Liver Transplant Immunosuppression and Rejection

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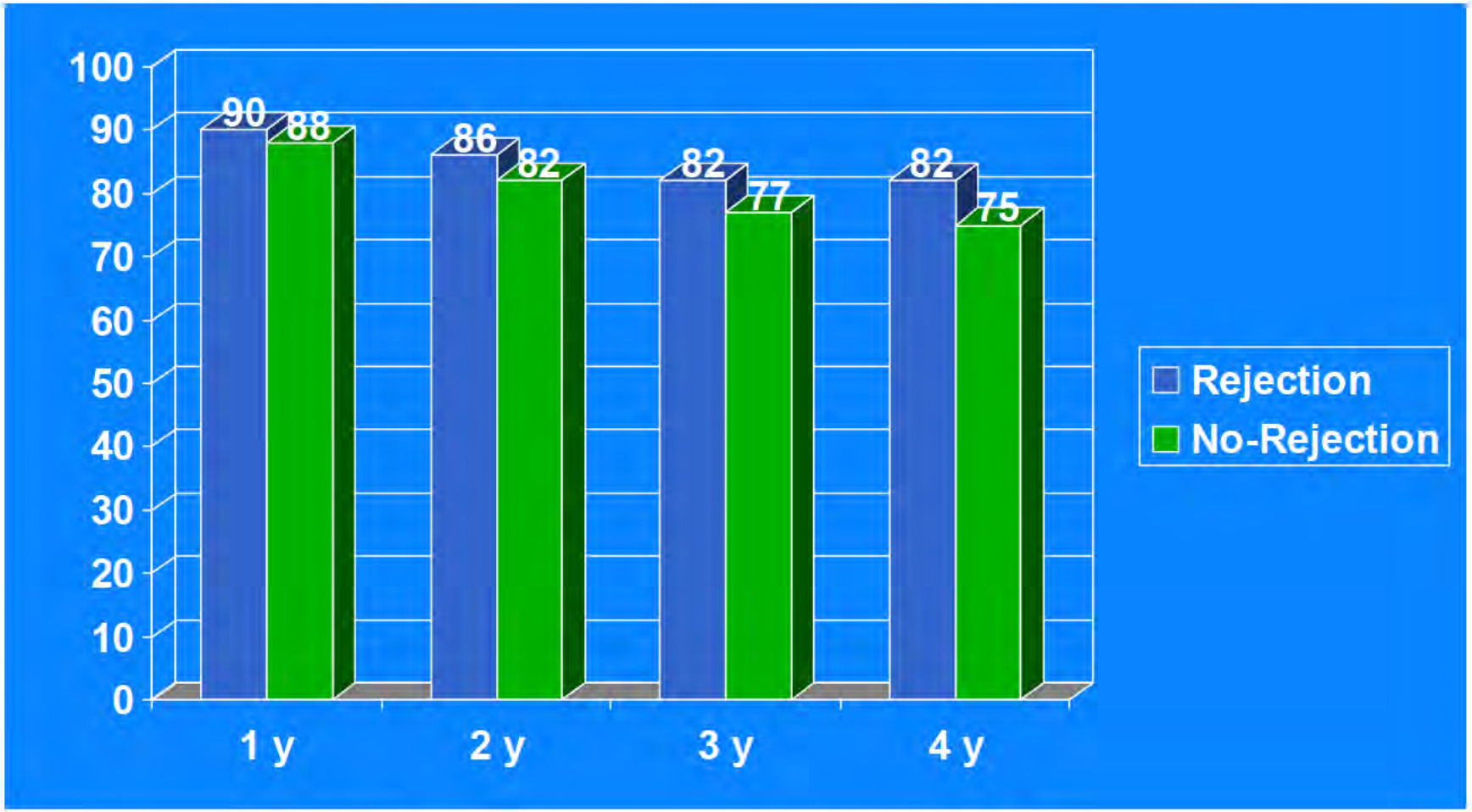
Background: Rejection, Graft loss and Survival

Survival vs Rejection in OLTx



Rejection/No-Rejection RR = 0.7

Impact of ACR Therapy on Survival

Patient	Therapy	RR Mortality
Non-HCV	Steroids	0.5
HCV	Steroids	2.9
HCV	OKT3	5.4

DO NOT TREAT MILD REJECTION IN HCV

Causes of Late Liver-Graft-Loss

Rejection	< 5 %
De-novo Malignancy	15 %
Infections	16 %
Cardiovascular Disease	20 %
Recurrent Disease	35 %

Excessive Immunosuppression causes more problems than rejection

Long-Term Complications of Immunosuppression

Renal Dysfunction	**80 %**
Hypertension*	**70 %**
Hyperlipidemia*	**50 %**
Diabetes Mellitus*	**20 %**
Bone Disease*	**20 %**
Skin Cancer	**40 %**
Lymphoma	**4 %**

* Less if Steroids are withdrawn shortly after 3 months

IMUNOSUPPRESSIVE REGIMENS

Immunosuppression Minimization (ISM)

Is best to personalize Immunosuppression considering recipient characteristics, etiology, and alloimmune activation.

Best started at or after 3rd post-Tx month if liver chemistries stable >/= 4 weeks.

Most patients should have steroid discontinuation by month 3 (keep low dose in immune-mediated liver disease; 5 mg Prednisone a day)

Avoid ISM in:

- Biopsy proven steroid-resistant rejection;
- Etiology is immune mediated disease (initial or re-transplant);
- Previous Antibody Mediated Rejection.

CNI Monotherapy

If initial 3 post-Tx months were uneventful, try CNI Monotherapy.

TAC trough:

- 0-3 months = 10-15 ng/mL (6 to 8 ng/mL if with MMF for renal sparing)
- 3-6 months = 6-10
- 6-12 months = 5-6
- > 12 months = 3

CSA trough:

- Week 0-2 = 250-350 ng/mL
- Week 3-4 = 200-300
- Week 5-12 = 150-250 (850-1400 2h post)
- 3-6 months = 150-200
- 6-12 months = 100-120
- > 12 months = 90-100

Dual to Monotherapy Conversion

If patient is on dual therapy after more than 1 year, consider change to monotherapy, if potentially beneficial and low immunological risk.

For renal dysfunction (using MDRD-6 or measured GFR) change to EVL + low dose TAC (</= 5 ng/mL) is better after 1 month and up to year 2.

Target trough level in Monotherapy:

- TAC = 5 ng/mL;
- EVL = 3 to 8 ng/mL +/- MMF 1 gm BID or low dose steroids (higher risk of rejection with EVL monotherapy).

Monitor closely after switch.

IS in Malignancy

Previous HCC:

SRL decreases recurrence in patients within Milan criteria, started 8 weeks after Tx.

EVL & SRL increases Recurrence Free Survival at 1 & 3 years with slight decrease at 5 y.

Initial target trough Tacro < 10 ng/mL and CSA < 300 ng/mL.

Previous Non-melanoma skin Cancer:

SRL decreases recurrence by 50% (in Kidney Tx).

Post NET and RCC:

EVL may be beneficial.

IS in Renal Dysfunction

Immediately post-LTx: anti-IL2R + MMF induction + delayed TAC or prolonged release TAC until day 5. Then TAC target 6-8 ng/mL + MMF/MPA.

For post-LTx CNI related renal dysfunction with GFR < 60 mL/min/1.73 m2 (CKD 3): after day 28, EVL target 3-8 ng/mL + TAC 3-4 ng/mL.

- Most benefit is obtained with change within the first post LTx year (after day 28).
- Some benefit up to year 2.
- Changes after year 2 do not help.

Triple Therapy Prednisone + CNI + MMF

- Improves patient & graft survival in HCV & Non-HCV.
- Lower ACR rate in HCV & Non-HCV
- Less renal toxicity with lower level of CNI.
- Does not increase risk of infection nor malignancy.

The Drugs

Cyclosporin A

From *Tolypocladium inflatum*; approved in 1983. Is calcineurin inhibitor.

Selective immunosuppression by inhibition of T-cell activation.

CyA forms complex with cytoplasmic receptor “cyclophilin” and inhibits calcium- & calmodulin-dependent phosphatase calcineurin.

Inhibition of Ca+ dephosphorilation of NFAT (Nuclear Factor of activated Tcells)

- Calcineurin is vital for the transcriptional process by which IL-2 and other cytokines are activated, which is needed for T-helper cell mediated graft rejection.

Cyclosporin A

Currently CyA comes as a microemulsion in lipophilic solvent which is less dependent in bile flow (**Neoral, Gengraf**).

CyA is metabolized in the liver by P450-3A pathway.

Cyclosporin A Toxicity

Nephrotoxicity: can be acute or long term; renal failure in up to 20%; can cause hyperkalemia and hypomagnesemia.

Hyperlipidemia, hyperglycemia, hypertension, gingival hyperplasia, hirsutism.

10-28% may have tremor, peripheral neuropathy, psychoses, hallucinations, motor weakness, or seizures.

May cause Hemolytic Uremic Syndrome.

Cyclosporin A Dose & Target Levels

Initial 10-15 mg/kg/d divided q 12h; check trough level after 24 h.

New data indicates that level 2-h post dose represents better “total exposure”.

Week 0-2:	trough 250-350 ng/mL (< 300 in HCC)
Week 3-4:	200-300
Week 5-24:	150-200 (850-1400 2h post)
Week 25-52:	100-120
Thereafter:	90 - 100

Cyclosporin A + Everolimus (3-8 ng/mL) Goal Trough Levels (Kidney Tx)

- Month 1 post-transplant: 100 to 200 ng/mL
- Months 2 and 3 post-transplant: 75 to 150 ng/mL
- Months 4 and 5 post-transplant: 50 to 100 ng/mL
- Months 6 to 12 post-transplant: 25 to 50 ng/mL

Cyclosporine A Side Effects > 10%

- Cardiovascular: Hypertension (8% to 53%), edema (5% to 14%)
- Central nervous system: Headache (2% to 25%), paresthesia (1% to 11%)
- Dermatologic: Hypertrichosis (5% to 19%)
- Endocrine & metabolic: Hirsutism (21% to 45%), increased serum triglycerides (15%), female genital tract disease (9% to 11%)
- Gastrointestinal: Nausea (2% to 23%), diarrhea (3% to 13%), gingival hyperplasia (2% to 16%), abdominal distress (<1% to 15%), dyspepsia (2% to 12%)
- Genitourinary: Urinary tract infection (kidney transplant: 21%)
- Infection: Increased susceptibility to infection (3% to 25%), viral infection (kidney transplant: 16%)
- Neuromuscular & skeletal: Tremor (7% to 55%), leg cramps (2% to 12%)
- Renal: Increased serum creatinine (16% to ≥50%), renal insufficiency (10% to 38%)
- Respiratory: Upper respiratory tract infection (1% to 14%)

Cyclosporine A
Side Effects </= 2%

- Cardiovascular: Chest pain (≤4%), flushing (<1% to 4%), glomerular capillary thrombosis, myocardial infarction
- Central nervous system: Convulsions (1% to 5%), anxiety, confusion, lethargy, tingling sensation
- Dermatologic: Skin infection (7%), acne vulgaris (1% to 6%), nail disease (brittle fingernails), hair breakage, night sweats, pruritus
- Endocrine & metabolic: Gynecomastia (<1% to 4%), hyperglycemia, hypomagnesemia, weight loss
- Gastrointestinal: Vomiting (2% to 10%), anorexia, aphthous stomatitis, constipation, dysphagia, gastritis, hiccups, pancreatitis
- Genitourinary: Hematuria
- Hematologic & oncologic: Leukopenia (<1% to 6%), lymphoma (<1% to 6%), anemia, thrombocytopenia, upper gastrointestinal hemorrhage
- Hepatic: Hepatotoxicity (<1% to 7%)
- Infection: Localized fungal infection (8%), cytomegalovirus disease (5%), septicemia (5%), abscess (4%), fungal infection (systemic: 2%)
- Neuromuscular & skeletal: Arthralgia, myalgia, weakness
- Ophthalmic: Conjunctivitis, visual disturbance
- Otic: Hearing loss, tinnitus
- Respiratory: Sinusitis (<1% to 7%), pneumonia (6%)
- Miscellaneous: Fever

Drugs that Increase Cyclosporin & Tacrolimus Levels

Calcium Channel Blockers	**Antifungals**	**Macrolide antibiotics**	**Pro-kinetics**	**Miscellaneous**
Diltiazem Nicardipine Nifedipine Verapamil	Fluconazole Itraconazole Ketoconazole Voriconazole Clotrimazole	Clarithromycin Erythromycin Troleandomycin Azithromycin Telithromycin	Cisapride Metoclo_pramide	Amiodarone Cimetidine Methyl-prednisolone Omeprazole Protease inhibitors **Nefazodone** **Ethinyl estradiol** **Grapefruit juice**

Drugs that Decrease Cyclosporin & Tacrolimus Levels

Anticonvulsants	Antibiotics	Herbal Preparations	Miscellaneous
Carbamazepine Phenobarbital Phenytoin Fosphenytoin	Rifabutin Rifampin Rifapentin	St. John's Wort	**Probucol** **Terbinafine**

Tacrolimus

- From *Streptomyces tsukubaensis*.
- It is 100-times stronger than CyA.
- Binds to FKBP12 and the complex inhibits calcineurin; this prevents transcription of IL-2, IL-3, IL-4, IL-8, and various chemotactic factors.
- It is absorbed in duodenum & jejunum without need for bile.
- Food decrease bioavailability.
- Metabolized via P450-3A pathway.

Tacrolimus Dose

- **Goal Trough levels**:
  - Monotherapy: 10-15 ng/mL early; 3-6 months = 6-10; 6-12 months = 5-6; > 12 months = 3.
  - In HCC, keep initial target < 10 ng/mL (will need MMF)
  - If with Everolimus: TAC goal 3 – 4 ng/mL.
- Immediate release (IR):
  - **Prograf**; Initial dose: 0.1 to 0.15 mg/kg/day in 2 divided doses, given every 12 hours
- Extended Release:
  - **Astagraf XL** is not approved for use in liver transplantation due to an increase in mortality in female liver transplant recipients receiving Astagraf XL.
  - **Envarsus PA** [Canadian product]: Initial: 0.11 to 0.13 mg/kg once daily concomitantly used with corticosteroids and/or mycophenolic acids or azathioprine; initiate within 24 hours of transplantation; titrate to target trough concentrations.
    - Conversion from immediate release to extended release: Patients stable on IR tacrolimus converted by initiating ER treatment in a 1:0.7 ratio (1 mg IR:0.7 mg ER) (or a 1:0.85 ratio for African American patients) using previously established total daily dose of IR product. Administer once daily.

Tacrolimus Toxicity

- More DM than CyA. More HUS than CyA.
- Less HTN, dyslipidemia, hirsutism (TAC causes hair loss), gum hyperplasia than CyA.
- Similar hyperkalemia, tremor, hypomagnesemia, infection, malignancies, & renal dysfunction than CyA.
- Nausea, vomiting, diarrhea, headache.
- Less rejection in 1st year in all, less steroid-resistant rejection, and longer graft survival in Hepatitis C than CyA.

Tacrolimus

Side Effects > 10%

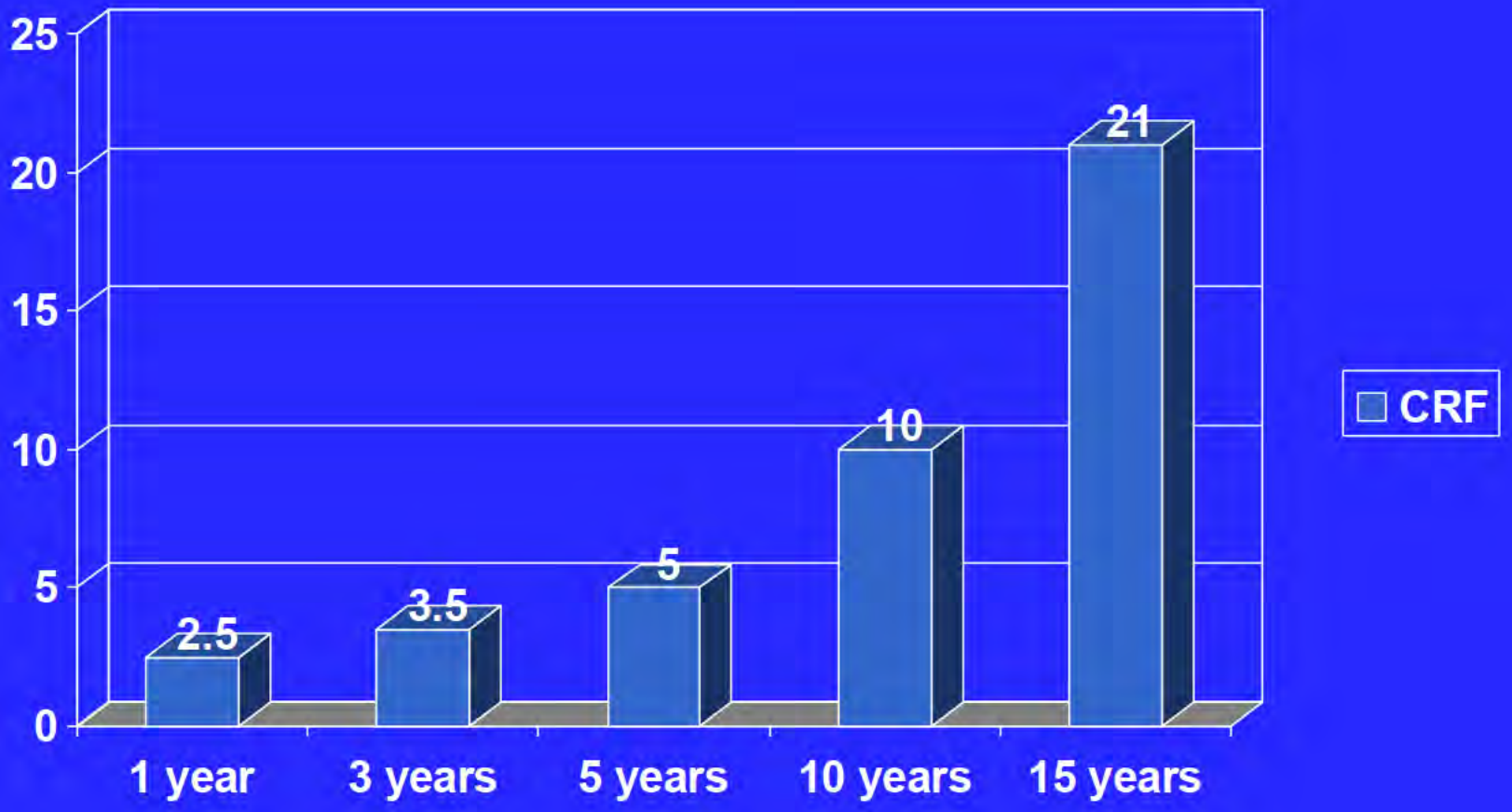
- Cardiovascular: Acute cardiorespiratory failure, angina pectoris, atrial fibrillation, atrial flutter, bradycardia, cardiac arrhythmia, cardiac failure, cardiac fibrillation, chest pain, deep vein thrombophlebitis, deep vein thrombosis, ECG abnormality (including abnormal QRS complex), edema, flushing, hemorrhagic stroke, hypertension, hypotension, orthostatic hypotension, peripheral edema, phlebitis, ST segment changes on ECG, syncope, tachycardia, thrombosis, vasodilation
- Central nervous system: Abnormal dreams, abnormality in thinking, agitation, amnesia, anxiety, ataxia, chills, confusion, depression, dizziness, drowsiness, emotional lability, encephalopathy, falling, fatigue, flaccid paralysis, hallucination, headache, hypertonia, hypoesthesia, insomnia, intolerance to temperature, mobility disorder, mood elevation, myoclonus, nerve compression, nervousness, neuralgia, neuropathy, neurotoxicity, nightmares, pain, paralysis (monoparesis, quadriparesis, quadriplegia), paresthesia, peripheral neuropathy, psychomotor disturbance, psychosis, seizure, vertigo, voice disorder, writing difficulty
- Dermatologic: Acne vulgaris, alopecia, cellulitis, condyloma acuminatum, dermal ulcer, dermatitis, diaphoresis, ecchymoses, exfoliative dermatitis, fungal dermatitis, hyperhidrosis, hypotrichosis, pityriasis versicolor, pruritus, skin discoloration, skin photosensitivity, skin rash
- Endocrine & metabolic: Acidosis, albuminuria, alkalosis, anasarca, cushingoid appearance, Cushing syndrome, decreased serum bicarbonate, decreased serum iron, dehydration, diabetes mellitus (including new-onset), gout, hirsutism, hypercalcemia, hypercholesterolemia, hyperkalemia, hyperlipidemia, hyperphosphatemia, hypertriglyceridemia, hyperuricemia, hypervolemia, hypocalcemia, hypoglycemia, hypokalemia, hypomagnesemia, hyponatremia, hypophosphatemia, increased gamma-glutamyl transferase, increased lactate dehydrogenase, metabolic acidosis, weight gain
- Gastrointestinal: Abdominal distention, abdominal pain, anorexia, aphthous stomatitis, biliary tract disease, cholangitis, cholestasis, constipation, diarrhea, duodenitis, dyspepsia, dysphagia, esophagitis, flatulence, gastritis, gastroenteritis, gastroesophageal reflux disease, gastrointestinal disease, gastrointestinal hemorrhage, gastrointestinal perforation, hernia of abdominal cavity, hiccups, increased appetite, intestinal obstruction, nausea, oral candidiasis, pancreatic pseudocyst, peritonitis, stomatitis, ulcerative esophagitis, vomiting
- Genitourinary: Anuria, bladder spasm, cystitis, dysuria, hematuria, nephrotoxicity, nocturia, oliguria, proteinuria, pyuria, toxic nephrosis, urinary frequency, urinary incontinence, urinary retention, urinary tract infection, urinary urgency, vaginitis

Tacrolimus

Side Effects > 10%

- Hematologic & oncologic: Anemia, benign skin neoplasm, decreased platelet count, decreased white blood cell count, disorder of hemostatic components of blood, hemolytic anemia, hemorrhage, hypochromic anemia, hypoproteinemia, hypoprothrombinemia, increased hematocrit, Kaposi sarcoma, leukocytosis, leukopenia, neutropenia, polycythemia, thrombocytopenia, thrombotic microangiopathy
- Hepatic: Abnormal hepatic function tests, ascites, cholestatic jaundice, granulomatous hepatitis, hepatitis (including acute and chronic), hepatotoxicity, hyperbilirubinemia, increased liver enzymes, increased serum alanine aminotransferase, increased serum alkaline phosphatase, increased serum aspartate aminotransferase, jaundice
- Hypersensitivity: Hypersensitivity reaction
- Immunologic: CMV viremia, graft complications
- Infection: Abscess, bacterial infection (may be serious), BK virus (including nephropathy), candidiasis, cytomegalovirus disease, Epstein-Barr infection, herpes simplex infection, herpes zoster infection, infection, opportunistic infection, polyomavirus infection, sepsis (children & adolescents), serious infection
- Neuromuscular & skeletal: Arthralgia, asthenia, back pain, lower limb cramp, muscle cramps, muscle spasm, myalgia, myasthenia, osteoporosis, tremor
- Ophthalmic: Amblyopia, blurred vision, conjunctivitis, visual disturbance
- Otic: Otalgia, otitis media, tinnitus
- Renal: Acute renal failure, hydronephrosis, increased blood urea nitrogen, increased serum creatinine, renal insufficiency, renal failure syndrome, renal tubular necrosis
- Respiratory: Acute respiratory distress syndrome, asthma, atelectasis, bronchitis, decreased lung function, dyspnea, flu-like symptoms, increased cough, nasopharyngitis, pharyngitis, pleural effusion, pneumonia, pneumothorax, productive cough, pulmonary edema, pulmonary emphysema, respiratory tract infection, rhinitis, sinusitis
- Miscellaneous: Abnormal healing, accidental injury, crying, fever, postoperative pain, postoperative wound complication, ulcer, wound healing impairment

Calcineurin Inhibitors in OLTx
Risk of Chronic Renal Failure



Risk Factors for CRF in Non-Renal Tx

	Relative Risk
Post-Op ARF	2.13
Diabetes Mellitus	1.42
Age (per each 10 years)	1.36
Hypertension	1.18
Hepatitis C	1.15

Sirolimus/Everolimus

Macrocyclic triene antibiotic with immunosuppressive, antitumor & antifungal properties

Binds to immunophilin FKBP12 but has different action than TAC: blocks cell-cycle progression at the “G1 – S phase” junction; mTOR.

No calcineurin inhibition, hence no increase in endothelin nor TGF beta that cause vasoconstriction and renal injury.

Suppresses cytokine driven T Cell proliferation.

Sirolimus increases risk of Hepatic Artery Thrombosis: “The safety and efficacy of Sirolimus…has not been established in liver transplant patients, and therefore such use is not recommended”.

Properties of mTOR inhibitors

- Synergism
- Anti-tumor effect (Kaposi, RCC, HCC).
- Vascular
- Potent inhibitor of proliferation.
- Anti fibrotic (TGFbeta)
- Tolerance (Tregs)

Sirolimus/Everolimus Toxicity & Dose

Anemia, hypercholesterolemia, hypertrigliceridemia, hyperlipidemia, leukopenia, thrombocytopenia, interstitial lung disease, peripheral edema, wound dehiscence, lymphocele, oral ulcers.

Dose Sirolimus: 2 mg/d, adjusted to maintain trough level of:

0-3 months: 8-10 ng/mL;

3-12 months: 6-8 ng/mL;

> 12 months: 5 ng/mL.

Everolimus given BID due to short half-life. Start 1 mg q12h.

Usually with low dose TAC (goal TAC 3-4 ng/mL) with EVL goal 3-8 ng/mL.

Monotherapy is not recommended (EVL goal 6-10 ng/mL.)

Sirolimus

- Macrolide antibiotic produced by Streptomyces hygroscopicus.
- Structurally similar to tacrolimus and binds the same target (FK-binding protein) but does not inhibit calcineurin.
- Blocks the transduction signal from the IL-2 receptor, thus inhibiting T- and B-cell proliferation.
- Its advantage over the calcineurin inhibitors (CNIs) is its freedom from nephrotoxicity and neurotoxicity.
- Side effects of sirolimus have relegated it to the status of an important **second-line drug**.

Everolimus
Zortress

- Derivate of Sirolimus and works as an mTOR inhibitor.
- Initial Dose: 1 mg twice daily (q12 h) in combination with reduced dose tacrolimus and a corticosteroid;
- Trough Goal: 3 – 8 ng/mL with reduced dose TAC (TAC goal of 3-4 ng/mL); EVL monotherapy goal is 6 – 10 ng/mL, but usually not recommended,
- Adjust maintenance dose if needed at a 4- to 5-day interval (from prior dose adjustment) based on serum concentrations tolerability, and response.
- Avoid concomitant administration of P-gp and strong CYP3A4 inhibitors.
  - If trough is <3 ng/mL: Double total daily dose (using available tablet strengths).
  - If trough >8 ng/mL on 2 consecutive measures: Decrease dose by 0.25 mg twice daily.

Everolimus
Side Effects > 10%

- **CV**: Peripheral edema (liver transplant: 18% to 20%), hypertension (17% to 30%);
- **CNS**: Headache (18% to 22%), insomnia (liver transplant: 6% to 7%), procedural pain (kidney transplant: 15%), fatigue (9% to 11%);
- **Endo**: Diabetes mellitus (new onset: liver transplant: 32%), hyperkalemia (renal transplant: 18%), hypercholesterolemia (9% to 17%), hypomagnesemia (kidney transplant: 14%), hypophosphatemia (kidney transplant: 13%), hyperglycemia (kidney transplant: 12%), hypokalemia (kidney transplant: 12%);
- **GI**: Constipation (kidney transplant: 38%), nausea (liver transplant: 14% to 15%), diarrhea (19% to 24%), vomiting (kidney transplant: 15%), abdominal pain (13% to 15%);
- **GU**: Urinary tract infection (kidney transplant: 22%), hematuria (kidney transplant: 12%), dysuria (kidney transplant: 11%);
- **Hem**: Anemia (kidney transplant: 26%), leukopenia (3% to 13%);
- **Infection**: Infection (liver transplant: 50%), viral infection (liver transplant: 17%), bacterial infection (liver transplant: 16%), hepatitis C (liver transplant: 11% to 14%);
- **Neuro, M-S**: Limb pain (kidney transplant: 12%), back pain (kidney transplant: 11%);
- **Renal**: Increased serum creatinine (kidney transplant: 18%);
- **Resp**: Upper respiratory tract infection (kidney transplant: 16%);
- **Miscellaneous**: Postoperative wound complication (liver transplant: 11%; includes incisional hernia, lymphocele, seroma, wound dehiscence), fever (13% to 19%)

Everolimus
Side Effects 1-10%

- **CV**: Chest pain (5%), tachycardia (3%), cardiac failure (1%), deep vein thrombosis (<1%)
- **CNS**: Depression (5%), paresthesia (5%), chills (4%), aggressive behavior (≤2%)
- **Derm**: Alopecia (10%), palmar-plantar erythrodysesthesia (5%), erythema (4%), onychoclasis (4%), skin lesion (4%), acneiform eruption (3%)
- Endo: Diabetes mellitus (10%; new onset: <1%), heavy menstrual bleeding (6% to 10%), menstrual disease (6% to 10%), decreased serum fibrinogen (8%), increased luteinizing hormone (1% to 4%), increased follicle-stimulating hormone (3%), ovarian cyst (≤3%), exacerbation of diabetes mellitus (2%)
- **GI**: Gastroenteritis (10%), hemorrhoids (5%), dysphagia (4%)
- **GU**: Vaginal hemorrhage (8%), dysmenorrhea (6%), uterine hemorrhage (6%), cystitis (3%), proteinuria (2%)
- **Hem/Onc**: Hemorrhage (3%)
- **Hepatic**: Increased serum bilirubin (3%)
- **Hypersensitivity**: Hypersensitivity reaction (≤3%; includes anaphylaxis, chest pain, dyspnea, flushing), angioedema (≤1%)
- **Infection**: Candidiasis (<1%), hepatitis C (<1%), sepsis (<1%)
- **M-S**: Muscle spasm (10%), jaw pain (3%)
- **Ophthalmic**: Eyelid edema (4%), conjunctivitis (2%)
- **Otic**: Otitis media (6%)
- **Renal**: Renal failure syndrome (3%)
- **Respiratory**: Streptococcal pharyngitis (10%), pleural effusion (7%), pneumonia (2% to 6%), bronchitis (4%), pharyngolaryngeal pain (4%), rhinorrhea (3%), sinusitis (3%)
- **Miscellaneous**: Postoperative wound complication (<1%; wound healing impairment)

Drugs that Increase Sirolimus or Everolimus Concentration

Calcium Channel Blockers	**Antifungals**	**Macrolide antibiotics**	**Pro-kinetics**	**Miscellaneous**
Diltiazem Nicardipine Nifedipine Verapamil	Fluconazole Itraconazole Ketoconazole Voriconazole Clotrimazole	Clarithromycin Erythromycin Troleandomycin Azithromycin Telithromycin	Cisapride Metoclo_pramide	Amiodarone Cimetidine Omeprazole Methyl-prednisolone Protease inhibitors **CyA** **Graprfruit juice**

Drugs that Decrease Sirolimus or Everolimus Concentration

Anticonvulsants	Antibiotics	Herbal Preparations
Carbamazepine Phenobarbital Phenytoin Fosphenytoin	Rifabutin Rifampin Rifapentin	St. John's Wort

Special Conditions to use Sirolimus/Everolimus

HCC	Anti-tumor Effect
HCV & PSC	Anti-fibrotic Effect
Renal Insufficiency	Spares CNI

Corticosteroids

- Block T-cell-derived and antigen-presenting cell-derived cytokine expression, decreasing IL-1, IL-2, IL-3, and IL-6
- Are used in reversing acute rejection and in maintenance.
- **Side effects**: hypertension, mental status changes, dyslipidemia, poor wound healing, hyperglycemia, gastric ulcers, myopathy, osteoporosis, Cushing S., fungal/bacterial infections, pituitary axis suppression, fluid retention, cataracts.
- Dose: 500-1000 mg pre-op; then taper from 50 to minimal dose over a few months.

Beneficial Effect of Steroid-Withdrawal after 3 months post OLTx

	Steroids	No-Steroids	P-value
Hypertension	58 %	15 %	0.0002
Diabetes	25 %	6 %	0.007
Infection	17 %	2 %	0.05
Bone Disease	9 %	0 %	0.05
Mean Cholesterol	253 mg/dL	183 mg/dL	0.001

Adverse Effects of Steroid-Withdrawal

- Recurrent AIH & PBC
- Worsens HCV if done before 3rd month.
- Flare up of Ulcerative Colitis
- Arthralgias
- Depression

Azathioprine (AZA)

Antimetabolite; antagonises purine metabolism. Inhibits synthesis of DNA, RNA, and proteins.

Used in < 5% US transplant centers.

Can cause myelosuppression and hepatotoxicity.

Side effects: nausea, vomiting, diarrhea, pancreatitis, anemia, leukopenia, thrombocytopenia, and weight loss.

Usual dose: 1-2 mg/kg/d

Mycophenolate Mofetil (MMF) Mycophenolic Acid (MPA)

- Inhibit de novo purine nucleotide synthesis by abrogation of inosine monophosphate dehydrogenase and production of guanosine nucleotides.
- Blocks DNA replication in T & B lymphocytes which are unable to use alternate salvage pathways.
- Liver dysfunction increase half life by decreasing conjugation; albumin levels change pharmacokinetics.
- More than 50% on transplant programs use them.
- Dose reduction and withdrawal are needed in 24-57%.

MMF & MPA Toxicity & Dose

Nausea, vomiting, abdominal pain, diarrhea, anemia, leukopenia, thrombocytopenia, hypercholesterolemia, hypokalemia, tremor, hypertension, edema.

MMF: 2-3 g/day, divide q 12h

MPA: 720-1440 mg/d divided q 12h.

MMF and MPA Toxicity

Side Effects > 10%

- **CV**: Hypertension (18% to 79%), edema (17% to 68%), hypotension (34%), tachycardia (22% to 23%), lower extremity edema (16%)
- **CNS**: Pain (25% to 79%), headache (11% to 59%), insomnia (24% to 52%), dizziness (34%), depression (20%), chills (3% to <20%), confusion (3% to <20%), drowsiness (3% to <20%), hypertonia (3% to <20%), malaise (3% to <20%), myasthenia (3% to <20%), paresthesia (3% to <20%)
- **Derm**: Skin rash (26%), ecchymoses (20%), cellulitis (3% to <20%)
- **Endo**: Hyperglycemia (44% to 48%), hypercholesterolemia (46%), hypomagnesemia (20% to 39%), hypokalemia (9% to 37%), hypocalcemia (11% to 30%), increased lactate dehydrogenase (24%), hyperkalemia (22%), acidosis (3% to <20%), weight loss (3% to <20%), hyperuricemia (13%), hyperlipidemia (10% to 12%), hypophosphatemia (9% to 11%)
- **GI**: Abdominal pain (22% to 63%), nausea (27% to 56%), diarrhea (24% to 53%), constipation (38% to 44%), vomiting (20% to 39%), decreased appetite (25%), dyspepsia (19% to 23%), esophagitis (3% to <20%), gastric ulcer (3% to <20%), gastritis (3% to <20%), gastrointestinal hemorrhage (3% to <20%), hernia of abdominal cavity (3% to <20%), intestinal obstruction (3% to <20%), stomatitis (3% to <20%), upper abdominal pain (14%), flatulence (10% to 13%)
- **GU**: Urinary tract infection (29% to 33%), hematuria (3% to <20%)
- **Hem/Onc**: Leukopenia (19% to 46%), anemia (20% to 45%), leukocytosis (22% to 43%), thrombocytopenia (24% to 38%), benign skin neoplasm (3% to <20%), disorder of hemostatic components of blood (3% to <20%), neoplasm (3% to <20%), pancytopenia (3% to <20%), skin carcinoma (3% to <20%; non-melanoma: 1% to 12%)
- **Hepatic**: Increased liver enzymes (25%), hepatitis (3% to <20%), increased serum alkaline phosphatase (3% to <20%)
- **Infection**: Bacterial infection (27% to 40%), viral infection (31%), cytomegalovirus disease (4% to 22%), fungal infection (11% to 12%)
- **M-S**: Asthenia (35% to 49%), tremor (12% to 34%), back pain (6% to 12%), arthralgia (7% to 11%)
- **Renal**: Increased serum creatinine (10% to 42%), increased blood urea nitrogen (37%)
- **Respiratory**: Dyspnea (31% to 44%), cough (41%), pleural effusion (34%)
- **Miscellaneous**: Fever (13% to 56%)

MMF and MPA Toxicity

Side Effects 1 - 10%

- **CV**: Exacerbation of hypertension (<10%), peripheral edema (<10%), phlebitis (4%), thrombosis (4%)
- **CNS**: Anxiety (<10%), fatigue (<10%)
- **Derm**: Acne vulgaris (<10%), pruritus (<10%)
- **Endo**: Diabetes mellitus (<10%)
- **GI**: Abdominal distension (<10%), gastroesophageal reflux disease (<10%), gingival hyperplasia (<10%), oral candidiasis (<10%)
- **GU**: Urinary retention (<10%)
- **Hem/Onc**: Lymphocele (<10%), severe neutropenia (2% to 4%), malignant neoplasm (≤2%), malignant lymphoma (1%), lymphoproliferative disorder (≤1%)
- **Hepatic**: Abnormal hepatic function tests (<10%)
- **Infection**: Influenza (<10%), wound infection (<10%), herpes simplex infection (6% to 8%), herpes zoster infection (4% to 5%), sepsis (2% to 5%)
- **M-S**: Muscle cramps (<10%), myalgia (<10%), peripheral pain (<10%)
- **Ophthalmic**: Blurred vision (<10%)
- **Renal**: Renal insufficiency (<10%), renal tubular necrosis (<10%)
- **Respiratory**: Dyspnea on exertion (<10%), nasopharyngitis (<10%), pneumonia (<10%), sinusitis (<10%), upper respiratory tract infection (<10%)

Drug-Drug Interaction
Azathioprine & Mycophenolate

Increases AZA	**Increases MMF**	**Decreases AZA & MMF**
Allopurinol	Probenecid	Cholestiramine
Methotrexate	Tacrolimus	Antacids
ACE inhibitors		Iron preparations

Antithymocyte Globulin (ATG)

ATGAM (equine) and Thymoglobulin (rabbit)

Polyclonal Ab against T-cells epitopes (CD2, CD3, CD4, CD8, CD28, & T-cell receptor), NK cells epitopes (CD16), and macrophages.

Cause T-cell depletion by: apoptosis, antibody mediated cytolysis, and internalization of cell surface receptors.

First dose can cause “cytokine release S”: fever, chills, tachycardia, chest pain, bronchospasm, GI disturbances, blood pressure changes. Steroids + Benadryl + acetaminophen helps.

Used in 6% of US transplant programs.

Dose: 1.5-5 mg/kg/d over 4-6 h infusion, for 3-5 days.

Muramonab-CD3 (OKT3)

Murine Ab against T-cell CD3 antigen; inactivates T-cell receptor.

Cytokine release syndrome is very common 1-3 h after first dose. Sometimes life-threatening with pulmonary edema and shock.

Re-exposure to OKT3 may decrease efficacy.

Dose: 5 mg IV q day x 10-14 days for steroid resistant rejection.

IL-2 receptor antibodies Basiliximab & Daclizumab

Basiliximab (Simulect) is chimeric, Daclizumab (Zenapax) is humanized;

Bind to IL-2R alpha-chain present in activated T-lymphocytes. Causes competitive antagonism of IL-2 induced T-cell proliferation.

Effect up to **3 weeks with Basiliximab**, and **10 weeks with Daclizumab**.

Side effects are mild.

Dose:

- a) Basiliximab: 20 mg IV pre-op + 20 mg 4 d later.
- b) Daclizumab: 1 mg/kg every 14 days x 5 doses.

UofL Liver Transplant Immunosuppression Protocol

UofL Induction Immunosuppression Therapy

No Renal Insufficiency	Renal Insufficiency (Cr Cr>2.0mg/dl or Cr CL ≤ 40 ml/min or requiring RRT) **Basiliximab will be used at the discretion of the transplant surgeon and hepatologist)**
IV Methylprednisone - 500 mg bolus at graft reperfusion. - 1 mg/kg per day Day 1-3 - 0.5 mg/kg per day Day 4-6	**IV Basiliximab 20 mg** - Day 0 - end of OR or in ICU - Day 4 - Day 10-14 (optional)

UofL Maintenance Immunosuppression Day 0-59

	No Renal Insufficiency		Renal Insufficiency	All Patients	
Day post OP	**Preferred Tacrolimus (FK)**	**Alternate Cyclosporine (CSA)**		**Prednisone Taper (day 0-120)**	**Mycophenolate mofetil (MMF) aka CellCept**
0	Start NG, SL or PO 0.05 mg/kg BID (see calculated weight-based dose table protocol)	Start NG or PO 4 mg/kg BID (or as a continuous IV infusion at 2 mg/kg/day)	See Basilixumab Induction above	50 mg q12h	1 gm IV q 12h (given as a 2 hr infusion per dose)
1				25mg q 12 h	
2				See prednisone taper protocol	1000mg BID ↓ dose 50- 100% if: • WBC < 1.5 • platelets < 40 K • acute CMV infection • diarrhea 2/2 MMF
3 – 59	Titrate dose for trough level of 7 – 10 ng/mL	Titrate dose for trough level of 200–250 ng/mL	See Basilixumab Induction above **Day 4 - 31** If Cr drops <2 mg/dl (GFR>40) start FK titrate 6-8 ng/ml. **Day 4 - 31** - If Creat>2 (GFR>40), may continue CNI free with slower steroid taper, consider lower goal CNI or +/- 20mg basiliximab at day 10-14. **Day 31+**- if Creat>2 (GFR<40) consider Everolimus monotherapy (goal 5-8) or Everolimus (4-8)/FK (3-6)	See prednisone taper protocol	

UofL Maintenance Immunosuppression
Day 60 and up

	No Renal Insufficiency		Renal Insufficiency	All Patients	
Day post OP	**Preferred Tacrolimus (FK)**	**Alternate Cyclosporine (CSA)**		**Prednisone Taper (day 0-120)**	**Mycophenolate mofetil (MMF) aka CellCept**
60	Trough of 5 – 8 ng/mL			See prednisone taper protocol	Withdraw at 2-4 months unless: • Pt at low levels of FK or CSA due to toxicity: Tac level < 6 CSA level < 100 • 1 rejection episode: treat through 4 months then reassess • 2 episodes of ACR or thymo: continue MMF x 1 year **SLKT continue CellCept indefinitely**
120	Trough of 4–7 ng/mL	Trough of 150–200 ng/mL		Discontinue unless tx for AIH, PSC, PBC or Re-tx - see prednisone taper protocol	**SLKT continue CellCept indefinitely**
1 year	Trough of 3-5 ng/mL	Trough of 50–150 ng/mL			**SLKT continue CellCept indefinitely**
2 years +	Trough of ~3 ng/mL	Trough 50–150 ng/mL			**SLKT continue CellCept indefinitely**

UofL Standard Prednisone Taper Protocol

Post-Tx day	Standard Adult Prednisone Taper	
	mg/dose	Interval
0	50	q12h
1	25	
2 – 3	20	
4 – 6	15	
7 – 14	10	
15 – 20	15	qDay
21 – 29	10	
30 – 60	7.5	
61 – 90	5*	
91 – 120	2.5	
121	Discontinue	

* Continue prednisone 5mg daily if re-transplant or special circumstances (AIH, PBC, PSC, idiosyncratic DILI)

Weight-based Calculations for Initial Tacrolimus Dose

Weight (kg)	Tacrolimus Dose
< 40	1.5 mg BID
40-49	2.0 mg BID
50-59	2.5 mg BID
60-69	3 mg BID
70-79	3.5 mg BID
80-99	4 mg BID
>=100	4 mg BID*

UofL Immunosuppression protocol for Everolimus or Sirolimus

Candidates for Therapy
-Patients with significant calcineurin inhibitor (CNI) toxicity without contraindication to mTOR therapy. -Patients at risk of CNI renal toxicity greater than 1-month post-transplant as a combination therapy approach with CNI dose reduction
Contraindications for Therapy
-Previous intolerance to mTOR inhibitor -Significant pulmonary disease especially interstitial lung disease or recurrent pneumonia -Severe anemia or other cytopenia not related to renal insufficiency -Significant proteinuria (>1 g per 24 hours)

UofL Protocol for Initiation of Therapy with mTOR

-Obtain baseline CXR, hematologic, liver, and renal profile, CNI drug level, UA for protein (24-hour collection if positive and Nephrology consult if confirmed), urine Protein/Creatinine ratio, baseline lipid profile, Doppler US of the liver

-UA for protein/creatinine ratio Q. 3 months for 1 year, unless otherwise directed by nephrology team based on pre-treatment studies. Consider extending interval of urinalysis to Q. 6 months if no proteinuria at 1 year.

-Monthly lipid profile for first 3 months of therapy, then obtain additional testing based on severity of hyperlipidemia and response to treatment

-If considering discontinuation of CNI, initiate 10 mg prednisone or equivalent additional immunosuppressive medication to decrease risk of rejection with everolimus or sirolimus monotherapy

-Follow up hepatic artery imaging after 3 months of therapy in absence of indication for earlier imaging. If within acceptable limits, image per standard post liver transplant protocol

UofL Protocol for Initiation of Therapy with mTOR

Everolimus (Zortress®) start at 0.5-1 mg by mouth twice daily
-Do not prescribe Afinitor - this is oncology dosing -Obtain everolimus levels 5-7 days into therapy. -Monitor everolimus levels weekly for 6 weeks after initiation of therapy, then every other week for 12 weeks, then monthly thereafter if stable -Adjust dose of everolimus by 0.5-1 mg twice a day to obtain target level of 4-8 ng/mL based on clinical indication -Once target level obtained, reduce CNI therapy to lowest tolerated level: -Tacrolimus target level 3-6 ng/ml -Cyclosporine target level 30-100 ng/ml
Sirolimus, start at 1-2 mgs by mouth daily
-Obtain sirolimus levels 5-7 days into therapy. -Monitor sirolimus levels weekly for 6 weeks after initiation of therapy, then every other week for 12 weeks, then monthly thereafter if stable

UofL mTOR Protocol Target Levels

Sirolimus				
	Day 0-21	**Day 22-120**	**Day 121-365**	**After day 365**
Sirolimus/Rapamycin (Rapamune®) – > day 31+				
Target level monotherapy (ng/ml)*	N/A	10-14	8-14	8-12
Target level dual therapy with CellCept** (ng/ml)	N/A	8-12	7-10	6-10
Everolimus				
Everolimus (Zortress®) do not start until day 31+				
Target level (ng/ml)†	N/A	4-8 (after day 30)	4-8	4-8

*Generally after 1 year, non-CNI monotherapy may be considered in patients at low immunological risk. During first year only if cytopenias forced MMF/MFA discontinuation.

**** reduce CellCept to 500 mg BID; discontinue if cytopenias develop rather than reducing rapamycin**

Discontinue everolimus/sirolimus therapy and resume previous immunosuppressive

- Severe rejection requiring multiple courses of intravenous corticosteroids
- Severe anemia or cytopenias requiring multiple transfusions or growth factor supplementation
- Severe mouth ulcers or GI distress
- Documented pneumonitis not due to infectious process
- Major surgery with risk for inhibition of wound healing (if elective surgery)
  - Initiate alternative immunosuppression and stop everolimus/sirolimus 3 weeks before surgery
  - May consider reinstitution of everolimus/sirolimus after recovery from surgery between 6 and 12 weeks based on tolerance of CNI therapy and extent of surgery
- Skin rash or allergic reaction suspected to be due to everolimus/sirolimus
- Medical complications of severe hyperlipidemia not responsive to therapy
- Abnormal imaging suggestive of hepatic artery disorders

UofL Treatment of Rejection

Management of Mild Rejection (Grade I & RAI < 4) HCV RNA positive patient (rare with DAA)
Increase tacrolimus dose to high therapeutic range (10- 15 ng/ml) For renal sparing protocol patient – start low dose tacrolimus (target levels 2-8) + MMF/MFA. If increased tacrolimus not tolerated, hold steroids at current dose/slow steroid taper.
Repeat Biopsy if not responding and rejection suspected
Management of Mild Rejection (Grade I & RAI < 4) HCV negative patient
Treat as per moderate to severe rejection protocol (see next). Change mTOR to FK/CNI, or increase CNI dose to higher therapeutic range, and/or add MMF/MFA. If renal sparing protocol, pulse steroids only, no additional tacrolimus.

Treatment of Mild, Moderate, or Severe Acute Allograft Rejection (ACR) #1

Treatment Day(s)	Solu-Medrol Dose	Oral Prednisone Dose	Diagnosting Testing QOD (M-W-F)
1	1000 mg IV	-	-
2		240 mg	Protocol (or alternate) labs
3		180 mg	Protocol (or alternate) labs
4		120 mg	Protocol (or alternate) labs
5		80 mg	Protocol (or alternate) labs
6		60 mg	Protocol (or alternate) labs
7		40 mg	Protocol (or alternate) labs; Repeat Liver Biopsy
8-14		20 mg	Protocol (or alternate) labs
15-21		15 mg	Protocol (or alternate) labs
22-28		10 mg	Routine
29-Indefinitive		5 mg	Routine

Protocol labs: CBC with diff, CMP, INR, Drug Level daily while hospitalized then at least QOD for duration of therapy, then twice weekly.

Day 7: Liver Biopsy

Infection Prophylaxis for Protocols #1, #2 and #3: Anti-infection Prophylaxis x 3 months: Valgancyclovir 900 mg/d + Bactrim 160/800 mg/d or Atavaquone 1500 mg/d + Fluconazole 400 mg/d (at discretion of provider based on infection risk and side effects including cytopenias)

Consider lower dose of steroids or alternative treatment for patients that are severely compromised or at treating physician's discretion

Consider lower dose of steroids (#1) or alternative treatment for patients that are severely compromised or at treating physician's discretion

Treatment of Mild, Moderate, or Severe Acute Allograft Rejection (ACR) #2

Treatment Day	Solu-Medrol Dose	Oral Steroid Dose	Diagnostic Testing QOD (labs typically on M-W-F)
1	1000 mg IV	As usual	None
2	1000 mg IV	As usual	None
3	1000 mg IV	As usual	Protocol (or “alternate”) labs
4	None	As usual	Protocol (or “alternate”) labs; repeat liver biopsy

Protocol labs: CBC with diff, CMP, INR, Drug Level daily while hospitalized then at least QOD for duration of therapy, then twice weekly.

Day 4: Liver Biopsy

Infection Prophylaxis for Protocols #1 and #2: Anti-infection Prophylaxis x 3 months: Valgancyclovir 900 mg/d + Bactrim 160/800 mg/d or Atavaquone 1500 mg/d + Fluconazole 400 mg/d (at discretion of provider based on infection risk and side effects including cytopenias)

Consider lower dose of steroids (#1) or alternative treatment for patients that are severely compromised or at treating physician’s discretion

Treatment of Mild, Moderate, or Severe Acute Allograft Rejection (ACR) #3

Treatment Day	Solu-Medrol Dose	Oral Steroid Dose	Diagnostic Testing QOD (labs typically on M-W-F)
1	1000 mg IV	As usual	
2	None	As usual	Protocol (or “alternate”) labs
3	1000 mg IV	As usual	None
4	None	As usual	Protocol (or “alternate”) labs
5	1000 mg IV	As usual	None
6	None	As usual	Protocol (or “alternate”) labs; repeat Liver Biopsy

Protocol labs: CBC with diff, CMP, INR, Drug Level daily while hospitalized then at least QOD for duration of therapy, then twice weekly.

Day 6: Liver Biopsy

Infection Prophylaxis for Protocols #1, #2 and #3: Anti-infection Prophylaxis x 3 months: Valgancyclovir 900 mg/d + Bactrim 160/800 mg/d or Atavaquone 1500 mg/d + Fluconazole 400 mg/d (at discretion of provider based on infection risk and side effects including cytopenias)

Consider lower dose of steroids or alternative treatment for patients that are severely compromised or at treating physician’s discretion

Consider lower dose of steroids (#1) or alternative treatment for patients that are severely compromised or at treating physician’s discretion

UofL Thymoglobulin Protocol for Steroid-Resistant Liver Allograft Rejection

THYMOGLOBULIN DOSE

Dose: 1.5 mg/kg IV. Consider reducing dose or holding if platelet count <50,000 or WBC <2000. Given on days 1, 2, 3, 5, 7

Route: Usually given by central line as a 4-6 hours infusion. Can be given peripherally over 6 hours if specially prepared with heparin and hydrocortisone. Notify pharmacy and indicate on the order.

Routine Prophylaxis: Steroids: 250 mg IV Solu-Medrol before first dose, 30 mg prednisone PO on days 2-3. Benadryl 50 mg IV or PO and Tylenol 650 mg PO 30 minutes before every thymoglobulin dose. The Benadryl dose may be reduced by 50% after symptoms subside in 2-3 days. Bactrim SS tabs daily during the course of thymoglobulin therapy, then 3 months thereafter. Valganciclovir for CMV prophylaxis during course and then 3 months thereafter.

POST-DOSE ORDERS

Day 1: VS q 15 min x 2 hrs, q 30 min for 2 hrs, q 4 hrs if stable. Temp hourly x 3, then routine prn.

Day 2: VS q 15 min x 1 hr, q 4 hrs. Temp q 4 hrs prn.

UofL Immunosuppression During Thymoglobulin

Immunosuppression during Thymoglobulin Therapy
Prednisone: Continue usual dose. May drop to 20 mg bid if the patient on higher dose **Mycophenolate/mofetil:** Continue as usual, adjusting as necessary if cytopenia or hold as clinically indicated **Tacrolimus or cyclosporine:** Continue as usual throughout the thymoglobulin course.
Follow-Up Tests
Protocol labs: Daily CBC with diff, CMP, INR Drug level while hospitalized then at least QOD for duration of therapy, then twice weekly. Check T+B Lymphocytes subsets after the third dose. **Day 8:** Liver Biopsy

Infection Prophylaxis

Infection Prophylaxis after Liver or Liver/Kidney Transplant

Reason	Regimen
Perioperative	Ampicillin/Sublactam 3 gm intra-op + 3 gm q 6 h x 24 h, or Vancomycin + Levaquin
Pneumocystis	Bactrim SS daily x 6 months, or Dapsone 100 mg/d, or Pentamidine 300 mg nebulization q month, or Atovaquone 1500 mg/d x 6 months
CMV	Ganciclovir 5 mg/kg IV daily while NPO, then Valcyte 900 mg/day x 3-6 months.
Fungus	Nystatin 5 mL S&S QID x 6 months, or Clotrimazole troches 10 mg QID, or Fluconazole 100 mg daily x 6 months

Liver Transplant Rejection

Immune System

Immune system:

- protects against infectious invasion, and
- provides self-nonself discrimination.

It has two sub-systems:

- Rapid response: done by innate immunity system.
- Specific response: classic T-cell response requiring days to weeks; this T-cells mediate graft rejection but also tolerance.

Both sub-systems are coordinated and provide immunological memory.

Hyperacute Rejection

- Extremely rare.
- Occurs hours to days after transplantation,
- Target is vascular endothelium.
- Antibody-mediated, & complement dependent graft destruction by ***coagulative necrosis***.
- Preformed antibodies specific to MHC.
- Lack of lymphocytic infiltration.

Acute T-Cell Mediated Rejection

Occurs in 45-70% of patients.

Days to months after transplant (usually initial 3 months).

Classical, cell-mediated rejection:

- Predominantly CD4 & CD8 T-cells.
- Directed against donor MHC antigens (cholangiocytes & vascular endothelium)

Target of current immunosuppression.

Diagnostic Triad:

- Portal inflammation
- Bile-duct damage
- Venular endothelium inflammation.

Banff Rejection Activity Index

Category	Criteria	Score
Portal Inflammation	Mostly lymphocytic inflammation involving, but not noticeably expanding, a minority of the triads	1
	Expansion of most or all of the triads, by a mixed infiltrate containing lymphocytes with occasional blasts, neutrophils and eosinophils	2
	Marked expansion of most or all of the triads by a mixed infiltrate containing numerous blasts and eosinophils with inflammatory spillover into the periportal parenchyma	3
Bile Duct Inflammation Damage	A minority of the ducts are cuffed and infiltrated by inflammatory cells and show only mild reactive changes such as increased nuclear:cytoplasmic ratio of the epithelial cells	1
	Most or all of the ducts infiltrated by inflammatory cells. More than an occasional duct shows degenerative changes such as nuclear pleomorphism, disordered polarity and cytoplasmic vacuolization of the epithelium	2
	As above for 2, with most or all of the ducts showing degenerative changes or focal lumenal disruption	3
Venous Endothelial Inflammation	Subendothelial lymphocytic infiltration involving some, but not a majority of the portal and/or hepatic venules	1
	Subendothelial infiltration involving most or all of the portal and/or hepatic venules	2
	As above for 2, with moderate or severe perivenular inflammation that extends into the perivenular parenchyma and is associated with perivenular hepatocyte necrosis	3

Acute T-Cell Mediated Rejection (TCMR)

Generally 5-30 days; 10-20% of patients.

- Early in initial 90 days;
- Late after 90 days (7.5 to 23% of patients); decreases graft survival.

Higher in: Autoimmune dz, Females, Young age, DR mismatch, Re-transplant.

Usually asymptomatic unless late diagnosis.

- Fever, Abdominal pain, Ascites, Leukocytosis, Eosinophilia

Biochemical abnormalities not specific (high GGT)

Diagnosis require liver Bx.

- Repeat Liver Bx if suboptimal biochemical response. No biopsy needed with good biochemical response.

Management of Acute T-Cell Mediated Rejection

Moderate to Severe: Corticosteroids.

Refractory:

- ATG;
- Consider Antibody Mediated Rejection (C4D staining + Donor Specific AB testing).

Investigate the reason of rejection.

Optimize immunosuppression.

If already optimized, add MMF, AZA or SRL.

Consider long term prednisone in:

- Autoimmune liver dz, or
- After 2 episodes of severe AR.

Treatment of Acute TCMR

Severity of Acute TCMR	Treatment
Mild Acute TCMR	Increase CNI level + /- add anti-metabolity (mycophenolate) or mTORi
Moderate and Moderate to Severe TCMR	-Pulse steroid SoluMedrol 500-1000 mg IV x 3 days + slow oral steroid taper + Increase CNI level +/- add anti-metabolity (mycophenolate) or mTORi, vs **-SoluMedrol 1000 mg IV x 1, then oral Prednisone (in mg) 240 x 1d, 180 x 1d, 120 x 1d, 80 x 1d , 60 x 1d, 40 x 1d, 20 x 7d, 15 x 7d, 10 x 7d, 5 daily.** Anti-infection Prophylaxis x **3 months** Valgancyclovir 900 mg/d + Bactrim 160/800 mg/d or Atavaquone 1500 mg/d + Fluconazole 400 mg/d
Failure to Respond or Severe Cholestatic TCMR	Anti-thymocyte globulin Anti-infection Prophylaxis x **6 months** Valgancyclovir 900 mg/d + Bactrim 160/800 mg/d or Atavaquone 1500 mg/d + Fluconazole 400 mg/d

Variants of Classic Acute TCMR

Plasma cell hepatitis:

- Early, often with (-) auto-Abs (> 60%)
- Centrilobular necrosis
- ? Antibody mediated Rejection

Idiopathic post-transplantation hepatitis:

- 5-15% with fibrosis progressing to cirrhosis over 10 y.
- Auto-Abs and plasma cell are associated with progression.

Late Acute Rejection (> 6 months after LT):

- May be histologically different.
- Lobular activity, interface hepatitis, central perivenulitis (without endothelitis); can mimic plasma cell hepatitis.
- Possible evolution to chronic rejection with perivenular hepatocyte drop out and loss of inflammation.

Antibody Mediated Rejection When to Consider

RISK FACTORS:

- Refractory Rejection (steroid resistant);
  - Monitor Donor Specific Antibodies (DSA) after severe TCMR, steroid-resistant TCMR, and in unexplained allograft dysfunction
- Re-transplant (sensitized).
- HLA mismatch, positive x-match.
- Necrosis or vascular injury.

TREATMENT: In evolution; plasmapheresis + IVIG + immunosuppression changes.

PROPHYLAXIS:

- Blood transfusion minimization in cirrhosis.
- Adherence to immunosuppression regimen.

Antibody Mediated Rejection Features

Clinical Features

- Unknown Incidence
- Due to ABO incompatibility and Donor Specific Antibodies (DSAs)
- May occur hours, days or months Post-OLTx
- Elevated liver enzymes, elevated PT/INR, decreased platelet count, low serum complement.
- High serum Donor Specific Antibodies (MFI > 1000).

Histologic Features

- Endothelial cell injury within the vasculature of the portal tracts (endothelial hypertrophy, endotheliitis with intraepithelial and marginating eosinophils, macrophages, lymphocytes, and neutrophils)
  - Bile ductular proliferation, scattered apoptotic bodies within the lobules, centrizonal swelling of hepatocytes, and canalicular cholestasis.
- Diffuse and strong C4d deposition in the portal veins, capillaries, and periportal sinusoidal endothelium involving most of the portal tracts.
- Exclusion of other causes of similar injury

Acute Antibody Mediated Rejection (AMR)

Clinical Manifestations	Histology	Definition Criteria
•Rapid allograft failure, hemorrhagic necrosis •Graft injury with refractory thrombocytopenia, hyperbilirubinaemia, low serum complement levels. •Elevated transaminases despite optimal donor liver quality	•Portal microvascular endothelial cell hypertrophy •Microvasculitis/capillaritis with monocytes, eosinophils and/or neutrophils •Portal/peri-portal edema •Microvascular injury involving central veins •Fibrin deposition, red blood cells seen in sub-sinusoidal regions •Cholestasis, ductular reaction	•Compatible Histology •Elevated DSA (> 1000); MFI > 3000 is associated with clinical outcomes. DSA with MFI Sum >20,000 has increased fibrosis risk. •Diffuse C4d deposition of microvasculature in ABO-compatible tissues, or portal stroma in ABO-incompatible tissues •Exclusion of other liver diseases or complications that can cause similar patterns of injury

Chronic Antibody Mediated Rejection (AMR)

Clinical Manifestations	Histology	Definition Criteria
•Can be associated with graft injury and/or advanced fibrosis without clear etiology, sometimes in the setting of cyclosporine use or HCV infection •Development of portal hypertension after transplantation •Abnormal liver tests during immunosuppression weaning	•Mild portal/peri-portal/peri-venular inflammation •Mild interface hepatitis •Dense portal fibrosis with collagenisation •Obliterative portal venopathy •"V" lesions (inflammation, necrosis or obliterative arteriopathy in the absence of significant portal or peri-venular activity). •Periductal fibrosis, ductopenia	•Compatible Histology •Positive DSA (> 1000) within 3 months of biopsy. MFI > 3000 is associated with clinical outcomes. DSA with an MFI Sum >20,000 has an increased risk of fibrosis. •Focal C4d positivity (>10% portal tracts) •Exclusion of other causes of graft injury

Antibody Mediated Rejection Treatment

Treatment of Acute Antibody Mediated Rejection

- First-line: conventional rejection therapy (tacrolimus and corticosteroid-based therapy) including IV Solu-Medrol 1 gm + tapper. Repeat biopsy and DSA.
- Second-line therapy: plasma exchange every other day and periodic low-dose IVIG (100–500 mg/kg) or High-dose IVIG (2-5 gm/kg) for a minimum of 5 sessions. Repeat biopsy and DSA.
- Third-line Therapy: In moderate to severe acute AMR persistent in biopsy after second-line therapy, consider anti-CD20 (Rituximab) agents or proteasome inhibitors (Bortezomib) up to 4 times each, alone or sequential. Repeat Bx and DSA to decide to repeat.
- Re-Transplantation: assess DSAs prior to consideration of re-transplantation in order to guide clinical management should allograft dysfunction, rejection, or other complications occur. Anti-Thymocyte globulin before Re-Tx?

Treatment of Chronic Antibody Mediated Rejection

- IV Solu-Medrol IV 1 gm + tapper + keep on Tacro > 5 ng/mL + Prednisone.
- In patients with plasma cell-rich rejection, treatment with a purine analogue or mycophenolate mofetil can also be considered.
- Focus on corticosteroid/tacrolimus compliance, more frequent laboratory and histologic surveillance, and the consideration of re-transplantation. Assess DSA before re-Transplant.

Risk of Rejection in OLTx with Different Regimens

Regimen	Acute (ACR)	Chronic (Ductopenic)
Pred + Aza	85 %	25 %
Pred + CyA	70 %	15 %
Pred + Tacr	55 %	6 %
Pred + CNI + MMF	45 %	1 %
Tacr + Rapa	18 %	1 %

Chronic T-Cell Mediated Rejection

Occurs to 2-5% of adult patients (up to 16% in children).

Evolves from severe or persistent Acute TCMR

Slow, indolent process months to years after transplantation.

Rise of cholestatic enzymes.

Has immune & non-immune components; poorly defined.

Causes ischemic injury and paucity of bile ducts.

Characterized by arteriole thickening & interstitial fibrosis.

Loss of small bile ducts +/- neo-intimal proliferation with ***obliterative vasculopathy*** (foam cell obliterative arteriopathy).

- Bile duct loss > 50%, or
- Bile duct atrophy/pyknosis in the majority of bile ducts, or
- Foam cell obliterative arteriopathy.

Risk Factors for Chronic TCMR

- Multiple or Severe A cute TCMR episodes.
- Severe AR with Centrilobular Necrosis.
- Non-compliance.
- Under immunosuppression.
- Autoimmune etiology
- Donor/ Recipient sex mismatch.
- Re-transplantation for rejection

Chronic TCMR Prognostic Factors

Bile duct loss > 50% of portal tracts

Foam cell clusters within the sinusoids.

Severe, bridging, perivenular fibrosis.

Severe hyperbilirubinemia (TB >/= 25)

- Bili of </= 4.6 has higher resolution.

Management of Chronic TCMR

Switch CyA to TAC while TB < 10 mg/dL

- 50% success
- Ductular reaction is a positive feature.

Higher TAC levels

Add mTOR-I or MMF

- Consider infection prophylaxis

Avoid over-immunosuppression with late cases of liver synthetic dysfunction.

Antibody Mediated Rejection (AMR)

Rare: less than 1% of Transplants (< 5% of sensitized patients).

Consider it if non-responding Acute TCMR therapy.

Mild Acute AMR is treated with Steroid boluses 500 – 1000 mg SoluMedrol/d IV x 3 + Steroid taper or lymphodepletion therapy.

Moderate-Severe Acute AMR treated with plasmapheresis, IV immunoglobulins or B-cell depletion with Rituximab or bortezomib.

No clear therapy for Chronic AMR.

Lesion Scoring of AMR

C4d-(immune)-score (formalin-fixed, paraffin-embedded) (C4d Score)

- 0. No C4d deposition in portal microvasculature
- 1. Minimal (<10% portal tracts) C4d deposition in >50% of the circumference of portal microvascular endothelia (portal veins and capillaries)
- 2. Focal (10–50% portal tracts) C4d deposition in >50% of the circumference of portal microvascular endothelia (portal veins and capillaries)—usually without extension into periportal sinusoids
- 3. Diffuse (>50% portal tracts) C4d deposition in >50% of the circumference of portal microvascular endothelia (portal veins and capillaries)—often with extension into inlet venules or periportal sinusoids

Lesion Scoring of AMR Histology Score (h-score)

- 1. Portal microvascular endothelial cell enlargement (portal veins, capillaries, and inlet venules) involving a majority of portal tracts with sparse microvasculitis defined as three to four marginated and/or intraluminal monocytes, neutrophils, or eosinophils in the maximally involved capillary with generally mild dilation.
- 2. Monocytic, eosinophilic, or neutrophilic microvasculitis/capillaritis, defined as at least 5–10 leukocytes marginated and/or intraluminal in the maximally involved capillary prominent portal and/or sinusoidal microvascular endothelial cell enlargement involving a majority of portal tracts or sinusoids, with variable but noticeable portal capillary and inlet venule dilatation and variable portal edema.
- 3. As above, with marked capillary dilatation, marked microvascular inflammation (10 or more marginated and/or intraluminal leukocytes in the most severely affected vessels), at least focal microvascular disruption with fibrin deposition, and extravasation of red blood cells into the portal stroma and/or space of Disse (subsinusoidal space).

Acute Antibody Mediated Rejection

- **Definite for acute/active AMR (all four criteria required):**
  - Histopathological pattern of injury consistent with acute AMR, usually including the following:
    - portal microvascular endothelial cell hypertrophy, portal capillary and inlet venule dilatation, monocytic, eosinophilic, and neutrophilic portal microvasculitis, portal edema, ductular reaction;
    - cholestasis is usually present, but variable;
    - edema and periportal hepatocyte necrosis are more common/prominent in ABO-incompatible allografts;
    - variable active lymphocytic and/or necrotizing arteritis
  - Positive serum DSA (Mean Fluorescent Intensity >/= 5000).
  - Diffuse (C4d score = 3)
    - microvascular C4d deposition on frozen or formalin-fixed, paraffin-embedded tissue in ABO-compatible tissues or
    - portal stromal C4d deposition in ABO-incompatible allografts.
  - Reasonable exclusion of other insults that might cause a similar pattern of injury. Most cases will score (C4d-score: 3+ h-score = 5 or 6).

Acute Antibody Mediated Rejection

Suspicious for AMR (both criteria required):

- DSA is positive (Mean Fluorescent Intensity >/= 5000).
- Non-zero h-score with: C4d-score + h-score of 3 or 4.

Indeterminate for AMR (requires 1+2 and 3 or 4):

- 1. C4d-score + h-score is ≥ 2.
- 2. DSA not available, equivocal, or negative.
- 3. C4d staining not available, equivocal, or negative.
- 4. Co-existing insult might be contributing to the injury.

Chronic AMR

Probable chronic active AMR (all four criteria are required):

- Histopathological pattern of injury consistent with chronic AMR; both required:
  - Otherwise unexplained and at least mild mononuclear portal and/or perivenular inflammation with interface and/or perivenular necro-inflammatory activity.
  - At least moderate portal/periportal, sinusoidal and/or perivenular fibrosis.
- Recent (for example, measured within 3 months of biopsy) circulating HLA DSA in serum samples;
- At least focal C4d-positive (>10% portal tract microvascular endothelia.
- Reasonable exclusion of other insults that might cause a similar pattern of injury.

Possible chronic active AMR:

- As above, but C4d staining is minimal or absent

GVHD post Liver Transplant

Less than 1%, but > 80% mortality.

Usually 2-8 weeks post-LT

TRIAD: Skin Rash + Cytopenia + Diarrhea, with normal Liver Enzymes and allograft function.

Diagnosis: FISH X-Y chimerism, PBMC donor-recipient chimerism, skin Bx, or rarely intestinal Bx.

Treatment:

- High dose steroids
- ? Lymphodepletion
- ? Stop immunosuppression
- Stem Cell transplant.

The Immune System and Liver Transplantation

T-cell Recognition of Alloantigen & T-cell Activation: Rejection

Recipient T-lymphocytes recognize a donor alloantigen by:

- a) ***Direct Path*** : native donor ***MHC*** molecule expressed ***in donor APCs*** ,
- b) ***Indirect Path*** : ***donor alloantigen peptides*** (from damaged cells or soluble MHC class I) ***presented by recipient APCs***.

"Direct path" dominates in "acute" rejection, and

"Indirect path" in chronic rejection and tolerance.

Costimulatory Pathways & Transplantation: Rejection

Optimal T-lymphocyte activation need TWO coordinated signals:

- **Signal 1**: T-cell Receptor (TCR) signal, which occurs after recognition of peptide/MHC on APC, (blocked by TAC, CyA, OKT3, Thymoglobulin, ALG, Alemtuzumab (CD52))
- **Signal 2**: occurs from interaction of "costimulatory T-cell molecule" with its "ligand" on the APC (blocked by CTLA4Ig, CD40L, CD28/B7, CD40/CD154)

If signal 1 & 2 occur, rejection develops.

- **Signal 3:** is blocked by IL2 receptor Monoclonal Ab, Rapamycin, AZA & MMF)

Effector Pathways of Graft Injury Rejection

There is not a single mediator or cell type that is absolutely required for allograft rejection; there are several redundant and compensatory mechanisms contributing to rejection.

After [T-cell Receptor signal + costimulatory signal, + cytokines], there is proliferation and maturation of $CD4^+$ or $CD8^+$ T-cells capable of graft injury; this will lead to:

- T-cell mediated cytotoxicity
- Delayed hypersensitivity
- Antibody-mediated damage

Effector Pathways of Graft Injury Rejection

- **<u>T-cell mediated cytotoxicity</u>**:
  - ***A) CD8+*** cytotoxic T-lymphocytes (CTLs) specific for donor ***class I***, cause apoptosis through biochemical mechanisms (perforin/granzyme B in a Ca^{++} dependent process, and Fas/FasL through caspase 8);
  - ***B) NK cells***, ***without*** need for ***activation*** or sensitization, which can cause apoptosis through FasL & granzyme B.

Effector Pathways of Graft Injury Rejection

- **Delayed hypersensitivity**:
  - ***CD4*⁺** T-lymphocytes specific for donor ***class II***, release IFN gamma activating macrophages and cellular mediators.
- **Antibody-mediated damage**:
  - Antibodies against liver sinusoidal endothelial cells (LSECs) indirectly promote acute rejection.

Immune System & Tolerance

- **Tolerance**: Absence of destructive response to an allograft in immunocompetent host.
- Tolerance is accomplished by T-cell suppression mediated by :
  - a) **cell-contact dependent mechanism**: $CD4^+CD25^+$ cells,
  - b) **cytokine mediated T-cell mechanism**: T regulatory-1 & T helper-3 (Th3),
  - c) **antigen presentation dependent mechanism**: by liver-derived Dendritic Cells (DCs) and by Liver Sinusoidal Endothelial Cells (LSECs) which behave as immature DCs causing incomplete activation, inhibiting T-cell response.
  - d) **NK cells** which give a “death signal” to recipient derived T-cell passing through the graft.

Costimulatory Pathways & Transplantation: Tolerance

- Optimal T-lymphocyte activation needs coordinated "signal-1" and "signal-2" stimuli.
- If only "signal 1" occurs, tolerance develops;
- To prevent rejection and induce tolerance, you can disrupt "signal 2":
  - a) CTLA4 (cytotoxic T lymphocyte antigen 4) can compete with CD28 for B7, and gives "negative costimulation"; CTLA4-Ig fusion protein has been used for this goal,
  - b) anti-CD154 disrupts CD40/CD154 pathway.
- Programmed death-1 (PD-1) is a molecule induced upon T-cell activation and causes a "negative signal" similar to CTLA4, causing spontaneous tolerance.
  - PD-1 binds to ligands PDL-1 & PDL-2.

Possible Mechanisms for Liver Tolerance

- 1) The liver produces large amounts of soluble MHC class I antigen, causing:
  - a) Passive blockade of alloantibodies & donor-specific effectors, or
  - b) Activation-induced apoptosis of allospecific CTLs.
- 2) Liver suppressor factor-1: is produced by spontaneously tolerant recipients and prolongs rat cardiac allograft survival.
- 3) Liver produces a soluble Fas “incomplete variant”, which inhibits anti-Fas induced apoptosis and inhibits CTL function in vitro.

Possible Mechanisms for Liver Tolerance

- 4) Graft-derived Stem-cells migrate out of the liver and establish “microchimerism” with clonal exhaustion/deletion of host alloreactive T-cells.
- 5) Inmature “Dendritic Cells” (DCs) and “Liver Sinusoidal Endothelial Cells” (LSECs) do not express enough costimulatory molecules, hence facilitate tolerance.
- 6) Kupffer cells (APCs) express FasL which can induce apoptosis of host T-cells.

Costimulatory Pathways & Transplantation: Autoimmunity

- Deficiency in “Programmed death-1” (PD-1) molecule and/or PDL-1 causes autoimmune disorders and autoimmune hepatitis with large amounts of CD8 T-cells in the liver.

Steroid Avoidance

Reason: Minimize osteoporosis, hyperglycemia, hypertension, hyperlipidemia, infections, Cushingoid features, and HCV recurrence.

TAC+MMF+Thymoglobulin vs. TAC+MMF+Steroids:

- F/U 1.5 y, graft survival 89% in both, rejection (20 vs 32%, $p<0.05$), recurrent HCV (50 vs 71%, p=ns)

TAC+daclizumab vs TAC+Steroids:

- F/U 3 months, daclizumab group had less steroid resistant rejection, DM, and CMV infection

Larger randomized studies with longer F/U are needed.

Renal Sparing Protocols

Up to 21% of LTx patients develop CRF within 5 years.

18% of patients have ***severe*** renal dysfunction after 13 years.

Adding MMF and reducing dose of calcineurin inhibitor (CNI) can improve GFR by 15% in 50% of patients even if done > 1 y post-OLTx; if CNI is D/C, rejection risk is increased.

Conversion from CNI to Sirolimus

28 patients with creatinine > 1.8 mg/dL were converted; mean time= 2y post-LTx.

Dose: 2 mg/d, titrated to 4-10 ng/mL.

14 (50%) had improvement in GFR;
7 progressed to ESRD, and
6 did not tolerate the change.

Large randomized trials are ongoing to evaluate proper time to change.