

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

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Co-promoter: Pr. Jean-Baptiste Demoulin

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# *ABCB1* Genetic polymorphisms:

- **SNP**

Single nucleotide polymorphism

Main source of interindividual variability

Natural variant

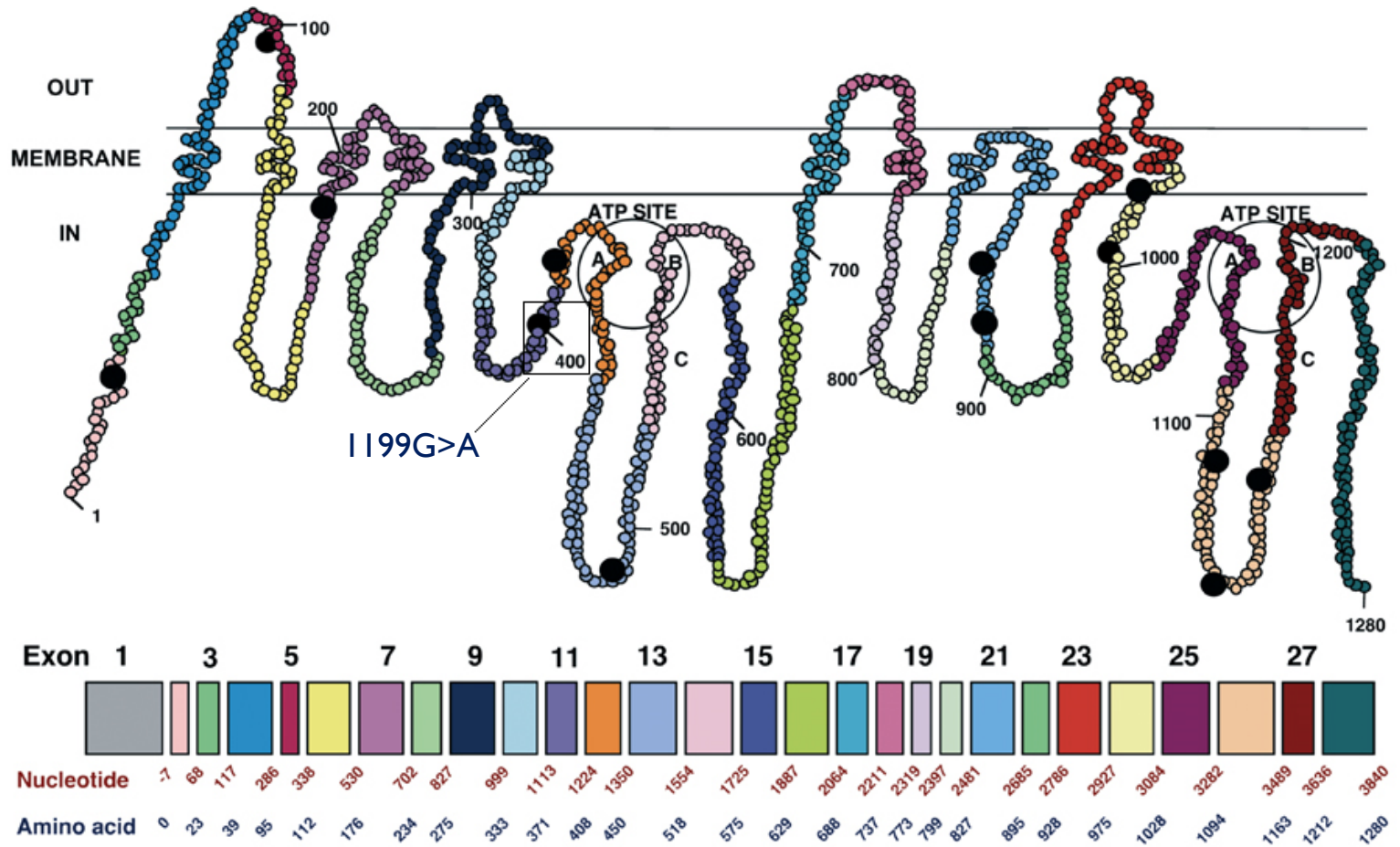
Allelic frequency > 1%

- **>50 SNPs**

- ***ABCB1* (exon 11) 1199G>A Ser400Asn**

Allelic frequency 6%

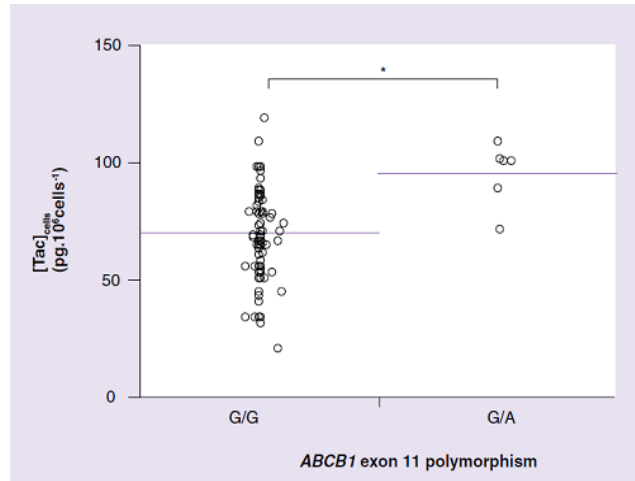
# ABCB1 I199G>A Ser400Asn



## ➤ Substrate recognition site

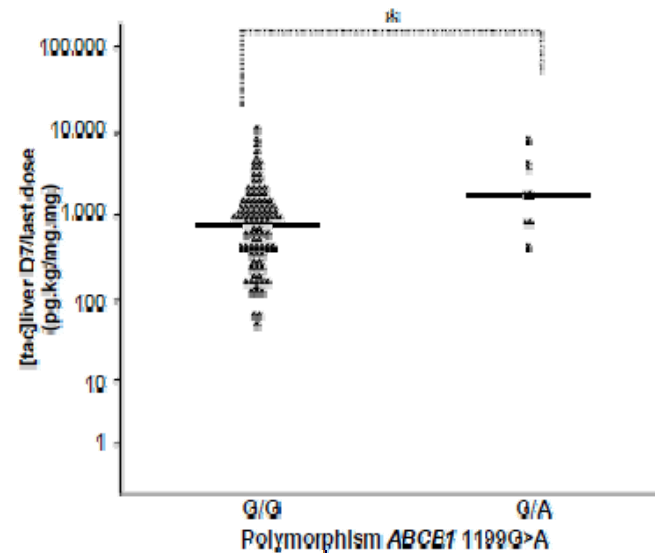
# ABCB1 I199G>A Ser400Asn

- *In vivo* studies



Tacrolimus trough peripheral blood mononuclear cell concentration 7 days post-transplantation ( $\text{pg}/10^6$  cells) according to ABCB1 exon 11 polymorphism, 1199G>A (1199A,  $n = 6$  and 1199G,  $n = 88$ ). [Tac]<sub>cells</sub> refers to the unadjusted tacrolimus trough peripheral blood mononuclear cell concentration. The mean values are indicated. \* $p < 0.05$ .

Capron A. et al., 2010



Elens L. et al., 2007

- I199A variant ↓ efflux activity ABCB1/tacrolimus

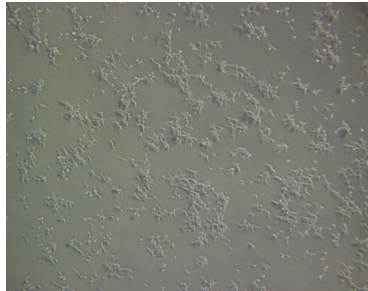
CycloporinA ↑ Crettol S. and al, 2008

Vinblastine, vincristine, vp16, paclitaxel ↑ Woodahl EL. and al, 2009

- Substrate-dependent impact of I199A

# Cell lines

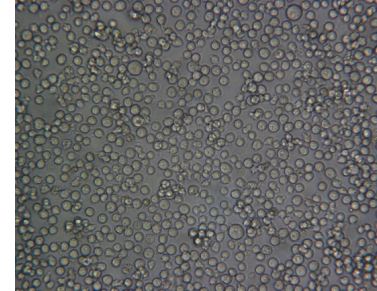
## HEK 293



### Human Embryonic Kidney

Adherent cells  
Undifferentiated cells

## K562



### Human Myelogenous Leukemia

Suspension cells

**Cells stable transfected by *ABCBI* c-DNA gene (pcDNA 3.1) wild-type I199G or mutated I199A**

Lipofectamine

Electroporation

Very good expression of exogenous proteins

Express BCR-ABL tyrosine kinase oncoprotein

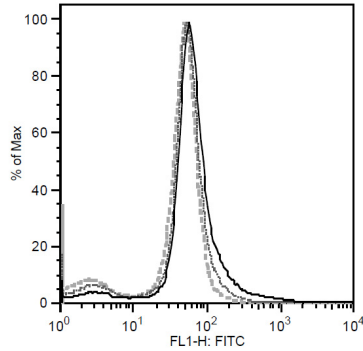


# Model characterization (I)

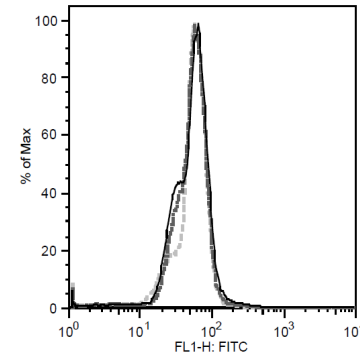
## Flow cytometry

----- Autofluorescence  
 ----- Isotype  
 ——— FITC

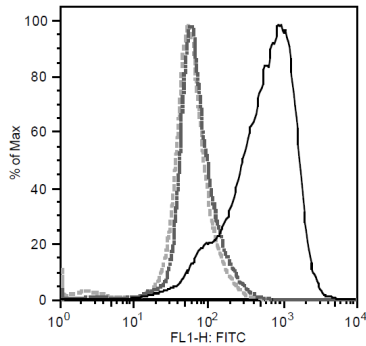
CTL HEK293



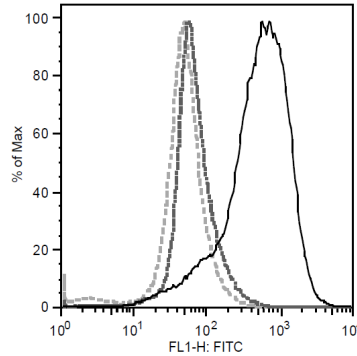
CTL K562 p.cDNA3.1



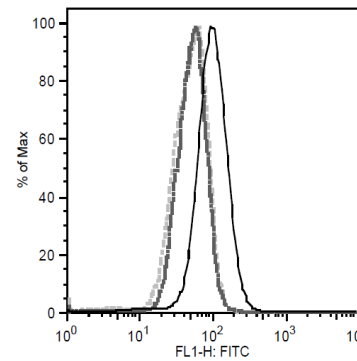
HEK I199G



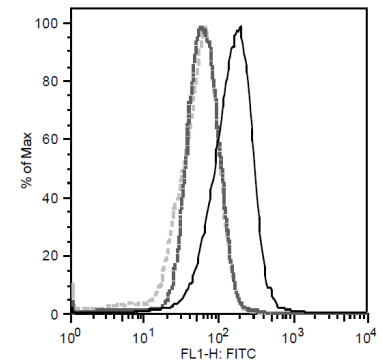
HEK I199A



K562 I199G



K562 I199A

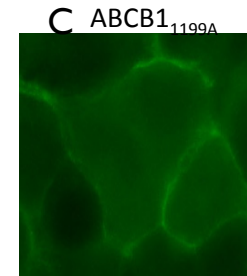
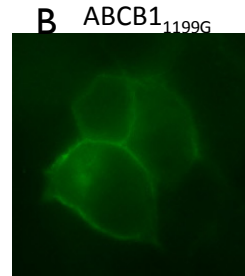
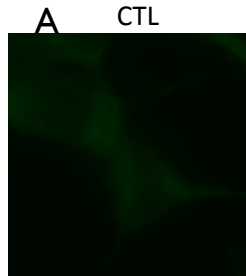


➤ I199G & I199A: ABCB1 overexpression

# Model characterization (2)

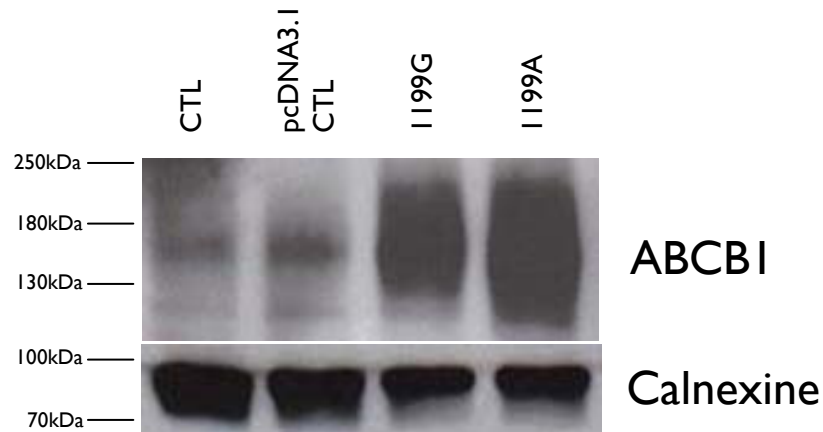
HEK293

Immunofluorescence



➤ Membrane overexpression 1199G & 1199A

Western blot



➤ Overexpression 1199G & 1199A

# Objectives

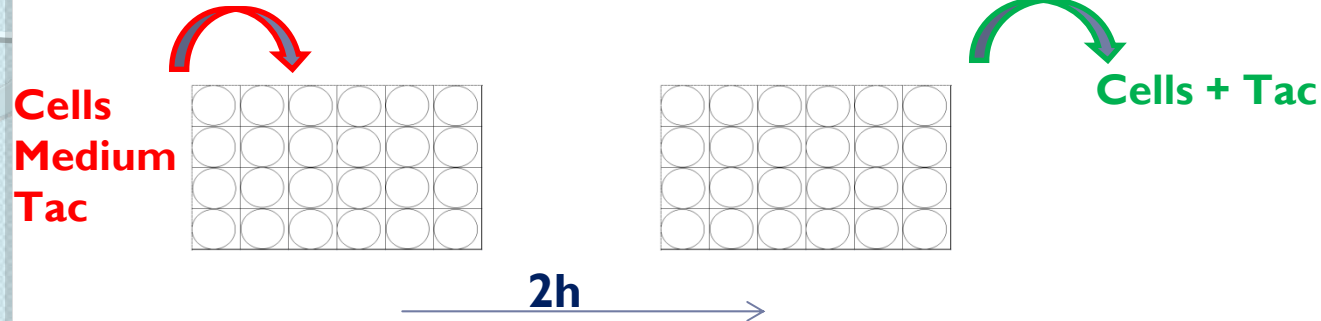
Impact *ABCB1* I199G>A on intracellular accumulation of an immunosuppressive agent (**tacrolimus**)  
in two recombinant cell lines



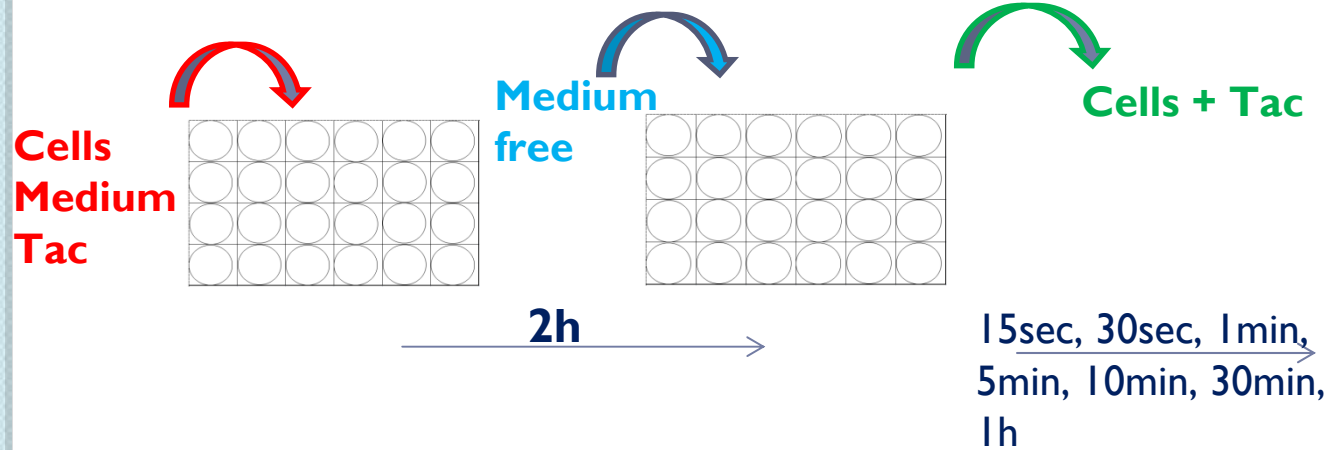
# Accumulation Kinetics

Experimental protocol:

[Tac] 0.05 $\mu$ M



Dosage Tac  
in cells

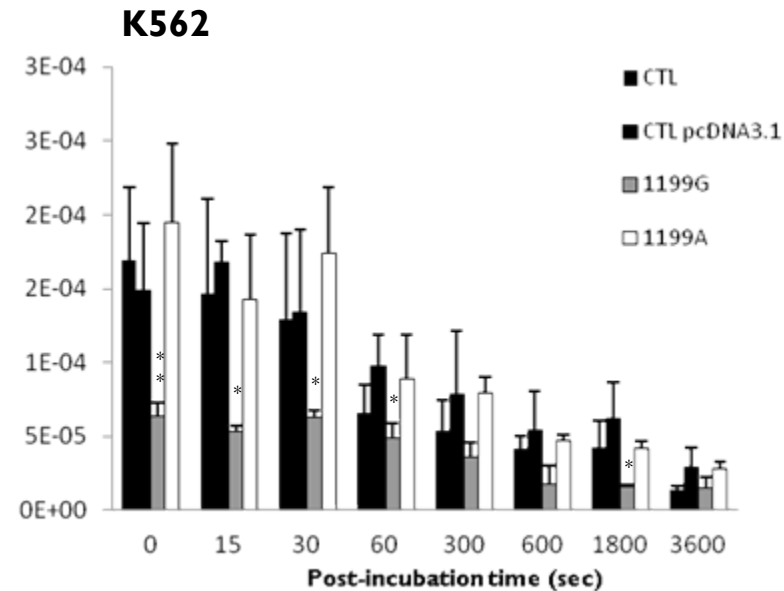
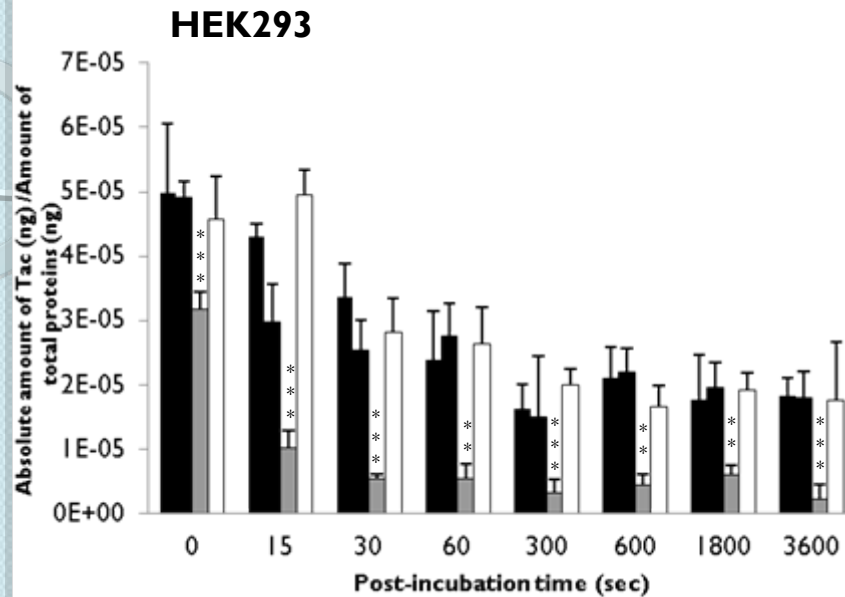


Dosage Tac  
in cells

Analysis LC-MS/MS

# Accumulation Kinetics

II. Development



- Intracellular [Tac] CTL ↑ vs I199G/WT => Tac = substrate of ABCBI
- Intracellular [Tac] I199A ↑ vs I199G
- I199A variant ↓ activity ABCBI/tacrolimus

# Conclusion

- Validated models to study ABCBI SNP: HEK293 and K562
- Tacrolimus export *in vitro* is decreased by the S400N I199A variant, in line with clinical data  
I199A ↓ activity ABCBI/tacrolimus



**Thank you for your attention**