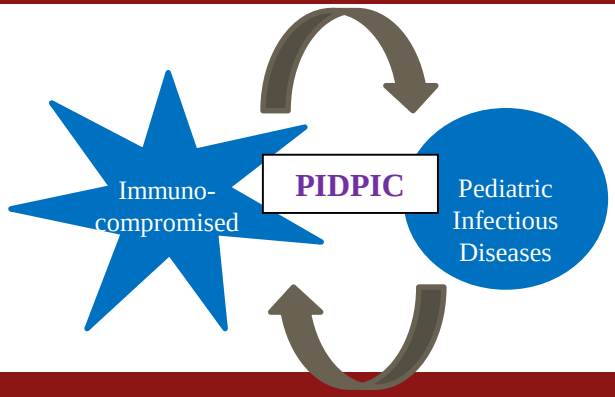

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The Tale of two Herpes Viruses: CMV and EBV

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Transplant Symposium March 28, 2014

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Cytomegalovirus

Sharon F. Chen, MD, MS

Stanford Children's Health | Lucile Packard Children's Hospital Stanford

Cytomegalovirus

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CMV is a childhood infection

After primary CMV infection, CMV transitions to latency.

Prevention Strategies – Pros/Cons

Strategy	Characteristic
Prophylaxis	Continuous anti-viral
Preemptive	Trigger used to start anti-viral
Hybrid	Mix use dependent on time

Prevention Strategies - Which one?

	CMV Syndrome & Disease	Late Onset CMV	Graft Loss, Rejection, Mortality	Leukopenia, Neutropenia
Prophylaxis	-	OR 6.21	-	OR 1.97 OR 2.07
Preemptive	-		-	
Comparison	No Difference	Prophylaxis higher risk	No Difference	Prophylaxis higher risk

Florescu DF. Clinical Infectious Diseases 2014, 58:785

Survey of Strategies Used

Strategy	% Use
Prophylaxis	46%
Preemptive	21%
Hybrid	33%

LePage AK. Transplantation 2013, 95:1455

Razonable. Am Journal Transplantation 2013, 13:93

CMV Immune Monitoring

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CMV is a problem in transplantation because the immune system is not normal.

Should we evaluate the immune response to CMV?

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CMV Immune Monitoring

Since a detectable specific T-cell response against CMV is correlated with an appropriate immune control of CMV of the recipient [10], the monitoring of cell-mediated immunity may be useful in establishing the real risk for developing CMV disease after transplantation and, therefore, for individualizing preventive strategies accordingly.

Manuel O. Current Infectious Disease Report. 2013, 15:491

CMV-specific T-cell Monitoring

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Rationale:

If left untreated, some patients with asymptomatic CMV "DNAemia" will never progress to CMV disease because their CMV-specific immune response spontaneously controls the virus.

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Manuel O. Current Infectious Disease Report. 2013, 15:491

Spontaneous CMV Clearance

CMV Cell-mediated Immunity	Spontaneous CMV Clearance
Positive	24/26 (92%)
Negative	5/11 (45%)

(p=0.004)

Lisboa LF. Transplantation 2012. 93:195

CMV-specific T-cell Monitoring Adult Lung Transplant Patients **D+/R-**

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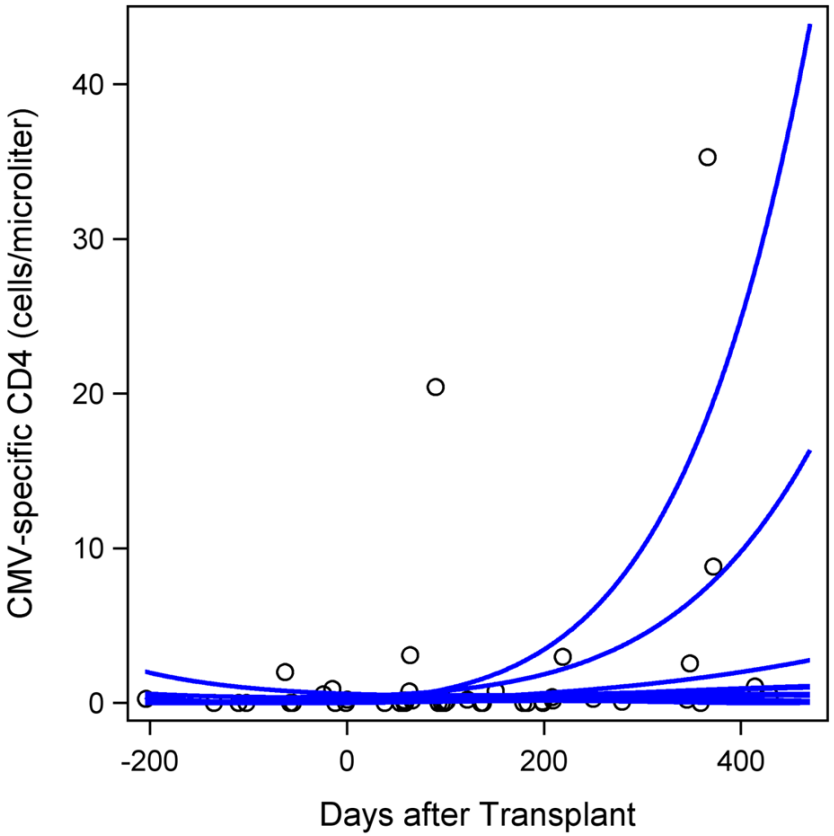

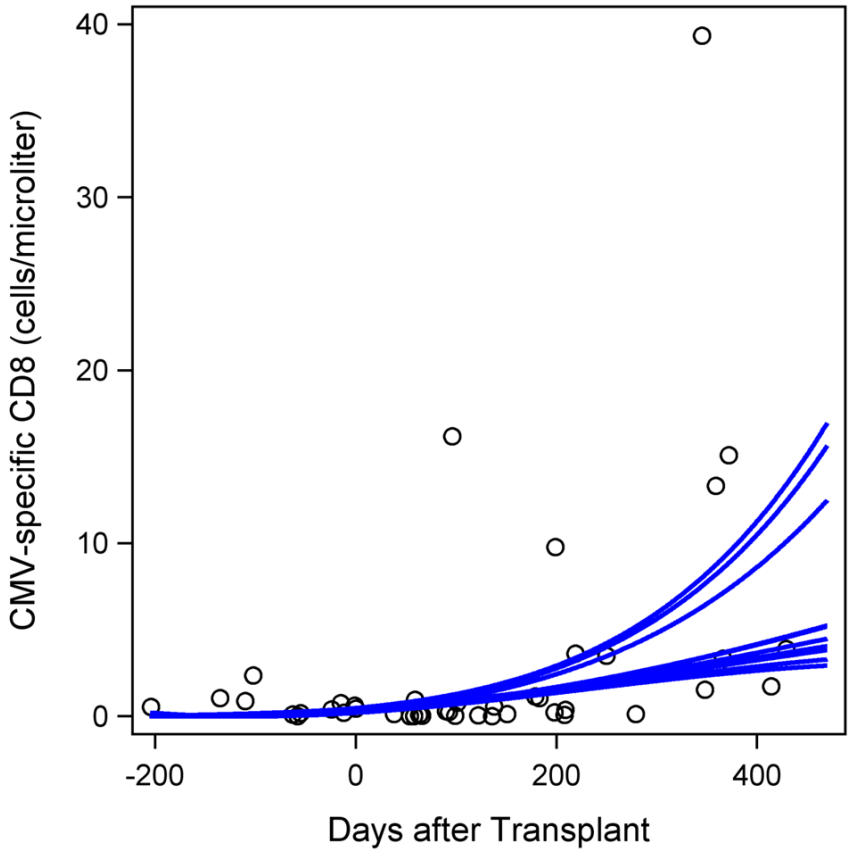

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CMV-specific T-cell Monitoring

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Clinical Aims:

✓To determine duration of CMV prophylaxis
✓To determine risk of developing CMV disease

Challenges:

✓Standardization of assays to monitor CMV-specific T-cells
✓Designing intervention trials

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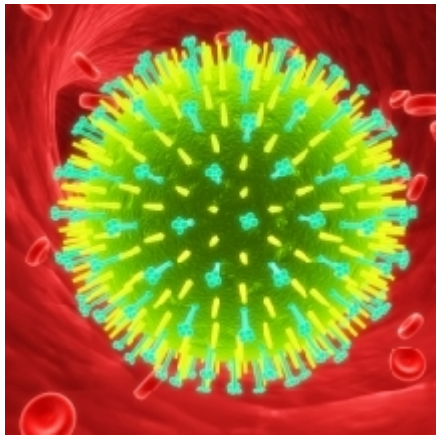
Epstein Barr Virus

Hayley Gans, MD



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The Virus

- In the herpes virus family: HHV4
- Ubiquitous, one of the most common human viral infections
  - 50% infected by 5 years, 90-95% by adulthood
- Spread through body fluids, predominantly saliva
  - Infectivity may last for weeks after primary infection, and can reoccur with reactivation
  - Spread through organ transplantation
- Once primary infection occurs, the virus establishes life long latency in a small population of B cells

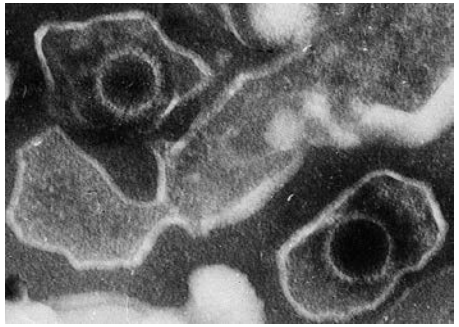


Table 1. Clinical spectrum of Epstein–Barr virus (EBV) disease in solid-organ transplant recipients

Category	Clinical symptoms	Histopathology
Non-specific viral infection	Fever Malaise Anorexia Abdominal pain Diarrhea	Not applicable
Mononucleosis	Fever Malaise Anorexia Adenopathy Tonsillitis/pharyngitis Hepatosplenomegaly	Proliferation of EBV-positive immunoblasts in the interfollicular region of lymphatic tissue in the absence of effacement and/or destruction of normal tissue architecture
Post-transplant lymphoproliferative disorders	Fever Malaise Anorexia Diarrhea Abdominal pain Hematochezia Tonsillitis/pharyngitis Hepatosplenomegaly	Proliferation of EBV-positive immunoblasts and atypical lymphocytes associated with effacement and/or destruction of normal tissue architecture. May be polymorphic or monomorphic in appearance.
Malignant lymphoma	Rapidly progressive growth of EBV-associated tumors despite reduction or withdrawal of immunosuppression	Indistinguishable from lymphoma in immunocompetent patients

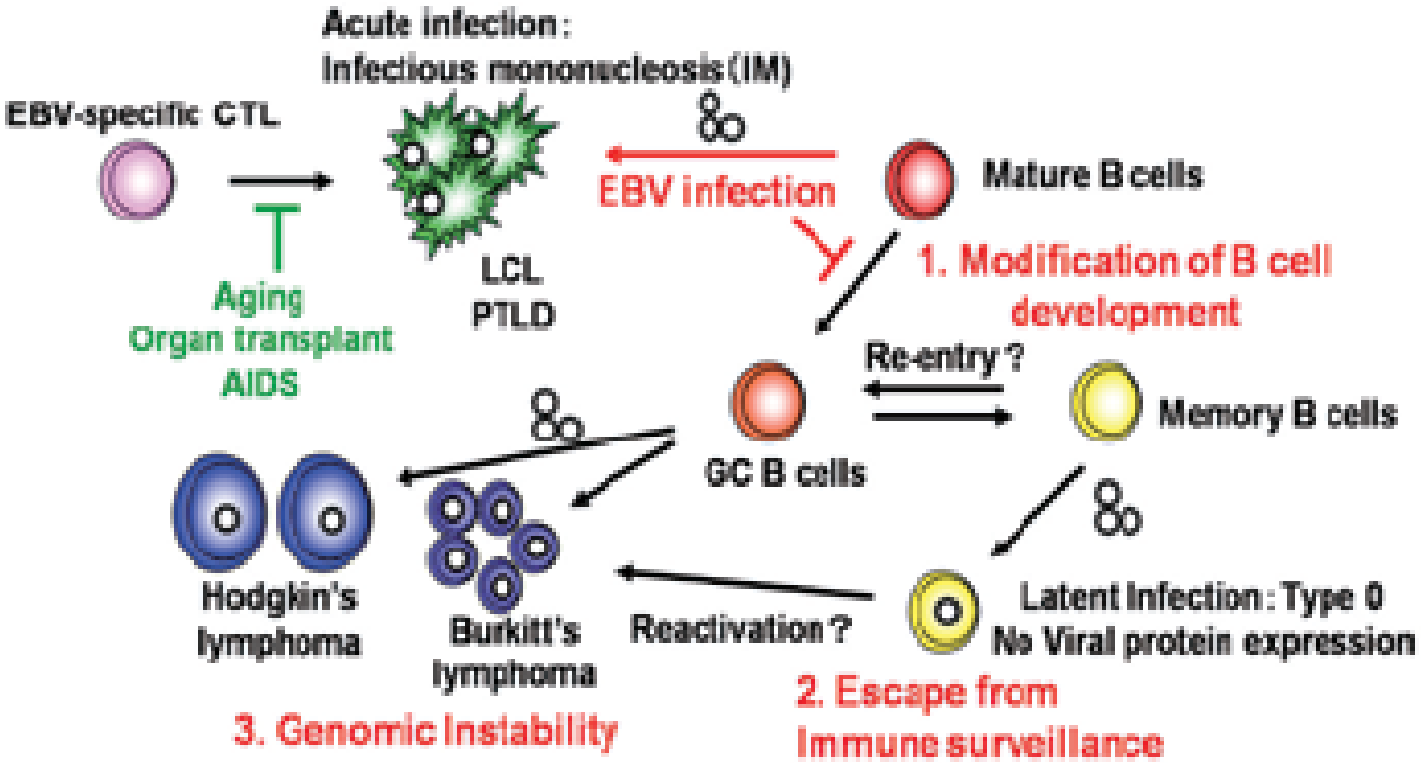
The importance of EBV

- Particularly problematic for pediatric organ recipients
  - Large number experience primary EBV infection post transplant
- The most important long term outcome is Post-transplantation Lymphoproliferative Disease (PTLD)
  - Occurs in 1-15% of liver/renal and up to 6-20% in lung /intestinal/heart
  - Outcomes of PTLD are variable to institutions and are organ-specific
    - Overall ~14%
    - 44% intestinal
    - 31% heart
    - 30% lung
    - 22% liver
    - ~0% renal
  - Prevention is key but early diagnosis is associated with better outcomes, low threshold of suspicion for disease

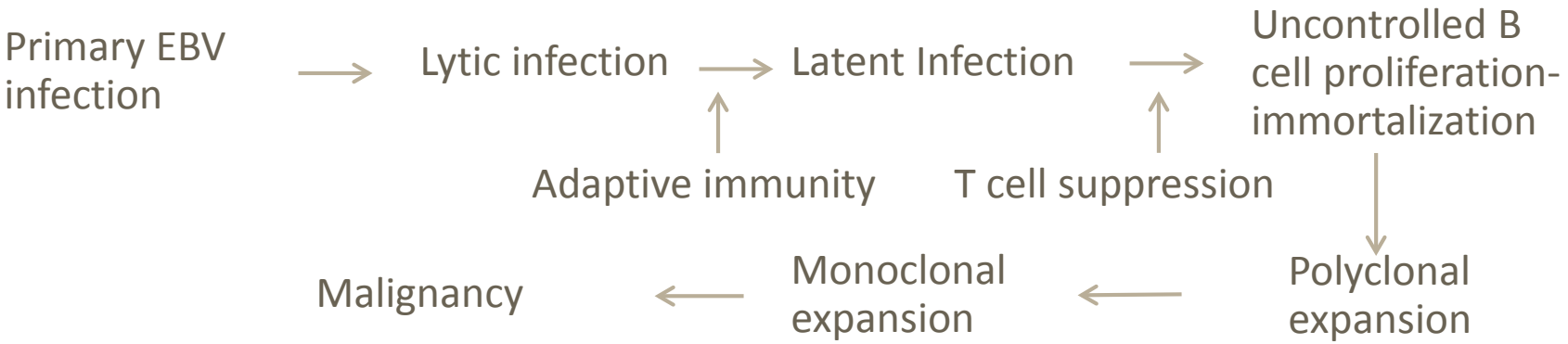
Risk Factors for PTLD

- Seronegativity at time of transplant-5-7 times increased risk
- Use of T cell suppressive therapy for transplant preparation and post-transplant
- Certain HLA types
- Extremes of age
- Circulating virus at time of transplant
- Viral co-infections
- Persistent low levels of viremia post-transplant

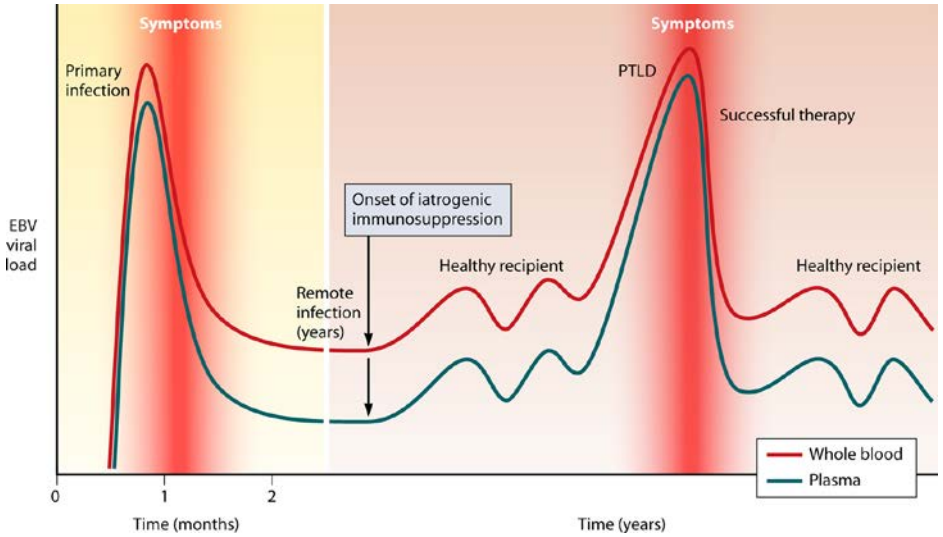
Life Cycle of EBV



PTLD Pathway



Surveillance

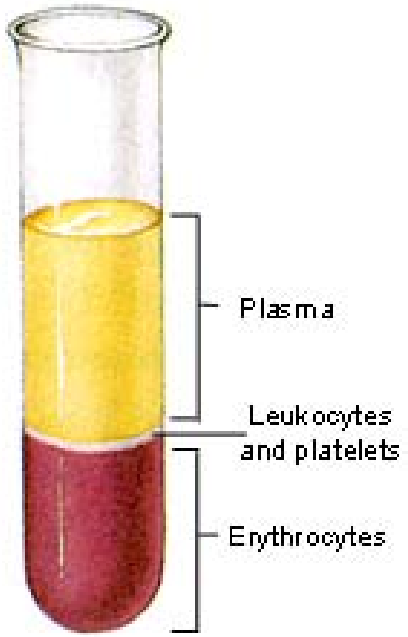


- No established cut-offs signifying PTLD disease or risk but usually detection in more than one sample prompts action
- 100% and 86% for PTLD using a cutoff of 2,000 copies/μg, 100% and
- 90% for 3,000 copies/μg, and 67% and 94% for 5,000 copies/μg.
- Only decreasing immunosuppression as preemptive therapy has been shown to be effective in reducing EBV disease including PTLD
- Older studies supported IVIg, but newer ones did not

Green, Am J of Transpl: 2006

Plasma vs Whole blood

- Both are good tests and can detect EBV genome by PCR
- Plasma is more specific while whole blood is more sensitive (contains EBV infected lymphocytes)
- Plasma is more stable if sample storage is an issue



Diagnosis

TABLE 3. Common laboratory tests to diagnose and manage EBV-related PTLD

Procedure	Specimen types[a]	Analyte	Indications for testing
Real-time PCR or similar EBV load assay	Blood, plasma	DNA	Predict current or impending PTLD or assess efficacy of therapy
	Biopsy, aspirate	DNA	Detect and semiquantify EBV
EBER *in situ* hybridization	Biopsy, aspirate	RNA	Detect and localize latent EBV
Immunohistochemistry			
LMP1, EBNA1	Biopsy, aspirate	Protein	Detect and localize latent EBV
CD20	Biopsy, aspirate	Protein	Classify PTLD and predict response to anti-CD20 immunotherapy

[a] Biopsy or cell aspirate is typically paraffin embedded and sectioned onto glass slides prior to testing.

Gully et al, Clin Microbiol Rev: 2010

Management

- Reduction of immunosuppression first introduced in the 1980s and remains the initial approach
  - 23-86% of organ recipients with non-malignant disease will respond in 2-4 weeks
  - Allows for host immune recovery and persistence for viral control
- Both acyclovir and ganciclovir show in vitro activity against the lytic phase of EBV (replicating), ganciclovir is ~ 10x more potent
  - Neither suppresses EBV-induced B cell proliferation or latent EBV within B cells
  - No prospective studies showing efficacy in treatment of PTLD disease, but routinely used
  - High peripheral viral loads associated with disease or PTLD are present despite anti-viral therapy and recent studies show that the majority of these cells are immortalized B cells without lytic EBV activity and therefore not susceptible to antiviral therapy
  - The only role may be by inhibiting the few cells with lytic infectious virus from spreading to new sites

Management

- IVIg including CMV-IVIg has been shown in vitro to be effective in controlling EBV infected cells
  - Studies have shown absence of antibodies to one of the EBNA proteins in patients with PTLD
  - Other studies have documented decreasing viral loads with increasing anti-EBNA antibodies (native or transfused)
- Rituximab or anti-CD20 monoclonal antibody is effective and should be used for PTLD that has a stron CD20 phenotype which is not routinely the case

Management

- Chemotherapy, radiation and surgery
  - Radiation and surgery of little value unless only localized disease but typically high viral loads signify systemic disease
  - Chemotherapy is immunosuppression and interferes with host immunity but appear to have a role in malignant forms of the disease
- Trials with cytokines IFN (α or γ) were initially promising with efficacy against the PTLD but increased rates of rejection and this has not been pursued further

The possibility of EBV-specific T cells

- Role for T cells in viral control has been established
- Studies have shown increased CD4+ and CD8+ EBV-specific T cells during treatment with reduced immunosuppression in PTLD patients*
- In addition, rebound viral loads after PTLD treatment were in the presence of strong T cell responses and well controlled
- Successful treatment of SCT recipients with EBV-specific T cells has been reported and in increasing use
- Issue for SOT are complex**
  - Unlike SCT recipients whose PTLD is derived from donor thus T cells from the original source can be obtained and are effective against disease, for SOT recipients the lesions are recipient derived and more likely from previous naïve patients. Therefore developing large quantities of T cells requires in vitro "immunization" of patients T cells before transfer
  - Studies are promising and ongoing

*Wilsdorf et al; Transplantation & Volume 95, Number 1, January 15, 2013
**Green, et al, Ped Trans 1999

Management

Table 2. Initial approach to pediatric transplant recipient with non-malignant Epstein–Barr virus–post-transplant lymphoproliferative disorders (EBV–PTLD) at Children's Hospital of Pittsburgh

	Liver	Kidney	Heart	Lung	Intestine
Immune suppression	Stop tacrolimus/CsA[1], AZA[2], MMF[3] Steroids at maintenance	Stop tacrolimus/CsA, AZA, MMF Steroids at maintenance	Stop tacrolimus/CsA, AZA, MMF Restart tacrolimus/CsA after level falls to achieve FK[5] level ~ 5 ng/ml or CsA[4] level 75–100 Steroids at maintenance	Stop tacrolimus/CsA, AZA, MMF Restart tacrolimus/CsA after level falls to achieve FK level ~ 7–8 ng/ml or CsA level 100–125 Steroids to maintenance	Stop tacrolimus/CsA, AZA, MMF Restart tacrolimus/CsA after level falls to achieve FK level ~ –8 ng/ml or CsA level 100–150 Steroids to maintenance
Antiviral therapy	Ganciclovir IV[6] CytoGam IV	Ganciclovir IV CytoGam IV	Ganciclovir IV	Ganciclovir IV	Ganciclovir IV CytoGam IV
Clinical F/U, Rejection Surveillance	Monitor LFTs Weekly EBV PCR Daily examination Liver biopsy for suspected ACR	Monitor renal function Weekly EBV PCR Daily examination Renal biopsy for suspected ACR	Echocardiograms (2–3 per week) Surveillance biopsies at 1 week then every 1–2 weeks Weekly EBV PCR Daily examination	TBB[7] 7–10 days into treatment Then PRN based on status Weekly EBV PCR Daily examination Frequent pulmonary function tests and CXR[8]	Endoscopy with biopsy every 1–2 weeks Weekly EBV PCR Daily examination

[1]CsA, Cyclosporin A; [2]AZA, Azathioprine; [3]MMF, Mycophenalate mofetil; [4]CsA level obtained by monoclonal assay; [5]FK, Tacrolimus; [6]IV, intravenous; [7]TBB, transbronchial biopsy; [8]CXR, chest radiograph.

Green, et al, Ped Trans 1999

Viral Load monitoring Post PTLD

- Use of viral load by PCR has been shown to correlate with disease regression and may predict time to rejection
  - Centers using 200 copies/10^5 PBL as threshold for PTLD diagnosis, when levels dropped to this level disease regression was seen and the start of rejection noted
  - The use of post-PTLD viral monitoring has been challenging as rebound levels are seen routinely without recurrent disease
- Repeat disease is seen in <10%