Analysis of circulating cell-free DNA in plasma of neuroblastoma patients

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Neuroblastoma

- Pediatric tumor of the peripheral sympathetic nervous system
- The most frequent extracranial solid tumor of childhood
- Rare tumor: 130 à 150 new cases per year in France

Criteria useful for the therapy at diagnosis:
- Age
- Stage
- Histology
- Status of MYCN gene
- Genomic profile

Localized NB and metastatic NB < 1year

MYCN without amplification

LINEs protocol

- Surgery +/- chemotherapy

MYCN amplified

HR protocol

Induction chemotherapy + surgery
- high dose of chemotherapy
- stem cells graft
- retinoic acid treatment

Metastatic NB > 1year

at relapse

- Status ALK gene for targeted therapy (AcSé protocol)
Analysis of circulating DNA in neuroblastoma patients

Why this study?
- The tumor is not always available for analyzing genomic alterations

Background

- Division of tumor cells
- Death
- Release of DNA in blood
Analysis of circulating DNA in neuroblastoma patients

- Collect of blood on EDTA tube
- Centrifugation 10 mn at 700g
- Freezing quickly in liquid nitrogen and stored at -80°C

- Extraction DNA : 200 µl of plasma on QIAmp DNA microkit (Qiagen) elution volume : 50µl

P=3.12e-06
**Study of MYCN amplification**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>2002-2014: analysis by qPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYCN</td>
<td>NAGK</td>
</tr>
<tr>
<td>targeted gene</td>
<td>Reference gene</td>
</tr>
<tr>
<td>FAM</td>
<td>Yakima Yellow</td>
</tr>
</tbody>
</table>

**2015-2017: analysis by ddPCR**

- Standard Curve (serial dilutions of a healthy donor's DNA)
- Dosage of each gene in the sample
- MYCN/NAGK ratio
- MYCN copy number

Results: analysis of >1000 samples

- MYCN DNA sequences can be detected at diagnosis in plasma of patients with MNA tumors

- Circulating MYCN DNA is not detected in plasma of patients without MYCN amplification

- Circulating MYCN DNA is detectable in plasma of patients with localized or metastatic neuroblastoma
  (sensitivity = 10% (% in stage I-II), 75% (stage III) and 85% (stage IV or IVS)

- Circulating MYCN DNA can be used to monitor disease progression

Study of status *ALK* gene by ddPCR

Mutations of *ALK* gene are detected in 8 à 10% NB cases. Presence of *ALK* mutation in Kinase domain 

![Activation of different pathways:
- Phosphoinoside 3-Kinase (PI3K)-Akt
- MAP kinase
- STAT3](image)

proliferation and survival of tumor cells.

Identification of 3 major hotspots involving amino acids 1174, 1245 and 1275

Mutations F1174L and R1275Q = 70% *ALK* mutations
RESULTS: DETECTION OF ALK MUTATION IN CIRCULATING DNA

Study population:
- 97 stade IV
- 7 stage II-III with MYCN ampl.
- 10 cases stage III w/o NMYC ampl.

114 circulating DNA samples

- 111 evaluable (97%)
- 87 WT (78%)
- 24 MT (22%)

2 MT (8%)
- F1174L (3520)

11# (46%)
- F1174L (3522)

15# (63%)
- R1275Q (3824)

# Concurrent mutations in 4 cases
Comparison of results obtained on circulating DNA and DNA tumor samples after ddPCR analysis

Selection of 60 patients with evaluable tumor DNA and circulating DNA samples

### F1174L (3520)

**Perfect concordance**

<table>
<thead>
<tr>
<th>Tumor DNA</th>
<th>Circulating DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>6</td>
</tr>
<tr>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

- Specificity = 100%
- Sensitivity = 100%

### F1174L (3522)

<table>
<thead>
<tr>
<th>Tumor DNA</th>
<th>Circulating DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>12</td>
</tr>
<tr>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

- Specificity = 92.4%
- Sensitivity = 85.7%

### R1275Q (3824)

<table>
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<th>Circulating DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>12</td>
</tr>
<tr>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

- Specificity = 97.9%
- Sensitivity = 92.3%
Conclusion

- **ALK** mutations detectable in circulating DNA from NB patients using ddPCR

- A good concordance between circulating DNA and DNA tumor samples after ddPCR analysis

- **ALK** mutations are detected in ~20% patients with high-risk neuroblastoma

- Analysis of cfDNA may help capturing tumor heterogeneity

ANALYSIS OF GENOMIC PROFILE FROM CIRCULATING DNA

70 circulating DNA:
- 13 stages 1-2
  - 11 stage 3
  - 39 stage 4
  - 7 stage 4s

- Analysis on ONCOSCAN array (affymetrix)

- Comparison with genomic profile from tumor samples

<table>
<thead>
<tr>
<th>Stage</th>
<th>Correlation</th>
</tr>
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<tbody>
<tr>
<td>Stage 1</td>
<td>0%</td>
</tr>
<tr>
<td>Stage 2-2b</td>
<td>50%</td>
</tr>
<tr>
<td>Stage 3</td>
<td>72%</td>
</tr>
<tr>
<td>Stage 4</td>
<td>84%</td>
</tr>
<tr>
<td>Stage 4s</td>
<td>86%</td>
</tr>
</tbody>
</table>

Stage 3
50% tumor cells in primary tumor
15.6 ng/ml plasma

Numerical profile
Stage 3
85% tumor cells in primary tumor
330 ng/ml plasma

Segmental case
Stage 4
90% tumor cells in primary tumor
80% tumor cells in bone marrow
1260 ng/ml plasma

Analysis of cfDNA may help capturing tumor heterogeneity
Conclusion

From blood sample, it is possible:

- To analyze circulating tumor DNA and to seek the genomic alterations
- To identify the presence of MYCN amplification or ALK mutations
- To help the clinicians in therapeutic decision
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