Anti-leukemic activity of a novel pegylated recombinant *Erwinia chrysanthemi* L-asparaginase *in vitro* and *in vivo*

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Post-doctoral program

Pathology of Lymphoid Cell team
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Mode of action of L-asparaginase

1953-1963
Discovery of the anti-tumor activity of L-asparaginase found in guinea pig serum

L-asparaginase

L-asparagine → L-aspartic acid + ammonia
asparagine synthetase

Survival of Normal cells

L-asparaginase

L-asparagine → L-aspartic acid + ammonia
asparagine synthetase

Death of Cancer cells

(Kidd, 1953; Broome, 1963)
Development of bacterial L-asparaginases

**Since 1964**
L-asparaginase derived from *Escherichia coli* or *Erwinia chrysanthemi* has been developed for the treatment of **Acute Lymphoblastic Leukemia**.

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Europe: native <em>E. coli</em></th>
<th>pegylated <em>E. coli</em></th>
<th>native <em>Erwinia chrysanthemi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>USA:</td>
<td>pegylated <em>E. coli</em></td>
<td>native <em>Erwinia chrysanthemi</em></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Native <em>E. coli</em> L-asparaginase</th>
<th>Pegylated <em>E. coli</em> L-asparaginase</th>
<th><em>Erwinia chrysanthemi</em> L-asparaginase (crisantaspase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trade Name</td>
<td>Kidrolase®</td>
<td>Oncaspar®</td>
<td>Erwinase®</td>
</tr>
<tr>
<td>Plasma half-life</td>
<td>1.1 - 1.24 days</td>
<td>5.8 - 6.0 days</td>
<td>0.65 - 0.77 days</td>
</tr>
<tr>
<td>Asn depletion</td>
<td>14 - 23 days</td>
<td>26 - 34 days</td>
<td>7 - 15 days</td>
</tr>
<tr>
<td>Immunogenicity</td>
<td>26 - 42 %</td>
<td>2 - 11%</td>
<td>33%</td>
</tr>
<tr>
<td>Hypersensitivity</td>
<td>41 %</td>
<td>11 - 32%</td>
<td>17%</td>
</tr>
<tr>
<td>Pediatric use</td>
<td>6,000-10000 IU/m² 3 times/week</td>
<td>2,500 IU/m² Twice</td>
<td>25,000 IU/m² 2-3 times/week</td>
</tr>
</tbody>
</table>

(Asselin BL, 1993; Avramis et al., 2002; Wang et al., 2003; Avramis and Tiwari, 2006; Dinndrof et al., 2007; Vrooman et al., 2010; Pieters et al., 2011)
Improved PD/PK properties of PEG-r-crisantaspase 

*in vivo*

<table>
<thead>
<tr>
<th></th>
<th>n-crisantaspase (250 U/kg)</th>
<th>PEG-r-crisantaspase (5 U/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(_{1/2}) of enzymatic activity</td>
<td>6 h</td>
<td>38 h</td>
</tr>
<tr>
<td>L-Asn depletion</td>
<td>48 h</td>
<td>72-96 h</td>
</tr>
</tbody>
</table>

B6D2F1-hybrid immune competent mice 

(Allas et al., abstracts #2033. 51st ASH annual meeting 2009)
Reduced immunogenicity of PEG-r-crisantaspase *in vivo*

**Graph:**
- **Y-axis:** Absorbance at 450 nM
- **X-axis:** Week
- **Legend:**
  - PEG-r-crisantaspase (5 U/kg)
  - n-crisantaspase (250 U/kg)

B6D2F1-hybrid immune competent mice

(Allas et al., abstracts #2034. 51st ASH annual meeting 2009)
PEG-r-crisantaspase has similar efficacy profile to n-crisantaspase *in vitro*
PEG-r-crisantaspase prevents leukemia development \textit{in vivo}

**A** Leukemia development (n=5/group)

**B** L-ASNase activity (n=2/group)

**C** L-Asn concentration (n=2/group)

**E** Survival rate (n=7/group)

immunodeficient NOD SCID mice
Conclusions

**PEG-r-crisantaspase**
- has improved PD/PK properties *in vivo*,
- presents reduced immunogenicity *in vivo*,
- has similar *in vitro* cytotoxic effect to n-crisantaspase,
- reduces the expansion of leukemic cells in leukemia-bearing mice,
- prolongs the survival of the treated animals.

These results support the clinical development of PEG-r-crisantaspase.

A phase I dose escalation study in adult patients with relapsed or refractory hematological malignancies has been recently initiated.
Acknowledgement

Hospices Civils de Lyon Sud

Gilles Salles, MD, PhD
Nicolas Rachinel, PhD
Céline Le Beux
Claire Lacroix

Alizé Pharma

Thierry Abribat, PhD
Soraya Allas, MD, PhD
Pierre Sahakian, Pharm D
Michel Julien, PhD