Chapter 7

Summary, General Discussion and Future Perspectives
Chapter 7

The interaction between CMV and its human host is complex and constitutes many levels. Millions of years of co-evolution have enabled CMV to manipulate the extent and quality of the host immune system, which allows it to establish latency and cycles of lytic infection [1]. CMV does not completely eliminate host immunity, but modulates it to overcome one of the broadest and strongest T cell responses analyzed to date [2]. This is essential in the life cycle of the virus, and ensures survival of both virus and host [1]. However, the success of this intricate and delicate balance between CMV and host is dependent on immunocompetence of the host. This prerequisite is clearly unfulfilled under immunosuppression, and results in significant morbidity and even mortality in recipients after renal transplantation [3].

Here we performed clinical and experimental studies to better understand the impact and mechanisms underlying human cytomegalovirus (CMV) infection, the most important infection in renal transplant recipients. These studies entail the level of the patient, tissue and cells, so to be able to explore both the functional outcome and the potential mechanisms. This provides additional insights into the effects of CMV that can ultimately contribute to maintain kidney function and improve the outcome of renal transplantation.

In chapter 2 we have demonstrated the intermediate and long-term irreversible and sustained loss of renal function after pronounced CMV DNAemia, the most generally applied method for detection and diagnosis of CMV in the clinic. A CMV peak viral load (PVL) exceeding 6310 international units/ml was associated with a lower eGFR (estimated glomerular filtration rate) independently of recipient, donor and transplantation characteristics. This indicated that recipients have long-term sequelae up to three years post-transplantation and that CMV-induced renal damage does not recover. It is important to consider that most likely not the infection in itself, but the extent of CMV DNAemia and its indirect effects affect renal function. Ideally, regular and intensive monitoring would allow physicians to contain CMV infection before it exceeds the critical threshold, which was defined by us as a PVL of 6310 IU/ml. The importance of containing CMV infection is also reflected in chapter 3, where we studied virus burden by quantifying CMV using the duration of infection. We found that prolonged CMV DNAemia was associated with increased risk for graft failure, both overall and death-censored, as well as with decreased eGFR at 12 months post-transplantation. This indicates that CMV-induced renal function decline can result from a higher extent of DNAemia, but alternatively from its extended duration.

This threshold of 6310 IU/ml (chapter 2) might be applied in the clinical setting, but commutability of CMV assays between institutes remains a challenge. This is due to the variability in for example specimens, amplification targets and instrumentation used amongst others [4, 5], and the collective of methods that are applied [6]. For example, the type of infection could affect in which blood compartment CMV is most productively detected, making it essential to monitor subjects using only a single type of specimen [7, 8]. Quantitative differences in limits of detection and reported results [5] lead to difficulties comparing findings between institutes and establishing
universal guidelines for intervention [6, 9]. This substantiates the necessity for comparable benchmark thresholds for CMV detection and intervention, so to ensure quality of patient care. Despite an international reference standard [6, 10] and FDA approval for a universal assay of CMV quantification in plasma [11], the challenges of harmonization remain. Focus on characterizing and improving commutability of CMV detection are thus essential to enable portability of results, standardized therapeutic thresholds and uniform interpretation [6].

Additionally, the lack of straightforward interpretation of detected viral loads for CMV disease and the absence of established consensus on the exact threshold for initiation of antiviral therapy, complicate interpretation of CMV infection [12]. This can result in overtreatment with accompanying unnecessary side-effects and high therapeutic costs on the one hand, while on the other hand it may cause undertreatment and progression towards symptomatic disease [13]. With that, the precise immunosuppressive regimen varies substantially between transplant institutions and consensus on the optimal immunosuppressive regimen is lacking [14]. It is therefore important to take the background of the recipient, immunological risk and drug characteristics into account when determining the specific threshold for every individual recipient.

Serological diagnosis of CMV infection after renal transplantation is complex since immunosuppression inhibits the immune response from developing according to its natural course. This contributes to preventing rejection of the transplanted organ, but additionally complicates interpretation of immunological parameters. The humoral and cellular arm of the immune system are in constant interplay, providing an important role for anti-CMV antibodies indirectly. Preformed CMV antibodies may aid immunosuppressed patients to prevent or limit dissemination during reactivation, but their exogenous administration showed only limited benefit [15]. It is important to realize that viral replication occurs intracellularly and therefore NK cells and the cellular immune response are pivotal to restrain infection, which cannot be directly attenuated by antibodies alone [16]. Nevertheless, antibody production is essential for the fetus in preventing congenital infection and for premature infants to impede transfusion-associated infection [15]. Also, positive results have been achieved with vaccines that stimulate neutralizing antibodies, for instance against the glycoprotein H complex [17].

Interestingly, our studies indicate that longer time-to-CMV IgM seroconversion, so a delayed CMV IgM seroresponse, was related to decreased eGFR and more pronounced graft failure (chapter 3). It suggests that the initiation of the CMV IgM response after infection provides information about the extent of renal damage, supposedly caused by CMV. Clinically, primarily CMV IgG antibodies are used, to determine previous history of CMV infection before transplantation. Now, measuring CMV IgM at 25 days post-infection (the median anti-CMV IgM seroconversion time) could provide an intermediate indication on the initiation and maturation of the adaptive immune response, and whether intervention is necessary. Although a direct role for CMV IgM in diagnoses is not likely, it could follow the CMV PCR
used to determine the recipient’s CMV infection, and supplement it regarding development of the immune response. It could indicate the necessity for adapting therapeutic intervention, for instance the anti-CMV medication and tapering of immunosuppression.

Reduction of immunosuppression, particularly MMF, is a strategy to potentiate and reinstate antiviral immunity of the host. This study demonstrated that prolonged duration of MMF treatment associated to extended CMV DNAemia, a delayed CMV IgM and CMV IgG seroresponse and decreased eGFR at 12 months post-transplantation (chapter 3). In this subpopulation of recipients, withdrawal of MMF is potentially beneficial for shortening CMV DNAemia and development of a CMV immune response without additional risk for cellular rejection. It could point towards an adapted strategy regarding MMF treatment to allow the recipient’s immune system to thrive against the viral infection. Regular measurements of CMV DNAemia and anti-CMV IgM could distinguish those subjects displaying a slow immune response who are more prone to infectious complications, and identify subjects eligible for withdrawal of MMF early.

Next to studying the effect of withdrawing immunosuppressive medicine, we were interested to explore the immunoregulatory potential of the body itself, specifically by regulatory B cells. Down-regulation of these cells could provide an alternative approach to withdrawal of immunosuppressive therapy, which we studied for MMF in chapter 3. In chapter 4 we evaluated B cell phenotyping as a measure of predicting transplant rejection in renal transplant recipients. Pre-transplant transitional CD24hiCD38hi or memory CD24hiCD27+ B cell subsets, presumably containing a regulatory B cell population, did not differ between subjects with biopsy-proven acute rejection (BPAR) and those without, neither by percentages, absolute numbers or survival free from BPAR. A potential reason is that it is still undetermined whether Bregs actually represent a dedicated lineage within the B cell population, or gain regulatory capacity in response to their distinct microenvironment or particular B cell receptor [18]. Alternatively they could result from the expansion of short-lived effector cells in response to inflammation, or differentiation secondary to resolution of the inflammatory response. Although cytokines and cell surface markers for Bregs have been identified, no lineage–specific molecular marker can uniquely identify regulatory B cell subsets [19]. This may result in biased model systems investigating heterogeneous populations of suboptimally defined regulatory cells, that are mixed with other B cell sub–populations from various developmental states, anatomic locations and functional capacity [18].

Although B cell subsets did not vary for CMV seropositive or seronegative recipients before transplantation, post-transplantation CMV infection was more prominent for patients with the highest percentage of transitional CD24hiCD38hi B cells (chapter 4). CMV peak viral load as measure for infection did not correlate to percentage of transitional CD24hiCD38hi or memory CD24hiCD27+ B cells. From
these association studies it is difficult to comment on the mechanism underlying the role of transitional CD24hiCD38hi and memory CD24hiCD27+ B cells on CMV infection post-transplantation. Nevertheless, we could speculate that negative regulation of the innate and adaptive immune responses by Bregs may indirectly also suppress protective host responses against pathogens and increase susceptibility to infections. For instance, the frequency of Bregs positively correlated with enhanced viral replication of chronic hepatitis B virus [20] and IL-10 levels were temporally closely associated with viral load [21].

Further studies exploring the relationship and mechanism between Breg homeostasis and CMV infection should be conducted to provide further insight. These could include studying Bregs in co-culture with CMV antibody-producing B-cells in vitro, the temporal IL-10 (and potentially cmvIL-10) production after transplantation and its correlation with peak viral loads. Adopting the theory that Bregs gain regulatory capacity in response to their distinct microenvironment, it would be very insightful to determine whether inhibition of this maturation is favorable for preventing CMV infection. The frequency or composition of the regulatory B cell compartment may serve as a minimally invasive monitoring tool to guide immunosuppressive strategy and identify recipients eligible for tapering of immunosuppression (for instance MMF in chapter 3) [22]. Personalization may prevent over-immunosuppression accompanied by malignancies, infections and cardiovascular events on the one hand, and under-immunosuppression with consequent acute and chronic rejection on the other [21].

Although it is unlikely to find large-scale practical application on the short term, immune regulation and principles learned from clinical tolerance are expected to provide important tools for transplantation in the future. Nevertheless, the effect of immunosuppression and the validity of tolerance biomarker signatures should be further characterized before introduction into clinical practice [22, 21]. This should be constrained by stringent quality restrictions considering the risk for acute rejection or graft damage [23]. While awaiting such comprehensive tolerance signatures, Breg characterization and cytokine profiling may provide surrogate markers to gauge immune regulation.

One of the characteristic properties of CMV is its immunomodulatory capacity, which allows it to modulate the host immune response. An alternative strategy to limit the detrimental effects of CMV infection would thus interfere with this capacity, to make it more vulnerable to clearance by the immune system. We decided to focus on CMV-encoded G protein-coupled receptor US28, given its most extensive characterization and potential as accessible target for intervention.

In chapter 5 we have demonstrated CMV-encoded viral G protein-coupled receptor US28 in vessels during productive and latent infection. Its expression was restricted to vascular smooth muscle cells and tubular epithelial cells after renal transplantation, while permissive infection was not restricted to a particular renal cell type. The segmental localization of US28 suggested that CMV regulates antigen
expression in response to, or interaction with, the specific microenvironment that the infected cell resides in. Against our expectations, US28 and immediate early antigen expression regularly did not overlap. This is an intriguing finding, for which conclusive insight into the underlying mechanism is currently lacking. However, immediate early antigen may be favored during the initial infection when the virus focuses on host cell control and immune evasion, while US28 expression in vascular smooth muscle cells might enable further infiltration of the underlying tissue or neointima.

By committing a substantial portion of its genome to immune modulation and evasion, CMV gains a temporary advantage that protects it from the antigen-specific host immune response and allows release of viral progeny, infection of neighboring cells and dissemination to more distant sites [15]. Recently, it was demonstrated that next to its multifunctional role during productive infection, US28 is also important during latency [24]. Despite vast limits to the viral transcription machinery during latency, a selection of proteins is expressed and profoundly affects the infected cells and cellular microenvironment. Intriguingly, expression partly overlaps with that during productive infection, which makes one wonder about why latently infected cells are immunoprivileged and not effectively targeted [25]. A mutant CMV solely expressing US28 (without the other three CMV-encoded GPCRs) could maintain latent infection, while a US28-deficient CMV induced the major immediate early promoter and production of infectious virus [24].

Development of suitable in vitro approaches for latency would yield great benefit for translating in vitro findings on CMV into clinical practice. It remains essential to determine whether CMV latency serves ‘just’ as shutdown of normal replication or as a process which is latency-specific. Is it interrupted active infection induced deliberately by the virus or is it forced upon the virus by the immune system? If the former is the case, does US28 play a role in this? What is the potential for therapeutic intervention? These considerations are expected to further expand our understanding of CMV latent infection and reactivation and explore it as important targets for future therapy, especially since latency often precedes clinical manifestation of disease [24].

We also showed that US28-deficiency significantly impeded viral spreading in vascular smooth muscle cells, particularly through cell-to-cell contact (chapter 5). US28 expression in VSMCs might enable spreading of CMV by binding to chemokines immobilized on neighboring cells, such as CX3CL1 (fractalkine) [26]. Since VSMCs in atherosclerotic lesions express membrane-bound CX3CL1 to a high extent, US28 could promote cell-to-cell interactions and promote viral spreading [27]. This would be a suitable focus for intervention, targeting the binding location for CX3CL1 and preventing US28 activation. Even though 26% of all registered drugs are directed against G protein-coupled receptors, these drugs modulate only 7% of all underlying targets and leave the large majority of targets unaffected [28]. Several non-peptide compounds can inhibit ligand binding to US28 directly or as allosteric modulators, or reduce the constitutive activity of US28 [29]. Although promising
as therapeutics for anti-viral intervention, their micromolar potency limits *in vivo* applicability [30]. The increased interest in llama-derived antibodies targeting GPCRs and more particularly chemokine receptors, so called nanobodies [31, 32] [33], provides opportunities to modulate (viral) chemokine receptor function.

From our findings on the localization of US28 in transplant biopsies and *in vitro* viral spreading experiments, we were interested to further characterize the functional effects of US28 on vascular smooth muscle cells (chapter 6). To study US28 in detail, an *in vitro* inducible US28 model system of vascular smooth muscle cells (VSMC-iUS28) was developed. The US28 expression levels in iUS28-VSMCs resembled those of WT CMV-infected VSMCs, suggesting it provides a suitable model system for US28 after CMV infection. Expression of US28 resulted in elevated secretion of VEGF and L-lactate, which suggests transition to a more dedifferentiated phenotype. Since upregulation of VEGF stimulates angiogenesis and the VEGF receptor, and angiogenesis in the intima could contribute to coronary artery vasculopathy, this supports a role for US28 in transplant vasculopathy [34, 35]. The suspected lower expression of α-SMA in CMV-infected compared to non-infected VSMC points towards dedifferentiation away from its contractile phenotype. Hence, these findings support a potential US28-dependent phenotypic switch of VSMCs to a more proliferative and synthetic phenotype.

US28 expression on the virion, cell surface and during latency make it an attractive and suitable target for intervention. High affinity (nM) US28-specific nanobodies could supersede the antigen-specificity of current compounds used to inhibit US28. An interesting recent technology utilizing the rapid and ligand-independent receptor internalization for US28 is that of fusion toxin protein [36]. Fusion toxin proteins utilize high-affinity receptor–ligand interactions to direct toxins towards their target. Nanobodies fused to toxins may access epitopes impenetrable to conventional antibodies and further strengthen the potency and efficacy of this antiviral strategy [36].

One concern for targeting viral GPCRs is selectivity and risk for aspecificity because of their homology to human cellular receptors. Obviously, preventing unintended modulation of cellular chemokine receptors is of the utmost importance. Biased signaling, or functional selectivity, occurs widely in the chemokine system and may contribute to specifically modulating viral GPCRs. This process describes preferential activation (bias) of a particular cellular signaling pathway and functional outcome depending on the specific ligand, receptor and cell combination. Functional selectivity allows a more focused approach to specifically target a particular receptor conformation, potentiate drug efficiency and limit side–effects [37]. The high selectivity of nanobodies may be beneficial in targeting viral GPCRs.

Interestingly, US28 expression in US28-deficient CMV-infected cells suggested increased lytic cell death. This could be due to US28 accelerating lytic replication of the virus, through which intervention could inhibit viral spreading and resulting CMV-induced effects. Although unlikely to completely prevent the viral spreading of CMV
over the monolayer, interfering with US28 could slow down infection of the cells. This allows for an interesting method for intervention, but additional experiments are required to further characterize its potential. For instance clarification of the affected intracellular signaling pathways can shed additional light on its role.

Interaction between CMV and the host

It is important to further elucidate the exact interaction between CMV and the host immune system that allows for cycles of latency and lytic infection [1]. On the one hand, the pro-inflammatory environment surrounding infected cells provides a benefit for viral replication or dissemination within the host [1, 38]. On the other hand, CMV employs multiple strategies to avoid efficient recognition by and activation of the immune system and thereby evade elimination [1]. One strategy of particular interest is cmvIL-10, the viral homologue of human anti-inflammatory cytokine IL-10 [25]. IL-10 is important during immune regulation by regulatory B cells, which points towards attempts of CMV to modulate the host immune response using similar mechanisms. It would be insightful to study the effects of cmvIL-10 on immune modulation, inflammation and infection and determine whether its mechanisms overlap with those induced by regulatory B cells.

We have found that both the extent of CMV DNAemia early after transplantation, as well as the duration of CMV DNAemia were associated with worse renal outcome after transplantation. This emphasizes the importance of restraining CMV in renal transplant recipients. Our findings may not only provide insight into CMV in the context of renal transplantation, but also indications for transplantation of the liver, heart, lung and pancreas that likewise necessitate immunosuppression [39]. CMV disease is a widespread complication after transplantation, with an incidence that varies extensively based on factors including donor–recipient serological matching, immunosuppressive regimen and type of organ [40]. For instance, the incidence is 50 – 75% for lung or heart–lung, 9 – 23% for heart and 22 – 29% for liver in absence of prophylaxis [40]. It remains to be determined whether findings in solid-organ transplant recipients can be extended to other groups of immunocompromised subjects, such as HIV-infected subjects and allogeneic hematopoietic stem cell recipients. The extensive group of potential beneficiaries further strengthens the imperative for improving prevention and treatment of cytomegalovirus inside and outside of the clinic.

On the cellular level it is important to untangle the potential different functions that US28 may perform depending on viral stage, environment of the infected cell or during in vitro and in vivo infection. Development of a suitable in vitro approach for latency would greatly benefit aligning experimental findings to the clinic. Furthermore, an essential future step involves the generation of a suitable in vivo model undergoing infection, which would allow characterization of US28 in interaction with the host immune system.
Summary, General Discussion And Future Perspectives

Unfortunately an ongoing challenge for research on CMV pathogenesis is the current lack of a suitable animal model. The extensive co-evolution between CMV and its host has allowed CMV to efficiently replicate and disseminate in its own (or closely related) host species, but also resulted in a limited host range. This substantially complicates the applicability of animal models for studying host receptors, viral entry, intracellular host defenses and specific cytokine responses [41]. Great progress may be achieved using human immune system mouse technology, which allows the study of human-restricted viruses directly in vivo. This approach enables engraftment of human cells into mice whose immune system was replaced by human hematopoietic progenitor cells. These models are increasingly implemented in studies of CMV latency and reactivation, its establishment and maintenance, viral genetics and antiviral drugs. However, only a small selection of the large variety of CMV infection-associated diseases has been recapitulated in humanized mice. Obviously it is challenging to reliably and reproducibly mimic human CMV infection in in vivo models, but further exploration holds attractive promise for the future [42]. Humanized mouse models could represent a valuable tool in the ongoing effort to characterize and modulate CMV infection and pathogenesis, for instance regarding the role of regulatory B cells in transplantation, but also to test efficacy of anti-US28 therapies in vivo.

Treatment of CMV infection

The preemptive therapy in the cohort of chapter 2 enabled CMV peak viral loads to be studied without anti-CMV treatment right after transplantation. Obviously, the very act of treating subjects is interfering with the potential rise in viral load, but this is intrinsic to the purpose of treatment, i.e. limiting productive CMV infection. Intrinsic difficulties with current anti-CMV treatment include toxicity of available drugs, as well as the necessity for intensive laboratory surveillance, CMV immunomodulatory effects and late-onset CMV disease [43]. On top of that, recipients with intensive immunosuppression and sustained ganciclovir treatment are especially vulnerable for resistance [44], and display more tissue-invasive disease and worse clinical outcome [45]. Development of new drugs with reduced adverse profiles and new targets are therefore of significant importance.

We have found that high peak viral load is associated with decreased eGFR (chapter 2), but have not studied the factors determining the height of PVL. Next to high PVL as a cause of decreased renal function, it additionally may be a consequence of it. Pre-existing donor and / or recipient characteristics could make renal cells increasingly susceptible to CMV infection. Factors such as longer ischemia times, more HLA mismatches or older age could predispose recipients to a quicker or more severe incline in viral load. Future studies should shed additional light on the factors responsible for the rise in peak viral load, and provide targets for intervention.
Although we cannot directly assess the effect of an immunosuppressive regimen without MMF, these findings suggest that MMF may be safely withdrawn in this population of primary infected renal transplant recipients. Nevertheless, it has been previously demonstrated that renal function, allograft survival and acute rejection rates benefit from treatment with daclizumab, corticosteroids and low-dose tacrolimus in combination with MMF [46]. Further research should thus indicate whether our findings can be safely translated to a broader population of patients, since rejection could still pose a risk.

Well-timed interference is of particular importance to restrain CMV and its detrimental effects after renal transplantation. Regular and extensive monitoring of the evolving CMV infection is necessary as well as compliance to antiviral medication. The occurrence of late-onset CMV disease and drug toxicity indicate that prophylactic CMV therapy may not be an optimal final solution, and warrants additional alternative strategies. One obvious strategy is that of “CMV matching”; exclusively selecting CMV-negative donors for donating to CMV-negative recipients to decrease the risk for CMV infection. Ideally, donor and recipient matching will contribute to graft survival, but the seroprevalence of CMV will inevitably extend the time on the waiting list, which is deemed unacceptable. At most, donor CMV-seronegativity could be included as preference criterion for living donation into CMV-seronegative recipients, when multiple suitable donor candidates are available.

An ultimate solution to prevent CMV infection altogether is still far from practical reality [47]. Immunotherapy with CMV vaccines provides an attractive option because of its effectivity and ease of administration [43]. Its high-priority status has supported cooperation within the fields of virology, immunology, epidemiology and clinical trials in an effort to decrease the disease burden, cost and human suffering associated with CMV [48]. These efforts contribute to alleviate CMV not only in post-transplant disease, but also other medical conditions including congenital infection, atherosclerosis, glioblastoma and immunosenescence [49]. Several candidate vaccines (e.g. against glycoprotein B) have reached preclinical and clinical testing [50], but none have been licensed so far [51]. Important questions that remain include to what extent suppression in viral load can be expected after vaccination, and what extent is necessary to prevent clinical manifestations of CMV [43]. With that, severe immunosuppression in the recipient may hamper effective immunization, which suggests that immunization before transplantation may be a more effective strategy [52]. An analysis in adolescent females prior to their first pregnancy indicated that CMV vaccination would be less costly and result in greater clinical benefits [53], suggesting it may be a viable approach to contain CMV infection.

Although among many immune modulatory strategies employed by CMV, US28 is one of the most widely characterized. Nevertheless, little is known about its role in (renal) transplantation. Since the vasculature is one of the components affected during chronic renal transplant dysfunction, US28 constitutes a potential target for therapeutic intervention. Targeting US28 could enable a larger therapeutic window by slowing down the initial viral dissemination, and containing CMV-induced damage.
A recent and promising strategy to overcome problems associated with CMV is the application of cameld-derived antibodies, so called nanobodies (Nbs). These have gained interest in the last decade for their suitable characteristics and therapeutic potential, providing a promising strategy in targeting human and viral proteins [54]. Nanobodies are recombinant antigen-specific variable domains that represent suitable candidates for modulating immune functions and targeting toxins and pathogens, both in research as well as clinical application [54, 55].

The therapeutic applications for nanobodies that are currently explored and developed range from diagnostic imaging, brain drug delivery, and targeting of receptors (CXCR4, CXCR7) and chemokines (CCL2, CCL5, CXCL11, CXCL12) [31 - 33, 56]. Although no nanobodies have actually been approved for therapeutic applications, research is rapidly developing into areas such as increasing stability of GPCR targets, tracking of proteins in live cells, and selective manipulation of specific signaling pathways [31]. Nanobodies could provide an interesting replacement, or complementation, for conventional antibodies in targeting G protein-coupled receptors in a variety of diseases (e.g. inflammatory diseases, metabolic disease and malignancies). US28 provides a suitable target for intervention, and may be complemented by other CMV antigens, including the other viral GPCRs and important structural proteins such as pp65, pp50, gB, and IE-1 [57]. Nanobody-based therapies could be used to complement and reinforce current antiviral strategies, before possibly replacing them altogether someday. It could be particularly suitable for those recipients experiencing resistant CMV, allowing for an additional angle to target the CMV infection.

First and foremost, despite the challenges to maintain renal graft function, renal transplantation remains the preferred choice of treatment for (pre)terminal renal failure due to substantial medical and economic benefits. Medically, kidney transplantation is associated with significantly lower mortality, lower risk for cardiovascular events and improved quality of life compared to chronic dialysis, and these factors relatively increase over time [58]. For instance, transplant recipients had a 48 - 82% lower long-term mortality rate compared to patients on the waiting list [59]. A rough estimation indicated almost €15.000 of savings per year for a patient after transplantation compared to dialysis [60] and lower costs per quality-adjusted life year [61].

One factor that nourishes optimism for the future of transplantation is the decreasing waiting list, increased total number of effectuated organ donors and increased number of transplantations from living donors between 2011 and 2015 [62]. These positive developments could be further reinforced by raising and stimulating societal awareness about transplantation. It remains of vital importance to involve and educate the public about the importance of transplantation and find new ways to engage potential donors in the attempt to further limit the shortage of donor organs.
REFERENCES

[38] Tedder TF. Introduction: Regulatory B Cell Special Issue—making all the pieces fit. Int Immunol 2015;27:467–70.