Multi Drug Resistance and its Reversal in Acute Myelogenous Leukemia

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MultiDrug Resistance Phenotype (MDR)

- Biedler & Riehm (Cancer Res 1970; 30:1174)
P388 cell line (rat AML) exposed to increasing doses of actinomycin D became resistant to this drug but is also cross resistant to adriamycin, vinblastine, colchicine...
- This phenomenon is reproducible on several cell lines (mice, human) resistant to one of these unrelated cytotoxic are also cross resistant to others.
- This resistance is pharmacologic: the content of the drug decreased compared to the parental cell line
  ==> this phenotype is the most frequent observed, is inter-species
  ==> the drugs involved are derived from natural products (xenobiotics)...
- It is énergie consuming (ATP)
- It is correlated with the expression of membrane glyco-proteins, called ABC proteins (for ATP Binding Cassette)
Structure of ABC transport proteins

(TMD-ABC)
Minimal structure
Usually intra-cytolasmic
TAP1/2, BCRP

(TMD-ABC)2
Typical structure
P-gp, MRP4-5

TMD0(TMD-BC)2
Atypical structure
MRP1-2-3-6
"ABC" Superfamily Transporter: highly maintained structures through species

- **ABC 2**: Bacteria
  - ATP → ADP
- **PDR5**: Yeast
  - NTP → NDP
- **pfmdr1**: Plasmodium
  - ATP → ADP
- **BCRP**: Human
  - ATP → ADP
- **MRP1**: Human
  - ATP → ADP

Musculoskeletal proteins are involved in the transport of xenobiotics.

ATP, ADP are involved in the transport process.
Cytostatiques chassés par la P-gp: Xénobiotiques

**Anthracyclines:** Extract from streptomyces

**Vinca- Alkaloids** Extract from Cantharanthus Roseus

**Taxanes** Extract from Taxus brevifolia

**Epipodophyllotoxines**
Extract from Mandragore
ABC Proteins in Acute Myelogenous Leukemia: a prognostic factor

Specific substrat for ABC pump

The efflux of the fluorescent probe inhibited by a «modulator» of ABC protein allows us to measure the ABC protein function in tumoral cells.

This efflux is HIGHLY predictive for response to chemotherapy in AML
(Legrand, Blood 97: 502-508, 2001)

The other prognostic factors in AML are
- Caryotype (clonal abnormalities)
- AML secondary to myelodysplasia

Survival without relapse

P=0.0004
**in vitro/in vivo** Resistance in AML tested with JC1: 3 prognostic groups

<table>
<thead>
<tr>
<th></th>
<th>Pgp (UIC2)</th>
<th>IC50</th>
<th>IC50</th>
<th>CD34</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DNR</strong></td>
<td>0.19</td>
<td>12 µM</td>
<td>839 µM</td>
<td>43%</td>
</tr>
<tr>
<td><strong>etoposide</strong></td>
<td>0.30</td>
<td>20 µM</td>
<td>1731 µM</td>
<td>66%</td>
</tr>
<tr>
<td><strong>etoposide</strong></td>
<td>0.40</td>
<td>94 µM</td>
<td>2131 µM</td>
<td>91%</td>
</tr>
</tbody>
</table>

Resistant disease after AML10 treatment
MDR1/Cytogenetic and secondary AML in 146 elderly AML (SWOG study)

Leith et al, Blood 1997
Cytotoxic potentiation of daunorubicin by verapamil and cyclosporin A in a P-gp+ cell line (Ross, Blood 1995)

Use of « modulators »
With cytotoxic to reverse MDR in tumor
Reversion of P-gp efflux: from bench to clinic

- Since 1981 (Tsuruo: verapamil), dozen of compounds were described as P-gp « modulators ». Mechanism of action is mainly competition with drug for P-gp.
- Many phases I and phases II of cytostatics + modulator were published in leukemia and solid tumors.
- During the last 10 years, several compounds with high reversal potency were developed specifically for clinical MDR reversal by pharmaceutical companies.
- Randomized phase III (cytostatics ± modulators) in AML are now completed and published
Pharmacological Drug Resistance Modulation: why AML is a good candidate for clinical trials

• MDR phenotype is easy to measure in circulating leukemic cells (flow cytometry)
• Intracellular pharmacokinetics of cytostatics (± modulator) could be checked in circulating leukemic cells during treatment
• Anthracyclines and VP16 are major drugs in the treatment of AML
• Results could be stratified according to MDR status before treatment
# Trials with Modulators

Originally developed for other therapeutic indications:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinine</td>
<td>phase III (AML)</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>phase III (AML)</td>
</tr>
</tbody>
</table>

Compounds specifically developed for modulation (derived from):

<table>
<thead>
<tr>
<th>Compound</th>
<th>Description</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSC833</td>
<td>(Cyclosporine D)</td>
<td>phases III (AML)</td>
</tr>
<tr>
<td>LY335979</td>
<td>(Quinoline (MS-073))</td>
<td>Phases III (AML)</td>
</tr>
<tr>
<td>GF 120918</td>
<td>(Acridocarboxamide)</td>
<td>phase I (solid T)</td>
</tr>
<tr>
<td>VX-170</td>
<td>(Pipecolinat)</td>
<td>Phases II (solid T)</td>
</tr>
</tbody>
</table>
Randomized trials in AML/MDS with Chemotherapy ± Quinine

- **QUININE**: 3 French trials in AML and MDS
  - In relapsing/refractory/secondary AL (E Solary, Blood 1996;88:1198):
    ID Ara-C+ Mitox±Q: 315 pts, Clinical Resistance: 40% vs 28% (p=0.03)

  - In high risk MDS (E Wattel, Br J Haematol 1998;102:1015):
    IDAra-C+Mitox±Q: 131 pts: CR: 41% vs 47% (NS),
    but 18% CR without Q vs 52% Cr with Q in P-gp (+) (p=0.02)

  - In de novo AML (E Solary, Blood 2003, 102:1202-10):
    Ara-C+Ida±Q: 435 pts,
    CR: 81% vs 82% (NS),
    but 46% CR without Q vs 80% CR with Q in pts with functional P-gp (p=0.02)
Ara-C+ Ida ± Quinine in de novo AML (GOELAM2)

E SOLARY et al, ASH2000 #2172

IDA 8mg/m2 D1-D5
Ara-C 200 mg/m2 D1-D7

IDA 8mg/m2 D1-D5
Ara-C 200 mg/m2 D1-D7
+ Quinine 30 mg/m2 D0-D5

CR

Age<45, BMdonor

HDARA-C Mitox ± G-CSF
AMSA VP-16 ± G-CSF

HDARA-C Mitox ± G-CSF
AMSA VP-16 ± G-CSF

CR

AMSA +Ara-C
Allograft

218pts

217 pts
GOELAM 02 - Initial response

<table>
<thead>
<tr>
<th></th>
<th>w/o quinine</th>
<th>with quinine</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All patients</strong></td>
<td>169/206 (81%)</td>
<td>169/208 (82%)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Function positive</strong></td>
<td>11/24 (46%)</td>
<td>24/30 (80%)</td>
<td>0.02</td>
</tr>
<tr>
<td>negative</td>
<td>46/53 (87%)</td>
<td>41/51 (80%)</td>
<td>0.54</td>
</tr>
<tr>
<td><strong>Gene positive</strong></td>
<td>35/40 (87%)</td>
<td>28/35 (80%)</td>
<td>0.57</td>
</tr>
<tr>
<td>negative</td>
<td>78/88 (89%)</td>
<td>72/89 (81%)</td>
<td>0.70</td>
</tr>
<tr>
<td><strong>Protein positive</strong></td>
<td>16/20 (80%)</td>
<td>29/33 (88%)</td>
<td>0.70</td>
</tr>
<tr>
<td>negative</td>
<td>79/92 (86%)</td>
<td>66/83 (80%)</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Quinine increases the CR rate in MDR+ patients when defined by Rh123 exclusion
GOELAMII de novo AML: Quinine rescued the Event Free Survival in P-gp(+) AML
Addition of CsA to HD Ara-C + DNR in poor risk AML
226 pts randomized (SWOG Study, Blood 2001, 98:3212)

Ara-C 3g/m2/d x 5
DNR 45mg/m2/d CI x 3d
± CsA 16mg/kg/d CI x 3d

P-GP « Neg » (129 pts) P-GP « Pos » (68 pts) All
HDA+Dnr - + CsA - + CsA - + CsA
CR 34% 39% 26% 46% 33% 39%
Res Dis 49% 36% 45% 30% 47% 31%

DNR (ng/ml) median 11.8 23.1

Overall Survival

Disease Free Survival

Overall Survival

Disease Free Survival

P=0.046

P=0.037
Randomized use of cyclosporin A (CsA) to modulate P-glycoprotein in children with AML in remission: Pediatric Oncology Group Study 9421; (Becton, D. et al. Blood 2006;107:1315-1324)

OS for 4 randomized groups excluding BMT patients: No difference

But: 1. only 14% of the patients were MDR1+ in children AML
2. CsA used only after complete CR
Modulator development: PSC833/ Valspodar (Novartis)

Cyclosporin A analog
- less toxique (nephrotoxicity, immuno-suppressive)
- better P-gp inhibition

Penetration Screening of CsA analogs: PSC833
No nephrotoxicity
No immunosuppressive effect
x10 to x100 times more potent as P-gp modulator than CsA
PSC833 increases the intracellular concentration of DNR in AML cells in vivo

Tidefelt et al, JCO 2000, 18:1837 (Karolinska Inst.)

• 10 pts with AML studied (7P-gp+, 3 P-gp-)

DNR 100 mg/m2 CI 72h
D1 D2 D3 D4
PSC833 IV 2mg/kg/2h
10mg/kg/36h

D1 D2 D3       D1 D2 D3
P-gp (+)          P-gp (-)

25
10
\( \mu M \)

D2 D3 D4

AUC leuk Cells/AUC plasma

D1 D2 D3
P-gp (+)
P-gp (-)
Novartis trial C301: **MEC (Mitox/Ara-C/VP16) ± PSC 833**

Patients refractory to tt or in early relapse (<1 year): 102 centres in 17 countries, 250 inclusions. 97-99.

<table>
<thead>
<tr>
<th>Day</th>
<th>Drug</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>PSC 833</td>
<td>IV 10mg/kg/d</td>
</tr>
<tr>
<td></td>
<td>Mitox 6 / 4.5 mg/m2/d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VP16 80 / 30 mg/m2/d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ara-C 1gm2 6h/d</td>
<td></td>
</tr>
</tbody>
</table>

Novartis trial C302: **DNR+AraC ± PSC833 in AML ≥65 years**

Induction treatment: 97 centres, 466 inclusions.

<table>
<thead>
<tr>
<th>Day</th>
<th>Drug</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>PSC 833</td>
<td>IV 10mg/kg/d</td>
</tr>
<tr>
<td></td>
<td>Ara-C</td>
<td>200 mg/m2/d</td>
</tr>
<tr>
<td></td>
<td>DNR 45 / 30 mg/m2/d</td>
<td></td>
</tr>
</tbody>
</table>
Pts >60yo with de novo AML (87) and post MDS AML (33) were included. The trial was hold in March 99 to assess toxicity.

120 pts analyzed: ADE: CR: 45%; Deaths: 27%
PSC833+ADE CR: 31%; Deaths: 54%
Trial was stopped due to excessive toxicity (infection++)
CALG B 9621: Dose escalation of DNR and VP16 + PSC833 and Ara-C in young untreated AML: 410 pts included

Estimation of maximum-tolerated dose of DNR

40 mg/m² (+PSC833) and 90 mg/m² were choosen for the phase III

Kolitz JE et al, JCO 2004, 21:4290
**de novo « young » AML:**

**CALGB9621**
(Kolitz, JCO 2004; 22:4290)

<table>
<thead>
<tr>
<th></th>
<th>Tt</th>
<th>Nb</th>
<th>CR in 1 course</th>
<th>ESF</th>
<th>EFS &lt;45a (220)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADE</td>
<td>394</td>
<td>85%</td>
<td>1 y</td>
<td>0,8 y</td>
<td></td>
</tr>
<tr>
<td>ADE-P</td>
<td>192</td>
<td>94% (p=0.02)</td>
<td>1,7 y</td>
<td>2,4 y (p=0.007)</td>
<td></td>
</tr>
</tbody>
</table>

- **R : PSC833 IV**
  - 10mg/kg/dx3d (during DNR and VP16)

- **Ara-C** 100 mg/m2/dx7d
- **VP16** 100->150/ 40->60 mg/m2/dx3d
- **DNR** 60->95 / 40->50 mg/m2/dx3d

- LTD finding:
  - without PSC833: 90 DNR + 100 VP16
  - with PSC833: 40 DNR + 40 VP16

- **Phase III stopped prematurely due to the decision to stop PSC833 development**
Evaluation of ABC transporter - Modulators Trials

• Very efficient modulators (like PSC-833) decrease the clearance of cytostatic drug(s) co-administered: to be able to evaluate the response and toxicity, the AUC of these drugs have to be the same in both arms (with and without modulator).

• Addition of modulator will benefit only to patients with functional P-gp: results have to be stratified according to functional tests.

• Addition of intermediate/high doses of Ara-C to anthracycline/VP16 ± modulator could mask the effect of P-gp modulation
Transport Mechanisms involved in Drug Resistance

cDNA transfection of de ABCB1, ABCC1, ABCG2, in sensitive cell lines gives a MDR phenotype
<table>
<thead>
<tr>
<th></th>
<th>Chimio Résistant (R)</th>
<th>Chimio Sensible (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P-gp (ABCB1)</strong></td>
<td><strong>MRP1 (ABCC1)</strong></td>
<td><strong>MRP2 (ABCC2)</strong></td>
</tr>
<tr>
<td>Anthracyclines</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Vinca-alkaloids</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Taxanes</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>VP16</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>
ABC Protein expression and pronostic value in AML
(C Marzac et al, Clin Cancer Research, 2005)
ABCA3 expression in AML:
Chapuy et al, Leukemia 2008; 22:1576
ABCA3 expression in AML: pronostic value on 86 AML
Chapuy et al, Leukemia 2008; 22:1576
ABCA3 is localized in the endosomal system
mRNA expression of 49 huABC proteins in « extreme » cohorts of AML
- « sensitive » AML to one standard treatment (CR>3y)
- « resistant » to such treatment (failures and CR<3months)

Patients features

<table>
<thead>
<tr>
<th>Age médian au diagnostic</th>
<th>Sensibles</th>
<th>Résistants</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 (17-78)</td>
<td>48 (17-78)</td>
<td>52 (19-73)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sexe</th>
<th>Sensibles</th>
<th>Résistants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femme</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Homme</td>
<td>21</td>
<td>11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sous-types FAB</th>
<th>Sensibles</th>
<th>Résistants</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M1</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>M2</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>M3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>M4</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>M5</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>M6</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>M7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cytogénétique</th>
<th>Sensibles</th>
<th>Résistants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>11 (22%)</td>
<td>11 (22%)</td>
</tr>
<tr>
<td>Intermédiaire</td>
<td>26 (50%)</td>
<td>14 (28%)</td>
</tr>
<tr>
<td>Défavorable</td>
<td>14 (28%)</td>
<td>6 (14%)</td>
</tr>
</tbody>
</table>
Taqman Low Density Array
7900Ht Fast real-time PCR system (Applied biosystems)

Principle: simultaneous quantitative PCR, in 1 µl microwells

- Probes (Taqman) are lyophilized *in situ* on microfluidic chip
- 12 to 384 well/transcripts including 1 housekeeping gene
- 1 to 8 samples
- The « mix » is dropped on top and diffuse in wells by centrifugation
- chip dedicated to human ABC mRNA
- Normalisation and quantification for each mRNA
ABC proteins in Acute Myeloid Leukemia: cDNA screening

Detection of differentiel expression (>2) of ABC mRNA in « extreme » populations

Close to ABCA3. Surexpressed in HL60/AR

Involved in doxorubicine transport in mélanoma

Surexpressed in MCF-7/CH1000

Non fonctionnal in mammals

<table>
<thead>
<tr>
<th></th>
<th>RQ moyen</th>
<th>Ct normalisé moyen du groupe R</th>
<th>P valeur</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA2</td>
<td>2,00</td>
<td>26,87</td>
<td>0,0108</td>
</tr>
<tr>
<td>ABCB1</td>
<td>3,32</td>
<td>28,88</td>
<td>0,0372</td>
</tr>
<tr>
<td>ABCB5</td>
<td>2,17</td>
<td>33,72</td>
<td>0,0071</td>
</tr>
<tr>
<td>ABCB6</td>
<td>2,23</td>
<td>27,54</td>
<td>0,0622</td>
</tr>
<tr>
<td>ABCC13</td>
<td>3,10</td>
<td>30,48</td>
<td>0,0354</td>
</tr>
<tr>
<td>ABCG1</td>
<td>2,11</td>
<td>28,30</td>
<td>0,0016</td>
</tr>
<tr>
<td>ABCG2</td>
<td>3,82</td>
<td>28,81</td>
<td>0,0033</td>
</tr>
</tbody>
</table>
ABC genes Expression in CD34+/CD38- (b m stem cells) and CD34+/CD38- (committed cells)

Differencial expression between CD34+/CD38- and CD34+/CD38+
In normal bone marrow

Leukemia (2006) 20, 750–754
Conclusions

• P-gp and BCRP are the most ABC pumps frequently expressed in « resistant » AML and are able to expel anthracyclines from leukemic cells.

• Randomized trials using potent P-gp modulators demonstrated benefit only in cases with functional P-gp.

• Numerous ABC pumps reduce the drug concentration in normal (and leukemic?) cells: to eliminate leukemic stem cell expressing several ABC pumps is elusive when using inhibitors.

• Hu mRNA chips in « extreme » AML populations could be useful for detection of ABC pumps of interest in drug resistance.