EuroClonality-NGS DNA capture panel for molecular biomarker detection in circulating tumor DNA. Preliminary results at baseline and MRD detection in NHL patients

M Alcoceba¹, J Gazdova², LG Díaz¹, M García-Álvarez¹, A Medina¹, P Tamayo¹, A Martín García-Sancho¹, R García-Sanz¹, D Gonzalez², ME Sarasquete¹

¹- Hospital Universitario de Salamanca, Spain
²- Queen’s University Belfast, UK

Euroclonality-NGS Group
Non-Hodgkin Lymphomas

• Diffuse large B-cell lymphoma, the most common form Non-Hodgkin Lymphomas (NHL), accounting for 30-40% of new diagnoses.

• DLBCL displays remarkable clinical and biological heterogeneity.

• Genetic alterations determine treatment and patient prognosis.
  ✓ Molecular subtypes based on the cell of origin: germinal centre B cell-like (GCB) and activated B cell-like (ABC) or other.
  ✓ All DLBCL at diagnosis tested for:
    - BCL2, BCL6, MYC and IgH traslocations by FISH (Double- triple – hit)
    - MYC, BCL2 and BCL6 expression by IHQ to check for double expressor

• Tumoral cells stay within lymph nodes, are not usually present in peripheral blood.

Liquid biopsy interesting!
How to monitor LNH disease

- Although therapy is curative in most cases, 30-40% ultimately relapse.
- Assessment of response and monitoring are therefore important for clinical care.

Liquid biopsy for MRD detection

**PET/TC recommended imaging strategy for response assessment**

Disadvantages:
- Low sensitivity
- Limited specificity
- Additional radiation exposure
Next generation sequencing approaches

**Immuglobulin based MRD**
- DLBCL patients TTP by ctDNA analysis of IgH rearrangements by NGS
- Ig-NGS tracks a *single* tumor-specific genetic aberration

**Genotyping-based MRD (CAPP-Seq)**
- Mutations, in/dels, traslocations involving genes (BCL2, BCL6, MYC , IgH) and IgH genes
- Higher sensitivity
- Clonal evolution and acquisition of molecular resistance

Roschewski M et al. Lancet Oncology 2016

F Scherer, Sci transl Medicine 2016
Molecular markers detection in LNH

Lymphoma fingerprints

1. Immunoglobuline and T-cell receptor gene rearrangement
2. Translocations
3. Single nucleotide variants
4. Copy number variations

1. PCR+ SANGER SEQUENCING
2. FISH
3. PCR+SANGER, qPCR
4. CGH-arrays, SNP arrays

Limited by sample restrictions

All tests in one by NGS
Objectives

• To assess the usefulness of the EuroClonality panel for molecular biomarker detection in circulating tumor DNA (ctDNA) from lymphoma patients at diagnosis. Comparison among ctDNA and lymph node paired tumor sample.

• To evaluate the performance of the EuroClonality panel for the detection of MRD in follow-up samples and compare with the monitoring standard of care and survival data.
**Material and methods**

### Patients

<table>
<thead>
<tr>
<th>Condition</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLBCL</td>
<td>n=24</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>n=4</td>
</tr>
</tbody>
</table>

**DLBCL follow-up samples:**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>FU1</td>
<td>n=17</td>
</tr>
<tr>
<td>FU2</td>
<td>n=16</td>
</tr>
</tbody>
</table>

**Samples**

- 20ml peripheral blood in EDTA (<1h)
- Double spun for plasma isolation

**ctDNA extraction:**

- Qiamp Circulating DNA

**ctDNA quality and quantity:**

- Qubit & Tape station

**Next generation sequencing:**

- Custom capture NGS Panel (Roche Nimblegen)
- ctDNA input at least 30ng

**Paired tumor samples (n=20)**

- LDCBG n=18
- LF n=2

**IGH/TCR Translocations Mutations CNVs**
Results 1: Single nucleotide variants (SNVs) in ctDNA at diagnosis

<table>
<thead>
<tr>
<th></th>
<th>21/24</th>
<th>Total SNV=127</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNV/patient</td>
<td>6</td>
<td>[1-19]</td>
</tr>
<tr>
<td>VAF</td>
<td>13%</td>
<td>[5-70%]</td>
</tr>
<tr>
<td>Alt allele depth</td>
<td>73</td>
<td>[25-724]</td>
</tr>
<tr>
<td>Total read depth</td>
<td>751</td>
<td>[126-1679]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>2/4</th>
<th>Total SNV=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAF</td>
<td>33%</td>
<td>[5-50%]</td>
</tr>
<tr>
<td>Alt allele depth</td>
<td>145</td>
<td>[28-342]</td>
</tr>
<tr>
<td>Total read depth</td>
<td>598</td>
<td>[327-697]</td>
</tr>
</tbody>
</table>
Results 2: IGH translocations in ctDNA

<table>
<thead>
<tr>
<th></th>
<th>BCL2</th>
<th>BCL6</th>
<th>MYC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concordant</td>
<td>17</td>
<td>Concordant</td>
<td>11</td>
</tr>
<tr>
<td>Only NGS</td>
<td>1</td>
<td>Only NGS</td>
<td>3</td>
</tr>
<tr>
<td>Only FISH/PCR</td>
<td>1</td>
<td>Only FISH</td>
<td>3</td>
</tr>
</tbody>
</table>

Gold standard
Lymph node biopsy with tumor cells analyzed by FISH and/or PCR
### Results 3: Overall detection rate SNVs & SV at dx in ctDNA

**IGH rearrangements detection rate:**
- 2/4 FL
- 13/24 DLBCL

<table>
<thead>
<tr>
<th></th>
<th>SNVs</th>
<th>Traslocations and/or IGH rearrangements</th>
<th>Overall detection rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLBCL</td>
<td>21/24 (87%)</td>
<td>17/24 (71%)</td>
<td>23/24 (96%)</td>
</tr>
<tr>
<td>FL</td>
<td>2/4 (50%)</td>
<td>3/4 (75%)</td>
<td>4/4 (100%)</td>
</tr>
</tbody>
</table>
Results 4: SNVs in ctDNA & paired tumor sample

**CASE 1**
- ctDNA: 0
- Tumor: 4
- 22% infiltration
- SNVs:
  - CREBBP: 5%
  - EZH2: 6%
  - KMT2D: 7%
  - KMT2D: 14%

**CASE 2**
- ctDNA: 4
- Tumor: 4
- 83% infiltration
- SNVs:
  - BCL2: 5%
  - CREBBP: 17%
  - KMT2D: 30%
  - TP53: 70%

N=2 FL CASES
Results 5: SNVs in ctDNA & paired tumor sample

N=18 DLBCL CASES

SNVs ctDNA 115
VAF ctDNA 13% (2%-70%)

SNVs tumoral 173
VAF tumoral 20% (2%-80%)

ctDNA
VAF: 7% [2%-30%]

100 (70%)

15 (5%)

73 (25%)

Tumor
VAF: 16%[2%-80%]
**Results 6: traslaciones ctDNA & paired tumor sample**

<table>
<thead>
<tr>
<th></th>
<th>NGS Tumor</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pos (11)</td>
<td>Neg (9)</td>
<td></td>
</tr>
<tr>
<td><strong>NGS ctDNA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pos (10)</td>
<td>Pos (10)</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Neg (10)</td>
<td>Neg (10)</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>

**Sensibilidad: 72%**

**Especificidad: 77%**

<table>
<thead>
<tr>
<th>Case</th>
<th>Infiltration (%)</th>
<th>FISH Tumour tissue</th>
<th>NGS Tumour</th>
<th>NGS ctDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>82%</td>
<td>IGHrr 70% (negative for BCL1 and BCL2)</td>
<td>BCL6 &gt; IGHMswc</td>
<td>Neg</td>
</tr>
<tr>
<td>15</td>
<td>34%</td>
<td>IGHrr 30% (negative for BCL1 and BCL2)</td>
<td>BCL6 &gt; IgHG3switch</td>
<td>Neg</td>
</tr>
<tr>
<td>7</td>
<td>28%</td>
<td>BCL6 translocation 40%</td>
<td>BCL6 &gt; IGHMswc</td>
<td>Neg</td>
</tr>
<tr>
<td>5</td>
<td>39%</td>
<td>T(14;18) 19%</td>
<td></td>
<td>BCL2 &gt; IGHD3-9</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>FISH negative for BCL2, BCL6, MYC.</td>
<td>Neg</td>
<td>BCL6 &gt; IGL</td>
</tr>
</tbody>
</table>
Objectives

- To assess the usefulness of the EuroClonality-NDC panel for molecular biomarker detection in circulating tumor DNA (ctDNA) from lymphoma patients at diagnosis. Comparison among ctDNA and lymph node paired tumor sample.

- To evaluate the performance of the EuroClonality-NDC panel for the detection of MRD in follow-up samples and compare with the monitoring standard of care and survival data.
Results 7: EMR (ctDNA) vs. (PET/TC)

<table>
<thead>
<tr>
<th>NGS-2</th>
<th>PET2</th>
<th>NGS-6</th>
<th>PET6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neg</td>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6</td>
<td>1</td>
</tr>
</tbody>
</table>

2-relapse
3-not relapsing

RCHOP: rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone
Results 7: Disease-free survival at 2-year according MRD values after 2-cycles

NGS

PET/CT

EMR -  EMR +

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\[ p = 0.015 \]

\[ p = 0.036 \]
Conclusions

- NGS allows the detection of a molecular marker in almost all LNH at diagnosis in ctDNA.
- High concordance in traslocation detection in ctDNA by NGS compared to gold-standard techniques.
- ctDNA is a robust surrogate for direct assessment of primary tumours genotype.
- Utility of ctDNA assessment for risk-stratifying LNH patients throughout disease course
Molecular Pathology Laboratory
Jana Gazdova
Peter Stewart
David González

Sacyl

Servicio Hematología HUS
Miguel Alcoceba  Isabel Prieto
María García-Álvarez  Belén Vidriales
Alicia Antón  Ramón García-Sanz
Norma C Gutiérrez  Marcos González
Verónica González  Dolores Caballero
Alejandro Medina  Alejandro Martín
Carmen Chillón  María Eugenia Sarasquete

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Servicio Medicina Nuclear
Luis G Díaz
Pilar Tamayo

Servicio Anatomía Patológica
Óscar Blanco

Servicio Anatomía Patológica