Review Article

Cell therapy in vascularized composite allotransplantation

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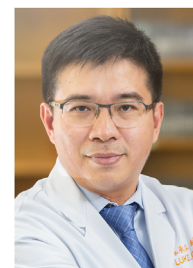
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ARTICLE INFO

ABSTRACT

Allograft rejection is one of the obstacles in achieving a successful vascularized composite allotransplantation (VCA). Treatments of graft rejection with lifelong immunosuppression (IS) subject the recipients to a lifelong risk of cancer development and opportunistic infections. Cell therapy has recently emerged as a promising strategy to modulate the immune system, minimize immunosuppressant drug dosages, and induce allograft tolerance. In this review, the recent works regarding the use of cell therapy to improve allograft outcomes are discussed. The current data supports the safety of cell therapy. The suitable type of cell therapy in allotransplantation is clinically dependent. Bone marrow cell therapy is more suitable for the induction phase, while other cell therapies are more feasible in either the induction or maintenance phase, or for salvage of allograft rejection. Immune cell therapy focuses on modulating the immune response, whereas stem cells may have an additional role in promoting structural regenerations, such as nerve regeneration. Source, frequency, dosage, and route of cell therapy delivery are also dependent on the specific need in the clinical setting.

Vascularized composite allotransplantation (VCA) has become a reliable treatment alternative for restoring form and function in patients with significant tissue loss. Various types of clinical VCA, including partial-face, full-face, hand, and knee allotransplantation, have been successfully performed with excellent short-to intermediate-term functional and immunological outcomes [1–4]. Unlike solid organ transplantation (SOT), VCAs usually contain multiple tissue types, including skin, muscle, bone, vessel and nerve. Therefore, the immunogenicity of different tissues in a single composite allograft must be considered. Lifelong IS regimens are required to maintain allograft survival. However, undesired

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side effects, such as opportunistic infection, metabolic complications, and malignant diseases occur frequently [5].

Alternative treatments to overcome immune rejection and to improve allograft outcome have been investigated in the past two decades. Cell therapy is one of the potential strategies to modulate immune systems and allow the recipients to develop tolerance to the allograft. In this review, the recent progress and efforts to induce and maintain allograft acceptance across the full major histocompatibility (MHC) barrier through cell therapy are summarized. The discussion parameters include timing, frequency, and infusion doses of various cell therapies; the comparisons of the autologous and allogenic source of cellular lineage used in cell therapy; the delivery dose and route of cell therapy and the need of the additional adjuvant IS and finally the mechanisms and outcome of using cell therapy in VCAs [Fig. 1].

Types of cells for therapy

Cell therapy is defined as the therapeutic use of cellular material through injection, grafting, or implantation into the recipient. The concept of cell therapy was first described in the 19th century with xenocelullar injection to human subjects [6]. Human cells were later developed for therapeutic purpose. Graft rejection is an immune cell-mediated phenomenon; therefore, immune and stem cell-based therapeutic approaches were highly regarded with great potential. These cells may modulate the immune system and induce graft tolerance, proposedly through reducing the graft destructive T cells or the allograft-reacting immune cells. In addition, the anti-inflammatory properties of stem cells via their secretion of anti-inflammatory molecules, such as interleukin(IL)-10, may also dampen the process of immune-mediated graft rejection.

Immune cell therapy

In the investigation of immune cell therapy, the use of dendritic cells (DCs) was the first to be explored. As an antigen-presenting cell, DCs are critical for initiating the immune response. Regulatory DCs (DCregs) play an essential role in maintaining tolerance. They have been applied as cell therapy since 1996. Similar to general DCs, DCregs uptake, process, and present soluble antigen (Ag) [7]. Meanwhile, regulatory T cells (Tregs) are also widely investigated as potential cell therapy in VCA. Tregs are known as a subpopulation of T cells with a function to suppress effector immune cells through activation-induced cell death, "ignorance" of self-antigens [8], and the induction of cell anergy. The later discoveries of CD8 Tregs and Type 1 regulatory T cells were identified as subgroups of Tregs [9,10]. Other regulatory cells with anti-inflammatory properties are regulatory B cells (Bregs) and regulatory macrophages (Mregs). However, to date, only limited VCA studies were performed to investigate these regulatory cells. The potential Bregs as cell therapy to induce tolerance was demonstrated in an islet model [11].

Stem cell therapy

Regarding their ability to differentiate, stem cells can be classified into totipotent, pluripotent, multipotent, oligopotent and unipotent, and all have self-renewal ability. Transferring the donor bone marrow (BM) cells that contain hematopoietic stem cells (HSCs) to the recipient is proven to induce tolerance through chimerism in the animal models [12,13] and clinical VCA studies [14]. Pluripotent HSCs have been used in transplantation since the 1960s and could improve allograft outcomes in SOT [15]. In VCA, these stem cells were studied extensively in small and large animals [16—18]. Other stem cells therapies used in VCA include BM-derived mesenchymal stem cells (BM-MSCs), and adipocytes derived mesenchymal stem cells (AD-MSCs). In addition to the immunological benefit in VCA, they also enhance the process of nerve regeneration [19,20].

Timing and frequency of cell therapy

In general, there are three critical phases of graft acceptance in transplantation: tolerance induction, tolerance maintenance, and rejection treatment. Timing and frequency of cell
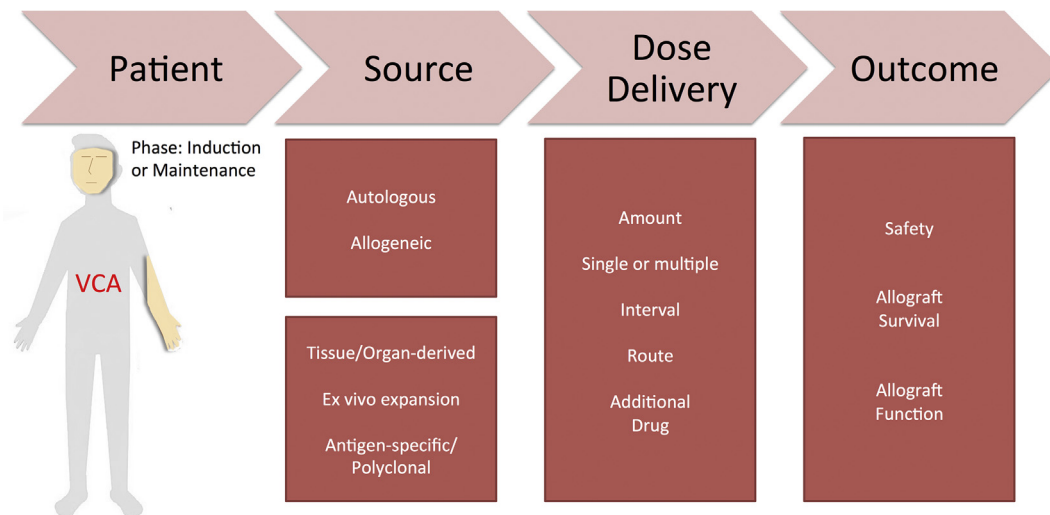

Fig. 1 Roadmap of technical considerations regarding the use of cell therapy.

therapy administration significantly affect allograft and recipient outcomes [21]. In animal studies, the cells are mostly infused on the same day of transplantation or given multiple doses within the first week post-transplantation. The induction phase is the most critical period for recipient immune system activation. Those infused cells are aimed to modulate the immune reaction, allowing the reduction of immunosuppressants dosage. Among the potential cellular candidates, BM cells [13] and DCregs [22] were studied comprehensively in immune tolerance induction. Several publications also reported the use of cell therapy in the maintenance phase and allograft rejection. In particular, Tregs [23] and umbilical cord derived mesenchymal stem cells [24] have been used to rescue rejections. The frequency of injections also affects the outcome of transplantation. Repetitive cell injections have cumulative beneficial effects. The extracellular vesicles and graft outcome are positively correlated with the number of cell injections [25,26]. Although several studies have demonstrated the effectiveness of a single bolus cell injection in graft tolerance induction [Table 1], the same quantity of AD-MSCs in the divided doses given repeatedly may lead to a superior VCA outcome [27]. However, conflicting results were reported [28,29]. A similar multiple injection approach with DCs was used in SOT clinical trials, which resulted in potentially conducive to modulate anti-donor immune reactivity [30].

Source and expansion

The manufacturing of the therapeutic cells should adhere strictly to Good Manufacturing Practices. The source of the cell can be derived either from allogeneic or autologous cellular lineages. Autologous cells are obtained from the recipient patient and cultured in smaller numbers, whereas allogeneic cells are obtained from unrelated donor tissues (such as BM) and manufactured in large quantities. In allogeneic cell therapies, cell manufacturing is aimed to treat on the community level with comprehensive product quality control. In contrast, the autologous cells are custom-made, and a small quantity is manufactured in hospital-affiliated laboratories. Autologous cell transfer might be a safer strategy than allogenic cell infusion for transplant recipients. It minimizes the risk of alloantigen-mediated attack on the infused cells generated from the recipient, thus providing better therapeutic efficacy. Studies using autologous cell infusions demonstrated a modest to significant beneficial effect in rat and swine VCA studies [27,31]. Clinically, the use of autologous cells was tried successfully in double hand transplantation [32]. Due to the limitation of the autologous cell number, the ex vivo cell expansion is required. However, allogenic cells could be used as the alternative to autologous cell therapy to increase the source of cell transplantation [17,18]. The tissue origins of cells also influence their immunomodulatory effectiveness, for example, AD-MSCs were shown to have greater immunomodulatory properties than BM-MSCs [33].

Besides the source, other factors could also affect the efficacy. In canines, age is an influential factor of BM-MSCs proliferation and immunomodulatory properties. With advanced age, the osteogenic gene expression is increased under

Table 1 Immune cell therapy in the VCA animal study.

Literature (Year)	Cell Type	Species	Model	Dosage (Day of administration), Route	Induction and IS (Day of administration)	Outcome	Mechanism
Kuo et al. (2009) [22]	Alloantigen-stimulated DCs	Rat	Hindlimb	$0.7\text{-}1\times10^7$ (7,14,21), IV	ALS (−4,1), CsA (0−20)	Prolong allograft >200 days	Induce T cell donor-specific hyporesponsiveness
Cheng et al. (2018) [39]	Alloantigen-stimulated CD4+CD25++Tregs	Rat	Hindlimb	$7\times10^5\text{-}2\times10^6$ (10), IV	ALS (−1,2) + CsA (0−7)	Maintenance of graft acceptance	Tregs graft engraftment
Anggelia et al. (2021) [23]	CD4+CD25++Tregs from tolerant recipients	Mouse	Osteo-myocutaneous	2×10^6 (30), IV	CoB (0,2) + RPM (0−28)	Rescue graft rejection	Suppress donor specific T cell proliferation
Lin et al. (2013) [67]	CD4−CD8+Tregs	Mouse	Osteo-myocutaneous	5×10^6 (0), IV	ALS	Prolong allograft >180 days	Induce T cell donor-specific hyporesponsiveness
Radu et al. (2012) [42]	Donor Mregs	Rat	Osteo-myocutaneous	5×10^6 (0), IV and IM	NA	Extended allograft survival from 5.6 to 7.4 days	Reduce infiltration of inflammatory cells

Abbreviations: ALS: anti-lymphocytes serum; CsA: cyclosporine A; CoB: costimulation blockade; DCs: dendritic cells; IM: intramuscular; IS: immunosuppression; IV: intravenous; Mregs: regulatory macrophages; Tregs: regulatory T cells; NA: not applicable; RPM: rapamycin.

proinflammatory conditions. It was suggested the BM-MSCs from young donors could be used as an alternative source to autologous BM-MSCs therapy for older canines [34]. The level of antigen specificity can also affect the suppressive capabilities of cell therapy. The treatment with antigen-specific Tregs is more effective than the treatment with non-specific Tregs [35]. A good example is that the activation of natural Tregs via the A2-CAR induced proliferation enhances the suppressor effect of the modified natural Tregs [36]. Modified microparticles to sustainably release IL-2, transforming growth factor(TGF)-β, and rapamycin also successfully induced Tregs differentiation from naïve T cells, demonstrated enhancement of Tregs associated cytokines expression and finally prolonged survival time of rodent VCA allograft [37,38].

Route of administration and additional immunosuppression (IS)

There are 2 main routes of cell therapy administration in VCA. One is via the circulation system, and the other is through the local injection. Intravenous (IV) administration of the therapeutic cells is more commonly used in transplantation due to the migratory nature of the immune and stem cells into the allograft, allowing adequate delivery of the cells to the targeted site. However, through IV injection, the cells can migrate to non-targeted organs such as the spleen, lymph nodes [39] or trapped in undesirable sites such as the lung [40,41]. The systemic delivery of the cells appears to be well tolerated; no significant adverse effects were reported despite the infusion of large cell quantity.

The local intramuscular injection has been used as an alternative route of infusion, and no significant difference was demonstrated in allograft outcome [42]. Considering the efficacy and efficiency of cell administration, the local administration might reduce the required therapeutic cellular number in cell therapy. A comprehensive study is needed to verify this hypothesis in VCA.

Cell therapy alone in the early stage of transplantation is not sufficient to induce long-term graft acceptance. Without immunosuppressants, Tregs infusion only prolonged skin allograft survival for 25 days. When T cell depletion induction agents and maintenance immunosuppressants were used in conjunction with Tregs infusion, heart allograft can survive beyond 125 days [43]. Thus, it is still mandatory to use induction agents (polyclonal or monoclonal antibodies) to deplete thymocytes, T- and B-cells in the induction phase despite the use of Tregs infusion. Interfering agents [anti-lymphocyte serum (ALS) or costimulation blockade in animal studies and thymoglobulin or alemtuzumab in clinical trials] can be used in the induction phase to interrupt the adaptive immune response against alloantigen in conjunction with Tregs infusion to achieve allograft tolerance [14,23,27,39,44]. Strategies to expand Tregs with a low dose of IL-2 administration after Tregs infusion [45,46].

Mechanism and outcome

The expected outcome with cell therapy in VCA is achieving donor-specific tolerance by targeting peripheral and central

mechanisms with minimize the use of immunosuppressants. Donor-specific tolerance could be achieved through chimerism that is defined as a single organism that contains cells derived from more than one origin. Several mechanisms of chimerism-induced peripheral and central tolerance have been proposed [47]. Many experimental studies from murine to large animals and clinical studies have suggested chimerism plays an active role in the induction and maintenance phase of allograft tolerance. Mixed chimerism could be achieved by conventional BM transplantation or vascularized bone marrow (VBM) transplantation. BM produces factors including chemokines, cytokines, adhesion molecules, and extracellular matrix proteins. The niche provided by BM is crucial for HSCs expansion and repopulation. Moreover, VBM transplantation tends to induce less graft-versus-host disease than conventional BM transplantation. Our recent study and other previous studies directly proved the superior efficacy of VBM transplantation to conventional BM transplantation [13,48,49]. However, its application in a clinical setting is far from realized. A clinical study by Schneeberger et al. showed the infusion of donor BM was ineffective to induce chimerism in patients who received VCA [14]. Donor BM cells migrate from the donor femur to recipients' organ, such as thymus [13,23,50], a place where T cell education occurs [51]. Chimerism in thymus indicates the central tolerance is developed, since the donor reactive Vβ5-expressing T cells in the Balb/c to B6 murine models were specifically suppressed in tolerant recipients [13,23,50]. The crucial roles of thymus in induction of donor-specific tolerance have been also reported, where thymectomy affected generation of donor specific chimerism in VCA recipients [52–54].

On the other hand, the immune cells that possess anti-inflammatory properties promote allograft acceptance through effector cells suppression [Figs. 2-4]. DCregs have been demonstrated in rat hindlimb models to promote transplantation tolerance through mitigation of T cell activation and induction of T cell anergy, T cell deletion, and Tregs conversion [55]. The evidence of Tregs involved in transplantation tolerance also has been widely reported. In SOT, increasing circulatory and graft-infiltrating Tregs are mainly related to donor-specific hyporesponsiveness [56,57]. The rodent VCA studies also support the importance of Tregs to promote allograft tolerance. $CD4^+CD25^+$ Tregs from the tolerant recipients were shown to have a higher suppressive capacity to effector T cells than those from the rejected. $CD4^+CD25^+$ Tregs from the tolerant recipients also migrated to the allograft to maintain the graft tolerance [39,58]. Tregs migration is associated with chemokine [59]. CCR7 involved in Tregs homing to secondary lymphoid organ while CCR4 responsible for Tregs migration to skin [60]. Learning from phenomenon in tumor microenvironment where tumor cells secrete CCL22 to recruit Tregs through interaction with CCR4 expressed on Tregs [61,62], strategy using synthetic of this chemokine to induce preferential Tregs migration to allograft and prolong the survival indefinitely might be a potential for clinical translation [63]. Although several conflicting results were reported in nonhuman primate studies [64] where Tregs infusion could increase memory T cell and alloantibody responses [65], the majority of evidences showed the advantage of Tregs infusion to prevent rejection. In a bilateral hand VCA
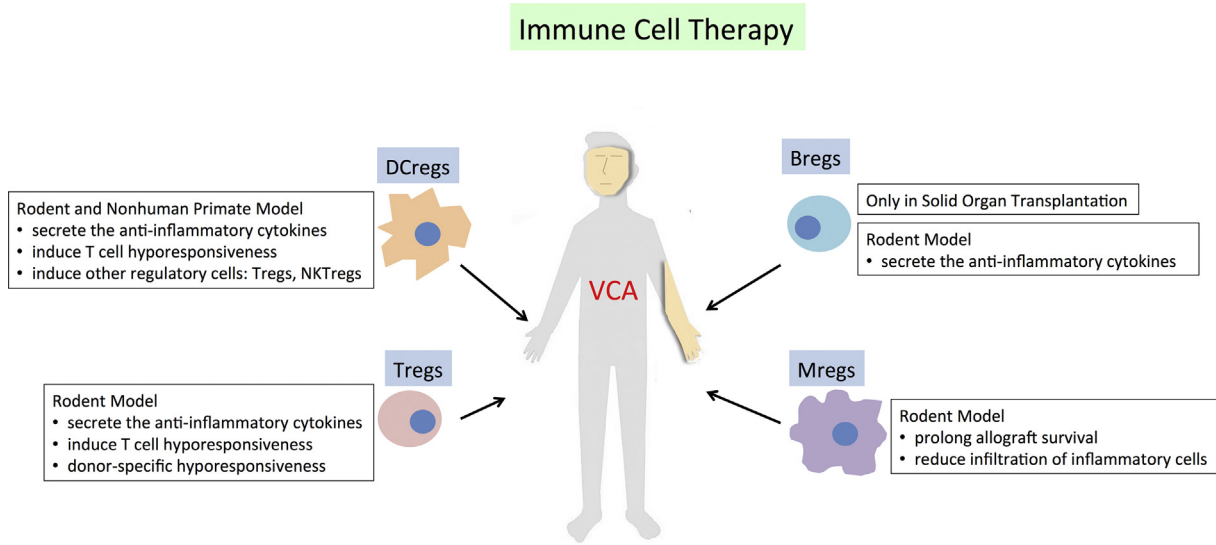
Immune Cell Therapy

DCregs

Rodent and Nonhuman Primate Model
- secrete the anti-inflammatory cytokines
- induce T cell hyporesponsiveness
- induce other regulatory cells: Tregs, NKTregs

Bregs

Only in Solid Organ Transplantation

Rodent Model
- secrete the anti-inflammatory cytokines

VCA

Tregs

Rodent Model
- secrete the anti-inflammatory cytokines
- induce T cell hyporesponsiveness
- donor-specific hyporesponsiveness

Mregs

Rodent Model
- prolong allograft survival
- reduce infiltration of inflammatory cells

Fig. 2 The proven immunomodulatory mechanisms of Immune cell therapy in the animal VCA study.

recipient, the number of Foxp3$^+$ cells increased in allograft skin over 6 years post-transplantation [66]. The utilization of non-CD4$^+$ Tregs (CD4$^-$CD8$^-$ Tregs) in osteomyocutaneous allotransplantation showed infusion induced donor-specific tolerance to allografts in mice that concurrently received ALS and short-term immunosuppressants [67]. However, the reliable and reproducible method for the isolation and expansion of non-CD4$^+$ Tregs are not established yet.

The potential use of stem cell therapy has also been demonstrated [Table 2 and Figs. 3 and 4]. Cellular and molecular studies showed their anti-inflammatory [68] and immunomodulatory activities [69]. In VCA, syngeneic HSC transplantation with conventional immunosuppressants effectively decreased donor-specific antibodies (IgG) and increased allograft survival in the sensitized rats [16]. In the canine model, simultaneous HSC transplantation and VCA

also induced allograft tolerance [17], and the additional use of granulocyte colony-stimulating factor can enhance HSC engraftment [18]. BM-MSCs are multipotent stem/progenitor cells. They are essential for the development of stable mixed chimerism, which is vital for donor-specific tolerance induction in the recipient. It has been hypothesized that BM-MSCs may prolong VCA survival through the increased levels of CD4$^+$CD25$^+$ Tregs, TGF-β1, and serum IL-10 [70]. A recent study illustrated the induced pluripotent stem cell-derived mesenchymal stem cells (MSCs) also prolonged VCA allograft survival through the induction of T cell hyporesponsiveness, reduction of proinflammatory cytokines (interferon gamma and tumor necrosis factor α), and elevation of the anti-inflammatory cytokine, IL-10 [71]. AD-MSCs also prolonged VCA allograft survival by suppressing effector T cells and enhancing Tregs [Table 2] [72,73]. Not only

Stem Cell Therapy

BM cells

Rodent, Swine, and Nonhuman Primate Model
- prolong allograft
- induce chimerism
- induce T cell hyporesponsiveness
- decrease donor-reactive T cell

VBM

AD-MSCs

Rat Model
- prolong allograft survival
- modulate T-cell regulation

VCA

HSCs

Rodent and Canine Model
- prolong allograft survival
- decrease donor-specific antibodies (IgG)

BM-MSCs

Rodent, Swine, Canine Model
- T cell hyporesponsiveness
- decrease proinflammatory cytokines
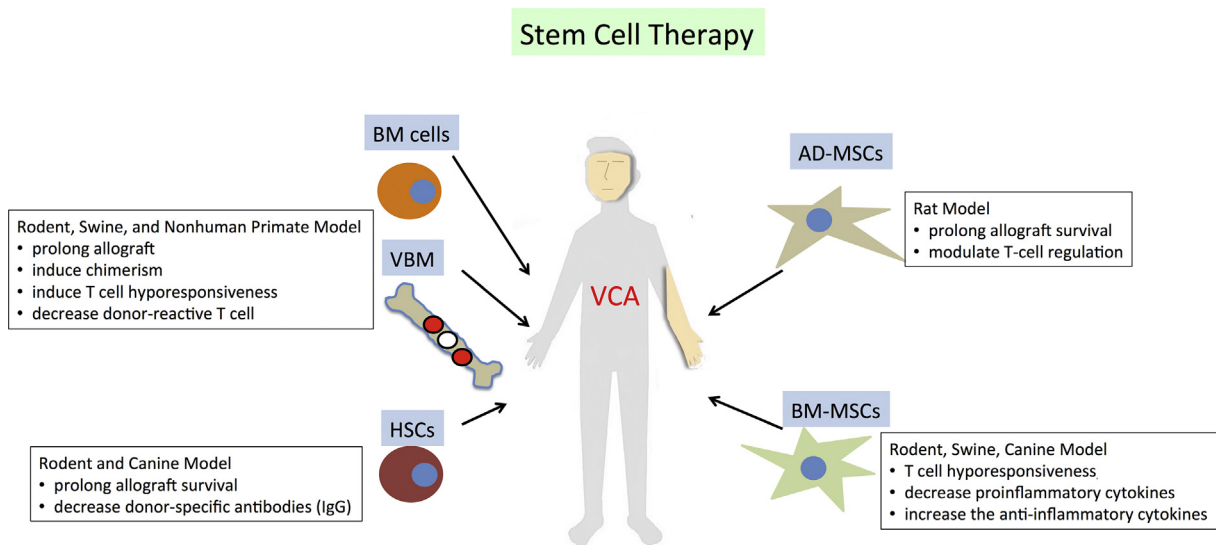- increase the anti-inflammatory cytokines

Fig. 3 The proven immunomodulatory mechanisms of stem cell therapy in the animal VCA study.
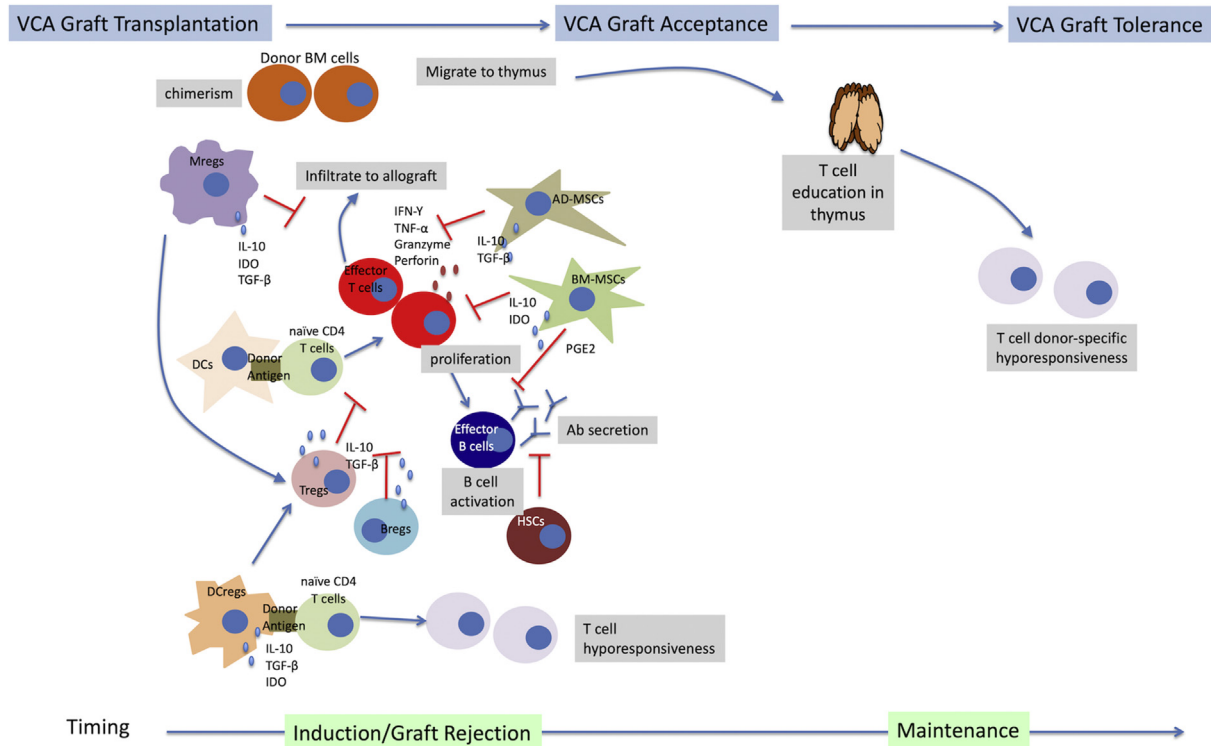
Fig. 4 Illustration of immune cell and stem cell immunomodulatory mechanisms in VCA transplantation.

preventing immune rejection, BM-MSCs and AD-MSCs were also reported to enhance nerve regeneration through the secretion of neuroregulatory factors and the ability to differentiate into Schwann-like cells [74].

Clinical trials: learning from solid organ transplantation (SOT)

Studies of SOT have been broadly performed since decades ago. The results provided significant insights into the mechanisms of rejection and tolerance. These experiences help delineate the immune responses to the allografts, and in the long-term, design optimal IS regimens in conjunction with cell-based therapies. DCregs, Tregs, and BM-MSCs are currently used in transplantation phase I/II clinical trials [30,75,76]. Although all these cells play the regulatory roles to suppress immune responses, only CD4$^+$ regulatory cells or Tregs consistently exert active tolerizing functions by inhibiting immune effector mechanisms [77]. Therefore, new IS protocols are developed to facilitate Tregs induction and expansion after transplantation [78]. The long-term results of these studies are highly anticipated with the hope to improve the lives of transplant recipients fundamentally. In recently studies from the phase I/II clinical trials in SOT, cell therapy showed good efficacy without adverse effects from short to mid-term monitoring [Table 3] [24,78,79]. Listed minor side effects include creatinine elevation, cytomegalovirus viraemia, urinary tract

infections that can be treated promptly. Nevertheless, the application of cell therapy in VCA remains limited [80]; this may be primarily due to small case number of VCA. But some studies have shown promising results. The Pittsburgh protocol demonstrated the use of BM cell therapy in VCA could induce chimerism. Thus, the dosage of IS monotherapy can be tapered [14]. The pilot study of MSCs therapy in bilateral hands also showed favorable results [32].

Summary

Conventional strategies to prevent allograft rejection in VCA rely on long-term IS. Recent data demonstrated the potential application of cell therapy in VCA. Animal studies elucidated the plausibility of using both immune cells and stem cells as the adjuvant treatment to improve VCA outcome and minimize the use of long-term immunosuppressants. Thus, side effects of long-term IS can be reduced. The selection of suitable cell therapy is clinically dependent. BM cells are more feasible for chimerism induction in the early phase after transplantation. Immune cell therapy focuses on modulating the alloreactive response. In addition, tissue-derived MSCs could be used to facilitate nerve regeneration. The source, frequency, dosage, and route of cell therapies depend on the clinical need. Although evidences from clinical VCA trials are still limited, the use of autologous cell therapy in clinical SOT trials with either bolus or divided doses is considered to be safe with good efficacy.

Table 2 Stem cell therapy in the VCA animal study.

Literature (Year)	Cell Type	Species	Model	Dosage (Day of administration), Route	Induction and IS (Day of administration)	Outcome	Mechanism
Xu et al. (2013) [12]	Donor BM cells	Rat	Osteo-myocutaneous	1.5×10^8 (0), IV	Anti-TCR (−3) + TBI (−1) + ALS (9) +Tac (−1−9)	Prolong allografts >200 days	Thymic deletion alloreactive T cell, superior donor-specific Tregs
Lin et al. (2021) [13]	Donor BM cells	Mouse	Myocutaneous	1.5×10^8 (0), IV	CoB (0,2) + RPM (0−28)	Prolong allografts >120 days	Induce chimerism and donor-specific tolerance
Mathes et al. (2014) [17]	Donor HSCs	Canine	Hindlimb	$1.7\text{-}5.1 \times 10^8$/kg (0), IV	TBI (−1) + CsA (-1-35) + MMF (0−28)	Prolong allografts >62 weeks	Increase Tregs engraftment, induce donor-specific tolerance
Leonard et al. (2014) [81]	Donor HSCs	Swine	Fascio-cutaneous	15×10^9 (0), IV	T cell depletion + non-myeloablatıve TBI + CsA (0−45)	Prolong allograft 85−100 days	Induce peripheral blood chimerism between 20 and 100%
Mitsuzawa et al. (2019) [71]	Syngeneic iMSCs	Rat	Hindlimb	2×10^6 (7), IV	Tac (0−6)	Prolong allografts >17−21 days	Induce T cell donor-specific hyporesponsiveness
Kuo et al. (2017) [31]	Autologous AD-MSCs	Swine	Hindlimb	1×10^6/kg (0−3), IV	TBI (−1) + Tac (0−4)	Prolong allografts >196 days	Modulate T cell regulation
Plock et al. (2017) [27]	Syngeneic AD-MSCs	Rat	Hindlimb	1×10^6 (4,8,15) IV	ALS (−4,1), Tac (0−21)	Prolong 50% allografts >100 days	Induce chimerism and donor-specific tolerance, elevate Tregs
Kuo et al. (2011) [73]	Donor AD-MSCs	Rat	Hindlimb	2×10^6 (7,14,21), IV	ALS (−4,1) + CsA (0−20)	Prolong allografts >200 days	Suppress T cell and enhance Tregs proliferation
Cheng et al. (2014) [29]	Syngeneic AD-MSCs	Rat	Osteo-myocutaneous	2×10^6 (1), IV	TBI (−1) + ALG (−1,10) + CsA (0−10)	Prolong 67% allografts >140 days	Induce donor-specific tolerance, elevated circulating Tregs
Schweizer et al. (2020) [82]	Syngeneic AD-MSCs	Rat	Hindlimb	1×10^6 (2,4,7,14,28), IV	CTLA4Ig (2,4,7) + ALS (1,5) + Tac (0−14)	Prolong 86% allografts >120 days	Induce chimerism and elevated systemic and skin Tregs

Abbreviations: AD-MSCs: adipose derived mesenchymal stem cells; ALG: anti-lymphocyte globulin; ALS: anti-lymphocytes serum; BM: bone marrow; CsA: cyclosporine A; CoB: costimulation blockade; DCs: dendritic cells; IM: intramuscular; iMSCs: induced mesenchymal stem cells; IS: immunosuppression; IV: intravenous; NA: not applicable; RPM: rapamycin; Tac: tacrolimus; TBI: total body irradiation.

Table 3 Cell therapy in clinical transplantation.

Literature (Year)	Cell Type	Model	Phase	Dosage (Days of administration), Route	Induction and IS (Days of administration)	Outcome
Schneeberger et al. (2013) [14]	Donor BM cells	Upper Extremity	I	$5-10 \times 10^8$/kg (14), IV	Alemtuzumab (0) + methylprednisolone (0) + tapered Tac	Safe and allows to use low-dose Tac monotherapy *Adverse event*: increase serum creatinine, hyperglycemia, hyperuricemia, minor wound infections
Del Bene et al. (2013) [32]	Autologous MSCs	Bilateral Hand Transplant	I	2×10^6 (1), IV	Basiliximab (4) + methylprednisolone (0−6) cortisone + Tac + MMF	Graft acceptance up to 10 months
Macedo et al. (2020) [30]	Donor-derived DCregs	Liver	I	$2.5\text{-}10 \times 10^6$ (−7, −3, 0), IV	Mycophenolic acid	Safe and induce systemic changes in recipient antigen presenting cells and T cells *Adverse event*: NA
Todo et al. (2016) [83]	Donor-specific Tregs	Liver	I	$0.23-6.37 \times 10^6$/kg (13), IV	Steroids + MMF (0−30) + Tac/CsA/RPM + CP (5)	Normal function 3/10 Patients show mild rejection after 1 year transplant *Adverse event*: CMV antigenemia, diabetic nephropathy
Sawitzki et al. (2020) [84]	CBMPs (DCregs, Mregs, Tregs)	Kidney	I/IIa	$2.0-2.5 \times 10^6$/kg (−1 or 7), IV	Basiliximab + tapered steroids, MMF, and Tac	Weaned IS *Adverse event*: Increase creatinine
Sánchez-Fueyo et al. (2020) [44]	AutologousPolyclonal Tregs	Liver	I	$1\text{-}4.5 \times 10^6$ (90), IV	Thymoglobulin (1−7) + Tac + methylprednisolone (700)	Reduced anti-donor T cell responses *Adverse event*: NA
Mathew et al. (2018) [78]	Polyclonal Tregs	Kidney	I	0.5, 1, and 5×10^9 (60), IV	Alemtuzumab (0,2)+ Tac (-1-30) + Sirolimus (replace Tac) + Mycophenolate	No rejection up to 2 years *Adverse event*: NA
Roemhild et al. (2020) [85]	Polyclonal Tregs	Kidney	I/IIa	0.5, 1.0, or 2.5-3.0×10^6/kg (7), IV	Prednisone (0−98) + MMF (0−336) + Tac	Enable minimization of IS *Adverse event*: CMV viraemia, urinary tract infection, and pneumonia
Hutchinson et al. (2011) [41]	Donor-derived Mregs	Kidney	I	5×10^7 (−6/-7) IV	Steroid (0−700) + Tac	Stable kidney function up to 3 years with minimal maintenance IS *Adverse event*: NA
Detry et al. (2017) [76]	Donor MSCs	Liver	I	3×10^6/kg (3), IV	ATG + Tac (0−270) + MMF (0−365) + Steroid	Did not promote tolerance *Adverse event*: NA
Shi et al. (2017) [24]	UC-MSCs	Liver	I	3×10^6/kg during acute rejection, IV	Basiliximab + tapered corticosteroids + prednisolone (0−90) + MMF + Tac	UC-MSC infusion for acute graft rejection is feasible *Adverse event*: NA up to 24 weeks

Abbreviations: ATG: anti-thymocytes globulin; CsA: cyclosporine A; CBMPs: cell-based medicinal products; CMV: cytomegalovirus; CP: cyclophosphamide; DCregs: regulatory dendritic cells; IS: immunosuppression; IV: intravenous; MMF: mycophenolate mofetil; Mregs: regulatory macrophages; MSCs: mesenchymal stem cells; NA: not applicable; RPM: rapamycin; Tac: tacrolimus; Tregs: regulatory T cells; UC-MSCs: umbilical cord mesenchymal stem cells.

Funding

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgement

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