ABCB1 1199G>A genetic polymorphism influences tacrolimus intracellular accumulation in HEK293 and K562 recombinant cell lines.

Presented by Geraldine Dessilly, PhD student
**ABCB1 Genetic polymorphisms:**

- **SNP**
  Single nucleotide polymorphism
  Main source of interindividual variability
  Natural variant
  Allelic frequency > 1%

- **>50 SNPs**
  - **ABCB1 (exon 11) 1199G>A Ser400Asn**
    Allelic frequency 6%
ABCB1  1199G>A  Ser400Asn

Substrate recognition site

I. Introduction

Pauli-Magnus C. and al, 2004
Stephen G. Aller and al, 2009
I. Introduction

ABCB1 1199G>A Ser400Asn

- In vivo studies

Capron A. et al., 2010

Elens L. et al., 2007

• 1199A variant ↓ efflux activity ABCB1/tacrolimus

CycloporinA ↑ Crettol S. and al, 2008
Vinblastine, vincristine, vp16, paclitaxel ↑ Woodahl EL. and al, 2009

➢ Substrate-dependent impact of 1199A
## Cell lines

<table>
<thead>
<tr>
<th>HEK 293</th>
<th>K562</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human Embryonic Kidney</strong></td>
<td><strong>Human Myelogenous Leukemia</strong></td>
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<tr>
<td>Adherent cells</td>
<td>Suspension cells</td>
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<tr>
<td>Undifferentiated cells</td>
<td></td>
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<tr>
<td><strong>Cells stable transfected by ABCB1 c-DNA gene (pcDNA 3.1) wild-type 1199G or mutated 1199A</strong></td>
<td></td>
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<tr>
<td>Lipofectamine</td>
<td>Electroporation</td>
</tr>
<tr>
<td><strong>Very good expression of exogenous proteins</strong></td>
<td><strong>Express BCR-ABL tyrosine kinase oncoprotein</strong></td>
</tr>
</tbody>
</table>

I. Introduction
Model characterization (I)

Flow cytometry

CTL HEK293

CTL K562 p.cDNA3.1

HEK 1199G

HEK 1199A

K562 1199G

K562 1199A

➢ 1199G & 1199A: ABCB1 overexpression
Model characterization (2)

II. Development

Immunofluorescence

HEK293

Membrane overexpression 1199G & 1199A

Western blot

Overexpression 1199G & 1199A
Objectives

Impact ABCB1 1199G>A on intracellular accumulation of an immunosuppressive agent (tacrolimus) in two recombinant cell lines
Accumulation Kinetics

Experimental protocol:

[Tac] 0.05µM

Cells  Medium  Tac

2h

Cells + Tac

Dosage Tac in cells

Cells  Medium  Tac

2h

Medium free

Cells + Tac

Dosage Tac in cells

15sec, 30sec, 1min, 5min, 10min, 30min, 1h

Analysis LC-MS/MS
Accumulation Kinetics

- Intracellular [Tac] CTL ↑ vs 1199G/WT => Tac = substrate of ABCB1
- Intracellular [Tac] 1199A ↑ vs 1199G
- 1199A variant ↓ activity ABCB1/tacrolimus
Conclusion

• Validated models to study ABCB1 SNP: HEK293 and K562

• Tacrolimus export *in vitro* is decreased by the S400N 1199A variant, in line with clinical data 1199A ↓ activity ABCB1/tacrolimus
Thank you for your attention