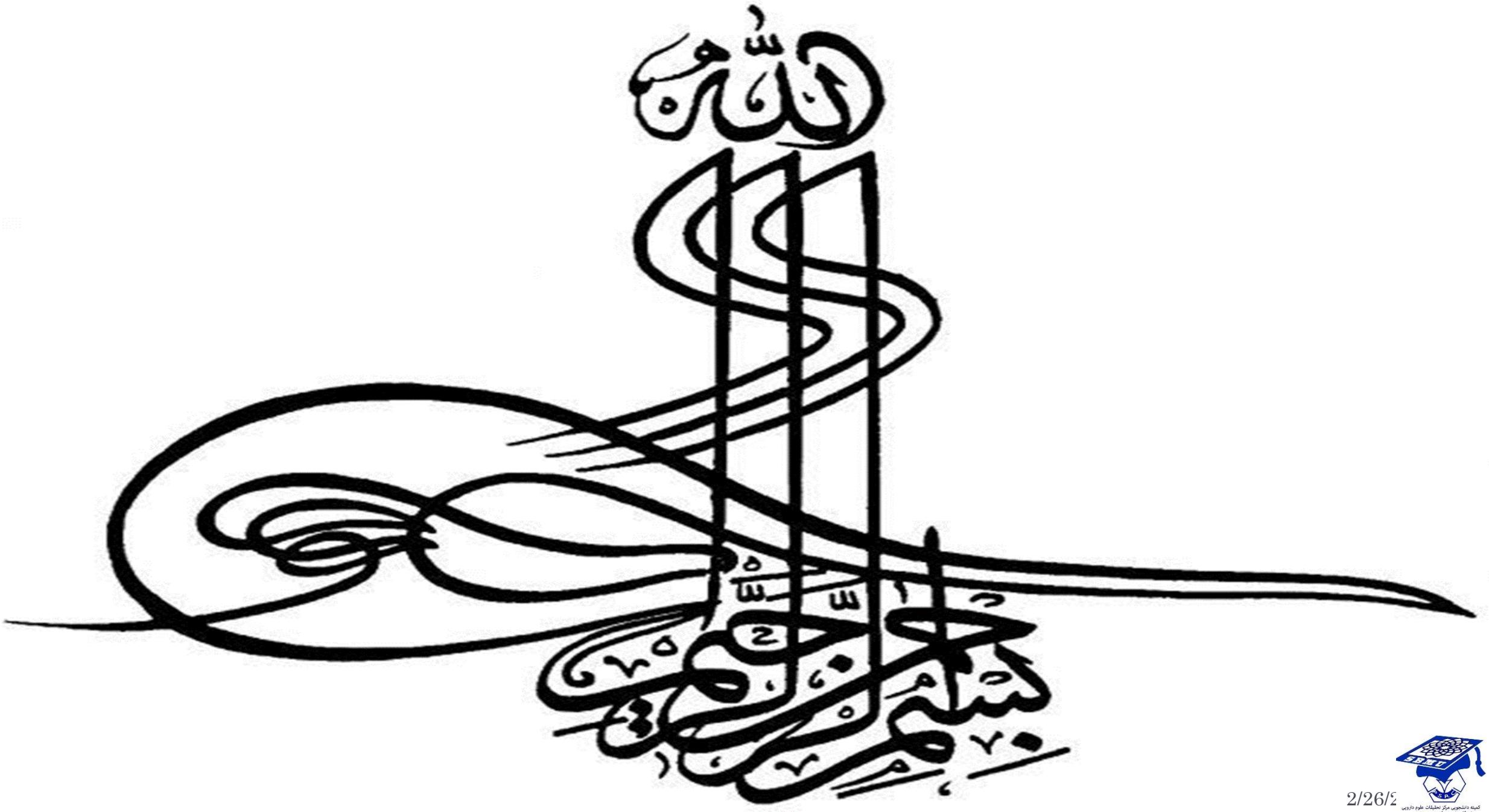

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Therapeutic Drug Monitoring (TDM) in

Patients with Kidney Transplant

Brief History of Transplant Immunosuppression

- The current era of transplantation was pioneered in 1954 at Harvard by Joseph Murray with the successful, Nobel Prize winning, living donor transplantation between the identical Herrick twins.
- The first attempts at immunosuppression used total-body irradiation
- Azathioprine was introduced in the early 1960s and was soon routinely accompanied by prednisolone
- The polyclonal antibody preparations antithymocyte globulin (ATG) and antilymphocyte globulin (ALG) became available in the mid-1970s.
- Azathioprine and prednisolone became the baseline regimen for maintenance immunosuppression following kidney transplantation, with ATG or ALG used for induction or for the treatment of steroid-resistant rejection.

- With this protocol, the success rate of kidney transplantation was about 50% at 1 year, acute rejection rates were approximately 60%, and the mortality rate was typically 10% to 20%.
- The situation was transformed in the early 1980s with the introduction of the cyclosporine.
  - Statistically significant improvement in graft survival rates to greater than 80% at 1 year and a marked reduction in rejection rates to 30% to 40%.
  - Mortality rates decreased with more effective immunosuppression, reduced use of corticosteroids, and overall improvements in surgical and medical care
  - Although the benefits of cyclosporine were clear cut, its capacity to produce both acute and chronic nephrotoxicity was soon recognized to be a major detriment.

In 1985, OKT3, the first monoclonal antibody used in clinical medicine, was introduced based on its capacity to treat first acute rejection episodes.

- The toxicity of the drug tended to restrict its use to episodes of rejection that were resistant to high-dose steroids and, in some programs, to use as an induction agent.

- With this limited armamentarium of medications—cyclosporine, azathioprine, corticosteroids, and the antibody preparations—the transplantation community entered the 1990s, achieving, with justifiable pride, success rates of up to 90% in many centers and minimal mortality.

- Because the number of available immunosuppressive medications was small, there was relatively little variation among the protocol options used in different programs.

Two Major Developments then Followed

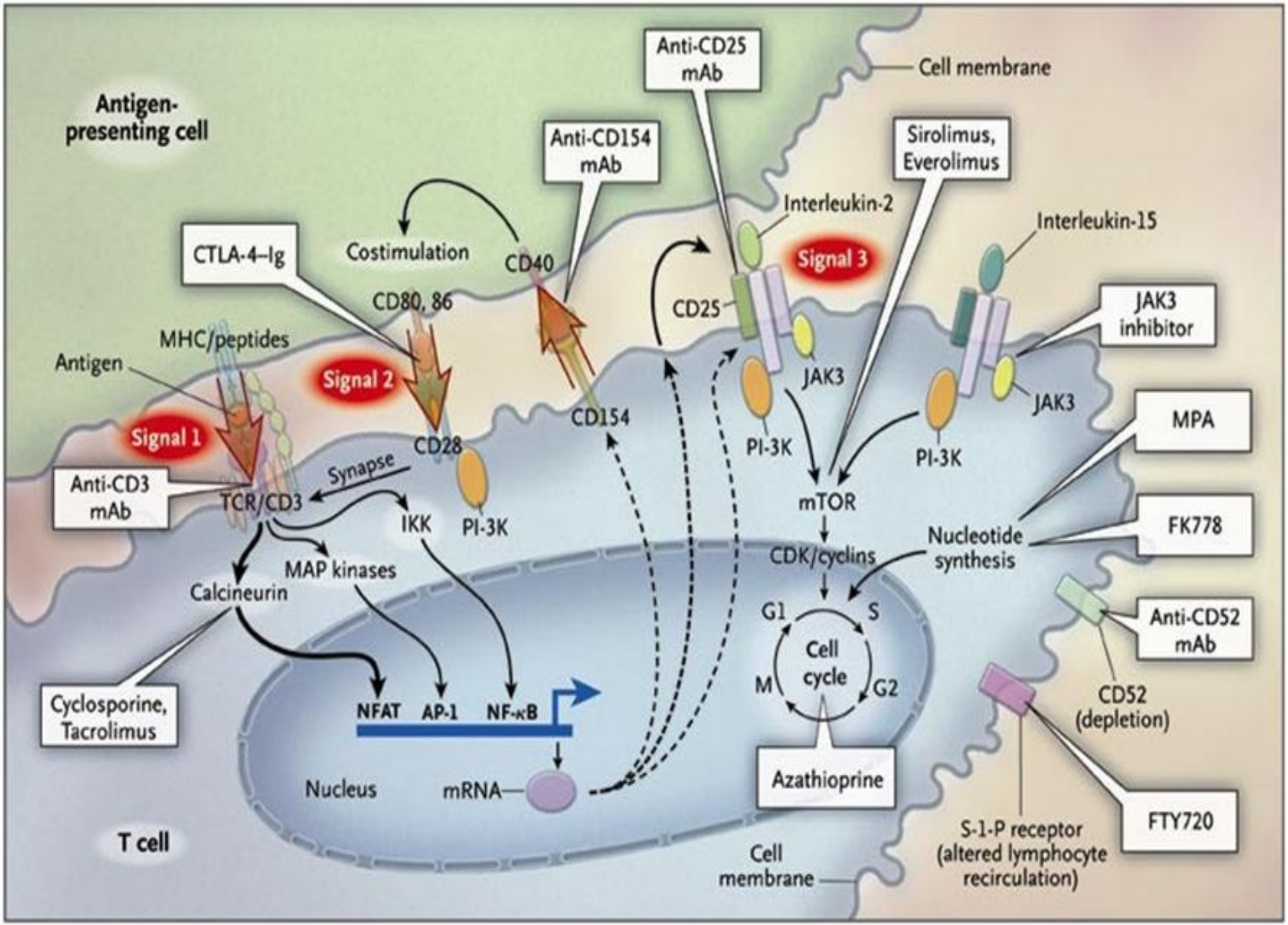
- **Tacrolimus** was introduced into liver transplantation and eventually into kidney transplantation as an alternative to cyclosporine because of its capacity to produce equivalent patient and graft survival
- **Mycophenolate mofetil (MMF)** was found to be a more effective agent than azathioprine by virtue of its capacity to reduce the incidence of acute rejection episodes when used with cyclosporine (and later with tacrolimus) and corticosteroids.

- **Basiliximab** and **Daclizumab**, two humanized monoclonal antibodies, were approved for use after kidney transplantation, also based on their capacity to reduce the incidence of acute rejection episodes

A polyclonal antibody, **Thymoglobulin**, available in Europe for several years, was approved for use in the United States for the treatment of acute rejection.

- In 1999, a class of new immunosuppressive medications, **the mTOR inhibitors**, was introduced. Initially, **sirolimus** was approved by the FDA; a similar drug, **everolimus**, was later introduced in Europe and gained FDA approval in 2010.
- The last major immunosuppressive medication to garner FDA approval for kidney transplantation was **belatacept** in 2011.

Immunosuppressive Agents in Current Clinical Use


Antigen-presenting cell
Anti-CD25 mAb
Cell membrane
Anti-CD154 mAb
Sirolimus, Everolimus
Interleukin-2
Interleukin-15
CTLA-4–Ig
Costimulation
CD40
Signal 3
CD25
JAK3 inhibitor
CD80, 86
MHC/peptides
Antigen
Signal 2
JAK3
JAK3
MPA
CD154
Signal 1
CD28
PI-3K
PI-3K
Synapse
Anti-CD3 mAb
TCR/CD3
IKK
PI-3K
mTOR
FK778
Nucleotide synthesis
MAP kinases
CDK/cyclins
Calcineurin
G1
S
Anti-CD52 mAb
Cell cycle
M
G2
CD52 (depletion)
Cyclosporine, Tacrolimus
NFAT
AP-1
NF-κB
Azathioprine
Nucleus
mRNA
FTY720
S-1-P receptor (altered lymphocyte recirculation)
T cell
Cell membrane

Calcineurin Inhibitors:

Cyclosporine and Tacrolimus

- Have served as the backbone of solid-organ transplant immunosuppression for the past 30 years.
- Although they are biochemically distinct, they are remarkably similar, not only in their mechanism of action, but also in their clinical efficacy and side-effect profile.

Some Comparative Features of Cyclosporine and Tacrolimus

Feature	Cyclosporine	Tacrolimus
Mode of action	Inhibition of calcineurin	Inhibition of calcineurin
Daily maintenance dose	About 3-5 mg/kg	About 0.15-0.3 mg/kg
Administration	PO and IV	PO, IV, and SL*
Absorption bile dependent	Sandimmune, yes; Neoral, no	No
Oral dose available (capsules)	100 mg; 25 mg	5 mg; 1 mg; 0.5 mg
Drug interactions	Similar	Similar
Capacity to prevent rejection	+	+ +?
Use with MPA	+	+†
Use with sirolimus, everolimus	+‡	+‡
Prolonged release formulations	-	+
Nephrotoxicity	+	+
Steroid sparing	+	+ +?
Hypertension and sodium retention	+ +	+

Pancreatic islet toxicity	+	+ +
Neurotoxicity	+	+ +
Hirsutism	+	-
Hair loss	-	+
Gum hypertrophy	+	-
Gastrointestinal side effects	-	+
Gastric motility	-	+
Hyperkalemia	+	+
Hypomagnesemia	+	+
Hypercholesterolemia	+	-
Hyperuricemia, gout	+ +	+

Data are based on available literature and clinical experience.

-, No or little effect; +, known effect; + +, effect more pronounced; + + ?, probable greater effect; IV, intravenous; MPA, mycophenolic acid; PO; by mouth; SL, sublingual.

* IV rarely needed because sublingual absorption is good.

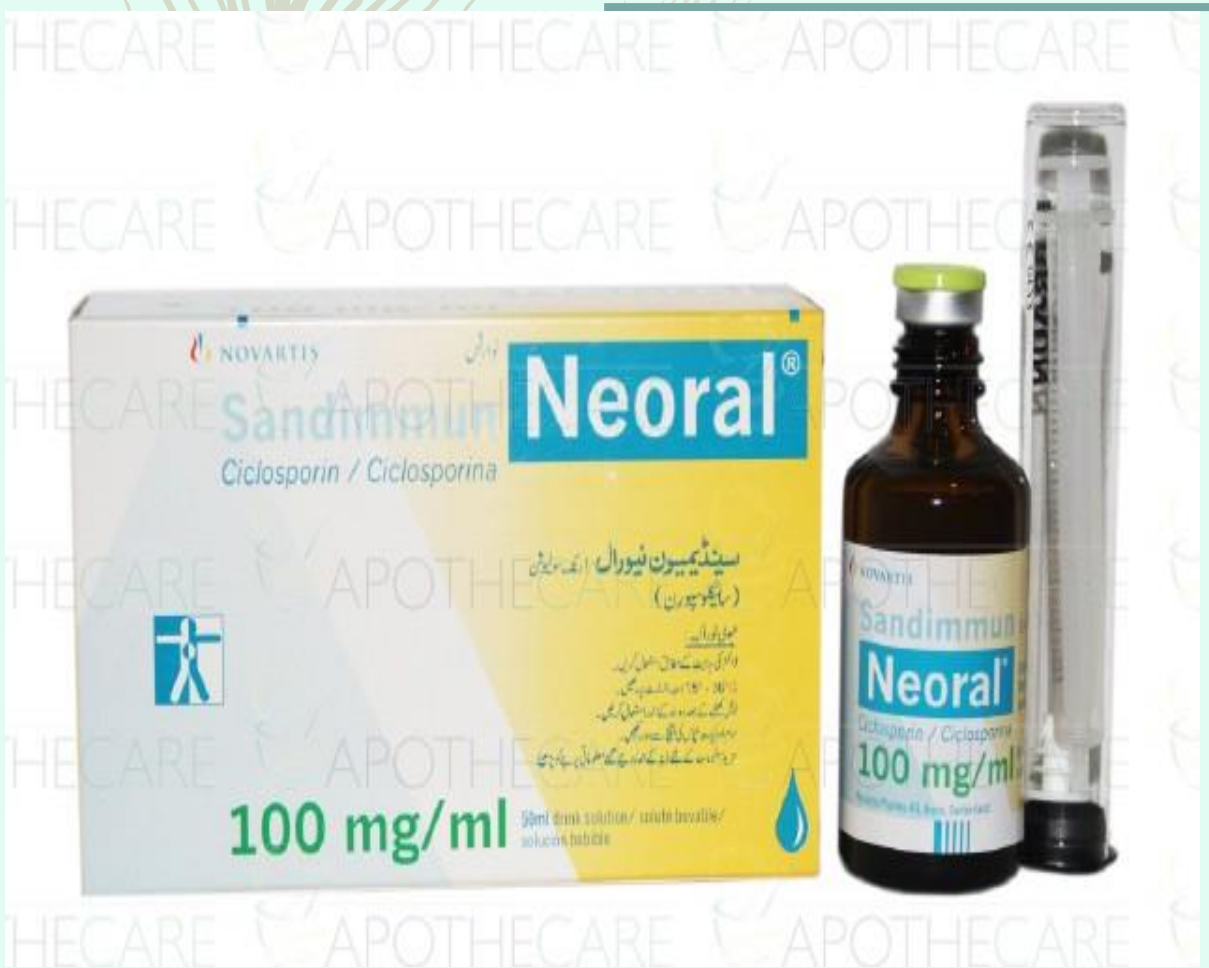
† Dose of MMF may be less when used with tacrolimus.

‡ Nephrotoxicity may be exaggerated when used in full dose.

– Patients receiving successful CNI-based immunosuppression maintain a degree of immune responsiveness that is still sufficient to maintain host defenses. This relative immunosuppression may be a reflection of the fact that **at therapeutic levels of these drugs**, **calcineurin activity is reduced by only about 50%,** permitting strong signals to trigger cytokine expression and generate an effective immune response. **In stable patients receiving cyclosporine, CD4+ T cells have reduced IL-2 production to a degree that is inversely correlated to drug levels**. The degree of inhibition of calcineurin activity and IL-2 production may be at the fulcrum of the delicate balance that exists **between too much and too little immunosuppression**.

Formulations

Cyclosporine




ZAHRAVI Pharm. Co.
Iminoral® 50
Ciclosporin
50 capsules
Each capsule contains:
Ciclosporin 50 mg


NEORAL
100mg


NOVARTIS
Sandimmun®
50 mg/ml Ampul
İmmünosupresif
1 ml x 10 Ampul


10 mL
Sterile Eye Drops
Lacrosporin®
0.05%
Ciclosporin
Each 100 mL contains:
Ciclosporin 0.05g
Store below 25°C
Protect from freezing
and light
Do not shake
Sina Darou
Tehran-iran
10 mL Sterile Eye Drop
Emulsion
Lacrosporin®
Ciclosporin
لاکروسپورین
http://irc.fda.gov.ir/nfi

Because of the pharmacokinetic properties of cyclosporine exhibiting inherent variability and the difference between therapeutic and toxic or ineffective concentrations being very small, the drug is considered to have a *narrow therapeutic window.*

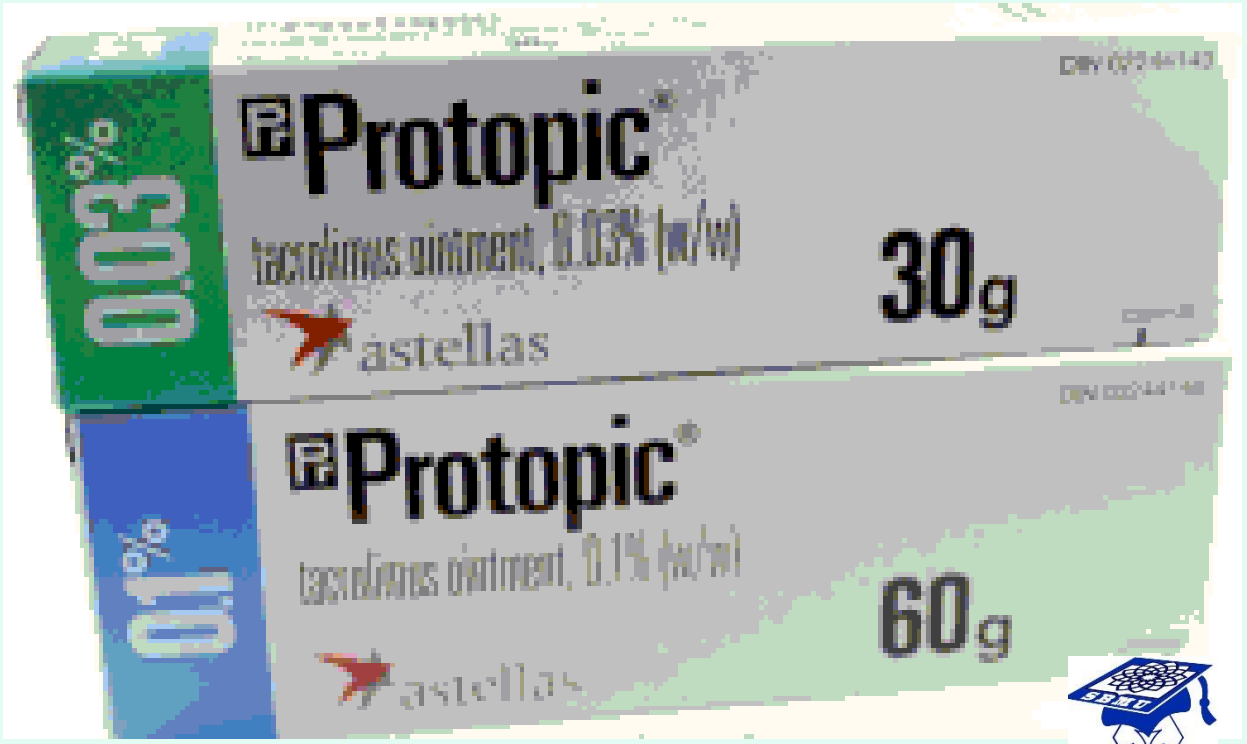
Several small studies show a **reduction in cyclosporine drug level by approximately 15% to 20% when using a 1:1 conversion between brand name and generic**. If generic formulations are used, it is probably better to use them consistently and to avoid switching formulations. **If conversions are made between the different formulations, it is wise to monitor drug levels and renal function**.

Patients should be counseled regarding the use of generic immunosuppressive medications to alleviate any potential anxiety regarding the use of nonbranded dosage forms and to enhance medication adherence. Extensive experience with generic formulations of cyclosporine has not demonstrated them to be inferior to the brand drug.

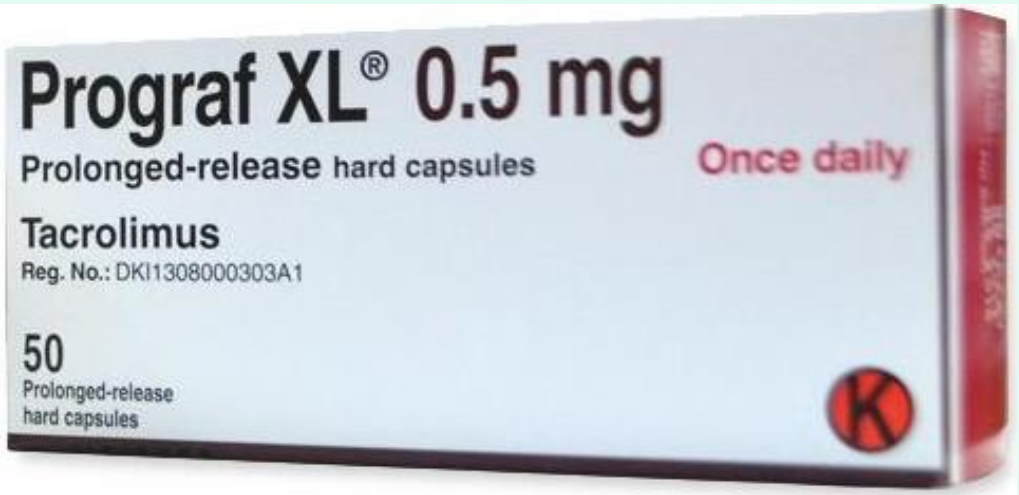
Tacrolimus


Prograf® 1 mg
Capsules
Tacrolimus
Immunosuppressant
50 Capsules
astellas
Prograf® 5 mg
Capsules
Tacrolimus
Immunosuppressant
50 Capsules
astellas
Prograf® 0.5 mg
Capsules
Tacrolimus
Immunosuppressant
50 Capsules
astellas


1 ml
Prograf®
Tacrolimus
Conc. for I.V.
astellas
PA 1241/14/4


Protopic®
tacrolimus ointment, 0.03% (w/w)
30g
astellas
Protopic®
tacrolimus ointment, 0.1% (w/w)
60g
astellas


Cograft® 1 mg
1 mg
Tacrolimus
Each capsule contains:
Tacrolimus (as monohydrate) 1 mg
48 Capsules


Prograf XL® 0.5 mg
Prolonged-release hard capsules
Once daily
Tacrolimus
Reg. No.: DKI1308000303A1
50
Prolonged-release
hard capsules


Prograf XL® 1 mg
Prolonged-release hard capsules
Once daily
Tacrolimus
Reg. No.: DKI1308000303B1
50
Prolonged-release
hard capsules

Because both CNIs are excreted in the bile with minimal renal excretion, drug doses do not need to be modified in the presence of kidney dysfunction.

The **pharmacokinetic parameters** of both drugs may **vary among patient groups**, and **these variations may have clinical consequences**. **Pediatric** and **African-American** transplant recipients may require **relatively larger doses** and **short dosage intervals**. **Longer dosage intervals** may be required in **older patients** and in the presence of **liver disease**.

- ✓ The **measurement of cyclosporine and tacrolimus levels** is an **intrinsic part of the management of transplant patients** because of **variation in interpatient and intrapatient metabolism**.
- ✓ There is also **a relationship**, albeit an inconsistent one, **between blood levels of the drug and episodes of rejection and toxicity**.

- ✓ **Drug-level monitoring** is the source of much confusion because of the **various assays available** and the **option of using different matrices** (i.e., plasma or whole blood) for their measurement.
- ✓ When Sandimmune was introduced, the trough level of cyclosporine (drawn immediately preceding the next dose, or Cmin), rather than the peak level, was measured because its timing was more consistent and appeared to correlate better with toxic complications. **More sophisticated techniques of monitoring** were suggested whereby a full, or abbreviated, pharmacokinetic profile is constructed to calculate the **AUC**, which reflects the bioavailability of the drug and **may theoretically allow for more precise and individualized patient management**. **Although attractive, these techniques never proved popular because of their cost and inconvenience**.

Evidence suggests that because of the **more consistent absorption of Neoral cyclosporine**, **its peak level (typically 2 hours after dosing) may correlate better with drug exposure and clinical events than the trough level**. So-called C2 monitoring is applied routinely in some centers and clinical trials.

For **tacrolimus**, the **trough levels are usually used for monitoring**, and **this level is an adequate approximation of drug exposure**.

Cyclosporine concentrations can be measured in **plasma or whole blood**. **Whole blood** (ethylenediaminetetraacetic acid [EDTA] anticoagulated) is the **recommended specimen** type because the **distribution of cyclosporine between plasma and erythrocytes is temperature dependent**. The clinician cannot begin to assess the significance of a cyclosporine level without knowing what kind of assay is being used.

- **Several methods are currently available to measure cyclosporine**, and **each differs in specificity for parent compound**. *High-performance liquid chromatography* (**HPLC**) is **the most specific method for measuring unmetabolized parent cyclosporine and is considered the reference method, But:**
  - Expensive
  - Labor intensive
  - Not available at all centers

- **Immunoassays**, which **use monoclonal antibodies against cyclosporine**, are **commonly used and have largely replaced HPLC**:
  - Fluorescence polarization immunoassay (FPIA)

- For monitoring of **tacrolimus** concentrations, most laboratories use the Abbott monoclonal antibody-based *microparticle enzyme immunoassay*
- *chemiluminescent microparticle immunoassay*
- *Electrochemiluminescence immunoassay*
- Liquid chromatography/tandem mass spectrometry (LC-MS/MS)

Approximate Therapeutic Ranges for Calcineurin Inhibitors

Post-transplantation Month	Cyclosporine HPLC and CMIA (ng/mL)	Cyclosporine FPIA (ng/mL)	Cyclosporine C2 levels* (µg/mL)	Tacrolimus CMIA (ng/mL)
0-2†	150-350	250-450	1.2-1.5	10-15
2-6	100-250	175-350	0.8-1.2	6-10
>6	100	150	0.5-0.8	4-8

CMIA, chemiluminescent microparticle immunoassay; FPIA, fluorescent polarization immunoassay; HPLC, high-performance liquid chromatography.

* Drawn within 15 minutes of 2 hours postdose. For C2 levels, no change in target levels is required for different assay types.

† In the first few days after transplantation, the trough cyclosporine level should not fall below 300 ng/mL by HPLC.

Mycophenolic Acid


500 mg
SUPRIMUN® 500
Mycophenolate Mofetil
50 F.C. tablets
Each tablet contains:
Mycophenolate Mofetil 500mg
ACTAVERGO
CLAUSEN


Citogan® 500
Mycophenolate Mofetil
50 F.C. Tablets
Each F.C. Tablet Contains:
Mycophenolate Mofetil 500 mg

Several studies have described **a relationship between the AUC for MPA and its clinical efficacy and side-effect profile**. **The relationship to random trough levels is less consistent and limited sampling strategies are not clinically feasible**. **Therapeutic drug monitoring is generally not required for routine clinical management**. In the event of side effects, the longer the period of drug-dose reduction or discontinuation, the greater is the subsequent incidence of episodes of acute rejection. Hence, the drug should be reintroduced as soon as possible and the clinical course carefully monitored.

Because therapeutic drug monitoring is not routinely performed during administration of these drugs, it will be difficult to determine their relative clinical effectiveness, and they should be used with caution.

Interestingly, **the AUC of MPA increases with time**; the same doses when used early postoperatively can produce much higher concentrations several months later. **Patients should be continuously monitored for adverse side effects and periodically be evaluated for an MPA dose reduction, if clinically appropriate**.

Enterohepatic cycling: This property also makes TDM of MPA difficult owing to the affect this secondary peak has upon the AUC.

mTOR Inhibitors: Everolimus and Sirolimus

Sirolimus has **a long half-life**, **averaging 62 hours**, and a **steady-state trough concentration can be achieved in most patients within 24 hours** by administering **a loading dose three times the size of the maintenance dose**.

Alterations of maintenance dosing can take 14 days before steady-state is re-achieved and has implications for therapeutic drug-level monitoring.

- Sirolimus is about 92% protein bound, mainly to albumin, while everolimus is less protein bound at approximately 74%.
- **Everolimus has a half-life of approximately 30 hours and is usually not administered with a loading dose.**
- **Renal excretion is minimal**, and **dose adjustment is not required in renal dysfunction but is required in hepatic dysfunction**. The majority of both agents are excreted in the feces via biliary elimination.

Therapeutic Drug-Level Monitoring

- **The target trough levels, using either chromatographic or immunoassay methodologies, vary between 5 and 15ng/mL, depending on the concomitant use of a CNI and the clinical circumstances and are a good reflection of drug exposure.**
- With the new CMIA methods, concentrations approximately 15% higher than those found with chromatographic methods are commonplace and should be considered when making dosing decisions.
- Because sirolimus has a long half-life, levels should be checked several days after a dosage adjustment is made, and once a steady-state has been reached, frequent monitoring may not be required.
- **Everolimus has target trough whole-blood concentrations of 3 to 8 ng/mL in renal transplant recipients**, also depending on the concomitant use of a CNI. The drug concentration is measured by LCMS/MS, a methodology that is not available at all reference laboratories and can limit the turnaround time of results.

Target blood levels similar to those described for tacrolimus.

Trough drug-level monitoring is now routine.

If sirolimus is given with tacrolimus, a combined trough level of 10 to 15 ng/dL is typically adequate.


Thanks for your Attention