi Hinoue
Hui Shen
Dramatic Rates of $PIK3CA$ Mutation in EBV+ GC

Amaro Taylor-Weiner
EBV and Identification of New Genomic Lesions in GC: Novel Amplifications
Focal Copy-Number Amplification Peaks Across 289 Gastric Cancers

Amplifications

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X

5 (1)
15q26.1 (8)
ERBB2 (1)
GATA6 (1)
CCNE1 (1)
ZNF217 (3)

9p Amplification

Andy Cherniack
Focal Amplification Peaks Across Molecular Subtypes

Andy Cherniack
Detailed Look at 9p24.1 Locus of Amplification

- JAK2
- CD274
- PDCLILG2

Genes and Amplifications:
- PD-L1
- PD-L2
Basics of immune checkpoints

Ribas et al
Elevated PD-L1 and PD-L2 Expression in EBV+ Gastric Cancer

Andy Cherniack
Vesteinn Thorsson
Expression Signatures of Immune Cell Signaling Enriched in EBV+

EBV | MSI | GS | CIN

- RANBP2-mediated transcriptional repression
- Caspase cascade in apoptosis
- IL12-mediated signaling events
- Integrins in angiogenesis
- Syndecan-1-mediated signaling
- Beta1 / Beta3 integrin cell surface interactions
- VEGFR3 signaling in lymphatic endothelium
- Fanconi anemia pathway
- ATM / ATR / BARD1
- TCPTP Signaling
- Regulation of Telomerase
- p53 pathway
- Regulation of Retinoblastoma protein
- Aurora A/B signaling
- FOXM1 / PLK1 signaling
- E2F / targets of cMyc activation
- BMP receptor signaling
- HIF-1-Alpha transcription factor network
- ARF6 trafficking events
- FOXA2 / FOXA3 transcription factor networks
Molecular Classification Scheme for Gastric Cancer

295

EBV Positive

64

EBV (EBV-CIMP)

MSI High

205

SCNA High Cluster

58

GS (Genomically Stable)

147

CIN (Chrom Instability)
CIN Tumors: Highly Recurrent Amplification of Oncogenes

Nils Wilheim
# MSI Tumors: Recurrent Mutations of Oncogenes

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>EBV</th>
<th>MSI</th>
<th>GS</th>
<th>CIN</th>
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<tbody>
<tr>
<td>EGFR</td>
<td>11%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERBB2</td>
<td>17%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERBB3</td>
<td>15%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JAK2/PD-L1/2*</td>
<td>6%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGFR2</td>
<td>9%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MET</td>
<td>6%</td>
<td></td>
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<tr>
<td>VEGFA</td>
<td>7%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KRAS/NRAS</td>
<td>17%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RASA1</td>
<td>6%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIK3CA</td>
<td>24%</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PIK3R1</td>
<td>3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td>11%</td>
<td></td>
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- Truncating Mutation
- Missense Mutation (recurrent or known in COSMIC)
- Missense Mutation (all other)
- Amplification
- Homozygous Deletion

Nils Wilheim
Recurrent Oncogenic Mutations in ERBB2/ERBB3

Activating HER2 Mutations in HER2 Gene Amplification Negative Breast Cancer

Oncogenic ERBB3 Mutations in Human Cancers
What About Genomically-Stable (i.e. Diffuse) Gastric Cancer

<table>
<thead>
<tr>
<th>RTK-RAS</th>
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Legend:
- Truncating Mutation
- Missense Mutation (recurrent or known in COSMIC)
- Missense Mutation (all other)
- Amplification
- Homozygous Deletion
Significantly Mutated Genes in Gastric Cancer (Excluding Hypermutators)

Amaro Taylor-Weiner
Highly Recurrent *RHOA* GTPase Mutations in Diffuse/Genomically Stable GC
RHOA: Roles in Invasion and Migration Could Contribute to ‘Diffuse’ Growth Phenotype
New Recurrent Fusion Gene Impacts RhoA Pathway and Adhesion

Angeliki Pantazi
Andy Mungall
Reanne Bowlby
Both RhoA and ARHGAP Fusions Enriched in Diffuse/GS Type GC
CLDN18-ARHGAP26....What are these genes?

Claudin 18: Component of tight junctions, cellular adhesion complex

ARHGAP26: a RHO-GAP, GTPase activating protein, something that should act to reduce RHOA activity...

???
Molecular Subtypes of GC and Key Features

**CIN**
- Intestinal Tumors
- TP53 Mutation
- RTK-RAS Activation

**EBV**
- *PIK3CA* Mutation
- *PD-L1/2* Overexpression
- EBV-CIMP
- *CDKN2A* Silencing
- Immune Cell Signaling

**GS**
- Diffuse Tumors
- *CDH1, RHOA* Mutations
- *CLDN18-ARHGAP* Fusion
- Cell Adhesion

**MSI**
- Hypermutation
- Gastric-CIMP
- *MLH1* Silencing
- Mitotic Pathways

Ryo Sakai
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Wei Zhang
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