Comprehensive molecular analysis of recurrence in gastroesophageal adenocarcinoma

PROMOREC project

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F Renaud, G Piessen, M Figeac, C Dejeante, M Messier, L Delattre, A Adenis, D Chatelain, C Boulagnon-Rombi, C Eveno, I Van Seuning, C Mariette, E Leteurtre, MP Buisine
Gastroesophageal adenocarcinoma

- High incidence in US and Europe
  - More than 1.6 million new cases
  - 1.3 million deaths expected in 2019
- Model of multi-level tumor resistance
- Recurrence in more than 50% of patients
- Major efforts have been made to understand the biology of these tumors

TCGA, Nature 2014, 2017
Cristescu et al, Nature Medicine 2015
Van Cutsem, Lancet 2016
Gastroesophageal adenocarcinoma

- However targeted therapies have had limited efficacy
- One potential reason could be genomic heterogeneity between primary and metastasis
- Biomarker profiling is routinely performed on a single site of GEA
- There is a critical need to understand the molecular features of the metastasis

✓ The objective was to assess the molecular profile of GEAs recurrence and to compare this profile with the one of the primary tumor

Robinson et al, Nature 2017
Pectasides et al, Cancer Discov 2018
Janjigian et al, Cancer Discov 2018
Methods

Oesophagus, gastroesophageal junction and gastric adenocarcinoma from Oct. 2013 to July 2018

Diagnosis
Primary tumour (PT)

Recurrence
Metastatic tumour (MT)

NCT02526095
RBD ID FREGAT 2013-A01281-44
Study population

 PRIMARY TUMOUR

Oesophagus, n = 37
GE junction, n = 27
Stomach, n = 20

n = 84

 METASTASIS

Distant, n = 35

Locoregional, n = 21

Peritoneal, n = 35

n = 91

Distant

Brain n = 5
Neck n = 1
Skin n = 1
Pleura n = 6
Liver n = 10
Adrenal gland n = 7
Scrotum n = 1
Testis n = 1
Inguinal n = 1
Bone n = 2
Eligible patients $n = 84$

Exploitable samples
Paired PT and MT $n = 74$

IHC / ISH $n = 74$
- HER2, EGFR, c-MET, MMR, EBV, p53, E-cadherin, ARID1A, MUC16, Mesothelin
- Analysis OK $n=74$

CGH array $n = 35$
- Pangenomic
- Analysis OK $n=33$

NGS $n = 49$
- 43 genes specific to GEA
- Analysis OK $n=42$

Pyrosequencing $n = 61$
- CDKN2A, RUNX3, CACNA1G, RASSF2, MGMT, MLH1, CDH1
- Analysis OK $n=61$
**IHC Results**

✓ Frequent co-expression of tyrosine kinase receptors in recurrence

<table>
<thead>
<tr>
<th></th>
<th>Non Tumor</th>
<th>PT</th>
<th>MT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HER2</strong></td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td><img src="image3" alt="Image" /></td>
</tr>
<tr>
<td><strong>Expression</strong></td>
<td>10/74, 13.5%</td>
<td>6 discordances out of 70 paired samples</td>
<td></td>
</tr>
<tr>
<td><strong>EGFR</strong></td>
<td><img src="image4" alt="Image" /></td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
</tr>
<tr>
<td><strong>Overexpression</strong></td>
<td>11/70, 16%</td>
<td>2 discordances out of 70 paired samples</td>
<td></td>
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<tr>
<td><strong>c-MET</strong></td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
<td><img src="image9" alt="Image" /></td>
</tr>
<tr>
<td><strong>Overexpression</strong></td>
<td>34/74, 46%</td>
<td>17 discordances out of 70 paired samples</td>
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</table>

*PT = Primary Tumor, MT = Metastasis*
IHC Results

 ✓ c-MET amplification limited to recurrence

FISH MET: Amplification in MT (ratio 27)

IHC Phospho-MET
IHC Results

✓ Frequent Mesothelin and MUC16 expression in recurrence

Mesothelin

MUC16

Non Tumor

PT

MT
Primary tumor and metastasis share the same molecular subgroup

IHC Results

- **EBER**
  - Negative: n = 4/74
  - Positive: n = 2/74

- **MLH1**
  - Normal: n = 23/74
  - Loss of expression: n = 4/74

- **E-cadherin**
  - Normal
  - Loss of expression: n = 23/74

- **p53**
  - Normal
  - Aberrant: n = 53/74

- **CIN**
  - Normal
  - Loss of expression

- **GS**
  - Normal
  - Aberrant: n = 2/74

- **MSI**
  - Normal
  - Loss of expression

- **EBV**
  - 4%

- **CIN**
  - 65%

- **GS**
  - 25%

- **MSI**
  - 7%

- **EBV**
  - 4%

References:
- TCGA, Nature 2014
NGS profile

- 42 patients, 91 samples: 45 PT, 46 MT
- Mutations
  - TP53 : 61/91 (67%)
  - CDH1 : 19/91 (21%)
  - ARID1A : 13/91 (14%)
  - SMAD4 : 12/91 (13%)
  - PIK3CA and KMT2C : 9/91 (10%)
  - APC : 7/91 (8%)
  - KRAS : 6/91 (6%)

Concordance Primary tumor / Metastatic tumor

<table>
<thead>
<tr>
<th></th>
<th>PT</th>
<th>MT</th>
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<tbody>
<tr>
<td>Primary tumor</td>
<td>79</td>
<td>57</td>
</tr>
<tr>
<td>Metastatic tumor</td>
<td>57</td>
<td>85</td>
</tr>
</tbody>
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Mutation% PT vs. MT

- TP53: 71% PT vs. 65% MT
- CDH1: 20% PT vs. 21.7% MT
- ARID1A: 15.5% PT vs. 13% MT
- SMAD4: 13.3% PT vs. 13% MT
- PIK3CA: 4.4% PT vs. 15.21% MT
- KMT2C: 8.8% PT vs. 11% MT
- APC: 8.8% PT vs. 6.5% MT
- KRAS: 4.4% PT vs. 6.5% MT

p = 0.001
CGH array

✓ Alterations more frequent in metastasis
CGH array

✓ Associated to distant metastasis
  +8q24, p = 0.03 (MYC)
  +11q13, p = 0.04 (CCND1)
  Liu et al, Gastroenterology 2017
  Mo et al, Cancer Res 2016
  Kuroda et al, Plos One 2011

✓ Associated to locoregional metastasis
  + 8q24, p = 0.03 (MYC)
  +16p11, p = 0.03 (DCTPP1, PRR14 - PI3K pathway)
  Yang et al, Oncogene 2016
  Kang, Oncol letter 2014

✓ Focal amplifications in both primary & metastasis
  e.g. KRAS, EGFR
  Pectasides et al, Cancer Discov 2018
  Janjigian et al, Cancer Discov 2018
  Liu, Sethi et al, Cancer Cell 2018
Conclusion

Molecular profiles are different between primary and recurrence in GEAs in ~1/2 of patients

Current tissue sampling practices for biomarker testing do not effectively guide precision medicine in this disease

We confirmed here... on paired samples
✓ Temporal heterogeneity
✓ KRAS: amplifications/gain in >20% of patients but mutations are rare
✓ Biomarkers of recurrence: co-occurring TKR alterations, +8q24 (MYC), +11q13 (CCND1)

New biomarkers of recurrence
✓ PI3KCA alterations
✓ MUC16 & mesothelin expression

Pectasides et al, Cancer Discov 2018
Janjigian et al, Cancer Discov 2018
Liu, Sethi et al, Cancer Cell 2018
Wong et al, Nature Medicine 2018
Acknowledgements

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Amiens University Hospital: Prof D Chatelain, Tumor bank team

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Fundings

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All the patients
Thank you