Comprehensive clonal mapping of hematopoiesis in vivo in humans by retroviral vector insertional barcoding

Luca Biasco
ASH meeting
San Francisco, 2014
Glaxo SmithKline (GSK) has licensed gene therapy for ADA-SCID and Wiskott-Aldrich Syndrome developed by TIGET

GSK has become the sponsor of ADA-SCID long-term follow study and financial sponsor of the WAS gene therapy trial

Gene therapy for ADA-SCID and WAS is still in development, it is not approved for use in patients outside of clinical trials or pre-approved compassionate use
Ex vivo GT approach to treat PIDs

ADA-SCID RV-GT
WAS LV-GT

- Ex vivo culture
- Transduction with γ-retroviral vector

Isolation of HSCs
- Conditioning of patient
- Infusion of modified stem/progenitor cells

a) Before therapy
b) After therapy

Long-term reconstitution of lymphoid lineages

Bone marrow
Thymus
Peripheral blood
Tissues
Results from WAS-GT clinical trial

Lentiviral Hematopoietic Stem Cell Gene Therapy in Patients with Wiskott-Aldrich Syndrome

- Robust gene transfer on infused CD34+ cells
- 7 patients treated (FU 2mo-4.5yrs)
- Persistent multilineage engraftment
- Restored expression of WASP in Lymphocytes and PLT
- Improved PLT number, normal PLT volume
- Improved immune functions and clinical conditions
- Well Tolerated, no leukaemia
Analysis of vector integration sites (IS)

How many clones?
Of what size?
Do they have common ancestors?
For how long do they last?
IS analysis on WAS GT treated patients

Days after GT

<table>
<thead>
<tr>
<th>Days after GT</th>
<th>Pts follow up</th>
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<tbody>
<tr>
<td>WAS1001</td>
<td>48mo - 5,754,095 seq reads – 27,318 unique IS</td>
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<tr>
<td>WAS1002</td>
<td>36mo - 10,062,533 seq reads – 25,609 unique IS</td>
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<tr>
<td>WAS1003</td>
<td>36mo - 5,806,171 seq reads – 24,632 unique IS</td>
</tr>
<tr>
<td>WAS1004</td>
<td>24mo - 6,916,615 seq reads – 11,814 unique IS</td>
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</tbody>
</table>

> 89,000 clones tracked from 7 PB and 6 BM distinct lineages including BM CD34+ cells
Clonal diversity of gene-corrected cells overtime

WAS Pt1 - PB population diversity

Months after GT

Numb. of IS

01 02 03 06 12 18 24 30 36 42 48 5958
Clonal diversity of gene-corrected cells overtime

WAS Pt1 - PB population diversity

Days after GT

Diversity index

Months after GT

Numb. of IS
Clonal diversity of gene-corrected cells overtime

- WAS1001
- WAS1002
- WAS1003
- WAS1004

Diversity Index

Days after GT
Mark-recapture of identical clones overtime

Estimated population size

Patients' range 1185-2884 clones
Clonal relationships among lineages overtime

Pearson Correlation

Number of cell types

Months after GT

01-03 months after GT

04-06 months after GT

09-36 months after GT
Detection of multipotent progenitors

CFU-assay
Clonal output of CD34+ progenitors
Clonal output of CD34+ progenitors

- SHARED CD34 IS
- CD34 IS
<table>
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<th>Months after GT</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>6</th>
<th>12</th>
<th>24</th>
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Relationship between HSPC and mature lineages

Input from CD34+ cells

Months after GT

Pt1  Pt2  Pt3  Pt4

BMCD14  PB CD14  BM CD15  PB CD15  BM CD19  PB CD19  BM CD3  PB CD3  BM CD4  PB CD4  BM CD56  PB CD56  BM CD61  PB CD8  BM Glyco
Relationship between HSPC and mature lineages

Unsupervised clustering

Input from CD34+ cells

PB Lymphoid T and B
PB myeloid + NK cells
BM myeloid and lymphoid
BM megakaryo-erythroid
Results from ADA-SCID GT clinical trial

- 18 patients treated, all alive
- Median follow up 6.9 years (range 2.3 – 13.4)
- Gene marking is persisting multilineage
- Effective clearance of dAXP
- T-cell counts progressively increased after GT
- Significant reduction in severe infections
- Well Tolerated, no leukaemia
IS analysis on ADA-SCID GT treated patients

Number of unique IS retrieved from different lineages

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<tr>
<th>Pts FU</th>
<th>years after GT</th>
<th>BM CD34</th>
<th>BM Glyco</th>
<th>BM CD15</th>
<th>BM CD19</th>
<th>BM CD56</th>
<th>PB CD4</th>
<th>PB CD8</th>
<th>PB CD15</th>
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<th>Total RIS</th>
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<td>53</td>
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<td>132</td>
<td>84</td>
<td>N.A.</td>
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Tracking specific HSPC clones overtime
Modeling hematopoiesis by IS analysis

BM
- CD 34
- CD 15
- Glyco
- CD 19

PB
- CD 15
- CD 19

VS

BM
- CD 34
- CD 15
- Glyco
- CD 19

PB
- CD 15
- CD 19

X_{t1}  X_{t2}  X_{t3}  X_{t4}  X_{t5}
0      1      1      1      1

time
Conclusions

- Survival and plasticity of long-term HSPC
  - In vitro activated HSPC could sustain long-term hematopoiesis in humans (independence from quiescence)
  - Multipotency could be exerted long-term with fluctuating outputs
  - Few thousands HSPC clones are responsible for the maintenance of steady-state hematopoiesis

- Early vs Late dynamics of HSPC after transplant
  - Evidences of a defined switch between Short term and Long term engrafting HSPC
  - Takeover of hematopoiesis by HSPC could occur between 6 and 12 months after transplant
  - Clonal diversity fluctuates and get stabilized at 12 months after GT

- Hierarchical structure of hematopoiesis
  - Origin of NK cells independent from lymphoid compartment
  - Evidence of a link downstream HSPC between myeloid and lymphoid lineages

(Biasco et al. in preparation)
Tracking individual T-cell clones by IS analysis

In vivo Tracking of T cells in Humans Unveils Decade-Long Survival and Activity of Genetically Modified T Memory Stem Cells

Serena Scala
Oral Presentation n.547
Session: 801. Gene Therapy and Transfer I
Monday, December 8, 2014: 2:45 PM-4:15 PM
South Building, Gateway Ballroom 104

(Biasco*, Scala* et al. Sci Trans Med - Under Revision)
# Acknowledgments

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<tr>
<td>Alessandro Aiuti</td>
<td>Clelia di Serio</td>
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<td>Francesca Dionisio</td>
<td>Danilo Pellin</td>
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<tr>
<td>Serena Scala</td>
<td>University of Groningen</td>
</tr>
<tr>
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<td>Ernst Wit</td>
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<tr>
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<td>GSK team</td>
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<tr>
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<tr>
<td>Eugenio Montini</td>
<td>Unit staff</td>
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<tr>
<td>Andrea Calabria</td>
<td>Collaborating Physicians</td>
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Caletta de Famara, Lanzarote, Canary Islands, Oct 2007