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Review

Acute Rejection of the Lung Allograft: Phenotypes and Management

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Abstract

Treatment options for end stage lung diseases are limited to stabilizing lung failure, decreasing disease progression, and symptom management, but significant reversal of lost lung function is often not possible. For well selected patients, lung transplantation may be a viable option to improve both longevity and quality of life. Though outcomes for lung transplant recipients have improved over several decades, long term survival still lags behind that of other solid organ transplant recipients. Longevity after lung transplantation is limited by chronic lung allograft dysfunction. Numerous insults to the allograft contribute to chronic rejection, alloimmune injuries including acute T-cell mediated and antibody mediated rejection are chief among them. Therefore, monitoring for and management of acute cellular and antibody mediated rejection are of paramount importance to those caring for lung transplant recipients. We provide an up to date and comprehensive review of acute rejection affecting lung allografts and attempt to highlight pathophysiology, risk factors, clinical presentation, rejection phenotypes, management strategies, as well as related from of acute allograft injury.

Keywords
Lung transplantation; acute rejection; lung transplant rejection; acute Cellular rejection (ACR); T-cell mediated rejection (TCMR); antibody mediated rejection (AMR); lung transplant ACR; lung transplant AMR; management acute lung transplant rejection; ACR treatment; AMR treatment; phenotypes acute lung transplant rejection; lung transplant immunosuppression management; post lung transplant monitoring

1. Introduction

Lung transplantation remains the only definitive restorative therapy for highly selected patients with end stage lung diseases who have exhausted options for medical therapy. Despite progress overtime as evidenced by an improved long-term survival when comparing earlier to recent eras (1996-2001 vs 2002-2007 and 2008-2013), it should be noted that survival over the two most recent eras has stagnated with an overall 1 year contingent 5 year survival of approximately 65% in North America [1]. Long term survival among lung transplant (LT) recipients significantly lags behind other solid organ transplant (SOT) recipients, with the most recent Scientific Registry of Transplant Recipients (SRTR) data reporting 5 years survival rates approximating >80%, ~80%, and ~80% for kidney, liver, and heart transplant recipients respectively [2-4]. Long term survival post LT is primarily limited by chronic lung allograft dysfunction (CLAD) which impacts 50% of recipients 5 years post-transplant and is the leading cause of death for those who survive the first post-transplant year [5]. Amongst the numerous allograft insults contributing to the development of CLAD overtime, acute alloimmune rejection is at the forefront. Herein, we provide a comprehensive clinically focused review of acute cellular and antibody mediated rejection, and attempt to characterize histopathologic and clinical sub-phenotypes, and review pathogenesis, risk factors, and management.

2. Acute Cellular Rejection

2.1 ACR Mechanism, Diagnosis, and Incidence

Acute cellular rejection (ACR) is characterized by a T-cell dependent alloreactive injury to the graft due to recognition of foreign donor major histocompatibility complex (MHC) antigens, and is identified histologically by a perivascular and/or peribronchiolar mononuclear cell infiltration [6, 7]. Effector T-cells, are recruited to the lung allograft through recognition of donor MHC antigens, leading to tissue injury and decreased allograft function. Several pathways of alloimmune recognition have been proposed. In the direct pathway, donor dendritic cells in the graft present the foreign MHC to the recipient's T-cells; the indirect pathway involves recipient's dendritic cells presenting processed allograft MHC proteins to the recipient's T-cells [6]. A semi-indirect pathway has also been described in which recipient dendritic cells acquire intact donor MHC molecules which may be recognized by recipient T-cells [8].

ACR is diagnosed based on histopathology obtained by transbronchial biopsies (TBBx), requiring at least 5 pieces of well alveolated lung parenchyma for adequate assessment. Standardized histologic classification, severity grading, and reporting nomenclature, have been outlined by an

ISHLT working group and last updated in 2007 [7]. The microanatomic location of the lesion defines the two histopathologic subtypes which can occur independently or concurrently. Perivascular inflammation involving primarily pulmonary venules is defined as acute rejection (AR), and peribronchiolar inflammation defines lymphocytic bronchiolitis (LB). Severity is based on the degree of extension and cellular composition of the mononuclear infiltrate. Table 1 summarizes AR and LB histologic features and grading schema, Figures 1-6 provide histopathology reference examples. AR and LB are treated similarly in clinical practice, when referring non-specifically to acute T-cell mediated alloimmune injury, we will use the term ACR. Where AR and LB are used, greater specificity is implied. Both AR and LB are well recognized risk factors for subsequent development of CLAD [9-11]. CLAD is a key limitation to long term success post lung transplantation and is the primary driver of mortality after the first post-transplant year [5], as such, timely recognition and management of ACR is of utmost importance.

Table 1 ACR and LB Histologic Features and Grading, adopted from Stewart et al [7].

Acute Cellular Rejection		
A Grade – perivascular inflammation	Severity	Histopathology
A0	None	Normal pulmonary parenchyma, no mononuclear cell infiltration, hemorrhage or necrosis
A1	Minimal	Scattered, infrequent, perivascular mononuclear infiltrates. Blood vessel lymphocytic cuff 2-3 cells in thickness, no eosinophils or endothelialitis
A2	Mild	More frequent perivascular infiltrates, eosinophils and endothelialitis may be present, no mono-nuclear cell infiltration into adjacent alveolar septa
A3	Moderate	Dense perivascular infiltrates, frequently with associated endothelialitis, eosinophils common, may have neutrophil infiltration as well, extension of inflammatory cell infiltrate into peribronchiolar and alveolar septa present
A4	Severe	Diffuse perivascular and air-space inflammatory infiltrates, prominent endothelialitis, epithelial cell necrosis, hyaline membranes, hemorrhage/necrosis may be present
Ax	Ungradable	Insufficient alveolated tissue
Lymphocytic Bronchiolitis		
B Grade – airway inflammation	Severity	Histopathology
B0	None	Normal bronchiolar tissue

B1R	Mild	Mononuclear cells within bronchiole sub-mucosa, may be infrequent, scattered, or forming small circumferential bands
B2R	Severe	Bronchiole submucosal mononuclear cells, increased eosinophils, epithelial damage, in most severe form may have fibrinopurulent exudate and neutrophils
BX	Ungradable	Insufficient bronchiolar tissue

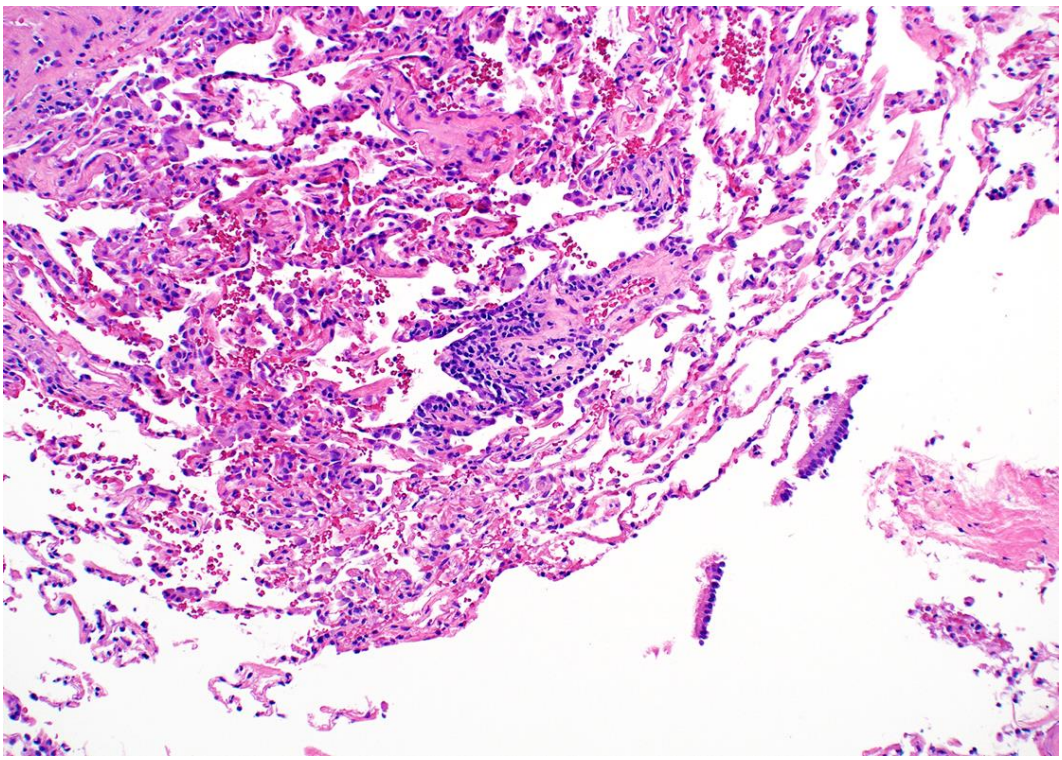

Figure 1 A1 ACR (minimal ACR): Focal perivascular mononuclear cell infiltrate of up to 3 layers of lymphocytes without endothelialitis or eosinophils (10× magnification).

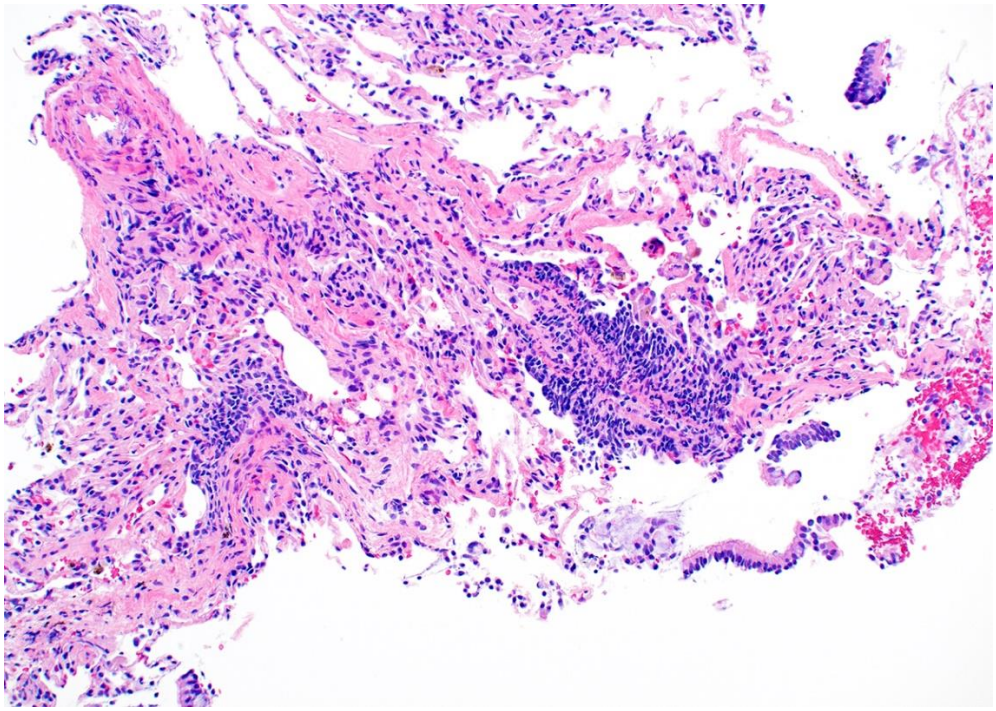

Figure 2 A2 ACR (mild ACR): More conspicuous mononuclear cell infiltrate with endothelialitis but no eosinophils. Extension to adjacent alveolar septa is not seen (10× magnification).

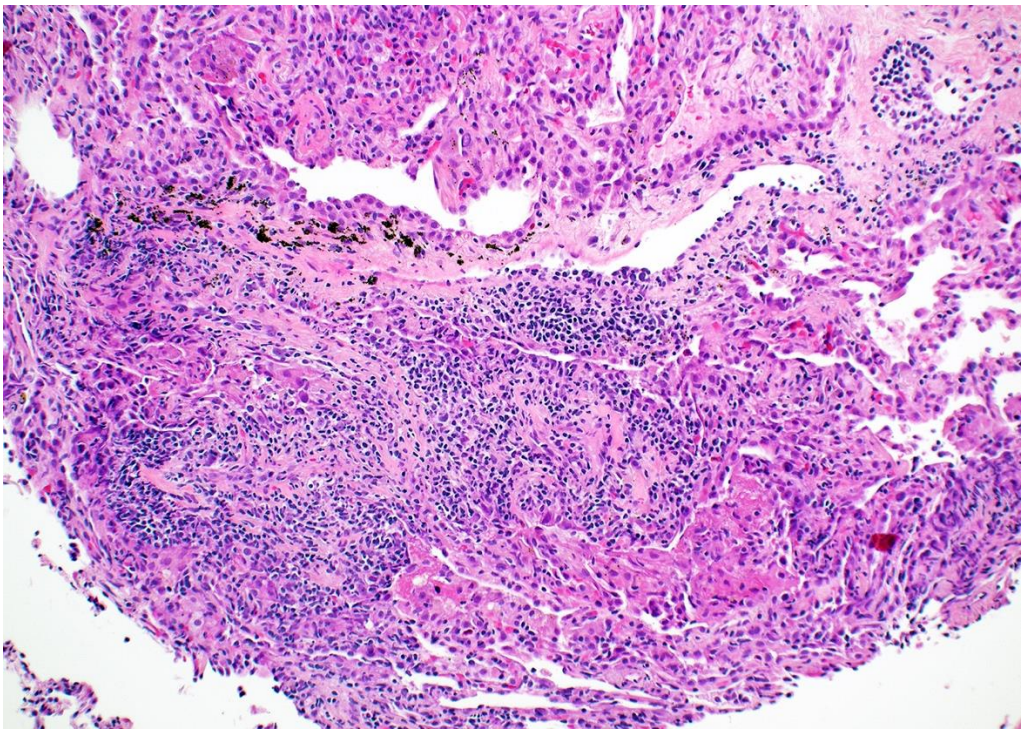

Figure 3 A3 ACR (moderate ACR): Conspicuous mononuclear cell infiltrate with extension into adjacent peribronchiolar alveolar septa and endothelialitis. Aggregates of intra-alveolar macrophages and reactive type II pneumocyte hyperplasia are seen adjacent to areas of alveolar septal expansion (10× magnification).

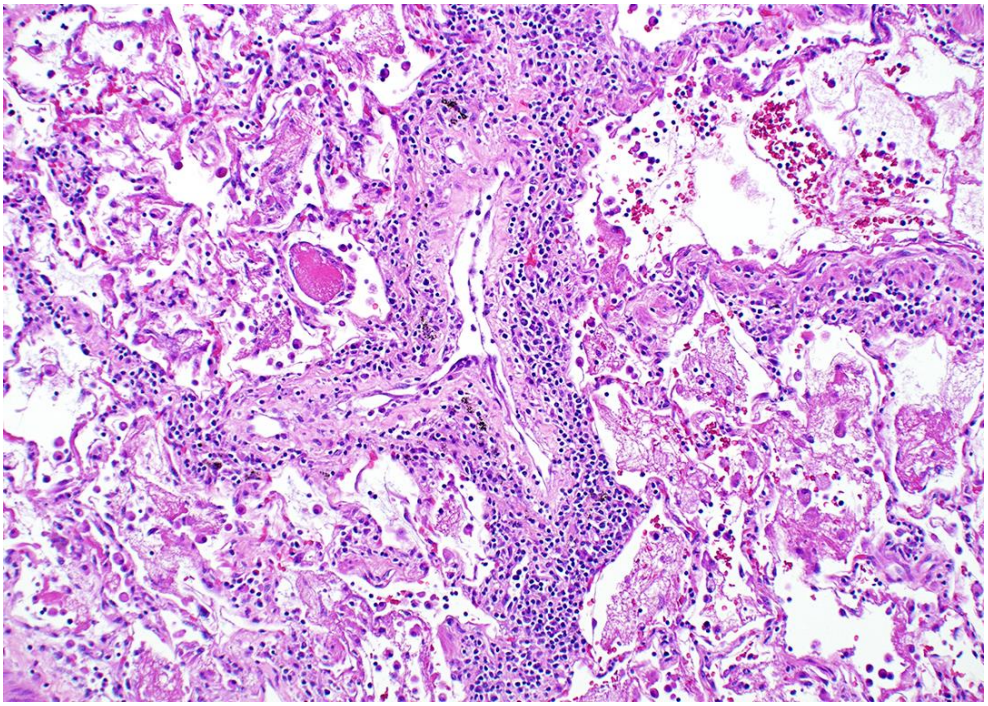

Figure 4 A4 ACR (severe ACR): Diffuse perivascular, interstitial and intra-alveolar aggregates of lymphocytes with diffuse pneumocyte damage and endothelialitis. Multiple intra-alveolar necrotic epithelial cells and hyaline membranes are seen (10× magnification). [Images courtesy of Dr. Sergio Pina-Oviedo, Duke University Hospital, Division of Pulmonary/Thoracic Pathology].

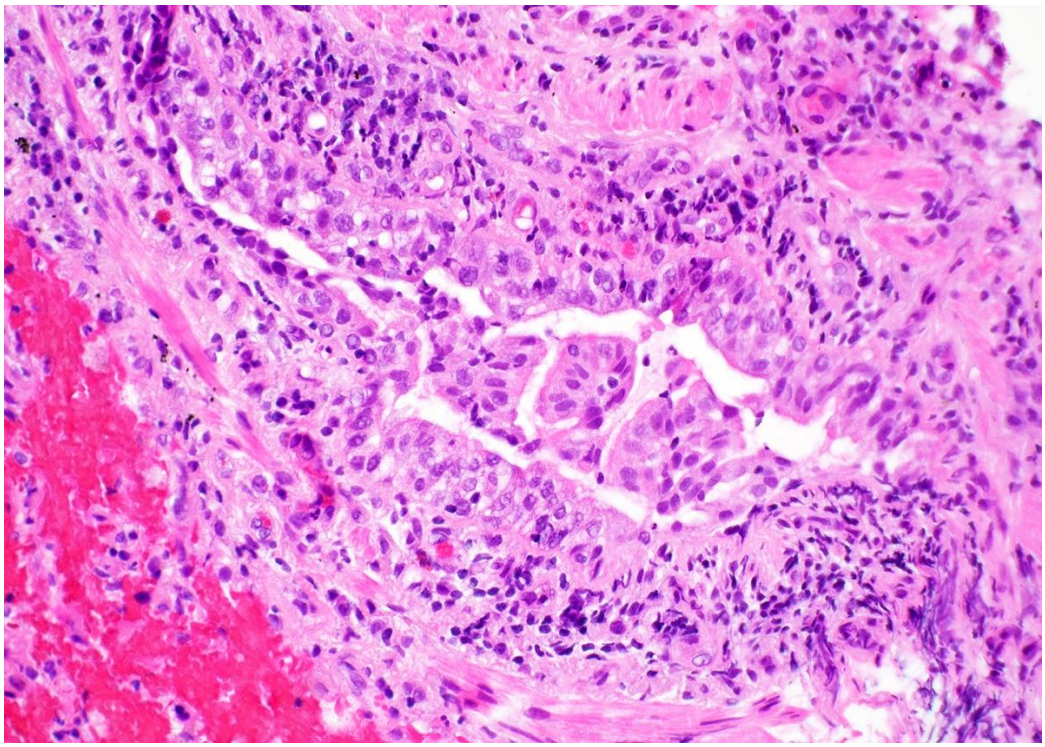

Figure 5 B1R (low-grade small airway inflammation): Sparse mononuclear infiltrate with rare eosinophils in bronchiolar submucosa with rare intraepithelial lymphocytes. No squamous metaplasia or epithelial damage is seen (20× magnification).

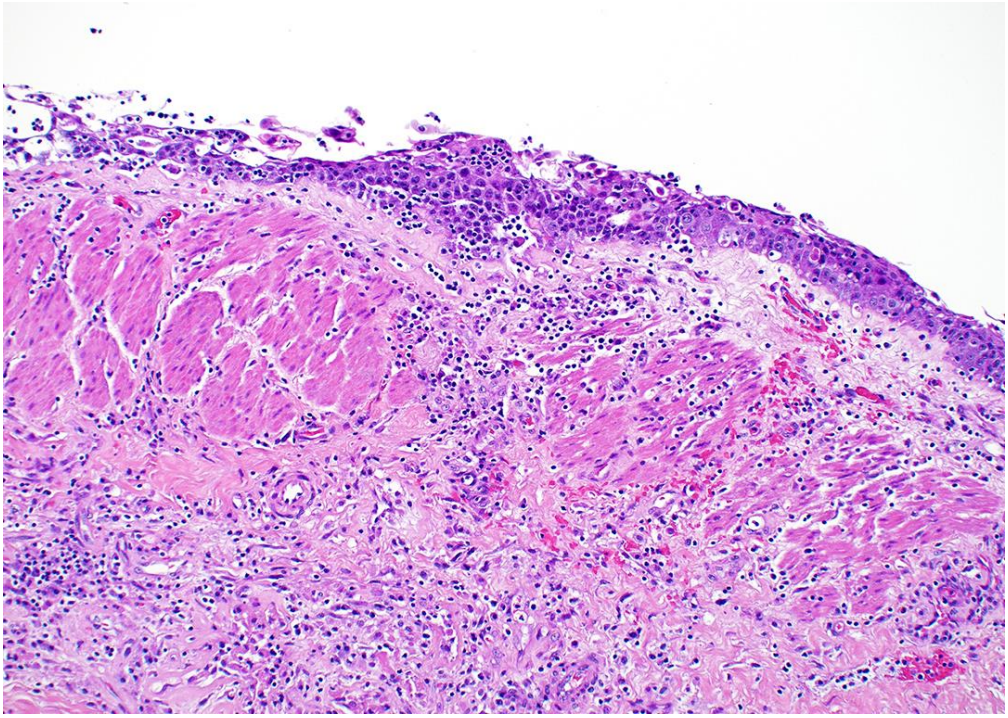

Figure 6 B2R (high-grade small airway inflammation): There are numerous intraepithelial lymphocytes and epithelial damage seen as squamous metaplasia with focal epithelial sloughing and early epithelial necrotic changes (10× magnification). [Images courtesy of Dr. Sergio Pina-Oviedo, Duke University Hospital, Division of Pulmonary/Thoracic Pathology].

While ACR is a common complication after LT, the true incidence is unknown. Estimates for the rates of ACR have ranged from 17-40% [12-14]. Differences in reported rates are likely explained by differenced in data source, ACR definitions used, immunosuppression utilization, and screening protocols. ISHLT registry data notes a declining incidence of treated ACR in the first post-transplant year overtime, impacting 26.6% of recipients between 2005-2018. The slight decline overtime is attributed to increased use of basiliximab induction as well as tacrolimus and mycophenolate for maintenance immunosuppression [5]. A recent multicenter prospective study reported 53.3% of subjects had at least one episode of AR, and 14.8% had at least one episode of LB, in the first post-transplant year [15].

2.2 ACR Risk Factors

The risk of ACR is greatest in the first months to one year after transplant and decreases thereafter [16, 17]. ACR risk factors may be broadly conceptualized into immunologic, donor/recipient, and environmental risks. Numerous risk factors have been implicated and summarized exhaustively by Renaud-Picard et al – notable risks are outlined in Table 2 [18]. In a prospective multicenter study of 400 patients undergoing surveillance transbronchial biopsies in the first-year post-transplant, increased degree of HLA mismatching, single vs. double LT, and decreased donor age were associated with increased ACR on univariate analysis. Only HLA mismatching and double vs. single LT remained significantly associated on multivariable analysis [15].

Table 2 ACR Risk Factors.

Immunologic	Donor/Recipient	Environmental
Increased degree of HLA mismatch	Younger recipient age	CMV infection
Allosensitization/Presence of DSAs	Single lung transplant	Bacterial/fungal infections (possible risk factor)
Genetic polymorphisms of innate and cellular immunity components	Early post-transplant period	CARV infection
Prior ACR episodes	GERD in recipient	
Immunosuppressive strategy	Younger donor age	
Subtherapeutic immunosuppression		

2.2.1 Induction and Maintenance Immunosuppression Impact on ACR

Induction immunosuppression utilization has increased overtime, with over 80% of recipients receiving induction therapy, the vast majority (>70%) with an interleukin-2 receptor antibody (IL-2RA), basiliximab [5], due to the perceived better safety profile in regards to cytokine release syndromes, prolonged lymphocyte depletion and infectious risk associated with alemtuzumab and antithymocyte globulin (ATG). ISLHT registry data demonstrates a minimal though statistically significant overall survival benefit for any induction agent utilization (IL-2RA, ATG, or alemtuzumab) vs. none; as well as a small but statistically significant decreased rate of treated ACR in the first post-transplant year with the use of IL-2RA only compared to alternatives [5]. Retrospective data supports decreased rates of early ACR when comparing: IL2-RA or ATG to no-induction (15% vs 22% and 17% vs 22% respectively, $p < 0.005$) [19], and decreased rates of AR with alemtuzumab compared to IL2-RA induction (39.1% vs 53.4% $p < 0.001$) [20]. Comparison of ACR rates between IL2-RA and ATG induction have been mixed with some retrospective studies noting no difference between the two [19, 21], and others reporting higher rates of AR with the use of IL2-RA [22]. A Cochrane meta-analysis published in 2013 that included 6 randomized controlled trials (RCT) found no significant differences in rates of ACR between any induction medication (IL2-RA, alemtuzumab, ATG, or Muromonab-CD3) or placebo; importantly no differences in adverse events related to infection or cancer were found either [23]. In contrast, the most recent published meta-analysis reviewing induction strategies in thoracic transplantation found that alemtuzumab was more effective at preventing ACR compared to ATG or IL2-RA [24]. Overall, the available data is conflicting and there is no consensus as to the optimal induction strategy to prevent ACR. Induction strategies continue to be determined by institutional experience and protocols, catered to individualized patient needs and risk assessments.

Triple immunosuppressive therapy with a calcineurin inhibiter (CNI), antimetabolite, and corticosteroid is the cornerstone of maintenance immunosuppressive (mIS) therapy. It is a-priori understood that maintaining appropriate immunosuppression levels decreases the risk of ACR. There is very limited and conflicting data on CNI level variability and ACR outcomes among LT recipients [25, 26], though it has been demonstrated that optimizing CNI levels may decrease ACR rates [27]. The optimal mIS therapy combination to minimize ACR remains a topic of debate. ISHLT registry data suggests a significantly lower rate of treated ACR in the first post-transplant year among those on tacrolimus vs cyclosporine-based regimens [5]. Interestingly, two metanalysis

published in 2009 and 2013 respectively come to somewhat differing conclusions as to whether a tacrolimus vs. cyclosporine-based regimen is associated with different ACR outcomes [28, 29]. Fan et al found lower rates of AR and LB with a tacrolimus based regimen; Penninga et al replicated the LB findings, but found no significant difference in rates of AR. A recent consensus document endorsed by the ISHLT does make a weak recommendation for tacrolimus as first line CNI based on moderate evidence supporting decreased rates of ACR and CLAD compared to alternatives [30]. A similarly weak recommendation for mycophenolic acid as the first line antimetabolite was made based on low level of evidence of better efficacy compared to azathioprine [30]. Whether mammalian target of rapamycin inhibitors (mTORi) such as sirolimus or everolimus, have a role in minimizing ACR risk is another area for which there is discordant data. Two small RCTs have found decreased rates of ACR when an mTORi is utilized in lieu of an antimetabolite [14, 31], though this was not a robustly positive result in the study by Glanville et al. Notably, this finding has not been replicated in a more recent RCT ("4EVERLUNG") evaluating mTORi as part of a quadruple mIS strategy, which found similar rates of ACR between quadruple and standard triple mIS groups [32]. The use of mTORI as part of mIS in LT recipients is best reserved for specific clinical scenarios, discussed further below. The optimal mIS to minimize ACR, and achieve other competing therapeutic goals, requires individualization on a case-by-case basis.

2.2.2 Infection and ACR

Community acquired respiratory viral infections (CARVs) may be concurrent with or antecedent to development of ACR. It is postulated that CARVs may lead to acute rejection through upregulation of immune mediators by activation of the innate immune system, or through epithelial injury and exposure of cryptic antigens [33, 34]. Data supporting the link between CARVs, particularly viral infection involving the lower respiratory tract, and increased CLAD risk [35, 36], is more robust than that for the association between CARVs and subsequent ACR, for which studies have shown inconstant results of association [37]. It is our clinical experience that post-viral ACR is in fact a true clinical entity. Though limited by the broad definition of ACR that was employed, a single center prospective study reported a 33.3% rate of ACR within three months of respiratory viral infection identified by BAL, compared to a 6.5% ACR rate among those without a preceding respiratory viral infection in the same time frame [38]. Rhinovirus, parainfluenza, and coronavirus comprised the majority of isolated infections. Other studies have reported a 1 year post viral ACR incidence of 3.8% and 6.5% after parainfluenza and human metapneumovirus respectively [39]. Dependent upon definitions used, organisms of interest, and follow up time considered, post-viral ACR has been estimated to impact anywhere between 5-55% of subjects [40]. Unsurprisingly, similar trends have been found among LT recipients with the novel SARS-CoV-2 virus. A single center retrospective review identified ACR in 10% of subjects who had persistently decreased allograft function 90 days post COVID-19 infection, another center reported 2/16 subjects developed ACR within 6 weeks after COVID-19 infection [41, 42]. Post COVID-19 ACR has not been a consistent finding, with another single center case-controlled study failing to identify any episodes of post COVID-19 ACR among 24 LT recipients within 90 days of infection [40]. Elucidating the impact of CARVs and COVID-19 on ACR and other outcomes of interest among LT recipients remains an active area of investigation.

Cytomegaly virus (CMV) viremia has also been identified as a risk factor for ACR among lung and other SOT recipients, possibly a result of the robust CD8 T-cell response generated to control this

pathogen [43, 44]. In fact, retrospective data and a prospective RCT evaluating extended CMV prophylaxis among LT recipients have demonstrated a tendency towards decreased rates of ACR within the extended prophylaxis groups [45, 46]. Notably, Todd et al failed to identify CMV infection, any respiratory infection (viral, bacterial, fungal, or mycobacterial), the use of induction immunosuppression, or choice of maintenance immunosuppression (Tacrolimus vs Cyclosporine) as significant ACR risk factors [15]. This further demonstrates the difficulties in clearly establishing ACR risk factors due to heterogeneity in the data reflecting differences in study design, changes in recipient characteristics, immunosuppression and infection prophylaxis strategies over time.

2.2.3 The Gastrointestinal Tract and ACR

Gastroesophageal reflux disease (GERD) and micro-aspiration are common problems for those with end stage lung disease, particularly IPF. Among LT recipients, its prevalence may even increase after transplant surgery due to vagal nerve injury, diminished cough reflex, impaired muco-ciliary clearance, and gastroparesis [47]. GERD and micro-aspiration of gastric contents, including bile acids and proteolytic enzymes such as pepsin, may lead to allograft injury through several mechanisms. Local inflammatory response stimulation due to direct cytotoxic effects of bile acid disruption of cell membranes. Loss of type II pneumocytes in this fashion may lead to alterations in surfactant homeostasis, all contributing to allograft injury [47]. There is also growing interest on the relationship between GERD and the composition of the allograft microbiome which may have implications for allograft injury risk [48]. Lung transplant recipients with GERD have been shown to have enriched concentrations of the bile acid, taurocholic acid (TCA), along with other inflammatory cytokines in BAL samples compared to those without GERD and were more likely to be treated for ACR within 3 months post-transplant [49]. Pepsin, another marker of gastric aspiration, has been observed to be enriched in the BAL fluid of subjects with A2 rejection or higher compared to allografts with lower grades or free from ACR [50]. Additionally, higher concentrations of TCA and glycocholic acid (GCA) in BAL samples correlated with increased rates of spirometrically defined acute allograft injury early post-transplant [49]. In a subset of GERD patients who underwent Nissen fundoplication, BAL fluid concentrations of TCA and other inflammatory cytokines decreased to levels comparable to non-GERD subjects [49]. While the association between increased rates of early ACR and GERD have been replicated [51], interestingly, in the study by Zhang et al, increased BAL concentrations of GCA and TCA (bile acids), rather than GERD itself, were associated with early acute allograft injury. This suggests that the presence of micro-aspiration, which may be mediated by other post-transplant foregut pathologies such as gastroparesis, esophageal dysmotility, hiatal hernias, or dysphagia, is also a key contributor allograft injury risk.

2.2.4 Optimizing ACR Risk

A detailed understanding of the risk factors associated with ACR is of key importance in the care of LT recipients. Our approach to all patients presenting with ACR, especially those with recurrent or refractory ACR, is to not only treat the rejection, but also elucidate if there is a modifiable risk factor that may be addressed. Those with recurrent respiratory infections may need additional guidance on safety precautions to limit infectious exposures, assessment of vaccination status, or testing and treatment for acquired hypogammaglobulinemia. Those with aspiration as a driving factor may be referred for further investigation of swallow mechanics and gastric emptying -

subsequent dietary modifications, Speech-Language Pathologist care, pyloric dilation with or without local botulinum toxin injection, or prokinetic medications may be indicated on a case-by-case basis. LT recipients with GERD may benefit from surgical reflux therapies such as fundoplication [52]. At our institution, all LT recipients undergo reflux and esophageal dysmotility screening with the Bravo Capsule pH and Endoflip systems [53, 54] within the first post-transplant year and are referred for evaluation of surgical reflux options if indicated.

Sub-optimal mIS is a key contributor to ACR, and identifying possibilities for optimization is critical for decreasing future risk. For those with inexplicably low or variable CNI levels, careful medication reconciliation evaluating for patient compliance and food-drug or drug-drug interactions that may impact CNI absorption or metabolism should be assessed. As addressed earlier, tacrolimus is the preferred CNI for mIS. In a single center RCT comparing tacrolimus vs cyclosporine on a background of basiliximab induction and azathioprine/prednisone maintenance, the tacrolimus group had a significantly lower burden of AR and LB [13], this finding has been in part supported by registry data, meta-analysis, and expert consensus [5, 28, 30]. In our experience, many transplant recipients are changed from tacrolimus to cyclosporine early post-operatively due to "neurotoxicity" that in retrospect is often difficult to discern from hospital acquired or critical illness encephalopathy or delirium. For those with recurrent ACR on cyclosporine, optimizing mIS by a trial of re-introducing tacrolimus on a case-by-case basis may be considered. The data supporting a change from azathioprine to mycophenolic acid in the setting of ACR or recurrent ACR is even less robust, however, this option may be considered as well for mIS optimization [30].

For LT recipients being managed with sub-optimal CNI levels, and/or dose reduced or absent antimetabolite due chronic kidney disease (CKD), CMV disease, or skin cancer, augmentation of mIS with a mTORi may be an option for optimization. In addition to their immunosuppressive effects, mTORi may have anti-neoplastic properties. When used as part of mIS in transplant recipients, they may have a beneficial impact in regards to incidence, progression and severity of pre-cancerous and cancerous skin lesions [55]. Multiple prospective studies have demonstrated decreased rates of CMV infection with the use of mTORi in place of an antimetabolite agent [14, 31, 56]. Additionally, in an RCT evaluating mTORi as part of a quadruple mIS strategy ("4EVERLUNG"), there was a decreased rate of CMV infection in the mTORi group, though the difference did not reach statistical significance [32, 57]. A strategy of renal preservation utilizing a quadruple mIS strategy with addition of an mTORi to limit CNI induced nephrotoxicity has been employed with some success. Several RCTs have demonstrated improved renal function compared to standard triple mIS with short term follow up [32, 58, 59]. However, it should be noted that in the 4EVERLUNG trial this benefit was not sustained at 5 years of follow up, and in the Nordic Certican Trial in Heart and Lung Transplantation (NOCTET) trial - which included both heart and lung thoracic transplant recipients - long term benefit beyond 5 years was only sustained among heart transplant recipients [57, 60].

Belatacept, a CTLA-4 fusion protein, is a novel mIS agent which works through co-stimulation blockade of the CD80/86 and CD28 interaction. It has been deployed with great success amongst Epstein Bar Virus (EBV) seropositive kidney transplant recipients as an alternative to, or with dose reduced CNI, to prevent long term nephrotoxicity [61, 62]. In clinical trials of kidney transplant recipients, when used in lieu of CNI, Belatacept was associated with an increased risk of ACR [63], it has also been demonstrated to decrease the rate of development of donor-specific antibodies (DSAs) [61]. Unsurprisingly, there has been great interest in the utilization of this agent among LT recipients for its renal sparing effects, or for those intolerant of CNIs due to severe complications such as

Posterior Reversible Encephalopathy Syndrome (PRES) or Thrombotic Thrombocytopenia Purpura (TTP) [64-66]. However, in the only RCT evaluating Belatacept among LT recipients, which randomized subjects to Belatacept plus reduced dose tacrolimus as part of a quadruple mIS strategy vs standard tacrolimus based triple mIS strategy, the trial was stopped after randomization of only 27 subjects due to an excess of deaths among the study arm (five vs none) [67]. In all cases of ACR, particularly persistent or refractory ACR with sub-optimal mIS, the reasons for poor optimization should be investigated and addressed wherever possible.

2.3 Eosinophilic ACR

In addition to histologic phenotypes, an eosinophilic phenotype of ACR is increasingly being recognized. Both peripheral eosinophilia and eosinophilic alveolitis have been associated with ACR, BAL eosinophilia >/= 1% in the first post-transplant year was also found to be a significant predictor of CLAD [68, 69]. Other investigators have also demonstrated an association between BAL or serum eosinophilia with CLAD, and specifically the restrictive allograft syndrome (RAS) phenotype which has a particularly poor prognosis [70, 71]. The presence of eosinophilia is of great interest as a predictive biomarker as is the potential for therapeutic trials utilizing already approved biologic drugs targeting the Th2/IL-5 pathway.

2.3.1 Related Forms of Acute Graft Injury

Azithromycin responsive allograft dysfunction (ARAD), previously referred to as neutrophil reversible allograft dysfunction, is a long recognized form of acute allograft injury. ARAD refers to a subset of possible or established BOS (bronchiolitis obliterans syndrome) patients who demonstrate at least 10% reversal in forced expiratory volume in 1 second (FEV1) decline in response to Azithromycin therapy [72]. Neutrophilic alveolitis in the absence of infection has been suggested to be a risk for future CLAD. While earlier studies identified BAL neutrophilia as a biomarker predictive of Azithromycin responsiveness, the later has not been reliably reproducible, hence the change in terminology [72-74]. An 8-week trial of Azithromycin therapy is advocated by the ISHLT guidelines as a possible therapeutic trial with new onset CLAD/BOS [75].

Acute Fibrinous Organizing Pneumonia (AFOP) is another form of allograft dysfunction that has been described histopathologically and may represent a form of acute, subacute, or fulminant allograft injury for which the mechanism of alloimmune injury has not yet been uncovered. AFOP was initially described in non-transplant setting as a histological variant of acute lung injury (ALI). It is characterized by a predominance of interalveolar fibrin conglomerates involving 25-90% of sampled air spaces, and must be differentiated from diffuse alveolar damage (DAD), with its hyaline membranes, or the classic organizing pneumonia (OP), which has fibroblast predominant granulation tissue airspace deposits [76]. In the non-LT setting, the AFOP injury pattern may be idiopathic but has been reported in association with infection, connective tissues disease, occupational exposures, and drug exposures [76]. AFOP has also been identified in lung allografts of recipients with a clinical picture of aggressive and rapidly declining graft dysfunction and hypoxia, accelerated CLAD, and progression to death [77, 78]. Later onset of AFOP, occurring over 90 days post-transplant, has been associated with worse survival and development of RAS [79]. Notably, histopathological examination of lung allografts from recipients with RAS found pathologic evidence of AFOP in 50% subjects [80]. The most common radiological corelates reported in association with

AFOB include diffuse (often basal predominant) bilateral infiltrates [77] but consolidative, ground glass opacities and nodular infiltrates have been reported [81]. The incidence of AFOP after LT is unknown, but retrospective studies have reported prevalence ranging from 1.7%-11% [78]. AFOP was initially described during histopathological review of non-BOS phenotype CLAD patients, but has since been identified in LT recipients after CARVs including H1N1 influenza [82], Adenovirus [83], and in the setting of DSAs [79]. Nevertheless, in the LT setting most cases remain idiopathic, and a definite causal association remains unknown. The prognosis of AFOP is grim despite attempts at treating associated infections, or with augmentation of immunosuppression with steroids or ATG [78]. Recently, a case was reported of successful ventilator liberation in an AFOP patient treated with tocilizumab and infliximab [83]. Ultimately re-transplantation may be the only option [84].

2.4 ACR Clinical Presentation

The clinical spectrum of acute rejection typically ranges from asymptomatic to non-specific respiratory and constitutional symptoms such as dyspnea, cough, or low-grade fevers. Crackles or wheeze may be heard on pulmonary auscultation, a decline in spirometry may be present as well [85, 86]. Higher grades of ACR (>/= A2) are correlated with increased symptom severity, however, symptom burden is unlikely to be clinically useful in distinguishing ACR grade, or between ACR and respiratory infection [85]. Rarely, those with severe ACR may present in extremis with acute hypoxic respiratory failure, though uncommon, we have seen such presentations among patients with very low or undetectable CNI troughs. Chest radiographs are frequently unrevealing in ACR, in a small single center retrospective study, normal chest radiographs were found in 77% of clinically or histologically determined ACR episodes occurring 30 days or later post-transplant [86]. Cross section high resolution CT scans may demonstrate ground glass opacites, consolidation, peri-bronchovascular or septal thickening, volume loss, or pleural effusions. However, all of these findings are non-specific abnormalities and have low sensitivity and specificity for detecting ACR [87]. Nevertheless, cross section imaging has a role in assessing severity and distribution of the disease process, excluding alternative diagnoses, and guiding TBBx targets [88, 89]. While a decline in numerous PFT parameters including FEV1, FEV1/FVC, FEF_{25-75}, TLC, and DLCO can be seen with ACR, these findings are reasonably sensitive but not specific, and typically signal a need for further bronchoscopic investigation [90]. Routine home spirometry is commonly employed for monitoring and early detection of graft dysfunction, home spirometers have been shown to correlate well with lab spirometry, generally, a decline in FEV1 of 10% from baseline is used to trigger additional investigation [91-93]. Donor derived cell free DNA (dd-cfDNA), which are fragments of double-stranded DNA released from allograft cells undergoing cell death, can be sequenced and measured in serum samples and quantitively compared to recipient derived cell free DNA. A relative increase of dd-cfDNA fraction (also referred to as %dd-cfDNA) is a novel biomarker of allograft injury [94], commercial testing options are currently available. Using a threshold of 1% relative increase to detect a composite of ACR and AMR (antibody mediated rejection), sensitivity approaches 100%, with a negative predicative value of up to 90% [94]. However, as dd-cfDNA is a general marker of graft injury, it lacks specificity. Its role in LT remains to be clarified given the multiple mechanisms of lung injury including infections, aspiration, ACR, AMR and BOS that can all cause a spillover of ddcfDNA. Further research to determine appropriate cut off values to distinguish between different

pathologies, and how best to incorporate this novel non-invasive testing into well-established allograft monitoring protocols is ongoing.

2.5 ACR and Surveillance Bronchoscopy

Though surveillance bronchoscopy (SB) with TBBx has not been demonstrated in the trial setting to be beneficial regarding overall survival or freedom from CLAD in comparison to clinically indicated bronchoscopies alone, it remains a frequently employed monitoring strategy [95]. In a survey of North American transplant centers published in 2004, 69% reported performing SB, though the procedure frequency and length of surveillance was not uniform between centers. Only 8% reported continued SB beyond 24 months [96]. Though the efficacy of SB with TBBx remains controversial, it has proven quite safe. In the largest published prospective cohort evaluating 1,235 surveillance and clinically indicated TBBx procedures, the complication rate was 6.5% - with 4% experience bleeding, 1.46% developing a pneumothorax, and 0.32% requiring temporary mechanical ventilation, no deaths were reported [97]. The advantage of SB with TBBx lies in detecting silent rejection or occult infection. Clinically silent A2 or higher rejection has been reported in up to 18.7% of SB procedures [98]. Importantly, SB often has therapeutic implications, a recent single center report found that 20% of their SB procedures resulted in actionable clinical management in the form of antimicrobial therapies, adjustment of mIS, or augmented IS [99]. See Figure 7.

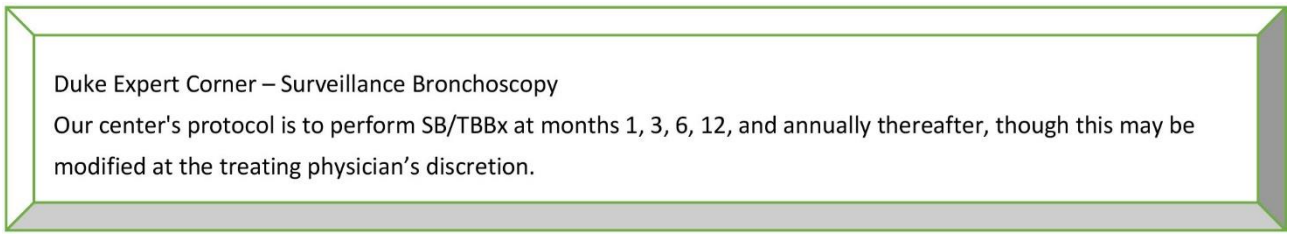

Figure 7 Expert Corner – Surveillance Bronchoscopy.

Both ACR and LB are well recognized risk factors for CLAD, which is the primary factor limiting longevity post LT [5]. Determining patient and graft survival estimates after CLAD onset is challenging as earlier reports exploring the natural history of CLAD typically did not differentiate between the bronchiolitis obliterans syndrome (BOS) and restrictive allograft syndrome (RAS) phenotypes, which were not formalized until the 2019 ISHLT consensus document [75, 100]. For example, in 2010, Copeland et al reported a median survival of only 2.5 years after BOS onset with earlier BOS onset (<2 years), and more severe obstruction at onset, portending the worse prognosis. However, BOS was defined per an earlier iteration of the ISHLT guidelines and therefor the cohort included both BOS and RAS subjects [101]. A later retrospective study evaluating survival after CLAD onset reported a median survival of 309 days (0.85 years) after RAS, much worse than median survival after BOS which was 1070 days (2.9 years) [102]. Significantly worse survival with RAS vs. BOS has been a reliably reproducible finding [103, 104]. There are currently no definitively effective therapies that significantly alter the natural history of CLAD. Given the poor prognosis, the importance of aggressively screening for and treating risk factors, namely ACR, is clear.

2.6 Treatment of ACR

There are no consensus documents to guide treatment of ACR, management is based on limited available data, clinical experience, and institutional protocols. There is consensus that higher grades, ≥A2, requires treatment, while treatment of LB minimal AR (A1), particularly when asymptomatic, remains controversial [6, 96, 105]. Most opt to treat clinically apparent rejection regardless of histologic severity as well [9, 11, 106]. Compared to clinically silent rejection, ACR with an FEV1 decline of at least 10% is associated with an increased risk of BOS and death, independent of frequency or severity of AR/LB episodes [107]. Initial therapy is typically a course of IV pulse steroids (500-1000 mg methylprednisolone over 3 days) followed by a steroid taper, dosing and length of treatment vary by center [6]. Repeat bronchoscopy with TBBx is typically performed 4-6 weeks after treatment to ensure resolution or identify those with refractory rejection that may require repeat or intensified therapy. Persistent >/= A2 rejection has been identified on up to 26% of follow up bronchoscopies despite treatment with pulse steroids (it should be noted that 47% had concurrent LB on the initial bronchoscopy), and was found to be associated with earlier BOS onset (median time to BOS 1.3 years vs 2 years) though no difference in overall survival [108].

2.6.1 Approach to Minimal AR

Potential approaches to asymptomatic A1 AR include treatment, monitoring with short interval follow up TBBx, or no management changes. However, there are several lines of evidence demonstrating the clinical significance of low-grade minimal rejection. When left untreated, A1 lesions frequently progress, with one study showing among 255 A1 lesions identified by SB, 25.1% progressed to >/= A2 and 15.7% developed new LB [11]. In contrast, among the 24 A1 lesions identified by clinically indicated bronchoscopy and treated with an oral prednisone burst and taper, only two progressed to a higher grade of ACR, and no new LB lesions were found at follow up [11]. Additionally, subgroup analysis demonstrated that those with 2 or more occurrences of A1 were more likely to develop BOS and had an earlier onset of BOS compared to those with at most one occurrence of A1 over the first transplant year (BOS: 68% vs 43%, mean onset: 599 days vs 819 days) [11]. Similarly, Khalifah et al demonstrated that A1 minimal rejection is an independent risk factor for BOS and that the risk for BOS may be abrogated by treating with augmented IS [9]. Additionally, even a single isolated episode of A1 minimal rejection, without recurrence or progression to a higher grade, has been identified to increase BOS risk compared to those without any ACR episodes in their history, with increased BOS risk remaining present even when asymptomatic and symptomatic A1 subjects were considered separately [106]. It should be noted that, the association between minimal rejection and CLAD risk has not been a universal finding. Biomarkers are emerging tools which may help identify which minimal rejection episodes may confer the greatest risk and warrant treating. One intriguing option is the chemoattractant CXCL9 which been shown to be elevated in BALF and serum in the setting of several underlying allograft injuries and predictive of future CLAD, though testing is not yet available in routine clinical practice and further study is needed [105, 109, 110].

Taken together, it seems that “ACR begets ACR.” Or, perhaps more accurately, “ACR begets worse ACR.” Though more frequent episodes, worse histologic severity, and greater degree of clinically apparent allograft dysfunction all correlate with increased CLAD risk, even a single episode of asymptomatic minimal ACR may increase the risk for subsequent CLAD. Augmenting

immunosuppression with high dose steroids frequently leads to ACR resolution and decrease the risk of persistent or worsening ACR severity. A general approach to ACR management is outlined in Figure 8, our institutions approach to minimal AR and LB is outlined in Figure 9.

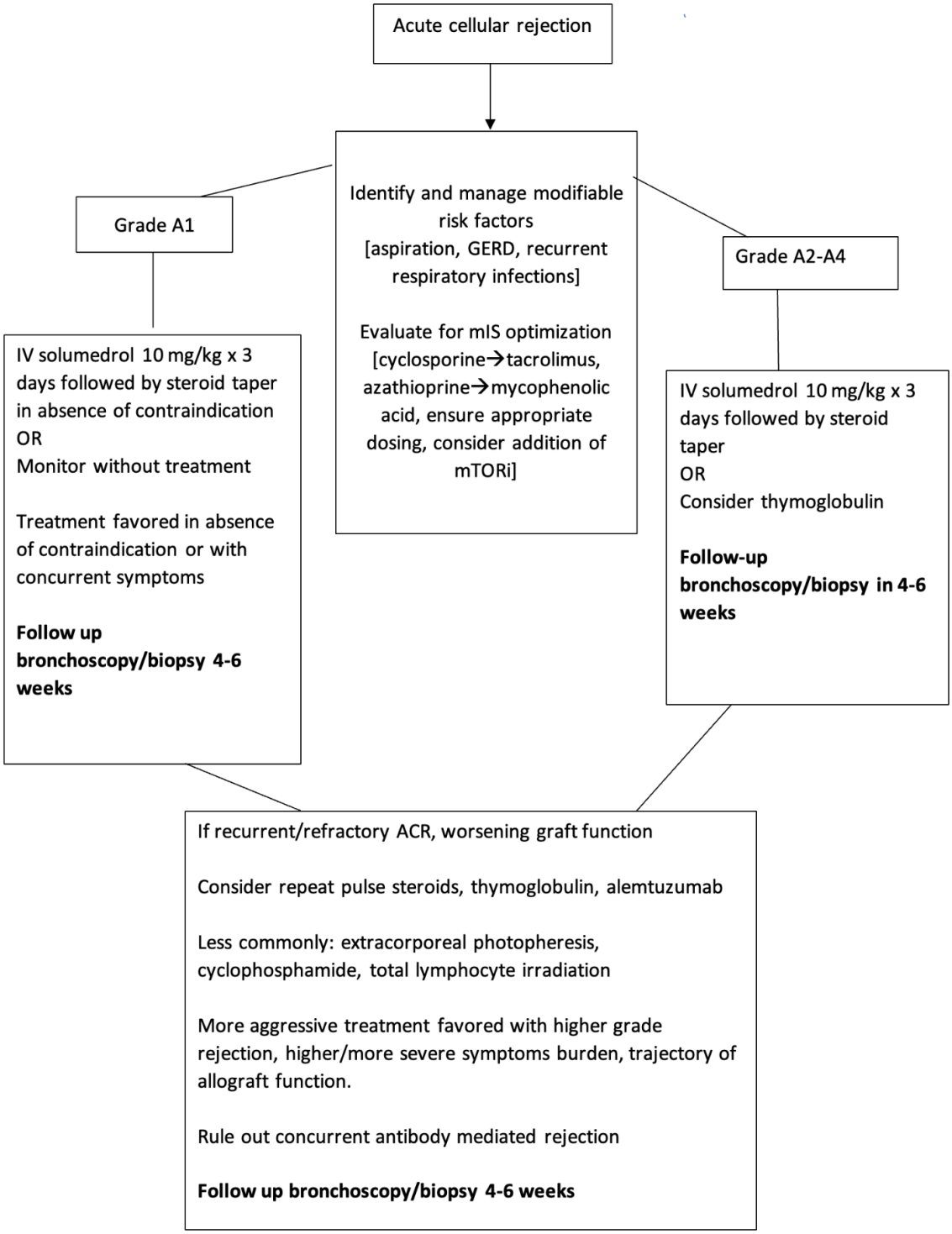

Figure 8 General approach to ACR management. *Thymoglobulin – rabbit anti-thymocyte globulin 1.5 mg/kg daily for 3-5 days is preferred. ATGAM – equine anti-thymocyte globulin 15 mg/kg daily for 3-5 days is an alternative.

Duke Expert Corner – minimal ACR and LB management

Our practice is to generally treat all A1, even when asymptomatic, unless there is a significant competing infectious issue that alters the risk profile. Standard therapy is 10 mg/kg of IV solumedrol for 3 days, followed by an oral prednisone taper from 60 mg, decreasing by 5 mg daily back to the prior maintenance prednisone dose. Follow up bronchoscopies are typically competed 4-6 weeks after augmented IS. Given the strong association between LB and BOS [10], we take a similar treatment approach.

Figure 9 Expert Corner – Recommended management minimal ACR/LB.

2.6.2 Refractory ACR

Refractory rejection (steroid or treatment resistant) is not specifically defined, but generally refers to rejection that persists despite two prior attempts at treatment with steroids. This is a challenging situation with poor quality of data to support treatment decisions. in one of the largest cohort of treatment for refractory ACR reported, 112 subjects were treated with ATG, 60% were complete responders with resolution of rejection on follow up bronchoscopies, and 22% were partial responders with decreased ACR severity [111]. Response was sustained for 1 year as determined by mean A grades over time. Responders had a lower mortality 1 year after therapy than non-responders and complete responders had significantly less new onset or progressive CLAD than partial responders and numerically less than non-responders [111]. Another cytolytic therapy, Alemtuzumab, has also been shown to decrease the total burden of rejection when used in this setting [112, 113]. Utilization of cyclophosphamide, inhaled aerosolized cyclosporine preparations, total lymphoid irradiation, and extracorporeal photopheresis have been reported as well, though experience with these treatment modalities for refractory ACR is even more limited [114-117]. ACR also commonly occurs in the setting of ongoing antibody mediated rejection (AMR), in this situation patients are much less likely to respond favorably to pulse steroids and the potential for concurrent AMR should always be considered and ruled out [118-120].

3. Antibody Mediated Rejection

3.1 AMR Molecular Mechanisms

AMR refers to acute alloimmune rejection mediated by the humoral arm of the immune system. It is only relatively recently that pulmonary AMR has become a well-accepted entity. The potential for acute allograft rejection of humoral origin was long postulated to exist based on histopathologic findings of endothelial capillaritis associated with severe graft dysfunction, even before the availability of modern HLA antibody testing [121]. Subsequent case series reported similarly severe graft dysfunction in the setting of complement deposition and later circulating DSAs [122]. The modern framework of AMR is activation of alloimmune B-cells and plasma cells resulting in the production of circulating DSAs with binding affinity for donor derived MHC molecules (also referred to as human leukocyte antigens, HLA); Class I MHC molecules are present on all nucleated cells,

Class II MHC molecules are present on antigen presenting cells, and are also found on pulmonary epithelial cells [123].

DSA binding to HLA antigens in the allograft vascular endothelium can promote local inflammatory and proliferative pathways through both complement dependent and independent mechanisms, which results in a cycle of tissue injury and allograft dysfunction [123]. Complement dependent cytotoxicity is thought to be the major mechanism of injury in AMR, and can involve classical, lectin or alternate pathways [124]. Additionally, there is ample evidence that humoral rejection can be injurious through complement independent pathways as well [125]. Wherein a "classic" DSA activates complement with terminal cytotoxicity resulting from assembly of the membrane attack complex, DSAs may alternatively result in antibody dependent cell mediated cytotoxicity (ADCC) if the crystalline fragment (Fc) portion of the DSA can interact and activate immune cells with cytotoxic capability, through Fc receptors [126]. In fact, natural killers (NK) cells have been proposed to have this capability through the CD16A-Fc receptor [126].

3.2 AMR Histopathology and Diagnostic Criteria

Histopathological findings in pulmonary AMR are non-specific injury patterns that may be supportive of but not specific for an AMR diagnosis. The classic pathologic changes reported in association with pulmonary AMR include neutrophilic margination, neutrophilic capillaritis, diffuse alveolar damage, persistent or high grade ACR or LB, arteritis, and acute fibrinous organizing pneumonia [127]. A consensus definition for diagnosing clinical pulmonary AMR was outlined by the ISHLT in 2016, the five criteria include: measurable allograft dysfunction, presence of circulation DSA, supporting lung histology, positive C4d staining on lung biopsy, and exclusion of other causes [125]. The diagnostic criteria were extrapolated from the Banff criteria for renal AMR [128]. Diagnostic certainty is graded based on the number of attributes present; definite AMR with all 5 attributes present, probable with 4, and possible with 3. A similar diagnostic framework was created for subclinical AMR, though excluding allograft dysfunction as these subjects are asymptomatic by definition. Though the presence of C4d staining, a marker of complement activation, was initially required to make a definitive diagnosis, it should be noted that interpretation of C4d staining in lung allografts is known to be difficult due to poor reproducibility, high background staining, and poor specificity; C4d+/definite AMR make up a minority of cases in recently reported cohorts [119, 129]. Additionally, AMR findings have been recognized in patients without any detectable donor specific HLA antibodies. In such cases the pathology is thought to be related to non-HLA allo or autoantibodies [130]. Some of these antibodies have been characterized (vimentin, AT1R, collagen V, and tubulin), but tests to detect them are not currently available in clinical practice [131, 132]. While the vasculopathy seen with AMR in lung and other solid organ allografts is similar, C4d staining characteristics, AMR in absence of HLA antibodies, the presence of varied pathological findings, as well as lack of clinical response to treatment in many cases, makes pulmonary AMR a unique entity. AMR in LT may have unique or additional pathogenetic factors in comparison to other solid organ allografts.

3.3 AMR Incidence and Risk Factors

The true incidence of pulmonary AMR is unknown, in part because prior to 2016 there were no consensus definitions for diagnosis. Additionally, HLA antibody detection technologies have evolved

greatly overtime to the highly sensitive, semiquantitative, solid-phase immunoassay platforms with optional functional assays for complement fixation (C1q and C4d assays) in current use [133]. A recently reported cohort of listed LT candidates found that 35% were allosensitized pre-transplant [134]. Sensitized patients experience increased wait times, increase waitlist mortality, and lower likelihood of transplant; the degree of allosensitization as determined by increasing calculated plasma reactive antibodies (cPRA) directly correlates with worse waitlist outcomes [134]. Higher Pre-transplant allosensitization is also associated with worse post-transplant outcomes, including decreased short term (30-day) and long-term survival, more post-surgical ventilator days, de-novo DSA development, AMR, and BOS [135-138]. In addition to allosensitization, primary graft dysfunction >/= grade 2 (PGD), redo transplantation, male sex, ex-vivo lung perfusion (EVLP), increased degree of HLA mismatching, high lung allocation score (LAS), and blood product transfusion, have been identified as risk factors for de novo DSA development post-transplant [118, 139-141]. De novo DSA development is quite common early post-transplant, with an incidence between 36-47%, the majority developing within 3 months of transplant and have specificity for Class II DQ antigens (60-67%) [118, 140]. Numerous studies have demonstrated a strong association between de-novo DSAs and subsequent CLAD/BOS, Tikkanen et al reported a two-fold increase in CLAD risk [140, 142, 143]. It is also clear that DQ antibody specificity is particularly problematic, compared to other de novo DSAs, DQ specificity is an even greater risk factor for CLAD, is more frequently the cause of clinical AMR, and results in worse graft survival [140, 144]. Recent publications utilizing the 2016 diagnostic criteria have found the rate of AMR development to range from 10.5%-28.7% for clinical AMR, and 18.5-40.3% for subclinical AMR [119, 145].

3.4 Clinical Presentation and Phenotypes of AMR

In contrast to ACR, which is frequently minimally or completely asymptomatic with benign chest imaging, and the risk of death rare with attributed mortality estimated to be low as 1.2% [146], the situation with AMR is quite different. Clinical AMR patients frequently present with dyspnea, hypoxia, and decline in spirometry, and occasionally hemoptysis [120, 122]. Abnormal imaging findings are very common, occurring in approximately 90% of those with definite, probable or possible AMR. Cross sectional chest imaging demonstrates ground glass opacities in more than 70% of cases, and pleural effusion in about 50% [119]. In small case series, fulminant respiratory failure necessitating hospitalization or invasive mechanical ventilation is not uncommon (18%-67%) [120, 122].

Hyperacute and subclinical AMR are additional AMR clinical phenotypes. Hyperacute rejection refers to nearly immediate and fulminant graft failure after implantation, mediated by pre-formed circulating DSA and subsequent complement pathway activity against the graft [147]. Hyperacute rejection is exceedingly rare in the modern era due to advances in lab technologies and the shift to "virtual cross matching," which allows for the avoidance of unacceptable antigens in a potential donor based on the recipients HLA-antibody profile [148]. Additionally, as the transplant community has gained experience with perioperative desensitization strategies, purposefully crossing historically unacceptable antigens has increasingly been demonstrated to be feasible for highly sensitized patients with acceptable short- and long-term outcomes [149-151]. Identifying which antigen/antibody pairs pose the greatest risk and should be avoided, as well as what specific therapeutic strategies are most effective, is an ongoing challenge that requires additional research.

Subclinical AMR is a common, and challenging, clinical phenotype defined by the presence of circulating DSAs in the absence of allograft dysfunction. Per the 2016 ISHLT guidelines, circulating DSAs, even without supportive lung histology or C4d staining, are sufficient to make a diagnosis of possible subclinical AMR [125]. In a retrospective cohort evaluating outcomes between subjects classified as having clinical or subclinical AMR, those with clinical AMR were far more likely to develop CLAD over 2 years of follow up (60% vs 11%), more likely to have DQ DSAs (95% vs 61%), a greater number of DSA specificities (3.4 vs 1.8), and higher immunodominant DSA mean fluorescence intensity (MFI), which semi-quantitative measure of DSA concentration in the serum [145]. There is also a growing body of evidence demonstrating the utility of pre-emptively treating sub-clinical AMR. However, predicting which patients with asymptomatic circulating DSAs will progress to clinical AMR remains a challenge, though the link between DSAs subsequent CLAD/BOS and AMR is clear [140, 142-144], the natural history of individual DSAs remains incompletely understood. Efforts to further risk stratify circulating DSAs and subclinical AMR is of utmost importance. In addition to DSA antigen specificities as seen with DQ antibodies, other DSA molecular characteristics such as complement fixing capability [129, 152, 153], MFI value [154], and DSA IgG sub-class [155, 156], may ultimately prove to be useful tools in clarifying DSA immunogenicity and risk of progression to clinical AMR. Higher %dd-cfDNA may help differentiate between pathologies with higher values being observed in AMR than ACR subjects, one study reported a median %dd-cfDNA of 5.4% vs 1.1% [157]. Similarly, %dd-cfDNA is higher in clinical vs subclinical AMR, with a median %dd-cfDNA of 5.4% vs 0.6%. Even more interesting, among those with asymptomatic circulating DSAs, a sustained rise in %dd-cfDNA may occurs up to 3 months prior to clinical AMR onset [157], and therefore may a be a useful risk stratifying or monitoring tool for those with asymptomatic DSAs. Another exciting development is the molecular assessment of rejection associated transcripts in lung biopsies to identify patterns consistent with rejection [158]. These patterns when used in conjunction with other information including HLA and non-HLA typing will provide substrate for machine learning and use of artificial intelligence in risk stratification of DSAs and diagnosis of AMR in the future.

With aggressive treatment more patients are surviving longer but may continue accruing ongoing damage from incompletely cleared DSA and ultimately progressing to CLAD. Chronic AMR is a phenotype of patients who survive the acute AMR episode but have persistent DSAs with ongoing decline including development of radiological or pathological pulmonary fibrosis and scarring after meeting CLAD criteria. More research is needed to study this group of patients.

3.5 AMR Treatment

3.5.1 Preemptive Treatment Sub Clinical AMR

Several earlier studies have attempted to evaluate the role of pre-emptive antibody depleting therapies with various combinations of intravenous immunoglobulin (IVIG), Rituximab and plasma exchange (PLEX). While some success with DSA clearance has been reported, these studies lacked appropriate controls making interpretation of results challenging [159, 160]. Additionally, these studies did not clearly specify that included subjects were asymptomatic consistent with the recent definition of subclinical AMR. One group treated 65 DSA positive patients with IVIG or IVIG and Rituximab and compared outcomes to 51 patients who screened negative for DSA. Similar rates of ACR, LB, and BOS were found between the two groups, however, as there was no untreated DSA

positive control group, the potential treatment effect is difficult to interpret [159]. Pre-emptive treatment of early DSAs with PLEX and Rituximab was demonstrated to increase DSA clearance compared to untreated DSA positive patients, however no difference in ACR rates, BOS rates, or mortality, was found [160]. However, many subjects in the untreated DSA group were selected not to be treated due to clinical risk factors, again making interpretation of treatment effect challenging [160]. A recent multicenter retrospective study analyzed outcomes between well-defined subclinical AMR patients, either treated or not, with non-standardized antibody depleting therapies. The most common pre-emptive therapies included IVIG, IVIG + Rituximab, PLEX + IVIG + Rituximab. Pre-emptive DSA therapy was protective against a combined endpoint of CLAD or death, and clinical AMR [161]. Furthermore, a subset of the untreated DSA positive cohort ultimately went on to develop clinical AMR and were treated at that time. Delaying therapy until clinical AMR was associated with a 3-fold increased risk of CLAD or death, and a shorter time to CLAD or deat (18.8 vs 22.9 months) overall suggesting that a preemptive treatment strategy is beneficial [161].

3.5.2 Treatment and Morbidity of Clinical AMR

There are no randomized clinical trials to guide treatment of pulmonary AMR, treatment modalities have been adopted from experience with other SOTs, as well as from chemotherapeutics directed at B-cell malignancies. The goals of treatment are to deplete circulating antibodies, prevent further antibody production, and limit ongoing inflammatory insult to the allograft. Multimodal therapy is usually employed, the backbone consisting of plasmapheresis, IVIG, and Rituximab. Addition of ATG preparations may be beneficial due to indirect effects on B-cell and immunoglobulin production stimulation, as well as for concomitant ACR which is common. Newer additions to the AMR armamentarium include proteosome inhibitors bortezomib and carfilzomib which specifically impact immunoglobulin secreting plasma cells – an important target that is not depleted with anti-CD20 antibody therapies. Belatacept, as mentioned earlier, has also been shown to decrease DSA production in clinical trials of kidney recipients. The existing literature surrounding treatment of clinical pulmonary AMR is limited to case series and cohort studies, deciphering therapeutic effect of any individual therapy, or regimen, is difficult. Common pitfalls include inadequate or no controls for comparison, and a lack of standardization of the therapeutic intervention [122, 129, 162]. It clear that pulse steroid monotherapy is insufficient treatment for AMR, with an estimated ~50% clinical response rate [120]. Among steroid non-responders' subsequently treated with PLEX, clinical improvement as determined by improvements in symptoms, hypoxia, or spirometry occurred in the majority (67%) [120]. However, PLEX itself will not decrease continued DSA production. 16 AMR patients were treated with an aggressive standardized protocol including PLEX, steroids, bortezomib, rituximab, and IVIG – by 6 months post therapy 5/16 subjects died and 7/16 had decreased allograft function from baseline; only 3 of the 11 survivors at 6 months cleared all DSAs [163]. The optimal combination of antibody depleting therapeutics and PLEX sessions to reduce DSA burden remains unknown [164]. Eculizumab, a compliment inhibitor which prevents C5 cleavage and prevents formation of the membrane attack complex, has been demonstrated to be useful for AMR treatment in kidney recipients, there is minimal experience reported with this agent in the LT literature [165, 166]. Imlifidase, an IgG degrading enzyme, has recently been reported to help facilitate HLA antibody depletion in a highly sensitized LT candidate refractory to other therapeutic trials [167]. The first series of pulmonary AMR patients treated with Tocilizumab was recently

published, there was a signal of greater DSA clearance and improved graft survival compared to AMR patients treated without Tocilizumab [168], further evaluation of this therapy in pulmonary AMR is warranted.

AMR treatment regimens are complex and ultimately determined by institutional experience and protocols, with adjustments made based on patient specific circumstances and the known risks and side effects associated with the individual treatment components, refer to Table 3. In general, more aggressive therapy is provided to those with a more severe clinical syndrome.

Table 3 AMR Therapeutic, Mechanism, Potential Contraindications.

Treatment	Mechanism	Concerns and potential Contraindications	Reference
Corticosteroid	Inhibition of inflammatory response, alteration of leukocyte movement, and influence leukocyte differentiation		[169]
Plasmapheresis	Removal of circulating antibodies	Coagulopathy, factor depletion, electrolyte disturbance	[164]
IVIG	Unclear multifactorial mechanism, complement modification, Antibody neutralization, regulation innate and adaptive immunity	Volume status, rare risk for clotting, kidney injury, anaphylaxis	[170]
Rituximab	Immune mediated cell destruction via complement and antibody cytotoxicity, depletion of B cells	Does not impact DSA secreting plasma cells	[171]
Bortezomib	Plasma cell depletion tough disruption of proteasome	Bortezomib – bone marrow suppression, thrombocytopenia, severe peripheral neuropathy, avoid in those with neuropathy or on azoles.	[172]
Carfilzomib		Carfilzomib – bone marrow suppression, thrombocytopenia, new on-set or worsening heart failure – avoid with low EF	
Eculizumab	Inhibition of membrane-attack complex via destruction of complement C5	Increased infectious risk encapsulated bacteria, Need meningococcal vaccine	[166]
Belatacept[1]	Selective T cell co-stimulation blocker binding CD80 and CD 86 on APCs	PTLD risk with EBV seronegative patient, infection risk	

Tocilizumab[1]	IL-6 antagonist	Increased GI perforation risk, avoid with diverticular disease	[168]
Tofacitinib[1]	JAK 1 inhibition		[173]
Belimumab[1]	B cell activating factor antagonist		[173]

Including novel agents[1]

Despite aggressive treatment, morbidity and mortality attributed to AMR is very high. In a recent study evaluating outcomes among 55 patients treated for AMR in the modern era, >70% required ICU care during the course of their AMR therapy, 50% required invasive mechanical ventilation (IMV), and 13% received VV ECMO [174]. Death attributed to AMR, complications of treatment, or CLAD occurred in 38% of subjects within 1 year of therapy, 10 deaths occurred throughout the course of the treatment hospitalization [174]. Similarly poor outcomes were noted in another modern cohort of 73 treated clinical AMR patients, where 75% were hospitalized, 38% required IMV, 26% died within 30 days of AMR therapy [129]. Overall, 84% either died or required re-transplantation with a median allograft survival time of 246 days from AMR diagnosis [129].

4. Conclusion

The screening, timely diagnosis, and management of ACR and AMR is the cornerstone of post LT management. In addition to timely treatment of ACR, every effort should be made to assess patients for risk factors that predispose to ACR given the risk of CLAD, which is the most important factor in predicting long term survival of LT recipients. AMR is not only a risk for CLAD but is highly morbid entity for which reliable and successful treatment options are needed, early aggressive antibody directed treatments may be warranted even in cases of subclinical AMR for patients who can reasonably tolerate augmented immunosuppression.

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Author Contributions

BM drafted the manuscript. All authors critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, read and approved the final manuscript.

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