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REVIEW

# Cell therapeutic approaches to immunosuppression after clinical kidney transplantation

**Christian Morath[1] · Anita Schmitt[2] · Florian Kälble[1] · Martin Zeier[1] · Michael Schmitt[2] · Flavius Sandra-Petrescu[3,4] · Gerhard Opelz[3] · Peter Terness[3] · Matthias Schaier[1] · Christian Kleist[3,5]**

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**Abstract** Refinement of immunosuppressive strategies has led to further improvement of kidney graft survival in recent years. Currently, the main limitations to long-term graft survival are life-threatening side effects of immunosuppression and chronic allograft injury, emphasizing the need for innovative immunosuppressive regimens that resolve this therapeutic dilemma. Several cell therapeutic approaches to immunosuppression and donor-specific unresponsiveness have been tested in early phase I and phase II clinical trials in kidney transplantation. The aim of this overview is to summarize current cell therapeutic approaches to immunosuppression in clinical kidney transplantation with a focus on myeloid suppressor cell therapy by mitomycin C-induced cells (MICs). MICs show great promise as a therapeutic agent to achieve the rapid and durable establishment of donor-unresponsiveness in living-donor kidney transplantation. Cell-based therapeutic approaches may eventually revolutionize immunosuppression in kidney transplantation in the near future.

✉ Christian Morath
christian.morath@med.uni-heidelberg.de

[1] Division of Nephrology, University of Heidelberg, Im Neuenheimer Feld 162, 69120 Heidelberg, Germany

[2] Department of Internal Medicine V, GMP Core Facility, University of Heidelberg, Heidelberg, Germany

[3] Department of Transplantation Immunology, University of Heidelberg, Heidelberg, Germany

[4] Department of Surgery, University Medical Center Mannheim, University of Heidelberg, Mannheim, Germany

[5] Department of Radiology, Division of Nuclear Medicine, University of Heidelberg, Heidelberg, Germany

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## Introduction

Kidney transplantation is the gold standard treatment for end-stage kidney failure [1]. However, long-term graft and patient survival are still limited. Death with a functioning graft and chronic allograft injury due to antibody-mediated rejection (ABMR) are the most common causes of graft loss during long-term follow-up after kidney transplantation. Death with a functioning kidney graft is often preceded by severe side effects of immunosuppressive medication, such as infection or malignancy. Thus, there is a need for the development of treatment strategies that enable sufficient immunosuppression to prevent ABMR, while avoiding the well-known deleterious side effects of immunosuppressive therapy. The ideal solution would be the induction of tolerance by establishing selective (donor-specific) unresponsiveness of the transplant recipient without a need for broad non-specific immunosuppression, thereby retaining full responsiveness of the patient's immune system against bacteria, viruses and other pathogens [2].

## Historical background to transplantation tolerance

Starting with the pioneering scientific work by Sir Peter Medawar and colleagues more than 60 years ago, it has become well known that alloantigens can not only activate but also inhibit immune responses. Billingham and colleagues induced tolerance in newborn mice by in utero injection of allogeneic bone marrow cells. A subsequently transplanted skin graft from the same donor was tolerated in adult mice

while third party grafts were rejected [3]. Today, tolerance is believed to be the Holy Grail of transplantation. However, tolerance is not uniformly defined, and various degrees of tolerance can be discriminated. Complete tolerance is the permanent and specific immunologic acceptance of alloantigens with full allograft acceptance; immunosuppressive medication is not needed [4]. Patients with clinical operational tolerance have well-functioning allografts without receiving immunosuppressive medication while an immune response against the transplanted alloantigens is still detectable [5]. Clinical operational tolerance often results from immunosuppression withdrawal many years after successful transplantation, such as in patients non-adherent to immunosuppressive medication. Clinical operational tolerance is seen at a higher frequency in pediatric patient populations than in adolescents. The prominent thymic function with the production of naïve T cells plays an important role in tolerance induction [6]. The rate of tolerance is generally higher if transplantation is performed in infants, illustrating the special role of tolerance induction in pediatric patients [7, 8].

## The recipient's immune system, regulatory T cells and tolerogenic dendritic cells

Discrimination between self and non-self is the key element of allorecognition. Recipient T cells recognize non-self-antigens from the foreign tissue present after transplantation. These activated T cells perform effector functions to reject the graft tissue. Artificial deprivation of T cells has been achieved in animal models and is associated with the avoidance of allograft rejection. To the contrary, T cells also play an important role as regulators of autoimmune responses. They are selected in the thymus whereby those with a high affinity for self-antigens are deleted, although some T cells escape thymus censorship and enhance the risk for autoimmunity. Regulatory T cells (Tregs) are the counterparts of T cells in that they are crucial for the maintenance of immunological tolerance. A major role of these cells is the limitation of T cell-mediated immunity towards the end of an immune reaction and the suppression of autoreactive T cells that escaped the process of negative selection in the thymus.

Two major Treg cell types can be distinguished, namely naturally occurring thymus-derived Tregs (tTreg), which develop in the thymus, and peripheral Tregs (pTreg), which develop by conversion from mature $CD4^+$ conventional T cells outside of the thymus [9, 10]. A reliable marker differentiating tTregs from pTregs has not yet been found. However, pTreg cells are believed to be an essential supplementary subset to tTreg cells by expanding T-cell receptor diversity within regulatory responses [11]. Tregs in general are detectable by their expression of the interleukin (IL)-2 receptor alpha-chain (CD25) and a low or negative expression of the IL-7 receptor alpha-chain (CD127) [12]. In addition, these cells express the transcription factor forkhead box (Fox) P3 which is essential for their function. Mutations of the *FoxP3* gene can prevent regulatory T-cell development, as illustrated by the lethal autoimmune disease IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome [13]. Treg-cell dysfunction caused by *FoxP3* gene mutation is the main pathogenic event leading to multiorgan autoimmune disease.

Tregs are divided into three major Treg subsets: central Treg (cTreg; resting Treg), effector Treg (eTreg; activated Treg) and memory Treg (mTreg) [14, 15]. Following thymic exit, cTreg cells express high levels of anti-apoptotic molecules and accumulate in lymphoid organs. cTregs differentiate into eTregs. eTreg cells are highly proliferative, predispose to apoptosis and are the dominant Treg cell population in non-lymphoid tissues [16]. eTregs develop into functional subsets such as T helper 1 Tregs ($T_H1$-Tregs), T helper 2 Tregs ($T_H2$-Tregs), T helper 17 Tregs ($T_H17$-Tregs) and T follicular helper Tregs ($T_{FH}$-Tregs) that can suppress specific T effectors [10]. MTregs show a low proliferative status and remain activated in the absence of ongoing antigen stimulation [15].

Tregs exert their immunoregulatory properties by targeting either T cells (e.g., cytolysis, release of suppressive cytokines, IL-2 consumption) or antigen-presenting cells (APCs), such as via reduced co-stimulation or antigen presentation [17]. In pregnant women, possible mechanisms by which Tregs induce tolerance can be examined. The role of Tregs in promoting tolerance to fetal alloantigens has convincingly been shown [18–20]. Pathologic changes within regulatory T-cell populations have been associated with preterm labor and pregnancy-associated diseases such as preeclampsia and HELLP syndrome [21–26]. These diseases show comparable pathologies to those seen in patients with biopsy-proven rejection after kidney transplantation. A distinct subset of human leucocyte antigen-antigen D related (HLA-DR)-positive Treg cells has a high impact on the suppressive activity of the Treg cell pool and their disappearance is a strong indicator for a rejection process [27, 28]. Aside from Tregs cells, dendritic cells (DCs), which are professional APCs, have the capability to induce immune suppression as well. The tolerogenic potential of DCs is indicated by the downregulation of factors related to boosting the immune response and the upregulation of inhibitory cytokines [29, 30]. Antigen presentation without co-stimulation inactivates effector T cells and impairs antigen processing by non-activated APCs, both of which contribute to the maintenance of self-tolerance [31, 32].

## Clinical protocols for tolerance induction

Clinical experience with the induction of tolerance has been obtained in adult kidney transplant recipients; however, studies investigating pediatric patients are rare. Reported cases of achieved (clinical operational) tolerance usually involve non-adherent patients who stopped their immunosuppressive medication but did not experience rejection of their allografts [5]. Prospective induction of tolerance has been attempted by combining living-donor kidney with bone marrow transplantation after the application of myeloablative or non-myeloablative conditioning regimens [33–35]. Other therapeutic strategies using cell products that are composed of immunoregulatory cell populations are currently being tested. The introduction of these newly developed strategies is accompanied by the need to overcome several hurdles. As with all other new medical therapies, cell-based products need to be validated in clinical trials. Strict governmental regulations are in place for the production and application of cell preparations [advanced therapy medicinal products (ATMPs); see section Clinical development and regulatory milestones for advanced therapy medicinal products] that are manufactured under conditions of Good Manufacturing Practice (GMP) [31, 36, 37]. The situation is often complicated because cell therapeutic approaches are usually combined with immunosuppressive medication that may interfere with the integrity of the applied cell population [38]. Table 1 gives an overview of all studies on cell therapeutic approaches to clinical tolerance that have been registered with www.clinicaltrials.gov as of December 2016.

### Combined kidney and bone marrow transplantation

The aim of combined kidney and bone marrow transplantation is the induction of (transient) mixed chimerism, a state in which bone marrow hematopoietic stem cells from two genetically different individuals coexist, representing a state of immunological tolerance [64]. Early data were provided by Spitzer et al. in patients with multiple myeloma given fully HLA-matched combined kidney and bone marrow transplants. Complete immunosuppressive drug withdrawal was achieved without evidence of acute or chronic kidney rejection or the occurrence of kidney disease in four of seven recipients [65].

Only a few centers have experience with inducing tolerance using a chimerism approach in living-donor kidney transplantation, namely Stanford University (Stanford, CA), Massachusetts General Hospital (Boston, MA) and Northwestern University (Chicago, IL). The main difference between the three different protocols used at these centers is the conditioning of the recipients. The Stanford group reported the use of a combination of total lymphoid irradiation (10 doses of 80 or 120 cGy), rabbit anti-thymocyte globulin (1.5 mg/kg at 5 daily doses) and intravenous methylprednisolone [56]. The MGH group initially used cyclophosphamide (60 mg/kg, days −5 and −4), humanized anti-CD2 monoclonal antibody (0.1 mg/kg day −2 and 0.6 mg/kg days −1, 0 and +1), intravenous calcineurin inhibitor (5 mg/kg day −1) and thymic irradiation (700 cGy day −1). During the course of the study rituximab and prednisone were added to prevent B-cell mediated rejections [34]. The Northwestern Group reported using a conditioning protocol consisting of total body irradiation (200 cGy day −1), cyclophosphamide (50 mg/kg day −3 and +3) and fludarabine (30 mg/kg days −4, −3, −2) [66].

The Stanford Group reported on 22 HLA-matched and 16 HLA-mismatched patients after combined living-donor kidney and $CD34^+$ hematopoietic stem cell transplantation [56]. Graft survival was 100% during the maximum observation period of 14 years. Of the 22 HLA-matched patients 19 demonstrated persistent chimerism for at least 6 months, among whom 16 were successfully weaned from immunosuppressive medication. The results of the HLA-mismatched transplants, however, are sobering. None of the patients developed chimerism beyond 3 months. Patients with transient chimerism, defined by the absence of rejection episodes, graft-versus-host disease (GvHD) and reactivity to donor cells were withdrawn from immunosuppression. All of these patients developed rejections, necessitating the reintroduction of immunosuppressive medication.

The Boston group recently published their experience with ten HLA mismatched kidney transplants after combined kidney and bone marrow transplantation [34]. All ten patients presented transient chimerism, and in seven of the patients immunosuppression was successfully discontinued for at least 4 years. To date, four patients remain free of immunosuppressive medication, ranging for periods of 4.5 to 11.4 years, while three have required reinstitution of immunosuppressive therapy due to recurrence of original disease or rejection. Two patients have lost their allograft, one due to antibody-mediated rejection and the other due to presumed tacrolimus-associated thrombotic microangiopathy.

At Northwestern University, 25 patients were transplanted after preconditioning in a HLA-mismatched living-donor kidney transplantation trial. Follow-up data of more than 18 months were available for 17 patients, of whom 12 developed persistent chimerism, four patients showed transient chimerism and one patient never developed chimerism. Results from Northwestern University of a non-chimeric operational tolerance protocol in ten renal transplant recipients, HLA-identical with their living-donor siblings, demonstrate an association between global RNA expression profiling and operational tolerance. Moreover, a time-dependent increase of circulating $CD4^+CD25^+CD127^-FOXP3^+$ Tregs in patients showing operationally tolerant versus a loss of Tregs in non-tolerant subjects was demonstrable [66].

**Table 1** Cellular treatment approaches for the control of allograft rejection and tolerance induction in clinical kidney transplantation

| Cell type | Clinical trial identifier | Trial phase | Start date | Number of patients | Cell treatment protocol | Location | Reference |
|---|---|---|---|---|---|---|---|
| Mitomycin-induced peripheral blood mononuclear cells | | | | | | | |
| Donor PBMCs treated with mitomycin C | NCT02560220 | I | 10/2015 | 10 | -MICs i.v. ($1.5 \times 10^6$ or $1.5 \times 10^8$/ kg BW) 2 or 7 days pre Tx | Heidelberg, Germany | [2, 39–41] |
| Mesenchymal stem/stromal cells | | | | | | | |
| MSCs derived from BM (BMSCs) | NCT02490020 | I | 01/2016 | 260 | -BMSCs i.v. ($2 \times 10^6$/ kg BW) 48 h pre Tx or 1 and 7 days post Tx<br>-BMSCs i.v. ($2 \times 10$ / kg BW) and i.a. ($5 \times 10^6$) 48 h pre Tx | Guangzhou, China | |
| Third-party allogeneic BMSCs | NCT02563366 | I / II | 11/2015 | 120 | -MSCs i.v. ($1 \times 10^6$/ kg BW), 4 doses at weekly intervals (early poor graft function / deceased donors) | Guangzhou, China | [42] |
| Allogeneic BMSCs from third-party donor | NCT02563340 | I / II | 11/2015 | 60 | -MSCs i.v. ($1 \times 10^6$/ kg BW), 4 consecutive doses every 2 weeks to patients with cAMR + desensitization therapy to decrease DSA (no HLA matches to organ donor and recipient) | Guangzhou, China | [42] |
| Allogeneic BMSCs from third-party donor | NCT02561767 | I / II | 10/2015 | 120 | -MSCs i.v. ($1 \times 10^6$/ kg BW) at day of Tx, 7, 14 and 21 days post Tx<br>-Patients with transplant from the same deceased donor randomly assigned to cell treatment and control group | Guangzhou, China | [42] |
| Third-party (healthy donor) allogeneic in vitro expanded BMSCs | NCT02565459 | I | 09/2015 | 22 | Recipients with transplant of deceased donor:<br>-Single MSC i.v. infusion ($1–2 \times 10^6$/ kg BW) | Bergamo, Italy | |
| Autologous ex vivo expanded BMSCs | NCT02012153 | I | 10/2013 | 6 | -MSCs i.v. ($2 \times 10^6$/ kg BW) 1 day pre Tx | Bergamo, Italy | |
| Autologous ex vivo expanded BMSCs | NCT00752479 | I / II | 05/2008 | 7 | -MSCs i.v. ($2 \times 10^6$/ kg BW) 1 day pre Tx or 7 days post Tx | Bergamo, Italy | [43, 44] |
| Third-party donor BMSCs, no sharing of HLA with mismatches of kidney donor and recipient | NCT02387151 | I | 03/2015 | 10 | -MSCs i.v. ($1–2 \times 10^6$/ kg BW) 25 and 26 weeks post Tx when immunosuppression is lowered | Leiden, Netherlands | [45] |
| Autologous BMSCs | NCT02057965 | II | 03/2014 | 70 | -MSCs i.v. ($1–2 \times 10^6$/kg BW) 6 and 7 weeks post Tx (graft from deceased donors) | Leiden, Netherlands | [46] |
| Autologous expanded BMSCs from HLA-DR mismatched patient | NCT00734396 | I / II | 02/2009 | 15 | -MSCs i.v. ($1–2 \times 10^6$/ kg BW) into patients with subclinical rejection or an increase in IF/TA in biopsy 4 weeks or 6 months post Tx | Leiden, Netherlands | [46] |
| Autologous SVF-derived, in vitro cultured MSCs | NCT02492490 | I / II | 12/2014 | 120 | -MSCs i.v. on day of Tx and 7, 14 and 21 days post Tx (deceased transplant donors) | Fuzhou, China | |
| Autologous SVF-derived, in vitro cultured MSCs | NCT02492308 | I / II | 12/2014 | 120 | -MSC i.v. infusion on day of Tx and 7, 14 and 21 days post Tx (living related Tx) | Fuzhou, China | |
| Autologous ex vivo expanded MSCs | NCT00659620 | I / II | 05/2008 | 20 | -MSCs i.v. as induction therapy on day of Tx (+ second dose of MSCs in some patients) | Fuzhou, China | |
| Autologous MSCs from BM | NCT00658073 | n.d. | 03/2008 | 165 | -MSCs i.v. as induction therapy on day of Tx and 2 weeks post Tx | Fuzhou, China | [47] |
| Autologous or allogeneic BMSCs | NCT02409940 | I | 09/2013 | 30 | In vitro expanded MSCs (autologous or allogeneic) 1 day pre and 30 days post Tx | Chandigarh, India | [48, 49] |
| Third-party donor BMSCs | NCT01429038 | I / II | 02/2012 | 20 | -MSC infusion ($1.5–3 \times 10^6$ / kg BW) 3 (±2) days post Tx (no HLA matching with organ donor and recipient) | Liege, Belgium | |

**Table 1** (continued)

| Cell type | Clinical trial identifier | Trial phase | Start date | Number of patients | Cell treatment protocol | Location | Reference |
|---|---|---|---|---|---|---|---|
| Hematopoietic stem cells | | | | | | | |
| BHSCs (HLA-matched sibling organ donor) | NCT02176434 | n.d. | 09/2015 | 5 | - Mobilized $CD34^+$ progenitor ($\geq 1 \times 10^7$/ kg BW) and T cells ($1 \times 10^6$ / kg BW) from peripheral blood 11 days post Tx | Zürich, Switzerland | [50] |
| BM of living haploidentical related donor | NCT02314403 | I | 02/2015 | 5 | -BM infusion simultaneously with Tx | Boston, MA, USA | [33, 34, 51, 52] |
| Donor HLA-matched BM | NCT02158052 | n.d. | 02/2015 | 10 | Patients with hematological malignancy or disorder and endstage renal transplant: -BM i.v. ($>2 \times 10^8$ nucleated cells / kg BW) after immunosuppressive and radiation conditioning | Boston, MA, USA | |
| BM of living haploidentical related donor | NCT01780454 | I / II | 03/2013 | 2 | -BM infusion simultaneously with Tx | Boston, MA, USA | [33, 51, 52] |
| BM of living donor | NCT00801632 | II | 12/2012 | 5 | -BM infusion simultaneously with Tx | Boston, MA, USA | [33, 53] |
| Haploidentical BM of first degree relative | NCT01758042 | n.d. | 11/2012 | 10 | -Infusion of BM after conditioning (hematological advanced disorder and chronic kidney disease) | Boston, MA, USA | |
| BM of living related donor | NCT00063817 | I | 06/2003 | 5 | -BM infusion | Boston, MA, USA | [33] |
| HLA-matched BM from related donor | NCT00062621 | I | 06/2003 | 5 | - Infusion of BM on day of Tx (patients with multiple myeloma and kidney failure) | Boston, MA, USA | |
| Donor HLA-matched BM | NCT00854139 | I | 08/2001 | 10 | Patients with multiple myeloma and endstage renal disease: -BM i.v. ($>2 \times 10^8$ nucleated cells / kg BW) after immunosuppressive and radiation conditioning | Boston, MA, USA | |
| BM of haploidentical related donor | NCT02029638 | II | 01/2014 | 3 | -Unprocessed, unmanipulated BM transplantation on day of Tx | Baltimore, MD, USA | |
| Donor BHSCs and graft facilitating cells | NCT02051673 | c.u. | 01/2014 | 4 | -Infusion of enriched hematopoietic stem cell transplant | Chicago, IL, USA | |
| BHSCs and graft facilitating cells from donor | NCT01649388 | I / II | 05/2012 | 30 | -Infusion of enriched cells from living transplant donor post Tx | Chicago, IL, USA | |
| DHSCs from HLA-identical sibling | NCT00619528 | n.d. | 07/2007 | 230 | -DHSC infusion pre Tx and 3, 6 and 9 months post Tx | Chicago, IL, USA | [54] |
| BHSCs and graft facilitating cells from donor | NCT00497926 | II | 03/2008 | 60 | -Application of enriched cells from living transplant donor | Chicago, IL; Durham, NC, USA | [54] |
| Donor BHSCs and graft facilitating cells | NCT00498160 | I / II | 01/2005 | 38 | -Infusion of cells derived from specially treated BM (living donor and deceased donor transplant recipients) | Chicago, IL; Louisville, KT, USA | |
| BHSCs from HLA-haploidentical allogeneic organ donor | NCT02199301 | n.d. | 12/ 2011 | 6 | -BM infusion immediate post Tx | Seoul, Republic of Korea | |
| Donor DBMCs | NCT00183248 | I / II | 09/2004 | 9 | -DBMCs i.v. infusion () 5 days and once between 4 and 6 months | Miami, FL, USA | [55] |
| BM of living related donor | NCT00018785 | I / II | 10/1998 | n.p. | - BM infusion | Miami, FL, USA | |
| HSCs and T cells from haploidentical donor | NCT01165762 | n.d. | 07/2010 | 25 | -Purified G-CSF-mobilized donor $CD34^+$ HSC and T cell infusion 2 weeks post Tx | Stanford, CA, USA | [50, 56–58] |
| HSCs and T cells from completely HLA-matched sibling donor | NCT00319657 | I | 07/2004 | 15 | -Purified G-CSF-mobilized donor CD34+ HSCs ($8–10 \times 10^6$/ kg BW) and T cells ($1 \times 10^6$/ kg BW) 2 weeks post Tx | Stanford, CA, USA | [50, 58–60] |
| Allogeneic BM | NCT00062712 | II | 06/2003 | 20 | -BM infusion 7 days post Tx (living and cadaveric kidney transplant recipients) | Bethesda, MD, USA | |

**Table 1** (continued)

| Cell type | Clinical trial identifier | Trial phase | Start date | Number of patients | Cell treatment protocol | Location | Reference |
|---|---|---|---|---|---|---|---|
| Regulatory T cells | | | | | | | |
| Autologous polyTregs and darTregs | NCT02711826 | I / II | 05/2016 | 45 | Patients with biopsy-proven subclinical inflammation of graft within 6 months post Tx:<br>-polyTreg infusion ($400 \pm 100 \times 10^6$/ kg BW)<br>-darTreg infusion ($400 \pm 100 \times 10^6$/ kg BW) | Birmingham, AL; Ann Arbour, MI;<br>Los Angelos, CA; San Francisco, CA; Cleveland, OH, USA | |
| Autologous polyclonally expanded $CD4^+CD25^+FoxP3^+$ nTregs | NCT02371434 | I / II | 01/2015 | 9 | - nTreg infusion ($0.5 \times 10^6$, $1 \times 10^6$ or $3 \times 10^6$) post Tx | Berlin, Germany | |
| Autologous darTregs | NCT02244801 | I | 11/2014 | 16 | -darTreg infusion ($3 \times 10^8$) and ($9 \times 10^8$) | San Francisco, CA, USA | [61] |
| Autologous ex vivo expanded polyclonal $CD4^+$ $CD127loCD25^+$ Tregs | NCT02088931 | I | 03/2014 | 3 | -Treg infusion ($3.2 \times 10^8$) to patients with biopsy-proven renal inflammation | San Francisco, CA, USA | |
| Autologous Tregs from ex vivo culture with donor PBMCs + belatacept | NCT02091232 | I | 05/2014 | 8 | -Treg infusion 7 days (+3 days) post Tx | Boston, MA, USA | |
| Autologous in vitro expanded nTregs from cryopreserved PBCs | NCT02145325 | I | 04/2014 | 10 | -$CD4^+$ $CD25^+$ Treg i.v. infusion 2 months post Tx | Chicago, IL, USA | |
| Autologous in vitro expanded nTregs | NCT02129881 | I / II | 04/2014 | 12 | -nTreg i.v. infusion ($1-10 \times 10^6$/kg BW) 5 days post Tx | Oxford, UK | [61] |
| Autologous Tregs expanded ex vivo from cryopreserved $CD4^+$ T cells | NCT01446484 | I | 10/2011 | 30 | Kidney Tx in children and adolescents:<br>-$CD4^+CD25^+CD127lowFoxP3^+$ T regulatory cells ($2 \times 10^8$, s.c.) 30 and 180 day post Tx | Moscow, Russia | |
| Myeloid regulatory cells | | | | | | | |
| Autologous tolerogenic DCs | NCT02252055 | I / II | 03/2015 | 16 | -In vitro generated ATDCs i.v. ($1 \times 10^6$/kg BW) 1 day pre Tx | Nantes, France | [61] |
| Regulatory Mregs | NCT02085629 | I / II | 07/2014 | 16 | -Donor-derived Mregs i.v. ($2.5-7.5 \times 10^6$/kg BW) 6–7 days pre Tx | Regensburg, Germany | [61] |
| Transplant-acceptance inducing cells | NCT00223093 | I / II | 02/2004 | 10 | -Infusion of TAICs generated from splenic mononuclear cells ($>1 \times 10^6$/kg BW) 5 days post Tx (deceased donor) | Kiel, Germany | [62] |
| TAICs of living related donor | NCT00223067 | I | 03/2005 | 10 | Infusion of TAICs generated from adherent PBMCs of living related donor cocultured with monocyte-depleted PBMCs of recipient ($>1.5 \times 10^7$/kg BW) 5 days pre Tx | Kiel, Germany | [63] |

The data on the clinical trials and corresponding references are based on the information provided in the web-based resource "ClinicalTrials.gov" maintained by the U.S. National Library of Medicine (NLM) at the National Institutes of Health (NIH), Bethesda, MD. Due to limited space, only the novel regimens comprising various cellular preparations are listed. These approaches are combined with standard or novel immunosuppressive induction and/or maintenance medication depending on the respective trial settings. Comprehensive data on each trial, including detailed treatment protocols and assignments to the different treatment groups, can be found within the database and related published references

ATDC, Autologous tolerogenic dendritic cell; BHSC, bone marrow-derived hematopoietic stem cell; BM, bone marrow; BMSC, bone marrow-derived mesenchymal stem/stromal cell; BW, body weight; cAMR, chronic antibody-mediated rejection; c.u., compassionate use; darTreg, donor-alloantigen-reactive regulatory T cell; DBMC, donor bone marrow cell; DC, dendritic cell; DHSC, donor hematopoietic stem cell; G-CSF, granulocyte-colony stimulating factor; HLA, human leukocyte antigen; HSC, hematopoietic stem cell; i.a., intra-arterial; IF/TA, interstitial fibrosis and tubular necrosis; i.v., intravenous; MIC, mitomycin C-induced cell; Mreg, regulatory macrophage; MSC, mesenchymal stem/stromal cell; n.d., not defined; nTreg natural regulatory T cell; PBMC, peripheral blood mononuclear cell; polyTreg, polyclonally expanded regulatory T cell; SVF, stromal vascular fraction; TAIC, transplant-acceptance inducing cell; Treg, regulatory T cell; Tx, transplantation

These results are encouraging. However, there are important issues that need to be addressed before the routine application of these approaches is feasible, particularly a reduction of toxicity of the current regimens and the reliable achievement of chimerism, to mention a few.

## Mesenchymal stem cell therapies

Pluripotent mesenchymal stem cells (MSCs) are naturally found in the bone marrow where they are precursors to bone, fat and other connective tissues. It has been postulated that this cell type also has immunosuppressive properties [67]. The exact mechanisms remain to be defined, however, an increase in Tregs after MSC infusion has been shown [68]. Tan and colleagues conducted the first randomized trial to assess the role of autologous MSC infusion as an induction agent for living-donor kidney transplantation [47]. A total of 159 living-donor kidney transplant recipients were divided into three treatment arms: (1) MSC treatment ($1–2 \times 10^6$ cells/kg body weight, on days 0 and 14) together with standard dose cyclosporine; (2) MSC treatment together with reduced dose cyclosporine (80%); (3) basiliximab (a monoclonal anti-CD25 antibody) instead of MSC induction therapy together with standard dose cyclosporine. Mycophenolic acid and glucocorticoids were administered at the same standard doses in all treatment arms. Patients treated with MSCs had better kidney function, a reduced risk of opportunistic infections and no rejection episodes, while four rejection episodes occurred in control group C. The overall rejection rate in recipients of a living-related kidney transplant during the first year after transplantation was relatively high (26%), and there are still concerns about a possible malignant transformation of MSCs. Further studies are therefore needed to assess this therapeutic approach.

Another study from China provided evidence for a safe use of MSCs. Six living-donor kidney transplant recipients received autologous MSC infusions (first infusion at the time of transplantation, second infusion 1 month later) and low-dose tacrolimus (mean dose 0.045 mg/kg), whereas six control group patients received tacrolimus (mean dose 0.077 mg/kg) as the primary immunosuppressant. All patients had good graft function during a follow-up period of 12 months. One control group patient suffered from an acute rejection episode while no such episode occurred in the study group [42].

In 2015, an Indian group published data from a pilot study with four patients who underwent living-donor kidney transplantation. These patients received low-dose anti-thymocyte globulin induction followed by calcineurin inhibitor-based triple drug immunosuppression. Autologous MSCs were isolated after bone marrow aspiration 4–6 weeks prior to transplantation. All patients received the first infusion 1 day before transplantation and a second infusion 30 days after surgery. During a follow-up of 6 months, none of the patients developed adverse events. No clinical or protocol biopsy-proven graft injury was detectable, while an increase of blood $CD4^+CD25^+FOXP3^+$-Tregs was noted [48].

## Treg therapies

Regulatory T cells are believed to play a key role in tolerance induction. Tregs have been implicated in the immunosuppressive mechanisms of all cell types discussed in this overview. The main rationale for a therapeutic Treg application is a shift in the naturally existing equilibrium between conventional T cells (T-con) and Tregs in favor of Tregs [36]. Although the exact dose for optimal immunosuppression is not known, it is widely accepted that enabling cell-based immunosuppression necessitates the infusion of billions of Treg cells. However, the required doses for different indications remain to be defined and appear to vary greatly. Published trials in type I diabetes mellitus and in hematopoietic stem cell transplantation report the use of doses between 0.1 and $20 \times 10^6$ cells/kg body weight [69]. Ex vivo expansion of isolated Tregs can be achieved when addressing CD28 costimulation. In combination with rapamycin a 1000-fold increase in Treg numbers over approximately 3 weeks of culture has been documented [70]. Alternatively, Tregs may be generated in the presence of IL-2 and transforming growth factor-beta (TGF-β) by conversion of T-cons [36].

To date, only a few clinical studies have assessed Treg cell therapy for GvHD prophylaxis in patients after hematopoietic stem cell transplantation. In a phase I study published in 2011, Tregs were expanded and administered to patients before stem cell transplantation. The procedure was safe, and GvHD rates were reduced compared to rates in control group patients [71]. Another study showed the prevention of GvHD by combined infusion of Tregs and T-con [72].

To date, no published results are available on the clinical use of Tregs after solid organ transplantation. The ongoing ONE Study, part of a collaboration between U.S. and European centers, focuses on living-donor transplant recipients. A phase I trial testing the safety of Treg application is also underway, and the results are expected in 2017 [61]. A trial from San Francisco evaluates Treg cell infusion after kidney transplantation as an adjunct immunosuppressive therapy, aiming at preventing biopsy-confirmed rejections during a 60 month follow-up period (see Table 1, Clinical trial identifier NCT02088931).

The total Treg pool consists of a variety of subpopulations with different functions. The ideal Treg subpopulations and number of Tregs needed for clinical application are yet to be defined [73].

### Regulatory myeloid cells

Myeloid cells derive from hematopoietic stem cells and may differentiate into various subsets. In human in vitro models, different regulatory myeloid cells can be generated from peripheral blood mononuclear cells (PBMCs), i.e., transplant acceptance-inducing cells (TAICs), regulatory macrophages (Mregs), dendritic regulatory cells (DCregs) and myeloid-derived regulatory cells [74].

Mregs are derived from peripherally isolated $CD14^+$ monocytes that are cultured together with macrophage colony-stimulating factor and interferon-gamma. Several murine studies have yielded evidence of their immunosuppressive properties. Inhibition of T-cell activation has been shown to be associated with inducible nitric oxide synthase [75]. The potential of Mregs to induce tolerance has been shown in rodent solid organ transplantation models. In humans, the TAIC-I clinical trial assessed the safety and tolerability of administering TAICs 5 days after transplantation to recipients of a deceased donor kidney graft; no adverse events occurred [76]. The TAIC-II clinical trial included five living-related kidney transplant recipients, and once again the administration of TAICs was found to be safe [63]. Of the five patients, two were withdrawn from steroids within 8 weeks, and tacrolimus trough levels were weaned to 2 ng/ml without signs of graft dysfunction during a follow-up of 36 months. One patient was excluded due to a biopsy-proven acute rejection which, however, was evident even before TAIC administration. Two years after transplantation this patient presented with a well-functioning allograft; HLA antibodies were no longer positive [63].

Since conducting these two trials, Hutchinson et al. have refined their Mreg purification technique and treated two additional living-donor kidney transplant recipients. Both patients were successfully transplanted and weaned to tacrolimus monotherapy [62]. Based on these results, it would appear that Mreg cell therapy might be a safe and efficient approach for achieving tolerance. Further proof of safety and efficacy, however, is needed. Currently, Mreg therapy as well as other cell therapies are being tested within The One study [61].

DCregs derive from PBMCs by costimulation of granulocyte/monocyte colony stimulation factor in addition to IL-4, IL-10 and TGF-β. A potential beneficial role has been shown by suppressing autoimmunity in type I diabetes [77], however, data in solid organ transplantation are lacking.

Myeloid-derived suppressor cells (MDSCs) are naturally occurring and expanded during inflammation. Most existing knowledge on these cells derives from cancer biology studies which have investigated immunosuppressive mechanisms [78]. Data on solid organ transplantation cases are rare. Vanhove and colleagues showed the induction of immune tolerance in a rat kidney transplantation model and an accumulation of these cells in the allograft [79, 80]. In vitro, MDSCs were able to induce T-cell apoptosis. A cross-talk between MDSCs and Tregs was noted [79, 80]. Clinical studies are lacking. Recent hematologic data suggest that GvHD can be controlled by MDSC treatment in mouse models [81]. In that study, MDSCs were shown to be associated with Treg induction and prevention of the initiation of an adaptive immune response. Elevated frequencies of circulating MDSCs were measured in patients after kidney transplantation, pointing to a possible role of MDSCs in tolerance induction [81].

## Induction of antigen-specific immunosuppression by mitomycin-induced cells

Manipulation of DCs by various chemical, pharmaceutical or biological means can convert these highly immunostimulatory APCs into cells with tolerogenic properties, making them capable of inhibiting or even actively suppressing immune responses. Therefore, tolerogenic DCs are considered for clinical application as a means to prevent rejection episodes in organ transplantation, as well as for the suppression of deleterious immune reactions in autoimmune diseases [29, 82, 83]. Mitomycin C (MMC), due to its cytostatic properties causing non-immunogenic apoptotic cell death and its usage for decades for treating various types of cancer (including bladder, metastatic breast, cervical, head and neck, non-small cell lung, gastric, pancreas and colon cancer), is thought to be a suitable candidate for modifying highly stimulatory immune cells (e.g. DCs) into cells exhibiting immunoregulatory properties [84, 85]. It had been shown in in vitro and in vivo studies in mouse and rat that tolerogenic MMC-treated DCs are capable of controlling both allograft rejection and autoimmune reactions. Protocols have been established to generate Good Manufacturing Practice-grade regulatory DCs in vitro [83]. However, there are still concerns that modified DCs regain their immunostimulatory properties when transfused into a living organism. PBMCs have been tested as an alternative to DCs. In a rat heart transplantation study, 50% of the recipient animals achieved long-term acceptance of the transplant, with >70 days of survival after the administration of a high dose of $1 \times 10^8$ MMC-induced PBMCs [mitomycin C-induced cells (MICs)] prior to transplantation [39]. Prophylactic treatment of the recipient resulted in donor-specific unresponsiveness. Infusing untreated blood cells instead of MICs or transplanting a heart from a third-party rat strain caused the graft to be rejected in an accelerated fashion [39]. Relevant preclinical treatment strategies using MMC-treated cell preparations in transplantation and autoimmune diseases are given in Table 2.

MIC therapy was applied for the first time to a human patient suffering relapses of acute lymphoblastic leukemia in an individual emergency treatment attempt. To control

**Table 2** Preclinical treatment strategies using mitomycin C-treated cell preparations in transplantation and autoimmune diseases

| Animal model | Mitomycin-induced cell population and treatment modality | Major observations of outcome | Reference |
|---|---|---|---|
| Rat | | | |
| Heart Tx | Donor PBMCs ($1 \times 10^8$), i.v., 7 days prior to Tx | -Significant prolongation of allograft survival<br>-Tolerance induction in 50% of recipients (>70 day survival)<br>-Donor-specific immunosuppression<br>-Induction of $CD4^+CD25^+FoxP3^+$ Tregs<br>-Increase of Tregs in lymphatic organs and cardiac allograft<br>-Induction of tolerance in naïve animals via adoptive transfer of peripheral immune cells from tolerant recipients<br>-Monocytes are necessary for tolerance induction | [39] |
| | Donor PBMCs ($1 \times 10^7$), i.v., 7 days prior to Tx | -Significant prolongation of allograft survival<br>-Induction of apoptosis in donor PBMCs | [86] |
| | MMC perfusion of allograft prior to Tx | -Significant prolongation of allograft survival | |
| | Donor DCs ($1 \times 10^6$), i.v., 7 days prior to Tx | -Significant prolongation of allograft survival<br>-Donor-specific immunosuppression<br>-Loss of allostimulatory capacity<br>-Active suppression of T-cell proliferation in vitro/in vivo<br>-Generation of tolerogenic DCs by downregulation of cell surface receptors CD80, CD86 and ICAM-1 | [88]<br>[87] |
| | Donor splenocytes ($5 \times 10^7$), i.v., 10 day prior to Tx | -Significant prolongation of allograft survival<br>-Donor-specific immunosuppression<br>-Antigen-specific suppression of T-cells in vitro | [89] |
| Composite tissue Tx (hind limb) | Donor PBMCs ($1 \times 10^8$), i.v./i.m., on day of Tx | -Significant prolongation of allograft survival | [90] |
| LiverTx<br>Heart Tx<br>Small bowel Tx<br>Skin Tx | Donor splenocytes ($3 \times 10^6$), i.p./i.v., 7 days prior to Tx | -Significant prolongation of allograft survival: liver (i.p./i.v.), heart, small bowel (i.v.)<br>-No prolongation of skin allograft survival<br>-Donor-specific immunosuppression<br>-Accumulation of donor splenocytes in the spleen of recipient | [91] |
| Mouse | | | |
| Experimental autoimmune encephalomyelitis (EAE) | Syngeneic splenocytes loaded with surrogate autoantigen glatiramer acetate ($2 \times 10^7$), i.v., on 3 consecutive days in remission phase after 1st relapse | -Significant reduction of relapse rate and duration of relapse<br>-Antigen-specific immunosuppression towards disease-inducing autoantigen<br>-Induction of $CD4^+CD25^+FoxP3^+$ Tregs<br>-Increase of Tregs in peripheral lymphatic organs and CNS | [92] |
| | Syngeneic DCs loaded with autoantigen MBP ($5 \times 10^6$), i.v., 5 days prior to EAE induction | -Resistance of mice towards EAE induction<br>-Protection of mice from autoimmune disease<br>In vitro suppression | [40] |
| Heart Tx | Donor splenocytes ($1 \times 10^7$), i.v., 7 days prior to Tx | -Significant prolongation of allograft survival<br>-Induction of apoptosis in donor splenocytes<br>-Increase of $CD4^+CD25^+FoxP3^+$ Tregs in the spleen | [93] |
| | MMC perfusion of allograft | -Significant prolongation of allograft survival | |
| Pancreatic islet Tx | Donor islets ($3.5–4 \times 10^2$) after 20 h in vitro culture | -Significantly prolonged survival (43% >100 days)<br>-Restoration of normoglycemia | [94] |
| Pig | | | |
| Kidney Tx | Donor PBMCs ($1 \times 10^8$) + CsA (10 mg/kg/day) day 14 to day 3 prior to Tx | -Significant prolongation of kidney allograft survival | [39] |
| Human | | | |
| Hematopoietic stem cell Tx | Haploidentical CD3/CD19-depleted peripheral blood stem cells | -Suppression of a starting rejection episode<br>-Efficient reduction of autologous B-, NK- and T cells<br>-Establishment of complete haematopoietic chimerism | [39] |

**Table 2** (continued)

| Animal model | Mitomycin-induced cell population and treatment modality | Major observations of outcome | Reference |
|---|---|---|---|
| Organ Tx (in vitro) | MMC-induced monocyte-derived myeloid suppressor cells (MICs) | -Active suppression of allogeneic T-cell responses in vitro<br>-Downregulation of stimulatory cell surface receptors CD80, CD83, CD86 and HLA-DR on MICs<br>-Induction of apoptosis in MICs | [41] |
| Multiple sclerosis (in vitro) | Mature monocyte-derived DCs | -Inhibition of autoantigen-specific T lymphocytes from MS patients in vitro<br>-Upregulation of expression of apoptotic and immunosuppressive genes | [40] |
| Rat-to-mouse (xenogeneic) Pancreatic islet Tx | Rat pancreatic islets ($3–4 \times 10^2$), 20 h to 7 days in vitro culture<br>Anti-donor ICAM-1 mAb / anti-recipient LFA-1 mAb | -Significantly prolonged survival of islet xenografts<br>-Restoration of normoglycemia<br>-Increased prolongation via blockage of ICAM-1 and LFA-1<br>-Optimal in vitro culture of islets for 40 h–3 days<br>-Induction of TGF-β expression in islets | [95] |

CNS, Central nervous system; CsA, cyclosporine A; EAE, experimental autoimmune encephalomyelitis; ICAM-1, intercellular adhesion molecule-1; i.m., intramuscular; i.p., intraperitoneal; LFA-1, lymphocyte function-associated antigen-1; mAb, monoclonal antibody; MBP, myelin basic protein; MMC, mitomycin C; MS, multiple sclerosis; TGF-β, transforming growth factor-β

recurrent therapy-resistant rejection of haploidentical stem cell transplants, the young patient received a transfusion of $10^9$ paternal MICs derived from CD3/CD19-depleted donor blood cells, mainly consisting of monocytes (about 53%), at the time when the onset of a rejection episode against the third transplant was noted. A second transfer of $2 \times 10^9$ MICs followed 1 week later, resulting in a decrease of autologous B, NK and T lymphocytes. Finally, stable complete hematopoietic chimerism was established for more than 1 year, supported by the additional administration of hematopoietic donor stem cell, mesenchymal stem cell and paternal cytomegalovirus-specific T-cell preparations. No adverse events attributable to donor MICs were noted [39].

A scheme of the mechanisms underlying immunomodulation achieved with MMC-treated peripheral blood cells is given in Fig. 1.

### The TOL-1 phase I study

The clinical application of MICs is presently being investigated in a single-center phase I trial in living-donor kidney transplantation. The prospective organ recipient is transfused with MMC-treated peripheral blood cells of the organ donor ($1.5 \times 10^8$ MICs/kg body weight) 1 week prior to transplantation (Fig. 2). In addition to MIC therapy, standard immunosuppressive medication consisting of cyclosporine A, enteric-coated mycophenolate sodium and methylprednisolone is administered to the kidney allograft recipients.

## Tolerance induction in pediatric patients

As described in the preceding sections, several approaches for induction of tolerance have been tested in adults. No tolerance protocol has yet been tested in pediatric patients, although there undoubtedly is great demand. Young patients can be expected to have many years of life before them and therefore, at least in principle, require a longer allograft survival time than adult recipients. It is therefore particularly important to minimize long-term allograft injury and the side effects of immunosuppression. Especially (very) young patients may have a better potential for tolerance induction due to their immature immune system. On the other hand, pediatric patients are more vulnerable to conditioning regimens, such as preconditioning for hematopoietic cell therapies. If less toxic regimens were to be identified they should be tested in pediatric patients as well.

## Clinical development and regulatory milestones for ATMPs

Advanced therapy medicinal products comprising somatic cell therapy medicinal products, gene therapy medicinal

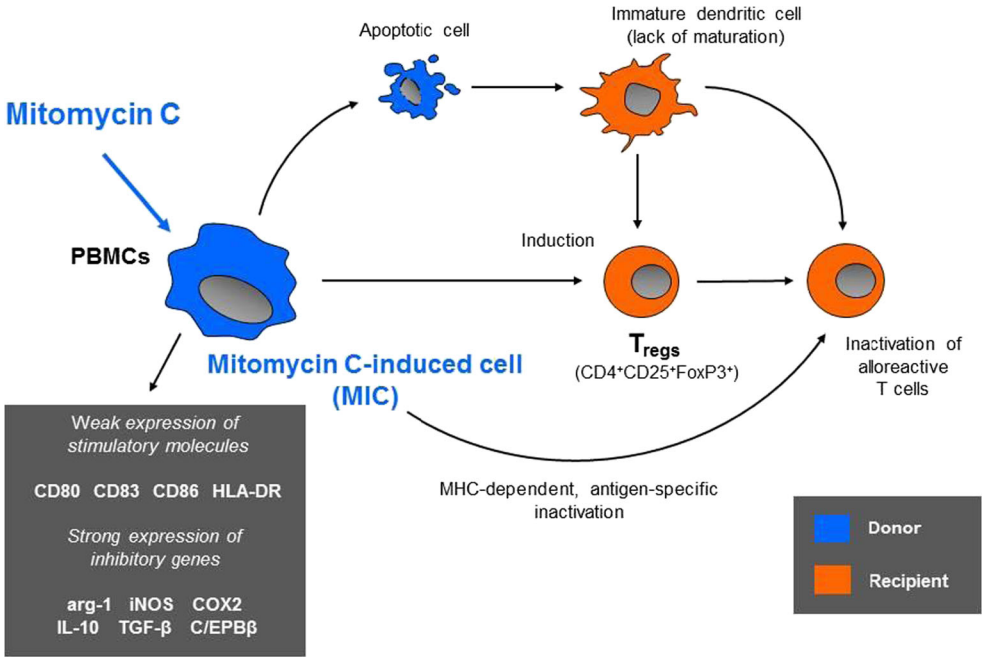


**Fig. 1** Mode of action of mitomycin C-treated peripheral blood cells [mitomycin C-induced cells (*MICs*] for the induction of donor-specific tolerance in allogeneic organ transplantation (adapted and modified from Morath et al. 2015 [2], used with permission). Short incubation of peripheral mononuclear blood cells (*PBMCs*) with mitomycin C (MMC) induces the generation of tolerogenic myeloid cells (MICs). These cells are characterized by low expression of immunostimulatory surface molecules, such as cluster of differentiation (*CD*) 80, CD83, CD86 and human leukocyte antigen–antigen D related (*HLA-DR*), as well as the upregulation of immunosuppressive genes, such as arginase-1 (*arg-1*), inducible nitric oxide synthase (*iNOS*), interleukin (*IL*)-10, transforming growth factor (*TGF*)-β, cyclooxygenase (*COX*)-2 and the transcription factor C/EBPβ. MICs directly inactivate alloreactive T lymphocytes and induce the development of $CD4^+CD25^+FoxP3^+$ regulatory T cells (*Tregs*) capable of suppressing harmful immune responses. In addition, MMC induces apoptosis in its target cells. MMC-treated apoptotic donor cells are taken up by recipient antigen-presenting cells (e.g., immature dendritic cells) preventing their maturation towards immunostimulatory cells. In turn, these immature myeloid cells exhibit an immunosuppressive phenotype inhibiting immune activation and promoting Treg formation

products and tissue-engineered products are attracting increasing interest for the treatment of patients with cancer, autoimmune diseases or orthopedic diseases. To ensure the high quality, biosafety and efficacy of ATMPs, harmonized regulations on the European Community level were established in 2008. In the USA, the Food and Drug Administration (FDA) regulates biological products for human use, both investigational and licensed. It regulates biological products, including gene

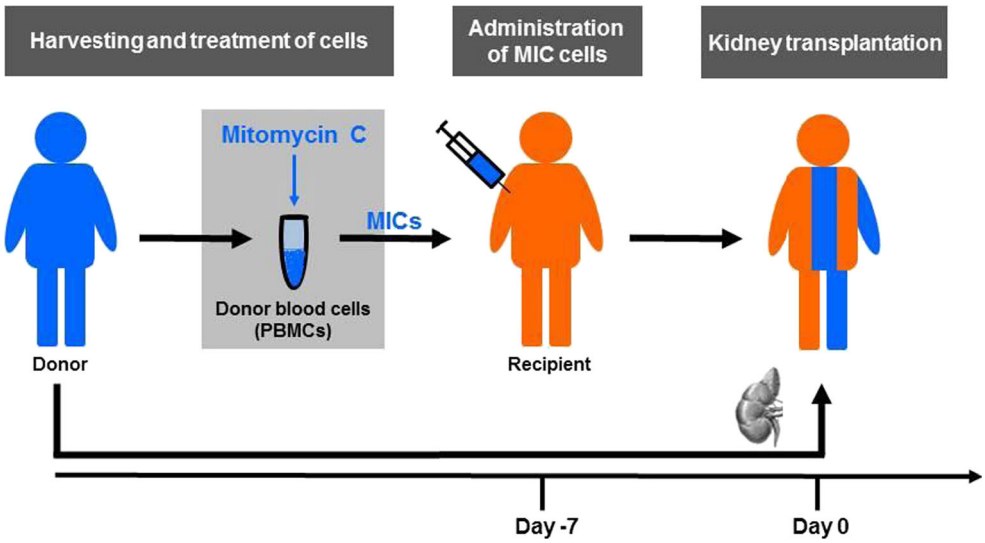


**Fig. 2** Protocol for the TOL-1 Study on MIC therapy (adapted and modified from Morath et al. 2015 [2], used with permission). Seven days before transplantation, PBMCs are retrieved from the kidney donor. After incubation of peripheral blood mononuclear cells (PBMCs) with Mitomycin C for 30 min, cells are washed. $1.5 \times 10^8$ MICs per kilogram body weight are infused to the recipient. Seven days later recipients receive a kidney allograft from the same donor

therapy, human tissue and cells, under applicable federal laws, including the Public Health Service Act and the Federal Food, Drug and Cosmetic Act. Both, Europe and USA have established legislative frameworks for the control of quality, manufacture, marketing and use of these complex cell therapy medicinal products [96]. To make the path of development of cell therapy medicinal products clear and transparent a harmonization of rules and requirements across the European countries as well as between the European Medicines Agency and the FDA is mandatory. For developers of ATMPs requests for and compliance with regulatory scientific advice and direct interaction with regulators is of great importance. The development of an ATMP is time-consuming and cost-intensive. After proof of concept by intensive preclinical studies, the investigational cellular approach can be translated from bench to bedside at a GMP unit in compliance with the legal requirements. In the next step, documents for the investigational medicinal product have to be filed for the appropriate governmental and institutional authorities to obtain (1) approval to perform a clinical phase I-II study from the competent authorities, (2) the manufacturing license and (3) the ethical vote from the Institutional Review Board at the study site. Therefore, documents including a detailed study protocol, an Investigator's Brochure, an Investigational Medicinal Product Dossier, extensive validation documents and complex Standard Operating Procedures are required.

For a broader availability of ATMPs, not only do regulatory requirements have to be fulfilled, but financial and economic support by health insurance programs/companies funds over the long term is also needed. These health insurance programs/companies issue directives specifying which services in their medical care coverage are reimbursed based at least in part on scientific reports that evaluate the benefits and risks of medical interventions.

Hence, it may take more than one decade to progress from the original notion of a cell-based therapy through to its proof of concept and translation into GMP-conform manufacturing and up to the final ATMP approval/authorization. This entire process translates into an enormous financial and regulatory effort and, due to a changing world, a complicated prognosis for the future development of products.

## Conclusion

Cell therapeutic approaches might revolutionize immunosuppressive regimens after kidney transplantation in the future. Early data on cell therapeutic strategies after kidney transplantation are encouraging. Reliable protocols that are capable of inducing stable clinical tolerance while at the same time avoiding side effects remain to be defined. The application of these cell therapies must be suitable for clinical routine. An elegant approach to immunosuppression by MIC cells had recently been introduced.

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**Compliance with ethical standards**

**Conflict of interest** None to declare.

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