In the name of GOD

Regenerative medicine in renal transplantation

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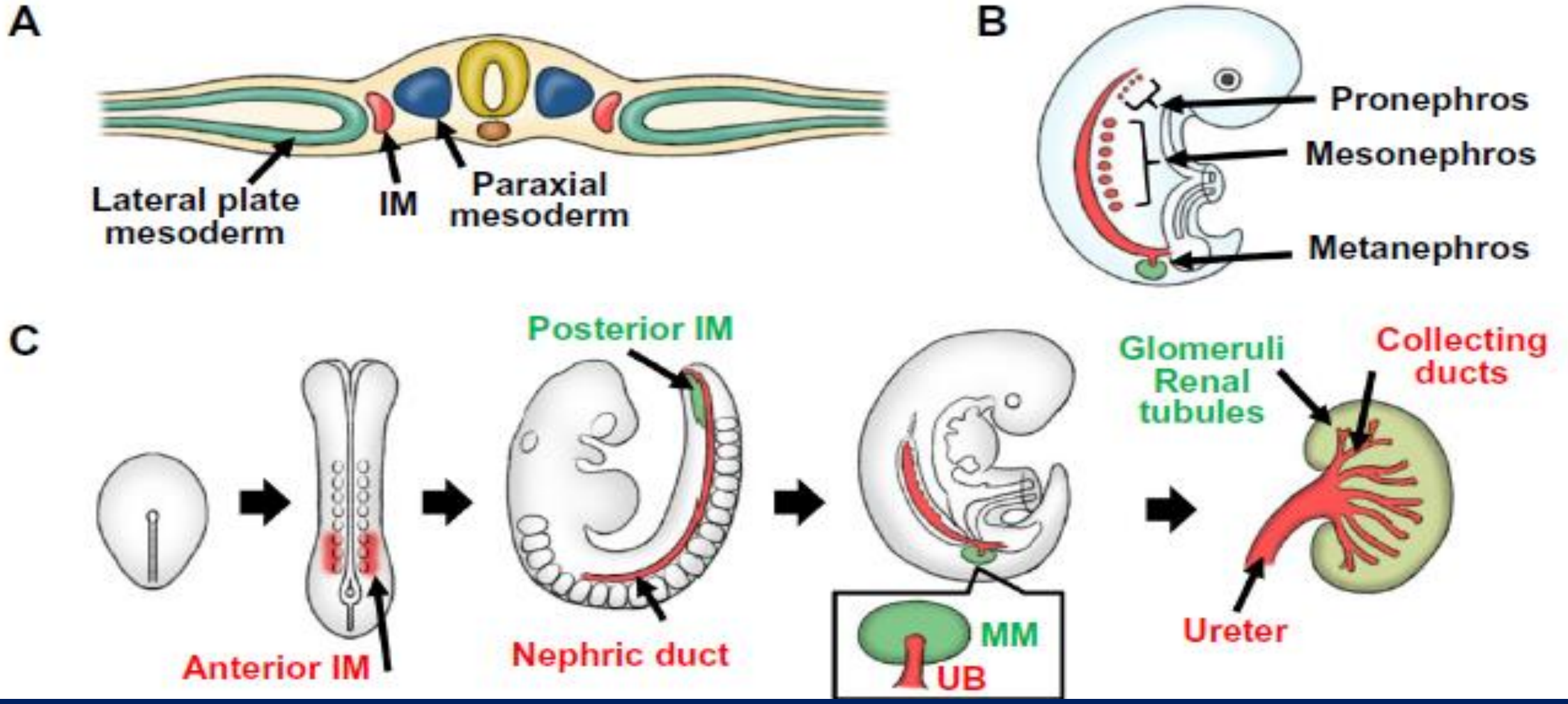
Fall 1400

Introduction

- **Kidney failure can be acute or chronic**
- **Both AKI and CKD may progress to ESRD a condition with very few effective therapies, except for supportive care**
- **Several strategies have been developed over the time:**
  - **Artificial kidney in the 1940s**
  - **Outpatient dialysis in the 1960s**
  - **kidney transplantation from a living and cadaveric donor**
  - **Discovery of drugs that delay the progression of disease, such as ACEi**
  - **Nevertheless, further strategies that remove patients from the transplant queue are still needed.**
  - **Therefore, the development of new therapeutic strategies is crucial and regenerative therapy has a promising field to achieve this goal**



Kidnies Formed by a reciprocal interaction between two IM derived embryonic tissues:

MM gives rise to the nephrons and interstitium of adult kidneys,

UB differentiates to elaborate the lower urinary tract from the collecting ducts to a part of the urinary bladder

Stem Cells and the Kidney: Where Do We Go from Here?

- **The kidney undergo mesenchymal-epithelial transition during development.**
- **kidney arise from reciprocal interactions between UB and MM.**
- **Contains more than 24 mature cell types arranged in distinct location.**
- **Unique organogenesis and structural complexity**

J Am Soc Nephrol 18: 2018–3020, 2007

[Organogenesis 3:1, 34-43, January/February/March 2007]; ©2007 Landes Bioscience

Review

Application of Regenerative Medicine for Kidney Diseases

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Previously published online as an *Organogenesis* E-publication:
http://www.landesbioscience.com/journals/organogenesis/abstract.php?id=3961

ABSTRACT

Following recent advancements of stem cell research, the potential for organ regeneration using somatic stem cells as an ultimate therapy for organ failure has increased. However, anatomically complicated organs such as the kidney and liver have proven more refractory to stem cell-based regenerative techniques. At present, kidney regeneration is considered to require one of two approaches depending on the type of renal failure, namely acute renal failure (ARF) and chronic renal failure (CRF).

The kidney has the potential to regenerate itself provided that the damage is not too severe and the kidney's structure remains intact. Regenerative medicine for ARF should therefore aim to activate or support this potent. In cases of the irreversible damage to the kidney, which is most likely in patients with CRF undergoing long-term dialysis, self-renewal is totally lost. Thus, regenerative medicine for CRF will likely involve the establishment of a functional whole kidney de novo. This article reviews the challenges and recent advances in both approaches and discusses the potential approach of these novel strategies for clinical application.

Different strategies toward replenishing the damaged renal parenchyma

- Harnessing the kidneys intrisic growth potential for renal cell therapy
  - Adult mammalian kidney is devoid of true regenerative potential, and ability to generate new nephrons
  - Exhaustion of the fetal pool of renal stem cells, which is depleted soon after (mice) or several weeks before (humans) birth.
- Transplantation of Fetal kidney grafts
- Fetal kidney stem cells
- Directed differentiation of pluripotent stem cells into kidney
- Establishment of renal progenitors from the adult kidney
- Genetic reprogramming of mature cells into a progenitor state.
- use of novel techniques : three dimensional developmental environment and organoid technology

Stem Cells International
Volume 2020, Article ID 8894590, 9 pages

Stem Cells and the Kidney: Where Do We Go from Here?

- The use of embryonic stem cells for organ regeneration is limited by formation of teratoma in the host.
- More differentiated stem cells may be safer for therapeutic use because lack of teratoma frmation.

J Am Soc Nephrol 18: 3018–3020, 2007

Adult stem cells as a tool for kidney regeneration

J Stem Cell Res Ther. 2020;6(1):1–9

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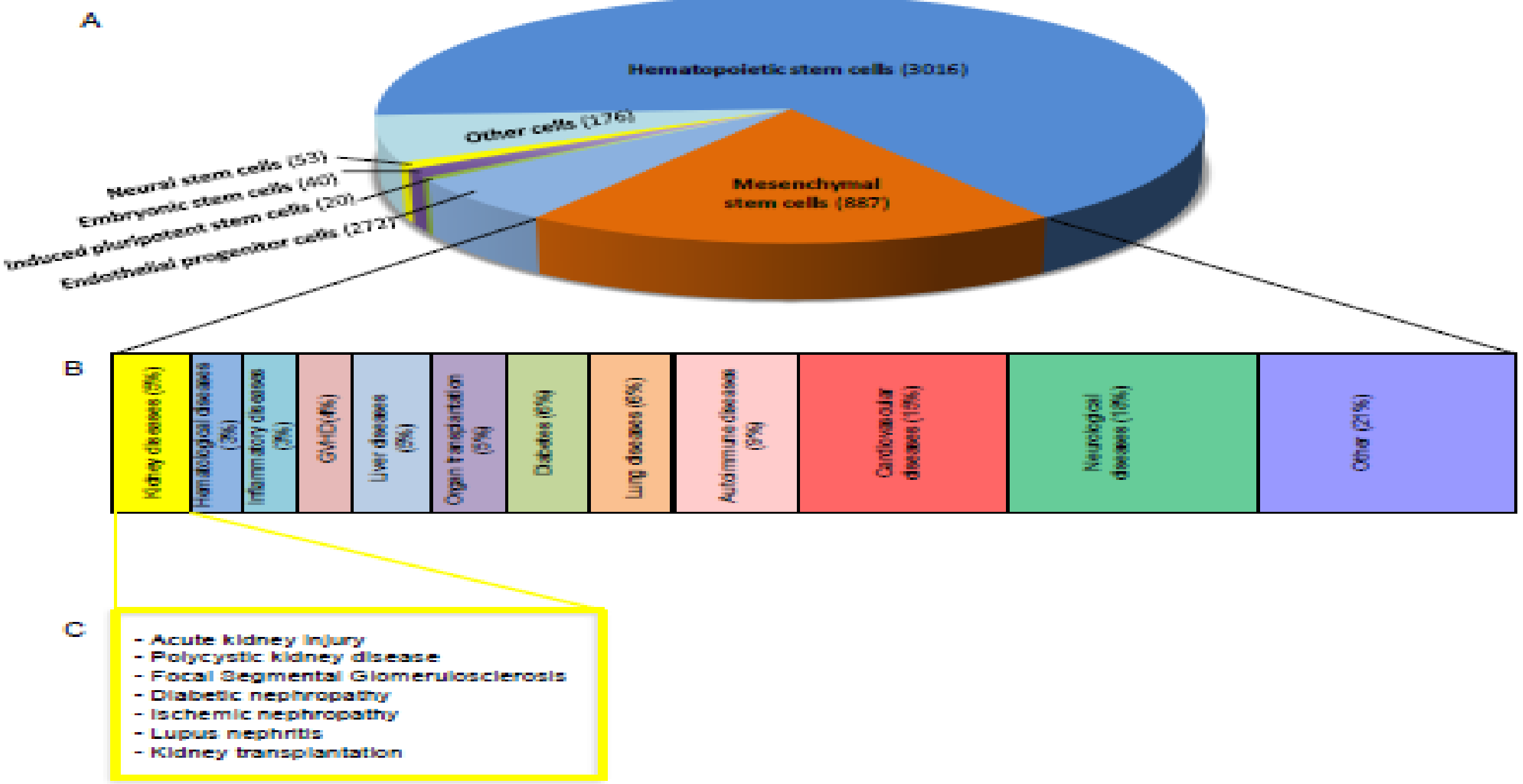
Conflict-of-interest statement: The authors declare no conflicts of interest regarding this manuscript.

of patients with ESRD. Adult stem cells are multipotent stem cells that reside in various tissues, such as bone marrow and adipose tissue. Although intensive studies to isolate kidney stem/progenitor cells from the adult kidney have been performed, it remains controversial whether stem/progenitor cells actually exist in the mammalian adult kidney. The efficacy of mesenchymal stem cells (MSCs) in the recovery of kidney function has been demonstrated in animal nephropathy models, such as acute tubular injury, glomerulonephritis, renal artery stenosis, and remnant kidney. However, their beneficial effects seem to be mediated largely *via* their paracrine effects rather than their direct differentiation into renal parenchymal cells. MSCs not only secrete bioactive molecules directly into the circulation, but they also release various molecules, such as proteins, mRNA, and microRNA, in membrane-covered vesicles. A detailed analysis of these molecules and an exploration of the optimal combination of these molecules will enable the treatment of patients with kidney disease without using stem cells. Another option for the treatment of patients with kidney disease using adult somatic cells is a direct/indirect reprogramming of adult somatic cells into



Clinical trials with cell-based therapy: embryonic and adult stem cells. (A) Pie chart showing the relative numbers of clinical trials using different types of stem cells as listed on the U.S. NIH website Int. J

Int. J. Mol. Sci. 2019, 20, 2790

Adult stem cells as a tool for kidney regeneration

- MSCs are effective to repair the kidney in various animal models; however, their beneficial effects can be due to paracrine factors that are secreted from MSCs.
  - MSCs release EVs that contain mRNA and micro RNA as well as proteins.
  - EV-derived molecules may play beneficial roles in the repair of the kidney.
- Ideal combinations of :
  - Use of molecules released from MSCs for stimulation of kidney repair without using stem cells.
  - Strategies to reprogram somatic cells to kidney progenitor cells to regenerate the kidney.

World J Nephrol 2016 January 6; 5(1): 43-52 .ISSN 2220-6124

Studies	No. of Patients (Follow-Up)	MSC (Source, Dose, and Timing)	Main Results
Kidney trasplantation			
Perico et al. NTC00752479	2 living donor kidney Tx recipients; (1 yr)	Autologous bmMSCs, single i.v. infusion of 1.7–2 × 10^6 cells/kg, day +7 post-Tx	MSC infusion was safe and well tolerated. Transient enhancement of serum creatinine levels after MSC infusion. Increased of the percentage of Treg and inhibition of memory $CD8^+$ T cell expansion
Perico et al. NTC02012153	2 living donor kidney Tx recipients; (1 yr)	Autologous bmMSCs, single i.v. infusion of 2 × 10^6 cells/kg, day −1 pre-Tx	MSC infusion was safe and well tolerated. No MSC-associated renal insufficiency. Decrease in circulating memory $CD8^+$ T and donor-specific $CD8^+$ T cell cytolitic response
Tan et al. NTC00658073	159 living donor kidney Tx recipients; n = 53 bmMSCs + std. dose CNI; n = 52 bmMSCs + low dose CNI (80% of std); n = 51 basiliximab + std. CNI; (1 yr)	Autologous bmMSCs, two i.v. infusions of 1–2 × 10^6 cells/kg, day 0 and day +14 post-Tx	MSC infusion was safe and well tolerated. MSC infusion showed lower incidence of acute rejection, descreased risk of opportunistic infections and had faster renal function recovery compared with controls.
Reinders et al. NTC00734396	6 living donor kidney Tx recipients; (6 mo)	Autologous bmMSCs, two i.v. injections of 0.1–1 × 10^6 cells/kg, 7 d apart at 6 mo post-Tx	MSC infusion was safe and well tolerated. Increased incidence of opportunistic infections. Resolution of tubulitis and interstitial fibrosis/tubular atrophy in two patients
Mudrabettu et al. NTC02409940	4 living donor kidney Tx recipients; (6 mo)	Autologous bmMSCs, two i.v. infusions of 0.2–0.8 × 10^6 cells/kg, day −1 and day +30 post-Tx	MSC infusion was safe and well tolerated. Increase in regulatory T cells and reduction in $CD4^+$ T cell proliferation
Pan et al.	32 living donor kidney Tx recipients; n = 16 MSC + low dose tacrolimus (50%of std), n = 16 std. tacrolimus dose controls; (2 yr)	Allogeneic bmMSCs, two infusions: 5 × 10^6 into renal artery at day 0 and 2 × 10^6 cells/kg at day +30 post-Tx	MSC infusion was safe and well tolerated. Comparable incidence of acute rejection and similar graft function and survival between patient groups. MSCs allow to use a lower dose of tacrolimus

Allogeneic mesenchymal stem cell as induction therapy to prevent both delayed graft function and acute rejection in deceased donor renal transplantation: study protocol for a randomized controlled trial

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Abstract

Background: Using kidneys from deceased donors is an available strategy to meet the growing need of grafts. However, higher incidences of delayed graft function (DGF) and acute rejection exert adverse effects on graft outcomes. Since ischemia-reperfusion injury (IRI) and ongoing process of immune response to grafts are the major causes of DGF and acute rejection, the optimal induction intervention should possess capacities of both repairing renal structure injury and suppressing immune response simultaneously. Mesenchymal stem cells (MSCs) with potent anti-inflammatory, regenerative and immune-modulatory properties are considered as a candidate to prevent both DGF and acute rejection in renal transplantation. Previous studies just focused on the safety of autologous MSCs on living-related donor renal transplants, and lack of concomitant controls and the sufficient sample size and source of MSCs. Here, we propose a prospective multicenter controlled study to assess the clinical value of allogeneic MSCs in preventing both DGF and acute rejection simultaneously as induction therapy in deceased-donor renal transplantation.

Methods/design: Renal allograft recipients (n = 100) will be recruited and divided into trial and control groups, and 50 patients in the trial group will be administered with a dose of 2×10^6 per kilogram human umbilical-cord-derived MSCs (UC-MSCs) via peripheral vein injection preoperatively, and a dose of 5×10^7 cells via renal arterial injection during surgery, with standard induction therapy. Incidences of postoperative DGF and biopsy-proved acute rejection (BPAR) will be recorded and analyzed. Additionally, other clinical parameters such as baseline demographics, graft and recipient survival and other severe postoperative complications, including complicated urinary tract infection, severe pneumonia and severe bleeding, will be also assessed.

Urine-derived stem/progenitor cells: A focus on their characterization and potential

World J Stem Cells 2020 October 26; 12(10): 1080-1096

Abstract

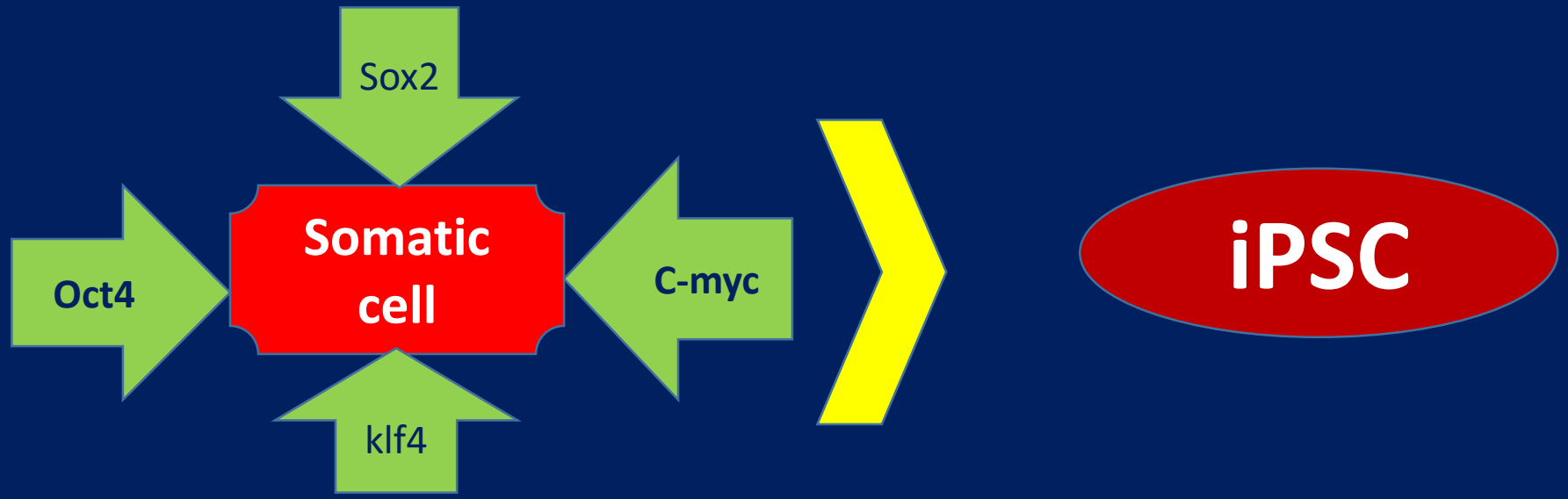
Cell therapy, *i.e.*, the use of cells to repair an affected tissue or organ, is at the forefront of regenerative and personalized medicine. Among the multiple cell types that have been used for this purpose [including adult stem cells such as mesenchymal stem cells or pluripotent stem cells], urine-derived stem cells (USCs) have aroused interest in the past years. USCs display classical features of mesenchymal stem cells such as differentiation capacity and immunomodulation. Importantly, they have the main advantage of being isolable from one sample of voided urine with a cheap and unpainful procedure, which is broadly applicable, whereas most adult stem cell types require invasive procedure. Moreover, USCs can be differentiated into renal cell types. This is of high interest for renal cell therapy-based regenerative approaches. This review will firstly describe the isolation and characterization of USCs. We will specifically present USC phenotype, which is not an object of consensus in the literature, as well as detail their differentiation capacity. In the second part of this review, we will present and discuss the main applications of USCs. These include use as a substrate to generate human induced pluripotent stem cells, but we will deeply focus on the use of USCs for cell therapy approaches with a detailed analysis depending on the targeted organ or system. Importantly, we will also focus on the applications that rely on the use of USC-derived products such as microvesicles including exosomes, which is a strategy being increasingly employed. In the last section, we will discuss the remaining barriers and challenges in the field of USC-based regenerative medicine.

iPSC against PSC

- Pluripotent stem cells:
  - Are self-renewing
  - Clonogenic, and
  - Able to commitment into the three different embryonic germ lines:
    - Ectoderm,
    - Mesoderm
    - Endoderm
  - Source of these cells is human embryos at blastocyst phase, namely, embryonic stem cells (ESCs
    - Use of ESC for cellular therapy is complex, considering:
      - Ethical conflicts concerning manipulation of human embryos
      - Safety concerns related to their immunogenicity
      - Risk of uncontrolled growth and teratoma formation

iPSC

- **In an attempt to overcome these issues, a new reprogramming technology has led to the generation of iPSC from somatic cells :**



- **iPSC share with ESCs many features including :**
  - **Pluripotency and high differentiation capacity**
  - **Representing a promising alternative as a source of pluripotent stem cells without**
    - **Ethical concerns**
    - **Immunorejection**
      - **Since they can be generated from patient derived adult cells**

iPSC New Approach to Treat Kidney disease

- Some limitations still persist and include :
  - The risk of tumor development following transplantation due to their high proliferative potential
  - Use of viral vectors for reprogramming
- ***Therefore, an alternative approach is to differentiate iPSC into a specific cell type before cellular transplantation***

iPSC-Derived Renal Cells as Cell Therapy for Kidney Diseases

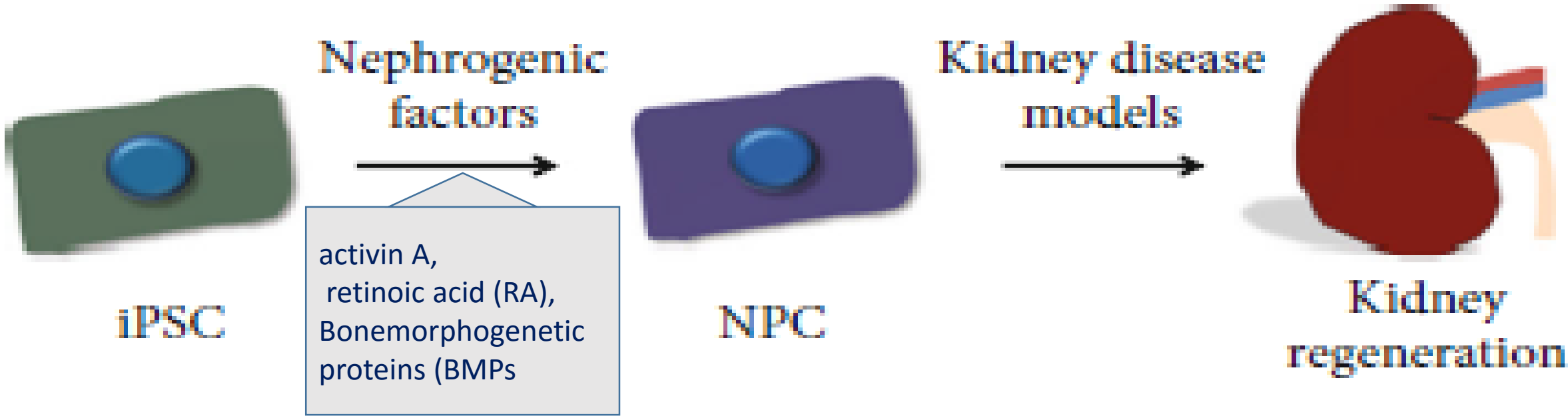


FIGURE 2: iPSC-derived renal cells in kidney diseases. iPSC: induced pluripotent stem cells; NPC: nephron progenitor cell.

iPSC technology-based regenerative medicine for kidney diseases

Clinical and Experimental Nephrology (2021) 25:574–584

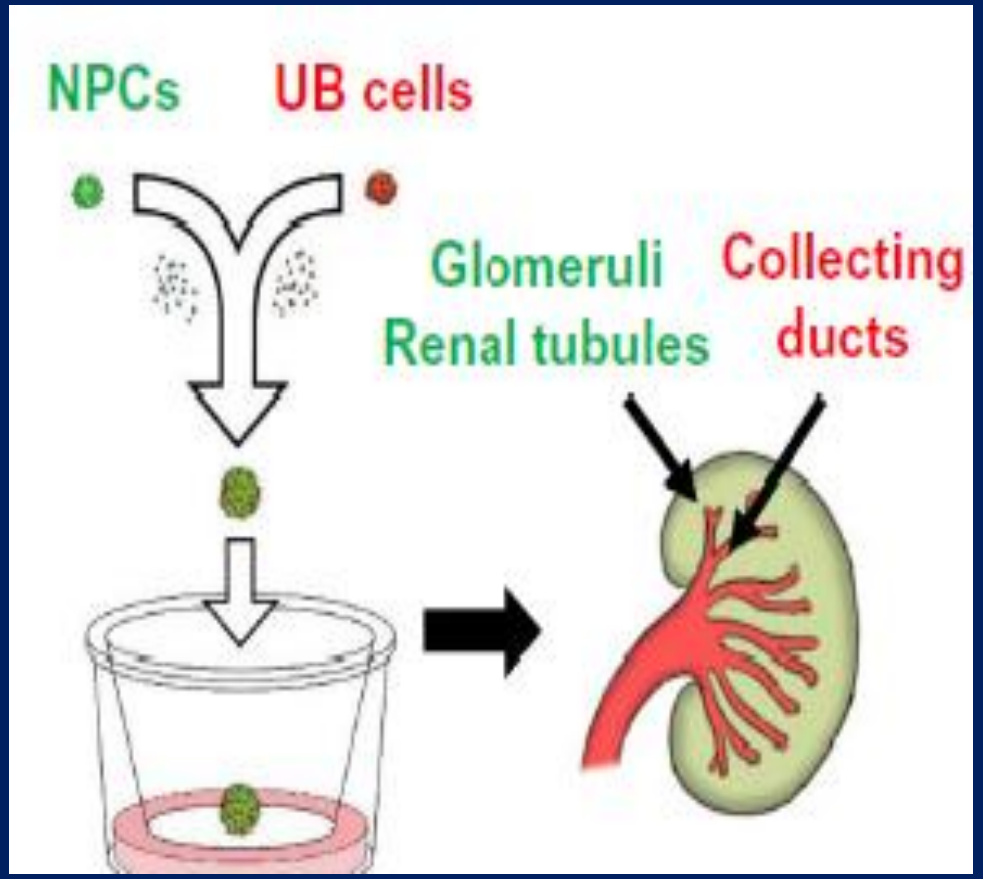
Kenji Osafune[1]

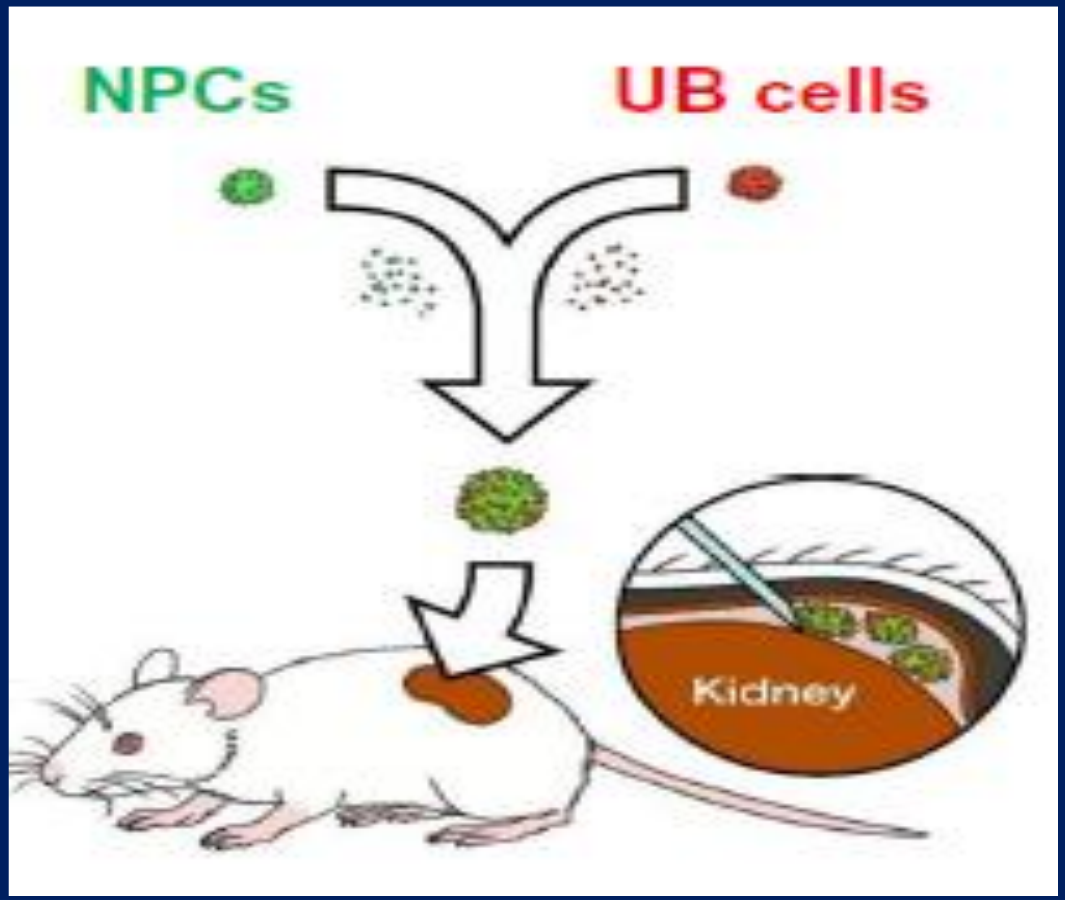
Received: 7 August 2020 / Accepted: 1 February 2021 / Published online: 3 March 2021

Abstract

With few curative treatments for kidney diseases, increasing attention has been paid to regenerative medicine as a new therapeutic option. Recent progress in kidney regeneration using human-induced pluripotent stem cells (hiPSCs) is noteworthy. Based on the knowledge of kidney development, the directed differentiation of hiPSCs into two embryonic kidney progenitors, nephron progenitor cells (NPCs) and ureteric bud (UB), has been established, enabling the generation of nephron and collecting duct organoids. Furthermore, human kidney tissues can be generated from these hiPSC-derived progenitors, in which NPC-derived glomeruli and renal tubules and UB-derived collecting ducts are interconnected. The induced kidney tissues are further vascularized when transplanted into immunodeficient mice. In addition to the kidney reconstruction for use in transplantation, it has been demonstrated that cell therapy using hiPSC-derived NPCs ameliorates acute kidney injury (AKI) in mice. Disease modeling and drug discovery research using disease-specific hiPSCs has also been vigorously conducted

iPSC technology-based regenerative medicine for kidney diseases
Reconstruction of kidney structures





Recent advances in the generation of nephron organoids from hiPSCs have enabled the modeling of kidney disorders, such as :

Nephronophthisis, congenital nephrotic syndrome , ARPKD

iPSC-EPO cells could be used to discover novel drugs and develop cell therapies against renal anemia

Cell Transplantation
Volume 29: 1–10

DOI: 10.1177/0963689719897066
journals.sagepub.com/home/cll
SAGE

Three-Dimensional Renal Organoids from Whole Kidney Cells: Generation, Optimization, and Potential Application in Nephrotoxicology In Vitro

Beichen Ding[1,2], Guoliang Sun[1], Shiliang Liu[1], Ejun Peng[1], Meimei Wan[3], Liang Chen[1,3], John Jackson[3], and Anthony Atala[3]

Abstract

The kidney function of patients with chronic kidney disease (CKD) is impaired irreversibly. Organ transplantation is the only treatment to restore kidney function in CKD patients. The assessment of new potential therapeutic procedures relies heavily on experimental animal models, but it is limited by its human predictive capacity. In addition, the frequently used two-dimensional *in vitro* human renal cell models cannot replicate all the features of the in vivo situation. In this study, we developed a three-dimensional (3D) *in vitro* human renal organoid model from whole kidney cells as a promising drug screening tool. At present, the renal tissue generated from human pluripotent stem cells (hPSCs) exhibits intrinsic tumorigenicity properties. Here we first developed a 3D renal organoid culture system that originated from adult differentiated cells without gene modification. Renal organoids composed of multiple cell types were created under optimal experimental conditions and evaluated for morphology, viability and erythropoietin production. As a novel screening tool for renal toxicity, 3D organoids

- As a novel screening tool for renal toxicity, 3D organoids were exposed to three widely used drugs: aspirin, penicillin G and cisplatin.

- The study results showed this 3D renal organoid model can be used as a drug screening tool, a new in vitro 3D human kidney model, and provide hope for potential regenerative therapies for CKD.

Overcoming kidney organoid challenges for regenerative medicine

Thomas Geuens[1], Clemens A. van Blitterswijk[1] and Vanessa L. S. LaPointe[1]

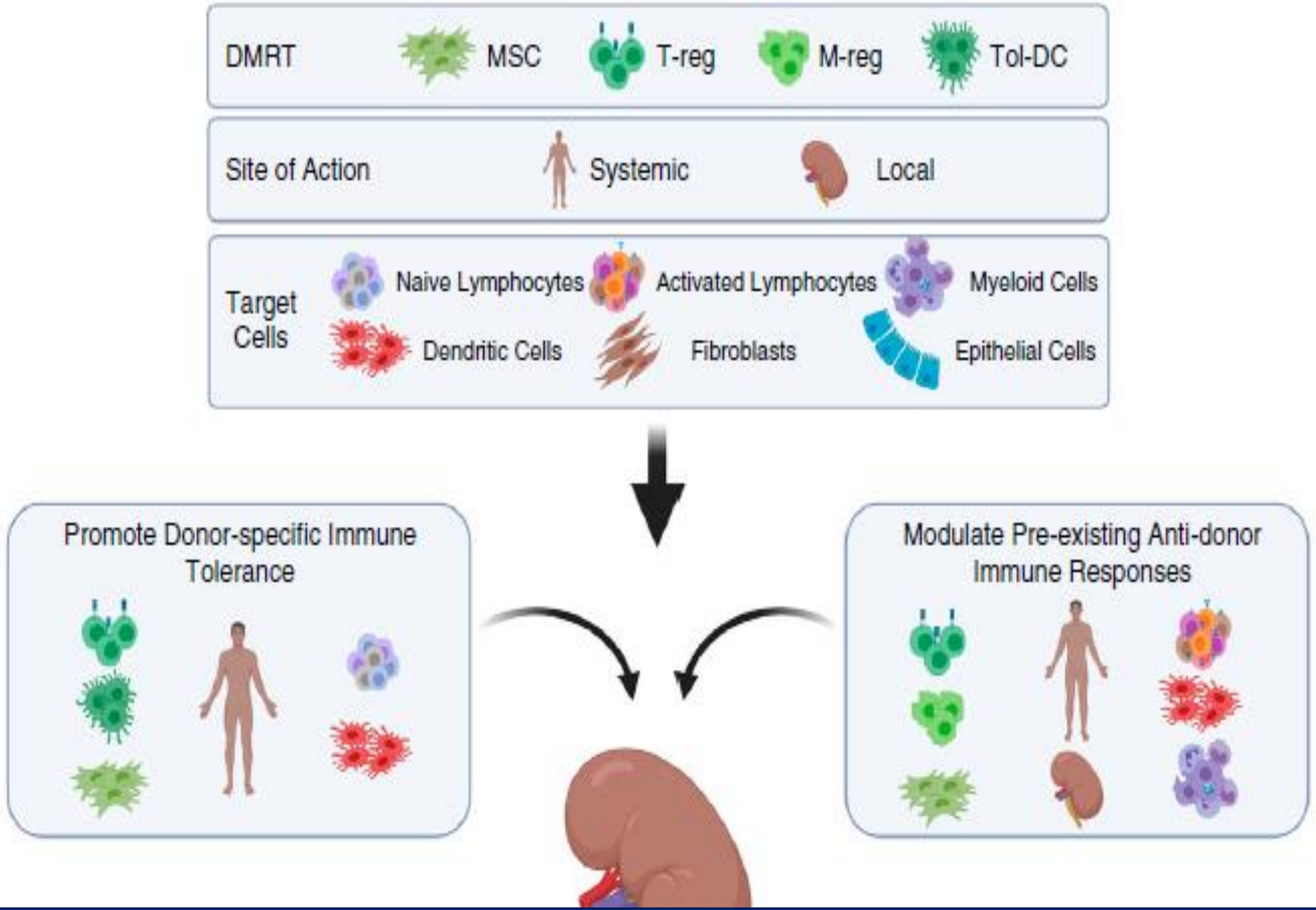
Kidney organoids derived from human induced pluripotent stem cells bear the potential to be used as a regenerative medicine renal replacement therapy. Advances in developmental biology shed light on the complex cellular regulation during kidney morphogenesis in animal models resulting in insights that were incorporated in the development of groundbreaking protocols for the directed differentiation of human pluripotent stem cells to kidney endpoints. Moreover, further optimization efforts to improve three-dimensional culture techniques resulted in the creation of kidney organoids. Before they can find their way to the clinic, there are critical challenges to overcome. Here, we will discuss recent advances and remaining challenges for kidney organoids to become successful in regenerative medicine. An innovative combination of tissue engineering techniques with more refined insights in the developing human kidney will ultimately lead to more mature and functional kidney organoids suitable as renal replacement therapy for patients with chronic kidney disease.

npj Regenerative Medicine (2020)5:8 ; https://doi.

Disease-Modulating Regenerative Therapies in Nephrology

Hickson et al. KIDNEY360 2: 542–557, March, 2021

- promoting disease resolution indirectly through local or systemic interactions with a patient's cells, without permanently integrating or directly forming new primary tissue.
- In four distinct areas of nephrology:
  - Renovascular disease (RVD)
  - Sepsis-associated AKI (SA-AKI)
  - Diabetic kidney disease (DKD)
  - Kidney transplantation (KTx).
- To date (2021), no DMRT has gained market approval for use in patients with RVD, SA-AKI, DKD, or KTx, and


DMRT
MSC
T-reg
M-reg
Tol-DC
Site of Action
Systemic
Local
Target Cells
Naive Lymphocytes
Activated Lymphocytes
Myeloid Cells
Dendritic Cells
Fibroblasts
Epithelial Cells
Promote Donor-specific Immune Tolerance
Modulate Pre-existing Anti-donor Immune Responses

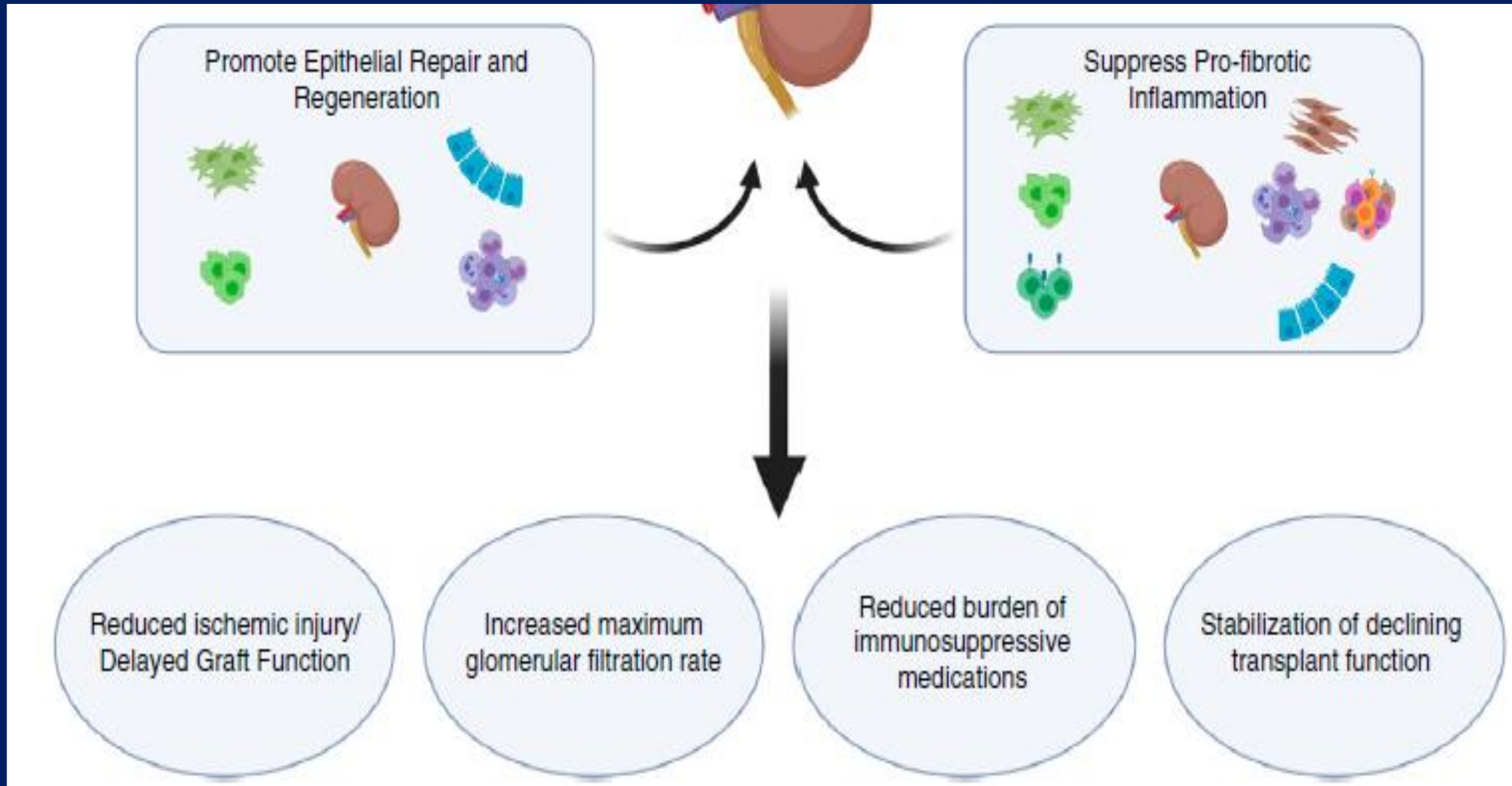


Figure 5. | Disease modulating regenerative therapies for kidney transplantation address diverse mechanisms and potential clinical benefits.

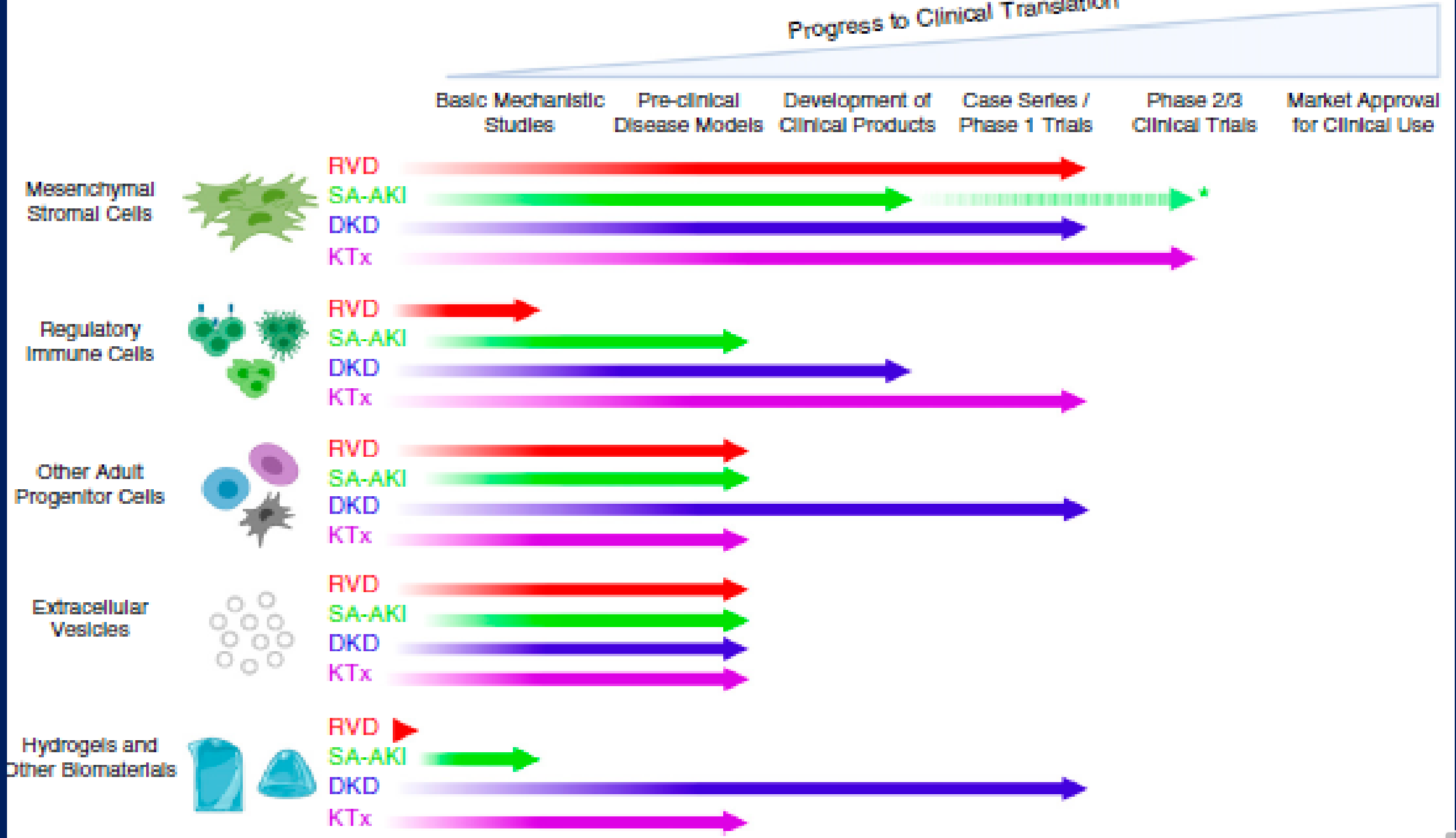


Figure 1. | Variable progress has been made toward clinical translation of five categories of disease-modulating regenerative therapies

Disease-Modulating Regenerative Therapies in Nephrology

Hickson et al.KIDNEY360 2: 542–557, March, 2021

- **To date (2021), no DMRT has gained market approval for use in patients with RVD, SA-AKI, DKD, or KTx, and**

- **clinical trials demonstrating definitive, cost-effective and patient benefits are needed.**

Effect of SVF Derived MSC in DCD Renal Transplantation

- Primary outcome :
  - Effects of autologous SVF derived MSC transplantation on reducing the dosage of CNI by 30% in Kidney Transplantation from Chinese Donation after Citizen Death [Time Frame: 1 years]
    - Changes of the immunosuppressant by reducing 30% of CNI dosage.
- Secondary outcome:
  - Changes in renal function as determined by eGFR and proteinuria (Time Frame: 1 year)

Review

Adipose-Derived Stem/Stromal Cells in Kidney Transplantation: Status Quo and Future Perspectives

Gabriele Storti [1,†], Evaldo Favi [2,3,*,†], Francesca Albanesi [3], Bong-Sung Kim [4] and Valerio Cervelli [1]

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Abstract: Kidney transplantation (KT) is the gold standard treatment of end-stage renal disease. Despite progressive advances in organ preservation, surgical technique, intensive care, and immunosuppression, long-term allograft survival has not significantly improved. Among the many peri-operative complications that can jeopardize transplant outcomes, ischemia–reperfusion injury (IRI) deserves special consideration as it is associated with delayed graft function, acute rejection, and premature transplant loss. Over the years, several strategies have been proposed to mitigate the impact of IRI and favor tolerance, with rather disappointing results. There is mounting evidence that adipose stem/stromal cells (ASCs) possess specific characteristics that could help prevent, reduce, or reverse IRI. Immunomodulating and tolerogenic properties have also been suggested, thus leading to the development of ASC-based prophylactic and therapeutic strategies in pre-clinical and clinical models of renal IRI and allograft rejection. ASCs are copious, easy to harvest, and readily expandable in culture. Furthermore, ASCs can secrete extracellular vesicles (EV) which may act as powerful

Citation: Storti, G.; Favi, E.; Albanesi, F.; Kim, B.-S.; Cervelli, V. Adipose-Derived Stem/Stromal Cells in Kidney Transplantation: Status

Mitochondrial transplantation

- Kidneys are second to the heart in oxygen consumption and mitochondrial number.
- Mitochondria dysfunction is a key contributor to renal tubular cell death during acute kidney injury
- Mitochondrial transplantation as a novel master key for treatment of various incurable diseases in the future.

Mitochondrial transplantation

- mitochondrial transplantation prolonged cold preservation time , might be improve organ transplantation outcomes
- The isolation of healthy and functional mitochondria is very important and crucial.
- Functioning for at least 28 days and had no pro-arrhythmic, inflammatory, immune or auto-immune response
- Currently, autologous transplantation is recommended for the clinical trial

Delivery methods of mitochondrial transplantation

- In situ or directly inject in to affected organ
  - Surgically in heart or steriotaxis for nervous system
  - The sonography-guided catheter , carotid system for brain and renal artery for kidney might be applicable
- Systemic transplantations

Cytotechnology (2019) 71:647–663

Mitochondrial transplantation practical view

- Recommended to obtain tissue biopsy from non-ischemic skeletal muscle mainly from pectoralis major or rectus abdominis.
- Usually, 6 mm tissue biopsy is suitable to take enough mitochondria for injection. Approximately, the number of mitochondria which can be obtained from a 6 mm biopsy is $2.4 \times 1010 \pm 0.1 \times 1010$
- Currently, the storage of mitochondria is impossible and it must be injected immediately after isolation.

Cytotechnology (2019) 71:647–663


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