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EUROPEAN RESPIRATORY UPDATE

Update on lung transplantation: programmes, patients and prospects

Tom Kotsimbos*,#, Trevor J. Williams*,# and Gary P. Anderson¶

The will is infinite and the execution confined…the desire is boundless and the act a slave to limit

Shakespere W. *Troilus and Cressida*. Act III, Scene ii.

Discovery consists of seeing what everyone has seen and thinking what nobody has thought

Anonymous

It has been over 30 yrs since the modern era of lung transplantation commenced and what was then the ''new frontier and exceptional'' has now become ''routine and expected''. Strong vision, bold action and creative thinking have led to outstanding progress in the management of end-stage lung and pulmonary vascular disease. The purpose of this article is to highlight what has been achieved, critically assess where we are in terms of a ''cure'' for severe lung disease, and (re)stimulate the creative thinking, action and vision that are still very much required to solve the ongoing impediments to achieving a durable lung allograft and long-term recipient survival following the life-giving procedure that lung transplantation is, and can be.

PROGRAMMES: MULTIDISCIPLINARY TEAMS

Where did we come from: where are we now?

History and programme evolution: from cottage industry to just expected

Lung transplantation has evolved over the last 60 yrs from a series of remarkable animal experiments to an established treatment of severe end-stage lung and pulmonary vascular disease fully funded in many jurisdictions. In its infancy it was swept along by the determination and drive of surgical pioneers. Amongst them the names Demikhov, Hardy, Schumway, Cooley, Reitz, Cooper and Patterson have exemplified the surgeons' drive to bring lung transplantation to clinical fruition. With long-term survivors of heart–lung transplantation (HLTx) from 1981 [1], single lung transplantation (SLT) from 1983 [2] and bilateral lung transplantation (BLTx) from 1985 [3], this decade saw the true genesis of clinical lung transplant programmes.

HLTx became the early option for lung replacement with Stanford University (Stanford, CA, USA) leading the way under the leadership of Bruce Reitz. A second key US centre in Pittsburgh was also active. Europe, under the leadership of Magdi Yacoub at Harefield Hospital (London, UK) and John Wallwork at Papworth Hospital (Cambridge, UK), developed very active HLTx programmes utilising donors from throughout the UK and the continent. Isolated lung transplantation (SLT and BLTx) grew initially out of the Toronto programme and with the move of Cooper and Patterson to St Louis (Barnes Hospital, Washington University, St Louis, MO, USA) by the end of the 1980's these were the dominant programmes. All these programmes in their infancy had a small nucleus of surgeons, physicians and co-ordinators who made transplantation happen, often without well-defined resources.

The most recent report of the International Society for Heart and Lung Transplantation (ISHLT) registry gives a clear view of the changes in lung transplant activity over 20 yrs [4]. From the mid-1980s, HLTx numbers reported to the ISHLT registry increased rapidly to a peak in 1990 of 276 HLTx in that year. This dropped steadily such that by the late 2000s, 85–100 HLTx are reported to the registry annually. SLT numbers rose rapidly from 1987, reaching a plateau by 1995. Since this time, SLT numbers have been relatively static (700–850) as outlined in the ISHLT registry reports. BLTx procedures have increased every year since 1985. This is now the most commonly performed lung transplant procedure. In 2009, approximately 2,300 BLTx were reported to the ISHLT registry; the greatest number to date. The number of centres now transplanting and reporting to the ISHLT registry is 158 for isolated lung transplants. For HLTx, 114 centres report activity to the ISHLT registry; however 95 centres report two or less HLTx per year [4].

It is fair to say that survival following lung transplantation started at a low base. More than 40 attempts were undertaken over a period of almost 18 yrs before the first long-term survivor was achieved. In 1988–1994, the ISHLT registry reported 1-, 2- and 5-yrs survival of 72%, 65% and 49%, respectively. In the most recent reported era, 2000–2009, this had improved to 80%, 72% and 54%, respectively [4]. The substantial improvement in survival is almost completely attributable to improved early survival post-lung transplant. Despite a concerted effort in multidisciplinary team-led care of transplant survivors little impact has been evident on survival

*Heart and Lung Transplant Service, Alfred Hospital, Monash University. #Dept of Allergy, Immunology and Respiratory Medicine, Alfred Hospital, Monash University, and ¶Dept of Pharmacology, University of Melbourne, Melbourne, Australia.

CORRESPONDENCE: T. Kotsimbos, Dept of Medicine, Central and Eastern Clinical School, Monash University, Alfred Hospital, Melbourne, Australia. E-mail: tom.kotsimbos@monash.edu

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beyond the first year [5]. The ISHLT registry reports survival contingent on surviving the first year. What is evident is that patients who had cystic fibrosis (CF) and pulmonary vascular disease have better survival after the first year compared to those with chronic obstructive pulmonary disease (COPD) or pulmonary fibrosis. Presumably this reflects the latter being older patients with more comorbidities. Overall, however, the ISHLT registry does not show any significant improvement in survival in patients having survived the first year post-lung transplant in more recent eras [4].

Surgical/anaesthetic/intensive care unit management

Surgical innovations

Incisions

There have been relatively minor changes in the surgical approach to lung replacement procedures since the original description of these procedures. HLTx is now a very uncommon procedure but midline sternotomy is still utilised. SLT is generally performed *via* a standard lateral thoracotomy; however, it is sometimes performed *via* a midline sternotomy facilitating surgical repair or revascularisation of the heart when this is simultaneously performed.

Double lung replacement was initially performed *via* a midline sternotomy utilizing a tracheal anastomosis. A high rate of tracheal anastomotic breakdown led to a redesign of the procedure to the ''bilateral sequential'' BLTx. In this procedure, a bilateral thoracotomy with transaction of the lower sternum (the so called ''clam shell incision'') is most often utilised. The clam shell incision can be particularly problematic in terms of post-operative pain control (generally mandating prolonged epidural anaesthesia) and its impact on respiratory muscle function. Variants that have evolved include bilateral thoracotomy with sternal sparing, as well as a reversion to the use of a midline sternotomy (especially where revascularisation of the bronchial anastomosis with an internal mammary artery pedicle is performed) [6].

BLTx *versus* SLT *versus* HLTx

As noted previously, HLTx remained the predominant technique for lung replacement into the early 1990s. From that time the number of SLT procedures grew until 2000, and from then onwards, BLTx became and remains the dominant procedure in lung replacement. Other innovations over the years include cut down lung transplant procedures (to overcome donor and recipient size discrepancies) where the lung volume is reduced (usually by lobectomy) *in situ* or on the back table [7, 8], and lobar transplant techniques that have been developed to allow either transplantation from live donors [9, 10] or splitting of a single large lung allowing bilateral replacement in a smaller recipient [11].

Anastomotic techniques

From the first successful HLTx procedure, anxiety was high regarding the viability of the airway anastomosis in the setting of a lack of bronchial arterial revascularisation. In reality this did not prove to be a major issue in *en bloc* HLTx procedures where collaterals from the coronary arteries *via* the posterior pericardium were thought sufficient to prevent major airway anastomotic breakdown. In isolated lung transplantation, wrapping of the anastomosis with the greater omentum was initially performed to expedite revascularisation and anastomotic healing. This proved to be unnecessary in SLT and BLTx. As noted previously, BLTx was initially performed as an *en bloc* procedure with a tracheal anastomosis but, unfortunately, the death rate due to anastomotic breakdown was unacceptably high [12]. The procedure was subsequently modified to the bilateral sequential lung transplant procedure with fashioning of the two telescoped bronchial anastomoses [13]. Airway complications have been commonly seen in up to 20% of anastomoses, although technical innovations seem to be reducing this incidence [14–16].

Post-operative care

The marked improvements in outcome from lung transplantation that have been seen in the last 30 yrs have predominantly been driven by improvements in early (3-month) survival. Better recipient selection and surgical improvements have contributed to this, but much of this improved survival appears attributable to the evolution of improved intensive care management [17, 18].

Fluid/inotropes

A clear understanding of the pathophysiology of the newly transplanted lung is crucial in developing post-operative fluid and inotrope strategies. An injured lung is prone to alveolar capillary leak and this situation is confounded by lack of lymphatic drainage of the newly transplanted lung allograft (due to cutting of lymphatics without re-anastomosis), low oncotic pressure (due to low serum albumin) and sometimes an elevated pulmonary capillary wedge pressure and a high cardiac output state (*e.g.* in the setting of left ventricle diastolic dysfunction). Thus, a rise in hydrostatic pressure readily produces pulmonary oedema, which, when present, can be very slow to resolve. Careful management of fluid and inotropes, as well as routine monitoring of the circulation utilising a central venous catheter, an arterial line and a Swan–Ganz catheter, is therefore needed to help minimise early allograft dysfunction syndromes [19, 20]. Echocardiography has also proven valuable in guiding appropriate setting of fluid input and ionotrope support and to assess post-transplant complications [21].

Ventilation

The continuing evolution of ventilator technology has allowed patients to be increasingly mechanically supported without deep sedation and paralysis in most cases. In addition, the improvements in noninvasive ventilation now allow for the extubation of patients who may still require a degree of ventilatory support.

ECMO

The availability of modern heparin bonded extracorporeal membrane oxygenation (ECMO) has allowed an unprecedented ability to support severe early graft dysfunction. Central and peripheral canulation techniques can be employed. For support of oxygenation and carbon dioxide clearance veno-venous ECMO can be utilised. In some patients veno-arterial ECMO may be required. Use of ECMO for early graft dysfunction seems to result in acceptable outcome, although the use of ECMO for late graft failure seems more questionable [22]. Some author's advocate a slow wean from veno-arterial ECMO post-operatively in all transplants with severe pulmonary hypertension to reduce early graft dysfunction but this has not been

systematically assessed. These patients often have a high cardiac output state with a stiff left ventricle compounding the alveolar capillary leak and lack of lymphatic drainage in the newly implanted lung allograft.

Recipient selection: waiting list issues

Recipient selection criteria have evolved greatly over the last 30 yrs as lung transplantation has matured from an experimental procedure to a standard approach in end-stage lung disease management (as evidenced by third party insurance funding). This has also seen a steady increase in recipient age and increasing medical comorbidities. To date, this has not resulted in a detectable adverse effect on transplant survival.

Several attempts have been made to develop international recipient selection guidelines [23, 24]. Due to quite large variation in donor rates, as well as quite marked differences in who may get access to lung transplantation from one country to another (*e.g.* lack of universal healthcare access/insurance in many countries), tailoring of these guidelines at national level has generally occurred. The purpose of the recipient selection criteria does differ depending on the approach to organ allocation. In some jurisdictions all patients who qualify (usually medically and financially) will be wait-listed for transplantation, often resulting in large lists with high rates of death on the waiting list. Another approach is to set up the selection process so that the most ''suitable'' patients get onto the waiting list, still ensuring that all suitable organs are utilised, but limiting transplantation to only those who will realistically be transplanted. This second pragmatic approach accepts that the number of potential recipients is many orders of magnitude greater than all available donors.

The major changes that have occurred over the last 20 yrs are liberalisation of recipient age, routine acceptance of patients with manageable comorbidities including diabetes, and acceptance of patients who have had previous surgery (including prior lung transplantation). The development of antiviral therapies (including highly active antiviral therapy and therapies for hepatitis B/C) have led some programmes to now no longer regard HIV and hepatitis B and C as absolute contraindications to solid organ transplantation. However, the decision for listing based on the recipient's underlying disease has not substantially changed. What is clear is that COPD patients without chronic hypercapnea are not survival advantaged, and thus lung transplantation (LTx) is only indicated to improve quality of life (QoL) where this indication can be accommodated. Bronchoalveolar cell carcinoma was previously regarded as the only primary lung malignancy where lung transplantation could be considered as a potentially curative procedure. Despite reports of high rates of recurrence post-transplantation, leading to many centres no longer accepting such patients, a recent report shows that there is no impact on 5-yr survival rates [25].

Donor selection/management issues

Donor networks

The desire to allow anonymity of donor and recipient has led to the development of independent organ procurement agencies servicing at regional, national and even multi-national levels. They generally commenced as renal donor networks but with the development of other organ transplants have extended their role as multi-organ and tissue donation agencies. Their role generally involves promotion of organ transplantation, initial assessment of potential organ donors, detailed further testing, confirmation of and/or obtaining consent from the next of kin and local jurisdictions (including the coroner if required). They are also responsible for contacting the recipient teams and co-ordinating the manpower and facilities required for multi-organ donation.

Increasingly greater resources have been invested as more borderline donors are evaluated. The demands of this irregular hour job, a desire to identify and utilise all suitable donor organs, as well as the increased requirements for auditing and reporting have led to much larger Organ Procurement Organisations (OPOs).

Donor organ allocation

Considerable differences are apparent in the approach to organ allocation both comparing contemporary OPOs and changes over time. The largest of these is the United Network of Organ Sharing (UNOS) in the USA. Initially, time on the waiting list was a key determinant of organ allocation once blood group and size compatibility were confirmed. However, this seriously disadvantaged patients with a rapidly progressive course (*e.g.* usual interstitial pneumonia (UIP)). Thus, recently allocation utilising a lung allocation score (LAS) has been instituted. This score tries to assess the utility of the transplant based on the likelihood of survival with and without lung transplantation [26, 27]. It does, however, strongly favour patients on mechanical ventilation or ECMO support and, as such, might be predicted to lead to a worsening of post-transplant survival and increasing cost [28, 29]. Other jurisdictions allocate the organ(s) to the transplant programme that internally decides on the best recipient [30]. These systems give greater flexibility and may assist the use of more marginal donors should circumstances dictate, but may be more difficult to defend if the allocation is challenged.

Cross-matching/virtual cross-matching

The role of an existing immunity directed against the human leukocyte antigen (HLA) in graft survival is well demonstrated in the classic study of TERASAKI *et al.* [31]. Even in an era utilising calcineurin inhibitor-based immunosuppression, the degree of HLA mismatch for both class I and class II antigens still has an impact on lung allograft and patient survival; although, this is difficult to predict at the individual level [32].

New nuclear and flow cytometry technology has provided a marked increase in sensitivity with the presence of a donor-specific antibody, which appears to impact on both short- and long-term survival [33]. Key questions remain as to what degree the quantitation and HLA specificity of these assays and/or their coupling with a functional readout will impact on recipient outcomes. Answers to these questions should allow a much greater understanding of the clinical relevance and role of these newer assays in organ allocation, as well monitoring post-transplantation.

Extending donor indications

Extended donors

At the outset, brain death donor criteria have been utilised for HLTx and LTx. The criteria for acceptable donors were initially very restrictive leading to <10% of all multi-organ donors being

used as lung donors. As well as standard multi-organ criteria, specific criteria such as cold ischemic time <5 h, age <45 yrs, non-smoker, no history of respiratory disease, no sputum on suctioning, clear chest radiograph, arterial oxygen tension (P_{a,O_2}) >300 mmHg on 100% and 5 cmH_2O positive end-expiratory pressure (PEEP) had been used to select suitable lung donors. Low donor numbers were the single major impediment to lung transplantation and slowly, liberalisation of these criteria has occurred. Donors falling outside these strict criteria have been referred to as ''marginal'' or ''extended indication''. Liberalisation of donor criteria, particularly in relation to donor age and smoking status [34], have seen substantial increases in lung transplant numbers and, while perhaps slightly impacting on the individual recipient's probable survival [35], this needs to be considered in the context of an overall increase in transplants performed [30].

Donation after cardiac death

Extension of the 5-h cold ischaemic time in lung transplantation well beyond the ''accepted'' 5 h limit did not seem to impact substantially on early graft function or longer term outcome. This led to an increasing understanding that the lung may be more resistant to the effects of warm ischaemia when contrasted to a highly metabolically active organ such as the heart. Animal and subsequent clinical work by STEEN *et al.* [36] has led to more widespread use of donation after cardiac death (DCD) [37, 38]. Recent reports from Australia show outcomes from ''controlled'' (Maastricht III) DCD donors that are at least comparable to donation after brain death [39, 40]. DCD from ''uncontrolled'' (out of hospital cardiac arrest) donors also appears feasible [41]. An increase in overall lung transplant numbers of 15–25% is reported with the development of DCD.

Donor management

Basic

As noted, very narrow initial criteria led to <10% of donor organs being utilised for lung transplantation. Liberalisation of donor criteria has led to increased lung transplant numbers. Complementary to this approach is the use of simple strategies which may convert potential donors to donors falling within standard criteria. Approaches such as bronchoscopic toilet, antibiotic strategies, physiotherapy and ventilation strategies are all reported [42, 43].

Ex vivo perfusion

Ex vivo perfusion of donor lungs in the clinical setting was first reported by STEEN *et al.* [36] in an attempt to assess the suitability of DCD lungs for transplantation. The concept has been extended to include assessment and management of ''borderline'' DCD lungs [44, 45] with lungs previously thought to be unusable being successfully transplanted [46]. One estimate is that 46% of lungs may be ''converted'' by *ex vivo* perfusion and management [47]. Outcomes including hospital length of stay seem comparable to conventional brain dead donors [48]. The still small experience, as well as the lack of data in using such organs without *ex vivo* reconditioning, makes it still very difficult to judge the true benefit of what is expensive and resource consumptive technology.

''Treatments'' to the donor lungs may include manipulation of ventilation, lung inflation, attempts to reduce lung water and also use of supra-pharmacological doses of antibiotic to resolve infection more rapidly. The initial lung function and response to therapy can be measured by the effects on oxygenation of the perfusate. Recognising that the perfusate has a low haematocrit is perhaps, at best, a crude measure at present. As the perfusion pump rate is fixed, pressure changes within the perfused lungs can be used to assess effects on pulmonary vascular resistance.

Donor procedure

En bloc versus individual organs

As noted earlier, lung transplantation was initially performed almost exclusively as an *en bloc* HLTx procedure. YACOUB *et al.* [49] described an approach to use the explanted HLTx recipient heart in a second recipient (the so called ''domino procedure''). With the development and proliferation of isolated lung transplant procedure, splitting of the heart–lung block into the heart and two separate lungs is almost universally the approach. The long standing urgent classification for cardiac allograft recipients in most jurisdictions has been a major factor in this change.

Perfusion solutions

As with much of LTx, lung allograft preservation evolved from preservation of the heart–lung block. An array of extracellular solutions (Eurocollins, low potassium dextran, University of Wisconsin, Perfedex) has been used with local additives and variations. The exact constituents were often based on a large number of animal experiments but limited human data; however it is a field that continues to generate studies [50]. Other groups have used more ''physiological'' approaches including blood products such as packed red blood cells (Cambridge solution). The various solutions have a lot of *in vitro* data as to their effectiveness but *in vivo* human data is uncommon. A recent study reported superiority of Perfedex solution over others [51] with respect to early graft dysfunction, but methodological issues make it difficult to come to a firm conclusion.

Donor/recipient matching: information* versus *decision making

Protocolised lung allocation score versus *physician weighted priority/utility decision making*

The decision to use a donor for lung transplantation has, to some degree, always presented a dilemma as to what is the ''optimum'' use of a heart–lung block. At one extreme is to perform HLTx in a single recipient and at the other extreme is to perform a heart transplant and two SLTs in three recipients. The factors determining what is the actual transplant(s) performed may depend on a strict formulaic approach to organ allocation through to systems giving substantial autonomy to transplant programmes which may have a more pragmatic approach. To some degree, however, the system is a spectrum that from one end reflects a strong societal individual rights view (a right to be transplanted) in an orderly fashion, with the other extreme being a utilitarian approach of ''doing the most good'' with the available donors. In most countries the availability of HLTx is severely limited as hearts are usually allocated on clinical urgency and lungs only are offered for lung transplantation.

As different countries and regions have a different position within the above spectrum, the system of lung allocation differs

substantially around the world. In the USA, for many years, once basic compatibility was confirmed (with priority given to transplant programmes in closer proximity to the donor) the UNOS system allocated based primarily on the length of time on the waiting list. In 2005, UNOS adopted a new system based primarily on the incremental effect of lung transplantation on survival. The LAS takes into account the likelihood of survival without a lung transplant relative to the likelihood of survival with a lung transplant over a 12-month horizon. A score of 0–100 is generated with scores >50 likely to be allocated an organ within weeks. Since implementing the system a change in the recipient mix has occurred with more acutely ill patients (including mechanically ventilated patients), often with interstitial lung disease being transplanted and fewer pulmonary hypertension patients being transplanted. A fall in time on the waiting list has occurred but this is probably mostly due to patients with low LAS not being listed until they deteriorate. Potential problems with the LAS include poorer overall survival rates as much sicker patients are transplanted. Furthermore, there may be a disincentive to utilise marginal donors where a high LAS means a high chance of a standard donor. The LAS approach has been adopted by other jurisdictions including Eurotransplant where out of country allocations are made according to LAS. All allocations within Germany are performed according to LAS but in other countries within Eurotransplant priority is determined by clinical urgency, balancing numbers and waiting time.

The Eurotransplant system does, however, also take into account other considerations. Equitable distribution of organs between Eurotransplant countries will affect the allocation of organs. Other parts of the world tend to leave the allocation to a specific transplant programme. In the UK and Australia, the closest transplant programme is offered the heart and lungs first; if they were unable to utilise organs they would be ''passed on'' according to a rotational system. Such systems allow considerable flexibility, in which transplants are performed, encouraging the referral of marginal organ where a risk benefit assessment can be made directly by physicians caring for the recipient. In Australia, such a system has led to very high rates of lung transplantation despite low donor numbers. Nevertheless, this approach may be viewed as arbitrary and is probably untenable in countries where individual rights and freedoms are seen as paramount.

End-stage lung disease and advances in management options

Management of severe lung disease

New therapies

The availability of new therapies over the last 30 yrs has impacted on the indication and timing of lung transplantation. In the commonest indication, COPD, surgical approaches including lung volume reduction surgery (LVRS) have been offered in patients who are severely symptomatic without lung transplantation offering a clear survival advantage [52]. A large randomised controlled trial of LVRS failed to show an overall effect on survival despite many patients having significant physiological and functional benefits. Many less invasive approaches to LVRS are reported [53, 54], but as yet they have not become standard therapy and have impacted minimally on the need for lung transplantation in COPD.

The increased availability of specific therapies for pulmonary arterial hypertension (PAH) combined with the generally poor outcomes from LTx and HLTx has seen some reduction in the proportion of patients receiving LTx with PAH. Combination oral therapy with or without the addition of parental prostanoids (epoprostenol, treprostenol or iloprost) are the mainstay of advanced disease treatment and are now recommended in major international guidelines [55]. The indication for transplantation has become the failure of these therapies to at least stabilise the condition.

The commonest form of interstitial lung disease presenting for transplantation is the UIP form of idiopathic interstitial pneumonia. Although nomenclature has changed over the years, very few patients appear to show favourable responses to immunosuppressing therapy. Indeed, a recent randomised controlled trial (RCT) of combination prednisolone, azathioprine and *N*-acetyl cysteine has been stopped prematurely because of inferior survival in patients on this combination therapy [56]. Some efficacy has recently been reported using the anti-fibrotic agent pifenidone [57] but this has not impacted on the need or timing of lung transplantation to date.

Continuing incremental improvements in CF management (*e.g.* nutritional, antibiotic therapies and strategies, airway clearance with DNAse and hypertonic saline) have led to an improved outlook for CF patients. However, LTx remains an important option in the care of CF patients with end-stage lung disease. Gene therapies and CF transmembrane conductance regulator (CFTR) modulators have been much anticipated in their application to reverse the core gene defect. To date, only one product, ivacaftor (modulates the effects of the G55ID mutation which is present in ~4% of CF patients), has moved into clinical practice [58]. It is hoped that this breakthrough will herald a new era of CFTR modulating therapies which, in turn, may dramatically change timing, and perhaps one day even the need, for LTx in these patients.

Current ethical framework for difficult decisions

Increasing activity versus *limited resources*

The move to utilise more ''marginal donors'', the increase in donors by DCD and the potential to ''resuscitate'' donor lungs utilising *ex vivo* perfusion all have impacted on the number of lung transplants performed. Despite this, the number of patients receiving lung transplantation is but a small fraction of those with end-stage pulmonary and pulmonary vascular disease who might benefit.

The imbalance between donor organ availability and potential recipient need continues to challenge programmes as to how to prioritise those requiring HLTx, those requiring re-transplantation and even those patients who may need true multi-organ transplantation (*e.g.* lung–kidney or lung–liver). Even at a simpler level, the dilemma is illustrated by the potential to perform SLT in many patients with interstitial lung disease or emphysema. Double lung transplantation undoubtedly results in better physiological outcomes, but not universally superior survival [59, 60]. However, the overall utility is better if SLT is performed where possible (most years of survival from the total donor pool) [60, 61].

As previously noted the different underlying societal attitudes (*e.g.* self-determinism *versus* utility) may result in nations and

regions coming to quite different approaches to the same question. A key factor for future projections is to what extent healthcare financiers will be prepared to fund increasing transplant numbers.

Bridge to transplantation

Continuous positive airway pressure/bi-level positive airway pressure

Increasingly, noninvasive ventilation has been used in the setting of hypercapneic respiratory failure as a bridge to transplantation. This is generally not supported by strong clinical trial data.

Mechanical ventilation

The utilisation of mechanical ventilation as a bridge to transplantation varies widely around the world. This is, in part, based on the ability to rapidly access donors and prioritisation of those who are mechanically ventilated (*e.g.* the LAS). Although mechanical ventilation at the point of LTx is a risk factor for increased post-operative mortality, the incremental effect on survival is often greatest in these circumstances. Mechanical ventilation in cystic fibrosis can be particularly problematic with the rapid accumulation of highly viscous secretions rapidly leading to severe sepsis. Perseverance with bi-level positive airway pressure, where possible, appears to be the preferred approach [62].

ECMO

As noted previously, technological improvements have improved the outcome of advanced life support with ECMO. There have been sporadic attempts to support patients to transplant over at least 20 yrs. A recent case series of 38 ECMO bridges to lung transplant reports an 89% successful bridge (median time 5.5 days) with 1-yr survival of 60% in those actually surviving to transplant [63]. A further recent study showed similarly inferior survival in ECMO bridges to transplant lung recipients [64]. Importantly a 2–4 week time window is required to find a suitable donor. In donor allocation systems with high priority categories and a large population base, finding a suitable donor in this time frame is feasible [65]. The ability to size match by cutting down donor lungs facilitates the ability to consider many more potential donors [66]. Evolving technologies, such as single twin lumen catheters that allow veno-venous ECMO or the ''Novalung'', may extend this time window by allowing mobility and some rehabilitation in the potential recipients receiving advanced life support [67].

Re-transplantation

Re-transplantation remains a very uncommon indication for lung transplantation. Re-transplantation for early graft dysfunction had particularly poor results and is now rarely offered. Patients without other comorbidities and $\geqslant$2 yrs after initial transplant have comparable outcomes to first time lung transplant recipients (LTR) [68]. As is a common issue with much LTx data there are many confounders. Generally, these re-transplant candidates are subjected to even tighter scrutiny than first time recipients in the selection criteria/organ allocation system. Thus, only a small proportion of patients dying of bronchiolitis obliertans syndrome (BOS) will receive re-transplantation, although it is likely to be a frequent occurrence in paediatric lung transplantation [69].

Age: paediatric lung transplantation versus *older recipients*

Pre-specified upper age limits for donors and recipients have been part of lung transplant practice from the earliest days. Although it is clear that the ageing process makes it more likely that other important comorbidities exist, in reality age limits have been used as a method of limiting the need to evaluate many patients who could conceivably benefit from transplantation. Indeed technical improvements have allowed successful lung transplantation despite serious comorbidities such as coronary artery disease [70].

Many jurisdictions have developed equal opportunity laws over the last several decades, which would prohibit excluding patients from consideration simply on chronological age. This, in part, explains the trend to increasing age of LTR. Often quoted is the lack of an effect of increasing age on transplant outcome. However, recipients >60 yrs (and particularly >65 yrs) probably represent only a small fraction of all patients with end-stage lung disease in those age ranges. Thus, it is probably fairer to say that in a highly selected subgroup, reasonable results can be achieved [71].

At the other end of the age spectrum paediatric LTx remains very uncommon with only ~100 being performed globally each year. Clearly improvements (*e.g.* CF) in prognosis have reduced the need for paediatric LTx. Nevertheless a shortage of paediatric lung transplant centres, the rapid downward trajectory on these patients and a great shortage of paediatric lung donors are contributory. Innovations such as cut down transplantation, lobar transplantation and living donor lobar transplantation have all been used to address the paediatric donor shortage.

The complexities of paediatric lung transplantation are reflected in inferior BOS-free survival. Thus, re-transplantation is often considered. At an objective level, an adult patient may have a superior median survival but in many countries the value of a year of survival in children may be valued higher than that of an adult. In publicly funded lung transplant programmes there may be an expectation that societal attitudes and beliefs are reflected in who ultimately receives a transplant.

Where we are now and the present challenges?

What is clear is that we can perform lung transplantation in many patients with the ability to improve survival and QoL (fig. 1). The majority of patients with severe lung disease do not receive a lung transplant, and even those who do generally have a period of improved QoL, a modest improvement in survival with significant complications (many iatrogenic) but ultimately still a substantially reduced life expectancy. Despite this, lung transplantation is now regarded as standard treatment of many end-stage lung diseases.

Patients' expectations are that they will have access to this complex and costly treatment and the ongoing intensive follow-up that is required. Healthcare funders have a strong desire to cap overall expenditure and reduce unit cost. Paradoxically, the more we move from ideal donors and ideal recipients the greater the likelihood that the unit price will actually increase. This revelation often leads to great consternation among the healthcare funders, who, while publicly supportive of increasing organ donation, struggle with the impact of this on the financial bottom line. Often under-appreciated by those who

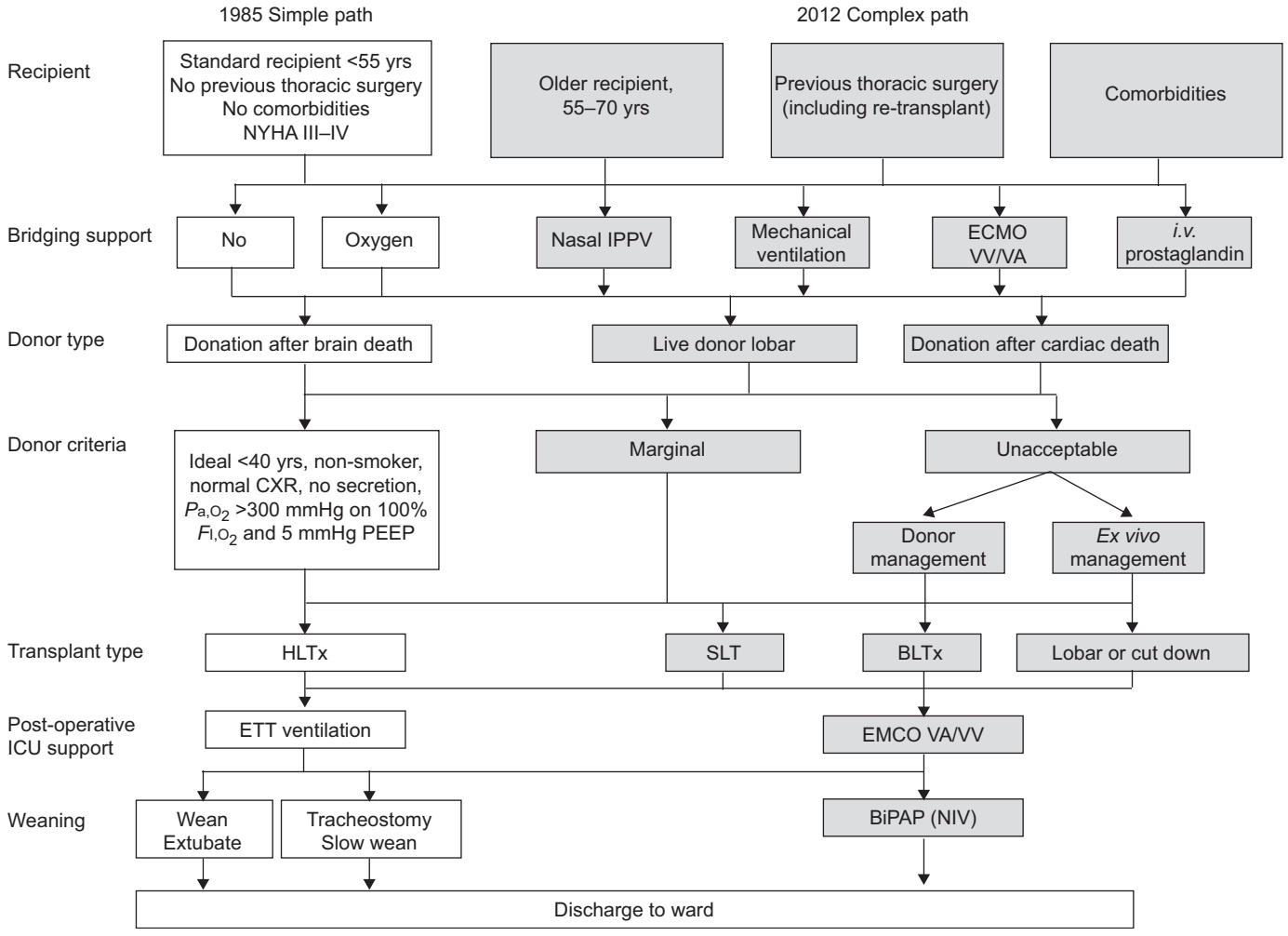

FIGURE 1. Increasingly complex pathways in lung transplantation (1985–2012). ICU: intensive care unit; NYHA: New York Heart Association; CXR: chest radiograph; P_{a,O_2}: arterial oxygen tension; F_{I,O_2}: inspiratory oxygen fraction; PEEP: positive end-expiratory pressure; HLTx: heart–lung transplantation; ETT: endotracheal tube; IPPV: intermittent positive pressure ventilation; ECMO: extracorporeal membrane oxygenation; VV: veno-venous; VA: veno-arterial; SLT: single lung transplant; BLTx: bilateral lung transplant; BiPAP: bi-level positive airway pressure; NIV: noninvasive ventilation.

fund healthcare is the cost of ongoing care of the still exponentially growing number of long-term survivors of LTx.

The road ahead is full of real challenges to increase the numbers of lung transplants performed, improving the QoL achieved and lengthen the survival of recipients. Controlling the costs of the transplant procedure, as well as the costs of ongoing care, will be critical in achieving extra proportional resources for the increasing in lung transplant activities.

PATIENTS: CURRENT CLINICAL MANAGEMENT/KEY QUESTIONS

Where are we now and where do we need to go?

What exactly is primary graft dysfunction and how can we understand it better?

A clear understanding of what primary graft dysfunction (PGD) is and isn't and what drives the underlying pathobiology as well as how it translates to downstream effects all have major implications for both short- and long-term outcomes in LTR [72–74]. After a relatively slow start, we are now making significant inroads into better understanding, diagnosing and managing this condition.

Definition, pathobiology and risk factor identification

PGD of the lung is a syndrome of ''acute lung injury'' that occurs within 72 h of LTx. It is characterised histopathologically by diffuse alveolar damage and physiologically by ''impaired oxygenation, diffuse pulmonary infiltrates and decreased lung compliance without an elevated left atrial pressure'' [72]. In 2005, an ISHLT-appointed working group on PGD added both a grading system (based on worsening P_{a,O_2}/inspiratory oxygen fraction (F_{I,O_2}) ratios) and a further time dependence to the definition (0–6 h, 24 h, 48 h and 72 h) of reperfusion with the dual aim of better delineating clinical risk factors and improving the predictive power for clinical outcomes [73].

It is widely believed that the ischaemia–reperfusion insult that necessarily accompanies all LTx procedures is at the core of PGD and that the variable clinical manifestations of this condition are largely due to critical differences in donor organ quality (including time of organ preservation and storage) as

well as variable contributions from recipient reperfusion processes, allo-associated inflammation and lung fluid balance dysregulation [72, 73]. In addition, all these donor, operative and recipient factors are, at least in part, management dependent. Therefore, it follows that ischaemia–reperfusion can be considered a multifactorial process whereby a variable number and magnitude of key disturbances in the organ donor, storage/transport conditions of the allograft and LTR immuno-inflammatory responses result in a spectrum of PGD that ranges from the very mild to the extremely severe. Finally, excessive fluid resuscitation may significantly confound the clinical syndrome of PGD in the setting of ''injured and inflamed lungs'' (irrespective of the cause), that no longer have the benefit of intact lymphatic drainage as a result of the transplant procedure.

The increasing appreciation of the impact of PGD on clinical outcomes post-LTx has led to efforts to better identify modifiable clinical risk factors despite the limitations posed by single centre studies with small numbers of patients spread over different treatment eras [74]. Given the previously mentioned pathobiological framework, it is not surprising that the factors that increase the probability and, more importantly, the severity of ischaemia–reperfusion injury are also the factors that have been generally acknowledged as being the key risk factors for PGD. These factors include ''marginal donor lungs'' for any reason (*e.g.* trauma, aspiration, pneumonia, and acute respiratory distress syndrome that may/may not be associated with brain death), prolonged ischaemic times and any degree of pulmonary arterial hypertension in the recipient [75, 76]. Interestingly, the time at which the $P_{a,O_2}/F_{I,O_2}$ ratio is at its worst within the first 72 h of reperfusion seems to vary considerably in different reports; resulting in minimal ''extra'' predictive power for lung allograft outcomes (most likely a function of the ''noise'' introduced when there are many interacting factors at play) [76]. In contrast, donor organ biology in the setting of brain death is increasingly of interest now that this is able to be compared to lungs that are DCD. Indeed, there is now very recent evidence that DCD lung allografts are generally associated with less PGD (''all else being equal'') than non-DCD lungs and better long-term outcomes [40]. These observations again highlight the mechanistic complexity that is hidden by clinically based definitions of PGD and risk factor identification and reminds us that a deeper understanding is dependent on more completely delineating the cellular processes associated with organ retrieval, storage and re-implantation of the lung allograft.

Clinical patterns: diagnostic considerations

The increasing standardisation of the definition and grading of PGD [73, 77] has had the immediate effect of increasing the number of milder cases being reported (grade 1 and 2) but attributing the major impact on early mortality to PGD severity of grade 3 or above (variably reported between 10–50%) [78, 79]. In addition, it is grade 3 PGD rather than milder grades of PGD that has been specifically associated with increased long-term BOS [74, 80] when the association has been present; perhaps in keeping with BOS heterogeneity and relatively small study cohort sizes [81–83]. Finally, although less stringent definitions of PGD were associated with an increased incidence they were not associated with increased mortality [84]. This finding has been confirmed and validated by the ISHLT grading system [85] thereby suggesting that an important ''cause and/or effect'' threshold may exist relating PGD to important clinical outcomes.

Given the variable contribution of PGD grade 3 to future BOS in LTR some further ''unbundling'' of this relationship is warranted. First, the ''effect'' size for this association is critically dependent on the probable mechanism at play. Is it the physiological derangement that is related to later BOS or is this derangement a marker of underlying processes that predispose to BOS, or both? Secondly, how can we better ''model'' what is going on pathobiologically, temporally and contextually? More detailed profiling of the development of grade 3 PGD is only now beginning, particularly regarding biomarkers of cell injury and inflammation [86]. However, only specific interventions in the ''controlled'' setting of a randomised clinical trial are likely to provide the best answers.

Management issues: prevention, treatment and late complications

An improved understanding of the key processes leading to the pathophysiology of PGD is clearly the first step in better preventing and treating this condition. Once PGD and its complications are established the clinical focus very quickly switches to supportive management in the form of more detailed attention to fluid balance, maintaining haemodynamic stability without excessive filling pressures, and minimising ventilator-induced lung injury in the early post-operative period. Even in this setting however, it would be very beneficial to delineate any alloimmune or other drivers of an ongoing injury process.

However, more difficult, but perhaps even more rewarding, will be an improved clarification of the risk factors and pathobiological processes that lead to PGD in the first place with an aim to prevent PGD of any severity that is associated with either a compromised allograft (excessive damage/impaired tissue repair) or problematic alloreactivity (excessive alloresponse/fragile tolerance), both of which may reasonably be linked to poorer short- and long-term outcomes.

As will be discussed later, although research efforts in this area have always been present, they are now escalating dramatically with the realisation that the key cellular processes that lead to PGD may also have a significant impact on lung allograft outcomes and survival in the long term. In particular, chronic allograft dysfunction (CLAD) rates have not improved in parallel with improvements in early acute rejection rates but may be more amenable to specific early interventions relating to prevention and better management of PGD. In particular, there is an increased focus on the links between ischaemia–reperfusion injury, reactive oxygen species formation, subsequent endothelial and epithelial cell injury, innate immunity and pro-inflammatory cascades [87]. Although the majority of work aiming to prevent PGD has focused on minimising cold ischaemia and improving lung preservation methods [75, 88], there is a parallel interest in reducing subsequent immuno-inflammatory pathway upregulation that could potentially become self-perpetuating (including anti-HLA allo-antibodies and auto-antibodies to collagen type 4) [33, 89–93]. Finally, *ex vivo* conditioning of the lung allograft not only has the potential to better delineate PGD pathophysiology but can also be harnessed to reduce ischaemia–reperfusion injury and block

immuno-inflammatory cascades by utilising specific treatment and delivery opportunities [94–97].

What does histopathological acute rejection (as currently defined) tell us or not tell us?

Acute rejection, as defined histopathologically, has proven to be a very useful concept over the last 20 yrs but the clinical exactness of the term does not completely reveal the full spectrum of cellular interactions and complexity that shapes an alloresponse, and for which immunosuppression is essential. In addition to the histopathological diagnosis of acute rejection being a relatively insensitive measure of alloreactivity, there is also the possibility that milder forms of an alloresponse, and even perhaps some cases of ''peri-vascular'' lymphocytic infiltration, may be predominantly tolerogenic rather than destructive; thereby raising the possibility in some LTR that ''alloimmune control'' risks of extra immunosuppression may outweigh the benefits.

Definitions, pathobiology and risk factor identification

Alloreactivity is defined as the host immune response to non-self-donor antigens and, as such, is a dominant feature of lung transplantation where donor organs are not routinely HLA-matched (because of organ scarcity and logistic constraints) [98]. Although HLA mismatching between donor organ and recipient is the primary basis for all allograft rejection syndromes, the exact pathobiology defining this process in an individual LTR is poorly understood and poorly monitored leading to a relatively heavy reliance on potent, broad-based immunosuppression strategies.

Despite our relatively limited understanding of the specifics of the alloresponse in any individual LTR, experimental evidence from T-cell depletion studies in animal models and the successful use of cyclosporin A in human lung transplantation strongly suggests that, at least initially, the alloresponse is predominantly T-cell dependent [99]. T-cells require a minimum of two signals for activation, antigen recognition and co-stimulation. Given the life history of all adult humans and, perhaps especially LTR, memory T-cells are ubiquitous and may be particularly problematic post-transplant because their activation is relatively less stringent than for naïve T-cells and of the potential of heterologous immunity [100]. The majority of B cells require help from T-cells to initiate antibody production and although T-cell independent antibody production is well described, this largely occurs in the setting of infection and/or later post-transplantation [101, 102]. In any case, donor-specific antibodies targeting HLA molecules on the various cell types in the allograft, as well as autoantigens, can contribute to graft loss especially if they are high affinity, complement binding and part of an integrated immune–inflammatory attack on the foreign tissue [100].

Alloreactive T- and B cells in the recipient can be initiated by so called direct, indirect or semi-direct pathways, depending on the interaction between donor major histocompatibility complex (MHC) and recipient antigen presenting cells [103, 104]. Although it has been reported that the direct pathways tend to dominate the early post-transplant period (relatively high precursor frequency) and the indirect pathway tends to dominate the later period (aimed at fewer donor MHC peptides displayed on recipient MHC molecules) [105], the exact contribution of either allorecognition pathway or even T- and B-effector cell pathway amplification [106–108] to chronic rejection in an individual LTR is difficult to ascertain [109]. Similarly, it is difficult to apportion the contribution of impaired regulatory control mechanisms [110–112], persistent autoimmunity [92, 113] and/or epithelial repair processes [114, 115] to chronic allograft loss.

In the above framework of alloreactivity-associated injury there are two extreme approaches to risk factor identification. At one extreme, there is the difficult task of identifying specific HLA combinations between donors and recipients factoring in the potential for heterologous immunity (*i.e.* recipient's infection history) that may predispose to an aggressive alloresponse. At the other extreme, the focus is on identifying early damage signals in the allograft that could act as a net integrator of pathways leading to acute and/or CLAD (see later section). In between these two extremes, the multi-dimensional complexity of the alloresponse makes it relatively difficult for the specific measurement of one dimension to sensitively and specifically capture all the information that is not measured in all its other dimensions. Having stated this, it is probable that an inter-relationship between activation of innate immunity, antigen presenting pathways, T-cell immunity, humoral responses and inflammation in the LTR exists even in the setting of immunosuppression and perhaps even gets stronger with increasing alloreactivity, thereby enabling the current spectrum of clinical tools to diagnose the risk and/or presence of significant alloreactivity (histopathology of lung tissue, donor-specific antibodies and even relatively non-specific signals from cellular immune assays) to be at least partly predictive of poorer allograft outcomes over time.

Clinical patterns: diagnostic considerations

In many ways, the management of life-threatening acute rejection has been a major success as it defined the modern era of lung transplantation. Calcineurin-based maintenance immunosuppression protocols and steroid-based augmented immunosuppression strategies have dramatically reduced the rate of histopathological acute rejection and hence graft loss from this condition over the last 25 yrs [116]. However, and perhaps equally important, despite the reduction in histopathological acute rejection, there has not been any significant improvement in chronic lung allograft dysfunction over the same time period [116, 117]. Is this because of the damaging effects of undiagnosed sub-clinical alloreactivity/fragile tolerance? Alternatively, is it because of other factors that may compromise the integrity of the allograft (*e.g.* infection, dysregulated inflammation/repair processes)? Or a combination?

The histopathological classification and grading of acute cellular rejection in LTR was first described in 1990 and has only minimally evolved since then [118–120]. Acute vascular cellular rejection on transbronchial biopsies is characterised according to the degree of perivascular lymphocytic infiltrate with minimal (A1), mild (A2), moderate (A3) and severe (A4) infiltration and variable infiltration with other cell types including eosinophils and neutrophils. Although the limitations of inadequate tissue sampling is always discussed, the lack of more detailed phenotypic analysis of the cellular infiltrate (*e.g.* CD4/CD8 T-cells, regulatory T-cells (Tregs) or B cells), particularly in relation to the total infiltrate, and also the resident cell profile (*e.g.* degree of endothelial damage,

apoptosis and/or proliferation) is not. Perhaps of even greater importance is the fact that this paucity of information coupled with the relative insensitivity of transbronchial biopsies to obtain adequate bronchiolar tissue with which to assess cellular rejection of the bronchioles makes it difficult to properly examine the potential link between the two. Acute cellular reaction grade B is characterised by lymphocytic inflammation of the small airways and perhaps not surprisingly is a stronger risk factor for the development of BOS than acute vascular rejection [119, 121]. For completeness, grade C rejection is characterised by features of obliterative bronchiolitis (including obstructive changes with or without mucostasis and/or endogenous lipoid pneumonia) but rarely diagnosed on transbronchial biopsy. Finally, grade D rejection is rarely seen but histologically is characterised by arterial and venous intimal fibrosis with or without inflammatory infiltrates [118, 119]. Finally, histopathological techniques to diagnose antibody-mediated rejection remain relatively problematic, although improving [82], thereby greatly limiting our ability to fully understand the potential relationships between circulating donor-specific antibodies, antibody- and cell-mediated rejection in the lung allograft and various clinical patterns of chronic lung allograft dysfunction [119, 120, 122–125]. From the clinical information perspective, the histopathological diagnosis of allograft rejection is associated with a clear trade-off between sampling/technical efficiencies and predictive power. Just as importantly, however, although there is a histopathological ''exactness'' associated with the diagnosis of allograft rejection types/grade and specific other patterns (*e.g.* bronchiolitis obliterans organising pneumonia (BOOP) or fibrinoid organising pneumonia (FOP)), the precise pathobiological relationships within and between the various vascular, bronchiolar and interstitial phenotypes central to these diagnoses remains unclear (fig. 2).

The current state of relatively insensitive and poorly specific tests for alloreactivity-associated organ damage relegates much of our diagnosis of acute rejection ''risk'' and ''presence'' to a relatively probabilistic analysis. This assessment then informs decision making pathways in a contextual framework where other important information such as infection risk and immunosuppression profiles are also factored in. In this setting, a clear understanding of both the certainties and uncertainties relating to ''acute rejection'', ''infection'' and ''drug toxicity'' are all critical.

Management issues: prevention, treatment and complications

In the context of ongoing long-term lung allograft loss, diagnostic uncertainty related to alloreactivity is dramatically compounded by the need to avoid under-management of this condition. Immunosuppression management is, therefore, understandably relegated to giving as much as possible without causing adverse events or overdoing the potential risk of adverse events (especially as there is really no viable back-up plan once the lung allograft is lost and re-transplantation is an option in only a handful of cases).

Although, it would then be acceptable to run lower immunosuppression levels if there was clear evidence of drug toxicity or significant infection, the exact ''thresholds'' for these decisions are not always clear, particularly where both damaging alloreactivity and infection are present to a varying degree and perhaps even compounding each other. The current lack of an accurate, robust, sensitive, predictive, treatment responsive and therefore meaningful measure of the alloresponse makes individual titration of maintenance immunosuppression, augmentation of immunosuppression strategies and management of established BOS all less than optimal. Better targeting immunosuppression may not only reduce graft loss from unchecked alloreactivity over time, but may also offer the possibilities of less fragile operational tolerance risk and limitation of excess infection and malignancy risk; both of which may also help to improve lung allograft outcomes as well as non-lung allograft morbidity and mortality. In addition, the development of novel targets for new classes of immunosuppressive drugs will be greatly facilitated by the identification of specific molecular pathways that trigger tissue injury-associated enhancement of the presentation of foreign antigens, activation of T-cells and amplification of immuno-inflammatory loops.

Although initial and subsequent risk stratification regarding alloreactivity and infection may be improved by utilising newer diagnostic and therapeutic tools to maximise benefit while minimising risks, a level of ''trade-off'' will always be required unless true tolerance of the allograft can be achieved at the same time as maintaining a fully potent host response to microbial pathogens!

How good are current immunosuppression strategies and how can they be better?

The relative potency and T-cell selectivity of cyclosporin A (CyA) revolutionised organ transplantation generally and lung transplantation specifically [1, 126]. Although CyA enabled effective immune suppression to be obtained without excessive myelosuppression, a ''standard'' triple regimen for immunosuppression (initially CyA+steroids+azathioprine) quickly became routine for LTR so as to maximise net immunosuppression whilst minimising specific toxicities [127]. This standard triple regimen has continued to be fine-tuned over the years by increasingly changing calcineurin inhibitor to tacrolimus, reducing the total steroid dose, and often using mycophenolate mofetil instead of azathioprine in an effort to increase the efficacy with which the alloimmune response is suppressed whilst keeping specific drug-related toxicities to an acceptable level. However, this ''trade-off'' can only be partially achieved with respect to infection risk given the many fundamental similarities with which an immune response attacks both foreign tissue and foreign pathogens. In the latter setting, better diagnostic tools for destructive alloreactivity would dramatically help to individualise care as would better diagnostic tools/therapeutic strategies for the various infection syndromes that LTRs are at risk from.

Calcineurin-based immunosuppression: a great start but still a way to go

CyA and tacrolimus specifically bind to cyclophilins (a family of cytoplasmic proteins) [128], and thereby inactivate calcineurin; a key intracellular protein phosphatase critical for cytokine gene transcription dependent T-cell activation and proliferation [128, 129]. As calcineurin inhibitors (CNIs) have significant inter- and intra-individual absorption variability, dosing needs to be titrated according to drug levels [130–132]. Maintaining CNI levels within a specified range helps to achieve acceptable immune suppression without excessive

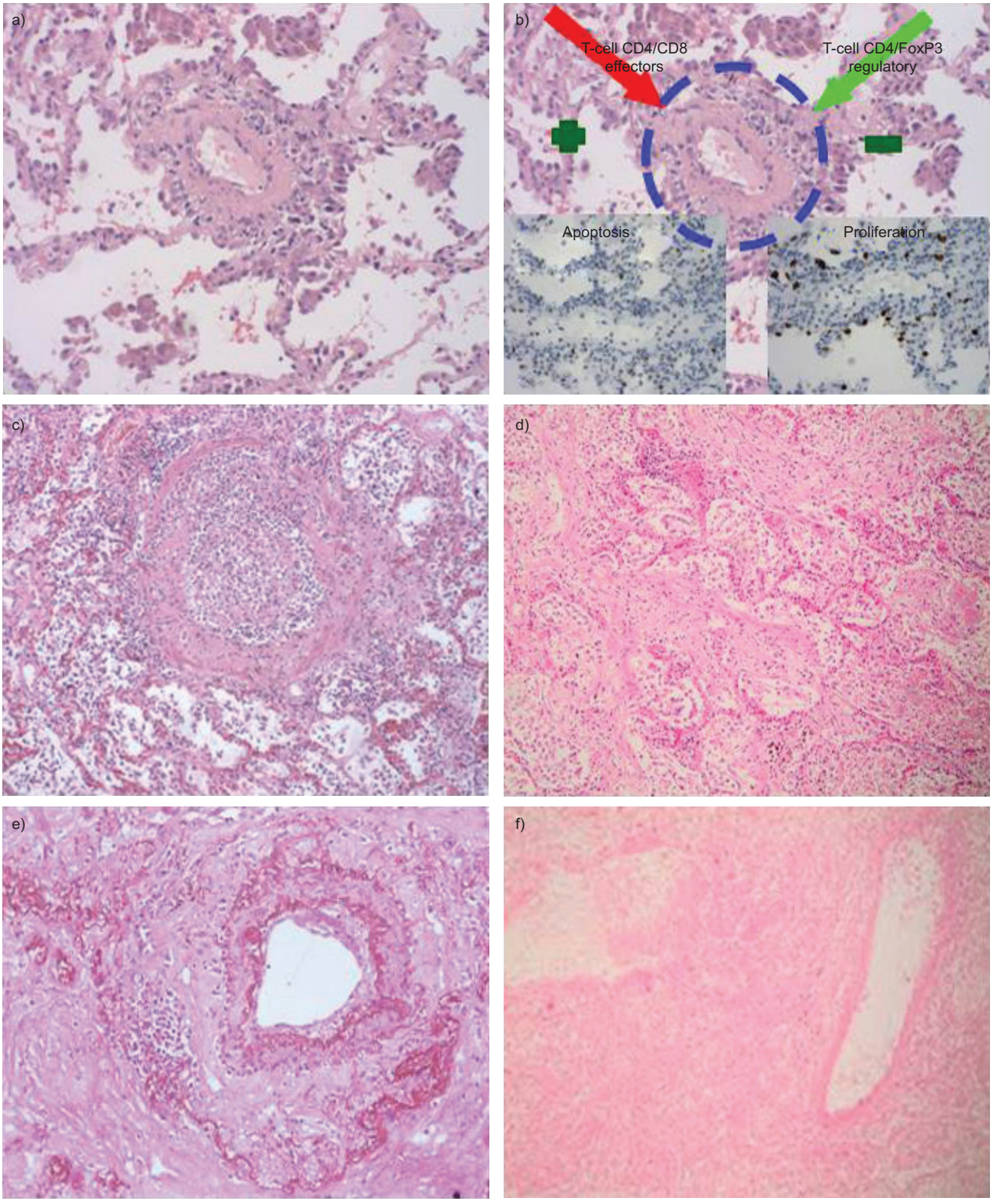

FIGURE 2. Histopathological phenotypes in the lung allograft. a, b) Vascular phenotypes, c, d) bronchiolar phenotypes, e, f) interstitial phenotypes. a) A2 cellular rejection, b) A2 plus immunohistochemistry for apoptotic (caspase 3) and proliferation (Ki67) markers, c) B-lymphocytic infiltrate, d) bronchiolitis obliterans organising pneumonia, e) obliterative bronchiolitis (OB), and f) OB with chronic vascular rejection/fibrosis. Other histopathological phenotypes not shown include: chronic, low grade pneumonitis; endothelialitis with/without complement staining; and fibrinoid organising pneumonia. In (b) the peri-vascular lymphocytic infiltrate is schematically shown to consist of a combination of CD4/8 T-effectors and CD4/FoxP3 T-regulatory cells.

toxicity and aides in the management of cytochrome P450 metabolism-mediated drug interactions in individual LTR.

According to the most recent ISHLT registry, tacrolimus is now the most common CNI being used by LTR (slightly more effective immunosuppression profile and a different side-effect profile) [4, 133, 134]. Over the years there has been much refinement in the dosing of both CyA and tacrolimus according to pharmacokinetic parameters [135]. This has been a necessary first step to better understanding the immune-related and off-target pharmacodynamic effects of these drugs. For both these CNIs, the most common serious adverse reaction is nephrotoxicity which may be acutely reversible or chronically progressive [136–138]. Indeed, chronic renal impairment and end-stage renal failure are now increasing problems in many long LTR survivors [132, 136]. Other severe but less frequent CNI side-effects include neurotoxicity syndromes and thrombotic thrombocytopenic purpura/haemolytic uraemic syndrome [139, 140]. Less severe but much more common side-effects include muscle dysfunction, hypertension, hypercholesterolemia, gingival hyperplasia and hirsutism [130, 141].

CNI dosing adjustment according to a pharmacokinetic target range is only partly predictive of pharmacodynamic effects (either allograft rejection syndromes or specific toxicities). The development of improved diagnostic assays for both alloreactivity and infection in the future is the critical next step to allow further titration of these drugs according to true pharmacokinetic/pharmacodynamic principles thereby maximising allograft specific immunosuppression whilst infection risk and organ toxicities are minimised across all time periods and clinical contexts.

Clinical protocols: maintenance versus *augmented immunosuppression*

The complexity of the immuno-inflammatory host response post-transplantation has led to the combination of several immunosuppressive agents with different therapeutic and toxicity profiles so that a ''net'' therapeutic index could be maximised. As already discussed, this led to the relatively standard triple agent immunosuppression regimen for LTR which has been modified over the years as evidence of improved efficacy or the need to reduce side-effects for specific agents has emerged [138, 141–143].

Steroids inhibit both humoral and cell-mediated immunity primarily by turning off gene transcription of multiple immune inflammatory genes [144]. These drugs are highly bioavailable and dosage is weight based but no drug level monitoring is available. Toxicities include well known acute and chronic gastrointestinal, metabolic and cardiovascular toxicity [4, 144]. Given the increasing appreciation of the chronic toxicity problem of corticosteroids, maintenance doses are usually reduced to baseline physiological levels within 6–12 h if possible. Paradoxically however, if a significant acute allograft rejection syndrome is suspected and no significant infection is present then pulse treatment with high dose methylprednisolone given over 3 days is one of the most effective ways to regain control.

Nucleotide blocking antimetabolites and agents such as azathioprine (AZA) and mycophenolate mofetil (MMF) usually complement CNIs and steroids in the standard triple regimen of immunosuppression routinely used in LTR. MMF is a powerful broad-spectrum immunosuppressant that inhibits T- and B cell proliferation [145]. As the synthesised form of mycophenolic acid, MMF has improved oral bioavailability [146] but drug monitoring is not routinely available. MMF dosing generally varies between 250 mg twice daily up to 1,000 mg twice daily being largely driven by the rejection/infection combined risk/benefit ratio as well as the common toxicity profile of this drug which includes gastrointestinal and haematologic side-effects [145, 146]. AZA is generally better tolerated than MMF especially at higher doses. Although it was initially thought that MMF was better at preventing acute rejection thereby making its increased toxicity profile acceptable, a more recent large RCT did not definitively confirm this [147].

As well as the changes to the first line immunosuppressants that have occurred over the last 5 yrs, the use of induction agents, such as the interleukin (IL)-2 receptor antagonist basiliximab and the mammalian target of rapamycin (mTOR inhibitors), sirolimus and everolimus are is now common [4, 142, 148]. The use of these agents is most commonly as CNI sparing agents (especially in the setting of nephrotoxicity risk) but they may also be used to combat an elevated risk of allograft rejection in specific settings (*e.g.* IL-2 receptor antagonists for high panel reactive antibodies (PRAs) in the immediate post-operative period and the mTOR inhibitors for recurrent/progressive late rejection). Sirolimus and everolimus exert their immunosuppressive effect by binding to intracellular immunophilin proteins (FKBP12) and blocking mTOR-associated cytokine mediated proliferation of T- and B cells resulting in apoptotic cell death [149, 150]. This anti-proliferative effect can also interfere with endothelial cell, fibroblast and vascular smooth muscle cell proliferation. Therefore, these drugs are generally not used in the first 3 months post-transplantation as they can potentially inhibit adequate healing following surgery with disastrous consequences [149, 151]. Variable bioavailability, the potential for drug–drug interactions and a relatively low therapeutic index (major toxicities include bone marrow suppression, gastrointestinal and pulmonary toxicity, including FOP), makes drug monitoring for these medications essential [151, 152]. However, even with their increased usage and blood level monitoring, the pharmacokinetics of the mTORs is not, as yet, as well understood as for the CNIs. In addition, given their broad anti-proliferative potential it is not clear whether there is a clear pharmacodynamic effect between immune cell suppression and non-immune cell regulated proliferation/healing for the lung allograft, and, if so, whether it is context dependent.

Although the relatively broad and aggressive triple immunosuppression regimen and its variations that are currently routinely used post-LTx have significantly reduced acute rejection syndromes, only a minimal impact has been made on long-term lung allograft function outcomes as has been previously discussed. This is an interesting paradox and suggests several possibilities which are not necessarily mutually exclusive. First, it is possible that despite reducing histopathological acute rejection rates, as diagnosed during surveillance bronchoscopies, undiagnosed subclinical alloreactivity persists to a degree that chronic rejection outcomes remain unabated. Secondly, current immunosuppression regimens may inhibit destructive alloreactive immune processes as much as operational tolerance and so over a longer time period there is little ''net'' gain, particularly if the destabilising effect of episodic infection is also taken into account. Finally, it is possible that the downstream effects of either

episodic or persistent sub-clinical alloreactivity or infection are greatly magnified by allo-related and/or immunosuppression-related dysregulated healing/repair mechanisms.

Individual patient management issues

All LTR more or less start with a relatively standard triple immunosuppression regimen which very quickly begins to be individualised according to routine clinical parameters relating, particularly, to rejection risk (PRAs, B and T-cell cross-match results, donor-specific antibodies), infection risk (especially primary donor sero-positive/recipient sero-negative mismatch (D+/R-) CMV/EBV mismatches and airway bacterial/fungal infections) and renal status. The use of induction therapy, early CNI target levels and the use of MMF rather than AZA are all dependent on this early assessment. From then on, there is a continuous evaluation of immunosuppression-related benefit/risk framework with changes being ideally made in parallel and in proportion to a changing risk context. The development of humoral rejection would, therefore, be managed by escalating therapy from increasing baseline immunosuppression to pulse methylprednisolone to courses of *i.v.* rituximab and/or plasmaphoresis and/or *i.v.* intragam as required, providing no significant infection was present [153, 154].

Although the availability of better immunosuppressive drug monitoring coupled with very sensitive, accurate and responsive diagnostic immune and toxicity assays would substantially improve immunosuppression-related risk management in LTR, it remains unlikely that these risks can be completely eliminated. Therefore, it follows that thoughtful, information-weighted and value-driven decision making will always be required to manage the intrinsic risk trade-off between alloreactivity and infection/toxicity that will never be able to be completely resolved with the use of these more specific but nevertheless still ''blunt'' immunosuppressant agents. These trade-offs are particularly relevant in the LTR who routinely receives a non-HLA matched donor organ which is necessarily fully exposed to aero-environmental irritants, allergens and infections [155, 156] and will, therefore, require a vigilant and indefinite review of immunosuppression strategy.

Given the above framework for potential pathways to ''chronic rejection'' it is perhaps not surprising that treatment of most patterns of persistent/progressive loss of lung allograft dysfunction in LTR do not respond dramatically to pulse steroids, an increase in baseline immunosuppression or even a course of antithymocyte globulin therapy. The proviso here of course is that acute cellular and/or humoral rejection have been excluded and as this is often difficult to do; a trial of increased immunosuppression is perhaps always warranted. It then becomes a self-fulfilling fact that the LTR that do not respond to an empirical trial of augmented immunosuppression have treatment resistant chronic rejection and those that stabilise have partially responsive disease. In either case, any further empirical increases in immunosuppression would have to be carefully weighed against the potential for greatly increasing infection-related problems, therefore accelerating the demise of the patient.

In the setting of established BOS, net immunosuppression should be reduced to minimise the possibility of further lung complications from either acute or chronic infection. The use of the immuno-modulating, non-immunosuppressing, antimicrobial agent azithromycin would also be reasonable in this setting [157–160]. However, the benefits of using azithromycin in both established BOS and/or even earlier remains to be confirmed in a large prospective RCT (the impetus for which would increase should this drug be associated with significant long-term toxicities).

Finally, although many newer agents are being developed with variable therapeutic indices (to decrease allograft rejection with minimal toxicity), perhaps the major hope lies with new biological agents and small molecule inhibitors that are strategically given at the time of transplantation to help promote long lasting operational tolerance [161, 162].

Are antimicrobial prophylaxis/treatment strategies adequate and where are the gaps?

The risks posed by ubiquitous DNA viruses that can reactivate from the latent state and common respiratory pathogens (viruses, bacteria and fungi) are ever present for the LTR. The reasons for this include: the HLA mismatched nature of the lung allograft, the broad spectrum of immunosuppression used to prevent both acute and chronic allograft rejection and the constant exposure of the lung allograft to the external environment, all of which make it very easy for the transplanted lung to become infected, for infection to progress and for antimicrobial treatment approaches to be relatively compromised.

Infection in the LTR can lead to clinically obvious septic syndromes that have direct and dramatic effects on the allograft or more subtle impacts associated with persistent, sub-clinical infection. These latter indirect effects may include breaking operational tolerance, contributing to low-grade airway damage and impairing well-regulated repair processes. The importance of preventing acute episodes of symptomatic infection as much as possible, and using treatment strategies that are quick and effective should they occur, is without question. What about low-grade persistent viral reactivation and/or airway infection? How should they be managed? Where does one draw the line between treatment or not? And when do the adverse side-effects of any antimicrobial intervention approach (whether it be prophylaxis or treatment) outweigh the perceived benefits? Also, how should we manage immunosuppressive strategies in individual case scenarios? Although these are difficult questions, it is clear that better diagnostic tools and therapeutic options for viral, bacterial and fungal infections in LTR being systematically applied to well phenotyped study cohorts with adequate longitudinal follow-up will provide the platform for future improvements in management. Finally, although annual influenza vaccine is recommended for all LTR unless there are contraindications, how should we think about the pros/cons of newer vaccine options being developed? All of the above is perhaps best exemplified by the transformation in CMV diagnostics and management over the last decade.

Viral infections

Although reactivating DNA viruses, such as CMV, have always been a well-recognised problem in LTR, the acute and chronic impact of episodic respiratory RNA viruses and adenovirus on the lung allograft are being increasingly appreciated as diagnostic testing for these viruses has become available and is more routinely used.

DNA viruses: CMV *versus* others

In the early days of lung transplantation, severe CMV disease, particularly in the form of CMV pneumonitis, was a major cause of early morbidity and mortality [163–166]. However, with the use of more tailored immunosuppression protocols, the increased availability of antiviral agents for prophylaxis and treatment protocols and the development of better molecular-based diagnostic tools to help guide pre-emptive intervention strategies, the rates of clinical CMV disease syndromes (including CMV pneumonitis) have reduced dramatically and the focus is now on how to best understand and therefore manage subclinical CMV reactivation [167–174].

Although the possibility of sub-clinical CMV reactivation is not difficult to conceptualise as ''filling the gap'' between a truly latent state and clinical disease, it was only after the development of quantitative molecular PCR-based assays for CMV and their systematic application in the peripheral blood and bronchoalveolar lavage of LTR study cohorts that the concept really took hold [170, 171, 175]. In addition, the sensitivity, quantitative power and dynamic range of these assays allowed for the early detection of CMV, viral load monitoring and assessment of treatment response [170, 171, 176].

The concept of sub-clinical reactivation raised two further issues related to CMV reactivation pathobiology. First, definitions for the CMV infection, sub-clinical reactivation and disease (including tissue invasive disease diagnosed by characteristic CMV inclusions on histopathology) had to be standardised and internationally accepted [177]. Secondly, the variable reactivation dynamics of CMV suggested that CMV specific immunity was variably able to control reactivation. Various measures of CMV-specific immunity are now an active area of research [178–181] and may further help to risk stratify patients thereby allowing for even further individualisation of antiviral and immunosuppression treatment approaches.

The current state-of-the art regarding CMV management in LTR combines a universal prophylaxis protocol for usually 3–6 months post-transplant coupled with protocols for routine diagnostic testing for CMV that can then guide pre-emptive intervention strategies, with full treatment protocols being reserved for break-through clinical disease [169, 172, 182, 183]. Although this extended approach has had the overall benefit of reducing CMV events in LTR, late CMV can still occur [172, 173, 184, 185] thereby raising the possibility that long-term prophylaxis may have additional benefits [183, 185, 186]. However, longer use of antiviral drugs will need to be balanced against the potential for increased toxicity, antiviral resistance and increased costs. These considerations are perhaps even more important if long-term prophylaxis is used to reduce the rate of BOS development in LTR. Although recent studies have shown that there is an association between sub-clinical CMV reactivation and BOS even in the modern era [186], a cause–effect understanding of this association can only occur through properly conducted randomised trials which would also be able to address a risk/benefit analysis of long-term antiviral prophylaxis for CMV.

Intravenous ganciclovir and oral valganciclovir are usually used in various combinations for both prophylaxis and treatment of CMV according to the risk of disease or its severity, respectively. For example, treatment of CMV is usually with *i.v.* ganciclovir but oral valganciclovir is often used for minor reactivation episodes, especially if there is also reduction of immunosuppression and viral load monitoring is ongoing. Anti-viral side-effects for both agents include neutropenia and prolonged use may predispose to anti-viral resistance (especially if high viral loads are present) which is difficult to treat [184, 187]. An exciting recent development is the possibility of a vaccine for CMV to help protect the particularly ''at risk'' sero-negative recipient [188–190].

Like CMV, other herpes viruses, such as human herpes virus 6 and 7, EBV, herpes simplex virus and varicella zoster virus, can also establish latency after primary infection and therefore reactivate with immunosuppression although the site, frequency and severity of this reactivation is very variable. Importantly, this reactivation variability is very dependent on the source cell for latency, the specific signals for reactivation and the anti-viral immunity thresholds required for controlling each of these viruses. Again, as for CMV, although excellent anti-viral agents currently exist, their optimal use is dependent on the development and systematic application of better diagnostic tools which would then enable an improved understanding of sub-clinical reactivation for each of these viruses and their association with various clinical syndromes [191–196]. This will be discussed in more detail for EBV (the second most problematic herpes virus for LTR) in relation to post-transplant lymphoproliferative disease (PTLD) [197–200]. Importantly, sub-clinical EBV reactivation may also specifically influence lung allograft outcomes through non-lytic virus effects [201, 202], stimulating an activated immuno-inflammatory phenotype [203] and the potential for generating cross-reactive antiviral memory T-cells [204]. Finally, these concepts are perhaps also important for non-herpes viridae DNA viruses such as human papilloma viruses, hepatitis B and C viruses and parvoviruses, all of which may cause significant problems in individual LTR [205, 206].

RNA viruses: influenza *versus* others

Community acquired respiratory viral infections are very common and so perhaps it is no surprise that the more routine application of molecular-based assay systems are increasingly diagnosing the presence of these viruses in upper respiratory tract (URT) and/or lower respiratory tract infection syndromes in LTR [207–213].

On average, up to 30% of all respiratory viral syndromes are now positively diagnosed using more recent, multiplex PCR tests with picornaviruses generally being the most frequently identified [207–209]. Influenza and the paramxyoviridae (parainfluenza, respiratory syncytial virus (RSV) and meta-pneumovirus) are not infrequently isolated and are particularly important as there are specific treatments available for these viruses [214]. It remains unclear, however, why in some cases the same virus may remain localised to the URT whereas in others it may quickly spread to the lower respiratory tract. Equally unclear is why the same virus in the lower respiratory tract of some LTR is associated with minimal symptoms whilst in other cases there is a rapid progression to severe infection, allograft injury and ultimately irreversible lung allograft dysfunction in the form of non-treatment responsive organising pneumonia (*e.g.* BOOP or FOP) [213–216]. Interestingly,

both BOOP and FOP have been previously described in the setting of acute community acquired pneumonia syndromes and are perhaps related to infectious pathogens [217, 218] in the setting of impaired immunity and dysregulated repair; both of which are more common in LTR.

Prevention is the best method of managing the potential problems from community viral infections in LTR and this involves strictly avoiding sick contacts/isolation procedures, appropriate hand hygiene and virus-specific precautions such as yearly influenza vaccinations [219–222]. The importance of community viral infections post-transplant and their varying presentations require a vigilant approach regarding risk stratification and diagnostic/management decision including the commencement of early treatment protocols for influenza (oseltamavir and zanamavir being the only current readily available antivirals in an outpatient setting) even whilst waiting for confirmatory test results. This is especially true during outbreaks, epidemics and pandemics such as the relatively recent H1N1/09 influenza pandemic where the pre-test probability of a positive test result in a specific clinical scenario is relatively high [219, 223, 224]. Effective treatment protocols based on parenteral or nebulised ribavarin are available for RSV and parainfluenza, although, in general, these are more complicated to administer [225, 226]. Unfortunately, therapies for picornaviruses are yet to become a clinical reality although research efforts in this area are continuing [227, 228].

HIV

Uniquely, HIV is an RNA virus that depends on a reverse transcriptase to synthesis its DNA form. Its preponderance for infecting CD4 T-cells, thereby leading to their depletion, is the major mechanism by which it causes severe immunodeficiency syndromes if unchecked. Over the last few years HIV infection has complicated a small number of immunosuppressed solid organ transplant recipients for various ''unplanned'' reasons. Of importance here is that nucleic acid testing for HIV in ''at risk'' donors can only reduce the negative window between HIV exposure and a positive test result to about 3 days and, therefore, can never eliminate all risks associated with using organs from such donors. Interestingly, the results of HIV in these cases are surprisingly not disastrously bad in the setting of appropriate management and, although there may be a reporting bias at play, the relatively positive outcomes offer unique pathobiological insights into the potential interaction(s) between newly and/or previously acquired HIV infection and a suppressed immune system [229]. Building on these observations, in South Africa, where there is a high prevalence of HIV and renal failure in the setting of limited resources for healthcare, there is now an active programme of renal transplantation using HIV positive donors and recipients with short- and long-term outcomes that are acceptable for that population [230, 231].

Bacterial infections

Bacterial infections are responsible for a major component of infectious complications in immunosuppressed LTR both in the lung allograft and in non-allograft tissues. Although acute bacterial infections can occur at any time post-LTx there is a biphasic peak in incidence early in the post-operative period and again following the development of BOS later. Early infections may begin in the donor lung or in the native airway of the recipient and therefore may complicate the bronchial anastomosis as well as the pleural and/or mediastinal space [232–234].

During the immediate post-transplant period the risk of aspiration pneumonitis is particularly increased in the setting of vagal injury-associated delayed gastric emptying and gastro-oesophageal reflux disease (GORD) [235]. Similarly, gastrointestinal infection with *Clostridium difficile* and intravenous line infections in the setting of prolonged parenteral antibiotic use are also not uncommon early following transplantation [234, 236, 237]. Late infections in the lung allograft are often due to impaired airway defences either acutely in the setting of a viral infection or chronically in association with BOS [121, 238, 239]. In both cases, nosocomial bacterial pathogens such as MRSA and multi-resistant Gram-negative organisms including *Pseudomonas aeruginosa* can be particularly problematic and ongoing immunosuppression can significantly compromise antimicrobial treatment regimens [234, 239–241].

The persistence of specific bacterial pathogens such as *Staphylococcus aureus* and *P. aeruginosa* in the lower airways of the lung allograft may be either a marker or a cause of lung allograft dysfunction or both. This is particularly relevant in LTR who have CF and the upper airway is already colonised with bacteria (*e.g. S. aureus*, *P. aeruginosa*). Although the distinction has important implications for pathobiology, its practical management often distils down to appropriate antibiotic treatment courses initially with a view to eradicating the bacteria and, if this fails and a chronic biofilm infection develops, the aim switches to maintenance suppression with control of acute exacerbations.

Community acquired bacteria including pneumococcus and atypical bacteria are always a possibility in LTR and they should always be covered in any empirical treatment regimen for community acquired pneumonia syndromes. In particular, the sero-dependence of *Mycoplasma* and *Chlamydia pneumoniae* diagnosis is problematic in immunosuppressed LTR. Interestingly, anecdotally it seems that relatively few cases of pneumococcal pneumonia and/or bacteraemia are diagnosed using standard tests in any one centre. However, multicentre systematic reviews examining the incidence of pneumococcal bacteraemia in LTR clearly indicate otherwise [242, 243]. These results suggest either that some LTR are not vaccinated prior to transplantation or that their vaccine-associated immune protection post-transplant is inadequate. Currently, it is recommended that at risk patients with severe lung disease are vaccinated with the 23-valent polysaccharide pneumococcal vaccine. The role of the newer conjugate pneumococcal vaccines in LTR with a T-cell dependent antibody production profile remains to be elucidated, but they may be particularly beneficial in the older LTx candidate prior to transplantation.

When bacterial sepsis does occur in immunosuppressed LTR, it can be devastating both because of uncontrolled sepsis and distal sight seeding [233, 242, 244, 245]. Early use of appropriate antibiotic treatment is critical in this scenario as high mortality rates are associated with delayed antibiotics, inadequate dosage and resistant pathogens [246–248]. Finally, the last comment is particularly relevant for less commonly diagnosed bacterial respiratory pathogens such as *Legionella*, *Mycobacteria*, *Nocardia*

and *Burkholderia pseudomallei* (meliodosis), especially if the acquisition is travel associated [249, 250].

Fungal infections

Pulmonary fungal infections are both a major cause of short- and long-term morbidity and mortality in immunosuppressed LTR. Between 15% and 30% of LTR will acquire a fungal infection and the incidence of this is again biphasic with an early peak post-operatively and a late peak following BOS [251–254]. By far the most common infection is *Aspergillus* in the airways of the lung allograft causing various degrees of tracheobronchitis with or without bronchial anastomotic involvement and/or tissue invasion [255, 256]. The incidence of severe invasive disease has been reduced with the increasing use of prophylactic antifungals, but mortality remains high should it occur [253]. Systemic candidiasis that is either an intravenous line or gastrointestinal related is also associated with significant mortality [257].

Aspergillus is a widely distributed filamentous organism (with septate hyphae whose conidia can be inhaled easily). The LTR is therefore always at risk from inhaling the conidia of *Aspergillus* and there is evidence that this is more likely in the setting of building-associated earthwork and seasonal factors [258]. In addition, some patients such as those with CF may be chronically colonised with *Aspergillus* in their native airway. In these patients, prophylactic antifungals for at least the first 6 months post-transplant are very important to prevent significant anastomotic or chronic airway infection [252, 259]. Later acquisition of infection, particularly if it persists despite antifungal treatment, may be both a marker of BOS and a cause of progressive loss of allograft function. In both cases, the risk of locally invasive or disseminated disease dramatically increases in the setting of increased immunosuppression [259–261].

The detection of *Aspergillus* in the airways of LTR is very sensitive using regular bronchoscopic sampling and fungal culture assays, such that the negative predictive value of these tests is excellent. Once *Aspergillus* is detected in the airways, however, the specificity with which colonisation, tracheobronchitis and tissue invasive disease are diagnosed is relatively poor. Whether galactomannan levels in the bronchoalveolar lavage, serum or urine [262–264] or *Aspergillus* PCR load in the blood [265] will prove useful in answering these questions remains to be seen.

The current prophylactic and treatment antifungal drug of choice for *Aspergillus* is voriconazole which has proven efficacy but significant bioavailability, drug–drug interaction and toxicity issues making therapeutic drug monitoring very important and useful [266–273]. A prophylaxis strategy is recommended when there is a high pre-test probability of *Aspergillus* being present and potentially causing serious complications as in LTR with *Aspergillus* in the sputum just prior to transplant surgery. However, a pre-emptive treatment course is preferred when *Aspergillus* is first isolated in LTR airways. Finally, antifungal combination therapy (voriconazole+caspofungin or amphotericin) is reserved for the most severe invasive forms of the disease [253, 274–276]. In all cases, however, the benefits *versus* risks of ongoing antifungal therapy in LTR have to be continually assessed.

Unlike *Candida*, *Cryptococcus* is a yeast that has a propensity for lung infection and early neurological involvement [277, 278], particularly in immunosuppressed LTR. *Cryptococcal* antigen testing is useful in both compartments and can also be used to monitor the effectiveness of specific antifungal therapy [277–279].

Non-aspergillus moulds that may affect the lung or sinuses of LTR include the *Zygomycetes*, *Scedosporium* and *Fusaerium* [278, 280]. Clinical presentation can vary from colonisation to invasive infections. *Scedosporium apiospermum* and *S. prolificans* often require combination therapy using voriconazole and terbinafine, reductions in immunosuppression and occasionally debulking surgery if possible [252, 275]. With increasing overseas travel, the endemic mycoses may need to be considered in a wide range of differential diagnoses given their variable clinical presentation [281, 282]. An initial awareness of the relevant epidemiology is key, coupled with appropriate sero-testing and culture diagnostic methods for blood and tissue. An early diagnosis is a prerequisite for aggressive management to commence otherwise mortality is likely to be high [282].

What do the labels chronic rejection/BOS/CLAD tell us and not tell us?

Although lung transplantation is associated with significant improvements in short-term patient survival and QoL in those with severe lung disease amenable to this treatment option, long-term survival remains limited. In particular, 5-yr survival rates are currently 50–60% with most late deaths being directly or indirectly associated with progressive allograft dysfunction. But what is progressive lung allograft dysfunction? What drives it? and what are the likely consequences/treatment implications? Moreover, how do the diagnostic concepts of ''chronic rejection'', ''BOS'' and ''CLAD'' help us answer these questions?

Definition: pathobiology and risk factors

Chronic rejection in the lung allograft has been conceptualised as the development of histopathologically defined obliterative bronchiolitis on the basis of strong observational and experimental evidence [118–121]. However, the insensitivity of histological diagnosis has led to a syndromic diagnosis of bronchiolitis obliterans, otherwise known as BOS, defined as the ''irreversible loss of forced expiratory volume in 1 s as a percentage of the best achieved post-transplant that is otherwise unexplained'' [283]. Although this clinical syndrome approach has clearly been very useful over the last 25 years, it has its limitations as it summarily dismisses the possibilities that non-obstructive progressive lung allograft dysfunction (*e.g.* restrictive lung pathophysiology or fibrosing/organising pneumonia) may also be a manifestation of chronic rejection in some circumstances and that other factors such as acute and/or chronic airway infections in particular may be important contributors to this process (either directly by breaking operational tolerance or indirectly by causing airway damage that is not appropriately repaired). These specificity issues with the diagnosis of BOS has led to the adoption of the term CLAD as a catch-all diagnosis irrespective of the various causes/processes that may have led to it [107, 284–287]. As always in medicine, each diagnostic approach has pros and cons meaning that in the absence of ''the perfect test'' there is an unavoidable ''trade-off'' for each diagnostic label that needs to be factored into decision making. In this setting, the key point will always be ''how well'' does the diagnostic label

answer the specific question being asked by the clinician, the scientist or the epidemiologist.

The complex interplay between donor antigens, recipient immunity and environmental stimuli that leads to the pathophysiology of BOS is likely to vary in individual LTR, to be dynamic and be contextual. Despite the compelling evidence for allo-recognition, inflammation and adaptive immunity amplification being important, it remains unclear why individual LTR develop early aggressive disease and others maintain excellent allograft function for many years. Most research studies in this area tend to focus on LTR who develop BOS/CLAD post-transplantation, but it may be equally fruitful to systematically study in detail those LTR who maintain excellent lung allograft function in the long term, or who recover quickly and fully after LRT viral infections. Although variations in HLA mismatching, PGD development, immunosuppression thresholds, the frequency/severity of episodic infections and the development of high-affinity alloantibodies and autoantibodies [107, 108, 121, 283, 288, 289] may partly explain the aggressiveness of alloreactivity or the robustness of operational tolerance, the predictive power of these associations lacks specificity for outcomes in an individual LTR. Although this complexity renders many animal models for lung transplant-associated chronic rejection relatively insufficient, they nevertheless provide a platform, albeit limited, for our understanding of this condition [290]. For example, in animal transplant models, initial epithelial loss occurs in both syngeneic and allogeneic heterotopic tracheal transplant grafts, but only in the allogeneic setting does fibro-proliferation occur. It remains unclear, however, how dependent the observed fibro-proliferation is on lymphocytic infiltration and whether it is a marker of epithelial loss and dysregulated repair processes or the result of excessive fibroproliferative pathways that may be amenable to treatment [291].

The effect of HLA mismatching in the clinical setting where most patients receive an average mismatch of two to three HLA antigens is relatively difficult to ascertain. Even using large registry databases, there are relatively few patients who have a high number of HLA matches, the HLA mismatches are all different and CNI immune suppression has a blunting affect [292].

Although the main identifiable clinical risk factors for BOS in previous studies have been acute rejection, CMV infection and lymphocytic bronchiolitis, the majority of BOS cases remain ''unexplained''. For example, acute rejection as currently diagnosed generally explains <5–10% of BOS outcomes [116, 121]. This may, in part, relate to the relative insensitivity of the tests used to diagnose the identifiable risk factors. Recent developments have led to increased diagnostic sensitivity either by definition (*e.g.* minimal acute rejection [116]), or technically (*e.g.* donor-specific antibodies, either pre-existing or *de novo* [293, 294] and subclinical CMV reactivation in the lung allograft [186]), with parallel improvements in the association with BOS [100]. However, diagnostic sensitivity for measures of alloreactivity and their potential association with BOS need to improve further. Such improvements in the near future may include better phenotyping of histological acute cellular rejection (*e.g.* effector T-cell/Treg and/or endothelial/epithelial damage signals) and better functional donor-specific antibodies assessment (*e.g.* affinity and complement binding).

Over the last decade, non-alloimmune risk factors for BOS have been increasingly identified both as the result of increased awareness, improved diagnostic testing and longer BOS-free follow-up periods. Such factors include common community acquired respiratory infections, persistent bacterial and fungal airway infections (previously discussed) [207–209, 214, 215, 239, 295], aspiration episodes related to GORD [81, 296, 297] and baseline graft function (either as a function of severe PGD [81] or due to a single rather than a double lung transplant procedure being performed [4, 296]).

Finally, we speculate that the clinical finding that lymphocytic bronchiolitis often precedes BOS may be a summary signal which encapsulates many other risk factors and may be both a strong biomarker of, or causally related to, the bronchiolar epithelial injury and/or dysregulated repair that is likely to be at the core of BOS development [298].

Clinical patterns: diagnostic considerations

As previously discussed, the current limitation to long-term survival for LTR relates predominantly to the development of progressive loss of allograft function whether it is defined as BOS or CLAD [4, 283, 284]. Indeed, plotting lung function status over time for individual LTR identifies several clear patterns of lung function loss from chronically progressive at one end of the spectrum to a prolonged period of stability before a large step down in function, and everything in between regarding stepped decreases in lung function interspersed with varying periods of stability. Interestingly, where temporal associations with these downward inflexion points can be identified, they most usually relate to ''respiratory infection'' episodes, other aero-environmental insults or low immunosuppression status. It is speculated that these factors may aggravate destructive alloreactivity at the same time as breaking any operational tolerance that may be active. In this framework of multiple risk factors that can be variably measured and with many potential interaction effects spread over time driving lung allograft dysfunction, perhaps the wisest approach is to focus on developing biomarker, physiological and imaging tests that can detect meaningful airway injury at the earliest time possible. Ideally, such a test could then be used to more accurately characterise BOS development and thereby potentially improve risk factor identification either singly or in combination.

Management issues: prevention, treatment and management of established disease

The management strategies for chronic progressive lung allograft dysfunction and established BOS with and without infection have already been addressed in terms of diagnostic decision making and therapeutic options. However, the ultimate management aim of this condition is prevention.

Ideally, prevention would mean the attainment of a truly tolerant state for the lung allograft without any need for maintenance immunosuppression. Although this remains a future hope, more realistic in the short term is perhaps the achievement of more robust operational peripheral tolerance that can better withstand the destabilising influences of infection and other injuries, and improvements in detecting and selectively targeting subclinical alloreactivity syndromes that damage the allograft (fig. 3) [299].

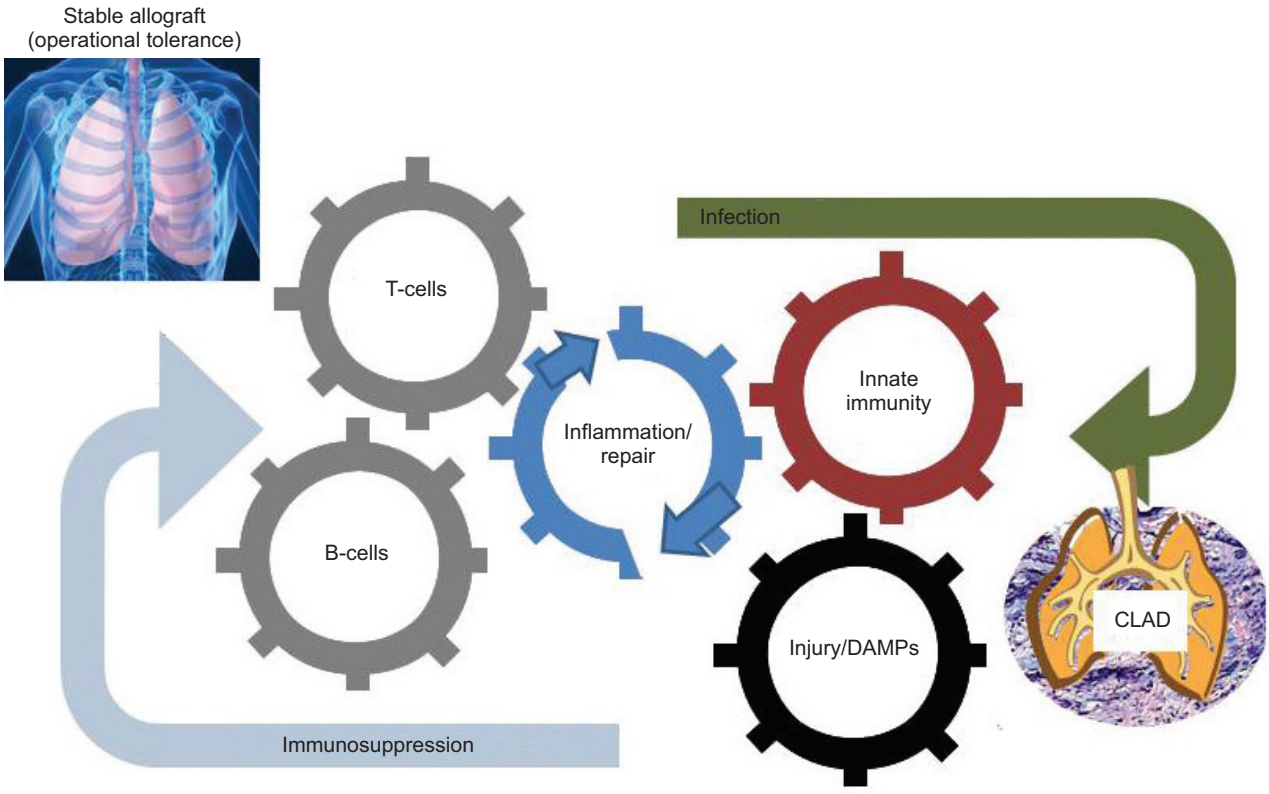

FIGURE 3. Conceptual schema for multi-dimensional, interacting, non-linear development of bronchiolitis obliterans syndrome/chronic lung allograft dysfunction (CLAD). DAMPs: damage-associated molecular patterns.

Finally, a more complete understanding of the mechanisms underlying the recent improvements in PGD outcomes and decreased BOS rates associated with DCD donors offers a potential opportunity to further modify the early transplant operation-associated factors that are critical for maximising the baseline integrity of the lung allograft and its initial set points regarding alloreactivity and operational tolerance. With this in mind, all the recent expansion in lung transplant activity options designed to increase the availability of this life giving procedure (fig. 1) needs to be continually balanced by the equally important need to improve long-term outcomes and QoL.

What is the impact of non-lung allograft disease on patient outcomes post-transplantation?

Although the management of allograft-related complications are an essential focus of post-transplant care, many non-allograft complications can still occur and cause significant morbidity and mortality. Non-allograft complications are particularly likely in older patients, severely ill patients that are also very deconditioned and malnourished, and in patients with severe end-stage lung disease due to a systemic process that can also variably affect other organ systems (*e.g.* CF).

Although LTx is now most commonly performed as a double lung procedure, the special situation of SLT can often be severely compromised by native lung complications such as dynamic hyperinflation (emphysema) or infectious complications (idiopathic pulmonary fibrosis). In addition, diaphragmatic impairment from either phrenic nerve injury or adhesions is particularly disastrous after a SLT. Finally, although rare, primary disease recurrence has been reported for sarcoidosis and lymphangioleiomyomatosis, most often as recurrent granulomatous disease in the allograft [300].

Post-transplant immunosuppression protocols might dramatically compound organ based pre-transplant comorbidities as well as contributing to the ever-present risk of drug–drug interactions and non-allograft infection and malignancy complications.

Lung allograft versus *comorbidities*

The awareness, timely detection and pro-active management of comorbidities and their complications are necessary to minimise their negative impact on post-transplant QoL and survival. Common comorbidities in older patients that can have a major impact post-LTx include cardiovascular disease, renal disease, osteoporosis and muscle wasting. This contrasts with the common pre-transplant comorbidities seen in CF patients (often younger) which include various gastrointestinal and nutritional disorders, liver disease, diabetes, osteoporosis and occasionally line-related chronic venous thrombosis problems. In both cases, immunosuppressants can aggravate pre-existing complications as well as leading to *de novo* organ toxicity problems.

By far the most important non-organ-specific complications post-LTx are immunosuppression-associated infections and malignancy. Allograft and non-allograft infections are a constant risk throughout the post-LTx period and have already been covered in detail. The prevalence of malignancy increases

with time post-transplant, from <5% to 10–15% and >20% in 1-, 5- and 10-yr survivors, respectively, with PTLD (especially in paediatric LTR and early post-transplant) and skin cancers (especially after 2 yrs and in those with prolonged sun exposure/drug-associated photosensitivity history) being the most common [4, 301]. PTLD is particularly problematic in the primary EBV D+/R- mismatch setting [302]. Although HLA matching status, level of immunosuppression and the absence of antiviral prophylaxis are also important risk factors in these patients, it is the routine use of EBV viral load monitoring that has the best predictive power for the development of this condition [197–199]. Other malignancy complications include all solid tumours, especially colon, breast and prostate, particularly in older LTR [4, 303]. Beyond the first year, malignancy accounts for ~10% of deaths [4, 301, 303].

In addition to LTR-associated medical comorbidities, there are also psychosocial and socioeconomic factors that can be very problematic. Although psychosocial problems can present difficult management issues in their own right, they can also indirectly influence patient outcomes by interfering with routine post-transplant management protocols. Pre-transplant multidisciplinary team assessment of potential LTR candidates is essential for screening patients for extreme psycho-behavioural issues. Nevertheless, there is an understandable bias towards avoiding the withholding of a potentially life giving procedure to young people with less than extreme problems in this area. Finally, for both medical and psychosocial comorbidities post-LTX, the large heterogeneity in LTR-associated conditions and severity status makes it almost impossible to conduct well-designed clinical trials to help guide difficult decision making in individual patients.

Clinical patterns of cumulative impact on key specific organs

Pre-existing renal disease and perioperative renal injury both significantly magnify the risk of post-transplant renal complications that can easily be exacerbated by even relatively minimal CNI toxicity [304–306]. If chronic renal impairment progresses to end-stage renal failure despite optimising nephro-protective management strategies, as much as possible, then the pros/cons of various dialysis options and even renal transplantation will need to be considered factoring in the total health status of the patient. In older LTR, cardiovascular complications are also common and include diastolic dysfunction, coronary artery disease, atrial fibrillation particularly in the perioperative transplant period and, later on, systemic hypertension and hyperlipidaemia [307]. Other significant complications that can be exacerbated by routine post-transplant medications include myelosuppression [308], glucose intolerance and diabetes [309], electrolyte disorders, gonadal dysfunction and osteoporosis [310–312].

Gastrointestinal disorders in LTR include GORD, which is relatively prevalent pre-transplant in many patient groups and has been linked to post-transplant lung function decline [235, 297, 313–319]. Although the observed association between GORD and CLAD is often difficult to separate in terms of cause and effect in later stages, if there is any evidence that GORD may be contributing to early loss of lung function, then appropriate medical and/or surgical therapies should be aggressively pursued [313, 320–323]. In this setting, BAL, pepsin or bile salts may be a useful biomarker especially in patients in whom there is a high pre-test probability of severe GORD, such as CF patients or any LTR with vagal nerve injury-associated decreased gastric emptying. CF patients are also at risk from distal intestinal tract obstruction, older LTR are at risk from colonic complications such as diverticulitis, and in both groups, prolonged antibiotic use is associated with *C. difficile* colitis which can be very severe and even life threatening [324–326].

Various acute and chronic neurological complications can occur in LTR, mostly in relation to CNI toxicity [139, 310, 326], that may or may not involve poorly measurable drug interactions with other neuro-active drugs and which are also dependent on liver metabolism, such as commonly used anti-anxiety and antidepressant medications. Although CNI toxicity may be difficult to diagnose, specific tests including computed tomography scan and magnetic resonance neuroimaging can be very helpful in excluding other potential causes. More frequent is CNI-associated peripheral muscle dysfunction and exercise limitation that has been described previously [327–329]. Although these findings were associated with a flurry of early research activity into the effects of CNIs on muscle and mitochondria [330–332], more recent research efforts have given us increasingly unique insights into this pathobiology [333]. Similarly, the high prevalence of osteopaenia and osteoporosis in patients with advanced lung disease (especially in those with low body mass index and chronic steroid use) and LTx was well recognised from the beginning. More recently, there has been an increasing interest in identifying different patterns of bone loss (as defined by bone densitometry) in the setting of post-transplant immunosuppression but ''adequate'' vitamin D, calcium and bisphosphonate therapy and then relating this information to overall bone strength and the development of fractures [311, 334–336].

Management issues: prevention versus *treatment*

The management of specific organ-based comorbidities in LTR necessarily involves both a pre and post- transplant focus. As already discussed, there are several absolute and relative contraindications related to the presence and severity of comorbidities for recipient selection. Once an LTx candidate is accepted on the waiting list there is a need to actively manage any potentially important comorbidities, as much as possible, whilst still maintaining a QoL perspective. In the extreme case, where the possibility of LTx is becoming increasingly unlikely, then a palliative care focus should increasingly dominate all management decisions including those related to comorbidities. However, once LTx occurs, pro-active management decisions to minimise the impact of comorbidities as much as possible is critical. This includes careful and individual titration of immunosuppressive and other drugs post-transplant, active risk factor and specific disease management of all comorbidities, and optimisation of psychosocial health and QoL issues.

Infection and malignancy are the major non-organ based comorbidities in LTR and both have specific pre-transplant and post-transplant risk profiles that are important for decision making and management. Management issues related to infection have already been discussed in a previous section. Current candidate selection guidelines consider malignancy within 2 yrs an absolute contradiction to lung transplantation and also recommend excluding patients with malignancy within 5 yrs of being assessed [23, 337]. Although there are

no routine screening guidelines for cancer post-transplant, ongoing awareness and vigilance is always required, especially in ''at risk'' patients with higher dose immunosuppression. Importantly, PTLD is the commonest cause of malignancy-associated morbidity and mortality early post-LTx whereas skin cancers dominate malignancy complications later on [4].

PTLD is predominantly EBV driven [197] with mortality varying according to age, extent of disease, response to initial treatment and the development of other complications [338–340]. Although histological diagnosis is essential for early treatment to commence [5, 15, 33], viral quantitative PCR assays are very helpful in predicting disease, guiding pre-emptive intervention (*e.g.* reduction in immunosuppression and antiviral therapy) and monitoring of disease risk, at least initially [341–343]. Although EBV virus-driven proliferation is a key initial driver in the development of PTLD, the failure of antiviral therapy (ganciclovir/valganciclovir) to treat this condition often necessitates further reductions in immunosuppression as well various other treatment strategies including intragam and rituximab, with more conventional combination chemotherapy approaches being reserved for refractory disease [338]. Skin malignancies are usually squamous cell carcinomas with poor prognosis being associated with older age, scalp site disease, extracutaneous spread and multiple tumours, as well as poorly differentiated histology, increasing tumour thickness and tissue invasion [344, 345]. Preventative management focuses on minimising immunosuppression doses and reducing the modifiable risk factors of current sun exposure and photosensitising drugs (*e.g.* voriconazole in at risk patients) recognising that previous exposures and latent infection with some DNA viruses (*e.g.* papillomaviruses) are no longer modifiable [346, 347]. Local surgery is the mainstay of treatment with increasing reliance on superficial ablative therapy, cryotherapy and photodynamic therapy to control both disease and at risk skin fields.

How do we measure QoL post-LTx?

The early focus of research post-transplant necessarily focused on establishing the surgical and technical feasibility of the transplant procedure, monitoring lung allograft function and getting the immunosuppression balance right in terms of preventing allograft rejection syndromes without excessive infection complications. Although achieving acceptable survival outcomes in both the short and long term was always a ''measurement'' priority, this was not necessarily the case for QoL after a lung transplant.

Measuring QoL

Health-related QoL (HRQoL) assessments after LTx are predominantly dependent on dyspnoea symptoms, exercise tolerance, medication burden and comorbidities which are only partly captured by lung function numbers and blood test results [348–351]. Although HRQoL is largely a function of an individual's sense of satisfaction and happiness related to their physical and psychological health, QoL can be more broadly defined as a complex interaction between a person's physical health, psychological state and personal beliefs, level of independence, social relationships and their relationship to their specific environment [352].

Clinical outcomes and HRQoL: BOS versus comorbidities versus others

Several longitudinal studies have shown that lung transplantation improves QoL post-transplant for many patients [353–358]. Anecdotally, this is not surprising given the precarious prognosis and QoL status of patients awaiting LTx almost by definition and the injection of hope and improved functional status that usually occurs once the operative ''hurdle'' of a lung transplant procedure has been passed. ''Usually'', however, does not mean ''always'' and neither does it mean ''forever''. In individual LTR, severe early allograft dysfunction or early loss of allograft function can occur either related to infection or BOS which, in combination with a chronic burden of complications post-transplant and/or a negative psychological state where expectations have not been met (whether or not they have been clearly stated), has major HRQoL implications.

BOS is the leading cause of mortality beyond the first transplant year [4] and the early development of this condition significantly reduces HRQoL [359]. Although, post-transplant HRQoL is very BOS dependent, other factors that may significantly compound problems with exertional dyspnoea and exercise intolerance are: peripheral muscle weakness; decreased mobility; treatment-related side-effects; anxiety/depression and the psychological effects of being diagnosed with a potentially life threatening illness, especially after having gone through the roller-coaster ride of hope for a better future with an LTx. However, a well-functioning graft post-transplant in combination with relatively minimal symptoms from comorbidities is likely to minimise the burden of post-transplant medication and lead to a more positive view of the future. What is clear here is that post-transplant HRQoL is dependent on key factors that act across multiple dimensions, are time dependent and may either compound or buffer each other.

It is therefore not surprising that several studies have shown differences in pre-transplant functionality and QoL status of LTR which contribute to post-transplant QoL [354, 360–363]. In particular, CF patients often have higher QoL measures when compared to other disease sub-groups perhaps due to the generally younger age at which they are transplanted, their life-long disease duration and potentially more finely tuned coping strategies [361, 362–364]. For all patients on the lung transplant waiting list, however, imminent death pre-transplant and the chance of survival post-transplant are likely to positively influence most measures of post-transplant QoL.

Managing the ''whole'' patient: are expectations being met post-transplantation?

As HRQoL in LTR is largely a function of physical wellbeing interacting with psychosocial health [358, 364], the cumulative impact of lung allograft dysfunction, severe comorbidities, significant anxiety/depression, other psychosocial issues and socioeconomic problems can quickly become overwhelming [362, 365, 366]. Psychological support, counselling and social work services (focusing on both pre and post-transplant issues coupled with appropriate pharmacologic treatment as required) are, therefore, an essential component of the multidisciplinary care of all LTR. As well as a focus on coping strategy improvement, the management of expectations post-LTx is equally important. For some patients, a wide pendulum swing between the ''hope'' of a completely new start post-transplantation and

the reality over time of another chronic disease state that requires constant management is very difficult to deal with and may lead to subsequent behavioural and psychosocial disturbances that can significantly impact on clinical outcomes thereby further exacerbating the problem and, of course, HRQoL. This may be the case in less mature individuals and paediatric LTR.

The complex risk/benefit calculation to help determine the potential prognostic and QoL benefits of LTx for any individual with progressive end-stage lung disease usually distils down to a question of ''when'' rather than ''if'' it is best to go onto the lung transplant waiting list. The overall improvement in HRQoL in late survivors post-transplant is therefore not surprising given the alternative, but QoL and mode of death are clearly also important in the non-survivors.

It is therefore not unreasonable to develop an approach to determine maximal net lung transplant benefit as this would guide more precise informed consent regarding lung transplantation and allow better targeting of early intervention strategies as appropriate in at risk individual LTR. Finally, such an approach may even allow for families, communities and society at large to also begin to more actively engage in the difficult questions relating to who should/shouldn't be considered for a lung transplant in the setting of limited healthcare resources.

PROSPECTS: FUTURE RESEARCH DIRECTIONS

Where do we need to go and how do we get there?

It is sobering that despite recent major advances in fundamental immunology, the goal of achieving consistent long-term lung allograft function following LTx remains frustratingly elusive. Given that the orthodox mechanism of allograft rejection, the mode of action of immune suppressive drugs and the many facets of infection in LTR have been already discussed, in this section we consider in more detail recent scientific insights and alternative mechanisms that might contribute to PGD, acute and chronic graft rejection, the inter-relationships between these conditions, and how such interactions may relate to new concepts in immune modulation and therapy. These advances, while still awaiting clinical translation into better graft outcomes, may also provide new insights into less invasive monitoring of rejection with biomarkers and into stem cell and regenerative strategies for growing new lung tissue. Additionally, we consider novel approaches to minimise immune suppression-associated lung infection risk and to reduce iatrogenic damage to non-immune organs such as skeletal muscle and bone, where loss of function directly contributes to patient immobility, morbidity and loss of QoL.

PGD and its relationship to alloreactivity and tolerance

PGD has been already discussed in detail. In brief, it is characterised by infiltration, oedema and severe hypoxia of the newly implanted lung allograft which reflects the aggregate of damage to the lung sustained during recovery, processing, surgery, reperfusion, haemodynamic instability and mechanical ventilation with or without further complications from pneumonia.

The inter-relationship between PGD, alloreactivity, tolerance, and acute and chronic rejection/BOS is an area of enduring and unresolved dispute. Although there is evidence linking early PGD to later allograft rejection syndromes the association is by no means simple and there is, to date, no convincing predictive algorithm to guide therapy.

Importantly, screening methods have reduced the incidence of hyper acute reaction, (where there is a pre-existing serological incompatibility that causes hyper acute antibody mediated lung damage). Similarly, harvesting improvements, such as very recently improved portable normothermic perfusion devices, have demonstrated superior outcomes in small limited pilot studies [367]. But it remains almost impossible to recover human lungs for transplantation without the organ sustaining some form of acute injury. PGD occurring within 72 h of transplantation remains relatively common with an incidence between 10% and 25% and the more severe grades are still associated with relatively high 30-day mortality rates despite advances in management [368].

Injury, innate immunity and alloreactivity

Experimental systems have provided important clues into the mechanisms that may lead to PGD but because of the diversity of injury types the clinical syndrome remains very hard to study mechanistically in humans. One area that is likely to be of major importance is innate immunity. Soon after the first innate immune Toll-like Receptor (TLR), TLR4, was discovered to be the Gram-negative bacterial endotoxin receptor, its close homologues were rapidly identified by homology screening and the family was recognised as innate immunity pattern recognition receptors that had evolved and diversified to detect distinct pathogen-associated molecular patterns (PAMPs). Almost immediately it was also discovered that TLRs were able to also respond to some self-proteins. This is especially true when the self-proteins are from immune privileged, mostly intracellular, sources normally hidden to adaptive immunity surveillance. Thus, the TLR receptors emerged as critical sensors not only of pathogens but also damaged tissue.

In experimental models it is now well established that the types of acute tissue damage known to occur in PGD can release intracellular cell contents collectively termed damage-associated molecular patterns (DAMPs) from injured cells [369]. These DAMPS are linked to the same innate immune effector pathways, such as TLRs, that mediate acute inflammation and prime the adaptive immune system. These receptors are coupled to their effector pathways *via* intermediates including MyD88 and IRF3 which recruit distinct cellular responses and inflammation. Accordingly, DAMPs are of interest both as potential mediators and biomarkers. Subsequently, additional damage and cellular stress sensing intracellular machinery has been discovered, such as the unfolded protein response system and the hypoxia response system [370, 371], important not only as candidate biomarkers but also as probable effector pathways in acute and chronic rejection.

Elevated levels of one such DAMP, RAGE (receptor for advanced glycosylation end products), is an epithelial injury marker whose measurement in donor lung bronchoalveolar lavage and recipient blood has been linked to PGD risk [372, 373]. Similarly, the level of long pentraxin 3 (a TLR agonist implicated in reperfusion injury) and endothelin-1 expression in both donors and recipients predicts PGD [374, 375]. Other researchers, noting the complexity of injury and the diversity

of material released from damaged cell and tissue responses to injury, have focused on gene transcript patterns or clusters of protein biomarkers [376]. While these DAMP biomarkers show promise as both diagnostic/prognostic biomarkers and treatment target none has been clinically validated to date.

DAMPs can also constitute neo-antigens because most are normally hidden from the host immune system inside cells. As well as validated antigen targets like collagen V, the intracellular DAMP content released on necrotic cell death, especially where innate signalling molecules including activin A are strongly activated, may trigger the formation of reactive T-cells and autoantibodies [377, 378]. Given that PGD can stimulate innate immunity which primes adaptive immunity, thereby shaping adaptive immunity-mediated T- and B cell effector pathways, it is perhaps not surprising that panels of acutely altered intracellular protein biomarkers correspond well to patterns of autoantibodies observed in chronic graft rejects [376]. In addition, recent reports have shown that specific autoantibodies are important risk factors for the development of BOS and CLAD [92].

However, activation of innate immune effector pathways is not a uniform pathway to inflammation and injury. Depending on the nature and degree of injury, concurrent mechanisms linked to immune tolerance and resolution can also be activated by events linked to causing PGD. ''Tolerance'' is a functional descriptor for the set of control mechanisms than contain overexuberant immune reactions and protect host tissue from damage. It operates at the T-cell level centrally with the elimination of self-reactive lineage committed thymocytes in the thymus and peripherally, for both T- and B-cells, *via* multiple checks and balances against excessive or inappropriate activation. In the peripheral T-cell compartment tolerance is particularly associated with induction of FoxP3+ Tregs. Emergence of Tregs is critically dependent on IL-6 and transforming growth factor (TGF)-β, factors also closely linked to induction of pathogenic IL17-producing T-cells [379, 380]. Given that genetic mutation studies have clearly shown the additional role of IL-6 (signalling *via* a component of its receptor complex called gp130) to promote fibrosis, this cytokine seems attractive for future research [381–383]. Moreover, the capacity of B cell derived IL-6 to trigger autoimmunity [384] further underscores this disease axis. IL-17 (and its closely related effector IL-23) is a potential specific therapeutic target for BOS. At least in experimental models, a TGF-β/Fc fusion protein that redirects TGF-β signalling has been used successfully to promote Tregs and diminish T-helper (Th)17 effectors [385].

Innate immune signals mediated by PAMPs and DAMPs prime the immune system at the molecular level by activating antigen presenting cells (APC) and upregulating co-stimulatory molecules such as those in the B7 family and CD40-CD40L interactions. Competent T-cell activation requires APC activation inducing IL-2 production to drive expansion and co-stimulatory molecules. When IL-2 signalling is interrupted strong suppression of T-cell expansion occurs; hence, the clinical efficacy of CNIs in clinical transplantation. However, where T-cell co-stimulation is interrupted, T-cells are pushed into either apoptosis or a persistently anergic or tolerant state. This form of inactivation is distinct from the suppressive effect of Tregs. This understanding has catalysed interest in understanding how PGD might shape the subsequent fate of a lung allograft over its entire life.

Much of the evidence for the innate immune system's role in human clinical lung rejection is inferential and based on the presence of known innate immunity effector molecules and the modulatory role of CD14 (which co-signals with TLR4) or TLR4 polymorphisms (where hypo-functional variants confer benefit) on graft outcome [386]. In model systems, innate immunity alone is insufficient to mediate chronic cardiac allograft rejection [387] which is consistent with clinical experience using CNIs. In LTx, high Tregs in peripheral blood suggest better allograft outcome [110] and, because Tregs work best in close proximity to effectors, this blood study may underestimate their true benefit. This suggests that manipulating low level or subclinical mechanisms linked to PGD might actually aid in promoting long-term engraftment and tolerance [388].

Lung allografts have one additional complexity not seen in revascularised allografts of other organs; the donor bronchial circulation is not connected with the recipient circulation because of the technical difficulty in making this anastomosis surgically. The bronchi are, therefore, chronically hypoxic leading to induction of hypoxia-induced factor dependent remodelling and fibrotic pathways that operate independently of innate and adaptive immunity and may contribute substantially to BOS [389].

Tolerance and intolerance: manipulating a multi-tiered system

PGD, and indeed tissue damage and inflammation from any cause, has the ability to break tolerance for both T- and B cell compartments due to the strong concurrent stimulation of innate immune pathways and immuno-modulatory growth factors/cytokines. This effect may be dramatically amplified in the setting of infection and reduced immunosuppression ultimately resulting in loss of lung allograft function that can quickly become irreversible (fig. 3).

In kidney allograft models, genetic inactivation of MyD88, a critical signalling intermediate linking TLRs with inflammatory gene induction, led to graft tolerance that was mediated by induction of regulatory FoxP3+T cells and reduced formation of damaging Th17 effector cells [388]. This raises the still untested possibility that it may be possible to subtly alter innate immune signalling after the initial transplant procedure in order to promote tolerance. If this strategy could be achieved as predicted in animal studies, it would affect both T-cell and B cell compartments. A second strategy that has been proven as an immune suppressing method is to block co-stimulation molecules such as CD40/L signalling. Recently, there has been specific interest in optimising the combination of anti-co-stimulation antibodies with IL-2 synthesis or activity inhibitors to drive a stronger, more tolerant state [161]. However, in human allograft medicine this latter strategy carries the risk of anergising or tolerising immunity to lung pathogens which would have profoundly detrimental effects. Indiscriminately targeting MyD88 or co-stimulation molecules carries a similar and substantial risk of triggering serious infection either by reactivating latent viral infection or worsening disease when the lung has already been colonised by bacterial or fungal pathogens.

Very recent studies have shown that *P. aeruginosa* can subvert tolerance in lung allografts by stimulating B7 expression on neutrophils [390]. This study points to the critical role that

infection or colonisation may have on long-term outcomes. This is further emphasised by studies in which neutrophils have been shown to act on dendritic cells to promote tolerance in the absence of an infectious pathogen [391]. Similar effects, exerted *via* the generation of cross reactive T-cell and activation of bystander effectors have been observed after *Listeria monocytogenes* infection [392]. Tregs exert their effects largely by interacting closely with T effector populations and usually need to be in relative excess. This has opened the possibility of promoting tolerance by simply depleting or suppressing the accumulation of a fraction of, but not all, T effector cells which can also be achieved by manipulating chemotactic recruitment signals [393]; an approach that also suppresses inflammation. There is also some evidence that the nature of injury and the duration of donor brain death, especially where PGD occurs, can suppress the development of tolerance [394] suggesting the possibility of still further improving graft harvesting and preservation methods. The improved short- and long-term outcomes seen with DCD LTx are perhaps an excellent example of this [40].

Although respiratory infection is a powerful inhibitor of tolerance, non-infectious respiratory insults may also be important. Cigarette smoke exposure, a noted immune damaging insult, suppresses tolerance at the level of indolamine 2,3-dioxygenase expression [395]. Interestingly, tranilast, a mast cell stabiliser that was initially examined in asthma but has now shown promise in arthritis and multiple sclerosis and induces tolerance [396], is believed to mimic anthranilic acid, formed by idoleamine oxidase from tryptophan. The role of idoleamine oxidase in LTx has been challenged with suggestions that it better marks inflammation than tolerance [397].

Given the importance of tolerance there has been increasing effort to develop viable methods to induce this state in both the T- and B cell compartments. In addition, the detection and monitoring of the ''tolerant state'' would be extremely useful in the clinical setting. Although decreased Tregs in the peripheral blood have been linked to BOS [398], they are not a useful biomarker in individual patients. Whether improved detection of Treg T-cell receptor specificities and/or phenotype characteristics will improve the biomarker potential of this T-cell subset will require proper longitudinal validation studies to be performed. In assessing these models and predictions it is, however, very important to consider that tolerance is almost certainly a labile and plastic state that can vary quickly in response to different clinical contexts.

Mesenchymal stem cells, once hoped to be a source of new lung tissue, have emerged as a viable strategy to induce tolerance because when they are grown *ex vivo* and reinfused, particularly with immune modulation targeting mTOR, a persistent tolerance-like state can be achieved [399]. An analogous strategy has been to infuse so called myeloid suppressor cells although the effects of these cells are less promising than Treg-targeted transfer protocols [400]. In skin allograft models, mast cell derived granulocyte macrophage-colony-stimulating factor plays a critical role in preconditioning dendritic cells to a tolerogenic state: whether this can translate to the lung, however, seems highly unlikely as lung granulocyte macrophage-colony stimulating factor has an intense immune priming effect and can break tolerance itself [401, 402]. Furthermore, mast cell degranulation, which occurs in response to DAMPs, also breaks tolerance [403]. Class II histone deactylase inhibitors epigenetically alter gene expression by regulating its acetylation promote FoxP3 activity and Treg induction. Since clinically useful histone deactylase have already entered practise in oncology it may be possible to create better tolerated variants as adjunctive therapy for LTR. Compared to T-cells and Tregs, B cell manipulation strategies have been much less studied as a means to tolerance induction even though it is known that the presence of inactivated B cells is a marker of tolerance and that these cells can adoptively transfer a tolerant state [404].

The acute lung: why does acute rejection not predict long-term lung outcomes?

Conventionally, there is a belief or assumption that the intensity of PGD or of acute rejection should predict the probability of chronic rejection leading to BOS and graft failure [405]. There is certainly observational evidence to support this view [80, 406]. But, on closer examination the concordances are weak and the features observed in the immunopathology and treatment response cannot be converted into a predictive algorithm that informs long-term allograft survival [74, 81, 89]. In any case, these conditions need to be aggressively treated in their own right because of the risk they pose to survival if uncontrolled. Most disappointingly, however, there is no clear evidence that, on an individual patient basis, therapeutic suppression of early manifestations of PGD or rejection beyond that required by clinical decision making related to risk management in the short term, can be achieved in a manner that would consistently reduce the probability or extent of BOS.

How specifically do earlier events relate the lymphocytic bronchiolitis that is observed to proceed and believed to, at least in part, cause subsequent obliterative bronchiolitis and therefore BOS? Given that lymphocytic bronchiolitis is patchy and easily missed on the biopsies needed to monitor its presence, and that BOS is defined as a clinical end-point that reflects already extensive damage and remodelling (and loss) of airways, what surrogates might be developed to better prevent its occurrence? Furthermore, given that available therapies are almost exclusively able to suppress immune alloreactivity and control some aspects of inflammation but are not specifically able to suppress or reverse fibrosis, what new drugs do we need? How will we then initially use anti-fibroproliferative agents to prove that excessive fibrosis is a causative factor for BOS rather than just a marker of a damaged epithelium that cannot heal in any other way? Alternatively, as is discussed in the field of lung fibrosis, does inflammation directly cause fibrosis in the first place? Or is inflammation a parallel process that overlaps in time with an entirely separate biology of airway destruction that is clearly refractory to current anti-inflammatory drugs? While this last point seems almost counter-intuitive based on current pathogenesis and treatment regimens there is clear evidence from other fibrotic diseases and from experimental models that inflammation can be dissociated from progressive fibrosis. For example, genetic manipulation of signalling from gp130 (a co-receptor essential for signalling of the IL-6 family cytokines which includes IL-6, IL-11, leukaemia inhibitory factor and oncostatin M) directly indicates that fibrosis and inflammation can be dissociated [382, 383]. Therefore, it follows that in LTx-associated obliterative bronchiolitis, there is a great difficulty in

knowing whether complete suppression of inflammation as a primary therapeutic goal is intrinsically able to reduce all future risk. This is compounded by a lack of proven biomarkers or imaging methods that detect very early, sub-clinical decline in graft integrity. One interesting interpretation of this poor concordance is that the fundamental mechanisms of PGD, acute and chronic rejection/BOS are not actually the same despite shared manifestations (like inflammation and alloreactivity).

A further aspect of the problem is that chronic rejection in the setting of adequate calcineurin inhibition is more likely to involve B-cell mediated anti-graft antibodies and non-T-cell/non-B cell mechanisms that are much less responsive to currently available therapies. Processes leading to fibrosis such as epithelial to mesenchymal transition, myofibroblast and fibroblast proliferation remain beyond the reach of current available therapies. There is more hope of shorter term progress targeting infectious risk factors that may amplify the processes described above particularly given the advances in strategies to target CMV and biota. But how specifically do latent viruses, especially CMV, airway colonisation with bacterial or fungal pathogens, or an altered meta-genome interact [74, 239]?

One area of striking deficiency in our current understanding is exactly how broad, potent immunosuppression regimens alter the lung microbiome. There is now very good evidence that even the healthy lung has a significant low level flora and that this microbiome shifts dramatically in lung disease [407, 408]. There is also emerging evidence from CF, asthma and COPD research that treatment, including steroids, shifts the composition of the microbiome substantially and that the microbiome varies with disease severity [409]. In health, the lung microbiome is a low-abundance mirror of what is found in upper airways [410]; this changes in both acute and chronic lung disease. There is considerable regional heterogeneity in the composition of the biome within lungs between lobular segments and there is emerging evidence that viruses, including low level viral infection that in its own right would not usually be considered a particularly damaging insult, can have a substantial and sustained effect on the patterns and virulence of the microbiome that is long lasting. These effects may result from an alteration in the complex pattern of micronutrients generated by low abundance pathogens that sustain more virulent bacteria [411–413]. Research into how the metagenome varies in lung allografts in relation to outcomes is currently in its infancy [414].

It is very likely that donor lung microflora/microbiome at transplant and over time will adversely affect allograft survival. *P. aeruginosa*, for example, has been shown to be capable of breaking allograft tolerance through the unusual paracellular mechanism of upregulating B7, a co-stimulation molecule usually found on dendritic cells and on neutrophils [390]. As *Pseudomonas* also has been shown to colonise damaged airways, both cause and effect possibilities exist for its presence in the airways being associated with a marked increased risk of BOS [239]. It seems increasingly likely that the well-established link between CMV and BOS will relate not only to classical mechanisms of upregulating innate natural killer cells [415], adaptive immunity effector pathways [416, 417] and reducing tolerance but also to a shift in the metagenome that may constitute a major driving insult. It is perhaps this insult that ultimately entrains treatment refractory pro-fibrotic pathways.

Given that the current potent immunosuppressive regimes that necessarily must be used to manage life-threatening rejection syndromes will almost certainly be shown to shift the lung metagenome, we are in urgent need for objective evidence of what really happens to the lung microflora in transplant medicine (and for that matter, the gastrointestinal microbiome as well). An ideal immune suppressor of the future would contain alloreactivity while promoting tolerance and maintaining normal host defence. For example, in a CMV positive recipient an ideal intervention would contain latent virus, preserve memory effect CD4 T-cell in the lung and yet still suppress alloreactivity. Such molecules are yet to be discovered.

How can we better individualise immunosuppression treatment and other management strategies post-LTx?

There are no proven biomarker strategies, surrogates or refined lung imaging methods that can detect incipient deterioration in the lung allograft and guide therapy. For example, new therapies such as novel antibodies that are highly effective immune suppressors and have been used as induction agents do not necessarily have a strong effect on subsequent BOS rates [418]. As such management remains largely empirical, changing or escalating in response to overt deterioration and crises. It is particularly disappointing that the successes for basic pharmacology, which has contributed so much to the discovery of novel agents, has not been equalled by clinical pharmacology where blunt pharmacokinetic blood levels remain the only guidance in the management for complex and subtle pharmacodynamic effects.

At all levels, LTx remains a major challenge in an era of personalised, stratified medicine; this is not to say the field is without progress. Pharmacogenomic approaches have revealed some mechanisms that are sensitive to immunosuppressive drugs, most notably export proteins [419, 420]. Large integrated studies, such as LARGO, have also pointed to gene variations that influence outcome, again pointing to ATP binding cassette transport proteins and cytochrome-mediated biotransformation variants as having the main influence on pharmacokinetic profiles and achieving stable blood levels in the clinical setting [421]. However, knowledge in the field remains indirect linking polymorphisms in inflammatory cytokines to outcomes rather than a more fundamental understanding of the effect of variants on the molecular loci of drug action.

Given that the types of immune suppressors that could control allograft rejection without damaging host tissues remain conceptual, there is also a pragmatic interest in better understanding the molecular basis of comorbidities common after lung allograft.

Decline in lung function is conventionally viewed as the cause of diminished exercise activity after transplantation but, and in a manner analogous to recent work in COPD, loss of skeletal muscle strength and mass can contribute substantially to this problem [422, 423]. Loss of muscle function and mass, together with fibre type switching result from lack of activity, steroid effects and the adverse effects of inflammation, which represses muscle growth at the level of satellite cells and myocytes, and promotes resorption of muscle proteins *via* the 26 proteasome. Given that long-term immunosuppressive regimens can impair nerve function, there is interest in

whether some loss of muscle might also result from a loss of trophic neuronal drive to the tissue.

Similarly, osteoporosis is a common clinical problem after LTx [424]. This is thought to reflect the intersection of direct effects of immune suppressing therapies, not restricted to steroids, to repress new bone formation together with the pro-osteoclastic effect of lung inflammation which is known to induce bone resorption. In model systems this is due to granulocyte-colony-stimulating factor produced in inflamed tissue feeding back to the bone marrow to induce proteases in the bone marrow endosteum essential to releasing tethered leukocytes into the circulation, as well as having a pro-osteoclastic effect and repressing osteoblast activity.

While direct neurotoxic effects of immunosuppressive regimens are very well known, it is surprising that so little attention has been paid to the possibility that affective disorders, which are over-represented in LTR, might, in some cases, have some underlying organic cause [360]. Severe peripheral inflammation has not only been linked to pain but also independently to depression and anxiety in other inflammatory conditions such as rheumatoid arthritis [425, 426].

Collectively, these advances and insights point to new strategies to refine clinical LTx, improve long-term allograft outcomes and better manage complex comorbidities. The extreme heterogeneity of transplantation medicine and biology means that progress will be slow but tangible advances seem within reach.

SUMMARY

When LTx started, its ultimate goal was to deliver on the hope of a longer and fuller life for selected patients with end-stage lung disease. Over the last 30 yrs we have learnt so much and applied it in a way that has extended thousands of lives in so many ways. We hope this article has clarified what remains to be done and opened up thinking to elucidate, address, and solve the remaining barriers to achieving a durable lung allograft with minimal comorbidities and as full a future as possible, for as many patients as possible, utilising this life-saving and life-giving procedure.

STATEMENT OF INTEREST

None declared.

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