Mesenchymal Stem Cell Therapy to Promote Corneal Allograft Survival – current status and pathway to clinical translation

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Abstract

Purpose of Review: To review literature on the therapeutic potential of mesenchymal stem cells (MSC) to prolong corneal allograft survival.

Recent findings: To date only a small number of studies have investigated MSC ability to modulate corneal allograft survival. Most reports showed positive results, which is encouraging, however as different MSC-application strategies (time point of injection, cell number/number of injections, route of injection, MSC source, MSC licencing) have been employed in various animal models it is difficult to compare and validate the results. MSC ability to promote graft survival has been attributed to their modulation of the recipient immune system, altering the Th1/Th2 balance, expanding Foxp3+ regulatory T cells, polarizing macrophages and inhibiting intra-graft infiltration of antigen presenting cells (APCs). More in depth analysis is required to elucidate the mechanism of MSC-immunomodulation *in vivo*.

Summary*:* MSC have shown potential to modulate corneal allograft rejection in various models using MSC from different species. In particular for high-risk patients with poor prognosis MSC-therapy might be a promising approach to promote corneal allograft survival. First-in-man clinical trials with MSC will hopefully shed new light on MSC-mediated immunomodulation *in vivo* and contribute to restore vision in patients receiving corneal allografts.

Keywords: corneal transplantation, mesenchymal stem cells, immunomodulation, regulatory cells, tolerance

Text of review

Introduction

Corneal transplantation (penetrating keratoplasty) is probably the oldest transplant procedure with scientific reports dating back for more than 100 years (1). With more than 100,000 procedures annually, cornea transplantation is also the most frequent transplantation procedure of human tissue. Interestingly, the survival rates of corneal transplants in low-risk patients with no history of neovascularisation or repeat transplantation are up to 90% despite the fact that donor-recipient tissue matching is not routinely performed in clinical centres. These high survival rates have been attributed to the so-called immune privilege of the eye and believed to be a result of the lack of antigen presenting cells (APCs), blood vessels and the expression of multiple anti-inflammatory or immunomodulatory molecules in the cornea and anterior chamber of the eye. Reports on the incidence of graft rejection after penetrating keratoplasty vary between 5% and 40 % (2-4). Despite this, the 5-year prognosis for cornea transplant survival is worse and estimated to be as low as 50%, which is poorer than that for solid organ transplants (5, 6). Topical treatment with corticosteroids is currently the gold standard in corneal transplantation. While this treatment is effective in many patients, it is much less effective in “high-risk” patients with previous episodes of neovascularisation or graft rejection. Immunosuppression can be induced in some patients by systemic administration of pharmacological agents such as Cyclosporin A or corticosteroids. However, their prolonged use can cause significant side effects. Therefore, alternative treatment protocols are urgently needed to significantly improve the prognosis of corneal transplants and/or to reduce potential adverse side effects of conventional therapy.

Cellular therapies to modulate corneal graft rejection

Cell-based treatment protocols have recently emerged as promising therapeutic strategy for modulation of immune-mediated diseases. Basically, two approaches with regard to how cellular therapies might work to modulate disease are currently discussed. The first one refers to the regeneration of damaged or lost recipient tissue by replacing tissue originating from transplanted cells. The second one refers to the stimulation of endogenous tissue repair mechanisms and/or the modulation of adverse recipient anti-donor immune responses through the application of tolerogenic cell therapies. It is now generally believed that cell therapy for treatment of immune-mediated diseases does not lead to the direct replacement of damaged host tissue through applied cells but, rather, facilitates endogenous tissue repair and/or modulates organ graft rejection by the secretion of anti-inflammatory molecules or the induction of endogenous regulatory cell populations. Cell-based therapies for patients receiving solid organ transplants are currently in clinical trials and include the use of regulatory T cells (Tregs) (7), myeloid derived suppressor cells, tolerogenic dendritic cells (DCs) (8) and, more recently, mesenchymal stem cells (MSC) (9, 10). Cell therapies for modulation of corneal transplant survival have – to the best of our knowledge – not progressed to clinical trials yet. This might be attributed to the fact that corneal transplantation is often considered as “unproblematic”. However, as previously pointed out, patients have an increased risk of rejecting their transplant in high-risk situations for which no adequate conventional immunosuppression has proven effective. Given the large number of corneal transplantations performed each year, the number of these patients is significant. While the potential application of cell therapies to modulate corneal allograft rejection in pre-clinical models using Tregs

and DCs has been recently reviewed (11), this article will focus on the application of immunomodulatory MSC to prolong corneal allograft survival and discuss the potential pathway to clinical translation.

Mesenchymal stem cells

MSC are non-hematopoietic stem cells with multi-lineage potential (12, 13). They can be isolated from bone marrow, adipose tissue, cord blood and various other adult tissues and have the capacity to extensively proliferate *in vitro*. In contrast to hematopoietic stem cells, which can be easily isolated based on the expression of lineage-specific cell surface molecules, no such single marker gene or protein exists which characterises a "true" MSC. This makes the isolation and subsequent characterisation of MSC more difficult and requires extensive surface profiling of isolated or cultured MSC before a cell should be considered to be a MSC for *in vitro* and *in vivo* research (14). One of the key features of MSC is that they have the potential to differentiate into various tissues of mesodermal origin, such as adipocytes, osteoblasts and chondrocytes which makes them, together with their proliferative potential, attractive targets for regenerative medicine applications. In addition to their differentiation capacity and proliferative potential, MSC have been widely shown to effectively inhibit both innate and adaptive immune responses *in vitro* (Figure 1)(15-17). They can modulate the maturation and expression profile of DCs and macrophages by reducing expression of the immune-relevant cell surface markers major histocompatibility complex (MHC) Class I molecules, CD80/CD86 and by inhibiting the production of pro-inflammatory cytokines (TNF-α and IL-12) (18-23). Moreover, MSC are shown to inhibit T cell and B cell proliferation and are able to inhibit pro-inflammatory cytokine producing CD4+ Th17 cells via CC

chemokine Ligand 2 dependent interaction (24). As it is well known that corneal graft rejection is mainly mediated by CD4+ T cells and with macrophages also playing an important role in the rejection process (25), the application of MSC to modulate corneal graft rejection is a reasonable approach.

Mesenchymal stem cells to modulate corneal injury and graft survival – pre-clinical research

There is already a plethora of data available on the therapeutic effects of MSCs *in vivo* (16). With regard to organ/tissue transplantation, first reports on beneficial effects of MSC therapy date back to 2002 when Bartholomew et al. reported that infusion of donor-derived baboon MSC ($20x10^6$/kg bodyweight on day 0) led to a modest but significant prolongation of survival of MHC-mismatched donor and third-party skin grafts in baboons (26). Even before MSC were tested as a therapeutic agent to prolong corneal allograft survival, they were investigated for their potential to improve corneal injury following alkali burn (27, 28). Oh and colleagues demonstrated that either local application (200ul of $2x10^6$ MSC solution) of MSC isolated from Fischer rats or 200ul MSC conditioned medium applied either once or daily for 3 consecutive days resulted in a reduction of corneal inflammation and neovascularisation in a Sprague-Dawley rat corneal injury model. Moreover, a reduction of pro-inflammatory cytokines IL-2 and IFN-γ and an increase in anti-inflammatory cytokines IL-10, TGF-β1 and Thormobospondin-1 in corneal tissue was reported. Interestingly, while the results with MSC conditioned medium were encouraging indicating paracrine therapeutic effects by molecules secreted from MSC in this model, the topical application of MSC onto the ocular surface was superior (27). However, only limited mechanistic information was provided. To identify the

mechanisms of MSC-mediated immunomodulation in ocular injury, this work was followed up by Roddy et al. who injected human MSC (hMSC) in Lewis rats with alkali burn (xenogeneic setting) (28). Immediately after injury, hMSC ($1x10^7$ cells/ml) were administered by either intraperitoneal (i.p.) or intravenous (i.v.) injection. The authors showed that both i.p. and i.v. injection of hMSC significantly reduced the development of corneal opacity 1 or 3 days after the injury. Of note, the therapeutic effect of hMSC seemed to have occurred without engraftment of hMSC in the injured corneas as measured by qRT-PCR specific for human GAPDH in rat corneal samples. Moreover, i.v. or topical application of recombinant TNF-stimulated gene 6 protein (TSG-6), shown previously to mediate - at least in part - therapeutic effects of MSC (29), was equally effective (28). Thus, application of MSC or TSG-6 has the potential to ameliorate ocular surface injury by modulating local and/or systemic immune responses but without the need for MSC engraftment. However, the injection of a large number of cells ($1x10^7$ MSC per rat equals approx. $3x10^7$ kg/body weight) and the injection of hMSC into a xenogeneic recipient are problematic, eg. i.v. administration of high cell numbers could lead to embolism.

In the following, recent work on the use of MSC to modulate corneal graft survival will be discussed (Table 1). In 2012 Jia et al reported that MSC-treatment prolonged corneal allograft survival in a rat corneal transplant model (30). MSC were isolated from allogeneic Wistar rats and analysed for differentiation potential and cell surface marker expression. The authors demonstrated that post-transplant i.v. injection of $5x10^6$ MSC (day 0, 1, 2) in Lewis rats receiving Wistar corneal transplants prolonged corneal allograft survival which could be further extended by co-application of Cyclosporin A. However, it is not described by the authors in this paper how graft quality and integrity was evaluated. Interestingly, only higher doses of Cyclosporin A

(2mg/kg) had an additive beneficial effect on graft survival and pre-transplant injection (day -3, -2, -1) of MSC did not result in prolongation of graft survival. Further, the authors claim that i.v. injection of MSC increased the ratio of CD4+CD25+Foxp3 cells compared to CD4+ cells in the draining lymph nodes and increased the production of anti-inflammatory cytokines (IL-4, IL-10) and reduced pro-inflammatory cytokine (IL-2, IFN-γ) secretion in lymphocytes isolated from MSC-treated animals. This report indicated that allogeneic MSC have the potential to modulate corneal graft survival but the time point of injection seems to be critical for the outcome of transplant survival. In 2012 Oh et al. also reported that hMSC-injection prolongs corneal allograft survival claiming that hMSC-application prevents early inflammation and late rejection in transplanted graft recipients (31). As previously investigated by this group, the beneficial effects of hMSC were tested this time in a mouse corneal transplant model. Two peri-transplant i.v. injections of $1x10^6$ hMSC at day -1 and day 0 (which were more effective than a single d0 injection) decreased early surgically-mediated inflammation but also reduced the activation of APCs in the cornea and in the lymph nodes. Consequently, immune rejection was prevented and graft survival was prolonged. Again the authors did not find evidence for engraftment of hMSC in the transplanted rat cornea as shown before in the alkali burn injury model (28). Mechanistically, the authors found that hMSC were trapped in the lungs of treated animals where they became activated and secreted TSG-6 which, in turn, modulated the early inflammatory response and attenuated graft rejection. Interestingly, knock-down of TSG-6 in hMSC followed by injection in animals receiving corneal transplants did not lead to a reduction of early inflammatory response and failed to prolong corneal allograft survival suggesting a dominant role of TSG-6 in modulating corneal allograft survival. As already pointed out before, the

application of human cells in rodent animal models needs to be considered with care, as it is not clear how xenogeneic immune responses in rodents toward MSC influence disease outcome. Moreover, it has not been investigated how MSC isolated from donors other than graft donor (allogeneic) or recipient (syngeneic) (i.e. third party-derived) will modulate corneal graft survival. To understand if third-party MSC have therapeutic efficacy would be important for a potential translation of this approach into a clinical scenario, as third-party MSC could be produced in large amounts before cell application as "off-the-shelf" therapy for corneal transplant recipients. We have established a fully allogeneic MHC-class I/II mismatched rat model in which Lewis rats received corneal allografts from Dark-Agouti (DA) rats. We showed that two i.v. injections of $1x10^6$ MSC/animal (day -7 and day 0) of both allogeneic (DA) and third-party (Wistar-Furth) derived MSC can successfully prolong corneal allograft survival in a fully allogeneic MHC-mismatched rat model (32). Mechanistically, treatment with allogeneic MSC lead to a reduction in frequency of all immune cell populations (CD4+CD25+ (activated T cells), CD11b/c+ (APCs), CD11b/c+MHCII+CD86+ (activated APCs), CD3+CD8+CD161+ (NKT Cells), CD3+CD8+ (CD8+ T cells) and CD45RA+ cells (B cells) in the corneal allograft and to an increase of CD4+Foxp3+ regulatory T cells in the spleen on the average day of rejection. Intra-graft pro-inflammatory cytokine expression (IL-1β, IL-6) was also reduced in allogeneic MSC-treated animals. These results suggested that injection of corneal transplant recipients with allo- and third-party MSC induces specific local and systemic protective mechanisms, however it is not clear if one or the other is sufficient to achieve the therapeutic effect or if both local and systemic immunomodulation is needed to achieve a therapeutic effect. Interestingly, syngeneic MSC applied with the same injection strategy (day -7, 0) did not prolong corneal allograft survival despite

reducing inflammatory cell populations in the corneal graft, however, no increase in regulatory CD4+ Foxp3 T cells was observed in the spleen of syngeneic MSC-treated animals indicating that allogeneic MSC-treatment induces an antigen-specific immunoregulatory effect leading to graft acceptance. By injecting syngeneic MSC into a naïve host (7 days before transplantation, the recipient has not seen the antigen) cells might not get the "inflammatory" stimulus to develop its full therapeutic immunomodulatory potential. Moreover, these results support other reports from the literature that MSC need to be immune-activated in order to exert their therapeutic effects to their full potential. In 2011 Duijvestein et al reported that mouse MSC treated with IFN-γ (500U/ml) (a procedure also called licencing) have enhanced immunosuppressive properties in murine colitis models compared to untreated MSC. In addition, induced Nitric Oxide Synthase (iNOS), one of the key molecules involved in MSC-mediated immunosuppression in rodents, was found to be up-regulated following treatment with IFN-γ which also resulted in enhanced inhibition of T cell proliferation (33). To investigate if IFN-γ treated syngeneic MSC promote corneal allograft survival in the rat, syngeneic Lewis rat MSC were licenced with IFN-γ (500U/ml) and injected (day-7, day 0) into corneal transplant recipients. Interestingly, no prolongation of graft survival was observed, indicating again that the timing of injecting MSC might be critical. Indeed more recent observations indicate that "licenced" MSC injected post-transplantation have enhanced therapeutic effects *in vitro* and *in vivo* (unpublished data). In 2014 Omoto et al. also reported on the homing of MSC to the inflamed ocular surface and suppression of allo-sensitisation in a murine model of corneal transplantation (34). In this work a mouse allogeneic transplant model (C57/BL6 corneal grafts transplanted on BALB/c recipients) was used. The authors injected syngeneic (BALB/c) MSC i.v. either on day 0 (single) or

on day 0 and day 7 (double) after transplantation and showed that MSC application modulates both direct and indirect rejection pathways and prevented corneal graft rejection with double injections being more effective. To investigate if MSC home to the transplanted cornea, the authors switched transplant model to C57BL/6 as recipient and BALB/c as corneal donor to take advantage of C57BL/6 GFP+ transgenic mice. Interestingly, in contrast to previous work, C57/BL6 GFP+ MSC could be detected in the allogeneic transplanted cornea and also draining lymph nodes but not to the ungrafted contralateral cornea. This novel observation will need to be further investigated to understand the importance of MSC-migration towards corneal injury.

In contrast to previous observations, Fuentes-Julian and colleagues reported in 2015 that i.v. injection of syngeneic MSC (4 injections of $2x10^6$ cells each at day-7, 0, day+3and day+14) from both human and rabbit adipose (AD) tissue significantly shortened corneal graft survival both in low-risk and high-risk rabbit allogeneic transplant models (35). Moreover, intrastromal single injection of hAD-MSC and rabbit AD-MSC ($2x10^6$ cells immediately after the transplant procedure was performed) also did not lead to prolongation of graft survival. Licencing of MSC with IFN-γ and TNF-α (20ng/ml) was investigated as well but did not result in prolongation of graft survival either. The reason why the application of MSC failed to promote graft survival in this model is unclear. In this work, AD-MSC from human or syngeneic donors have been used which might have less immunomodulatory potential compared to bone marrow MSC although described otherwise in the literature (for review see (36).

Most of the work reviewed so far has high-lighted the importance of CD4+ Foxp3+ regulatory T cells induced by MSC-therapy. However, in 2015 Ko and colleagues

reported on another regulatory cell type enriched following MSC-injection (37). hMSC or controls were injected in BALB/c mice (day-7/-3) receiving C57BL/6 corneal grafts. Results indicated prolongation of graft survival in the hMSC treated groups with reduced corneal edema and reduced number of CD4+ IFNγ+ T cells in the lymph nodes. While no hMSC could be found in the lung of hMSC-treated animals the authors describe the appearance of a novel myeloid cell expressing MHC class II+B220+CD11b+ markers in the lungs on day 0 of hMSC-treated animals. Sorting of MHC class II+B220+CD11b+ cells and subsequent T-cell stimulation experiments indicated that these cells inhibit T-cell proliferation and reduced proinflammatory cytokine expression. Moreover, adoptive transfer of MHC class II+B220+CD11b+ sorted cells into naïve animals followed by corneal transplantation did not lead to graft rejection indicating transfer of tolerance or unresponsiveness by this regulatory cell population.

Conclusions from these studies:

So far, only a limited number of reports have been published investigating the immunomodulatory potential of MSC in corneal transplantation. Different MSC-application strategies (time point of injection, cell number and number of injections, mode of injection, species, licencing, animal model) have been investigated to explore the therapeutic potential of MSC in corneal transplantation (and in other transplant models as well) which makes it difficult to validate the results. However, mostly positive results have been reported so far and, while encouraging, there are also reports showing that MSC application is not beneficial in promoting corneal graft survival. The induction of CD4+ Foxp3+ regulatory T cells following MSC-injection seems to be critical for corneal allograft survival although other regulatory cell

populations have been recently described. This would facilitate the clinical application of MSC with an “off-the-shelf” treatment protocol with third-party MSC produced in large stocks e.g. by pharmaceutical companies involved in stem cell research. Indeed, under the umbrella of a project funded by the European Union FP7 programme (VISICORT), a Phase 1 clinical trial is planned in 2018 for treating “high-risk” corneal transplant patients with third-party MSC, with the main focus on being on safety but also investigating potential efficacy. To our best knowledge this will be the first clinical trial using MSC to prevent corneal graft rejection. While we still have much to learn about the mechanism by which MSC can modulate ocular immune-mediated diseases, this clinical trial will hopefully generate beneficial data on MSC safety and immunotherapeutic effects in this disease setting, which could be of importance for other transplant and ocular disease models as well.

Key points (3-5 key bullet points)

- MSC promote corneal allograft survival and ocular injury
- Involvement of CD4+ Foxp3+ regulatory T cells and other regulatory cells in immunomodulation described
- Different application strategies and source of MSC described but no clear correlation between cell dose, MSC source or route of application

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Conflict of interest

None declared

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*This is the first report showing efficacy of allogeneic and third-party MSC to modulate rat corneal allograft survival. This finding is potentially important for translation of MSC-therapy using third-party MSC. Moreover this report also high-lights the importance of timing of immunomodulatory MSC injection.

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*In this work, the authors show for the first time that i.v. injected MSC migrate (syngeneic model) into the transplanted cornea but not in contralateral tissue. This could be important as it shows that the precence of MSC in the corneal transplant may be required for immunomodulation.

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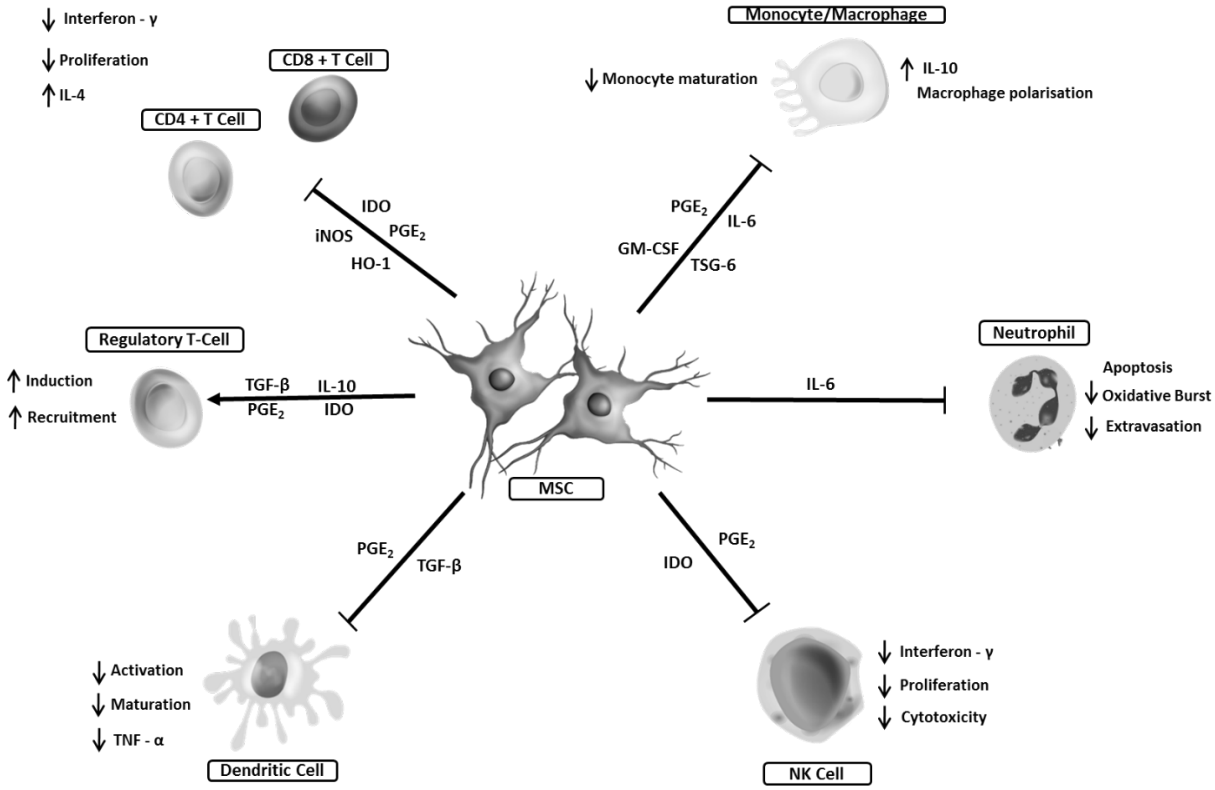
**Very intersting paper showing that injection of hMSC generates a new tolerogenic monocyte/macrophage in the lung of recipients which in turn is able to prolong graft survival upon injection into naïve hosts. This indicates that more than one regulatory cell type may contribute to prolong corneal allograft survival after MSC administration.

Figure 1. **Proposed mechanisms of MSC interaction with immune cells.** MSC respond to local environmental cues and communicate with immune cells from both the innate and adaptive immune system through the secretion of immunoregulatory molecules. Via the secretion of Interleukin-6 (IL-6), MSC reduce neutrophil oxidative burst potential, decrease extravasation and increase apoptosis. MSC prevent monocyte/macrophage maturation or shift it towards a tolerogenic phenotype, MSC derived prostaglandin E2 (PGE_2), interleukin-6 (IL-6) and GM-CSF are key factors in this effect. Tumour necrosis factor-inducible gene 6 (TSG-6) decreases nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signalling and reduces the secretion of pro-inflammatory cytokines by inflammatory macrophages. Many different soluble and cell contact-dependent molecules have been accredited to MSC inhibition of both CD4+ and CD8+ T-cell activation and proliferation *in vitro*. These molecules can act via antigen presenting cells or on T cells directly and include but are not limited to PGE_2, the tryptophan depleting molecule Indoleamine 2,3-dioxygenase (IDO), inducible nitric oxide synthase (iNOS) and heme oxygenase (HO-1), a molecule shown to be important in MSC-mediated suppression of allo-activated T cells. MSC have been shown to induce and recruit regulatory T-cells via transforming growth factor beta 1 (TGF-β1), IDO, PGE_2 and the anti-inflammatory Interleukin-10 (IL-10). MSC mediate their suppressive effects on dendritic cells via PGE_2 and TGF-β1, resulting in decreased levels of CD80, CD86, MHCI, MHCII and secreted tumour necrosis factor-α (TNF-α). MSC limit NK cell cytolysis, inhibit proliferation and decrease the secretion of interferon-γ (15-17).

Table 1: **The application strategy and major findings from the most recent MSC treatment in cornea transplantation studies are collated**. IV, intravenous; SI, stromal injection; NR, normal risk; HR, high risk; MSC, mesenchymal stem cell; BM, bone marrow derived; dLN, draining lymph node. Number of MSC per kg body weight was calculated by assuming the weight of the animal: Oh et al. and Ko et al. - an 8 week old female BALB/c mouse was estimated to be 17g; Omoto et al. – the average weight of a 6-8 week old male C57BL/6 mouse estimated to be 21g; Fuentes-Julias et al. – the average weight of New Zealand White rabbit estimated to be 3kg as indicated by the authors; Jia et al. – the average weight of a female Wistar Furth rat was estimated to be 200g as indicated by the authors; Treacy et al. – the average weight of an 8-12 week old Lewis rat was estimated to be 253g.


↓ Interferon - γ
↓ Proliferation
↑ IL-4
CD8 + T Cell
CD4 + T Cell
IDO
iNOS
PGE2
HO-1
Monocyte/Macrophage
↓ Monocyte maturation
↑ IL-10
Macrophage polarisation
PGE2
IL-6
GM-CSF
TSG-6
Regulatory T-Cell
↑ Induction
↑ Recruitment
TGF-β
IL-10
PGE2
IDO
MSC
IL-6
Neutrophil
Apoptosis
↓ Oxidative Burst
↓ Extravasation
PGE2
TGF-β
PGE2
IDO
↓ Activation
↓ Maturation
↓ TNF - α
Dendritic Cell
↓ Interferon - γ
↓ Proliferation
↓ Cytotoxicity
NK Cell

Paper	Source	Recipient Species *(Xeno-, allo- or syn-geneic)*	Normal (NR) or *High Risk (HR) Model*	Route (Timing of Administration)	Number of MSC Per Injection *(Number per kg body weight)*	Outcome	Mechanism of action
(30)	Wistar Furth Rat BM MSC	Lewis Rat **(Allogeneic)**	NR	IV administration (d-3, d-2, d-1) IV administration (d0, d1, d2)	$5x10^6$MSC **($25x10^6$/kg)**	Allograft survival was prolonged by post-surgical but not pre-surgical MSC administration Co administration of MSCs with low dose CsA (1mg/kg) accelerated graft rejection while administration with 2mg/kg CsA prolonged allograft survival	Post-operative MSC administration inhibited T cell proliferation both i*n vitro* and *in vivo* decreasing expression of Th1 associated cytokines IFN-γ and IL-2 and increasing Th2 associated IL-4, while expanding CD4+CD25+Foxp3+ regulatory T cells
(31)	Human BM MSC	BALB/c Mouse **(Xenogeneic)**	NR	IV administration (d-1) IV administration(d-1, d0)	$1x10^6$ MSC **($58.8x10^6$/kg)**	hMSCs through TSG-6 reduced the surgery induced inflammation prolonging allograft survival in a dose dependent manner	hMSC treatment reduced IL-6, IL-1β and IL-12 levels and infiltrating APCs in the cornea and dLN subsequently inhibiting the adaptive CD4+ and CD8+ T cell immune response The effect was mediated by TSG-6 as demonstrated by TSG-6 siRNA knock down and recombinant TSG-6 IV administration
(32)	Lewis Rat BM MSC Wistar Furth Rat BM MSC Dark Agouti Rat BM MSC	Lewis Rat **(Syngeneic)** **(Allogeneic)**	NR	IV administration (d-7, d0)	$1x10^6$ MSC **($3.95x10^6$/kg)**	MSCs derived from allogeneic sources (WF BM MSC and DA BM MSC) significantly prolonged allograft survival while recipient derived (syngeneic) Lewis BM MSC failed to prolong allograft survival	Allo-MSC inhibited the intra-graft infiltration of CD3+CD8+CD161+ NKT cells, CD11b/c+ APCs, CD4+CD25+ activated T cells and CD45RA+ B cells, while also expanding a population of CD4+CD25+Foxp3+ splenic regulatory T cells DTH experiment demonstrated allo-MSC specifically inhibit corneal donor alloantigen sensitization
(34)	BALB/c Mouse BM	BALB/c Mice C57BL/6	NR	IV administration (d0) IV administration (d0,	$1x10^6$MSC **($47.62x10^6$/kg)**	BALB/c MSC prolong allograft survival in a C57BL/6 host in a	IV administered MSCs migrated to the transplanted cornea, conjunctiva and

	MSC GFP^{+}C57BL /6 Mouse BM MSC	Mice **(Syngeneic)**		d7)		dose dependent manner	draining lymph nodes where they suppressed the maturation of CD11c+MHC II+ APCs, consequently inhibiting DCs direct and indirect allo-sensitization of CD4+ T cells
(35)	Human AD MSC Rabbit AD MSC	New Zealand White Rabbit **(Xenogeneic) (Syngeneic)**	NR + HR	NR – SI administration (d0) HR – IV administration (d-7, d0, d3, d14)	NR/HR – $2x10^{6}$ MSC **($0.666x10^{6}$/kg)**	SI in the NR model and IV injection in the HR model lead to more rapid allograft rejection compared to controls	SI or IV administration of hAD-MSC or rAD-MSC lead to an increase in corneal edema and a higher number of infiltrating CD45+ leukocytes which could be attributed to MSCs production of inflammatory cytokines IL-6 and IL-8
(37)	Human BM MSC	BALB/c Mouse **(Xenogeneic)**	NR	IV administration (d-7, d-3)	$1x10^{6}$ MSC **($58.8x10^{6}$/kg)**	hMSCs prolong allograft survival by enriching a population of regulatory myeloid cells in the lung mediated by TSG-6	hMSCs in a TSG-6 dependent manner induced a MHC class $II^{+}B220^{+}CD11b^{+}$ population of myeloid cells in the lung which remained in circulation for up to 7 days post MSC administration This specific myeloid cell population suppressed T cell proliferation in vitro and could prolong allograft survival when adoptively transferred