Comparative genomic analysis of *PML* and *RARA* breakpoints in paired diagnosis/relapse samples of patients with acute promyelocytic leukemia treated with ATRA and chemotherapy

Licia Iaccarino

Department of Biomedicine and Prevention

University of Tor Vergata Rome

Rome, September 23, 2016
“Therapy-related” acute promyelocytic leukemia has been reported in cancer patients treated with:
- Topoisomerase II inhibitors
- Radiation therapy

Primary neoplasms before t-APL has been reported in patients who received chemotherapy for a non-malignant disorder.
Breast cancer

Autoimmune diseases
- Multiple sclerosis
- Inflammatory bowel disease
- Rheumatoid arthritis

Non Hodgkin lymphoma

Genitourinary system
Molecular insights in therapy-related APL

DNA Topoisomerase II in Therapy-Related Acute Promyelocytic Leukemia

Anita R. Mistry, Ph.D., Carolyn A. Felix, M.D., Ryan J. Whitmarsh, B.A.,
Annabel Mason, B.Sc., Andreas Reiter, M.D., Bruno Cassinat, Pharm.D.,
Anne Parry, Ph.D., Christoph Walz, Joseph L. Wiemels, Ph.D., Mark R. Segal, Ph.D.,
Lionel Adès, M.D., Ian A. Blair, Ph.D., Neil Osheroff, Ph.D., Andrew J. Peniket, B.A.,
Marina Lafage-Pochitaloff, Ph.D., Nicholas C.P. Cross, Ph.D.,
Christine Chomienne, Ph.D., Ellen Solomon, Ph.D.,
Pierre Fenaux, Ph.D., and David Grimwade, Ph.D.

Breast cancer and multiple sclerosis

6 t-APL arising after mitoxantrone

Multiple sclerosis

12 t-APL arising after mitoxantrone

Molecular analysis of t(15;17) genomic breakpoints in secondary acute promyelocytic leukemia arising after treatment of multiple sclerosis

Syed Khizer Hasan, Ashley N. Mays, Tiziana Ottone, Antonio Ledda, Giorgio La Nasa, Chiara Cattaneo, Enrica Borlonghi, Lorelle Mellilo, Enrico Montefusco, Jose Cervera, Christopher Stephen, Gnamam Satchi, Anne Lennard, Marta Libura, Jo Ann W. By, Neil Osheroff, Sergio Amador, Carolyn A. Felix, Maria Teresa Voso, Wolfgang R. Sperr, Jordi Esteve, Miguel A. Sanz, David Grimwade and Francesco Lo-Coco
DNA Topoisomerase II in Therapy-Related Acute Promyelocytic Leukemia

Anita R. Mistry, Ph.D., Carolyn A. Felix, M.D., Ryan J. Whitmarsh, B.A., Annabel Mason, B.Sc., Andreas Reiter, M.D., Bruno Cassinat, Pharm.D., Anne Parry, Ph.D., Christoph Walz, Joseph L. Wiemels, Ph.D., Mark R. Segal, Ph.D., Lionel Adès, M.D., Ian A. Blair, Ph.D., Neil Osheroff, Ph.D., Andrew J. Peniket, B.A., Marina Lafage-Pochitaloff, Ph.D., Nicholas C.P. Cross, Ph.D., Christine Chomienne, Ph.D., Ellen Solomon, Ph.D., Pierre Fenaux, Ph.D., and David Grimwade, Ph.D.

Figure 1. Identification of a Breakpoint Hot Spot in PML Intron 6 in Mitoxantrone-Related APL.

Molecular analysis of t(15;17) genomic breakpoints in secondary acute promyelocytic leukemia arising after treatment of multiple sclerosis

Syed Khizer Hasan, Ashley N. Mays, Tiziana Ottone, Antonio Ledda, Giorgio La Nasa, Chiara Cattaneo, Erika Bolenghi, Lorella Mellito, Enrico Montefusco, Jose Cervera, Christopher Stephen, Gnannam Satchi, Anne Lennard, Marta Libura, Jo Ann W. By, Neil Osheroff, Sergio Amadori, Carolyn A. Felix, Maria Teresa Voso, Wolfgang R. Sperr, Jordi Esteve, Miguel A. Sanz, David Grimwade and Francesco Lo-Coco

DOI: 10.1182/blood-2007-10-115600 originally published online July 23, 2008
Two main classes:

1. Catalytic inhibitors: Anthracylines (epirubicin and daunorubicin)
2. TOPO-IIA poisons: Etoposide and Mitoxantrone
918 de novo APL patients treated with ATRA + anthracycline-based CHT

17 patients developed a t-MN (MDS, AML, ALL), after a median of 43 months from CR. The 6-year cumulative incidence of t-MN was 2.2%

Despite t-MN is relatively infrequent after first-line treatment of APL with ATRA and standard CHT, therapeutic strategies to avoid / minimize this severe complication are warranted.
Topo-II inhibitors and APL development

The incidence of t-APL after mitoxantrone in multiple sclerosis is 2%.

The rate of relapse of APL is ~10% after combined ATRA + anthracycline-based chemotherapy.
Hypothesis

Disease Recurrence in APL $\xrightarrow{}$ Therapy related APL or true relapse?

Investigate possible switches of breakpoints in \textit{PML} and/or \textit{RARA} between diagnosis and relapse with potential involvement of therapy-related “hotspot” regions at “relapse”
Methods

• 30 APL paired diagnosis/relapse cases with available DNA

• Identification of PML/RARA isoforms

• Long-range PCR to amplify the genomic PML/RARA rearrangement

• Direct sequencing to identify the exact location of the breakpoint
# Clinical characteristics of APL patients (n=30)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range)</td>
<td>36 years (5-77 years)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>16/14</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LPA99 (n=4)</td>
</tr>
<tr>
<td></td>
<td>AIDA2000 (n=16)</td>
</tr>
<tr>
<td></td>
<td>IC-APL (n=3)</td>
</tr>
<tr>
<td></td>
<td>ICC-APL01 (n=2)</td>
</tr>
<tr>
<td></td>
<td>MRC (n=5)</td>
</tr>
<tr>
<td>Mitoxantrone (total median dose)</td>
<td>90 mg (20-90 mg)</td>
</tr>
<tr>
<td>Anthracyclines (total median dose)</td>
<td>144 mg (51-756 mg)</td>
</tr>
<tr>
<td>Median latency (range)*</td>
<td>19 months (5-105 months)</td>
</tr>
</tbody>
</table>

* Latency between APL diagnosis and relapse
Primer positioning for long-range PCR

PML  PML/RARA  RARA

bcr3  bcr2  bcr1

Intron 2

Exon 3

Forward primers

Reverse primers

16.9kb

Chromatogram obtained revealing the breakpoint junction sequence

Results: distribution of *PML/RARA* breakpoints

The location of the breakpoint was in all cases identical at diagnosis and at relapse.
Conclusions

- **The molecular profile** of the breakpoints at the t(15;17) translocation was **identical** at diagnosis and relapse in 30 analysed pts.

- **PML** breakpoints were never localized within the **hotspot region** at position 1482-1489, previously identified in t-APL developing after mitoxantrone treatment.

- Considering the rarity of APL relapse, a larger series of patients analysed at diagnosis and relapse are needed to better investigate whether a “relapse” may mask t-APL.
Acknowledgements

Tiziana Ottone
Syed Khizer Hasan
Mariadomenica Divona
Laura Cicconi
Serena Lavorgna
Valentina Alfonso
Claudia Ciardi
Adriano Venditti
Sergio Amadori

David Grimwade
Giuseppe Basso
Esperanza Such
Monica Boccia
Eduardo Magalhaes Rego

Maria Teresa Voso
Francesco Lo-Coco