Nephrology and Transplantation Department
Labbafinejad Medical Center

Shahid Beheshti University of Medical Sciences

Social Security Organization of Islamic Repoblic of Iran

# “Beyond Trough Levels: The Role of IPV and Pharmacogenomics in Tacrolimus Monitoring”

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2026.06.12

# OUTLINES

- Tacrolimus therapeutic drug monitoring
- Interapatient Variability (IPV)
- Pharmacogenetics
- Pharmacokinetic model to predict starting dose

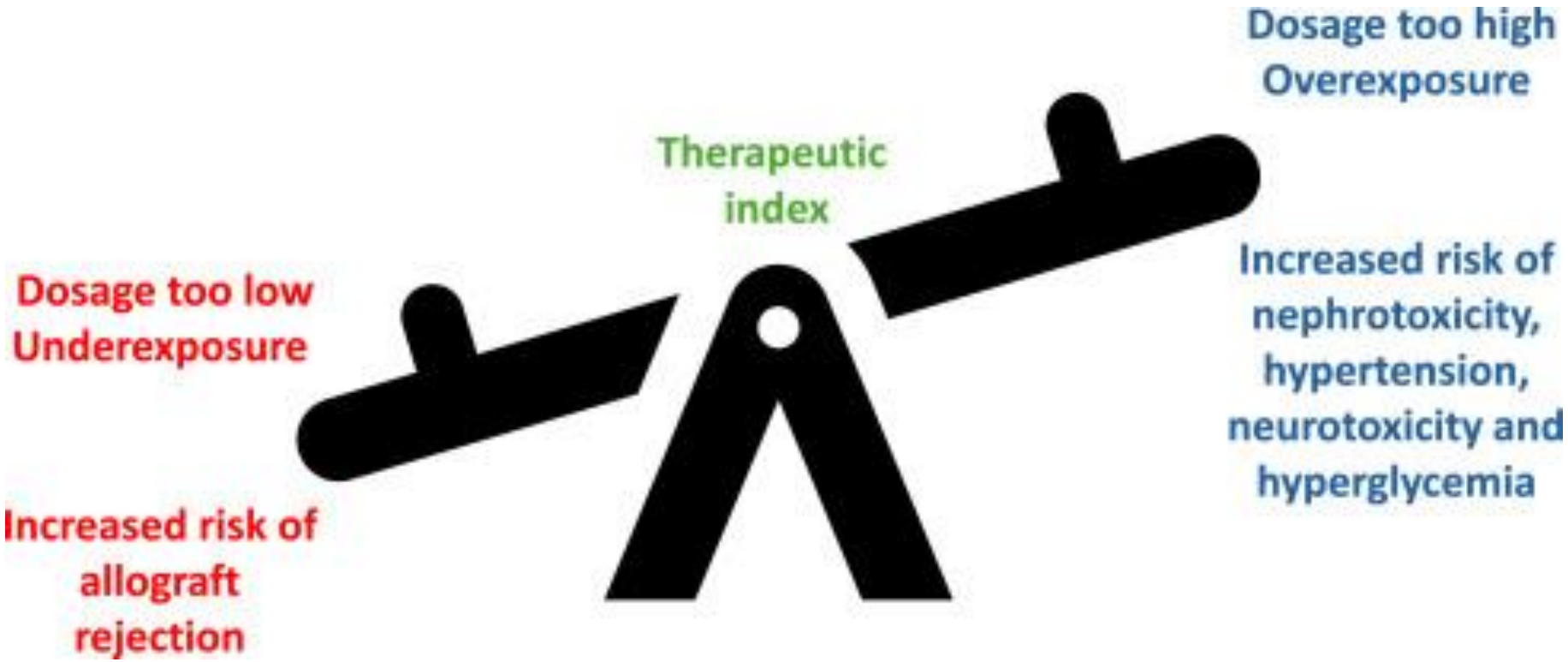
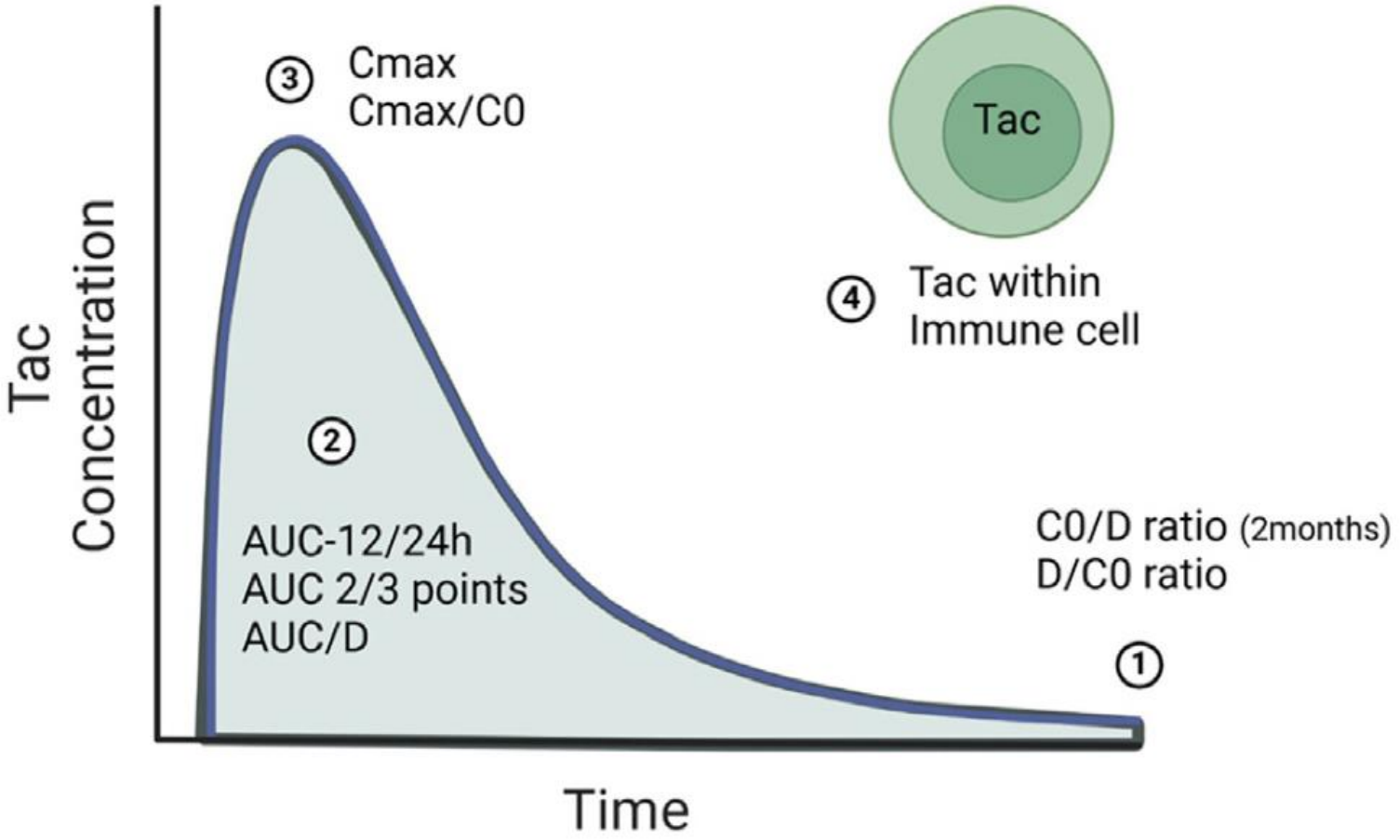

Dosage too high
Overexposure
Therapeutic
index
Dosage too low
Underexposure
Increased risk of
nephrotoxicity,
hypertension,
neurotoxicity and
hyperglycemia
Increased risk of
allograft
rejection

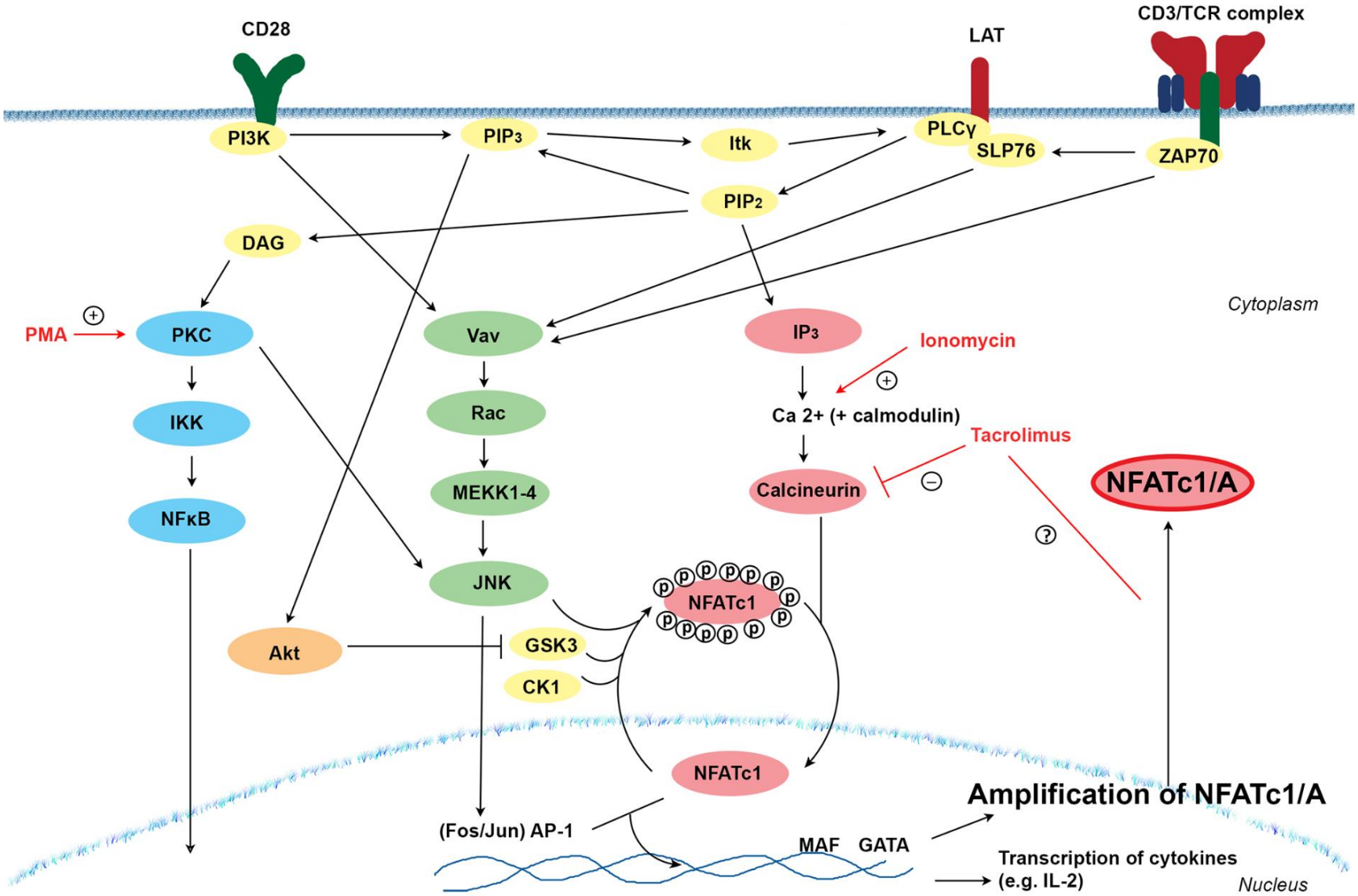
**TABLE 5.2** Key pharmacokinetic parameters of small molecule immunosuppressants relevant for TDM assay development.

| Immunosuppressant | Absolute oral bioavailability | Distribution into blood cellular components | Matrix of choice for TDM | PK parameters used for TDM | Typical target concentrations in transplant patients | Major immunosuppressant metabolites | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Metabolite | Immunosuppressive activity in % of parent drug | C0 concentration in % of parent drug |
| Tacrolimus | 17%–23% | approx. 85% | EDTA whole blood | C0 | 3–15 ng/mL | 13-O-desmethyl (M-I) | 6 | 6.4 |
| | | | | | | 31-O-desmethyl (M-II) | 100 | Unknown |
| | | | | | | 15-O-desmethyl (M-III) | 0 | 5.3 |
| | | | | | | 12-hydroxy (M-IV) | 3.5 | Unknown |
| | | | | | | 15,31-O-didesmethyl (M-V} | 0 | Unknown |
| | | | | | | 13,15-O-didesmethyl (M-VII) | 0 | 1.7 |

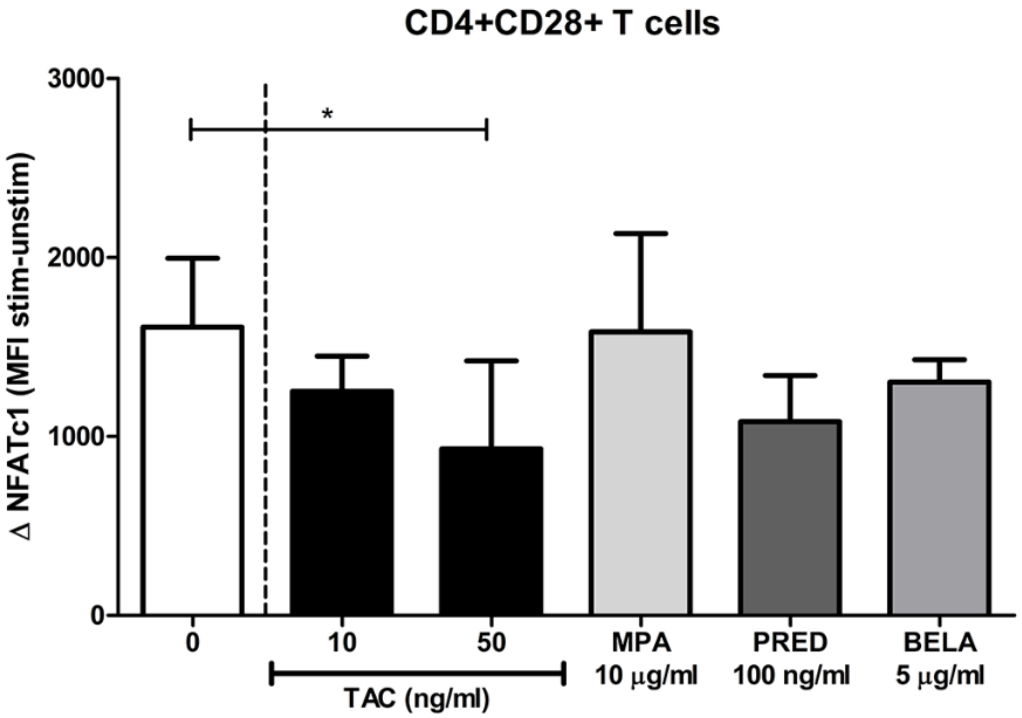
# Different modalities of tacrolimus therapeutic drug monitoring




Kidney International Reports (2025) **10,** 1326–1328

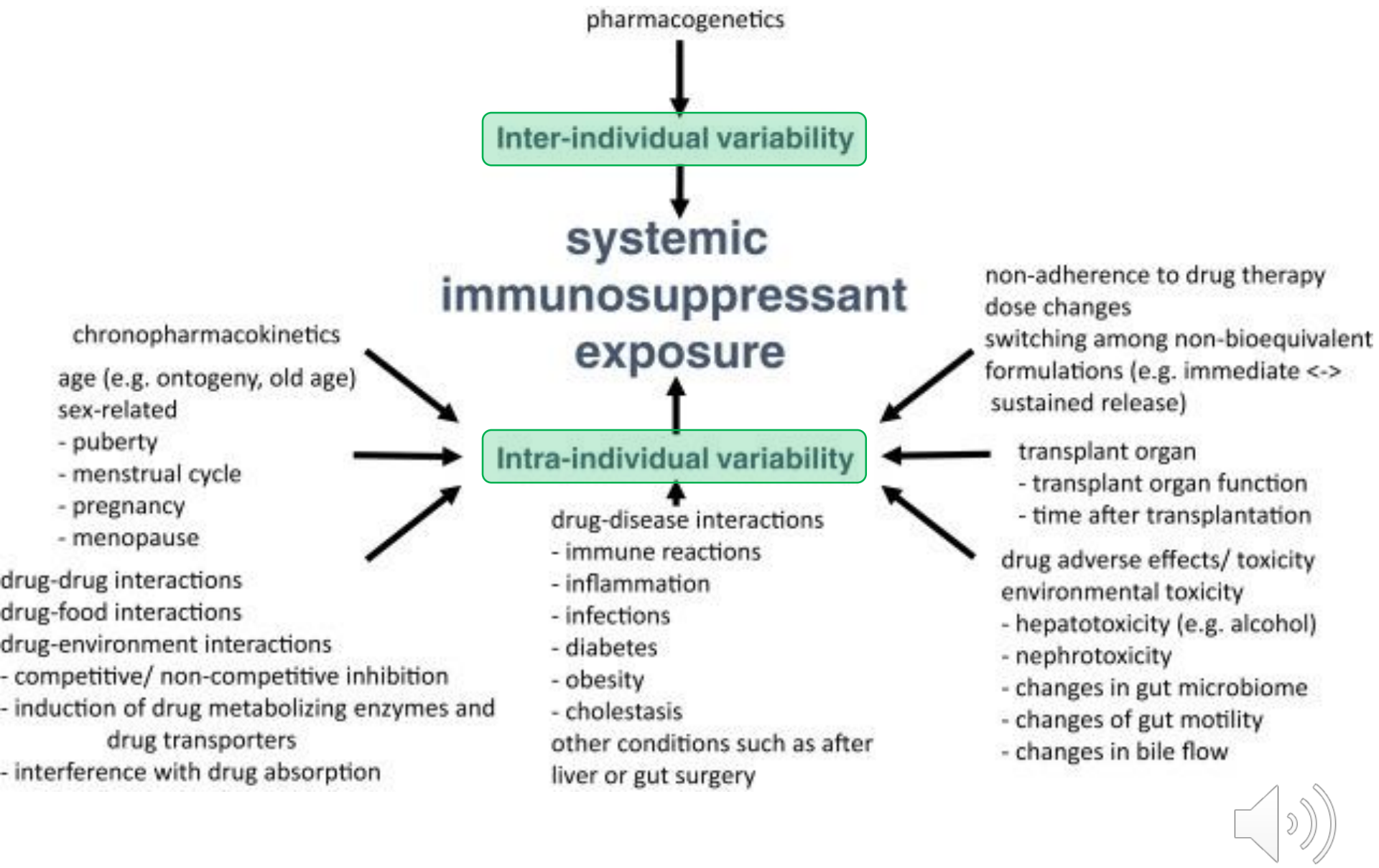

CD28
LAT
CD3/TCR complex
PI3K
PIP3
Itk
PLCγ
SLP76
ZAP70
PIP2
DAG
Cytoplasm
PMA
PKC
Vav
IP3
Ionomycin
Ca 2+ (+ calmodulin)
Tacrolimus
IKK
Rac
Calcineurin
NFATc1/A
NFκB
MEKK1-4
JNK
NFATc1
Akt
GSK3
CK1
NFATc1
Amplification of NFATc1/A
(Fos/Jun) AP-1
MAF
GATA
Transcription of cytokines
(e.g. IL-2)
Nucleus

# Analysis of NFATc1 amplification in T cells for pharmacodynamic monitoring of tacrolimus in kidney transplant recipients




## Conclusion

In conclusion, measuring NFATc1 amplification is a direct tool for monitoring biological effects of tacrolimus on T cells in whole blood samples of kidney transplant recipients. This technique has potential that requires further development before it can be applied in daily practice.




**Biomarkers in Kidney Transplantation. DOI:** https://doi.org/10.1016/B978-0-12-824069-4.00009-4

# What is intra-patient variability (IPV)?

- The tendency for the same dose of tacrolimus to provide fluctuating trough levels over time within a single patient

**How is intra-patient variability assessed clinically?**

## Standard deviation (SD)

$$TacSD_j = \sqrt{\frac{\sum_i (C0_{ij} - \overline{C0}_j)^2}{n_j - 1}}$$

- Measures spread of values around the mean
- Higher value of SD = higher variability

## Coefficient of variation (CV)

$$\text{CV (\%)} = \frac{SD}{mean} \times 100$$

- Ratio of the SD to the mean, expressed as %
- Higher % CV = higher variability

Cervelli M, Russ G. Aus J Pharmacy 2012;93:83-86

# Intra-patient variability

**CV% calculation**

**High variability**

| | Visit 1 | Visit 2 | Visit 3 | Visit 4 | Visit 5 |
|---|---|---|---|---|---|
| Tacrolimus daily dose (mg/day) | 5 | 5 | 5 | 5 | 5 |
| Target trough (ng/mL) | 6.2 | 7.9 | 5.2 | 8.5 | 4.9 |

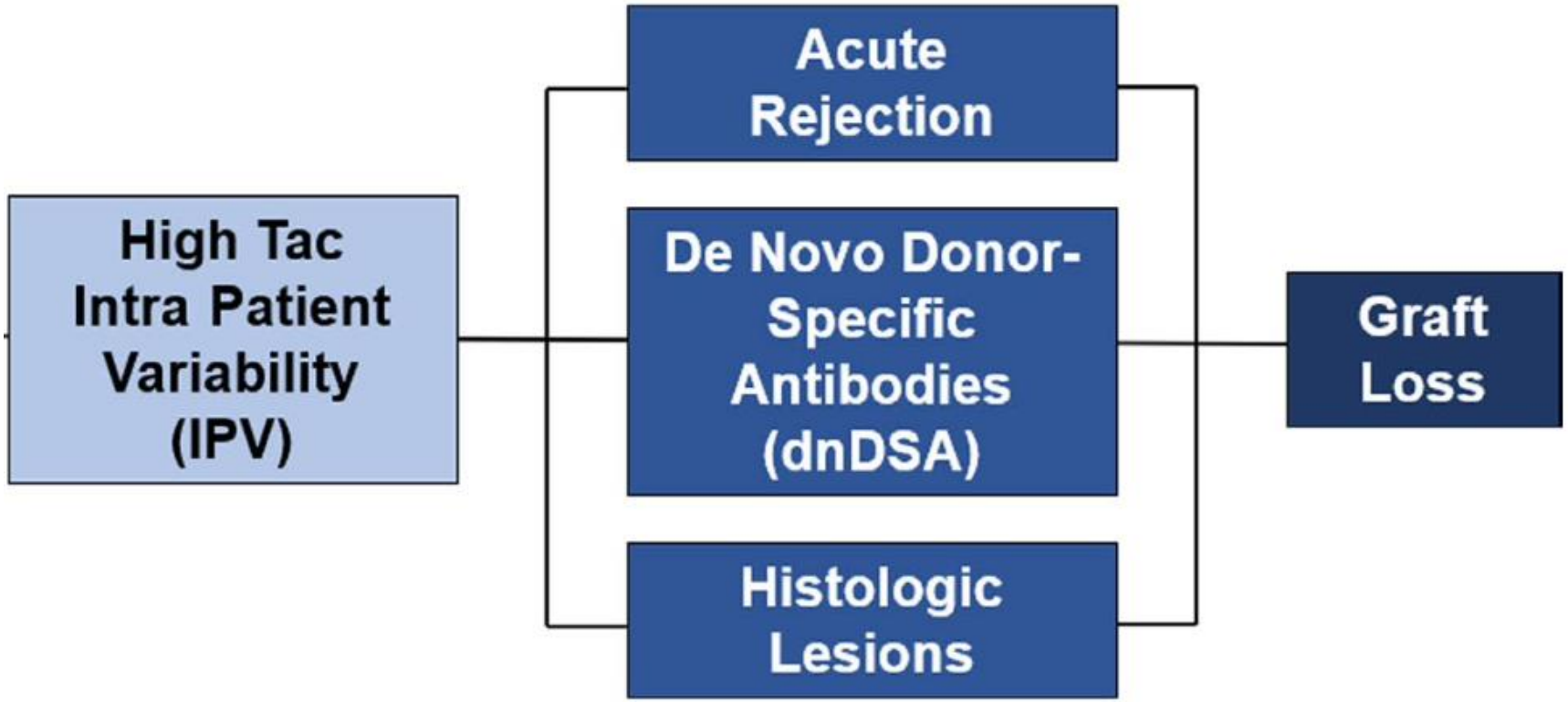
| | |
|---|---|
| SD | 1.60 |
| MEAN | 6.54 |
| CV | 25% |

**Low variability**

| | Visit 1 | Visit 2 | Visit 3 | Visit 4 | Visit 5 |
|---|---|---|---|---|---|
| Tacrolimus daily dose (mg/day) | 5 | 5 | 5 | 5 | 5 |
| Target trough (ng/mL) | 6 | 6.8 | 5.5 | 5.8 | 5.3 |

| | |
|---|---|
| SD | 0.58 |
| MEAN | 5.88 |
| CV | 10% |

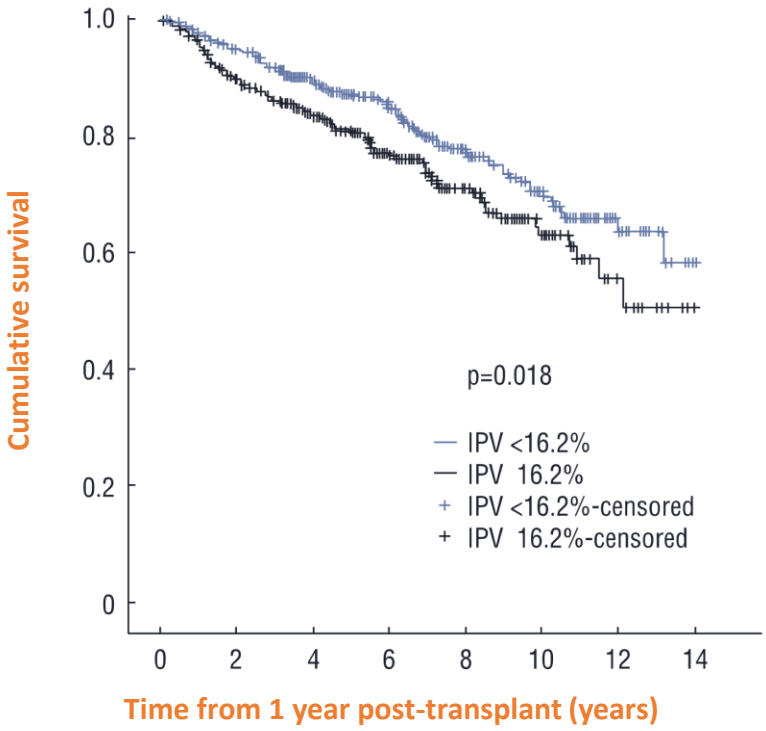
# Clinical Implications




*Am J Transplant*. 2020;20:1969–1983.

# High IPV is associated with poor long-term outcome

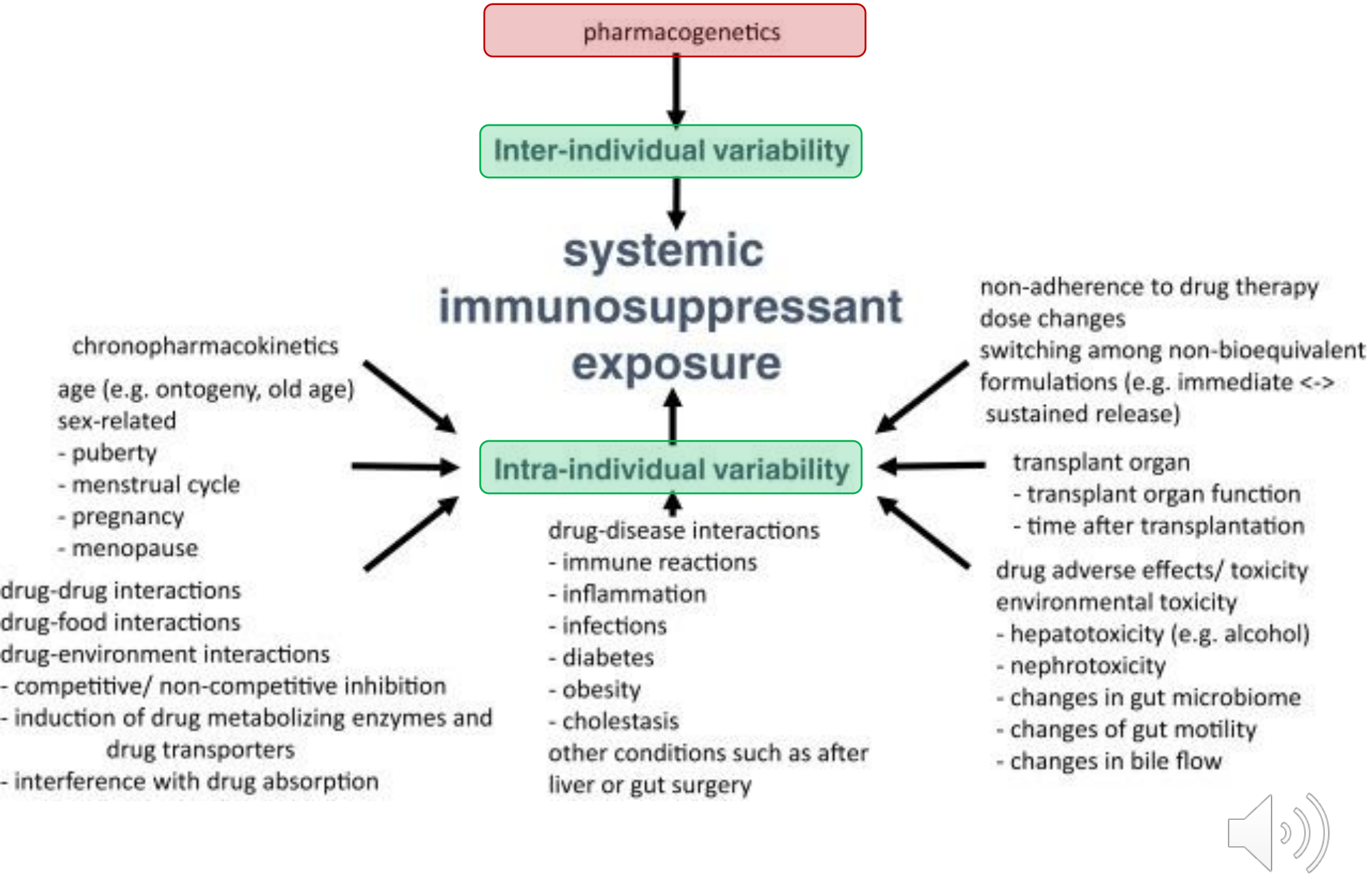
- Retrospective cohort study in which tacrolimus IPV was calculated from pre-dose concentrations measured 6-12 months post-transplantation (N=808)




> **Long-term transplant outcomes were significantly worse in patients with high IPV in tacrolimus exposure (>16.2%) (p=0.018)**

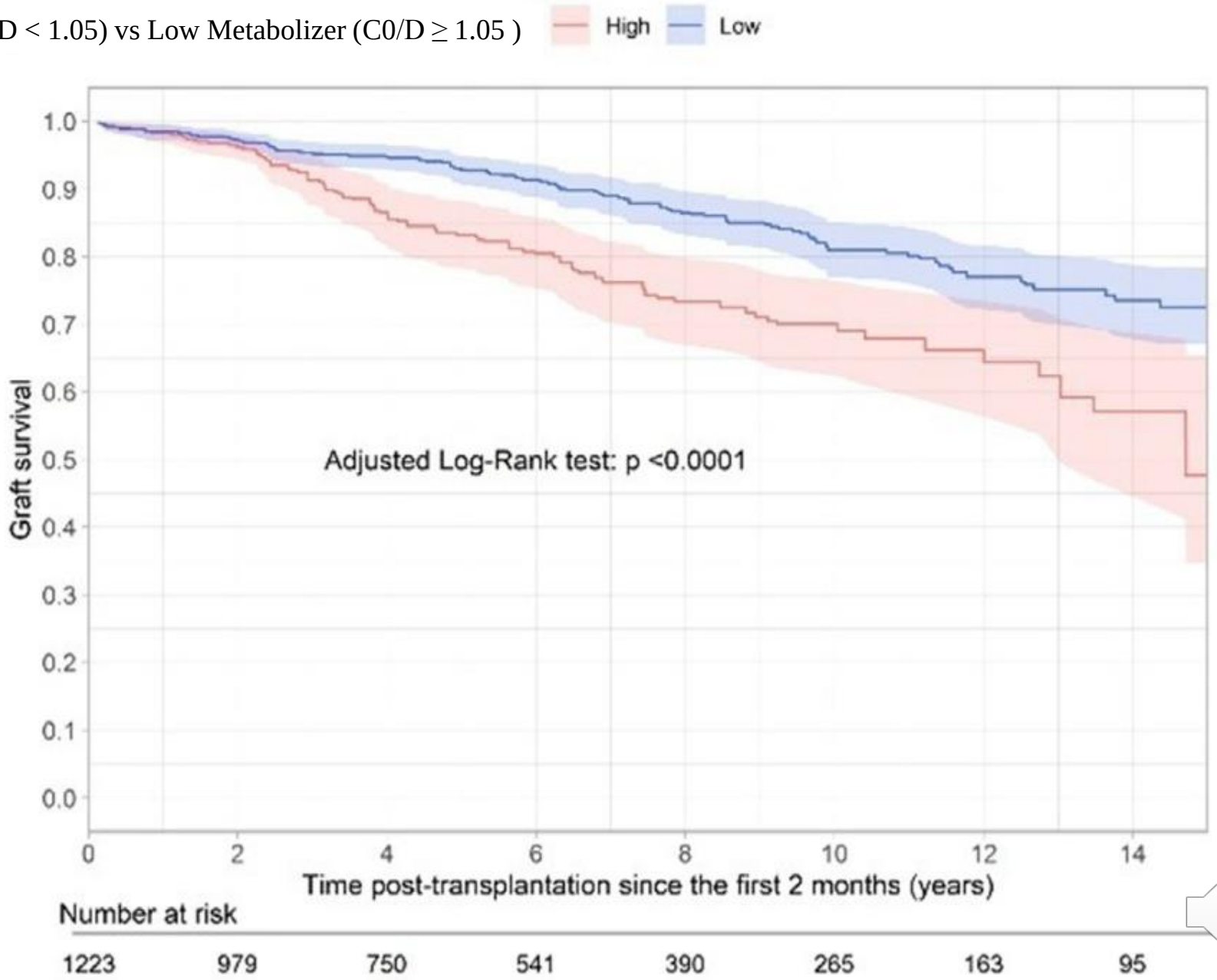
Shuker N, et al. Transpl Int 2016;29:1158-1167.

Intra-patient variability (IPV) in tacrolimus C0 concentrations can be considered a potential biomarker of treatment outcome.



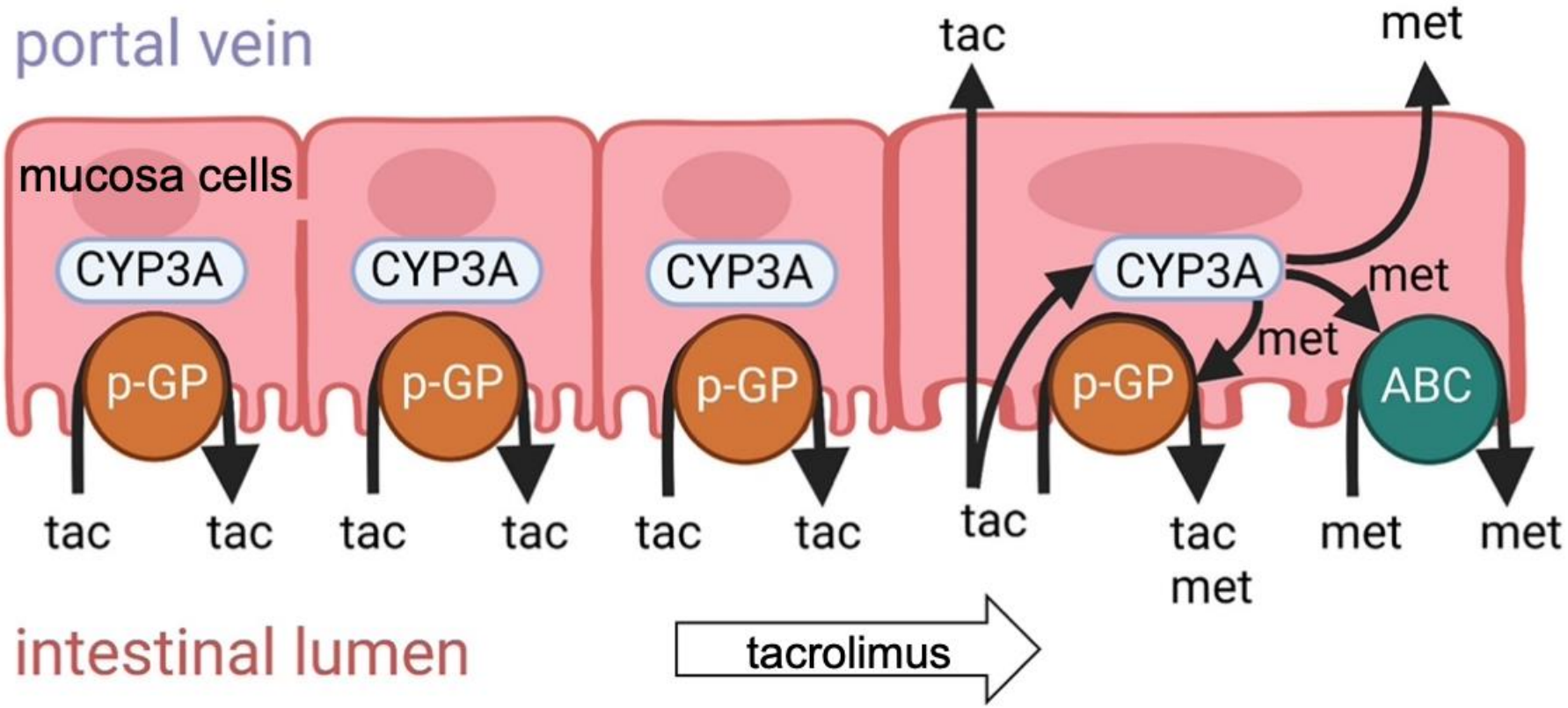

**Biomarkers in Kidney Transplantation. DOI:** https://doi.org/10.1016/B978-0-12-824069-4.00009-4

High (C0/D < 1.05) vs Low Metabolizer (C0/D ≥ 1.05 )




Kidney International Reports (2025) **10,** 1428–1440

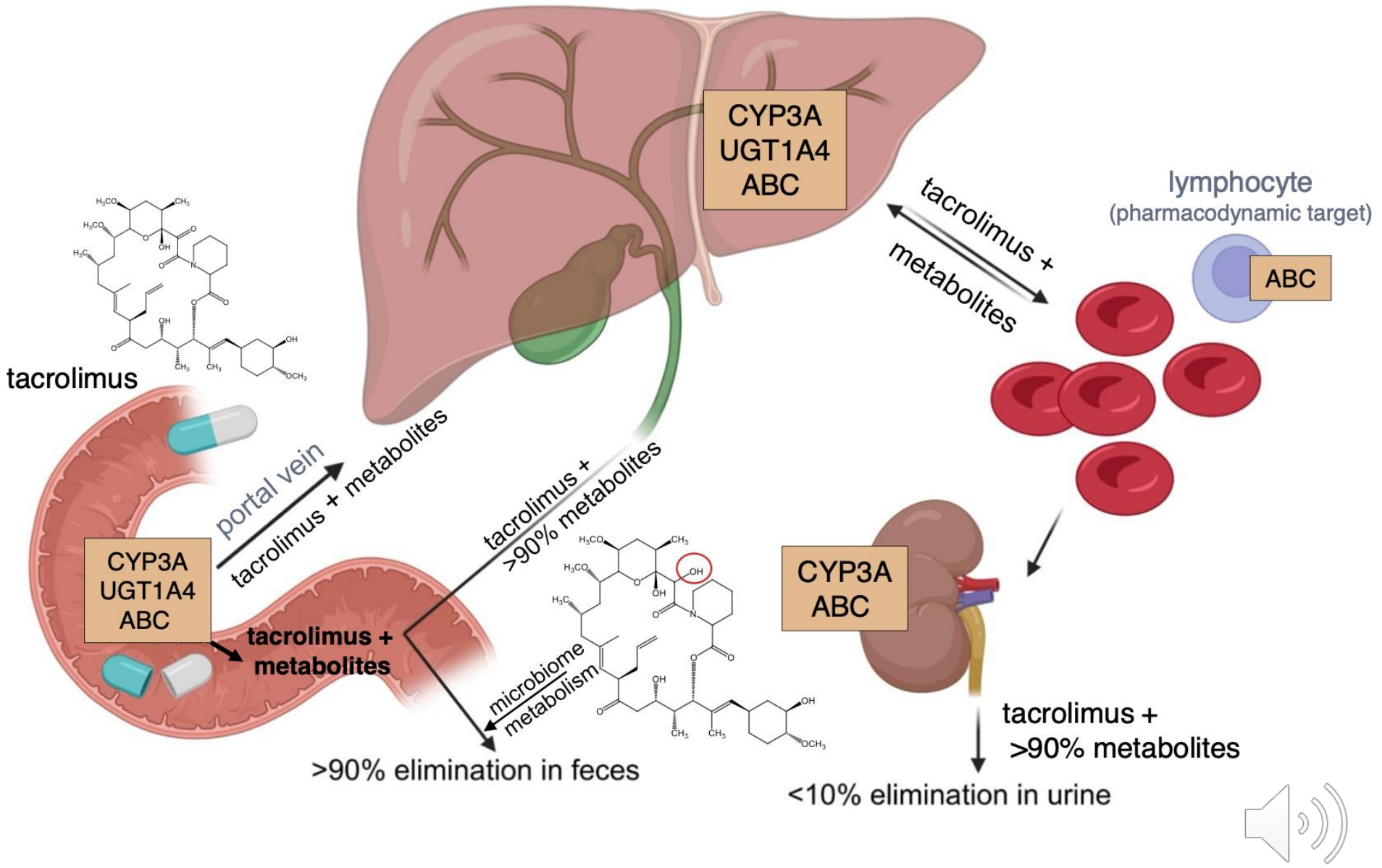
# Tacrolimus Metabolization




The main enzyme involved is CYP3A5, followed by CYP3A4.

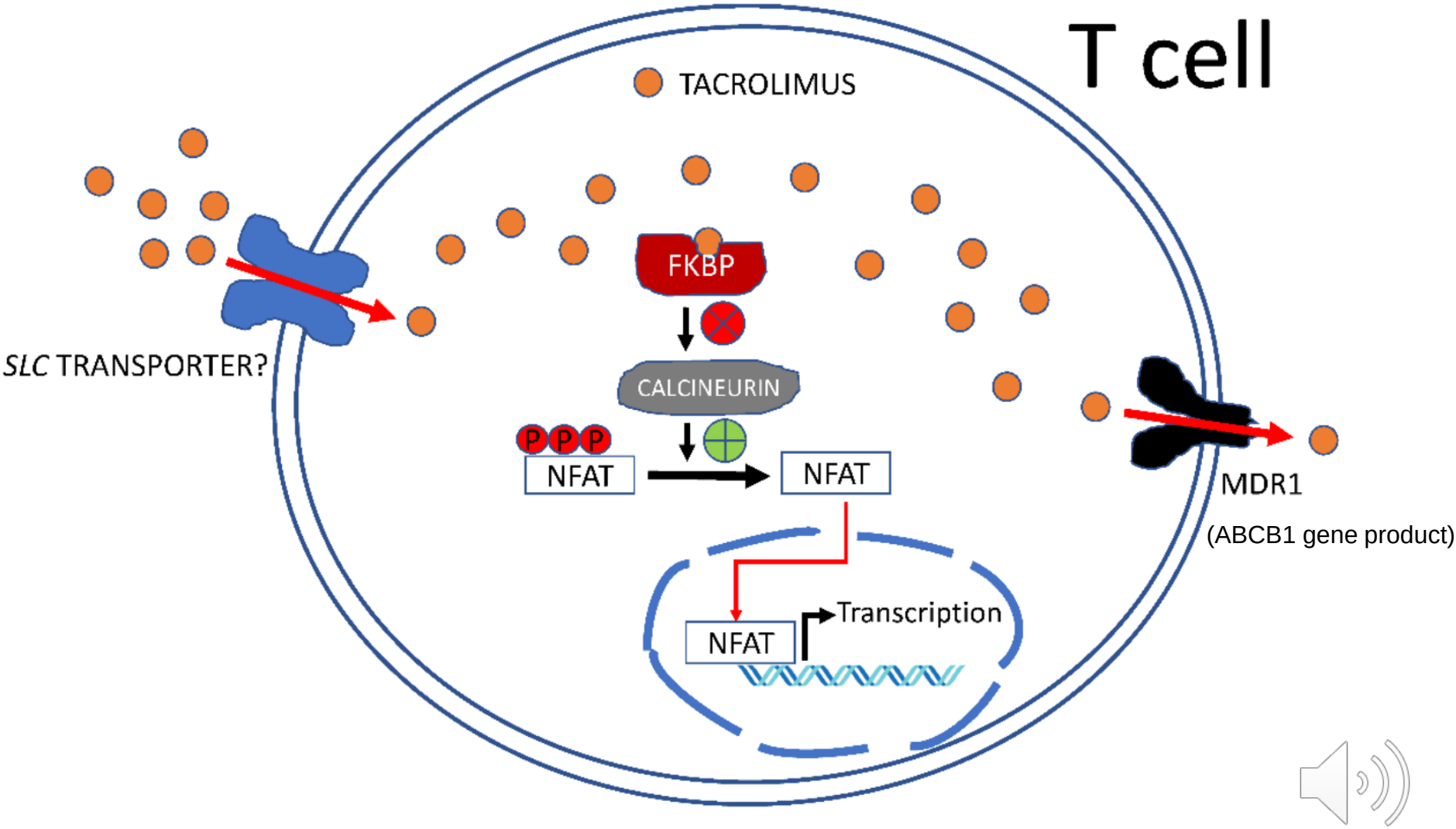
**Biomarkers in Kidney Transplantation. DOI:** https://doi.org/10.1016/B978-0-12-824069-4.00009-4

CYP3A5*3, CYP3A4*22, CYP4A4*1B, ABCB1




**Biomarkers in Kidney Transplantation. DOI:** https://doi.org/10.1016/B978-0-12-824069-4.00009-4

Several genetic polymorphisms in selected SLC and ABC transporter encoding genes have been reported to be associated with tacrolimus exposure and its toxicity.




*Pharmaceutics* **2022**, *14*, 1755. https://doi.org/10.3390/pharmaceutics14091755

# The Influence of CYP3A Pharmacogenetics on Tacrolimus Pharmacokinetics, IPV, C0/D Ratio, and Clinical Outcome

**Table 1 Assignment of likely metabolism phenotypes based on *CYP3A5* diplotypes**

| Likely phenotype | Genotypes | Examples of diplotypes[a] |
|---|---|---|
| Extensive metabolizer (CYP3A5 expresser) | An individual carrying two functional alleles | *1/*1 |
| Intermediate metabolizer (CYP3A5 expresser) | An individual carrying one functional allele and one nonfunctional allele | *1/*3, *1/*6, *1/*7 |
| Poor metabolizer (CYP3A5 nonexpresser) | An individual carrying two nonfunctional alleles | *3/*3, *6/*6, *7/*7, *3/*6, *3/*7, *6/*7 |

[a]Additional rare variants, such as *CYP3A5*2*, *8, and *9 may be found, which are of unknown functional significance. However, if a copy of *1 is present, expected phenotype would be intermediate metabolizer.

| Genes | rsID | Alleles | Pharmacokinetic Parameters |
|---|---|---|---|
| CYP3A5*3 | rs776746 | *3/*3 | ↓ D[a,b] ↓ CL/F ↑$C_0$/D ↑ $C_{max}$/D ↑$AUC_{12}$/D ↑$C_0$ |
| CYP3A5*6 | rs10264272 | *6/*1 *6/*6 | ↑$C_0$/D |
| CYP3A5*7 | rs41303343 | *7/*1 *7/*7 | ↑$C_0$/D |
| CYP3A4*1B[c] | rs2740574 | *1B/1 *1B/*1B | ↑D ↓$C_0$/D |
| CYP3A4*18[c] | rs28371759 | *18/*1 *18/*18 | ↑CL/F ↓$C_0$/D ↓$C_2$/D |
| CYP3A4*22 | rs35599367 | *22/*1 *22/*22 | ↑$C_0$/D ↓D |

| Genes | rsID | Genotype | Tac-induced Adverse Events Risk | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Acute Rejection | Nephrotoxicity | DGF | PTDM | Hypertension | Neurotoxicity |
| **CYP3A5*3** | rs776746 | *3/*3 | | a↑[31, 34]<br>↓[30] | ↑[58] | | ↓[35, 36] | ↓[70] |
| **ABCB1 3435C > T** | rs1045642 | T-T | | ↑[50] (D&R)<br>↓[37] (D) | | | | ↓[38] |
| **ABCB1 2677G > T/A** | rs2032582 | G-(T/A) or<br>(T/A)-(T/A) | | | | | | ↑[38]<br>↓[11] |
| **ABCB1 1236C > T** | rs1128503 | C-C | | | | | | ↑[11] |

**TABLE 6 |** Association of CYP3A5 expressor status with post-transplant complications.

| Complication | Occurrence | CYP3A5 non-expressor | CYP3A5 expressor | OR (95%CI) | *p* value |
|---|---|---|---|---|---|
| NODAT | YES | 39 | 28 | 2.22 (1.14–4.33) | 0.018[a] |
| | NO | 30 | 48 | | |
| Rejection | NO | 58 | 52 | 2.43 (1.08–5.44) | 0.028[a] |
| | YES | 11 | 24 | | |
| Tacrolimus toxicity | YES | 22 | 18 | 1.51 (0.72–3.15) | 0.266 |
| | NO | 46 | 57 | | |

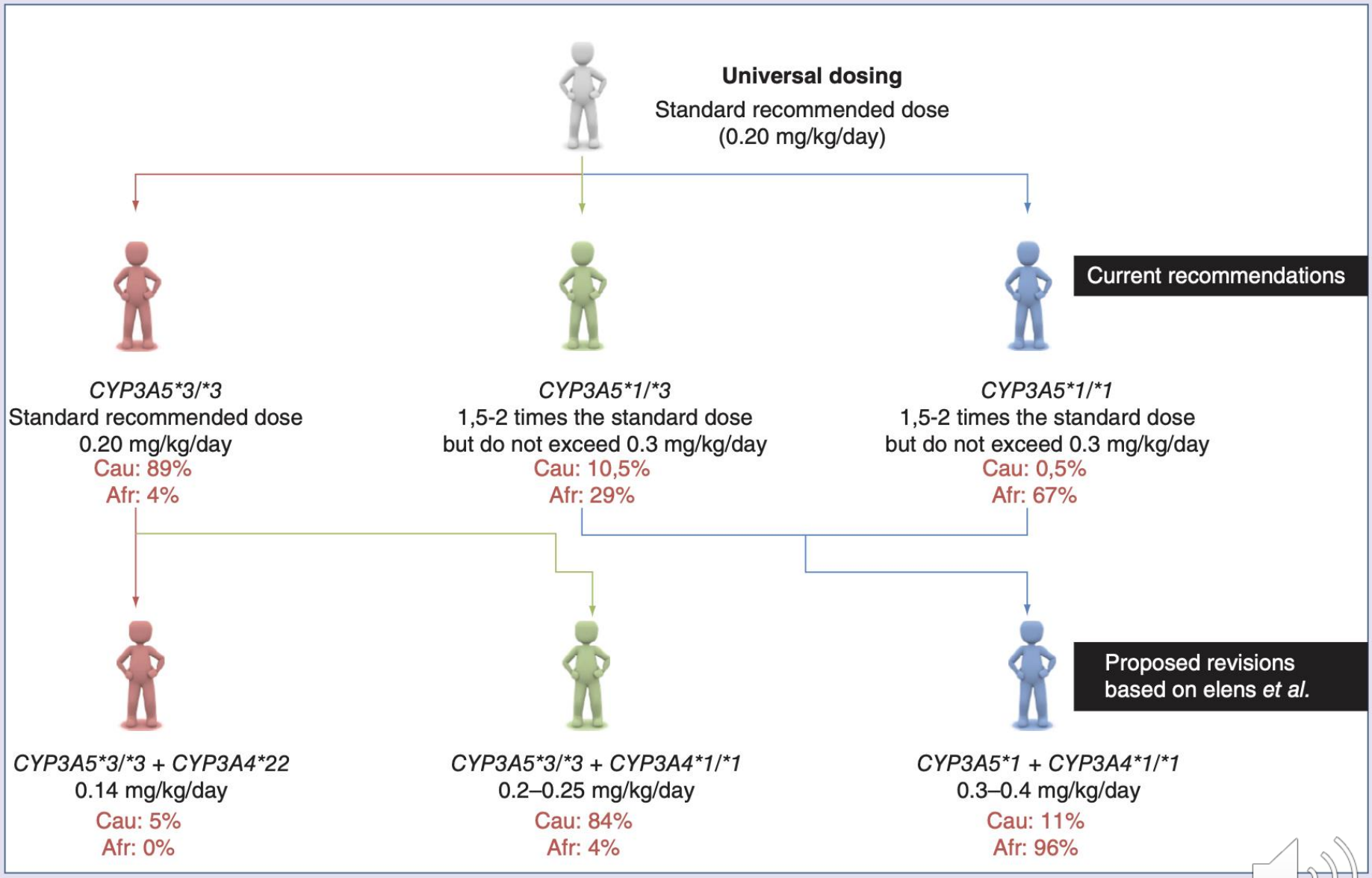
[a]*Statistically significant.*

**Table 2 Dosing recommendations for tacrolimus based on *CYP3A5* phenotype**

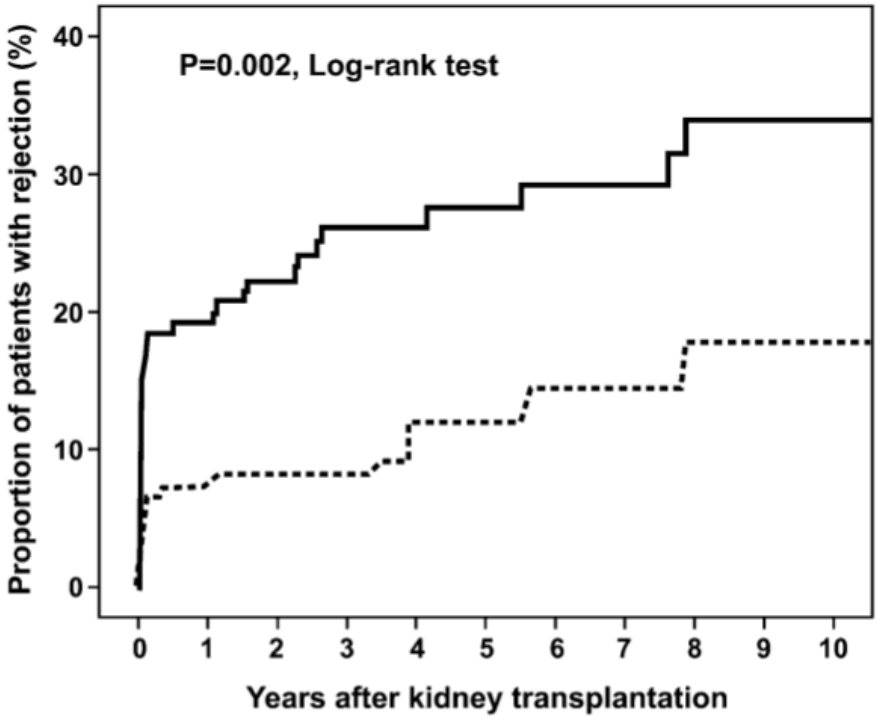
| *CYP3A5* phenotype[a] | Implications for tacrolimus pharmacologic measures | Therapeutic recommendations[b] | Classification of recommendations[c] |
|---|---|---|---|
| Extensive metabolizer (CYP3A5 expresser) *1/*1 | Lower dose-adjusted trough concentrations of tacrolimus and decreased chance of achieving target tacrolimus concentrations. | Increase starting dose 1.5–2 times recommended starting dose.[d] Total starting dose should not exceed 0.3 mg/kg/day. Use therapeutic drug monitoring to guide dose adjustments. | Strong |
| Intermediate metabolizer (CYP3A5 expresser) | Lower dose-adjusted trough concentrations of tacrolimus and decreased chance of achieving target tacrolimus concentrations. | Increase starting dose 1.5–2 times recommended starting dose.[a] Total starting dose should not exceed 0.3 mg/kg/day. Use therapeutic drug monitoring to guide dose adjustments. | Strong |
| Poor metabolizer (CYP3A5 nonexpresser) *3/*3 | Higher ("normal") dose-adjusted trough concentrations of tacrolimus and increased chance of achieving target tacrolimus concentrations. | Initiate therapy with standard recommended dose. Use therapeutic drug monitoring to guide dose adjustments. | Strong |

**TABLE 4.** CYP3A Combined Genotype Classification[258]

| | ***CYP3A4*22* Carriers (*CYP3A4*1/*22* or **22/*22*)** | ***CYP3A4*22* Noncarriers** |
|---|---|---|
| CYP3A5*1 noncarriers or CYP3A5 nonexpressers (eg, CYP3A5*3/*3) | CYP3A poor metabolizers PM | CYP3A intermediate metabolizers IM |
| CYP3A5*1 carriers or CYP3A5 expressers (eg, CYP3A5*1/*3 or *1/*1) | CYP3A intermediate metabolizers IM* | CYP3A normal metabolizers NM |

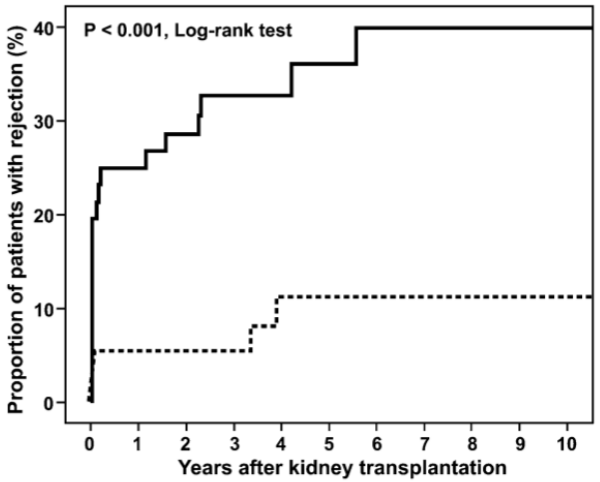

Universal dosing
Standard recommended dose
(0.20 mg/kg/day)
Current recommendations
CYP3A5*3/*3
Standard recommended dose
0.20 mg/kg/day
Cau: 89%
Afr: 4%
CYP3A5*1/*3
1,5-2 times the standard dose
but do not exceed 0.3 mg/kg/day
Cau: 10,5%
Afr: 29%
CYP3A5*1/*1
1,5-2 times the standard dose
but do not exceed 0.3 mg/kg/day
Cau: 0,5%
Afr: 67%
Proposed revisions
based on elens et al.
CYP3A5*3/*3 + CYP3A4*22
0.14 mg/kg/day
Cau: 5%
Afr: 0%
CYP3A5*3/*3 + CYP3A4*1/*1
0.2–0.25 mg/kg/day
Cau: 84%
Afr: 4%
CYP3A5*1 + CYP3A4*1/*1
0.3–0.4 mg/kg/day
Cau: 11%
Afr: 96%

# CYP3A5 Genotype and Tacrolimus IPV



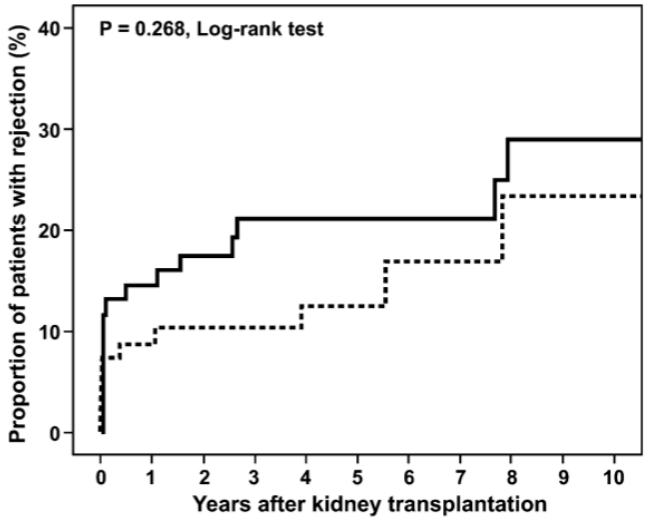

| Patients at the risk | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| High Tacrolimus variability —— | 125 | 101 | 90 | 67 | 60 | 47 | 43 | 34 | 28 | 20 | 16 |
| Low Tacrolimus variability ---------- | 124 | 115 | 111 | 89 | 64 | 40 | 34 | 27 | 23 | 15 | 9 |

**A**
**CYP3A5 expressers**
**(n = 111)**




| Patients at the risk | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| High Tacrolimus variability ——— | 56 | 42 | 37 | 26 | 22 | 18 | 16 | 11 | 10 | 5 | 4 |
| Low Tacrolimus --------- | 55 | 52 | 51 | 38 | 28 | 18 | 16 | 13 | 11 | 7 | 5 |

**B**
**CYP3A5 non-expressers**
**(n = 138)**




| Patients at the risk | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| High Tacrolimus variability ——— | 69 | 59 | 53 | 41 | 38 | 29 | 27 | 23 | 18 | 15 | 12 |
| Low Tacrolimus variability --------- | 69 | 63 | 60 | 51 | 36 | 22 | 18 | 14 | 12 | 8 | 4 |

**Conclusions:** The IIV of tacrolimus trough concentrations had a significant impact on rejection-free survival. The effect was influenced by *CYP3A5* polymorphism, although the tacrolimus variability itself was not determined by the *CYP3A5* polymorphism.

# Pharmacogenetics Based Dose Prediction Model for Initial Tacrolimus Dosing in Renal Transplant Recipients

*Lekshmy Srinivas[1]*, Noble Gracious[2] and Radhakrishnan R. Nair[1]**

[1]*Laboratory Medicine and Molecular Diagnostics, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India,*
[2]*Department of Nephrology, Government Medical College, Thiruvananthapuram, India*

**Equation 1** Dosing equation to calculate starting tacrolimus dose/kg to attain optimal initial post-transplant period tacrolimus level

$$\textit{Required tacrolimus dose/kg} = \frac{\textit{Desired tacrolimus level on Day } 6}{159 - (40 \times \textit{CYP3A5 genotype})} \quad (1)$$

*CYP3A5* genotype = 0 for *3/*3, 1 for *1/*3, and 2 for *1/*1

Frontiers in Pharmacology doi: 10.3389/fphar.2021.726784

# FACTORS AFFECTING TACROLIMUS TROUGH LEVEL ($C_0$) AND PHARMACOKINETIC ACTIVITY

## 1. ALBUMIN (Hypoalbuminemia)

Tacrolimus binds mainly to albumin

Albumin ↓

↓

Protein binding ↓ (mainly to albumin)

↓

Unbound (free) fraction ↑

↓

→ Pharmacologically active tacrolimus ↑

## 2. HEMATOCRIT (Low)

Hematocrit ↓

↓

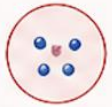
Erythrocyte binding ↓ (RBCs act as a binding site)

↓

Whole-blood tacrolimus concentration ($C_0$) ↓

↓

Apparent clearance ↑



Tacrolimus in RBC

## 3. AGE (Young)

Young age

↓

CYP3A activity / metabolic capacity ↑

↓

Tacrolimus clearance (CL) ↑

↓

Tacrolimus $C_0$ ↓

## 4. BSA (Body Surface Area)

BSA ↑

↓

Metabolic mass ↑ (liver size, blood flow, organ function)

↓

Tacrolimus clearance (CL) ↑

↓

Dose requirement ↑ (to achieve target $C_0$/AUC)

## 5. DRUG–DRUG INTERACTIONS

### CYP3A INHIBITORS
(decrease tacrolimus clearance)

- Azole antifungals (e.g., voriconazole)
- Macrolides (e.g., clarithromycin)
- Protease inhibitors
- Calcium channel blockers (e.g., diltiazem)
- Amiodarone, grapefurit juice

↓

CYP3A inhibition ↑

↓

Tacrolimus clearance ↓

↓

Tacrolimus $C_0$ ↑ (risk of toxicity)

### CYP3A INDUCERS
(increase tacrolimus clearance)

- Rifampin, rifabutin
- Phenytoin, carbamazepine
- St. John's wort

**STEROIDS (e.g., Prednisone)**
Induce CYP3A expression/activity

↓

Tacrolimus clearance ↑

↓

Tacrolimus $C_0$ ↓ (risk of rejection)

ELSEVIER

Transplantation Proceedings

Volume 48, Issue 4, May 2016, Pages 1176-1178


# Correlation of Hematocrit and Tacrolimus Level in Liver Transplant Recipients

S. Limsrichamrern, C. Chanapul, P. Mahawithitwong*, Y. Sirivatanauksorn, P. Kositamongkol, S. Asavakarn, C. Tovikkai, and W. Dumronggittigule

$$\text{Corrected tacrolimus level} = (0.21 \times \text{tacrolimus}) + [0.89 \times \text{tacrolimus} \times (40/\text{Hct})]$$

- Approximately 70–80% of tacrolimus is distributed in erythrocytes, low hematocrit will reduce the whole-blood concentrations of tacrolimus
- Patients with a lower hematocrit had higher CL/F.

British Journal of Clinical Pharmacology

Br J Clin Pharmacol (2019) **85** 601–615 601

# ORIGINAL ARTICLE

# A population pharmacokinetic model to predict the individual starting dose of tacrolimus in adult renal transplant recipients

- In the first 3 months post-transplantation, age, albumin, body surface area, serum creatinine, *CYP3A5* genotype, *CYP3A4* genotype, haematocrit and lean bodyweight significantly influence the pharmacokinetics of tacrolimus in adult renal transplant recipients.
- A separate model for the starting dose was developed:

$$\begin{aligned} Dose\ (mg) = {} & 222\ \text{ng h ml}^{-1} * \ 22.5\ \text{l h}^{-1} \\ & *[(1.0, \textit{if}\ \text{CYP3A5}*3/*3)\ \textit{or}\ (1.62, \text{if CYP3A5}*1/*3 \text{ or CYP3A5}*1/*1)] \\ & *[(1.0, \textit{if}\ \text{CYP3A4}*1 \text{ or unknown})\ \textit{or}\ (0.814, \text{if CYP3A4}*22)] * \left(\frac{Age}{56}\right)^{-0.50} * \left(\frac{BSA}{1.93}\right)^{0.72} /1000 \end{aligned}$$

- The tacrolimus starting dose should be higher in *CYP3A5* expressers, younger patients and those with a higher body surface area (BSA). It should be lower in patients carrying the *CYP3A4*22* allele.
- The starting dose model can be used to individualize the tacrolimus starting dose following kidney transplantation.

C0 of 10 ng ml–1 ≈ an AUC0-12h of 222 ng h ml–1

| Tacrolimus | Time after transplantation | Target $AUC_{0\text{-}12h}$ | Corresponding target range C0 |
|---|---|---|---|
| (Prograft, Adport) | <6 weeks (triple therapy MPA + PRED) | 160 μg*h/L | 8–10 μg/L |
| | >6 weeks (triple therapy MPA + PRED) | 120 μg*h/L | 5–7 μg/L |
| | >6 months (triple therapy MPA + PRED) | 80 μg*h/L | 3–5 μg/L # |

*Note*: # C0 levels <5 μg/L should only be accepted when corresponding $AUC_{0\text{-}12\ h}$ is >75 μg/L (see Meziyerh *et al.*). Recommendations for these targets are based on: <6 weeks: Ekberg *et al.*[48] and Moes *et al.*[49]; >6 weeks: Moes *et al.* (in preparation); >6 months: Meziyerh *et al.*[26]

| Tacrolimus | Time after transplantation | Target $AUC_{0\text{-}24h}$ | Corresponding target range C0 |
|---|---|---|---|
| (Advagraf, Dailiport) | <6 weeks (triple therapy MPA + PRED) | 320 μg*h/L | 9–11 μg/L |
| | >6 weeks (triple therapy MPA + PRED) | 240 μg*h/L | 7–8 μg/L |
| | >6 months (triple therapy MPA + PRED) | 160 μg*h/L | 4–6 μg/L # |

*Note*: # C0 levels <5 μg/L should only be accepted when corresponding $AUC_{0\text{-}24\ h}$ is >150 μg/L (see Meziyerh *et al.*[26]).

Basing the tacrolimus starting dose on the patient's CYP3A genotype may be beneficial:

1. The targeted C0 was achieved sooner.
2. Required fewer dose modifications.

# The CYP3A5 Genotype and Tacrolimus C0/D Ratio

- Patients with a C0/D ratio of <1.05 ng/ml/mg were characterized as fast metabolizers.
- Patients with a C0/D ratio of 1.05–1.54 ng/ml/mg were characterized as intermediate metabolizers.
- Those with a C0/D ratio of >1.55 ng/ml/mg were defined as low metabolizers.

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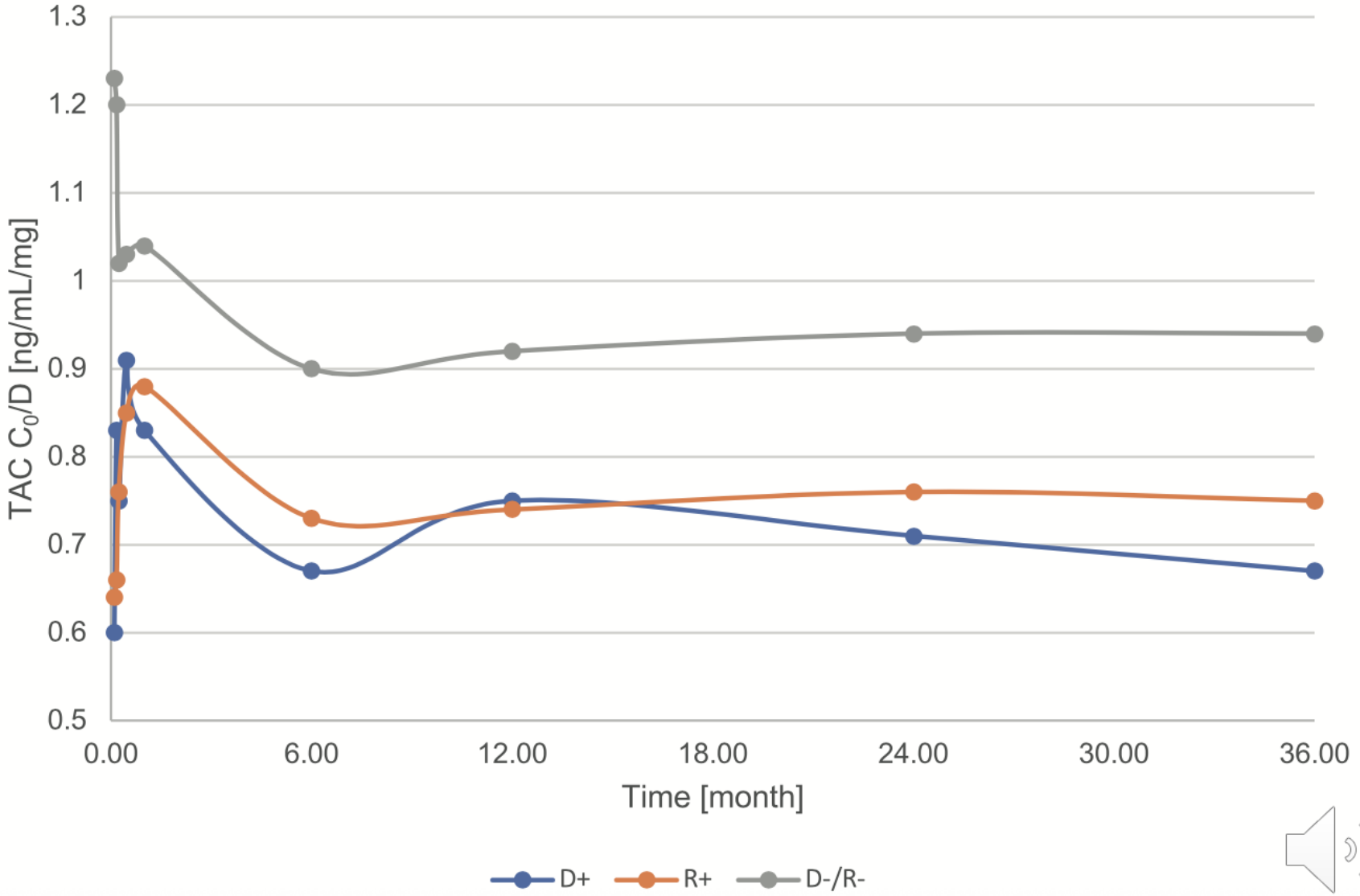
# The Effect of Donor-Recipient CYP3A5 genotype on TAC $C_0$/D

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# Tacrolimus Intrapatient Variability on Graft Outcomes in Adherent Renal Transplantation Patients: A Cross-Sectional Study

82.1% patients had C0/D > 1.55 ng/mL/mg.

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**Results.** Of 202 transplant recipients, 128 were included with a mean age of 45.48 ± 13.14 years. The median Tac-IPV was 13.28% with 43.75% of patients with Tac-IPV > 15%. There were no significant differences in graft function, rejection, CNI toxicity, and CMV viremia among the groups during the 24-month study ($P > .05$). However, BK viremia was significantly higher among patients with Tac-IPV > 15% (13 vs. 2.9%, $P = .042$). The risk of antibody-mediated rejection alone (22.7 vs. 2.9%) or any kind of rejection (22.7 vs. 11.8%) was significantly higher in patients with higher Tac-IPV, and in those who had mean trough levels below 7 ng/mL ($P = .015, .032$; respectively).

**Conclusion.** Tac-IPV is low in adherent patients (with the median of 13.28%) and maintaining tacrolimus trough level above 7 ng/mL can overcome the adverse graft outcome of Tac-IPV in compliant kidney transplant recipients.

# Take Home Message

- Pharmacogenetics helps explain “how much tacrolimus the patient needs.”
- IPV explains “how stable tacrolimus exposure remains over time.”
- The C0/D ratio in post-transplantation period explains “metabolizer phenotype”

# Take Home Message

- Precision immunosuppression is an integrated approach that combines pharmacogenomic insights with phenotypic measures, clinical context, laboratory data and a deeper understanding of tissue-level drug exposure.


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Dose = CL/F * AUC.

$$
\begin{aligned}
{}^{CL}\!/_{F} = 22.5 * [&(1.0, \text{if CYP3A5} * 3/*3) \; \textit{or} \\
&(1.62, \text{if CYP3A5} * 1/*3 \text{ or CYP3A5} * 1/*1)] \\
&*[(1.0, \text{if CYP3A4} * 1 \text{ or unknown}) \; \textit{or} \\
&(0.814, \text{if CYP3A4} * 22)] * \left(\frac{Age}{56}\right)^{-0.50} * \left(\frac{BSA}{1.93}\right)^{0.72}
\end{aligned}
$$

# Will Membrane Transporters Ever Become Pharmacogenetic Biomarkers of Tacrolimus Pharmacokinetics and Pharmacodynamics?

- In patients with a faster metabolism (CYP3A5*1/*3, CYP3A5*1/*1), the dose required to reach target tacrolimus trough concentrations is higher.
  - A low tacrolimus C0/D.
  - High IPV

**TABLE 5.** PD Biomarkers and Assay Platforms to Assess the Effect of Tacrolimus

| Biomarker | Assay |
|---|---|
| CaN activity | $^{32}P$ release from a synthetic phosphorylated peptide |
| | Dephosphorylation of a synthetic peptide by HPLC |
| | Dephosphorylation of synthetic peptide by LC-MS/MS |
| Dephosphorylated proteins in signal transduction pathways | Phosphoflow cytometry |
| Nuclear translocation of NFAT | Flow cytometry |
| NFAT-regulated gene expression | Real-time PCR |
| Intracellular cytokines and chemokines | Flow cytometry |
| Cytokine production by T cells | ELISPOT |
| T-cell subsets (regulatory T cells) | Flow cytometry, qPCR. |
| T-cell surface marker expression | Flow cytometry |
| T-cell proliferation | PCNA expression by qPCR, CSFE staining by flow cytometry |
| Graft-derived cell-free DNA | Digital droplet PCR |
| ATP release from $CD4^+$ T cells | Luminescence |

ATP, adenosine triphosphate; CaN, calcineurin; CFSE, carboxyl fluorescein diacetate succinimidyl ester; ELISPOT, enzyme-linked immunospot; HPLC, high-performance liquid chromatography; PCNA, proliferating cell nuclear antigen; qPCR, quantitative polymerase chain reaction.

**Table 2.** Demographic factors affecting tacrolimus pharmacokinetics and associated recommendations.

| FACTOR | PK IMPACT | STRENGTH | RECOMMENDATIONS |
|---|---|---|---|
| Age (˃ 65 years)[27,80] | ↑ concentration (up to 50%) normalized to dose ↓ Cl/F | Strong | Consider dose reduction in elderly, based on regular TAC monitoring. Consider follow-up of TAC-induced neurotoxicity and nephrotoxicity. |
| Gender[25,26] | / | Weak | Consider dosage adjustment on an mg/kg basis sufficient to limit gender variability. |
| Ethnicity[87–90] | Lower TAC concentration in Afro-American population<br>Higher TAC bioavailability in Asian population | Strong | Ethnic factors highly correlate to genetic polymorphisms, (see pharmacogenetic section). |

TAC = tacrolimus, ↑ = increased, ↓ = decreased, Cl/F = apparent clearance.

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