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Calcineurin Inhibitor Sparing in Paediatric Solid Organ Transplantation

Managing the Efficacy/Toxicity Conundrum

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Abstract

Despite their efficacy, the calcineurin inhibitors (CNIs) ciclosporin and tacrolimus carry a risk of debilitating adverse effects, especially nephrotoxicity, that affect the long-term outcome and survival of children who are given organ transplants. Simple reduction in dosage of CNI has little or no long-term benefit on their adverse effects, and complete withdrawal without threatening graft outcome may only be possible after liver transplantation. Until the last decade, the only option was to increase corticosteroid and/or azathioprine doses, which imposed additional long-term hazards. Considered here are the emerging genera-

tion of new agents offering an opportunity for improving long-term graft survival, minimizing CNI-related adverse events and ensuring patient well-being.

A holistic, multifaceted strategy may need to be considered – initial selection and optimized use and monitoring of immunosuppressant regimens, early recognition of indicators of patient and graft dysfunction, and, where applicable, early introduction of CNI-sparing regimens facilitating CNI withdrawal. The evidence reviewed here supports these approaches but remains far from definitive in paediatric solid organ transplantation. Because *de novo* immunosuppression uses CNI in more than 93% of patients, reduction of CNI-related adverse effects has focused on CNI sparing or withdrawal.

A recurring theme where sirolimus and mycophenolate mofetil have been used for this purpose is the importance of their early introduction to limit CNI damage and provide long-term benefit: for example, long-term renal function critically reflects that at 1 year post-transplant. While mycophenolic acid shows advantages over sirolimus in preserving renal function because the latter is associated with proteinuria, sirolimus appears the more potent immunosuppressant but also impairs early wound healing. The use of CNI-free immunosuppressant regimens with depleting or non-depleting antibodies plus sirolimus and mycophenolic acid needs much wider investigation to achieve acceptable rejection rates and conserve renal function.

The adverse effects of the alternative immunosuppressants, particularly the dyslipidaemia associated with sirolimus, needs to be minimized to avoid replacing one set of adverse effects (from CNIs) with another. While we can only conjecture that judicious combinations with the second generation of novel immunosuppressants currently in development will provide these solutions, a rationale of low-dose therapy with multiple immunosuppressants acting by complementary mechanisms seems to hold the promise for efficacy with minimal toxicity until the vision of tolerance achieves reality.

Organ transplantation became a practical reality more than half a century after the first experimental organ transplant was performed by Alexis Carrel and Charles Guthrie in 1905.[1] However, it was the introduction of ciclosporin (cyclosporine) in the early 1980s that ensured favourable long-term outcome by providing the long-sought immunosuppressive specificity against transplant antigens. By inhibiting calcineurin and preventing migration of transcription factors activating nuclear targets, ciclosporin provided unprecedented efficacy in inhibiting lymphocyte proliferation without the frequent life-threatening infections that characterized less specific treatments such as bone-marrow ablation and high-dose corticosteroids in combination with mercaptopurine or later azathioprine.[2] Tacrolimus joined the immunosuppressive pharmacopoeia in 1989 and provided further evidence of the benefits of calcineurin inhibition.

Protocols based on primary maintenance immunosuppression with a calcineurin inhibitor (CNI) normally in combination with other immunosuppressants still dominate solid organ transplantation more than 25 years later and have empowered additional surgical and management advances that particularly benefit paediatric solid organ transplantation. Tacrolimus is now the CNI preferred for maintenance immunosuppression in paediatric solid organ transplantation[3] but supplementation with newer agents has become increasingly common.[4] As a result, acute rejection episodes across all transplant indications have fallen to record lows[4] and cause few graft losses,[5] chronic immune injuries to grafts have declined, and modest improvements in longer-term graft and patient survival continue.[3] There is an exception as children approach adulthood, with 17% of liver, 42% of kidney and 30% of

cardiac adolescent transplant recipients losing their grafts probably through nonadherence.[6]

With the benefit of effective calcineurin inhibition has come the attrition of CNI-related adverse effects, which are now a major focus in transplant management.[7] In renal transplantation, CNI-induced renal impairment is a key risk factor for reduced long-term allograft survival.[8] Renal impairment also occurs in up to 60% of paediatric nonrenal graft recipients[9] and is liable to progress to end-stage renal disease. This and other adverse effects of CNI medication are considered in more detail in section 1. Minimization of adverse effects is now possible by modifying immunosuppressive therapy in response to individual requirements and this embraces a number of strategies. The first is by withdrawal or reduction of CNI dosage (the process of 'CNI sparing'), usually in parallel with the introduction of drugs from the growing repertoire of new immunosuppressants. This approach is considered in section 2. The second strategy considers optimized CNI usage through appropriate selection of agent, tailoring of therapy and monitoring both patient and graft (covered in section 3). Managing appropriate dosage of both CNI and supplemental or replacement agents is the subject of section 4. Finally, we can aim for true individualization in the selection of immunosuppressive regimens based on patient susceptibility defined in studies of genetic association with outcome markers of transplantation and immunosuppression (covered in section 5). Nowhere is this more important than in children, for whom there are potentially lifetime consequences of immunosuppression relating to malignancy, growth, development and fertility. Largely beyond the scope of this review, but also affecting paediatric transplant recipients, are the complications of ontogeny, which may affect drug disposition and response,[10] as well as the management challenges arising from childhood obesity and diabetes mellitus.[11,12]

1. Adverse Effects of Immunosuppression

Immunosuppressant adverse effects may be generic or specific to a class of agents, such as the CNIs, and probably related to their mechanism of action. The major generic risks of immunosuppression are infections and malignancy, but cardiovascular disease is also common, even in children.

In paediatric graft recipients, infections accounted for 34.5% of deaths in intestinal,[13] 30.1% in renal[14] and 28.4% in liver transplantation.[15] Their prevalence may increase in the very young and following antibody induction,[14] and certain agents have been associated with increased risks of specific infections, e.g. cytomegalovirus (CMV) with muromonab CD3.[16,17] There are recent proposals by the American Society of Transplantation for unified monitoring of infection in trials of immunosuppression.[18] Bacterial infections occurred in approximately 60% of children within the first year after liver transplantation.[19] In paediatric renal graft recipients, 23% were hospitalized within 2 years for bacteraemia[14] and 36% developed urinary tract infections.[20] Fungal infections developed within 1 year in 40.5% of children receiving liver transplants and were associated with increased mortality.[21] Viral infections occurred in 40–50% of paediatric liver recipients[21] and caused hospitalization of 23.9% of renal graft recipients.[14]

Younger age and antibody induction are risk factors for bacterial and viral infection in paediatric renal transplantation.[14] Younger age (infants), Hispanic race, split or reduced-size cadaveric graft, ciclosporin (rather than tacrolimus) use, transplantation pre-2002, serum bilirubin level and anhepatic surgical time were significant independent predictors of bacterial or fungal infections in a recent multicentre study of 2291 paediatric liver transplants.[22] Polyoma BK virus infection appears especially deleterious in paediatric renal recipients[23,24] and adenovirus in bowel recipients,[25] while human herpesviruses 6 and 7, CMV and Epstein-Barr virus (EBV) infections affect all graft recipients.[26] Viral infections may compromise graft function in paediatric renal and liver recipients,[27,28] and post-transplant rejection, reduced-size grafts, year of transplant before 2002 and recipient EBV status contributed to the increased risk of infection in paediatric liver recipients.[22] In paediatric kidney recipients, CMV infection and donor CMV positive serostatus increased the risk of acute rejection.[29] EBV seronegative recipients of EBV-positive grafts are especially at risk from post-transplant lymphoproliferative disorder (PTLD). PTLD occurred in 6.3–20% of

paediatric recipients of liver grafts,[30] 3.5–5% of cardiac grafts[31,32] and 1.7% of renal grafts.[33] Unacceptably high rates of 6.9% in one paediatric renal cohort were attributed to 'robust' (over-) immunosuppression with basiliximab, calcineurin inhibitor, sirolimus and corticosteroids.[34] Major sites are the gastrointestinal tract and lungs,[32] although a high incidence of sino-nasal PTLD was recently reported in children receiving lung transplants for cystic fibrosis.[35] Younger age and the use of tacrolimus or equine antithymocyte globulin (ATG) for induction may be positive risk factors[30,31,34,35] but EBV polymerase chain reaction-positivity is not.[36] PTLD may benefit from immunosuppression withdrawal,[37] and its associated B-cell lymphomas from the emerging therapies of rituximab[38] or EBV-specific cytotoxic T lymphocytes.[39]

The risk of solid organ malignancies is increased several-fold in adult graft recipients,[40] and colon and skin cancer are particular risks.[41] However, malignancies other than PTLD appear to be relatively rare in children, maybe at least partly reflecting shorter follow-up.[42] For example, a recent report in 98 children with transplants showed that they were spared malignant skin lesions in the first 5–16 years post-transplant.[43]

Cardiovascular disease is a major cause of death in approximately 10% of paediatric renal transplant recipients[44] and cardiopulmonary deaths occurred in 12% of paediatric liver graft recipients.[22] In adult liver graft recipients, tacrolimus is associated with fewer deaths and cardiovascular events than ciclosporin.[45] Risk factors for cardiovascular disease assessed in children at 3.5 years after renal grafting[46] showed that >40% had elevated blood pressure, serum triglyceride and homocysteine levels, and low haematocrit, while 20% had elevations in serum cholesterol levels. In children receiving renal grafts, blood pressure and serum cholesterol were disproportionately higher in regimens containing corticosteroids and sirolimus in one study,[46] and with ciclosporin rather than tacrolimus in another[47] (see also CNI-related hypertension in section 1.1).

1.1 Calcineurin Inhibitor (CNI)-Specific Adverse Effects

Ciclosporin and tacrolimus share a common profile of adverse effects, which is probably related to their inhibition of endogenous processes involving calcineurin. Of these, nephrotoxicity, diabetes, hypertension, lipid abnormalities and neurotoxicity are the most frequent, and some are more prevalent in children than in adult graft recipients.[48]

CNI-induced nephrotoxicity probably has the greatest impact on post-transplant renal dysfunction in nonrenal graft recipients and it is the effect of ciclosporin and tacrolimus on renal function that is considered here. The emerging consensus is that ciclosporin and tacrolimus cause a quantitatively similar deterioration in glomerular filtration rate (GFR).[9,49] Nephrotoxicity initially results from vasoconstriction of glomerular arterioles induced reversibly by high CNI concentrations[50] and manifest by reverse diastolic intrarenal blood flow.[51] Acute injury translates into chronic renal impairment through the ischaemic consequences of vasoconstriction, activation of the renin-angiotensin system and transforming growth factor-β upregulation.[9] In paediatric recipients of renal grafts, such changes contribute to chronic allograft nephropathy together with immune injury and the consequences of infection.[52,53] Renal dysfunction/impairment is frequently expressed as GFR <70 or 80 mL/min per 1.73 m^2 (calculated using the Schwartz or less perfectly the modified Counahan-Barratt formulae). In 117 paediatric liver graft recipients receiving ciclosporin-based triple therapy, 32% had renal dysfunction at a mean of 7.6 years post-transplant.[54] According to other data, the prevalence may be as high as 57% in paediatric cardiac or liver graft recipients.[55-58] In a small cohort of ten liver graft recipients followed-up for more than 10 years, 30% showed a creatinine clearance below 60 mL/min/1.73 m^2.[59] From 3% to 10% of paediatric heart recipients developed end-stage renal failure.[9] The consequences are severe: dialysis because of all causes of graft failure carries a 3.4-fold increase in mortality risk over that pre renal transplant.[60]

Diabetes is induced by CNIs as a result of inhibition of insulin production in islet cells by both ciclosporin and tacrolimus,[61] and this is dose-related.[62] However, disorders in glucose metabolism are frequent after transplantation and hyperglycaemia has been associated not only with CNI use but also with acute rejection, infection and, particularly, corticosteroids,[62] which profoundly increase insulin

resistance.[63] After adult solid organ transplantation, meta-analysis has shown an overall incidence of new-onset diabetes of 13.4% and a higher frequency with tacrolimus (16.6%) than ciclosporin (9.8%).[64,65] Hyperglycaemia is around 30% more prevalent with tacrolimus.[66,67] In contrast, diabetes occurs in only 3% of children after transplantation,[68,69] with one exception,[64] and several studies have reported no increased risk of developing diabetes with tacrolimus versus ciclosporin in children.[69-71] However, the incidence of new-onset diabetes in paediatric transplant recipients may be increasing[72] and pre-transplant glucose intolerance has been recorded in 33% of children with end-stage renal disease.[72] The distinction between adults and children may relate to the unmasking by the CNI of an underlying metabolic syndrome that causes the hyperglycaemia[73] and is less prevalent or severe in children. Certainly other cofactors for insulin resistance and diabetes suggest their protracted development and include African ethnicity, obesity, family history, hepatitis C infection, the number of transplants and increasing age.[65,74] Consequently, hyperglycaemia may be seen as a prompt towards assessing glucose tolerance,[75] which may be valuable in the selection of CNI pre-transplant[72] or in sparing CNI dosage post-transplant to limit progression to diabetes. Further inducements may be that strict blood glucose control has a beneficial effect on the development and progression of diabetic nephropathy,[76] and that diabetes is adversely associated with graft rejection, graft loss and infection.[75]

Dyslipidaemia is associated with the CNI, sirolimus and corticosteroid components of immunosuppressive regimens, as well as with diabetes. A high prevalence characterizes paediatric heart, liver and renal transplantation with both hypertriglyceridaemia and hypercholesterolaemia found in 30–50% of transplant recipients.[63,77,78] Ciclosporin medication was associated with higher serum cholesterol levels in paediatric renal graft recipients than tacrolimus.[47,70] Hypercholesterolaemia can be corrected by statins,[79] early use of which conferred survival benefit by preventing coronary artery disease in paediatric heart grafts,[80] possibly by involving immunomodulation.[81]

Hypertension associated with CNIs may also be exacerbated by corticosteroids and is an adverse risk factor for cardiovascular disease and progressive renal dysfunction.[82,83] Post-transplant hypertension was not only associated with GFR inversely[53,84] but also with end-organ damage, early cardiomyopathy and premature atherosclerosis if uncontrolled in paediatric renal graft recipients.[82] Graft survival itself was impaired in such children with early post-transplant systolic hypertension.[85] Recent studies have emphasized the link between increased systolic blood pressure at transplant or in the 3 successive months with hypertension and impaired GFR at 1 year – and the corresponding need for early antihypertensive intervention.[53] In paediatric renal graft recipients, around 80% required antihypertensive medication,[82] but comparable requirements in 185 paediatric liver transplant patients were around 35%.[19] The prevalence of new-onset hypertension in this liver cohort was greater with ciclosporin than tacrolimus (47% vs 39%),[19] and a significant disadvantage with ciclosporin was the elevation in both systolic and diastolic blood pressure in paediatric renal graft recipients.[47] In contrast, high tacrolimus levels and the use of prednisolone plus sirolimus combination therapy were risk factors for hypertension in a paediatric heart transplant cohort.[86]

Neurotoxicity may range from tremors to seizures and more commonly occurs in the first 30 days post-transplant when blood concentrations of ciclosporin or tacrolimus are high.[19,87] Between 20% and 35% of paediatric liver transplant recipients show these symptoms but similar disorders are a common feature of children with acute liver failure, up to 20% of whom may receive transplants.[62]

Both ciclosporin and tacrolimus may increase bone loss and resorption.[88] Hirsutism and gingival hyperplasia occur more commonly with ciclosporin than tacrolimus[19] and the latter appears to relate to a genetic variant in interleukin (IL)-1A.[89] Hyperuricaemia was also noted to be 4-fold more frequent in paediatric liver graft recipients treated with ciclosporin than with tacrolimus.[90] Both ciclosporin and tacrolimus cause cholestasis, the former by inhibition of multidrug-resistance protein 2.[91] Tacrolimus induces cholestasis particularly in paediatric liver recipients with steroid-resistant rejection and may resolve on conversion to ci-

closporin.[92] Resolution of other tacrolimus-specific adverse events, such as cardiomyopathy,[93,94] haemolytic anaemia[95] and sebaceous neoplasms,[96] may also be achieved on conversion to sirolimus. Recent reports also implicate tacrolimus in food allergy and polysymptomatic eosinophilia,[97,98] and the development of hearing loss.[99,100]

2. CNI Sparing

Avoiding CNI-related adverse events is now an achievable objective given the expanding pharmacopoeia of immunosuppressants (table I). Detailed discussion of the mechanisms of action of these agents is beyond the scope of this article and the interested reader is referred to comprehensive reviews appearing elsewhere.[101,102] These describe ciclosporin[103] and tacrolimus,[104] the inosine monophosphate dehydrogenase inhibitor mycophenolate mofetil (MMF),[105] the mammalian target of rapamycin (mTOR)/tyrosine kinase inhibitors sirolimus[106] and everolimus,[107] as well as corticosteroids and azathioprine.[103] There are additional data on the newer agents such as the sphingosine derivative fingolimod (FTY720),[108] FK778 (a leflunomide derivative), gusperimus (deoxyspergualin) and its analogue anisperimus.[109-111] There is increasing application of T- and B-cell depleting antibodies, including IL-2 receptor (IL-2R) antibodies, alemtuzumab, and fusion proteins such as belatacept (LEA29Y) for blocking co-stimulation during antigen presentation.[109-111] Finally, there are innovative agents, such as the protein kinase C inhibitor AEB071 and the Janus kinase 3 inhibitor CP690550, which are under evaluation.[101,112]

As is frequently the case, there is a wider initial experience of novel approaches such as CNI sparing in adult than paediatric solid organ transplantation. Two recent reviews are particularly pertinent and complement the information presented here.[113,114] The dominant use of CNIs for primary immunosuppression places exacting demands on alternative immunosuppressive regimens so that neither efficacy is sacrificed (increasing acute rejection episodes or chronic immune graft injury), nor are alternative debilitating adverse effects introduced. The adverse effects of non-CNI agents have been considered elsewhere[40] but new complications of existing agents continue to emerge such as the recent association of MMF with an increased risk for first trimester pregnancy loss and congenital malformations.[115] Two basic strategies have been adopted to minimize CNI exposure: first, reductions in CNI doses without introducing additional agents; and, second, use of supplemental immunosuppression with novel immunosuppressants to spare or replace CNIs. The related strategy of improved usage of CNIs is considered in section 3 after this discussion of CNI sparing.

2.1 Without Novel Immunosuppressants

CNI dosage reduction may be an initial component of all strategies of CNI sparing. However, the experience of Rice and colleagues[116] in 25 paediatric heart transplant recipients is typical of its influence on CNI-related adverse events: there was no evidence that customary progressive reductions in CNI dosage influenced renal function beyond 1 year post-transplant.

Total CNI withdrawal may have greater benefit. A meta-analysis of ten trials performed in adult renal graft recipients concluded that "cyclosporine withdrawal in selected patients seems to impart little risk of long-term graft failure".[74] However, complete withdrawal without adding new agents can only be realistically considered late after liver transplantation where 19–42% of adult liver grafts develop tolerance 'spontaneously'[6] and a successfully treated acute rejection episode has seemingly no adverse sequelae to graft function.[117] A recent paediatric study reported withdrawal of ciclosporin from a ciclosporin plus corticosteroids plus azathioprine triple regimen under a routine protocol where there was good liver graft function, no sign of rejection and no history of rejection episodes during year 2 after transplantation.[118] Azathioprine and prednisolone doses were increased before ciclosporin withdrawal over 2 weeks. Of 53 eligible patients, no rejection episodes were noted in 35 (66%) together with an improvement in renal function. The remainder experienced acute rejection but this was successfully treated with methylprednisolone and restarting ciclosporin. No grafts were lost and successful withdrawal was most frequently achieved in children whose bodyweight was below 10 kg.

Table I. Classification of immunosuppressive drugs and their registration status

Class/agent	Registration status in transplantation	Proprietary name[a] [manufacturer website]
Calcineurin inhibitors		
Ciclosporin	Rejection prophylaxis in renal, liver, heart transplantation	Neoral® (microemulsion formulation) [www.novartis.com]
Tacrolimus	Rejection prophylaxis in renal, liver, heart transplantation	Prograf®; Advagraf® (sustained-release formulation) [www.astellas.com]
Voclosporin (ISA 247)	In development for transplantation	[www.roche.com]
mTOR inhibitors		
Everolimus	For preventing rejection of kidney and heart transplants in EU only	Certican® [www.novartis.com]
Sirolimus (rapamycin)	Rejection prophylaxis in renal transplant recipients aged >13 y	Rapamune® [www.wyeth.com]
Antiproliferative agents		
Azathioprine	Organ transplantation	Imuran®/Azasan® [www.gsk.com]
Mycophenolic acid		
mofetil ester	Rejection prophylaxis in renal, cardiac and liver transplantation	CellCept® [www.roche.com]
sodium salt	Rejection prophylaxis in renal transplantation	Myfortic® [www.novartis.com]
Antibodies and receptor blockers/antagonists		
Alemtuzumab (anti-CD52)	Licensed for non-transplant indications; used in phase II clinical trials in organ transplant recipients	Campath®/MabCampath® [www.genzyme.com] [www.bayerhealthcare.com]
ATG (rabbit, equine)	Treatment of acute rejection after organ transplantation	Thymoglobulin®, Atgam® [www.genzyme.com]
Basiliximab (anti-IL-2R)	Rejection prophylaxis in renal transplants treated with ciclosporin	Simulect® [www.novartis.com]
Belatacept (LEA29Y) [anti-CTLA-4]	In phase III trials in renal, liver, cardiac and cell transplantation	[www.bms.com]
CD40 monoclonal antibody	In development for transplantation	[www.astellas.com]
CP 690550 (JAK3 inhibitor)	In development for rheumatoid arthritis and transplantation	[www.Pfizer.com]
CRB 15 (anti-IL-15R)	In development for rheumatoid arthritis and transplantation	[www.roche.com]
Daclizumab (anti-IL-2R)	Rejection prophylaxis in renal transplants treated with ciclosporin	Zenapax® [www.roche.com]
Efalizumab	Licensed for psoriasis after progression to phase I/II trials in transplantation	Raptiva® [www.genentech.com]
Muromonab CD-3 (anti-CD3)	Treatment of acute rejection of renal, cardiac and liver grafts	Orthoclone OKT3® [www.orthobiotech.com]
Tocilizumab (leflunomide) [anti IL-6R]	Early studies in transplantation not pursued; now in phase III trials in rheumatoid arthritis	Actemra™ [www.roche.com and www.chugai-pharm.co.jp]
Visilizumab (anti CD3)	Used for graft-versus-host disease in phase II trials	Nuvion® [www.pdl.com]
Others		
AEB071	In development for transplantation	[www.novartis.com]
Alefacept (ASP 0485)	Licensed for psoriasis phase II trials in transplantation	Amevive® [www.astellas.com]
Anisperimus (LF15-0195)	Analogue of gusperimus in phase I/II studies	[www.solvaypharmaceuticals.com]
BCX 4208	In development for autoimmune disease and transplantation	[www.roche.com]
Fingolimod (FTY 720)	Phase II trials in transplantation discontinued. Now in phase III trials for multiple sclerosis	[www.novartis.com]
FK 778	Phase II trials in transplantation and further development discontinued in June 2006	[www.astellas.com]
Gusperimus (deoxyspergualin)	Licensed for transplantation only in Japan	Spanidin® [www.nipponkayagu.co.jp]
SBR 759	In development for transplantation	[www.novartis.com]

a The use of trade names is for identification purposes only and does not imply endorsement.

ATG = anti-thymocyte globulin; **IL-*x*R** = interleukin-*x* receptor; **JAK3** = Janus kinase 3; **mTOR** = mammalian target of rapamycin.

Table II. Use of depleting antibodies for calcineurin inhibitor sparing in paediatric transplantation

Transplant cohort	Regimen	Outcome	AR (%)	Other	References
Paediatric renal (n = 34)	ATG (8) or ALE (26) then low-dose TRL	Estimated GFR stable to 1 y: 91 mL/min/1.73m^2	9 at ≥6 mo	Infections in 21%, neutropenia in 29% of patients	129,131
Paediatric renal (n = 26)	ALE then low-dose and spaced TRL therapy in 69% – sustained in 46%	Patient/graft survival: 96%/88% at 2 y	12	Mean CL_{CR} at 25 mo: 83 mL/min/1.73 m^2; no BKV, CMV, PTLD; diabetes mellitus in 8% of patients	130
Paediatric liver (n = 10)	ALE induction + low-dose TRL ± CS; AIH subset of 6 vs 10 no ALE (historic)	100% patient and graft survival at mean 1.6 y	70	In AIH subgroup: AR 66% vs 100%; CR 0% vs 30%	132
Paediatric small bowel (high risk) [n = 11]	ALE + low-dose TRL + CS	Patient survival 64% (short follow-up)	57	No opportunistic infections	133

AIH = autoimmune hepatitis; **ALE** = alemtuzumab (Campath®); **AR** = acute rejection episodes; **ATG** = antithymocyte globulin; **BKV** = Polyoma BK virus; **CL_{CR}** = creatinine clearance; **CMV** = cytomegalovirus; **CR** = chronic rejection; **CS** = corticosteroids; **GFR** = glomerular filtration rate; **PTLD** = post-transplant proliferative disorder; **TRL** = tacrolimus.

2.2 With Novel Immunosuppressants

The two major approaches are (i) induction with antibodies depleting T and/or B cells or targeting lymphocyte receptors and inhibiting function; and (ii) replacement of the CNI with, for example, MMF, sirolimus or adjunctive immunosuppressants with distinct mechanisms of action and adverse effect profiles.

2.2.1 Depleting Antibodies

The proportion of paediatric renal, lung and heart recipients receiving antibody induction in the US has progressively increased over the last decade to 77%, 59% and 50%, respectively, in contrast to paediatric liver and intestine transplants, where no antibodies are used in 80% and 60%, respectively.[3] Kidney transplantation favours lymphocyte depletion with rabbit ATG, while IL-2R antibodies are preferred in heart or lung transplantation.[3] Tan and colleagues[119] recently reviewed the experience with induction therapy. Recent reports examining efficacy and safety have demonstrated high rates of patient and/or graft survival in paediatric renal, heart and liver cohorts treated with ATGs, but acute rejection rates vary from 0% to 50% because comparisons generally involve dissimilar immunosuppressant regimens.[120-124] Clearly, an acute rejection incidence approaching 50% with ATGs represents no advance, while a low incidence of acute rejection associated with higher renal impairment in paediatric kidney recipients receiving ATG was an inferior outcome to that in children given basiliximab or no antibody induction.[123] Data from a small study with alemtuzumab in paediatric renal recipients showing high acute rejection rates of 75%,[125] and the failure of muromonab-CD3 induction to show any improvement over ciclosporin[126] were also discouraging. Noteworthy in small studies in paediatric heart[127] and liver transplantation[128] were low rates of PTLD using ATG.

Very few studies report the use of ATG and alemtuzumab for CNI sparing (table II). In paediatric renal recipients, tacrolimus dosage was significantly reduced with subsequent benefit to renal function, and the incidence of acute rejection remained low (9–12%) with both agents.[129-131] Alemtuzumab caused seemingly fewer infectious episodes.[130] Similar reductions in tacrolimus dosage

Table III. Use of interleukin-2 receptor antibodies for calcineurin inhibitor (CNI) sparing in paediatric transplantation

Transplant cohort	Regimen	Outcome	AR (%)	Other	Reference
Paediatric renal (n = 43)	CNI ± AZA ± MMF + CS with vs without BSX	Patient/graft survival (%): 100/100 vs 96/87 (1 CR)	7 vs 26 at 1 y	Mean GFR 98 mL/min/m^2 vs 75 mL/min/m^2 at 1 y	151
Paediatric renal (n = 34)	DCL induction + early MMF + SRL	100% patient survival; 93% graft survival	22 at 6 mo; 32 at 1 y	Stable GFR post-transplant; PTLD 6% of patients	152
Paediatric liver (n = 3)	Use of BSX with CNI + CS ± AZA ± MMF for GFR <65 mL/min/m^2	All survived; 1 with CR was re-transplanted	1 with AR; 1 with CR	GFR increased by 69%; CNI targeted to low trough concentrations	153

AR = acute rejection episodes; **AZA** = azathioprine; **BSX** = basiliximab; **CR** = chronic rejection; **CS** = corticosteroids; **DCL** = daclizumab; **GFR** = glomerular filtration rate; **MMF** = mycophenolate mofetil; **PTLD** = post-transplant proliferative disorder; **SRL** = sirolimus.

were possible in liver[132] and small-bowel recipients[133] but at the cost of unacceptably frequent acute rejection episodes (in 70% and 57%, respectively) and there were no data on renal function.

2.2.2 Non-Depleting Antibodies

The specificity of the IL-2R antibodies basiliximab and daclizumab confers theoretical benefit in suppressing selective immune responses over antibodies depleting entire lymphocyte populations. Recommended doses for basiliximab are 12 mg/m^2 on post-transplant days 0 and 4, and for daclizumab are 1 mg/kg on the day of transplant and on 1 day of each of 4 successive weeks.[132] A daclizumab trough concentration >5 mg/L has been suggested for CD25 saturation.[134] Two reports have investigated optimal dosage.[135,136] An additional (third) dose of basiliximab resulted in an improved incidence of acute rejection (37% vs 64%) in 11 patients at 6 months after paediatric renal transplantation, with seemingly no influence on viral infections but a greater incidence of allergic reactions.[135] In contrast, two doses of daclizumab appeared as effective as five in a cohort of 109 adult heart transplant recipients.[136] The safety and efficacy of these agents has been widely assessed in paediatric transplantation,[57,109,137-140] with recent reports describing usage in several different maintenance regimens in paediatric renal, liver and heart transplantation.[13,123,141-151] The majority of these studies show improvements in efficacy (a reduced incidence of acute rejection) with basiliximab or daclizumab without increases in adverse events, except where additional immunosuppressants were also introduced. One recent retrospective study in 88 children receiving renal grafts showed improved renal function in children receiving basiliximab versus those given ATG or no antibody induction, but no benefit on survival or acute rejection incidence.[123]

Although used frequently for corticosteroid avoidance, only a few studies report the use of basiliximab and daclizumab for CNI sparing (table III). In 43 paediatric renal graft recipients, addition of basiliximab induction to a CNI-based regimen with or without azathioprine or MMF reduced the incidence of acute rejection (7% vs 26% at 1 year) and spared renal function (GFR 98 vs 75 mL/min/m^2).[151] In a study of CNI avoidance, daclizumab

induction was used with sirolimus, MMF and prednisone maintenance immunosuppression in 34 paediatric renal transplant recipients.[152] Acute rejection rates were 32% at 1 year, and GFR reached a nadir of 60 mL/min at 1 year and then rose thereafter. In three paediatric liver transplant recipients, basiliximab was used to reduce CNI dosage and target low trough concentrations and minimize nephrotoxicity.[153] GFR increased by 69% over baseline GFR <65 mL/min/m^2 to around normal values but one patient experienced acute rejection and one chronic rejection (table III).

There are a number of newer immunosuppressants that have yet to be used in paediatric transplantation for CNI sparing. These include rituximab, a B-cell depleting monoclonal antibody,[154,155] belatacept (LEA29Y), a fusion protein derivative of cytotoxic lymphocyte antigen A-4 (CTLA4-Ig) inhibiting the co-stimulation pathway,[101] and efalizumab, an anti-lymphocyte function-associated antigen-I co-stimulatory blocker.[156]

2.2.3 Mycophenolic Acid

Mycophenolic acid was first licensed in 1995 and has progressively become a key component of many immunosuppressive regimens.[4] It is used both as the mofetil ester (MMF) and increasingly as the enteric-coated sodium salt formulation. Mycophenolic acid inhibits inosine monophosphate dehydrogenase 2, a mediator of guanine synthesis and thereby inhibits lymphocyte proliferation. A number of older studies demonstrating the efficacy and safety of MMF were reviewed by Ettenger and Sarwal.[157] Corresponding studies with enteric-coated mycophenolate sodium are emerging, mainly relating to use in adult renal and heart recipients.[158] Recent reports have confirmed the value and safety of mycophenolate used for supplemental immunosuppression.[159-164] A recurring theme was improved efficacy, judged from a lower incidence of rejection and/or longer graft life, when MMF replaced azathioprine in immunosuppressant regimens. Two studies also showed an improvement in renal function as judged by improved creatinine clearance (CL_{CR})[161] or GFR.[159] None of these studies described reduction in CNI dosage.

However, reductions/withdrawal of CNI medication have been increasingly described in recent reports using MMF and frequently associated with low rates of acute rejection (table IV). Many reports describe introduction of MMF late (4–8 years) post-transplant. In 18 paediatric renal graft recipients with renal dysfunction treated with MMF, Filler and colleagues[165] reported ciclosporin withdrawal in 11 (61%) and a 61% mean ciclosporin dosage reduction in the remainder. Serum creatinine levels fell by 33%. David-Neto et al.[166] withdrew ciclosporin in 31% of 13 children with renal grafts and reduced doses by 28% in the remainder, with a 32% improvement in serum creatinine levels. In two reports from the same paediatric renal transplant centre targeting ciclosporin blood concentrations to 60 μg/L, 50% reductions in dosage were possible following MMF introduction and GFR was stabilized.[167,168] In a group of 19 children with chronic allograft nephropathy, introduction of MMF at 3–84 months post-transplant allowed a 22% reduction in ciclosporin dosage and an 80% restoration of the 32.7 mL/min/1.73 m^2 loss in GFR experienced previously.[169] The benefit of earlier conversion was emphasized in some of these studies (table IV).

In two separate studies of 14 children with liver grafts, Aw et al.[171] associated 75% reductions in ciclosporin concentrations with improvements in GFR in 92%, while Ferraris et al.[172] showed corresponding reductions of 45% in ciclosporin concentrations with 59% improvements in CL_{CR}. In a later study, the same group achieved an approximate 75% reduction in blood ciclosporin and tacrolimus concentrations in 11 patients and reversed CNI-induced renal dysfunction in 82% of these patients.[174] Evans et al.[173] achieved CNI discontinuation in 83% of their cohort of 48 paediatric liver graft recipients and again observed improvements in GFR in 92%. In 14 children with heart grafts introduction of MMF at a mean of 8 years post-transplant allowed a 50% reduction in CNI dosage and a 67% increase in inulin clearance 1 year later[175] (table IV).

Two studies in children receiving renal grafts describe early use of MMF. Harmon and colleagues[152] used induction with IL-2R blockade and a CNI-free regimen of MMF and sirolimus in 34 children and achieved stable GFR post-transplant with 93% graft survival and 32% acute rejection at 1 year. In a recent multicentre trial of paediatric renal graft recipients receiving a ciclosporin plus

Table IV. Use of mycophenolate mofetil (MMF) for calcineurin inhibitor (CNI) sparing in paediatric transplantation

Transplant cohort	Regimen	Outcome	AR (%)	Other	Reference
Paediatric renal (n = 18)	Add MMF to CsA + AZA + CS ± ATG at mean 6.2 y post-transplant	↓ mean Cr 188 to 127 μmol/L; stop AZA	6	CsA dosage at mean 9 mo, ↓ by 61% in 7, withdrawn in 11	165
Paediatric renal (n = 13)	Add MMF to CsA + AZA + CS at mean 5 y post-transplant	↓ mean Cr 2.2 to 1.5 mg/dL; stop AZA	0	CsA dosage ↓ by 28% when MMF added and 50% by mo 18; CsA withdrawn in 4	166
Paediatric renal (n = 19 with CAN)	Add MMF to reduce CsA in regimen ± AZA	GFR increased by 26.7 mL/min/1.73 m^2 6 mo after adding MMF	0.26 vs 1.11 episodes per patient	CsA dosage ↓ by 22%	169
Paediatric renal (n = 34)	DCL induction + early MMF + SRL	100% patient survival; 93% graft survival at 1 y	22 at 6 mo; 32 at 1 y	Stable GFR post-transplant; PTLD 6%	152
Paediatric renal (n = 19)	CsA (12) or TRL (7) ± CS; add MMF late	CNI doses/levels ↓ by 50%	1 late AR	Stable GFR; infection, lipids, BP unchanged	168
Paediatric renal (n = 17)	CsA monotherapy; + late MMF where GFR ↓	CsA doses/levels ↓ by 50% (60 μg/L target)	6	Prevented further ↓ in GFR	167
Paediatric renal (n = 44)	Randomized withdrawal of CsA or MMF for 2 y; CS retained	GFR ↓ >10 mL/min/1.73 m^2 in 73% of patients with CsA vs 29% with MMF	MMF 9, CsA 9 during withdrawal; MMF 11, CsA 0 by 2 y	↑↑ cholesterol mean levels at 2 y: MMF −13%; CsA +3%; haemoglobin ↓ with MMF; BP, serum triglycerides unchanged	170
Paediatric liver (n = 14)	Late addition (mean 5 y post-transplant) of MMF for renal sparing	GFR improved at 6 mo + 1 y in 92% of patients	21	With MMF ± CS, CsA levels ↓ by 75%	171
Paediatric liver (n = 14)	MMF added to CsA + CS	Mean CL_{CR} ↑ by 59% at 2 y	0	CsA dosage ↓ by 37%, levels ↓ by 45%	172
Paediatric liver (n = 48)	Late addition (mean 4 y post-transplant) of MMF for renal sparing in CNI regimens	GFR improved at 1 + 2 mo in 92% of patients	4	CNI discontinued in 83%, ↓ in remaining 17% of patients	173
Paediatric liver (n = 11 of 191)	Add MMF late to CNI regimen where NEF; then reduce CNI dosage	Biochemical renal function improved in 82% of patients	None	No infections; CsA and TRL levels ↓ by >75%	174
Paediatric heart (n = 14)	CNI sparing with MMF at mean 8 y post-transplant	Mean 67% ↑ in inulin clearance at 1 y	NS change	50% ↓ in CNI dosage	175

AR = acute rejection episodes; **ATG** = rabbit antithymocyte globulin; **AZA** = azathioprine; **BP** = blood pressure; **CAN** = chronic allograft nephropathy; **CL_{CR}** = creatinine clearance; **Cr** = serum creatinine level; **CS** = corticosteroids; **CsA** = ciclosporin; **DCL** = daclizumab; **GFR** = glomerular filtration rate; **NEF** = CNI-induced renal dysfunction; **NS** = not significant; **PTLD** = post-transplant proliferative disorder; **SRL** = sirolimus; **TRL** = tacrolimus; ↑ indicates increased; ↑↑ indicates greatly increased; ↓ indicates decreased.

MMF plus corticosteroid triple regimen, randomized withdrawal of ciclosporin or MMF was attempted under increased corticosteroid cover in 44 children.[170] There were two steroid-sensitive episodes of acute rejection in each group during drug withdrawal, which had to be abandoned in 14.3% receiving MMF and 21.7% receiving ciclosporin. Decreases of more than 10 mL/min/1.73 m^2 in GFR at 2 years were noted in 73% of the children continuing to receive ciclosporin and in 29% receiving MMF. Over the same interval, mean cholesterol levels fell by 13% in the MMF group but rose by 3% with ciclosporin, and haemoglobin was lower with MMF than ciclosporin. However, at 2-year follow-up, two patients had experienced a first acute rejection episode after ciclosporin withdrawal; one lost renal function and the other the graft with chronic rejection. The essential message was of short-term benefit with MMF, but not without risk[170] (table IV).

Starting doses of MMF in children of 300–450 mg/m^2 twice daily have been recommended with tacrolimus-based regimens in renal[157] and heart[175] graft recipients, and 10–15 mg/kg twice daily in liver recipients.[171,174] Two-fold higher doses are required during ciclosporin co-therapy because of its potent inhibition of the biliary excretion and enterohepatic recirculation of mycophenolic acid and its metabolites,[91] leading to a seemingly greater total mycophenolic acid clearance.[176] Younger children also require higher MMF doses because of faster clearance.[177] For enteric-coated mycophenolate sodium, 400–450 mg/m^2 twice daily is suggested.[178] Case reports[178,179] indicate that conversion from MMF to this enteric-coated formulation reduces the adverse effects of diarrhoea, nausea and gastrointestinal dysfunction,[158] but it is not yet clear whether the clinically silent weight loss recently reported with MMF in paediatric renal graft recipients might also be reduced with enteric-coated mycophenolate sodium.[180] Concentrations of mycophenolic acid are reported to relate to efficacy and adverse effects, and are considered in greater detail in section 4.[181,182] Pre-dose plasma mycophenolic acid concentrations of 1–3.5 mg/L or post-dose areas under the concentration-time curve (AUC) from 1 to 12 hours (AUC_{1-12}) for mycophenolic acid of 30–60 mg • h/L are recommended.[183-185]

2.2.4 Sirolimus and Everolimus

Sirolimus and its structural analogue everolimus are potent inhibitors of mTOR and downstream events in cell-cycle progression, thus inhibiting lymphocyte proliferation. Sirolimus was first licensed in 1999 and is available in the US for renal graft recipients aged 13 years or older, but its efficacy and safety have been evaluated in many paediatric solid organ transplants.[186-188] Everolimus is licensed in the EU but not the US, and few reports exist on its use. Eisen[189] reviewed its use in adults. Recent reports have evaluated the efficacy of everolimus and sirolimus in paediatric transplantation.[27,152,190-196] An emerging theme is improved efficacy with sirolimus following antibody induction. Two such renal studies reported 100% patient and graft survival, and very low acute rejection incidences (<5%),[190,192] but in a third there was 33% acute rejection and a high (39%) frequency of treatment failures due to adverse events.[152] When used with antibody induction, corticosteroids and tacrolimus in 18 paediatric small-bowel recipients, sirolimus improved graft survival but particularly acute rejection (16.7% vs 73.7% at 30 days).[196] Early initial CNIs may be used to delay treatment with sirolimus and avoid the complications of impaired wound healing associated with its inhibition of fibrogenesis. In a cohort of 66 paediatric renal transplant recipients where sirolimus was added to CNI plus corticosteroids dual therapy, acute rejection occurred in 10.6% and graft survival was 98% at 6 months.[27] Addition of sirolimus also rescued 50% of 16 paediatric liver transplant patients with chronic rejection while receiving a tacrolimus-based regimen.[194] Improved graft function and survival also resulted when sirolimus was added to a tacrolimus-based regimen in 39 paediatric liver and/or small-bowel recipients.[195] Hoyer and colleagues[193,197] found everolimus to be safe and well tolerated in 19 paediatric renal graft recipients.

Significant improvements in renal function have been obtained using sirolimus in CNI-sparing regimens (table V). In four studies in paediatric renal graft recipients, [162,198-200] replacement of CNI with sirolimus late (mean 2.7–4.9 years) after transplant achieved significant improvements in GFR of up to 70% in the short term. One study concluded that earlier conversion to sirolimus and milder chronic

Table V. Use of sirolimus (SRL) and everolimus (ERL) for calcineurin inhibitor (CNI) sparing in paediatric transplantation

Transplant cohort	Regimen	Outcome	AR (%)	Other	Reference
Paediatric renal (n = 18); 50% with CAN	SRL replaced CsA-based regimens mean 4.1 y post-transplant where GFR ↓↓	Mean GFR significantly improved by 32%	6	Adverse effects in 17%; 1 death; no withdrawal of SRL required	199
Paediatric renal (n = 19)	CNI replaced with SRL + MMF/AZA + CS vs minimized-dose CNI + AZA/ MMF + CS in NEF	GFR improved by >20% in both arms	0	CNI dose reduced 39% in minimization arm; 70% of SRL group with dyslipidaemia	162
Paediatric renal (n = 8)	SRL replaced CNI-based regimens at mean 2.7 y post-transplant where GFR ↓	GFR ↑ in 5/8, unchanged in 2	13	Transient HypL; infections in 50% of patients	198
Paediatric renal (n = 29)	Replace CNI with SRL in CNI + MMF + CS	GFR improved for 6 mo after conversion but deteriorated thereafter; final GFR higher with earlier conversion	10	No deaths, no graft loss; SRL withdrawn in 31% of patients	201
Paediatric renal (n = 13)	Late conversion from CsA + MMF + CS to ERL + low-dose CsA ± CS in patients with CAN and ↓ GFR	5% ↑ in mean GFR at + 1 y to 47 mL/min/1.73 m^2	0	50% ↓ in CsA dose; CS withdrawn in 77%; serum cholesterol remained >200 mg/dL	203
Paediatric renal (n = 21)	Late conversion to SRL from CsA ± AZA ± MMF + CS	Mean GFR ↑ at 18 mo in grade I CAN, stable in grade II CAN	0	Mean trough SRL levels of 7 μg/L; 1 withdrawal of SRL; no AEs in 43% of patients; diarrhoea and mouth ulcers common in rest	200
Paediatric liver/kidney (n = 3)	SRL replaced CNI in mixed regimens	Serum creatinine ↓ in each case	0		202
Paediatric liver (n = 45)	SRL added to low-dose TRL ± ATG ± CS – for AR, NEF etc.	TRL withdrawn in 80% of those experiencing bad AE	14	Also enabled CS withdrawal in some; no renal data	204
Paediatric liver (n = 9)	SRL added to mixed CNI-based regimens; 3 with CR, 6 with NEF	Mean GFR ↑ 45%; TRL levels ↓ 50%, withdrawn in 1	0	SRL discontinued in 33% (AE serum cholesterol ↑↑); CR resolved in 3	205
Paediatric liver (n = 38)	SRL added to TRL ± CS; NEF in 11 patients	TRL levels ↓ 44%; mean CL_{CR} rose 33%	No data	SRL withdrawn in 53% due to AE	206
Paediatric liver/bowel/ combined (n = 39)	SRL replaced TRL in various regimens	78% converted to SRL successfully; ≤5 μg/L trough levels	5	Renal improvement if early conversion; little dyslipidaemia	207
Paediatric bowel (n = 16)	SRL used to reduce TRL dose by ≈50% in 15 with NEF	Mean serum creatinine fell from 1.5 to 0.6 mg/dL	Not in NEF cohort	Neutropenia in 25%; HypL in 56%; SRL withdrawn in 8 patients, 5 permanently	208
Paediatric heart (n = 16)	SRL replaced TRL at mean 2.7 y post-transplant	Mixed indications; GFR ↑ in 2 of 3 with NEF	No data	SRL withdrawn in 18.8%; HypL in 38% of patients	209
Paediatric heart (n = 15)	SRL used to reduce CNI in mixed regimens	Mean CL_{CR} ↑ 19.3% at 30 days after conversion	13	CNI discontinued in 33.3% of patients	210

AE = adverse events; **AR** = acute rejection episodes; **ATG** = rabbit antithymocyte globulin; **AZA** = azathioprine; **CAN** = chronic allograft nephropathy; **CL_{CR}** = creatinine clearance; **CR** = chronic rejection; **CS** = corticosteroids; **CsA** = ciclosporin; **GFR** = glomerular filtration rate; **HypL** = hyperlipidaemia; **MMF** = mycophenolate mofetil; **NEF** = CNI-related renal impairment; **TRL** = tacrolimus; ↑ indicates increased; ↑↑ indicates greatly increased; ↓ indicates decreased; ↓↓ indicates greatly decreased.

allograft nephropathy were essential for achieving improvements in renal function.[200] Complete CNI withdrawal was achieved with a low incidence of acute rejection episodes, but with 17–70% experiencing adverse events, especially dyslipidaemia. Recently, Powell and colleagues[201] have attempted earlier conversion (at <12 months post-transplant) in a subgroup of 17 of 29 children with renal grafts where sirolimus was used for CNI sparing. GFR was higher at 6 months after introducing sirolimus the earlier conversion was attempted, but deterioration in GFR was observed in all subjects after 6 months. Larger cohorts may be needed to establish whether adverse effects (acute rejection in 10% and adverse events prompting withdrawal in 31%) are responsible or whether deterioration of renal function is associated with sirolimus-based regimens long term. However, proteinuria was uncommon (unlike in adults) and hypertension was improved.[201] In a small series of three liver-kidney transplants, introduction of sirolimus within the first 3 months post-transplant also abrogated CNI renal impairment[202] (table V).

In liver graft recipients, introduction of sirolimus has allowed 40–50% reductions in tacrolimus concentrations[205,206] or complete drug withdrawal in 80% of those with severe CNI-induced adverse effects.[204] Improvements in renal function were associated both with earlier[205] and delayed[206] introduction of sirolimus, but frequent adverse effects prompted sirolimus withdrawal in 33–50% of subjects. In an extension of one of these studies to include paediatric small-bowel (± liver) graft recipients, 78% were successfully converted from CNI to sirolimus-based regimens, with sirolimus withdrawn in the remainder.[207] Again, earlier conversion favoured relief of renal dysfunction, while adverse effects such as dyslipidaemia were not common. This contrasted with a 56% incidence of hyperlipidaemia in 16 paediatric small-bowel recipients given sirolimus from 13 days to 4.5 years post-transplant for sparing CNI-impaired renal function.[208] Eleven of 15 with CNI-induced nephrotoxicity showed a median 60% fall in serum creatinine levels after tacrolimus dosage reductions, but sirolimus could be continued in only four because the remainder experienced severe complications. Finally, sparing of tacrolimus with sirolimus has been reported in two paediatric heart transplant cohorts. Balfour and colleagues[210] achieved CNI withdrawal in 33% of patients and there was a mean increase of CL_{CR} from 88 to 105 mL/min/1.73 m^2 at 30 days after conversion. Lobach et al.[209] replaced tacrolimus at a mean of 2.7 years after cardiac transplantation for various indications including renal dysfunction. GFR increased in two of three such patients from 43 to 67 and 32 to 106 mL/min/1.73 m^2, but adverse effects in the complete cohort were again frequent and prompted sirolimus withdrawal in 18.8% (table V). Use of everolimus for halting the loss of graft function after renal transplantation in 13 children receiving a ciclosporin plus MMF plus corticosteroids triple regimen was recently described by Pape and colleagues[203] (table V). Ciclosporin dosage was reduced to 50% with the introduction of everolimus, MMF was stopped, and prednisolone was tapered and withdrawn in ten children by 6 months. GFR was marginally but significantly improved, there was a trend to albuminuria, and high serum cholesterol levels were not reduced.[203]

Increased experience with sirolimus has prompted dose reductions to avoid frequent adverse effects and current recommendations for maintenance doses in children are as low as 1 mg/m^2[186] following a higher loading dose. This is much reduced from the 15 mg/m^2/day tapered to 9 mg/m^2/day by 3 months recommended in earlier reports.[211] Corresponding reductions in target sirolimus trough concentrations from 15–25 μg/L to 3–12 μg/L prevail, but changes in dose dependency with age and the prevalence of drug interactions mandate for frequent therapeutic drug monitoring (section 4). A pharmacokinetic interaction with ciclosporin causes elevations in sirolimus levels by up to 85% and has led the manufacturer to recommend delaying sirolimus doses (by >4 hours) when the two agents are co-prescribed.[212] Terminal half-lives for sirolimus are considerably lower in children than in adults (10–24 vs 49–70 hours),[211] rising with age across the paediatric population.[195,211] This has prompted a suggestion for twice-daily dosage in children,[211,213] especially the <5-year age group.[214]

There are fewer data with everolimus, but maintenance doses of 0.8–1.3 mg/m^2 twice daily are recommended together with target trough concen-

trations of 3–8 μg/L[189,193,203] (see also section 4). Similar drug interactions to sirolimus are likely.

2.2.5 Other Agents

Fingolimod (FTY720) is a sphingosine homologue that inhibits lymphocyte trafficking from the thymus and lymph nodes to organ grafts when phosphorylated.[108] However, when tested in ciclosporin versus low-dose ciclosporin plus MMF regimens in a large cohort of 668 adult kidney recipients, it did not support a 50% reduction in ciclosporin dosage and resulted in worse renal function and an increased incidence of macular oedema.[215] Future use for CNI sparing in children is unlikely and fingolimod is now being investigated primarily in multiple sclerosis.

FK778 is derived from the active metabolite of leflunomide, an agent used for treatment of rheumatoid arthritis. It inhibits pyrimidine synthesis via dihydroorotate dehydrogenase and shows synergism with CNIs.[216] Development for transplantation was halted in June 2006.

Gusperimus (deoxyspergualin) is an antiproliferative agent. It is licensed in Japan to treat steroid-resistant acute rejection in renal graft recipients and has been used with plasmapheresis to treat acute humoral renal rejection.[217] No paediatric transplant data are available on its use in CNI sparing, nor on its analogue anisperimus.

Other new agents such as the protein kinase C inhibitor AEB071 and the Janus kinase 3 inhibitor CP690550 simply offer the promise of future benefit in CNI sparing.[101,112]

3. Improved CNI Usage

The choice of CNI, their tailored use in the individuals/populations concerned and optimal monitoring of patients receiving the drugs is considered here. Section 4 discusses the complementary process of immunosuppressant drug monitoring.

3.1 Choice of Primary CNI

Every centre will develop their favoured immunosuppressant regimens based on experience but these should be frequently reviewed to accommodate growing knowledge of existing and novel immunosuppressants, and innovations in their management. For example, the Cochrane Review of randomized controlled trials in adult and paediatric renal transplantation demonstrated the superiority of tacrolimus over ciclosporin in improving graft survival and preventing rejection, but at a cost.[218] The conclusion was that "treating 100 recipients with tacrolimus instead of cyclosporin would avoid 12 experiencing acute rejection and two losing their grafts, but cause an extra five to become insulin-requiring diabetics". Similar findings in liver transplantation were reported recently by McAlister and colleagues.[219] Choices may also be influenced by specific considerations such as the pathogenesis of end-stage disease,[220] the type of graft[221] or the relative frequency of adverse effects (see section 1). However, it is uncertain what are the consequence of changes in drug formulation on such outcomes – as exemplified by the earlier replacement of the conventional formulation of ciclosporin with a microemulsion formulation and the introduction of an extended-release alternative for tacrolimus.[222,223]

3.2 Tailored Use of CNIs

Despite the improved outcome cited for tacrolimus in section 3.1, recent reports specific for paediatric transplantation emphasize that improved efficacy may be achieved with ciclosporin by increasing the dosage to accommodate for inferior drug exposure in liver recipients younger than 12 years.[224] A means of improving ciclosporin delivery using inhalation therapy remains to be tested in a paediatric lung transplant population.[225] For tacrolimus, dosage requirements in the <5- and 5- to 12-year age groups may also be increased by 2.7- and 1.9-fold, respectively, relative to those in patients older than 12 years[226] or from 0.15 mg/kg twice daily to 0.23 mg/kg twice daily in recipients younger than 6 years.[227,228] Conversely, reduced doses of tacrolimus 0.1 mg/kg twice daily have been suggested in paediatric renal graft recipients weighing >40 kg.[229] There are salutary reminders that presumptions about the equivalent efficacy of split drug regimens used for minimizing adverse effects may be counterintuitive.[230]

3.3 Optimal Patient Monitoring

Using conventional renal and liver function tests as an adjunct to histopathology for assessing graft

rejection and organ function may be convenient, cheap and rapid but has significant deficiencies. Improving patient monitoring using markers operating with high sensitivity and specificity is overdue and one such candidate needing wider evaluation is cystatin C as a marker of renal function.[231,232] The new technologies of proteomics and metabololomics offer even more exciting opportunities. Not only do they detect markers at low concentrations with new sensitive technologies but they consider profiles of multiple compounds, so endowing greater specificity than with single markers. Their emerging promise has been summarized in two recent and pertinent reviews.[233,234] Particularly attractive is their potential for monitoring sequential changes within a particular patient, so providing the benefit of early detection of clinical dysfunction in both graft and patient. Such individualization is explored further in section 5.

4. Immunosuppressant Drug Monitoring

The measurement of ciclosporin and tacrolimus drug concentrations has been a valuable adjunct to the clinical management of patients prescribed these agents since their initial introduction.[235] Immunosuppressant drug monitoring (IDM) of mycophenolic acid, sirolimus and everolimus is more recently introduced but nonetheless necessary because of the lack of accurate, comprehensive and real-time measurements of 'immunosuppression', as well as the narrow therapeutic index for these agents and the relationship of drug levels both to efficacy and toxicity. Monitoring sirolimus concentrations is also a condition of its European licence. The process of IDM is imperfect, nevertheless. A recent review highlighted the following three deficiencies: (i) the variable relationships of drug concentrations (pharmacokinetics) to immunosuppressant efficacy and toxicity (pharmacodynamics); (ii) the widespread use of nonspecific immunoassay techniques for immunosuppressant measurements; and (iii) the limited utility of population therapeutic ranges.[40] An additional problem is how best to effectively monitor combinations of immunosuppressant agents. This discussion focuses more on obtaining the best from IDM and in a cost-effective way.

4.1 Concentration Monitoring for Immunosuppressant Drug Monitoring

Immunosuppressant concentrations in peripheral blood are assumed to reflect those in the graft, where they exert their effects, but a recent report showed tacrolimus concentrations in tissue correlated better to histological events.[236] Nevertheless, drug concentrations in both graft and blood show substantial interindividual variability due to the following three major determinants: (i) differences in the extent of absorption, metabolism and excretion; (ii) the impact of the function of both transplanted and other organs; and (iii) quantitatively unpredictable interactions with a plethora of interacting agents used commonly in transplant recipients.[109] Variability in drug concentrations may also have a genetic component (section 5). All affect immunosuppressant exposure, typically calculated from the AUC of concentrations in blood or plasma over one interval between successive doses. This AUC is most accurately calculated from a series of 8–12 blood samples but its measurement in this way is both impractical on a routine basis and prohibitively expensive, so surrogate markers of AUC are used instead. These are often single samples whose concentration is proportional to AUC, or combinations of small numbers of samples that estimate AUC with greater accuracy but at a greater cost of time and resources. The most commonly used single surrogate samples, 'trough concentrations' are those taken just pre-dose – a corresponding 12 or 24 hours after once- or twice-daily drug administration, respectively – and termed C_0 (or C_{12} or C_{24}) samples. Immunosuppressant drug trough samples are taken at a time when the absorption and distribution of the drug is usually complete and clearance decreases concentrations of the drug in proportion to time. This gives trough concentrations some practical benefit because they change relatively slowly and with some predictability (a proportional decrease) over increasing time. Therefore, reporting the time of the sample post-dose assists interpretation of results.

A second single surrogate marker of AUC is the peak drug concentration (C_{max}) and this correlates with AUC more closely than does C_0.[237] However, the exact time of C_{max} is not known unless measured, and assumptions of the time post-dose can

provide considerably discrepant results at a time when drug concentrations are changing rapidly and unpredictably. The final surrogate is an abbreviated AUC based on estimation using a reduced (usually 2–4) number of samples, termed a limited sampling strategy (LSS). The timing of these samples is defined in a trial group of patients and usually statistically validated in a second similar cohort.[238]

The frequency at which any type of IDM is performed generally decreases with increasing time post-transplant but increases when the clinical condition of the patient deteriorates. In children, where shorter immunosuppressant drug half-lives and rapid changes in well-being are frequent, daily IDM may be indicated in the early post-transplant interval decreasing to 3- to 6-monthly if stable. Otherwise, changes in any of the following parameters may indicate a requirement for IDM, and regular testing should continue until stability returns: (i) immunosuppressant dosage; (ii) acute rejection or other type of graft dysfunction; (iii) evidence of immunosuppressant-related adverse effects; (iv) impaired renal, liver, bowel or haematological function (affecting drug absorption, distribution, metabolism and excretion); and (v) changes in co-medication (especially those affecting cytochrome P450 [CYP] 3A or multiple drug resistance protein 1 [MDR1] {P-glycoprotein} for the CNI and mTOR agents and uridine diphosphate-glucuronyltransferase [UGT] for mycophenolic acid). Increasing age and growth spurts may also change immunosuppressant requirements. The following sections describe specific considerations for IDM of ciclosporin, mycophenolic acid, sirolimus plus everolimus and tacrolimus.

4.1.1 Ciclosporin

Trough concentration monitoring was the standard method for ciclosporin[239] until the replacement of the conventional formulation in the mid 1990s with a microemulsion formulation. The microemulsion formulation provides enhanced and more consistent ciclosporin absorption and prompted several studies of outcome after renal and liver transplantation related to monitoring concentrations at 2 hours post-dose (C_2 monitoring) and a guide for implementation.[240] C_2 is a surrogate estimating early ciclosporin exposure ($AUC_{0–4}$) and provides an assessment of ciclosporin absorption that associates well with both rejection risk and adverse effects in adults and children.[240-242] The recommended therapeutic ranges clearly differ from those using C_0[242] and may need local refinement to accommodate preferred regimens. Practical disadvantages are the requirement for strict compliance with blood sampling at 2 hours ± 15 minutes and the inability to differentiate poor absorbers (with low C_2 results) from those with delayed but otherwise normal absorption ('slow absorbers').[240] Such characteristics are frequent after paediatric liver[224] and heart[243] transplantation. A number of LSS for ciclosporin (listed in Ting et al.[238]) also exist, but a critical evaluation of the benefit to patient outcome of approaches other than C_0 monitoring of ciclosporin concluded that the supporting evidence was weak.[244]

4.1.2 Mycophenolic Acid

Mycophenolic acid concentration monitoring has been advocated on the basis of the criteria discussed at the start of this section, including extensive intraindividual variability in its pharmacokinetics[245,246] and genetic variants in UGT, its major metabolic pathway.[247] In contrast, categorical (fixed) dose administration has also been recommended (in the initial MMF package leaflet[115] and cited in Holt[248]). Consequently, formal trials started in 2003–4 to compare the impact on outcome of these two approaches. Three large studies in renal transplantation have compared MMF usage in fixed dosage versus concentration-controlled regimens – the FDCC (Fixed Dose vs Concentration Controlled) international trial in 69 centres,[249] the OPTICEPT trial in 65 centres in the US[250] and the APOMYGRE (Adaptation de Posologie du MMF en Graffe Renale) study in 11 French centres.[251] In these studies, outcomes using fixed dosage of MMF (2 g/day) plus CNI plus corticosteroids ± antibody induction were compared with those in cohorts in which MMF dosage was adjusted on the basis of exposure – trough concentrations or AUC estimated by LSS. Final results are available only for the APOMYGRE study and this showed significantly fewer treatment failures and acute rejection episodes in the monitoring arm with no significant differences in adverse event frequency.[251] Mycophenolic acid exposure and MMF doses were higher in the concentration-controlled arm, in which dose adjust-

ment was based on Bayesian forecasting using three blood samples over the first 3 hours post-dose. This estimation model is accessible electronically.[252] A cost analysis also demonstrated effective neutrality in terms of monitoring costs versus those incurred in managing additional complications in the fixed-dose cohort.[253] Interim results of the FDCC study have shown no benefit of monitoring, but also no increased dosage adjustments in the monitoring arm.[249] Similarly, the OPTCEPT trial also showed no benefit of monitoring, but no differences in trough mycophenolic acid concentrations between the different cohorts.[250] These contrasting negative results may therefore underlie some resistance to making dose changes on the basis of mycophenolic acid results alone. There may also be a cogent demand for more frequent monitoring than the three occasions during the first month used in these trials because mycophenolic acid pharmacokinetics may be especially susceptible to the impact of a number of known modulators during this early post-transplant interval.[183] These include changes in CNI comedications,[254,255] corticosteroids,[256] oral antimicrobials,[257] serum albumin concentrations,[183] and renal[183] and liver function.[258] Ontogeny is another variable of significant influence in children.[183]

Further evidence in favour of mycophenolic acid monitoring derives from associations of systemic mycophenolic acid concentrations with both efficacy in preventing rejection[181,182,259] and toxic adverse effects[181,182,260] in liver and renal graft recipients, including children.[261] AUC monitoring (with a target of 30–60 mg • h/L) appears more sensitive than C_0 (target of 1–3.5 mg/L) in assessing outcome,[183] but its practical constraints have led to a wider consideration of LSS, many of which were reviewed recently.[185,238] Willis and colleagues[262] have identified differences between theoretical and actual timing of key features in the AUC profiles (such as C_{max} and peaks of enterohepatically recirculated mycophenolic acid) as major sources of estimation error in LSS, which may reduce their comparability. This enforces potential dangers in using LSS for mycophenolic acid monitoring in a patient population other than the one in which it was developed.[238]

A final question with mycophenolic acid monitoring is its utility during prescription of enteric-coated mycophenolate sodium, the enteric-coated formulation which exhibits delayed and unpredictable multiple peaks of absorption.[263] These may make LSS monitoring impractical and interfere with conventional C_0 monitoring at 12 hours post-dose. Further evidence is needed to clarify monitoring requirements and optimal techniques with this formulation.

4.1.3 Sirolimus and Everolimus

Sirolimus and everolimus have both shown concentration-related efficacy and toxicity in clinical trials[264,265] and IDM is recommended in children.[186,193] Everolimus is usually given twice daily[107] and targeted to trough concentrations at 12 hours post-dose of 3–8 μg/L, but sirolimus is administered once daily because of its longer half-life and 24-hour trough concentrations of 3–12 μg/L are targeted.[264,265] However, twice-daily administration of sirolimus should be considered if such therapeutic concentrations are difficult to achieve because of faster metabolism in children,[211,213] particularly the <5-year age group.[214] An LSS for sirolimus based on samples at 0, 2 and 6 hours after sirolimus administration was devised in adult renal transplant recipients but a target AUC was not defined and the approach remains unvalidated in paediatric populations.[266] Variables affecting drug concentrations include hepatic impairment[267] and fatty food, which affects sirolimus absorption and dictates that food should be taken at consistent times in relation to therapy.[268] Also affecting sirolimus concentrations are corticosteroids and mycophenolic acid,[269] and ciclosporin.[212] The converse influence of sirolimus on tacrolimus bioavailability is particularly influential in children[270] and more persistent than in adults.[237]

4.1.4 Tacrolimus

Trough concentration monitoring is the customary technique for monitoring tacrolimus. Samples taken at 12 hours post-dose are used during conventional dose administration and at 24 hours with the sustained-release formulation. In 18 paediatric liver graft recipients, conversion to the sustained-release formulation was achieved using a 1 : 1 ratio of daily doses and this provided AUC equivalence and trough blood concentrations 92% of those with the conventional formulation.[271] The same therapeutic

range of tacrolimus concentrations up to 12–15 μg/L is used with both formulations depending on time post-dose, assay and transplant indication. Although early studies demonstrated no consistent benefit of alternative single times to C_0 in predicting AUC,[237] recent reports have shown the opposite.[238,272] The use of LSS provides even closer prediction of AUC, notwithstanding use in different patient populations.[272] However, only one estimation based on the mean of C_0 and C_2 values has been used in paediatric graft recipients[226] and none of the LSSs have been validated using the sustained-release formulation.

4.2 Pharmacodynamic Monitoring for Immunosuppressant Drug Monitoring

Pharmacodynamic assays have so far failed to live up to the promise that these functional indicators of immunosuppression imply. They are frequently limited both by the availability of suitable tissue (ideally from the graft) and an appropriately rapid turnaround time – especially where lymphocyte cultures are used. In addition, assays such as *in vitro* calcineurin inhibition[273] may fail to consider the multiple immunosuppressive consequences of the agent to which they relate. However, new candidates with some interesting potential are the Immunknow™ assay for quantitating immunity mediated via stimulated CD4+ T cells[274,275] and IL-2 messenger RNA synthesis in isolated lymphocytes incubated with CNI and determined by real-time quantitative polymerase chain reaction technology.[276]

5. Genetic Markers for Individualizing Therapy

Associations between single nucleotide polymorphisms and outcome are reported with increasing frequency in disease and therapeutics. Those in transplantation are not routinely considered in patient management but have considerable potential for individualizing therapy and selecting immunosuppressant regimens. Examples relating to the efficacy and adverse effects of immunosuppressants, as well as favouring the induction of tolerance[277] are listed in table VI. The vast majority of this work emanates from studies in adult graft recipients.

Gene variants have been associated with many aspects of transplant outcome[234,279,306] including the immune response,[278,283] cell signalling,[280] cell injury and death,[281,296] and immunosuppressant efficacy.[282,284,285,307] Genetic associations also influence the generic adverse effects of immunosuppression such as infection and malignancy. For example, recent reports suggest that variants of Toll-like receptor genes *TLR 2* and *TLR 4*,[286,287] and mannose-binding lectin[288] increase bacterial and viral infection risk, and may also affect graft loss and mortality. Squamous cell carcinoma incidence in renal transplant recipients[289] and the recurrence of hepatocellular carcinoma[290] also show a variability related with genetic associations in metabolism and drug transport (table VI).

CNI-related adverse events show genetic associations independent of or linked to drug concentrations. Ciclosporin-specific gingival overgrowth was more frequent in renal transplant recipients with an IL-1A high activity variant.[89] Renal toxicity and dysfunction have been linked to high activity variants of angiotensin-converting enzyme[291] in liver graft recipients, to high producers of transforming growth factor β in paediatric heart transplant recipients[308] and to low activity expression of methylene tetrahydrofolate reductase in chronic allograft nephropathy.[293] Khan and colleagues[279] comprehensively reviewed other genetic factors predisposing to renal allograft failure.

Concentrations of CNIs within tissue are linked to the activity of CYP3A5 and MDR1 (P-glycoprotein), both of which show genetic polymorphism in their respective genes *CYP3A5* and *ABCB1*. For example, a startling odds ratio of 13.4 for ciclosporin-induced nephrotoxicity was recorded in renal transplant patients who accumulate ciclosporin after receiving kidneys from donors with the low activity MDR1 variant *ABCB1 3425TT*.[292] The large number of additional studies recording the impact of *ABCB1* and *CYP3A5* variants in both donor grafts and the recipients of heart, liver, lung and renal transplants have been reviewed recently.[309] There are comparable data on UGT variants impacting on mycophenolic acid kinetics[305,310] but this does not support a relationship to gastrointestinal adverse effects[311] unlike the well recognized

Table VI. Genetic variants associated with transplant outcomes

Variant protein/gene	Population at risk	Consequences	Risk	References
Immunosuppressant efficacy				
Cytotoxic T lymphocyte antigen 4 *CTLA-4* (A49G and G6230A)	Liver graft recipients	Increased risk of post-transplant rejection	RR 1.34 per allele	278
Immune response genes, cytokines, chemokines/receptors, adhesion molecules, growth factors, tissue injury, etc.	All transplant recipients and others prescribed immunosuppressants	Variability in rejection, tissue damage and repair	Various	234,279-282
Metabolic/receptor variants glutathione S-transferase T1/ glucocorticoid receptor *IMPDH2 3757C*	All transplant recipients and others prescribed corticosteroids; renal graft recipients	Steroid resistance? Increased rejection risk	Qualitative; OR 2.99	283-285
Immunosuppressant-related adverse effects				
Infections				
Toll-like receptors *TLR 2* (Arg753Gln) *TLR 4* (D299G and T399I)	HCV-infected liver graft recipients; CMV-exposed graft recipients	753Gln increases graft loss/mortality; variants increase risk of CMV infection/disease	RR 5.2; OR 5.84	286,287
Mannose-binding lectin *MBL2* (coding and non-coding variants)	Liver graft recipients	Infection risk increased where liver donor *MBL2* variants present	× 3.8-fold	288
Neoplasia				
Methylene tetrahydrofolate reductase *MTHFR* (C677T)	Renal graft recipients	Increased risk of squamous cell carcinoma with *MTHFR 677T*	OR 2.54	289
Multiple drug resistance protein 1 (MDR1) [P-glycoprotein, ABCB1] *ABCB1* – exon 21: G2677A	Liver graft recipients	Reduced recurrence of hepatocellular carcinoma in carriers of *2667A*	OR 0.374	290
Renal dysfunction				
Angiotensin-converting enzyme *ACE* (intron 16 deletion)	Homozygous carriers of the angiotensin-converting enzyme deletion	Greater susceptibility to CNI-nephrotoxicity and CRF	× 4 risk of CRF	291
MDR1 (P-glycoprotein, ABCB1) *ABCB1* – exon 26: C3435T	Renal graft recipients	CsA nephrotoxicity more prevalent where donor genotype is *ABCB1 3435TT*	OR 13.4	292
Methylene tetrahydrofolate reductase *MTHFR* (C677T)	Renal graft recipients	Predisposes to chronic allograft nephropathy	OR 3.91	293
Transforming growth factor 1 $TGF_{\beta 1}$	High $TGF_{\beta 1}$-producing organ graft recipients	Greater susceptibility to renal dysfunction	Significant difference	9

Continued next page

Table VI. Contd

Variant protein/gene	Population at risk	Consequences	Risk	References
Miscellaneous				
Interleukin (IL)-1A *IL1A* (A-889T)	Transplant recipients treated with CsA	Gingival overgrowth	Significant difference	89
CTLA-4 *CTLA-4* (A49G)	Kidney transplant recipients	Gingival overgrowth: decreased with 49G allele	2-fold	294
IL12 (IL12 p40) *IL12B* (rs 3212227): A1188C	Kidney transplant recipients	CMV reactivation increased in 1188C low IL12 expressors	OR 1.52	295
Fas (CD95) Apo-1/Fas (A-670G)	Liver transplant recipients	Impaired graft survival in –670AA homozygous recipients at >11 mo post-transplant	Significant difference	296
Various immune and cell cycle genes	Liver transplant recipients	Gene signatures define tolerance vs immunosuppression dependence	Qualitative	277
Drug exposure				
Cytochromes P450 3A *CYP3A4*1B* vs *CYP3A5* 3*	All transplant recipients and others prescribed immunosuppressants	Altered metabolism of CsA, ERL, SRL, TRL (?FK778); Africans more prevalent for 3A5*1 – faster metabolism and need higher doses	Qualitative	297-299
Multiple drug resistance protein 2 *MRP2* (C-24T and C-3972T)	All transplant recipients and others prescribed MPA	Higher MPA exposure (leads to reduced liver dysfunction/greater gastrointestinal adverse effects) in renal graft recipients with variants (–24T and –3972T)	Qualitative	300
MDR1 or P-glycoprotein (*MDR1 or ABCB1*) *ABCB1* – exon 12: C1236T *ABCB1* – exon 21: G2677AorT *ABCB1* – exon 26: C3435T	All transplant recipients and others prescribed immunosuppressants	Reduced MDR1 expression in variants; increased exposure to drugs because transport impaired; increased tissue concentrations	More variable responses to CsA, ERL, SRL, TRL	283,298,301,302
Thiopurine methyltransferase (TPMT) [297] *TPMT*2*, *TPMT*3A*; *TPMT*3C* and less common variants	Homo- and heterozygous and compound carriers of TPMT variants	Impaired TPMT activity and clearance of its substrates, e.g. azathioprine, mercaptopurine, leading to toxicity	Variable with gene load	303,304
Uridine diphosphate-glucuronosyltransferase *UGT1A9* promoter variants *UGT1A9*3* *UGT2B7*	Recipients of mycophenolate (MMF or MPA)	*UGT1A9* promoter variants increase MPA glucuronidation; *UGT1A9*3* carriers show slower rates of MPA glucuronidation; *UGT2B7* variants affect acyl MPAG production	Qualitative	247,305

CMV = cytomegalovirus; **CNI** = calcineurin inhibitor; **CRF** = chronic renal failure; **CsA** = ciclosporin; **ERL** = everolimus; **HCV** = hepatitis C virus; **MPA** = mycophenolic acid; **MPAG** = mycophenolic acid glucuronide; **OR** = odds ratio; **RR** = relative risk; **SRL** = sirolimus; **TRL** = tacrolimus.

association of variants in thiopurine methyltransferase[303,304] with myelosupression (table VI).

6. Conclusion

Evidence continues to grow for both the safety and efficacy in children of regimens incorporating the new generation of immunosuppressant agents. Following the precedent set in adult solid organ recipients, studies are now reporting paediatric experience of these agents for CNI sparing or replacement and demonstrate encouraging benefit.

Some of the principles emerging may echo those established from the more abundant data in adults. For example, early introduction of CNI-sparing protocols – for example at 3 months after transplantation – ensures better responses before nephrotoxicity becomes chronic, irreversible and progresses to a major loss of renal function. So far, the corresponding factors predictive of a favourable response to CNI sparing in children are ill defined. Whether recognized problems with medication adherence in paediatric patients might be an additional problem in changing drug regimens also remains unclear.[312] In adults, ciclosporin sparing with MMF is best attempted while CL_{CR} is above 45 mL/min, and proteinuria <0.3 g/24 h, while conversion later than 6 years post-transplant appears futile.[313] Further guidelines in adults remaining to be proven in children suggest that at least some CNI exposure is appropriate when using MMF or sirolimus for CNI sparing early after transplantation.[314] However, residual corticosteroid use may enable replacement of CNI with MMF in paediatric liver graft recipients[173] and substantial CNI dosage reductions are proven using MMF in children (table IV).

The adult experience with sirolimus in CNI sparing reported a slight benefit to CL_{CR} (+6.4 mL/min in a meta-analysis), but with a 3–4% incidence of acute rejection.[315] The relatively few paediatric studies with sirolimus have encountered a high incidence of adverse events (table V) and prompt a reconsideration of appropriate dosage, not least in the context of the distinct pharmacokinetic features of sirolimus in children, as well as a delayed introduction to avoid impairment in wound healing. Depleting and non-depleting antibodies are still poorly evaluated, with the limited paediatric experience suggesting that CNI avoidance after antibody induction may be associated with an unacceptably high incidence of acute rejection episodes (table II).

With sirolimus and indeed with all the additional agents emerging, there is a need for well controlled trials, probably undertaken at multiple centres to achieve the required weighting. Those centres unable or preferring not to participate might instead consider adopting improved practices for CNI usage and monitoring. None should ignore the potential for tailoring individual therapy according to the susceptibility for adverse outcomes as determined from a growing list of genetic determinants.

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