Unsupervised clustering of protein expression data

- **Reactive**
  - ‘activated’ stromal cells

- **Invasive**
  - Low expression of cell adhesion proteins

- **Proliferative**
  - High expression of cell cycle proteins
Protein expression data by TGCA molecular type

EBV  MSI-H  GS  CIN

CASP7 (deaved)
EEF2
PCNA
LCK
PREX1
ETS1
BAX
TGM2
SYK
CLDN7
VHL
TFRC
FASN
CCNB1
ASNS
EIF4EBP1
RPS6KA1
GAPDH
CTNNA1
RUVS

MYH11
RICTOR

C4AV
KIT
HSP70
MYC
PRKCA
PRKCa, pS657
CCND1
EIF4EBP1, pS65
ACVRL1
BCL2
TUBA, acetyl-Lys40
COOLBA1
PKC-α/δ, pS660
PEA15
AKT
CCNE1
ACACA
EGFR, pY1068
EIF4EBP1, pT37
NDRG1, pT346
TP53
BAD, pS112
RPS6KA1, pT359/363

Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes

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Gastric cancer, a leading cause of cancer-related deaths, is a heterogeneous disease. We aim to establish clinically relevant molecular subtypes that would encompass this heterogeneity and provide useful clinical information. We use gene expression data to describe four molecular subtypes linked to distinct patterns of molecular alterations, disease progression and prognosis. The mesenchymal-like type includes diffuse-subtype tumors with the worst prognosis, the tendency to occur at an earlier age and the highest recurrence frequency (63%) of the four subtypes. Microsatellite-unstable tumors are hyper-mutated intestinal-subtype tumors occurring in the antrum; these have the best overall prognosis and the lowest frequency of recurrence (22%) of the four subtypes. The tumor protein 53 (TP53)-active and TP53-inactive types include patients with intermediate prognosis and recurrence rates (with respect to the other two subtypes), with the TP53-active group showing better prognosis. We describe key molecular alterations in each of the four subtypes using targeted sequencing and genome-wide copy number microarrays. We validate these subtypes in independent cohorts in order to provide a consistent and unified framework for further clinical and preclinical translational research.

n=300
principal component analysis of gene expression data, association investigated of first three principal components with predefined EMT signature, MSI, cytokine signalling, cell proliferation, methylation, TP53, mutations, copy number
ACRG molecular subtypes of gastric cancer

MSS/EMT type related with younger age, diffuse type and advanced stage
MSI type related with location in the antrum, intestinal type and early stage
MSS/TP53+ group related to EBV positivity

ACRG molecular subtypes in other cohorts

ACRG gastric tumors

Singapore gastric tumors

TCGA gastric tumors

Proliferation-sig
MSI/MSS-sig
MLH1-mRNA
MSI-α assay status
hypermethylation
EMT-sig
CDH1-mRNA
TP53-sig
TP53-mut (33%)
EBV (6%)

Proliferation-sig
MSI/MSS-sig
MLH1-mRNA
EMT-sig
CDH1-mRNA
TP53-sig

Proliferation-sig
MSI/MSS-sig
MLH1-mRNA
MSI-α status
hypermethylation
EMT-sig
CDH1-mRNA
p53-sig
TP53-mut (46%)
EBV (10%)

Eventless probability

Overall survival (months)
ACRG mutational landscape
Summary molecular classification

- 3 ‘comprehensive’ molecular studies - similar subgroups and aberration patterns, but not identical
- substantial inter-tumour molecular heterogeneity, no information on intra-tumour heterogeneity
- Only ACRG groups have prognostic value
- some suggestions of potential drug targets
- CAVE: all locally advanced resectable disease, ethnicity
- CAVE: very costly assays on fresh, ‘large’ samples
Molecular based treatment of gastric cancer?
ESMO gastric cancer treatment guideline 2014

Early
- Operable Stage T1N0
  - Consider endoscopic/limited resection
  - Preoperative chemotherapy
    - Surgery
    - Post-operative chemotherapy

Locally advanced
- Operable Stage >T1N0
  - Preoperative chemotherapy
  - Surgery
  - Adjuvant chemotherapy

Gastric Cancer (Adenocarcinoma)
- HER2 positive
  - Trastuzumab + CF/CX
    - HER2 negative
      - Platinum+ fluoropyrimidine-based doublet or triplet regimen

Recurrent, metastatic or inoperable
- Inoperable or metastatic
  - Palliative chemotherapy
  - Best supportive care if unfit for treatment

Preferred pathway
Re-assess

2nd line chemo Clinical trials if adequate PS
Consider clinical trials of novel agents
‘Molecular driven’ gastric cancer surgery
Predicting peritoneal spread

Diffuse

Intestinal

‘Molecular driven’ gastric cancer surgery
Predicting peritoneal spread

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>MSS/TP53-</th>
<th>MSS/TP53+</th>
<th>MSI</th>
<th>MSS/EMT</th>
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</thead>
<tbody>
<tr>
<td>No. of documented recurrences/No. of total subjects per subgroup</td>
<td></td>
<td></td>
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<tr>
<td>ACRG cohort</td>
<td>47/107 (43.9%)</td>
<td>31/79 (39.2%)</td>
<td>16/68 (23.5%)</td>
<td>31/46 (67.4%)</td>
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<tr>
<td>SMC-2 cohort</td>
<td>38/88 (43.2%)</td>
<td>30/85 (35.3%)</td>
<td>10/49 (20.4%)</td>
<td>33/55 (60.0%)</td>
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<tr>
<td>TOTAL</td>
<td>85/195 (43.6%)</td>
<td>61/164 (37.2%)</td>
<td>26/117 (22.2%)</td>
<td>64/101 (63.4%)</td>
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<tr>
<td>Pattern of recurrence&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Peritoneal seeding (with malignant ascites)&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>ACRG cohort</td>
<td>11/47 (23.4%)</td>
<td>6/31 (19.4%)</td>
<td>2/16 (12.5%)</td>
<td><strong>24/31 (77.4%)</strong></td>
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<tr>
<td>SMC-2 cohort</td>
<td>9/38 (23.6%)</td>
<td>9/30 (30.0%)</td>
<td>2/10 (20.0%)</td>
<td><strong>17/33 (51.5%)</strong></td>
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<tr>
<td>TOTAL</td>
<td>20/85 (23.5%)</td>
<td>15/61 (24.6%)</td>
<td>4/26 (15.4%)</td>
<td><strong>41/64 (64.1%)</strong></td>
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<tr>
<td>Liver metastases only&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>ACRG cohort</td>
<td>6/47 (12.8%)</td>
<td>3/31 (9.7%)</td>
<td>4/16 (25.0%)</td>
<td>0/31 (0.0%)</td>
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<tr>
<td>SMC-2 cohort</td>
<td>12/38 (31.6%)</td>
<td>2/30 (6.7%)</td>
<td>2/10 (20.0%)</td>
<td>3/33 (9.1%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>18/85 (21.2%)</td>
<td>5/61 (8.2%)</td>
<td>6/26 (23.1%)</td>
<td>3/64 (4.6%)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Of all recurrences per group.  
<sup>b</sup>Of first site of recurrences with peritoneal seeding/malignant ascites/no. of all recurrences per group.  
<sup>c</sup>Of first site of recurrences with limited liver metastases/no. of all recurrences per group.
T4, diffuse type + MSS/EMT or GS type = High risk of peritoneal metastasis

Treatment decision:
Neoadjuvant chemotherapy + D2plus/D3 gastrectomy + prophylactic HIPEC
Strong prognostic value of microsatellite instability in stage II and III intestinal type non-cardia gastric cancer.
‘Molecular driven’ gastric cancer surgery
Predicting regional lymph node spread

<table>
<thead>
<tr>
<th>Level</th>
<th>MSI</th>
<th>MSS</th>
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<tbody>
<tr>
<td>First level</td>
<td>38%</td>
<td>57%</td>
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<tr>
<td>Second level</td>
<td>10%</td>
<td>22%</td>
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<tr>
<td>Third level</td>
<td>3%</td>
<td>10%</td>
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</table>

Personal communication Prof Roviello
T1N0/T2N0, non-cardia, intestinal type + MSI = low risk of nodal metastasis, good prognosis

Treatment decision:
Distal/subtotal gastrectomy + limited D1 lymphadenectomy
# Molecular driven oncological treatment

<table>
<thead>
<tr>
<th>TCGA classification</th>
<th>Promising molecule in TCGA</th>
<th>Frequency of alteration in all cases</th>
<th>Drug development</th>
<th>Clinical trial identifier</th>
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<tr>
<td></td>
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<td>Drugs</td>
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<tr>
<td>EBV 9%</td>
<td>PI3K/Akt</td>
<td>24%</td>
<td>MK-2206</td>
<td>NCT01260701</td>
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<td>PD-1/PD-L1, PD-L2</td>
<td>6%</td>
<td>BYL719</td>
<td>NCT01613950</td>
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<td>Everolimus in combination with other drugs</td>
<td>NCT01049620, NCT01248403</td>
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<td></td>
<td>GSK2636771</td>
<td>NCT01458067</td>
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<td>Pembrolizumab (MK-3475)</td>
<td>NCT01848834</td>
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<td></td>
<td>Nivolumab (BMS-936558)</td>
<td>NCT02267343, NCT01928394</td>
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<td>MPDL3280A</td>
<td>NCT01375842</td>
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<td>MEDI 4736</td>
<td>NCT01693562</td>
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<td>AZD1480</td>
<td>NCT01219543</td>
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<td>MSI 21%</td>
<td>MLH1</td>
<td>15%</td>
<td>Pertuzumab</td>
<td>NCT 01774786, NCT01702558, NCT01641939</td>
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<td>ERBB3</td>
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<td>Trastuzumab emtansine(T-DM1)</td>
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<tr>
<td>Genomically stable 20%</td>
<td>CDH1</td>
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<td></td>
<td>RHOA</td>
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<td></td>
<td>CLDN18-ARHGAP26</td>
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<tr>
<td>Chromosomally unstable 50%</td>
<td>FGFR2</td>
<td>9%</td>
<td>Dovitinib (TKI258)</td>
<td>NCT01719549, NCT01576380, NCT01921673</td>
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<td>c-MET</td>
<td>6%</td>
<td>Rilotumumab (AMG102)</td>
<td>NCT01443065</td>
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<td>Onartuzumab</td>
<td>NCT01662869</td>
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</table>
Distribution of potential drug targets within TGCA groups

EBV
- JAK2: 11%
- KRAS: 7%
- HER3: 4%
- HER2: 11%

MSI
- PIK3CA: 67%
- JAK2: 11%
- MET: 3%
- KRAS: 25%
- FGFR2: 2%
- HER3: 14%
- HER2: 5%

GS
- Unknown: 61%
- PIK3CA: 9%
- HER2: 7%
- FGFR2: 9%

CIN
- PIK3CA: 10%
- Unknown: 19%
- HER2: 24%
- HER3: 18%
- KRAS: 18%
- FGFR2: 8%
conclusions

- Milestone in our understanding of the biology of gastric cancer highlighting heterogeneity
- Currently no impact on routine clinical care or clinical trial design
- Functional significance of new aberrations unknown
- Some promising therapy approaches are broader than molecular subtypes
  (PARP inhibition, immune modulatory therapy, angiogenesis, combination therapies etc)