Introduction

- Kidney transplantation is currently the preferred treatment for patients with end-stage kidney disease (ESKD).
- long-term kidney allograft survival has been limited by chronic immune-mediated rejection due to both cellular and humoral pathways, innate immune factors and possibly other pathways.
- Immune therapy in solid organ recipients is the reduction of immunosuppressive treatment and defining more individualized immunosuppressive protocols rather than tolerance.

History

- Since the discovery in 1969 of the suppressor T cells, the regulatory T cells (Tregs) research field has undergone an incredible boom over the years.
- The breakthrough in this field dates back to the discovery, in 1995, of a subset of thymus derived CD4+ T cells expressing high levels of **IL-2Ra (CD25)** able to protect thymectomized mice from autoimmunity.
- Tregs have a crucial role in maintaining immune homeostasis and preventing autoimmunity

Nishizuka Y, Science, 1969
Sakaguchi S, Journal Immunology, 1995

Types of Tregs: Phenotyping

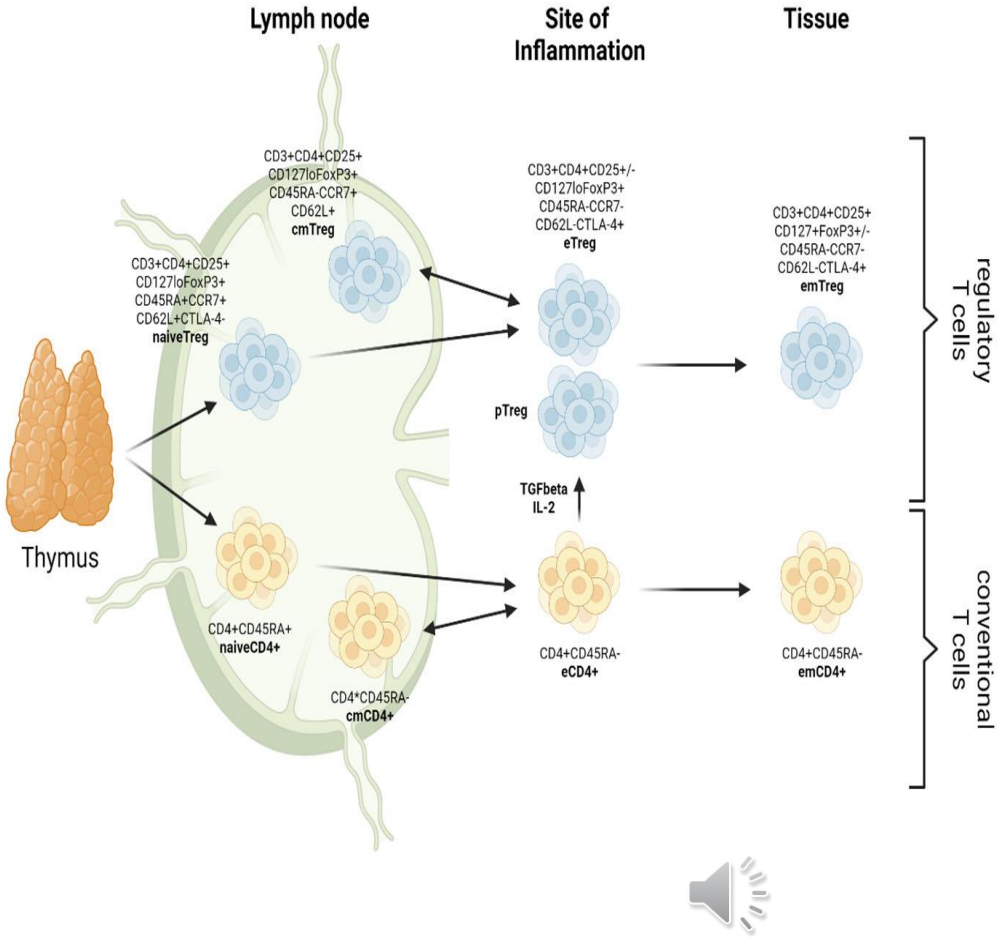
- **$CD4^+$ Treg cells :**

1. High levels of the IL-2 receptor α chain CD25
2. Transcription factor Foxp3, methylation shows a stable form.
3. Surface marker **CD127** is inversely correlated with Foxp3 expression
4. Other markers

CD45RA	**Naïve cell**	CD103	Effector and memory cells
CD45RO	Memory cell	GITR	Costimulatory
ICOS	Production of IL-10 or TGF-β	CD31	Adhesion and transmigration
CD39/73	cAMP- or adenosine-mediated suppression	CD147	Activated Treg
LAP	LAP/TGF-β complex in activated cells	Activated Treg	Migrate to the graft
CD161	Proinflammatory potential	CTLA-4	Coinhibitory molecule
CD71	Activated Treg	CD137	Antigen-specific T cells
GARP	Binds to TGF-β in activated cells	CD49d	Part of lymphocyte homing receptor, useful for purification Tregs with CD127
CD62L(low)	Naïve cells/ effector and memory cells		Clin J Am Soc Nephrol. 2018 Nov 7; 13(11): 1760

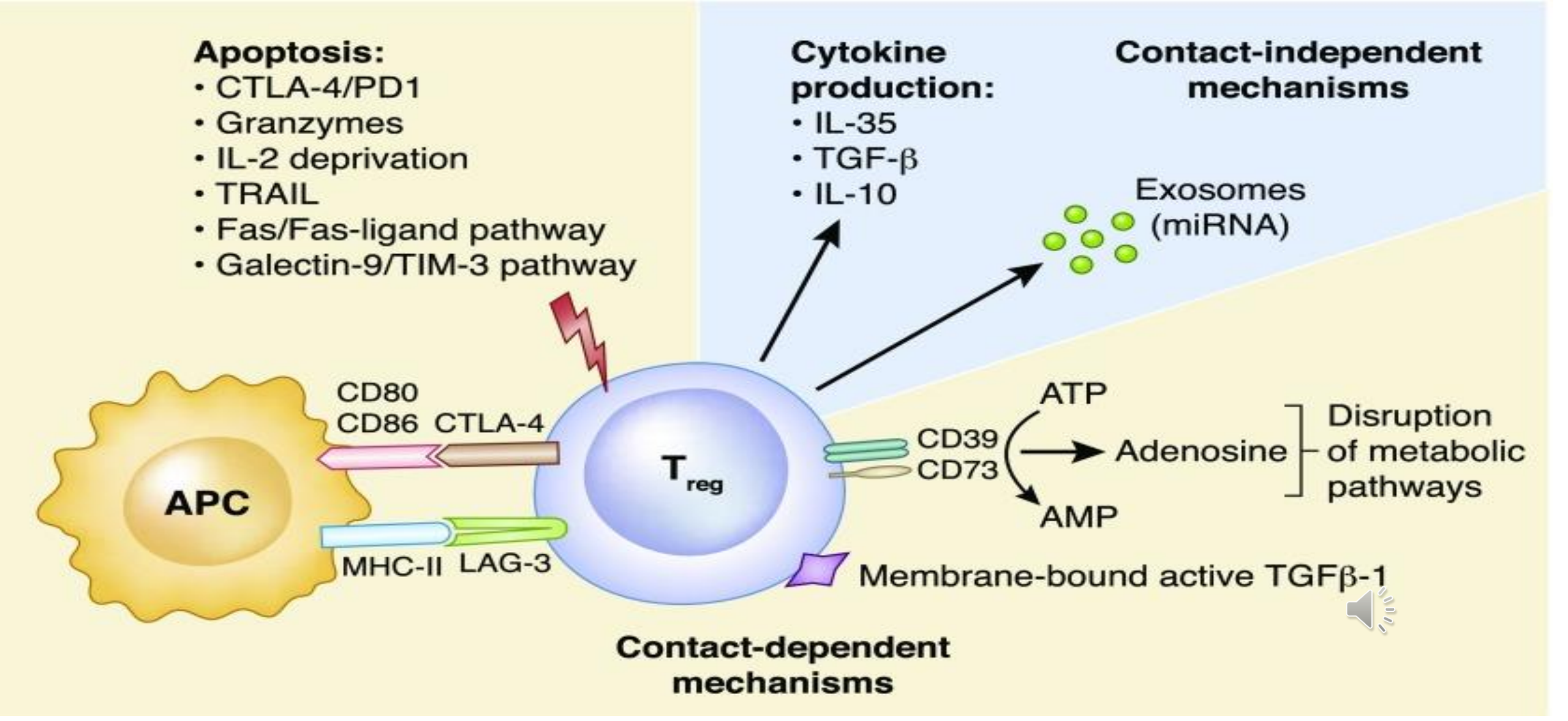
Differentiation dynamics and plasticity of (regulatory) T cells

- Regulatory T cells are broadly classified as thymus derived (natural) Tregs, or peripheral inducible Tregs.
- Inducible Tregs can be generated from natural Tregs or naïve $CD4^{+}CD25^{-}$ cells upon T cell receptor stimulation in the presence of cytokines such as TGF-*β* and IL-2
- Memory Treg subdivided into central memory (cmTregs) and effector memory with a long lifespan.
- $CD4^{+}CD25^{+}FOXP3^{+}$ Tregs constitute 5%–10% of all circulating $CD4^{+}$ cells

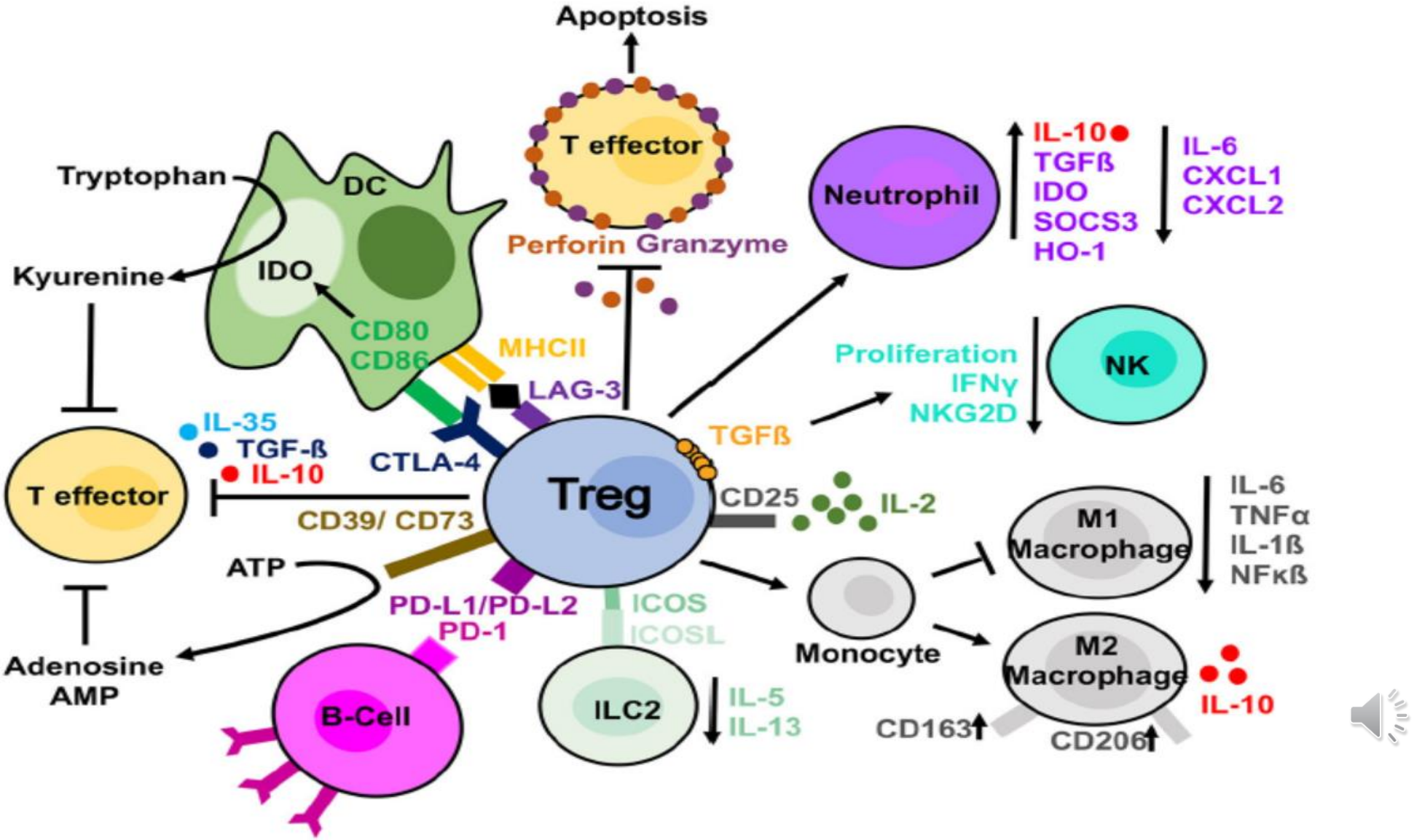


Nina Pilat, Int. J. Mol. Sci. 2023, 24, 1752

Mechanism of Actions, Interactions with Other Cell Types



Treg suppressive mechanisms



The Effects Of Immunosuppressive Drugs On Tregs

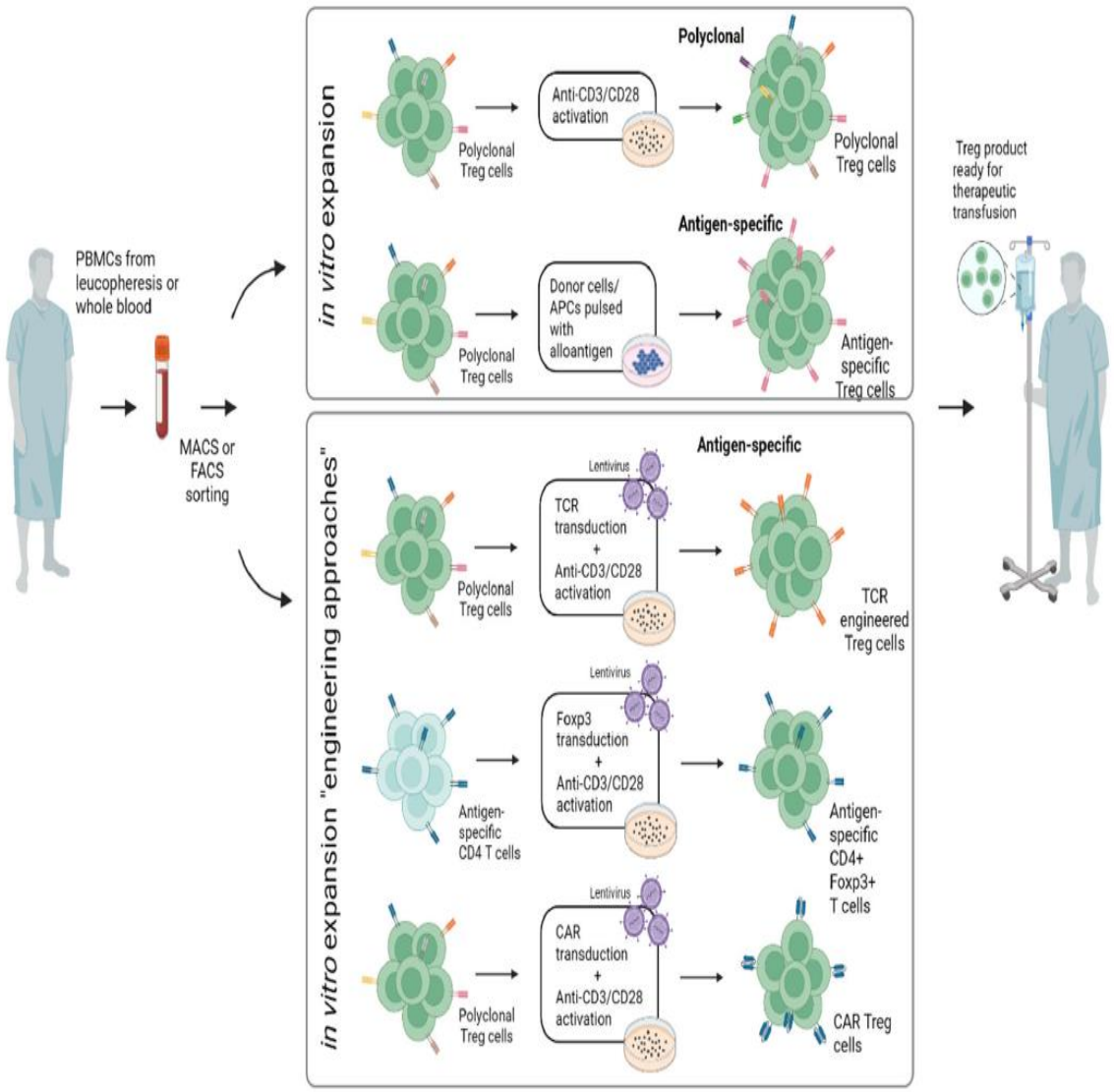
Calcineurin inhibitors	Decreases treg viability and proliferation
Mycophenolic acid	Variable
Glucocorticoids	No effect
Mtor inhibitors	Promote the differentiation and expansion of tregs , increase foxp3 expression
Thymoglobulin	Shift the treg to T effector ratio
Alemtuzumab	Generation/expansion of tregs
Anti-cd25 (basiliximab)	Deleterious effect on treg populations
Costimulatory molecule blocker belatacept	Reduce their number
Metformin	Phosphorylation of p-stat5 and FOXP3, increase treg populations
Erythropoietin	Inhibit proliferation of conventional T cells, while simultaneously sparing treg proliferation
Low-dose recombinant IL-2	Potential means of expanding tregs, risk of stimulating natural killer cell activity

Sources of human Treg cells

- ✓Peripheral blood
- ✓Umbilical cord blood (UCB)
- ✓Treg cells can also be isolated from discarded thymuses removed during paediatric cardiac surgery.

Approximately 300 × 106 CD4+CD25+ Treg cells can be isolated from thymuses from one donor. paediatric thymuses may be an attractive source of non-autologous Treg cells.

Different approaches for in vitro expansion of Tregs.

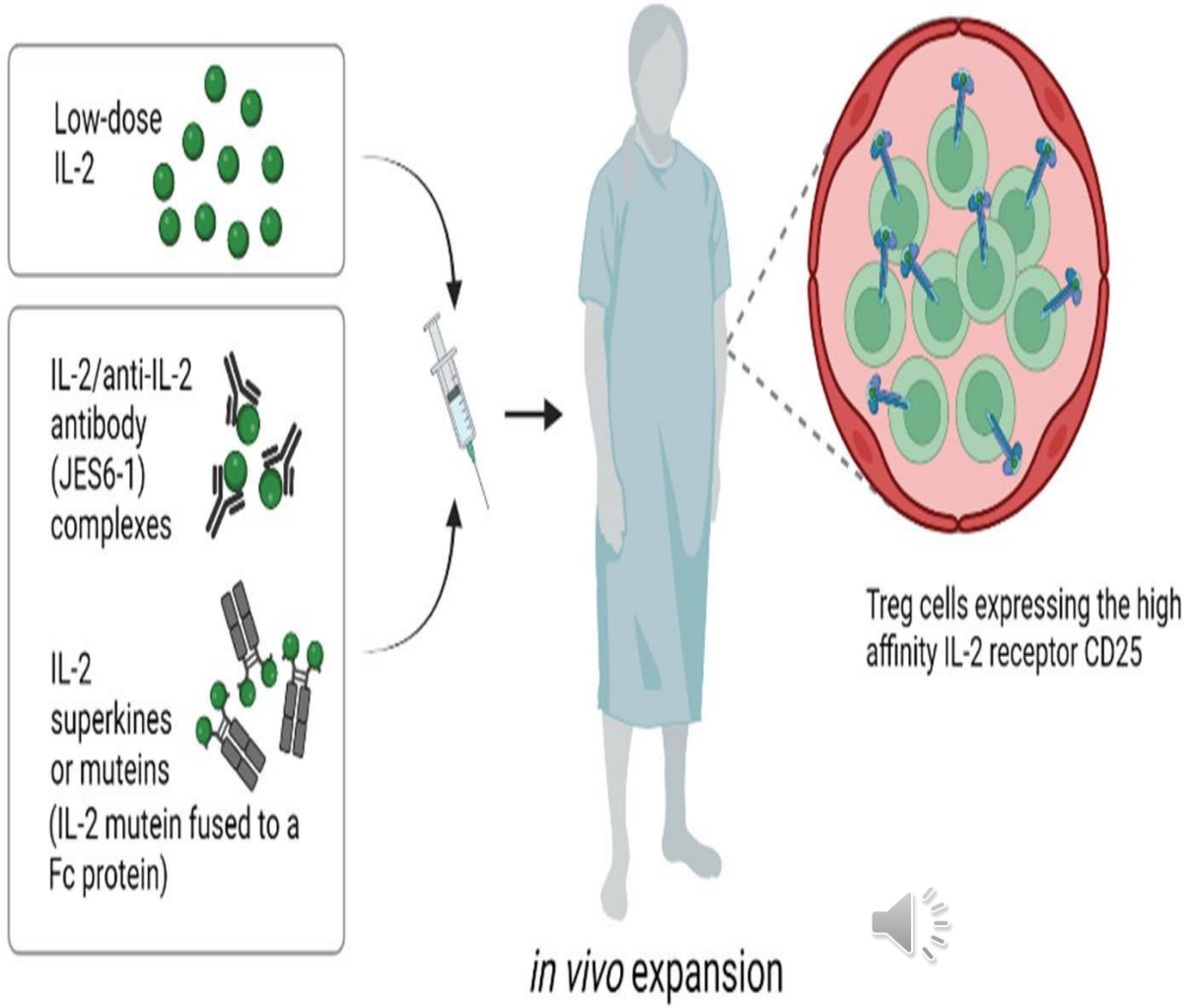


- Polyclonal stimulation via CD3 and CD28 (mostly coated on plates or expander beads)
- Antigen-specific Treg: enriched by in vitro coculture with donor cells/APCs pulsed with donor.
- Transduce normal Tregs with a donor-specific transgenic TCRs.
- Prepare Tregs from antigen specific normal Foxp3- CD4 T cells by transducing these cells with Foxp3.
- Cells engineered to express a CAR treg.

Pilat et al, International Journal of Molecular Sciences, 2023

Approaches for in vivo expansion of Tregs.

- Expanding Treg in vitro is time consuming and demanding.
- Injection of IL2 increases the number of Tregs, however low half life and also increase another tcells and can cause rejection
- Complex of IL2, IL-2/mab complexesTregs, JES6-1, high-affinity IL-2R receptor, i.e., Tregs, but not typical CD8 cells and NK cells.
- Genetic engineering to prepare IL-2 “superkines” or “muteins” that bind selectively to certain components of the IL-2R.



Pilat et al, International Journal of Molecular Sciences, 2023

Treg Cell-based Therapies In Clinical Trials

- Graft-versus-host disease (GvHD)
- Solid Organ Transplantation
- Hematopoietic stem cell transplantation
- Autoimmune diseases

Clinical Immunology (2009) 133, 22–26

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

RAPID COMMUNICATION

First-in-man clinical results of the treatment of patients with graft versus host disease with human ex vivo expanded CD4+CD25+CD127− Tregulatory cells

Piotr Trzonkowski [a,*,1], Maria Bieniaszewska [b,1], Jolanta Juścińska [c], Anita Dobyszuk [a], Adam Krzystyniak [a], Natalia Marek [a], Jolanta Myśliwska [d], Andrzej Hellmann [b]

They concluded that the adoptive transfer of expanded Tregs might be a good option as an adjuvant therapy in chronic and acute GvHD

- Background: The low number of Tregs in bone marrow transplantation is associated with GVHD.
- Source donors, ex vivo proliferation
- A Caucasian woman, aged 44, underwent BMT due to myelodysplastic syndrome in 2005. At the day +137, developed symptoms GVHD, In May 2008, adoptive transfer of Treg, improvement in chronic GVHD
- A Caucasian male, aged 40, with blast crisis in the course of chronic myelocytic leukemia. GVHD day 22, day 75 treg infused and slight decrease in lab tests.

HEPATOLOGY

HEPATOLOGY, VOL. 64, NO. 2, 2016

A Pilot Study of Operational Tolerance With a Regulatory T-Cell-Based Cell Therapy in Living Donor Liver Transplantation

Satoru Todo,[1*] Kenichiro Yamashita,[1*] Ryoichi Goto,[2] Masaaki Zaitsu,[2] Akihisa Nagatsu,[2] Tetsu Oura,[2] Masaaki Watanabe,[2] Takeshi Aoyagi,[2] Tomomi Suzuki,[2] Tsuyoshi Shimamura,[3] Toshiya Kamiyama,[2] Norihiro Sato,[4] Junichi Sugita,[5] Kanako Hatanaka,[6] Hisashi Bashuda,[7] Sonoko Habu,[7] Anthony J. Demetris,[8] and Ko Okumura[7]

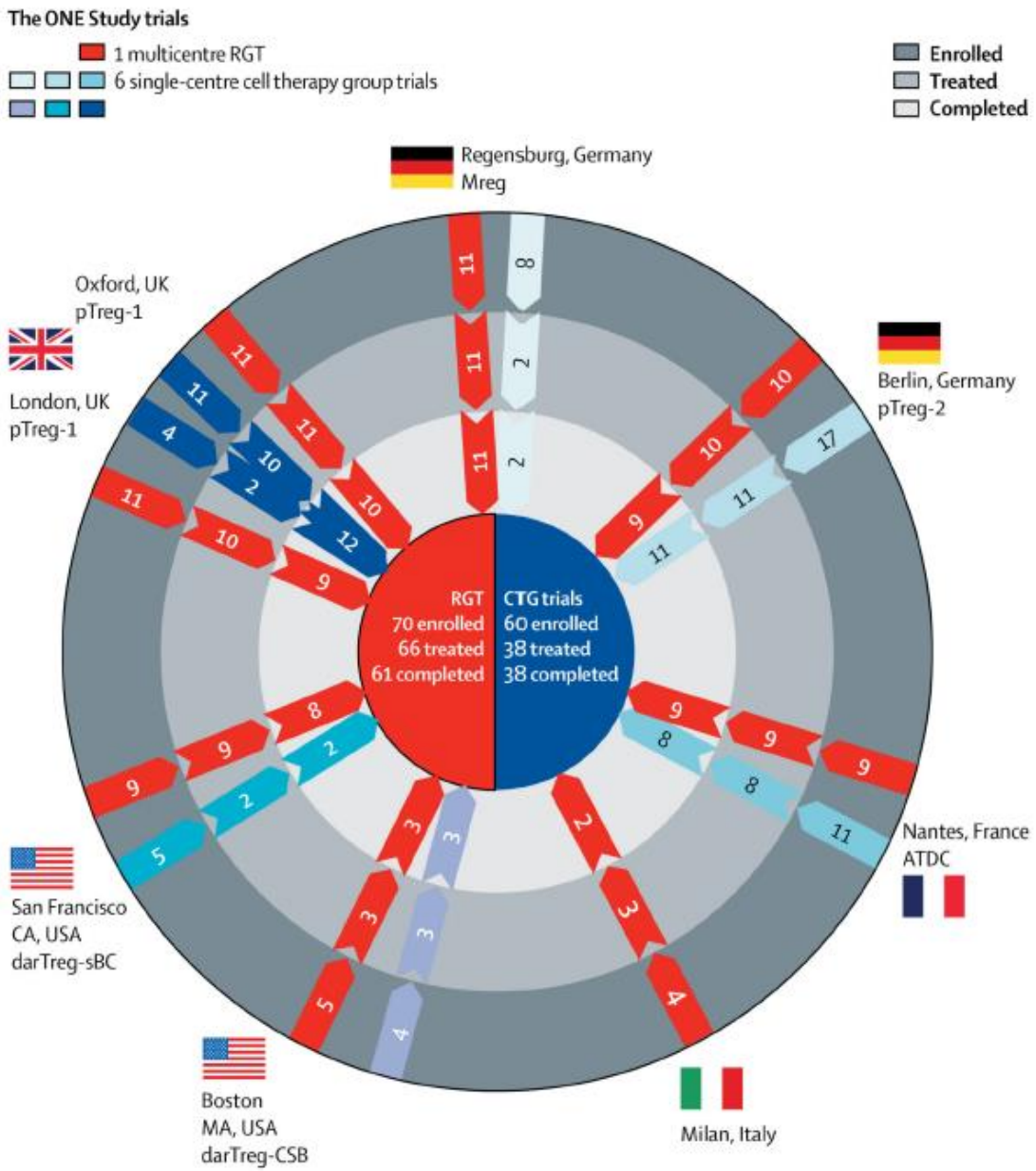
Conclusion: A cell therapy using an ex vivo-generated regulatory T-cell-enriched cell product is safe and effective for drug minimization and operational tolerance induction in living donor liver recipients with nonimmunological liver diseases.

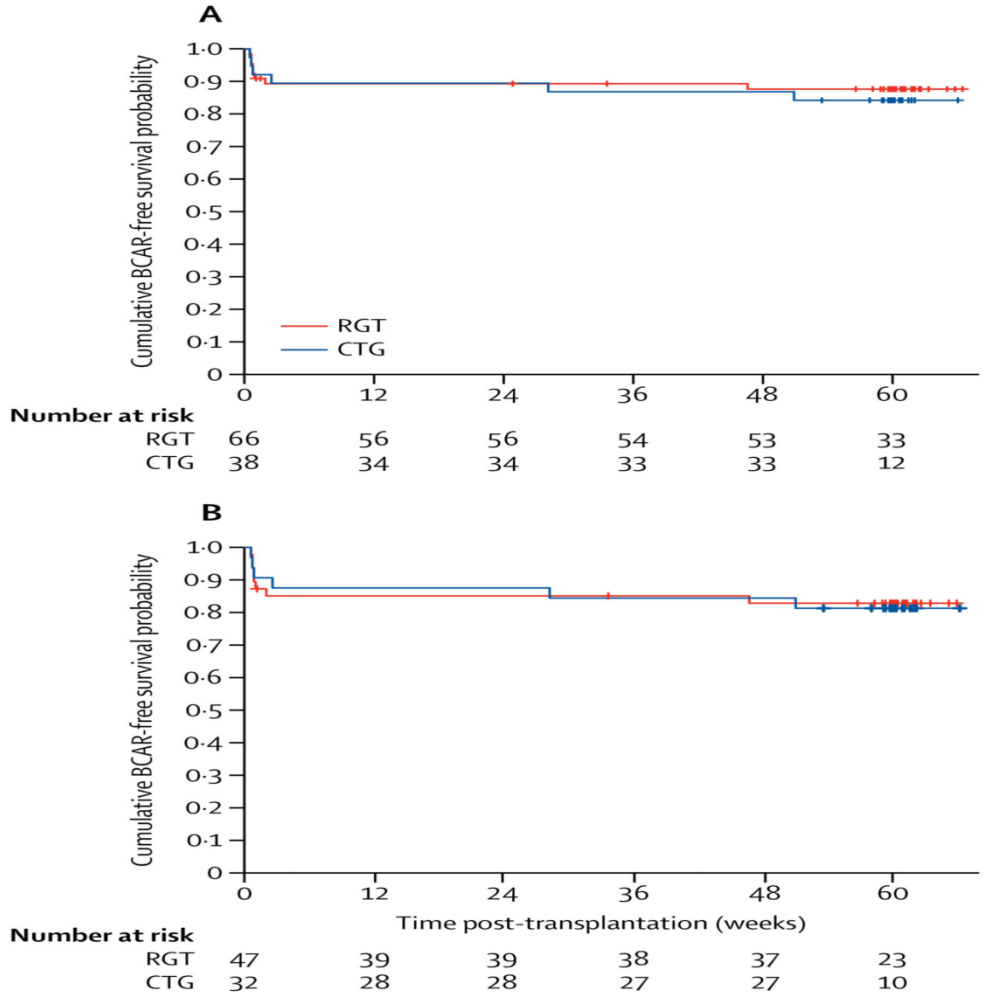
- Adoptive transfer of an ex vivo-generated regulatory T-cell-enriched cell product was conducted in 10 consecutive adult patients early post-LT
- Cells were generated using a 2-week coculture of recipient lymphocytes with irradiated donor cells in the presence of anti-CD80/86 monoclonal antibodies
- Immunosuppressive agents were tapered from 6 months, reduced every 3 months, and completely discontinued by 18 months.
- Seven patients have completed successful weaning and cessation of immunosuppressive agents. At present, they have been drug free for 16-33 months; 4 patients have been drug free for more than 24 months. The other 3 recipients with autoimmune liver diseases developed mild rejection during weaning and then resumed conventional low-dose immunotherapy.

Regulatory cell therapy in kidney transplantation (The ONE Study): a harmonised design and analysis of seven non-randomised, single-arm, phase 1/2A trials

- The ONE Study: seven investigator-led, single-arm trials done internationally at eight hospitals in France, Germany, Italy, the UK, and the USA.
- Patients: living-donor kidney transplant recipients aged 18 years and older, from, Dec 11, 2012, and Nov 14, 2018, 60 weeks followup
- Reference group trial :Basiliximab, tapered steroids, MMF, Tacrolimus
- Six non-randomised phase, unmasked, 1/2A cell therapy group : One of six containing regulatory T cells, dendritic cells, or macrophages
- Immunosuppression: Except basiliximab induction, cell therapy and MMF tapering
- Primary endpoint: Biopsy confirmed acute rejection

Lancet. 2020 May 23; 395(10237): 1627–1639





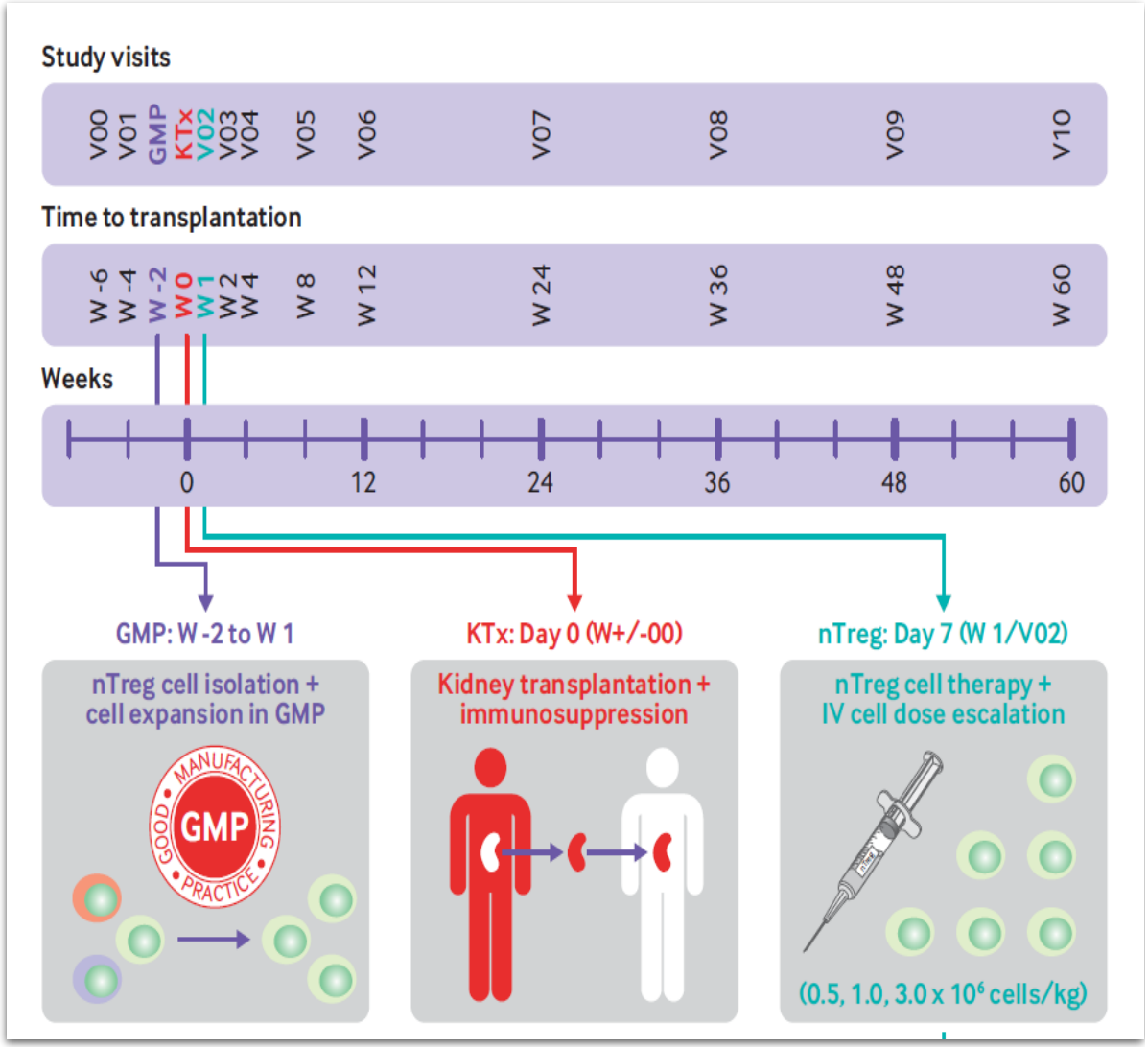
Conclusion: Regulatory cell therapy is achievable and safe in living-donor kidney transplant recipients, and is associated with fewer infectious complications, but similar rejection rates in the first year.

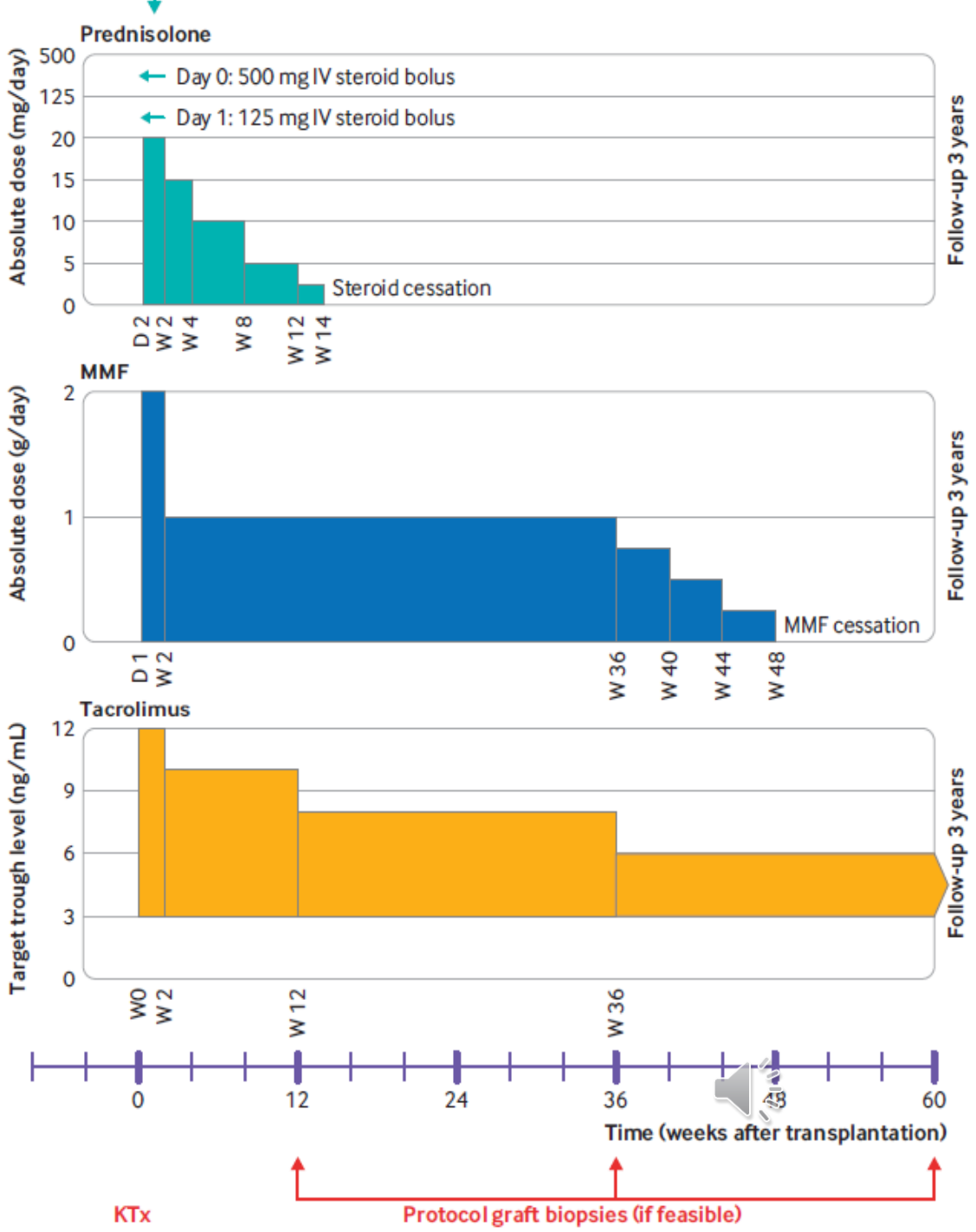
Regulatory T cells for minimising immune suppression in kidney transplantation: phase I/IIa clinical trial

- Recipients of living donor KTx ,(ONEnTreg13, n=11) and corresponding reference , group trial (ONErgt11-CHA, n=9).
- CD4+ CD25+ FoxP3+ nTreg products were given seven days after kidney transplantation
- Endpoint: Primary, safety consisting of adverse infusion related effects, infections, acute rejection, and graft function or failure, secondary, safe tapering immunosupression 48 weeks after Tx.
- Isolation and expansion of **autologous polyclonal nTregs** with good purity and sufficient yield from 40-50 mL of peripheral blood taken two weeks before kidney transplantation and infused after one week post Tx.

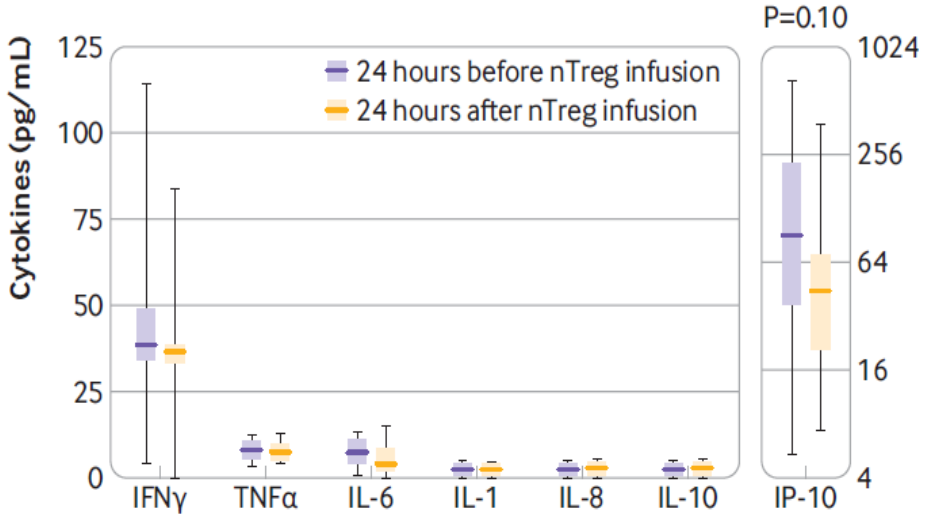
Andy Roemhild et al. BMJ 2020, 371

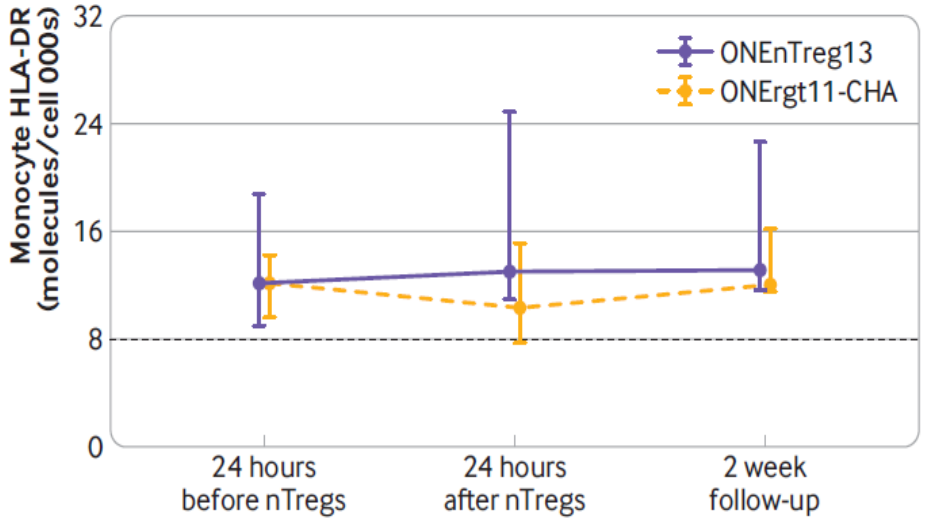
ONE study ONEnTreg13 clinical trial design

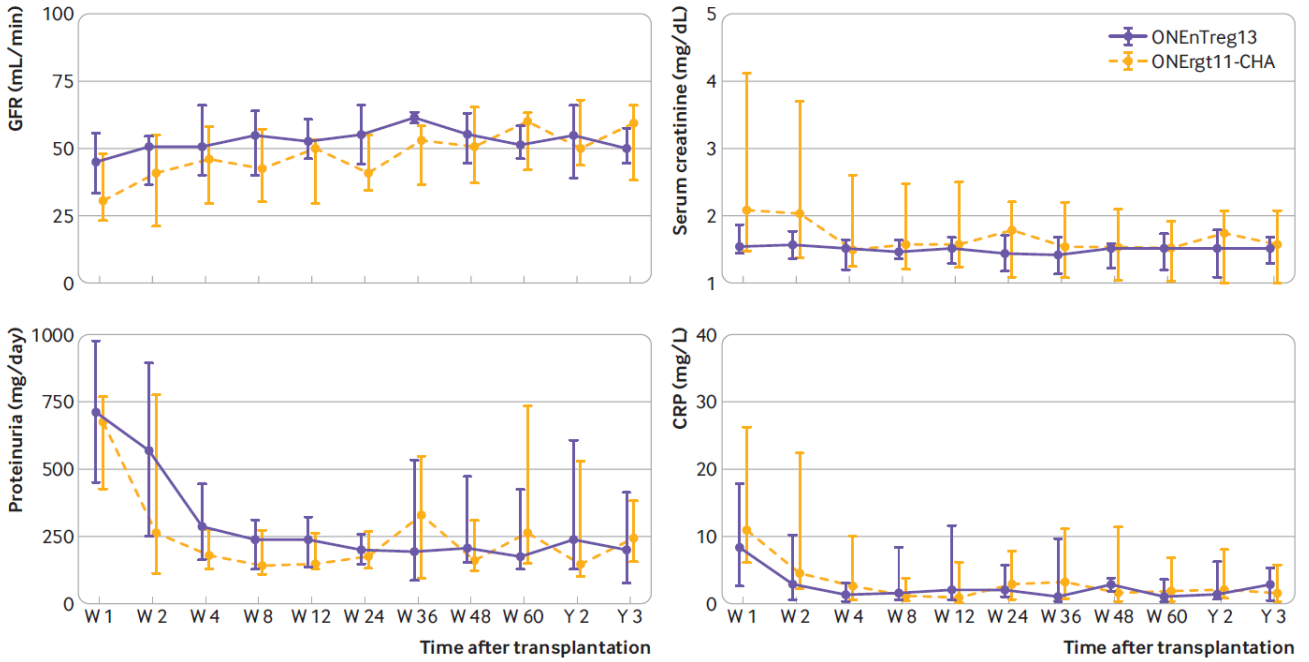




Immune Complications After Infusion & Long Term Follow-up Of Renal Allograft Function







The application of autologous nTregs was safe and feasible even in patients who had a kidney transplant and were immunosuppressed. These results warrant further evaluation of Treg efficacy and serve as the basis for the development of next generation nTreg approaches in transplantation and any immunopathologies.

Transplantation Without Overimmunosuppression (TWO) study protocol: a phase 2b randomised controlled single-centre trial of regulatory T cell therapy to facilitate immunosuppression reduction in living donor kidney transplant recipients

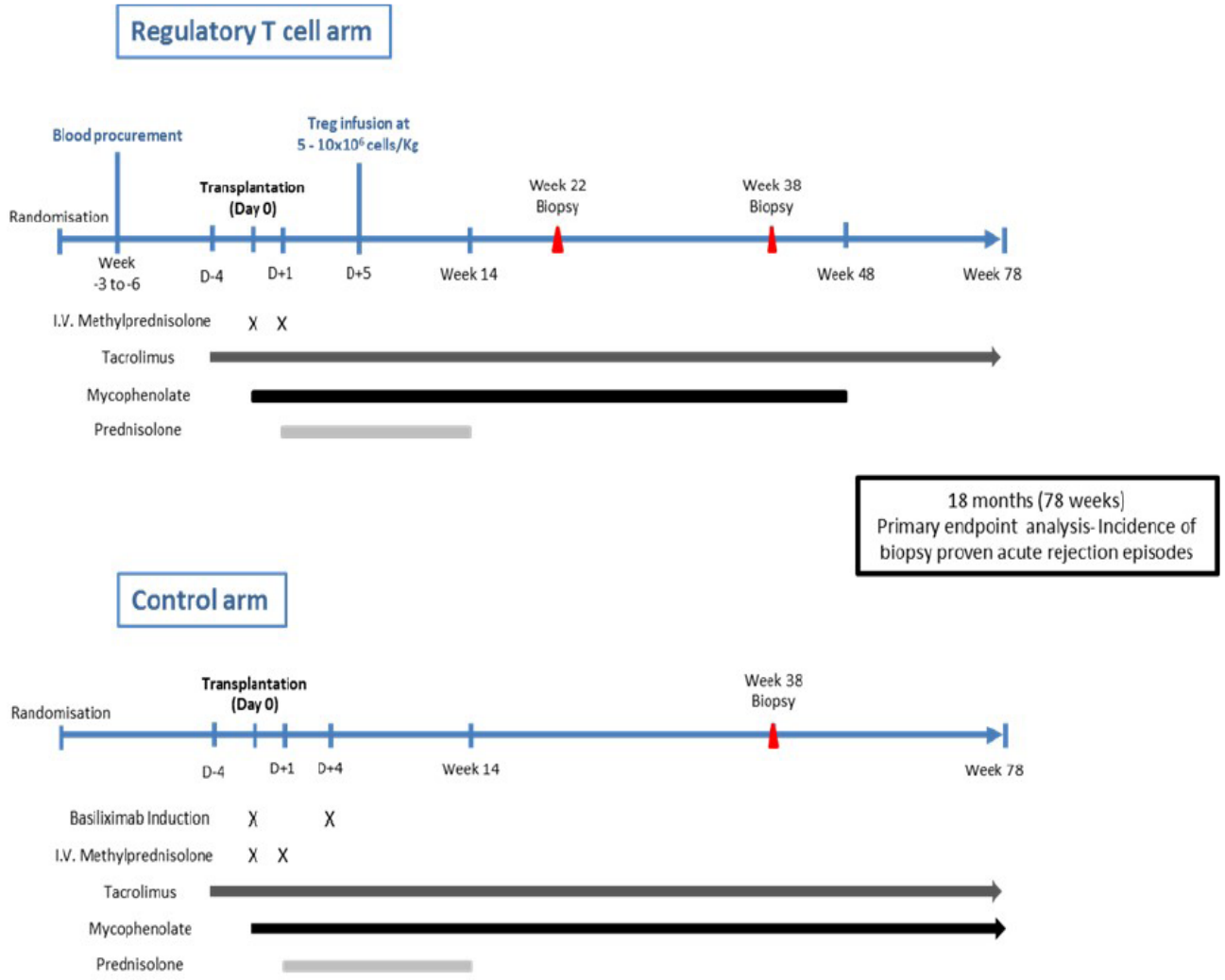
Matthew Oliver Brook ,[1,2] Joanna Hester,[1] William Petchey,[2] Ines Rombach,[3] Susan Dutton,[3] Matthew James Bottomley,[1,2] Joanna Black,[3] Seetha Abdul-Wahab,[1,4] Andrew Bushell,[1] Giovanna Lombardi,[4,5] Kathryn Wood,[1] Peter Friend,[1,2] Paul Harden,[2] Fadi Issa[1]

Introduction:Regulatory T cell (Treg) therapy has been demonstrated to facilitate long-term allograft survival in preclinical models of transplantation and may permit reduction of immunosuppression and its associated complications in the clinical setting

- **Methods:** Phase IIb, 60 patients, RCT, 1:1, single center, living donor Tx
- Treg therapy (TR001) vs. standard clinical care (control).
- **TR001 arm** :Infusion of autologous polyclonal ex vivo expanded Tregs **,**5 days after Tx, no monoclonal Ab induction.
- **Control participants**: receive a standard basiliximab-based immunosuppression regimen with long-term tacrolimus and MMF
- **Primary endpoint:** biopsy proven acute rejection over 18 months.
- **Secondary endpoints**: immunosuppression burden, chronic graft dysfunction and drug-related complications.

BMJ, 2022

Diagrammatic representation of the immunosuppressive regimen used in The TWO study



Regulatory T cell arm: 370 mL of whole blood a minimum of 3 weeks prior Tx

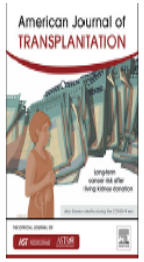
Autologous Treg product, negative selection of CD8+ cells, and positive selection of CD25+ cells resulting in enrichment of CD4+CD25+FOXP3+

Treg Polyclonal: three rounds of stimulation with anti-CD3 and anti-CD28 bead stimulation in the presence of IL-2**,** **Rapamycin** is added to the culture to promote Treg stability

Contents lists available at ScienceDirect

American Journal of Transplantation

journal homepage: www.amjtransplant.org



Original Article

Donor antigen-specific regulatory T cell administration to recipients of live donor kidneys: A ONE Study consortium pilot trial

Eva C. Guinan [1,†], Laura Contreras-Ruiz [1,†], Kerry Crisalli [2], Charles Rickert [2], Ivy Rosales [2], Robert Makar [3], Robert Colvin [2], Edward K. Geissler [4], Birgit Sawitzki [5], Paul Harden [6], Qizhi Tang [7], Giles Blancho [8], Laurence A. Turka [2], James F. Markmann [2,*]

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[4] University Hospital Regensburg, Department of Surgery, Regensburg, Germany
[5] Institute of Medical Immunology, Virchow – Klinikum, Berlin, Germany
[6] Oxford Transplant Centre, Oxford University Hospitals NHS Foundation Trust, Oxford, UK
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[8] Centre of Research in Transplantation and Immunology, Nantes University, Nantes, France

Article in Press

Phase I-II clinical trial

Purified donor antigen reactive (dar)-Tregs

(CD4+CD25+CD127low)

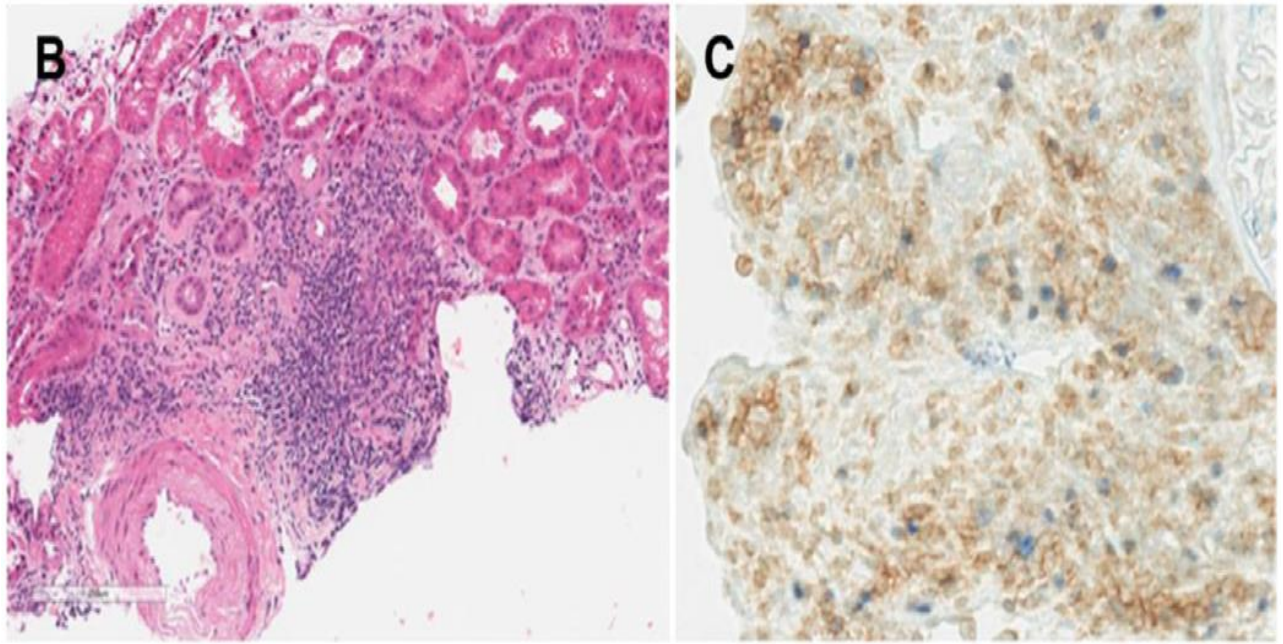
3 patients, 7 to 11 days after live donor renal transplant. Modified immunosuppression regimen, without induction therapy: TAC, MMF, Prednisolone

Prednisolone: stopped 14 weeks after Tx

MMF: stopped 11 -13 months after Tx

dar-Treg infusion after Day 5

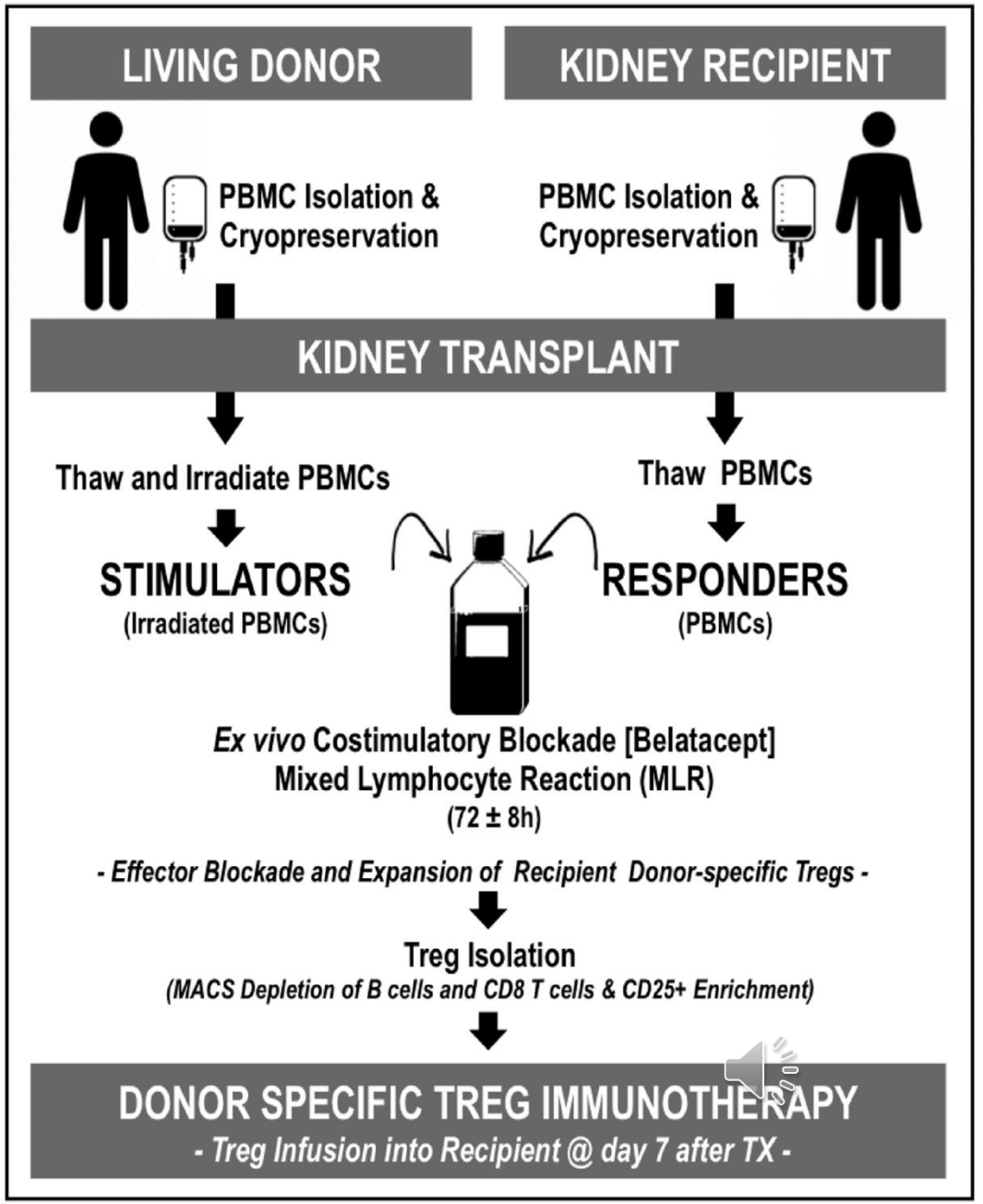
Conclusion: the patients are now all >6 years posttransplant on tacrolimus monotherapy with excellent graft function.



B: High magnification image of a representative infiltrate showing periarterial mononuclear cell aggregates (H&E, 200).

C: The aggregates are enriched in CD4+ T cells of which 27% were Foxp3+

Biopsy: 12 days after Tx

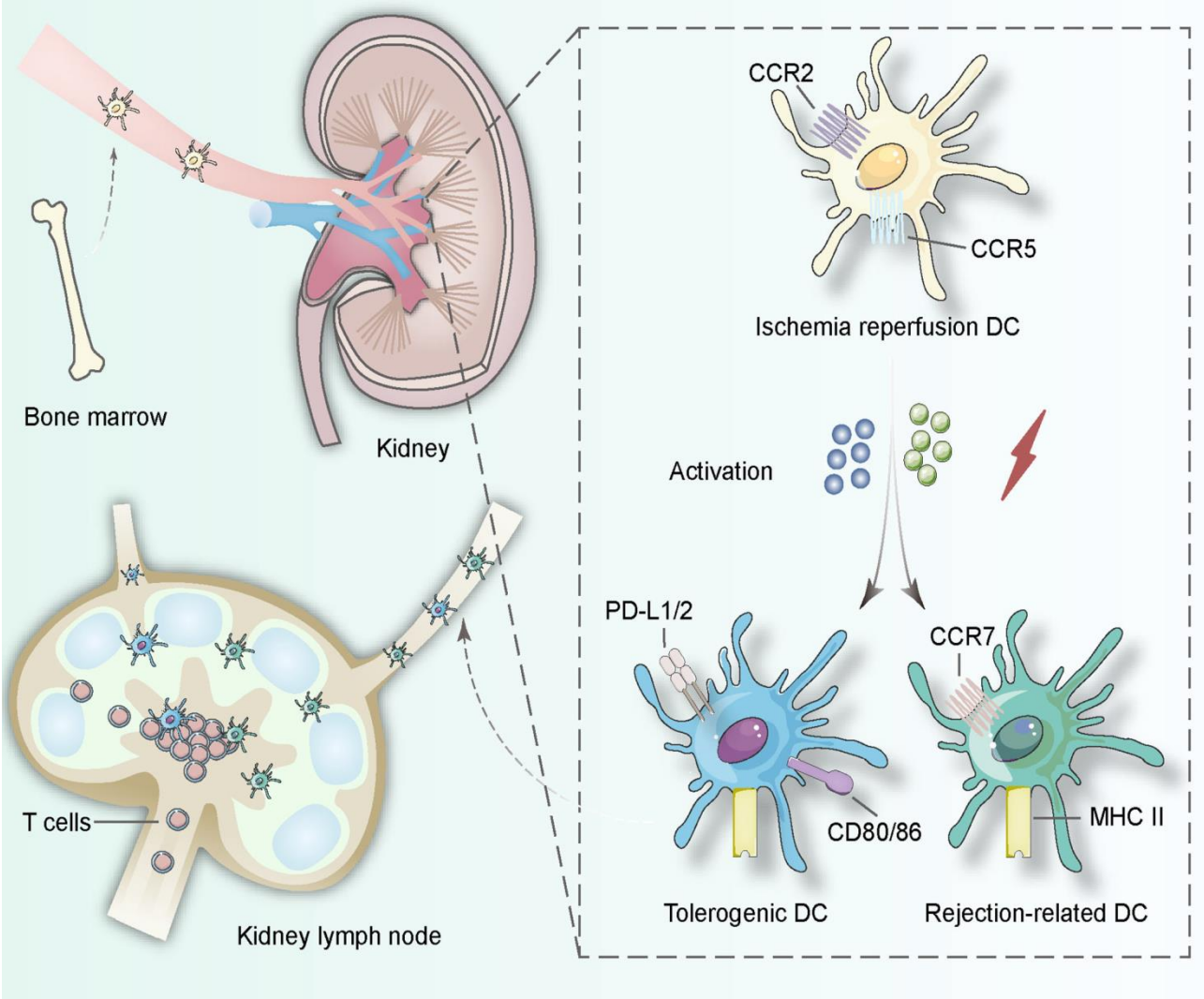


Cell Types With Immunosuppressive Function

- **CD4+FOXP3+ Treg cells**
- CD8+ treg cells
- Il-10-producing type 1 treg cells (TR1 cells),
- Transforming growth factor-β (tgfβ)-producing CD4+ TH3 cells
- Regulatory γδ T cells
- Regulatory B cells (breg cells)
- Myeloid-derived suppressor cells
- Immunosuppressive plasmocytes
- Regulatory invariant natural killer (NK) T cells
- Subsets of innate lymphoid cells

To date, FOXP3+ Treg cells are the only known cell lineage arising in the thymus that is exclusively dedicated to inducing and maintaining immune tolerance.

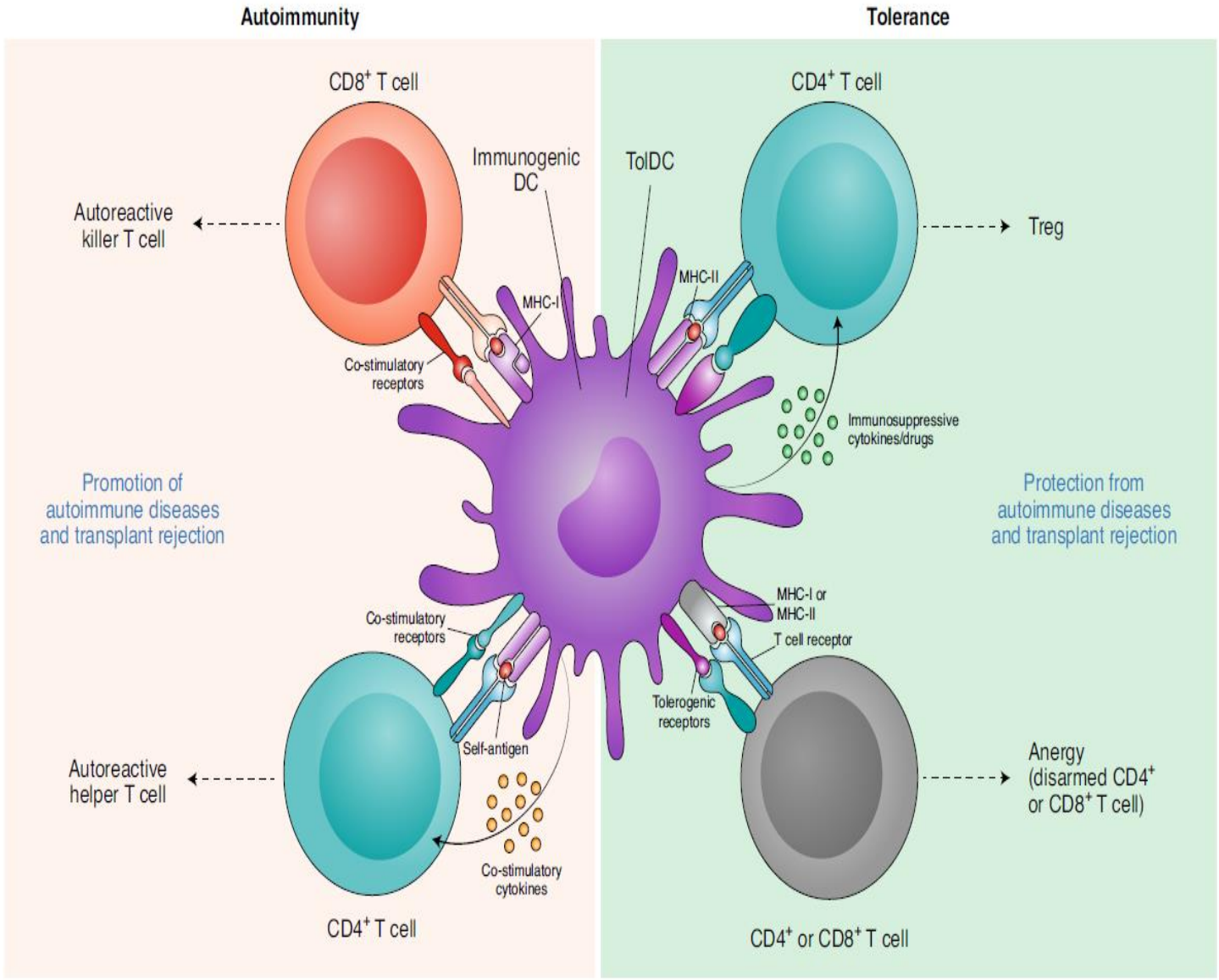
Dendritic Cells: Versatile Players in Kidney Tx



- DCs in the kidney originate from bone hematopoietic stem cells and involve in lymphatic recycling in vivo.
- When ischemia–reperfusion occurs, immature DCs start to search for interactions with T lymphocytes and change their surface proteins including CCR2, CCR5 to induce tolerance procedure (expressing PD-L1/2 and CD80/86) or rejection procedure (expressing CCR7 and MHC class II).
- The activation can be derived from pathogen-associated molecular patterns and danger-associated molecular patterns in the procedure of ischemia-reperfusion.

Jinwen Lin et al , Frontiers in Immunology 2021

Dendritic cell function in autoimmunity versus tolerance



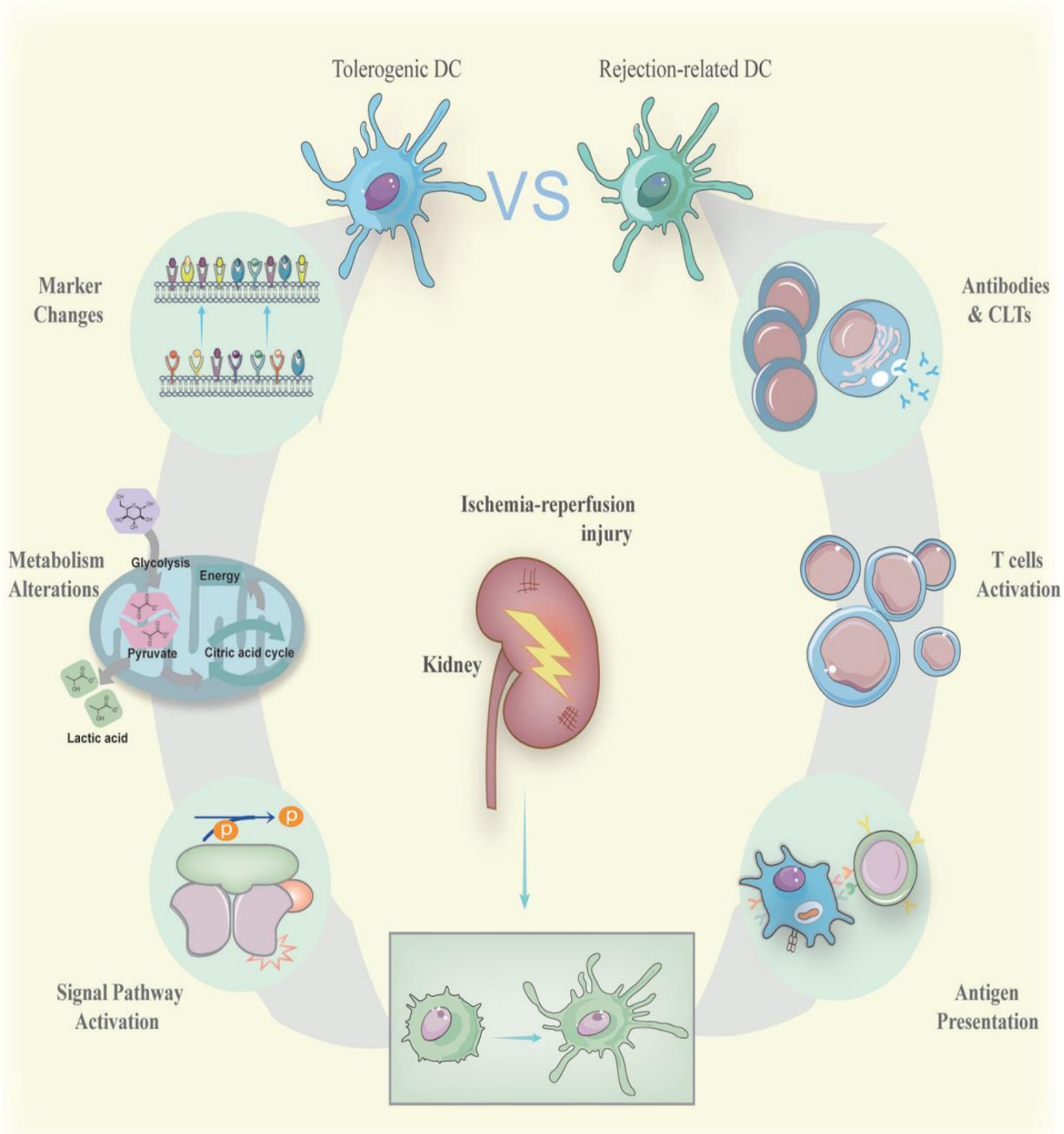
Dendritic cells (DCs) specifically interact with T cells through major histocompatibility complex (MHC)-I and II.

The balance of signalling through co-receptors and cytokines determines the outcome of antigen recognition.

Activated, pro-inflammatory DCs presenting self-antigen can prime auto-reactive killer clusters of differentiation (CD)8+ and CD4+ helper T cells.

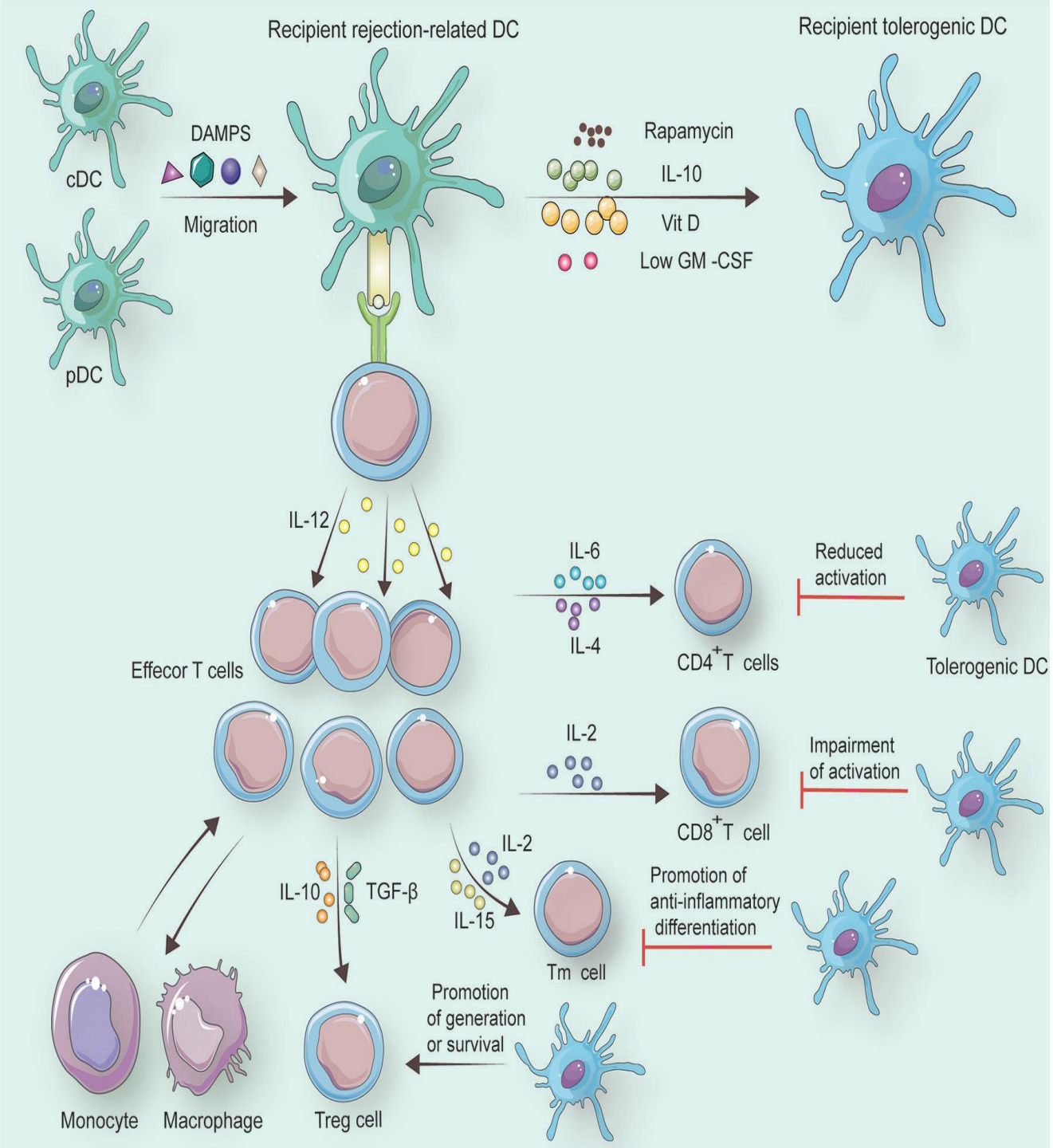
Tolerogenic DCs (tolDCs) express different co-receptors and cytokines, and can induce regulatory T cells (Tregs) (upper right), as well as disarm autoreactive CD4+ and CD8+ T cells.

Anna Cifuentes-Rius , Nature Nanotechnology, 2021



- Immature DCs can be activated by antigens derived from ischemia-reperfusion and act as the role of powerful antigen-presenting cells to trigger antibody-mediated rejection and cell-mediated rejection.
- The result of antibody-mediated rejection is activated B cell releasing harmful antibodies while active cytotoxic T cells kill donor cells forming cell-mediated rejection.
- On the contrary, when treated by specific drugs, immature DCs can also maintain their surface markers to suppress possible inflammation caused by transplantations via signal pathways activation regulating metabolism alterations

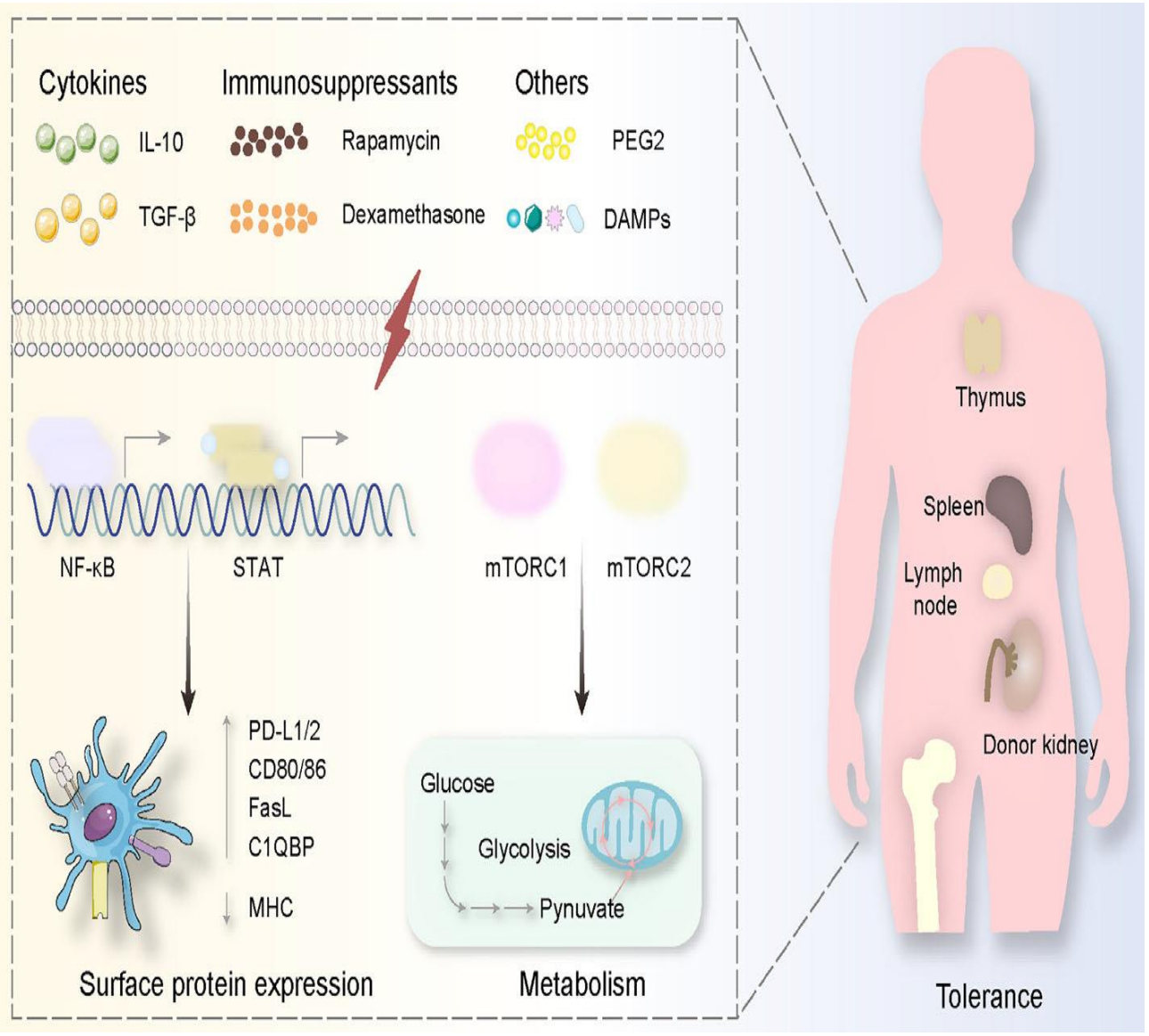
Jinwen Lin et al , Frontiers in Immunology 2021



- In response to specific factors including DAMPs, recipient cDCs and pDCs change into recipient rejection-related DCs.
- If rapamycin, IL-10, Vit D, or alow dose of GM-CSF is employed to treat recipient rejection-related DCs, recipient tolerogenic DCs can be generated.
- Under the control of recipient rejection-related DCs, naive T cells differentiate to CD8+ T cells with the help of IL-2 and differentiate to CD4+ T cells assisted by IL-6 and IL-4.
- Memory T cells (Tm cells) also originate from naïve T cells, and this alteration is associated with IL-2 and IL-15.
- Treg cells can occur when IL-10 and TGF-b are secreted by recipient rejection-related DCs.
- Tolerogenic DCs reduce CD4+ T cell activation, and they can impair active CD8+ T cells.
- Tm cells tend to be anti-inflammatory promoted by tolerogenic DCs. Treg cells survive for a longer period with tolerogenic DCs than with rejection-related DCs.

Jinwen Lin et al , Frontiers in Immunology 2021

Substances generate Tolerogenic DCs



- These stimulations derived from cytokines (IL-10, TGF-b), immunosuppressants (Rapamycin, Dexamethasone), and others (PEG2, DAMPs)
- They mediate signal pathways involving NF-kB and mTOR activtion, causing surface protein expression alterations (highly expressing PD-L1/2, CD80/86, FasL C1QBP while decreasing MHC expression) and metabolism changes (from glucose to pyruvate).
- All these procedures happen in the donor kidney and the immune organs including the thymus, spleen, lymph node, and bone marrow.

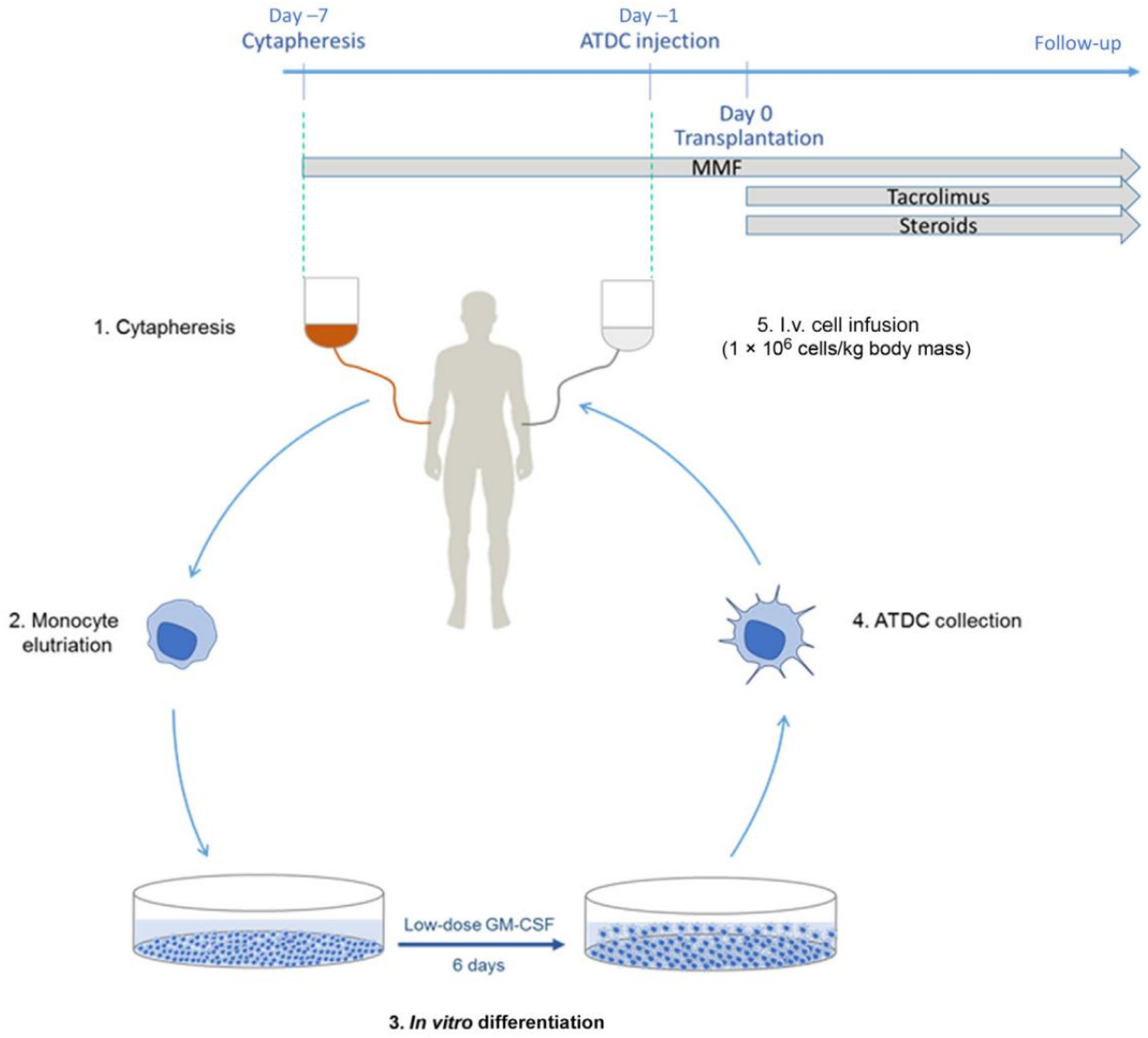
Jinwen Lin et al , Frontiers in Immunology 2021

A Phase I/IIa study of autologous tolerogenic dendritic cells immunotherapy in kidney transplant recipients

- Eight patients received ATDC the day before Tx, with standard steroids, MMF, TAC with an option to taper MMF.
- A control group of nine patients received the same standard immunosuppression, except basiliximab induction replaced ATDC therapy and MMF tapering was not allowed.
- During the three-year follow-up, no deaths occurred and there was 100% graft survival.
- Episodes of rejection were observed in two patients from the ATDC group and one patient from the control group.

Aure´ lie Moreau, Kidney International, 2023

Protocol of autologous tolerogenic dendritic cell (ATDC) generation and administration



- Cytapheresis was performed on recipient 7 days before Tx.
- Monocytes were isolated and differentiated in ATDCs after a 6-day culture with granulocyte-macrophage-colony-stimulating factor (GM-CSF).
- ATDCs were then collected and infused i.v. to patients at a dose of 1 million/kg body mass 1 day before transplantation.
- MMF treatment began 7 days before the surgery, whereas the other treatments (Tacrolimus and Steroids) were administered on day 0.

Aure´ lie Moreau, Kidney International, 2023

Ongoing Clinical Trials Adopting Tregs In Autoimmunity

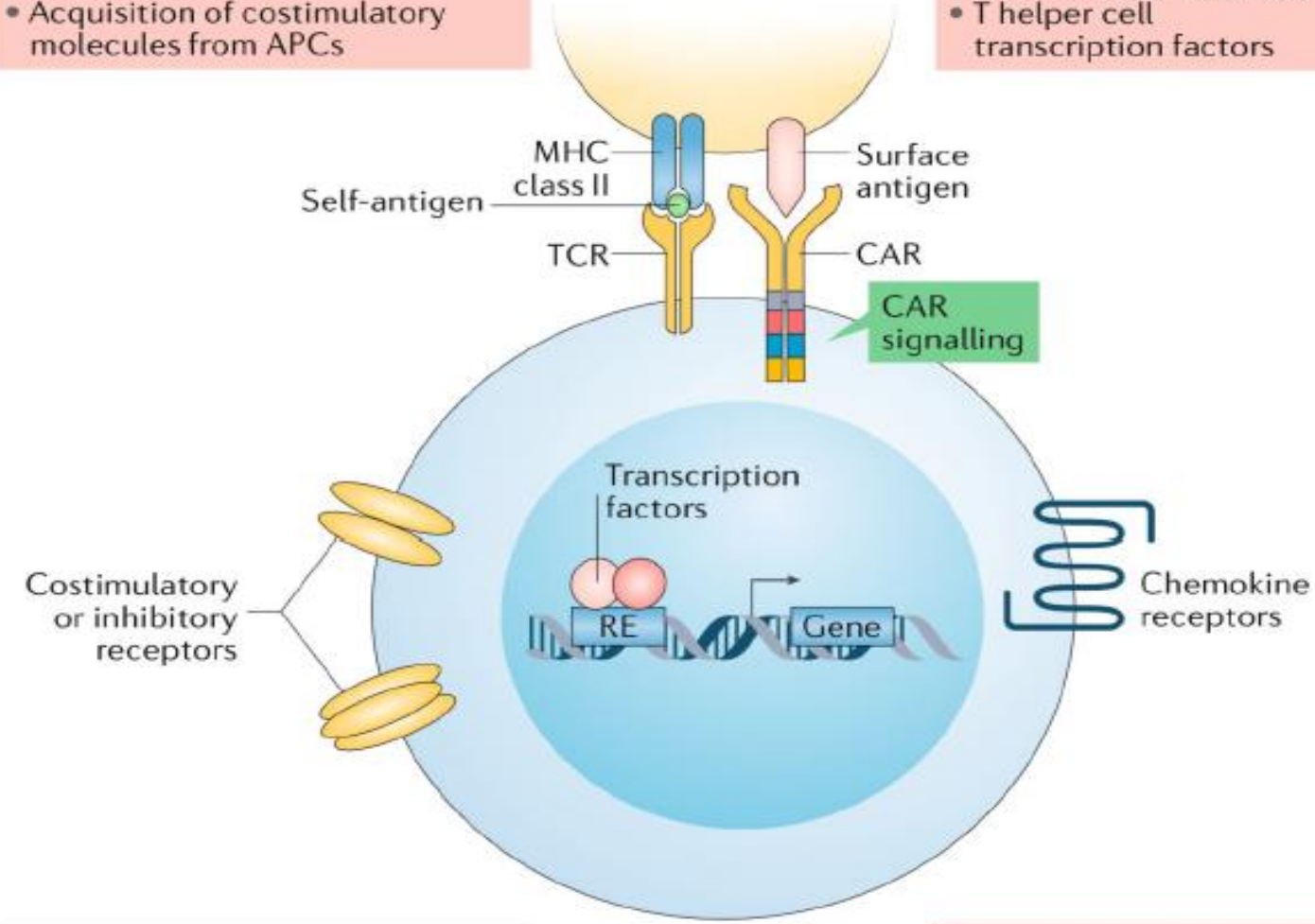
Study ID	Phase	Indication	Enrollment/ Age	Product	Dose	Status
ISRCTN06128462	I	Type 1 Diabetes	12/ range 5–18	Polyclonally expanded tTregs **(A)**	10 and 30 × 10^6/kg	Completed
NCT02691247	II	Type 1 Diabetes	113/ range 8–17	Polyclonally expanded tTregs **(A)**	2.5 and 20 × 10^6/kg	Active, not recruiting
NCT02772679	I	Type 1 Diabetes	16/ range 18–45	Polyclonally expanded tTregs **(A)**	3 and 20 × 10^6/kg	Recruiting
NCT02428309	I	Cutaneous Lupus	NA/ range 18–60	Polyclonally expanded tTregs **(A)**	1, 4 and 16 × 10^8	Active, not recruiting
NCT03239470	I	Pemphigus	12/ range 18–75	Polyclonally expanded tTregs **(A)**	2.5 × 10^8 and 10 × 10^8	Recruiting
NCT03011021	I/II	Type 1 Diabetes	40/ >18	Polyclonally expanded tTregs **(UCB)**	2 × 10^6/kg	Recruiting
NCT02932826	I/II	Type 1 Diabetes	40/ range 6–60	Polyclonally expanded tTregs **(UCB)**	2 × 10^6/kg	Recruiting
NCT02704338	I/II	Autoimmune hepatitis	30/ range 10–70	Polyclonally expanded tTregs **(A)**	10–20 × 10^6/kg	Unknown
NCT03185000	I/II	Crohn's Disease	20/ range 18–80	Polyclonally expanded naive tTregs **(A)**	0.5–1, 3–5 and 8–10 × 10^6/kg	Not yet recruiting

Pilat et al, International Journal of Molecular Sciences, 2023

Regulatory T cells as living drugs

Suppression
- Secretion of inhibitory signals
- Secretion of regulatory cytokines
- Sequestration of IL-2
- Acquisition of costimulatory molecules from APCs

Specificity
- TCR expression
- CAR expression
- Expression of SynNotch
- T helper cell transcription factors



Stability
- Overexpression of FOXP3, HELIOS, BACH2 or STAT5-CA
- Ablation of CHIP, DBC1 or PKCθ
- Cytokine converters

Survival
- Metabolic requirements
- PI3K–AKT signalling
- JNK1 signalling
- Requires exogenous IL-2

Leonardo M. R. Ferreira et al.Nat Rev Drug Discov. 2019, Oct, 18

Conclusion

In conclusion, although current data suggest that Treg therapy alone might be insufficient for the induction of full immune tolerance in transplantation.

There is now optimism that Treg therapy will eventually become a valuable method for substantially reducing the need for continuous immunosuppression in transplant patients