FUSION IN GI TUMORS

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DISCLOSURE

Consulting for
AMGEN; ASTRAZENECA; BIOCARTIS; BOEHRINGER INGELHEIM; BMS; MERCK SERONO; MSD; ROCHE; SANOFI; SERVIER
What is a fusion gene?

A fusion gene is a gene that results from the joining of two different genes. This can happen through a variety of mechanisms, such as translocation, inversion, or gene duplication. Fusion genes are often involved in cancer, as they can alter the function of the involved genes.

How it can be detected?

By WGS or by RNA sequencing:

A. Insert and read

5' insert 3'

B. Pair-end

5' insert read 3'

C. Discordant read pairs and junction spanning reads

"Discordant read pairs"

"Junction spanning reads"

Adapted from https://tumorfusions.org/
What is a fusion gene?

- **Gene A**
  - DNA: exon-exon-exon-exon
  - Junction point
  - Fusion transcript

- **Gene B**
  - DNA: exon-exon-exon-exon
  - Junction point
  - Fusion transcript

How it can be detected?

**FISH**

- **Positive pattern**
  - DNA: exon-exon-exon
  - Fusion gene

- **Negative pattern**
  - DNA: exon-exon-exon

Adapted from [https://tumorfusions.org/](https://tumorfusions.org/)
What is a fusion gene?

A fusion gene is a new gene that is formed when two different genes are joined together. This can happen due to a break in DNA, which causes the genes to be separated, and then they can be rejoined in a new way. This process can result in a new RNA transcript that combines exons from both original genes.

How it can be detected?

SPLIT FISH

SPLIT FISH is a method used to detect fusion genes. It involves the use of fluorescent probes that bind to specific regions of the DNA. When the DNA is broken, these probes will label both ends of the break, but the labeled ends will not be close enough to each other to form a stable hybrid. This results in a negative pattern, which is indicative of a fusion event.

In contrast, a positive pattern would result if the labeled ends of the DNA are close enough to form a stable hybrid, indicating the presence of a fusion gene.

Adapted form https://tumorfusions.org/
How it can be detected? from RNA

KNOWN PARTNERS

Partner A
Partner B
Partner C

UNKNOWN PARTNERS

Partner X

Gene B
Gene B
Gene B
Gene B

Fusion breakpoint
cDNA generated by Reverse transcription

KNOWN PARTNERS

UNKNOWN PARTNERS
Use of specific primer for each partner for a PCR reaction

Generation of high copy number of fusion
Easy to detect
If you mix different primers in your PCR reaction you are able to detect different known fusion partners.
Thanks to this new approach you are able to detect known and unknown partners of fusion gene in one reaction.
POTENTIALLY TARGETABLE or RECURRENT FUSION GENE IN GASTROINTESTINAL MALIGNACIES
Recurrent Fusion in gastric cancer

- **CLDN18-ARHGAPs** (ARHGAP26/6) fusion in gastric cancer
  - a fusion between a tight-junction protein claudin and a regulator of small G proteins
  - Prevalence of 8.8% of a large series of gastric cancer (Chinese)
  - Associated with genomically stable tumors (15%)
  - Is associated with young age, female gender
  - With diffuse type and the content in gastric signet-ring cells
  - Get no benefit from oxaliplatin/fluoropyrimidines-based chemotherapy

Recurrent Fusion in diffuse gastric cancer

- 80 patients with diffuse gastric cancer <45 years
  - Systematic screening of fusion genes
  - Enrichment for RHOGAP fusion genes
- Validation in a series of 305 patients with DGC
  - Older than 46 years (249 patients)
- 4.4% of the patients harbored a fusion including a gene containing a RhoGAP domain
- Patients with RhoGAP domain fusion-containing DGCs (n = 17) had a significantly worse prognosis than those without such fusions (n = 367)

Yang H Nature Commun 2018;9:4439
Recurrent and potentially targetable fusions in hepatocellular carcinoma

- Cyclin A2/E1 target of structural rearrangement by fusion or viral insertion ~7% of HCC
- CCN-HCC were enriched in large tumors
- poor prognosis
- developed in younger patients
- with a non-fibrotic liver (fibrosis stage F0-F1)
- These tumors are characterized by RB1 and PTEN inactivation, and by the absence of CTNNB1 and TERT promoter mutation and by an accumulation of structural rearrangement
- A targetable vulnerability?
  - chemotherapy
  - PARP inhibitors
  - ATR pathway inhibitors

Bayard Q et al. Nature Commun 2018;9:5235
Targetable Fusion in intra hepatic cholangiocarcinoma

• **FGFR2** rearranged iCCA
  • FGFR2–PPHLN1 fusion, FGFR2–BICC1 fusion, FGFR2–TXLNA fusion FGFR2–KCTD1 fusion, FGFR2–KIAA1598 fusion, FRFR2–FRK fusion, FGFR2–ZMYM4 fusion, FGFR2–SORBS1, FGFR2–INA fusion FGFR2–NRAP fusion and several others

NRG1 is a ligand of the ERBB3 and ERBB4 receptors. Fusion leads to enhanced expression of the EGF-like domain of NRG1, which serves as ligand for ERBB2/ERBB3 receptor complexes. NRG1 fusion are present in KRAS wildtype pancreatic adenocarcinoma.

- 2 series: 64 pts, 8 pts KRAS wild-type (12.5%)
- NRG1 fusion are present in 6 out of the 8 patients
  - ATP1B1–NRG1, APP–NRG1, ATP1B1–NRG1, SAR-NRG1-CDH6, APP-NRG1-APP
- Response observed in the 4 patients treated by afatinib, a panERBB inhibitor

Targetable NRG1 Fusion in GI tumors

21,858 tumor specimens profiled from Sept 2015 to Dec 2018, 41 cases (0.2%) harbored an NRG1 fusion.

0.5% of pancreatic adenocarcinoma

When considering RAS KRAS WT subset → ~5%
Targetable Fusion in Colon Cancer

- **ALK, ROS, NTRK** rearranged mCRCs (n = 27)
  - 11 NTRK1, 2 NTRK3, 11 ALK, 3 ROS1 fusions *Pietrantonio F JNCI 2017;109:1-10*

- **RET** rearranged mCRCs (n=24) *Pietrantonio F Ann Oncol 2018;29:1394–1401*

- Compared to 319 patients ALK ROS and NTRK negative patients

- Compared to 291 patients RET negative patients
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Univariate HR: 4.59 [95%CI:3.64–33.66] P<0.001
Multivariate HR: 2.97 [95%CI:1.25–7.07] P=0.014
Targetable Fusion in Colon Cancer

~ 21,000 CRC tissue specimens characterized by Foundation Gene panels
18,107 patients tested for MSI (4.5% of MSI CRC tumors)

Prevalence of the different fusion genes involving a potentially targetable kinase gene

<table>
<thead>
<tr>
<th>KINASE</th>
<th>N (%) 18107</th>
<th>%MSI</th>
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</thead>
<tbody>
<tr>
<td>ALK</td>
<td>17 (0.09%)</td>
<td>14.3%</td>
</tr>
<tr>
<td>BRAF</td>
<td>23 (0.12%)</td>
<td>16.7%</td>
</tr>
<tr>
<td>FGFR2</td>
<td>4 (0.02%)</td>
<td>75%</td>
</tr>
<tr>
<td>NTK1</td>
<td>26 (0.14%)</td>
<td>84.6%</td>
</tr>
<tr>
<td>NTK3</td>
<td>3 (0.0016%)</td>
<td>100%</td>
</tr>
<tr>
<td>RET</td>
<td>28 (0.15%)</td>
<td>50%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>101 (0.5%)</td>
<td>46%</td>
</tr>
</tbody>
</table>

ALK, BRAF NTRK1 and RET are the most prevalent involved kinase genes in fusion
Microsatellite instability was ten time more frequent in KRE+ CRC as compared to KRE- CRC
Microsatellite instability was enriched in cases with RET and NTRK1 fusions and to a lesser extent in cases with BRAF and ALK fusions.

Madison R ESMO 2018 n° 3509
Targetable Fusion in Colon Cancer

KRE POSITIVE CRC harbored fewer mutation in TP53, APC, KRAS and PIK3CA

Madison R ESMO 2018 n° 3509
<table>
<thead>
<tr>
<th>Potentially Targetable</th>
<th>Gastric cancer</th>
<th>Pancreatic cancer</th>
<th>Cholangiocarcinoma</th>
<th>Liver cancer</th>
<th>Colon cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGFR2</td>
<td>+</td>
<td></td>
<td>+++ up to 45%</td>
<td></td>
<td>+</td>
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<tr>
<td>NTRK3</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>ALK</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
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<tr>
<td>ROS1</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
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<tr>
<td>RET</td>
<td></td>
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<td></td>
<td>+</td>
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<tr>
<td>BRAF</td>
<td>+</td>
<td></td>
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<td>+</td>
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<tr>
<td>NRG1</td>
<td>++ up to 5% in KRAS WT</td>
<td>+ %</td>
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<td>+</td>
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<tr>
<td>CCNA2/CCNE1</td>
<td></td>
<td></td>
<td>++ up to 7%</td>
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<tr>
<td>CLDN18-ARHGAPs</td>
<td>+++up to 15% of GS</td>
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