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# Report of the second joint meeting of ESOT and AST: current pipelines in biotech and pharma

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## Summary

Following the first joint meeting organized by the European (ESOT) and American (AST) Societies of Transplantation in 2010, a second joint meeting was held in Nice, France, on October 12–14, 2012 at the Palais de la Mediterannee. Co-chairs of the scientific advisory committee were Dr. Flavio Vincenti (AST) and Dr. Teun Van Gelder (ESOT). The goal was to discuss the key unmet needs in solid organ transplantation with the opportunity to interrelate current basic research efforts with clinical translation. Thus, the topic of this second meeting "Transformational therapies and diagnostics in transplantation" was devised and a summary of this meeting follows.

## Cell therapy in transplantation

Edward Geissler (Regensburg, Germany) opened the meeting with a lecture on the potential role of cell therapy in the field of organ transplantation. He noted that significant technological progress had been made in the isolation and purification of cell products of high quality. Using magnetic bead sorting and ex vivo expansion, the cell type can be selected and purity ensured with sufficient volume for human dosing applications. Examples of such cell types are natural T-regulatory cells (Tregs), alloantigen-exposed donor-specific Tregs, tolerogenic dendritic cells, suppressive macrophages, and mesenchymal stem cells. Purity is of course crucial, as contamination and expansion of alloreactive cells would be detrimental to the outcome of cell therapy. An example of the proof of concept in an experimental setting is the study of Sagoo *et al.* [1]. Here, human Tregs were isolated and expanded and then reinfused in a humanized mouse model of skin transplantation, with suppression of this stringent rejection response. This study suggests that cell-based medicinal products can be developed for use as an adjunct immunosuppressive therapy in organ transplantation.

An EU-funded consortium including both academic and industry partners called the "One Study" was recently established with the goal of manufacturing distinct populations of hematoregulatory cells to prevent immunological rejection of transplanted organs without the need for the long-term use of pharmacological immunosuppression. Each partner is focusing on a unique cell type in proof of concept studies in man. Unique to this collaboration, all clinical trials must include the same protocol of management with the exception of the cell type infused; hence the term the "One Study". The different cell therapy products will be tested side by side using living donor renal transplant recipients. In the control arm, immunosuppression


 

will include basiliximab induction followed by tacrolimus, mycophenolate, and 3 months of corticosteroid therapy. In the experimental group, basiliximab will be eliminated and replaced by cell therapy and mycophenolate will be weaned during the first post-transplant year. This cooperative project aims to develop successful protocols utilizing various immunoregulatory cell products in organ transplantation recipients, allowing a direct comparison of the safety, clinical practicality, and therapeutic efficacy of each cell type.

## Costimulation blockade

In the next section of presentations, the focus was on pathways to mediate immune regulation. Recognizing that T cells require not only engagement of the T-cell receptor but also a positive costimulatory signal to become activated, numerous laboratories have focused efforts on either interfering with positive or enhancing negative costimulatory signals. Following a short introduction of the mechanism of action of belatacept (CTLA4Ig) which inhibits the positive stimulus, Dr Lionel Rostaing (Toulouse, France) reviewed the BENEFIT study results. Despite a higher incidence of acute rejection in the belatacept-treated group, renal function at one and 3 years after transplantation was better compared with the cyclosporin-treated control group [2,3]. Projected mean half-life of the graft in the belatacept group is calculated to be increased by 2 years compared with the control group. Similar results were seen in the BENEFIT-EXT study utilizing extended criteria donor kidneys, in which the 3 year data show sustained better renal function in the belatacept-treated patients [4]. Belatacept is now registered for the prevention of acute rejection after kidney transplantation in patients with a positive Epstein–Barr virus serology.

The experience with a belatacept conversion trial was also presented. In this phase 2 study, kidney transplant recipients on maintenance calcineurin inhibitor (CNI) therapy were randomized for the continuation of CNI, or switch to belatacept treatment [5]. At month 12, the mean improvement in GFR from baseline was higher in the belatacept group versus the CNI group. Six patients in the belatacept group had acute rejection episodes, all within the first 6 months; all resolved with no allograft loss. Dr. Rostaing also suggested that the potential benefit of using belatacept in those recipients at risk of CNI-mediated post-transplant diabetes.

In the next presentation, Dr. Allan Kirk described the exciting developments of costimulatory blockade for the induction of tolerance, a presentation that demonstrated effectively how the science in this field has gone from bench to bedside and back again. Costimulatory blockade has been used effectively for the induction of tolerance in rodent models of transplantation, but has been less effective in larger animals and humans. In primates, belatacept has shown limited immunosuppressive capacity as mono-therapy. Only when combined with other immunosuppressive drugs does belatacept have effective anti-rejection activity. This is similar to studies in man reported above in which recipients on belatacept maintenance therapy had higher early rejection rates compared to those on CNI maintenance.

Our understanding of costimulation has evolved substantially and now encompasses not only the recognition of positive signals involved in T-cell activation but also negative signals inhibiting T-cell activation and promoting T-cell tolerance. As an example, the consequences of B7:CD28 blockade by hCTLA4Ig on regulatory T-cell (Treg) generation in various major histocompatibility complex (MHC) mismatch mouse heart transplant models were investigated. Administration of hCTLA4Ig significantly decreased the number of Tregs in C57Bl/6 wild-type recipients and this effect was predominant in thymus-induced Tregs that expressed the marker Helios, a marker that discriminates naturally occurring thymus-derived Tregs from those induced from peripheral naïve T cells. Although hCTLA4Ig prevented rejection in a fully allogeneic mismatch model, it accelerated rejection in a MHC class-II mismatch model (MST = 26, $P < 0.0001$), in which long-term allograft survival is dependent on Tregs. This accelerated rejection was associated with a marked reduction in thymus-induced Tregs and led to a higher effector/regulatory T-cell ratio in secondary lymphoid organs and in the allograft. This study confirms the importance of the B7:CD28 pathway in Treg homeostasis in an *in vivo* transplant model and suggests that hCTLA4Ig therapy may be deleterious in circumstances where engraftment is dependent on Tregs [6].

Newly identified costimulatory pathways may prove to be attractive therapeutic targets for organ transplantation [7]. Memory T cells are largely CD28 negative, and therefore unaffected by belatacept. However, this cell population can be targeted and depleted with alefacept, a monoclonal antibody that blocks the costimulatory interaction of LFA-3/CD2. Targeting CD28 directly would avoid the dilemma of inducing stimulatory or blocking effects. However, the use of this drug in man has been associated with marked cytokine release [8].

Dr. Flavio Vincenti (San Francisco, US) presentation focused on the CD40 costimulatory pathway. CD40 ligand (CD40L, CD154) is expressed on activated $CD4^+$ T cells. Prior studies targeting CD40L in man at UCSF were halted after inclusion of five kidney transplant recipients, because of the occurrence of thromboembolic events in 3 of these 5 patients. These events were the result of expression of CD154 not only on T cells, but also on platelets [9]. Therefore, targeting CD40 is being accomplished using a

new approach with the anti-CD40 compound ASKP1240, developed by Astellas [10,11]. This compound, previously known as 4D11, is able to prolong graft survival in experimental models when combined with MMF and/or tacrolimus. Moreover, in a high shear stress condition model, ASKP1240 does not destabilize thrombus formation. A recent phase 1B study with ASKP1240 in 50 patients receiving kidneys from living donors has been completed and demonstrated no significant adverse events. Additional phase 2 testing is pending utilizing both a CNI-containing and CNI-free arms. This is an exciting new opportunity to assess a potent costimulatory pathway.

## B cells and antibody-mediated rejection

A significant problem in transplantation is the role of alloantibody in late allograft injury and effective therapies to mitigate this injury. In a session on B cells and antibody-mediated rejection (ABMR), Menna Clatworthy (Cambridge, UK) stressed the increasingly recognized role of B cells in transplantation, not just as the precursors of antibody-producing plasma cells, but also as T cell-activating antigen presenting cells and as a source of pro-inflammatory cytokines. More recently, focus has turned to B cells as immune regulators in tolerance induction. Thus, a better understanding of factors which control the activation and inhibition of B cells will be critical to target B cells therapeutically [12].

Dr. Clatworthy described the array of coactivating receptors expressed by B cells, including CD19 and CD21, and the signaling cascade initiated by ligation of the B cell receptor with these molecules. In addition to activating receptors, the B cell also expresses a number of inhibitory receptors, which can increase the activation threshold of B cells, including FcγRIIb (CD32B) and CD22. The importance of these receptors is illustrated by studies in knockout mice, which show B-cell hyperactivity and heightened antibody responses. She also mentioned the potential for CD19 antibodies in transplantation, supported by some interesting data from Thomas Tedder and colleagues, suggesting that in contrast to CD20 antibodies, anti-CD19 treatment may allow elimination of some bone marrow and lymph node antibody-secreting cells [13]. The role of the B-cell cytokine BAFF (BLyS) was also discussed, particularly with respect to recent data suggesting involvement in the development of ABMR [14]. Thibault–Espitia found that stable patients with high BAFF-R levels had a higher risk of developing graft dysfunction, and that patients with lower levels of BAFF transcripts or a higher level of soluble BAFF had a significantly higher risk of developing donor-specific antibodies (DSA). These data suggest that BAFF constitutes a risk factor for renal graft dysfunction and development of DSA, and agents targeting BAFF–R interactions may offer new therapeutic opportunities in transplantation. Belimumab, an anti-BAFF antibody now registered for use in lupus, may prove to be helpful in intervening in this process [15]. Future attempts to target B cells will need to address the problems similar to T cells in how to inhibit effector B cells, while enhancing those with regulatory capacity.

In recent years, there have been major advances in our understanding of rejection mechanisms and particularly the demonstration of the destructive power of anti-HLA alloantibodies associated with ABMR. In addition, there is compelling evidence that donor-specific anti-HLA antibodies are also largely responsible for the chronic deterioration of allografts. The emergence of sensitive techniques for detecting DSA together with the implementation of molecular approaches and better assessment of graft pathology have expanded the spectrum of what constitutes ABMR. In a presentation by Alexander Loupy (Paris, France), the many forms of ABMR were discussed. Antibody-mediated injury to allografts often consists of indolent ABMR, ABMR without C4d deposition, and antibody-mediated vascular rejection. C4d positivity is not an absolute requirement for microcirculation inflammation [16]. In addition, arteriosclerosis, previously thought to be a bystander lesion related simply to aging of the donor, is accelerated in ABMR, and forms a part of the rejection process [17]. Further, Dr. Loupy stressed that the recognition of these entities in our diagnostic system is important for the clinical management of patients.

A recently published review of 12 controlled clinical trials of the treatment of ABMR demonstrated the efficacy of treatments for ABMR in renal allografts, but of low or very low quality [18]. Larger randomized controlled trials and dose-response studies are required. Based on current data, the consensus is to use a combination of plasma exchanges (PE)/repeated doses of intravenous immune globulin (IVIg) and in some practices rituximab (anti-CD20 antibody). Christophe Legendre (Paris, France) expects that a French study will soon be published that will more definitively determine whether the use of anti-CD20 in conjunction with the other therapies is more efficacious in preventing ABMR (Clinical trials.gov identifier NCT01350882). The proteasome inhibitor bortezomib is also currently under study in a well-designed controlled trial, as it has been difficult to evaluate the efficacy based upon the already performed studies. A more effective strategy in patients may therefore be preventive efforts to avoid ABMR. Table 1 summarizes a number of these approaches.

## Individualized immunosuppression

In the last few years, the contribution of CNI on the development and progression of chronic allograft damage has

  

**Table 1.** Strategies for the prevention of the occurrence of antibody-mediated rejection (ABMR).

1. Waiting for a highly HLA compatible kidney (with the disadvantage that the time spent on dialysis is detrimental to the patients).
2. Improving the chances to get a cross-match negative donor either with the acceptable mismatch programs (F Claas, this meeting) or exchange donor programs (R Gaston, this meeting) (efficient and good results but not possible for all patients).
3. Desensitizing the patients: (a) Before transplantation using either plasma exchange (PE)/IVIg (R Montgomery) or IVIg/rituximab (S Jordan); (b) Adding a cycle of bortezomib before transplantation to increase the efficacy of PEs (Mayo clinic); (c) Using immunoadsorption just prior to transplantation with IA (Vienna protocol); (d) Using eculizumab postoperatively (M Stegall); (e) Treatment of patients with positive DSA but a negative CDC cross-match with either IVIg or a combination of PEs/IVIg and anti-CD20 (Necker protocol).

been debated [19,20]. To avoid or minimize the use of CNIs, mTOR-inhibitors (mTORi) were postulated as feasible alternatives as effective but non-nephrotoxic immunosuppressive regimens in renal transplantation. Josep M. Grinyó (Barcelona, Spain) noted that the use of mTORi over the last decade has been relatively limited despite data from well-designed randomized clinical trials showing clear improvements in renal allograft function compared to maintenance regimens with CNI [21]. The relatively poor tolerability of mTORi may partially account for their low use [22]. The ability to decide which patient should remain on full dose CNI treatment, or be converted from CNI to mTORi, or simply weaned from CNI can only be fully achieved when biomarkers reflecting the individual immune reactivity are available to guide these decisions.

Of special interest for the transplant community is whether such markers could be used to i) identify pretransplant immunologically "high risk" patients likely to develop severe acute rejection episodes shortly after transplantation, ii) monitor drug-dependent reconstitution or functional changes in repopulating lymphocytes, iii) delineate and predict deterioration in graft function or rejections after transplantation or iv) identify potentially tolerant patients in whom immunosuppressive drugs can be safely withdrawn. Dr. Birgit Sawitzki (Berlin, Germany) suggested that collaborative efforts should be maintained in large prospective multicenter trials in which the relevance of such types of biomarkers, including monitoring Tregs, can be further validated [23,24]. Such efforts are being undertaken by RISET, a multinational European project financed by the European Commission within the Sixth Framework Programme.

An overview of the government-funded US consortia of transplant centers, the clinical trials in organ transplantation (CTOT), was presented by Dr. Donald Hricik (Cleveland, US). Currently, 16 trials (CTOT-01 through CTOT-16) have been designed and implemented. CTOT-01 is a prospective observational study of primary kidney transplant recipients designed to analyze the predictive value of serum and urinary biomarkers, including mRNAs for nine candidate chemokines, and two candidate proteins measured by ELISA. The combination of CXCL9 (MIG) protein and CXCL10 (IP-10) protein function at least as well as any combination of urinary mRNAs, with a high positive predictive value (73.3%) and extremely high negative predictive value (91.3%) in diagnosing acute rejection. Moreover, the urinary proteins are easier to measure than the more cumbersome measurements of mRNA using RT-PCR techniques. Compared to a control group without biopsy proven rejection, those with rejection exhibit elevated levels of CXCL9 protein up to 30 days prior to the rejection episode and exhibit a decline to the normal range following treatment. CXCL9 protein measured at 6 months post-transplant was predictive of a >30% decline in eGFR between 6 and 24 months post-transplant, independent of biopsy proven acute rejection, suggesting that it is a marker for subclinical immune injury that may lead to graft dysfunction. Absence of urinary CXCL9 protein at 6 months was strongly associated with stable kidney function over the ensuing 18 months. The results of CTOT-01 were instrumental in designing CTOT-09. This latter study is an ongoing randomized prospective trial of nonsensitized, living donor kidney transplant recipients treated with rabbit anti-thymocyte globulin, tacrolimus, an MPA product, and low-dose steroids to test the hypothesis that the combination of tacrolimus withdrawal and subsequent reinstitution of tacrolimus in patients with urinary biomarkers suggestive of early immune reactivation will lead to better renal histology and function in the experimental. In this study, recipients that are free of rejection or BK infection undergo randomization to tacrolimus withdrawal versus being maintained on standard therapy while being intensively monitored using the markers noted in CTOT-01. Allograft biopsies are performed at 6 months and at 24 months and when further indicated by the level of urine chemokines, to ascertain the need for further biopsies and/or renewal of tacrolimus.

## Current pipeline in biotech and pharma

The ongoing needs for innovative therapies were emphasized throughout the meeting, although it was clear that a number of promising candidates for immunosuppression were no longer undergoing clinical development. An appli-

 

cation wide open for innovation is in the prevention and mitigation of ischemia-reperfusion injury (IRI). Several promising agents were discussed.

At the time of the meeting, Astellas was developing diannexin, a recombinant homodimer of the endogenous human Annexin V protein that was designed to prolong its half-life in the circulation and increase its affinity for phosphatidylserines on cell surfaces. Binding of diannexin to phosphatidylserines that has translocated to the surface of hypoxic cells inhibits the cascade that leads to IRI. By phosphatidylserines blockade, diannexin inhibits adhesion and activation of the mononuclear cells, platelets, and factor XII that promote the downstream inflammatory and thrombotic pathways leading to microvascular occlusion and cellular damage [25]. Dr. Roy First (Chicago, US) demonstrated that in a phase 2a study ($n = 50$ patients) in extended criteria kidneys, diannexin reduced the incidence of delayed graft function (DGF) and was associated with improved renal allograft function as well. Further human studies were pending the conclusion of toxicity data in rodents. However, while writing this meeting report (March 2013), Astellas made the decision to discontinue the development of diannexin based on the recent findings in a preclinical study conducted during the clinical phase II/III study. Unexpected cardiac and pulmonary toxicities were noted in rats and monkeys during this preclinical study. No such findings have been observed to date in human subjects receiving diannexin.

Another therapy to prevent DGF is being developed by Catalyst Biosciences (represented by Edwin Madison). Proteases are naturally occurring protein-cleaving enzymes that regulate a wide variety of biological processes. These agents can be used as biopharmaceuticals by either improving marketed protease-based drugs or redirecting them to cleave specific proteins that promote disease. These redirected engineered proteases are called "Alterase™ Therapeutics" and complement component C3 has been selected as the initial target [26]. The anti-C3 program is currently in preclinical nonhuman primate efficacy studies using a hind limb ischemia model. Tolerability and pharmacodynamic efficacy have been demonstrated, while being able to fully deplete circulating C3 for a therapeutically relevant time period. An anti-C3 alterase development candidate has been selected for further preclinical development, and phase I studies are expected in the second part of 2013.

Innovations in transplant diagnostics are a critical need in the field. This includes biomarkers to guide the immunosuppressive burden in individual patients. In addition to the institutional initiatives mentioned above (RISET and CTOT), industry is active in developing new methods to assist in patient management. The FDA has approved the ImmuKnow™ assay (Cylex Inc., Columbia, MD, USA) which is being used by a number of transplant centers over the last decade to monitor the extent of immunosuppression. In this assay, an aliquot of recipient whole blood is incubated with phytohemagglutinin, a nonspecific mitogen which stimulates proliferation of T-lymphocytes, resulting in increased ATP production which is measured in CD4T cells using magnetic bead capture and a bioluminescence assay. A profile of responses has been identified for those at risk for infection and rejection [27]. Brad Stewart (Cylex Inc.) reported the initial results of a multicenter interventional in liver transplant. Patients were randomized to standard clinical management or had the ImmuKnow™ assay included with their testing. A significantly reduced incidence of infectious complications was seen in the ImmuKnow™ group, with no difference in the incidence of acute rejections. Following the meeting in Nice Cylex Inc. was bought by Viracor-IBT Laboratories, but the ImmuKnow™ assay is still on the market.

Scott Batty presented a novel method to analyze an individual's specific immune profile using the iCHIP technology developed by ImmunArray (Rehovot, Israel). The iCHIP platform measures serum auto-antibody repertoires using microarray technology [28]. Global patterns of antibodies are detected via proprietary sets of antigens, and analyzed to provide a diagnostic and prognostic profile of the individual. Proprietary software analyzes the measured antibody-binding signal and compares it with the system's database to provide a probability value for the indication tested (diagnosis, prognosis etc.). The first iCHIP-based products to be launched are tests for diagnosis and prognosis of autoimmune diseases (lupus) and organ transplantation rejection, and may assist in early diagnosis, disease sub-classification and prognosis and monitoring response to treatment [29].

With the realization that tacrolimus is currently a first choice immunosuppressive drug in large numbers of transplant centers throughout the world, it is no surprise that innovative formulations for this drug are being developed. Veloxis Pharmaceuticals (Horsholm, Denmark, represented by John Weinberg) is developing a once-daily dosage tablet version of tacrolimus (LCP-Tacro™). The pharmacokinetics of LCP-Tacro are characterized by a "flat" pharmacokinetic curve, reducing the peak concentrations associated with standard tacrolimus formulations. Compared with the twice-daily dosage capsule version of tacrolimus, LCP-Tacro has potential benefits which include once-daily dosing, improved systemic absorption, improved bioavailability and thus a lower dose of tacrolimus, and limited variability in the concentration of tacrolimus in the blood ("peak-to-trough" fluctuation). The benefits of these flatter, less variable blood levels may include a reduction in side effects, an improvement in efficacy, and/or greater convenience to patients, including a less frequent need for dose adjustments. A first phase III

  

study in stable kidney transplant patients is already completed, and a second phase III study is ongoing [30]. Results are eagerly awaited.

## Small molecules of interest

A number of small molecule agents for immunosuppression are under study. Klemens Budde (Berlin, Germany) reviewed the clinical and experimental data of sotrastaurin. Sotrastaurin is the first oral, selective protein kinase C (PKC) inhibitor, which blocks early T-cell activation and the NF-κB signaling pathway. The PKC family plays a key role in the adaptive immune system and was shown to be involved in rejection processes after solid organ transplantation [31]. Sotrastaurin has been effective in early clinical studies of T-cell driven autoimmune diseases such as psoriasis, and demonstrated a good tolerability and safety profile. In the phase II study in kidney transplantation, further evidence for its efficacy in transplantation was found. However, sotrastaurin in a CNI-free regimen did not achieve sufficiently lower acute rejection rates and renal function was not substantially improved compared to those on CNI [32–34]. Ultimately further development of this interesting novel compound in transplantation was terminated, as the data were not sufficiently supportive to start phase III trials comparing sotrastaurin regimens with the standard of care consisting of tacrolimus and MPA. Development in liver transplantation was similarly stopped. Novartis, the company responsible for the agent stressed that this decision was not for safety concerns. Further development in treatment of B-cell lymphoma and metastatic uveal melanoma is still ongoing. The discontinuation of the development of sotrastaurin for transplantation highlights the difficulties of drug development in the current environment in transplantation.

Flavio Vincenti (San Francisco, US) reviewed the mechanism of action and important clinical trial data in renal transplantation for the Janus kinase (JAK) 3 inhibitor tofacitinib, formerly known as CP-690,550 [35]. JAKs are cytoplasmic tyrosine kinases that participate in the signaling of a broad range of cell surface receptors, particularly members of the cytokine receptor common gamma (cγ) chain family. JAK3 inhibition has immunosuppressive effects and treatment with tofacitinib in clinical trials has demonstrated efficacy in autoimmune disorders such as psoriasis and rheumatoid arthritis (RA) [36,37]. Renal transplant clinical trials in humans have demonstrated tofacitinib to be noninferior to cyclosporine in terms of rejection rates and graft survival [38,39]. There was also a lower rate of new-onset diabetes after transplant. However, there was a trend toward more infections, including cytomegalovirus and BK virus nephritis, indicating the potent immunosuppressive action of this agent. In the phase IIb study, tofacitinib was equivalent to cyclosporine in preventing acute rejection, was associated with improved renal function and less chronic allograft histological injury. However, tofacitinib-treated patients did suffer from more side effects (including anemia, neutropenia, serious infections, and CNS located post-transplant lymphoproliferative disorder) [40]. These complications clustered in the patients exposed to higher tofacitinib concentrations, suggesting that therapeutic drug monitoring may help in avoiding toxicity. The optimal therapeutic window, however, needs to be determined in prospective trials. Following the meeting Pfizer has announced that it will not further develop tofacitinib for transplantation.

## Unfulfilled targets in transplantation

While it has been about 25 years that the group of Lucien Aarden from the University of Amsterdam showed that high levels of IL-6 in the urine are found in the first days after transplantation and that these levels rise shortly before acute cellular rejection is diagnosed [41], little additional study was published. More recently, Dr. Carla Baan (Rotterdam, The Netherlands) has demonstrated some exciting data demonstrating the emerging importance on the role of B cells in alloreactivity. IL-6 is a B-cell stimulatory factor key for the differentiation into antibody-producing plasma cells to T-cell dependent antigens. Therefore, blocking the signal that is induced when IL-6 binds to the IL-6R may provide an attractive therapeutic approach to reduce antibody production. Tocilizumab, a humanized anti-IL-6R that binds to both soluble and membrane bound forms of this receptor, has been approved by the FDA for the treatment of adult patients with moderately to severely active RA and with an inadequate response to one or more disease-modifying anti-rheumatic drugs or TNF antagonists [42]. A first study with this drug in highly sensitized patients awaiting kidney transplantation has been developed (see Clinical Trials.gov).

Another novel potential therapeutic pathway may be by blocking IL-17 and Th17 cells. These cells have been shown to play an important role in autoimmune disease, although their role in alloimmunity remains under speculation. Secukinumab (AIN457) is a human antibody neutralizing IL-17A. In patients suffering from RA, inflammatory bowel disease and psoriasis local high levels of IL-17 are detected. High levels of IL-17 may be crucial in psoriasis patients who develop arthritis. Treatment with Secukinumab in psoriasis patients showed good results while those in RA reported conflicting outcomes [43,44]. At present, no studies in organ transplantation are scheduled.

As we move away from CNIs to avoid drug-related toxicity, it has become apparent that other agents targeting less central signaling pathways often fail to inhibit T cells that

are antigen experienced (so-called memory T cells). In his talk, Dr. Alan Kirk (Atlanta, USA) discussed the properties of memory T cells that relate to their susceptibility to immunosuppressive manipulation and proposed selective targeting of memory T cells. Here, alloresponsive $CD8^+$ effector memory T cells express high levels of CD2 [45]. Addition of alefacept, the CD2-specific fusion protein, to these T cells in culture inhibited memory T-cell proliferation and successfully targeted belatacept-resistant proliferation. These studies provide a rationale for translation of an immunosuppression regimen pairing alefacept and belatacept to human renal transplantation.

There is an increasing evidence of interaction between the innate immune response, including complement, in the control or development of alloreactivity. Work by several groups has shown that complement is also produced by cells within transplanted organs and that local, transplant-derived complement activation is a key mediator of ischemia reperfusion injury. Results of studies published since the mid-2000s by Peter Heeger (New York, USA) uncovered the unanticipated finding that T cells and antigen-presenting cells (APC) produce complement proteins. Moreover, this immune cell-derived complement activates spontaneously via the alternative pathway, yielding local, but not systemic, production of C3a and C5a. These anaphylatoxins bind to their respective G-protein coupled receptors, C3aR and C5aR, expressed on the T cells and APCs. The complement-induced T cell and APC activation drives T-cell differentiation, expansion and survival. Complement deficiency or blockade of complement function attenuates allograft rejection in mice and limits human alloreactive T-cell responses, while increasing complement activation, achieved by genetic removal of the complement regulatory protein accelerates murine allograft rejection, and augments human T-cell responses *in vitro*. These findings support the need for design and testing of complement inhibitors as adjuvant therapies to prevent human transplant rejection and prolong transplant survival. Similarly, anti-C5 monoclonal antibody suppresses alloreactivity and prolonged graft survival was shown in an experimental heart transplant model [46] and C5 blockade inhibited T cell-mediated responses like IFN-$\gamma$ production and proliferation. These data support the concept that C5 blockade may constitute a viable strategy to prevent and/or treat T cell-mediated allograft rejection in humans. Currently, the humanized antibody eculizumab provides anti-C5 blockade and has been given to presensitized kidney transplant patients for the prevention of ABMR. With the growing data that complement also influences the function of T cells, anti-C5 therapy may also suppress T-cell reactivity in unsensitized allograft patients. Trials with anti-C5 to prevent the T cell-mediated rejection process are therefore warranted.

## Challenging medical decisions

### Transplanting the sensitized patient

There are now several options and effective therapies for sensitized patients, some of which have already been outlined above and in Table 1. Additional strategies were discussed in this session. Living donor kidney exchange programs (kidney paired donation [KPD]) offer incompatible donor-recipient pairs the opportunity to be transplanted [47]. In such programs, patients who cannot directly receive a kidney from their intended living donor, because of ABO blood type incompatibility or a positive cross-match, exchange donors with other incompatible donor/recipient pairs to receive a compatible kidney [48]. KPD and desensitization have traditionally been considered competing strategies and patients have been offered one or the other regardless of the probability of a successful outcome. However, a newer approach undertaken analyzes the donor/recipient phenotype and attendant beneficial modalities [49]. KPD should be favored among patients with immunologic phenotypes that are likely to match without prolonged waiting times. However, as many as 50% of patients with incompatible donors will fail to find a match in a KPD pool and many of these patients may be better off by undergoing desensitization to their donor. Patients who are both difficult-to-match because of broad sensitization and hard-to-desensitize because of strong donor reactivity can often be successfully transplanted through a combination of desensitization and KPD. Through the use of these various modalities, it is estimated that most patients with incompatible live donors can undergo successful renal transplantation. Robert Gaston (Birmingham, USA) presented the case of a highly sensitized female patient, with blood type B. She had ABO-incompatible siblings, and with her children she had a positive cross-match who eventually was transplanted using combined approaches.

### Acceptable and unacceptable mismatches

The presence of DSA detectable in complement-dependent cytotoxicity (CDC) before transplantation is a contra-indication for transplantation [50]. Patient sera are therefore screened before transplantation to define those HLA mismatches that may be unacceptable because of preformed antibody. This prevents potential allocation of an organ where a positive cross-match is likely. For this reason transplant exchange organizations like Eurotransplant and the United Network of Organ Sharing (UNOS) routinely introduce these so-called unacceptable HLA mismatches in the patients' profiles [51]. As a consequence, highly sensitized patients accumulate on the waiting list as they have DSA

  

against the majority of the donors. For patients waiting for a deceased donor organ, the acceptable mismatch program of Eurotransplant has shown to be very efficient for highly sensitized patient [52]. For patients with a living donor toward whom they have formed DSA can take advantage of a paired donor exchange program [53].

The introduction of more sensitive solid phase assays for HLA antibody screening using single antigen bead (SAB) testing has led to an increase in detection of the number of sensitized patients. Conflicting data in the literature lead to the conclusion that the presence of DSA by SAB testing is a not always a contra-indication for transplant but rather a risk factor for worse outcomes [54,55]. If all DSA detected by SAB would be considered unacceptable mismatches, highly sensitized patients have no chance to be transplanted. Considering the high mortality on the waiting list, this would have an enormous impact. Therefore, the group of Dr. Frans Claas (Leiden, The Netherlands) tested the incidence of preformed DSA detectable by Luminex SAB in a cohort of highly sensitized patients transplanted via the acceptable mismatch program. More than half of the transplanted patients had DSA and indeed the incidence of acute rejection was higher in patients with DSA. However, long-term graft survival was similar in the $DSA^+$ and $DSA^-$ patients. If these DSA would be classified as unacceptable mismatches half of the patients would not have been transplanted. In conclusion, DSA detectable in CDC are a contraindication for transplantation and should be considered unacceptable mismatches. DSA detectable by SAB are a risk factor for graft rejection and more complicated outcomes. Depending on the prognosis of the patient, the clinician should decide when it is acceptable to take this risk to proceed with transplant recognizing the presence of DSA. In case of highly sensitized patients, DSA detectable by Luminex SAB should not be considered the sole determinant of unacceptable mismatches [56].

## The unmet needs

### New molecules in delayed graft function

Delayed graft function occurs commonly following kidney transplantation, with an incidence as high as 50% in some series involving organs from deceased donors. The consequences of developing DGF are significant. In addition to the acute complications related to renal failure and the associated economic impact of prolonged hospitalization, the development of DGF increases the risk of chronic allograft nephropathy and shortens allograft survival. New therapies seek to suppress the inflammatory response in organs, limit cell death and/or interrupt detrimental signaling of necrosis, and inflammatory homing of adaptive immune cells to the site of injury. Dr. Bernd Schröppel (New York, US) reviewed a number of novel targets and therapeutics, especially within the innate immune response, including toll-like receptors, complement, and autophagy (shown in Table 2). At present, we lack selective blockers and inducers of autophagy, but low levels of autophagy are protective for tubular cells during stress. Dr. Schröppel emphasized that predicting organs at risk and a clear definition of clinical endpoints will be critical in designing interventional trials in the future.

### New molecules for fibrosis

In the lung and kidney, long-term graft failure is manifested by airway obstruction and fibrosis (BOS) and by

**Table 2.** New molecules under the development of delayed graft function.

| Agent | Target | Mechanism | Reference |
|---|---|---|---|
| E5564 (TAK-242) | TLR4 antagonist | Reduction in TLR4-dependent HMBG1-induced inflammation | Wu [61] |
| Microcept | CD35 | Complement regulatory protein CD35 (CR1) | Pratt [62] |
| Eculizumab | Complement protein C5 | Binding C5 inhibits its cleavage by the C5 convertase, which prevents the generation of the terminal complement complex C5b-9 | NCT 01403389 |
| rPSGL-Ig (YSPSL) | Fusion protein of P-selectin ligand and IgG1-Fc | Blocks leukocyte adhesion and protects against ischemia reperfusion injury (IRI) in animal models | Gaber [63] |
| siRNA to p53 | p53 is a pivotal protein in the apoptotic pathway | Attenuation of p53-mediated apoptosis | Molitoris [64] |

**Table 3.** New molecules under development as anti-fibrotic therapies.

| Agent | Target | Mechanism | Reference |
|---|---|---|---|
| Pirfenidone | TGF-β | Inhibition of TGF-β production and consequent matrix deposition | Sharma [65] |
| Decorin | TGF-β | Regulation of matrix mineralization by modulating collagen assembly | Mochida [66] |
| PHI | Prolyl-hydroxylase | Prolyl-hydroxylase inhibitor | Franceschini [67] |
| Klotho | FGF23 | Klotho competes with TGF-β1 for binding to TGF-βR2 | Krajisnik [68] |

 

interstitial fibrosis and tubular atrophy (IF/TA), respectively. In the heart, late graft failure is manifested by coronary vasculopathy. The sites of injury may include epithelial or endothelial cells or both. Following one of these injuries, the response includes both a proliferation response and an inflammatory response mediated by chemokines, cytokines, and growth factors. This process culminates in the deposition of matrix and associated chronic inflammation. While fibroblast activation is associated with fibrosis, the etiology of these cells is not completely known [57]. A number of preclinical models in rats and mice have been developed to study late graft failure. These models have provided a platform to investigate the mechanisms of fibrotic injury and identify possible therapies. Targets have included global anti-inflammatory approaches, and alternatively direct anti-fibrotic therapies [58]. These include blockade of matrix molecule synthesis both at the molecular and protein levels (overview in Table 3). Dr. Roslyn Mannon (Birmingham, US) discussed the potential of these therapies for clinical transplantation.

The last presentations of the meeting were focused on how to improve long-term outcome after kidney transplantation. Both Dr. Christophe Legendre (Paris, France) and Dr. Barbara Murphy (New York, US) again stressed the fact that we need biomarkers to better target the level of immunosuppression in individual patients. At present most patients are treated with the same immunosuppressive therapy, and we are unable to identify patients in whom immunosuppression may be tapered to lower levels to avoid drug-related toxicities that affect long-term outcome such as infection and malignancy. The role of CNIs in the development of chronic renal allograft dysfunction may have been over-estimated in the past, but at the same time may still be an existing threat to transplanted (and native) kidneys. Histologic features of chronic allograft nephropathy are already visible early post-transplantation, also in patients in whom clinical deterioration becomes evident only years later [59,60]. The ongoing Genomics of Chronic Renal Allograft Rejection ("GoCAR") study aims to determine the role of cell- and antibody-mediated responses in chronic rejection of transplants, to determine the gene expression profile associated with the development of chronic rejection, and to determine whether variants of specific genes cause susceptibility to rejection (NCT00611702). In this presentation, it was suggested that the ability to better predict chronic rejection, and with a better understanding of the pathophysiology interventions can be developed, then chronic rejection should no longer be considered inevitable. The insights from these final presentations demonstrated the vast array of investigation across the world, and opportunities for new therapeutics moving forward.

## Authorship

All authors have contributed substantially to the writing of this manuscript.

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