HEIDELBERG UNIVERSITY HOSPITAL

Donor-Derived Modified Immune Cell Therapy in Kidney Transplantation

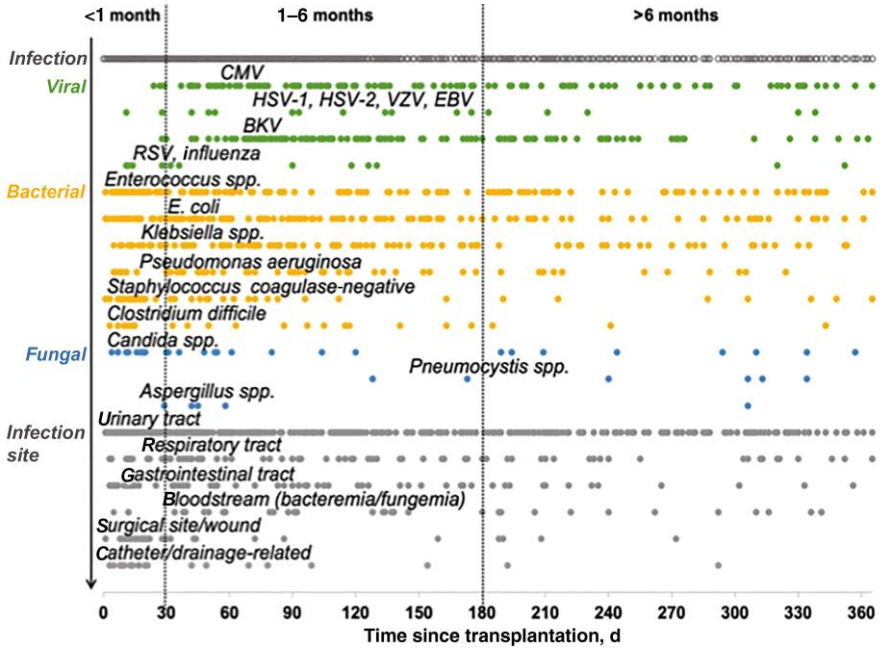
April 19, 2024 Christian Morath

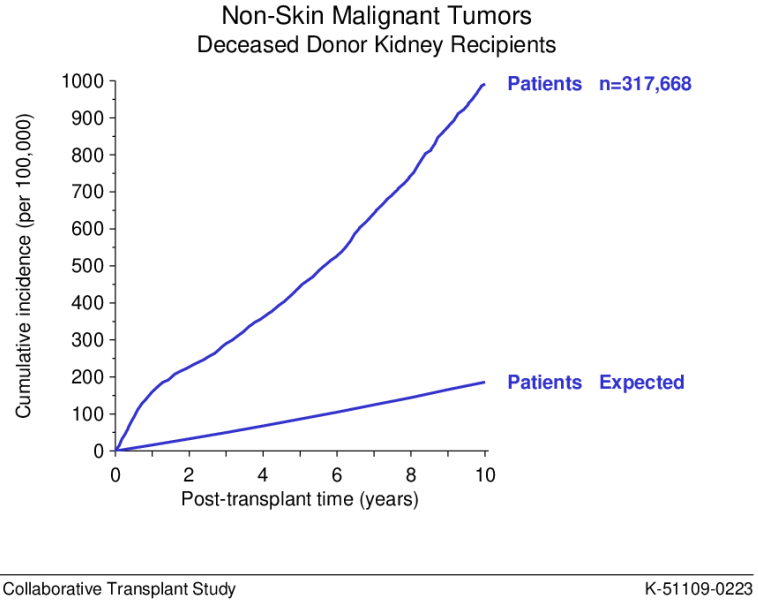
UK HD

Conflict of interest statement

Company association: TolerogenixX GmbH

Other: The authors hold patents and licences for the use of modified immune cell (MIC) therapy in the treatment of transplant patients and autoimmune diseases.

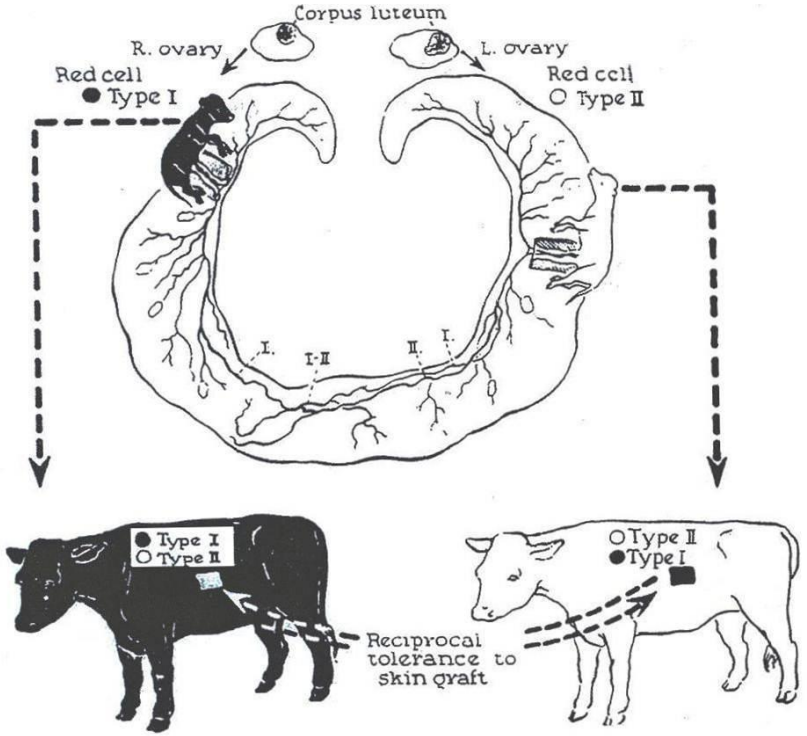




Key problems with conventional immunosuppression

Inadequate prevention of chronic rejection, increased incidence of infectious complications, increased incidence of malignancy

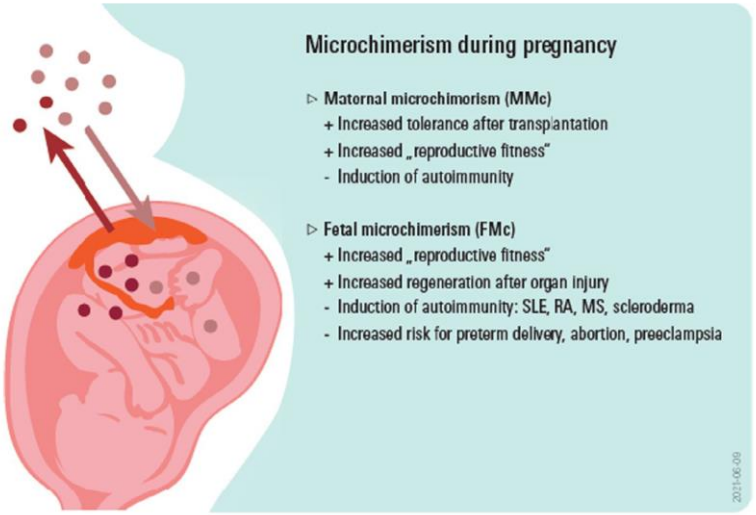
Sommerer (DZIF) *Open Forum Infect Dis 2022,* ctstransplant.org

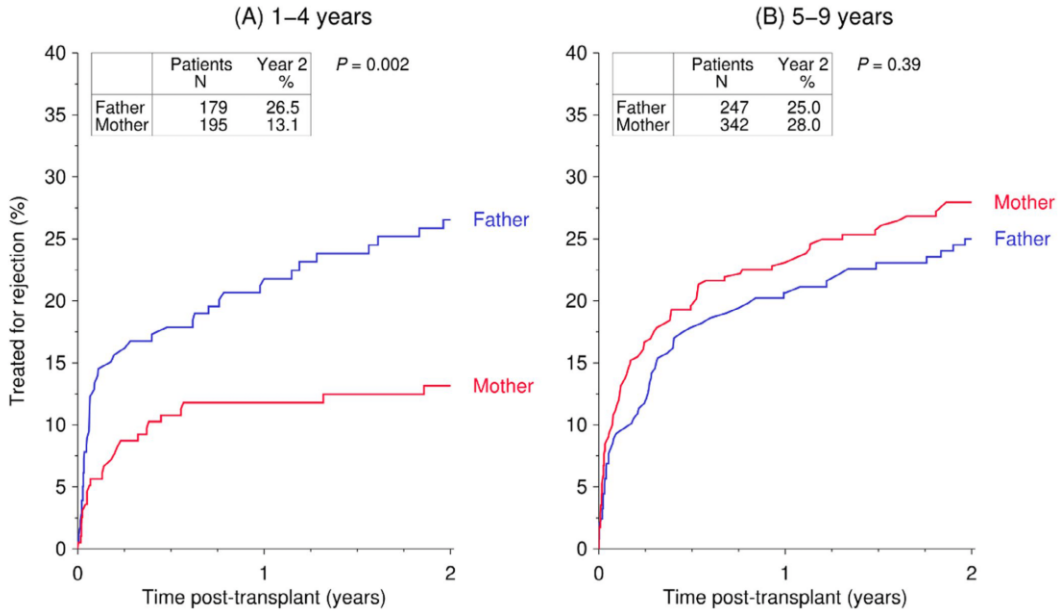


What would the ideal immunosuppression look like?

Learning from nature: illustration of the "natural tolerance" of Freemartin cattle, so-called Freemartinism

https://www.starzl.pitt.edu/transplantation/immunology/chimerism.html





What would the ideal immunosuppression look like?

Microchimerism results in a 50% reduction in rejections requiring treatment due to temporary "tolerance" to NIMAs.

Engels *Pediatr Transplant* 2022

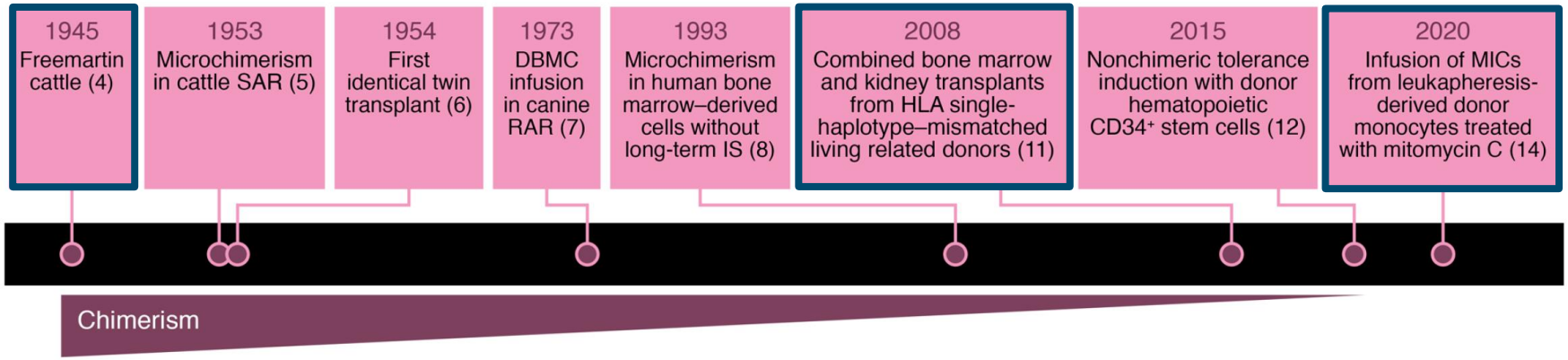


Figure 1. A brief history of time: tolerance from discovery to application. Timeline detailing the evolution of understanding immunologic tolerance and subsequent efforts to achieve it. RAR, renal allograft recipient; SAR, skin allograft recipient.

From Freemartinism to MIC cell therapy

Is permanent chimerism a prerequisite for the successful establishment of immunological tolerance?

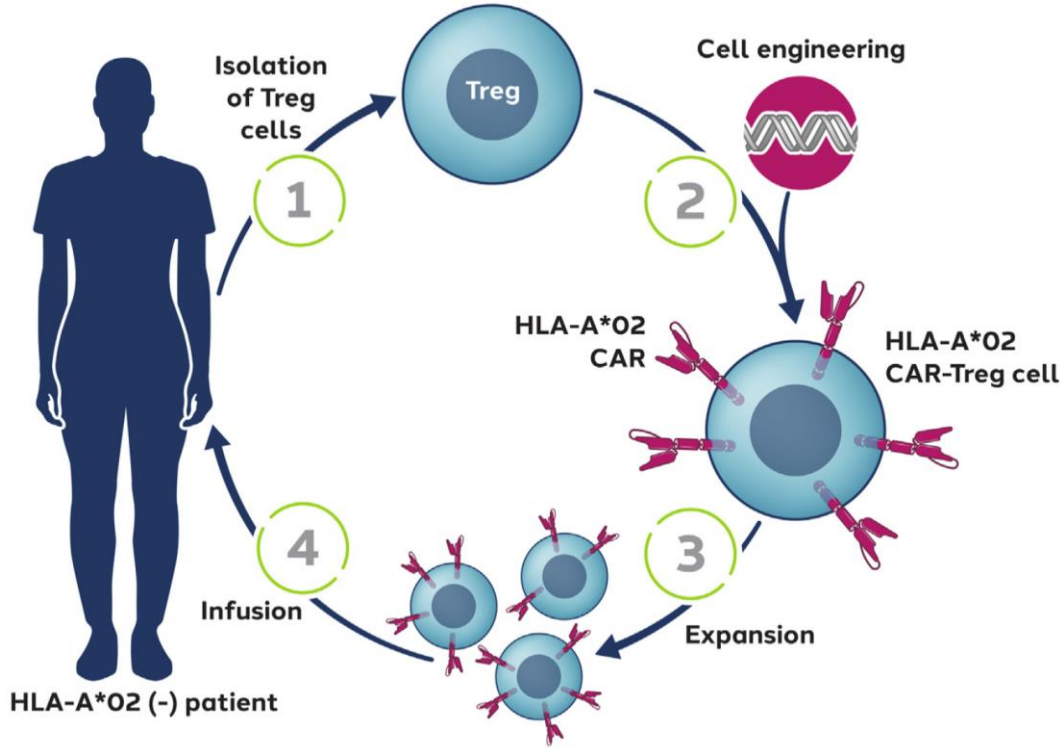
Kant and Brennan *J Clin Invest* 2020

Cell product	Cell type	Clinical findings	Immunological findings	Ref
MDR-101	Enriched $CD34^+$ hematopoietic stem cells and defined dose of $CD3^+$ T cells	Interim analysis of 18 HLA-matched patients: 100% graft survival, no rejection, no DSA, no GvHD, 5 patients w/o IS on day 365	9 patients with mixed chimerism on day 180	12
FCRx	$CD34^+$ hematopoietic stem cells and $CD8^+$/TCR-facilitating cells	Interim analysis of 37 patients: 2 GvHD, 2 graft loss at 1 year, 2 deaths, 22 w/o IS	23 patients with stable chimerism	13
Treg (Berlin)	Autologous polyclonal regulatory T lymphocytes	3-year data: 100% graft survival, 3/11 patients with rejection >BANFF Borderline, 8/11 patients on Tac monotherapy	Less $HLA\text{-}DR^+CD4^+$ T cells, more marginal zone B lymphocytes	15
Treg (London/ Oxford)	Autologous polyclonal regulatory T lymphocytes	4-year data: 100% graft survival, no rejection, 4/12 patients on Tac monotherapy	Less $CD14^{high}CD16^+$ pro-inflammatory monocytes, more $CD4^+CD25^{high}CD127^{low}$ T lymphocytes, more marginal zone B lymphocytes	16
MIC	Allogenic PBMC treated with an alkylating agent	3-year data: 100% graft survival, no rejection, no DSA with LD-CyA, LD-MPA in pat. of group C	Increased $CD19^+CD24^{hi}CD38^{hi}$ transitional B lymphocytes, Reduced antidonor T cell response	20

From Freemartinism to MIC cell therapy

Combined bone-marrow kidney transplantation (MDR-101, FCRx), regulatory T-cell therapies (Treg) and modified immune-cell therapy (MIC)

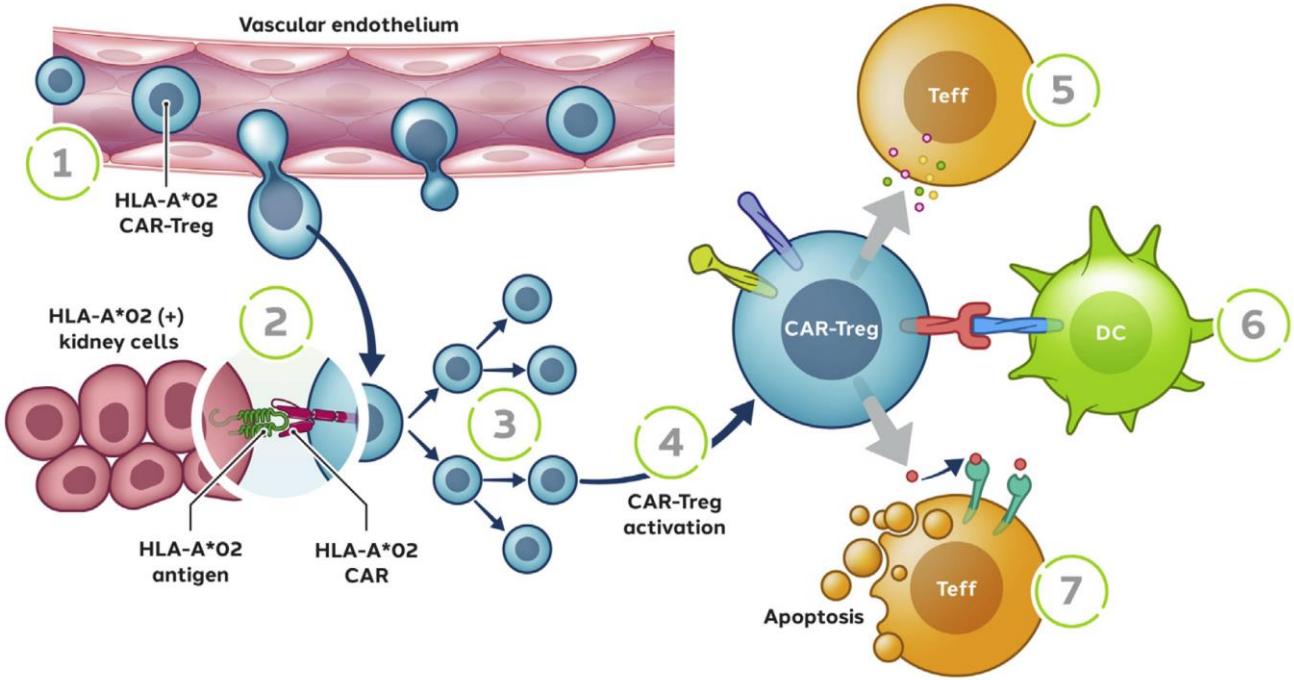
Modified from Morath *Der Nephrologe* 2021



Increasing Treg effectiveness by introducing a CAR

Tregs are isolated, modified by introduction of a HLA-A*02 CAR, expanded and reinfused into the HLA-A*02-negative patient.

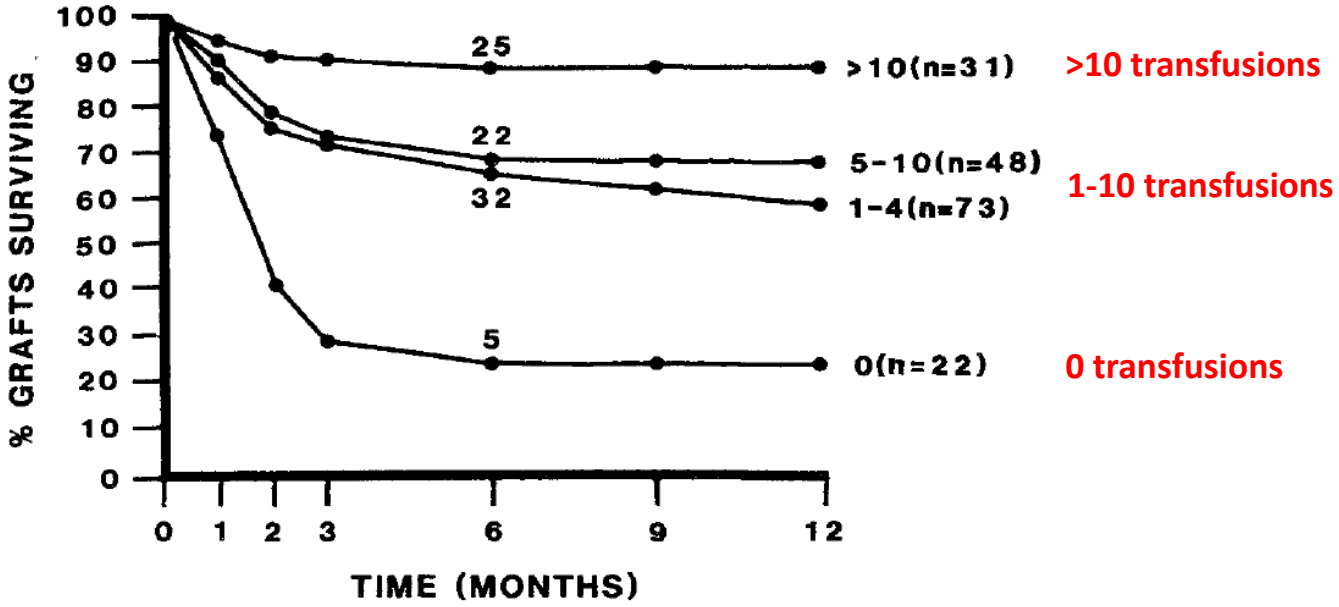
Schreeb *KIReports* 2022



Increasing Treg effectiveness by introducing a CAR

HLA-A*02-negative patient receives HLA-A*02-positive organ, HLA-A*02 CAR-Treg accumulate in the organ bind to the antigen and are activated.

Schreeb *KIReports* 2022



Early clinical observation "*transfusion effect*"

Improved kidney graft survival with increasing number of pre-transplant whole blood transfusions in the pre-cyclosporine era

Opelz *Lancet* 1981

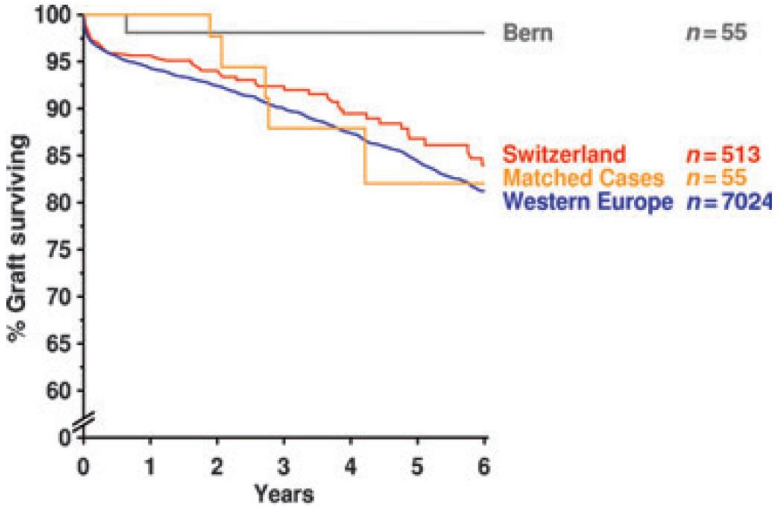


Figure 1 Graft survival after living renal allograft transplantation in patients with donor-specific transfusions (DST) ('Bern') and without DST ('Switzerland', 'Western Europe', 'Matched Cases').

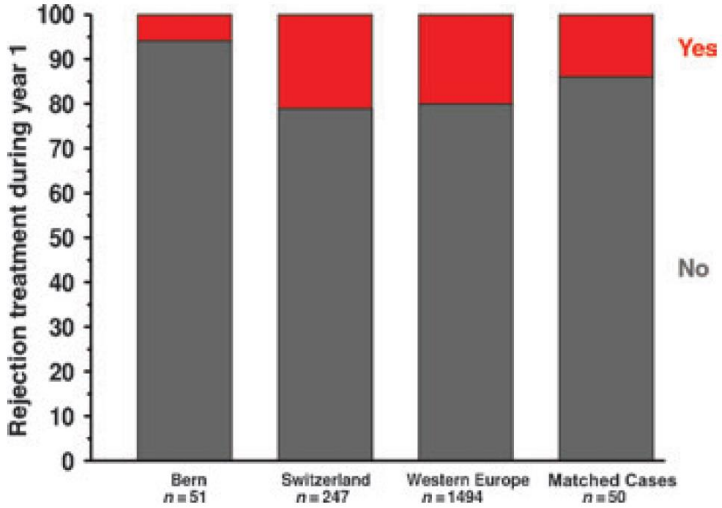
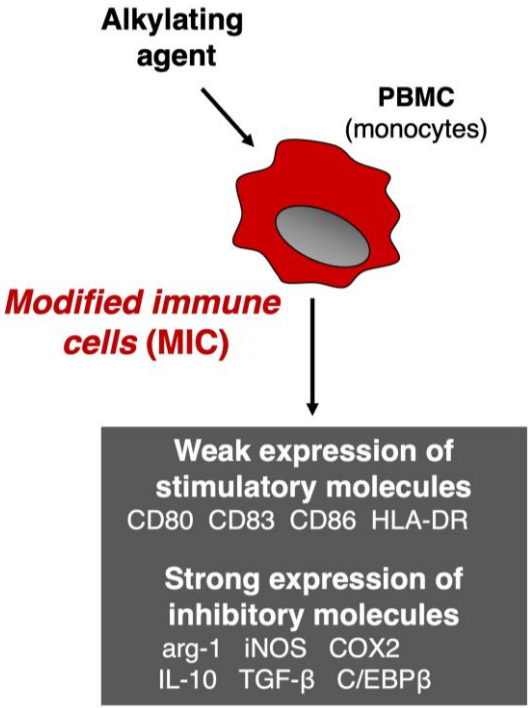


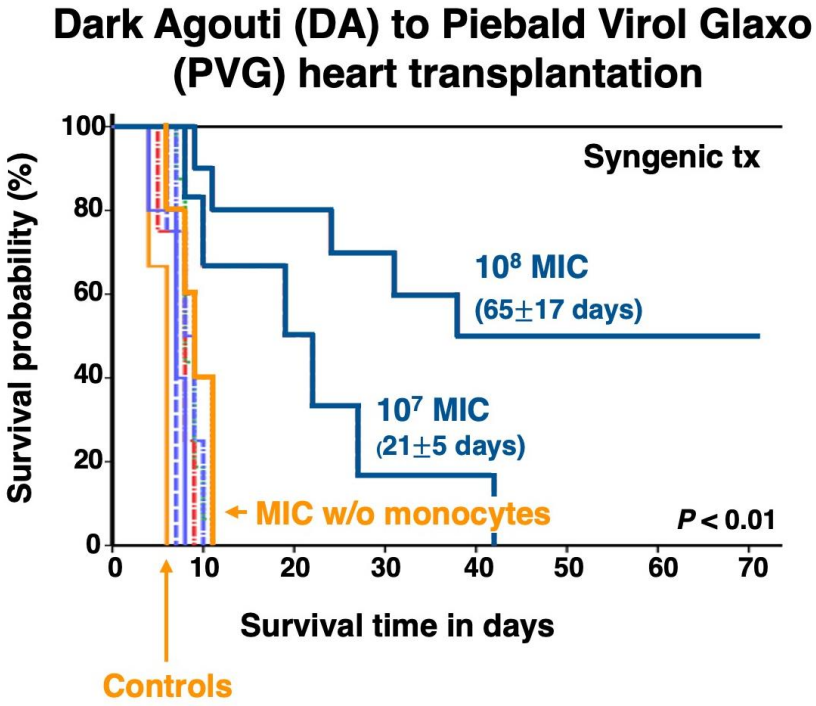
Figure 2 Percentage of patients treated for allograft rejection during the first year after living renal allograft transplantation. Patients were treated with donor-specific transfusions (DST) ('Bern') and without DST ('Switzerland', 'Western Europe', 'Matched Cases').

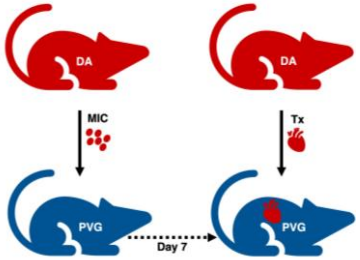
Donor-specific transfusions

1993-2003, N = 61, 2x 200 mL whole blood or mononuclear cells from living donor, 55 transplanted, 6 sensitized (not transplanted)

Marti *Transpl Int* 2006



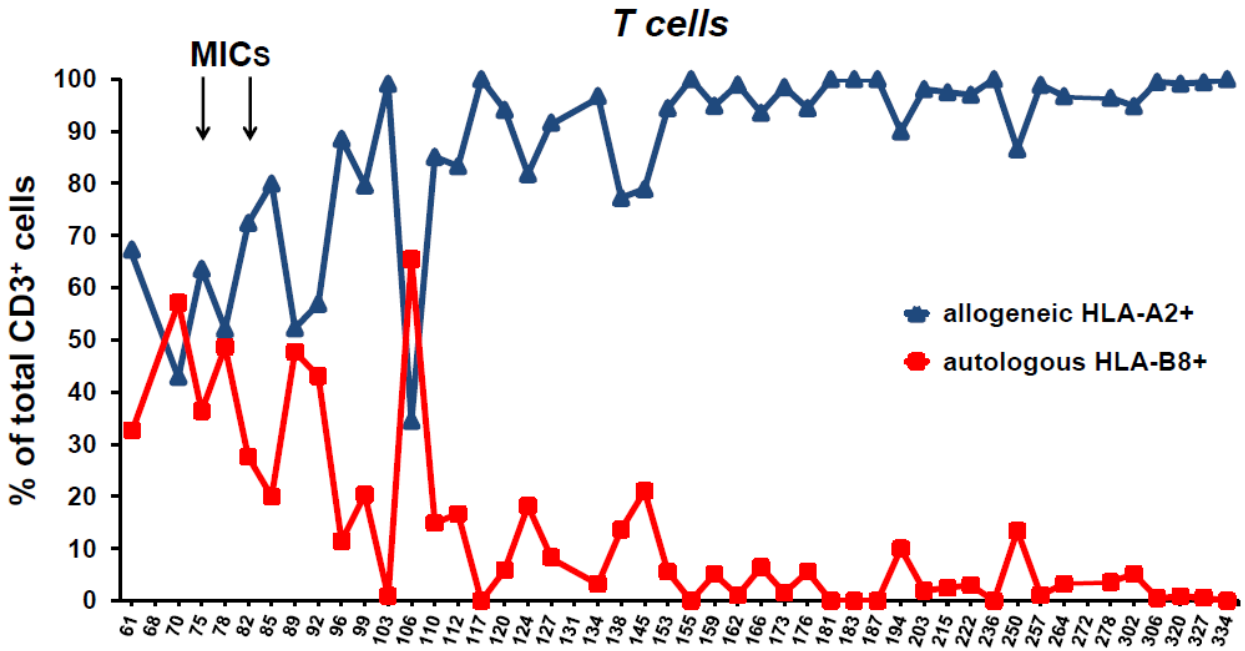




What are MIC (= *Modified Immune Cells*)?

Monocytes (PBMC) that are replication-incompetent after a short treatment with an alkylating agent and have immunosuppressive properties.

Terness *Proc Natl Acad Sci USA* 2008, Kleist *Clin Sci (Lond)* 2015, modified from Morath *Pediatr Nephrol* 2018

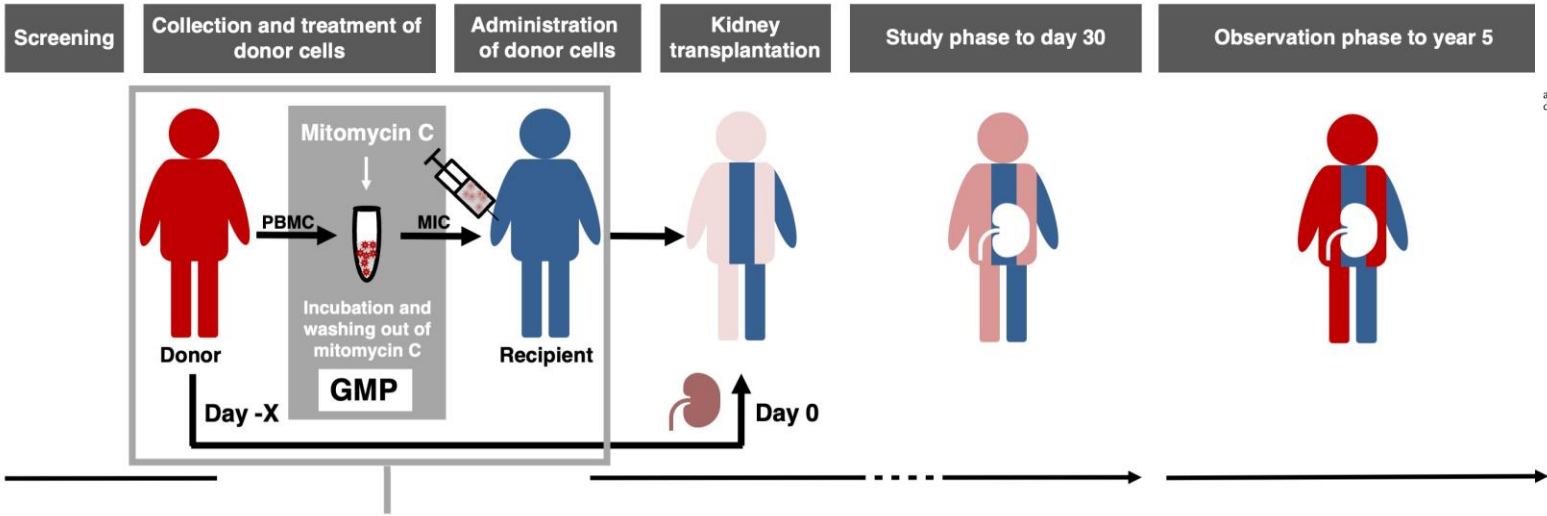


„First-use-in-men“

Complete cellular donor-chimerism after MIC-treatment in a child with a haplotype-identical SCT who suffered from repeated rejection

Kleist *Clin Sci (Lond)* 2015

Gefördert durch:
Bundesministerium für Wirtschaft und Energie
aufgrund eines Beschlusses des Deutschen Bundestages
EXIST Existenzgründungen aus der Wissenschaft
ESF Europäischer Sozialfonds für Deutschland
EUROPÄISCHE UNION



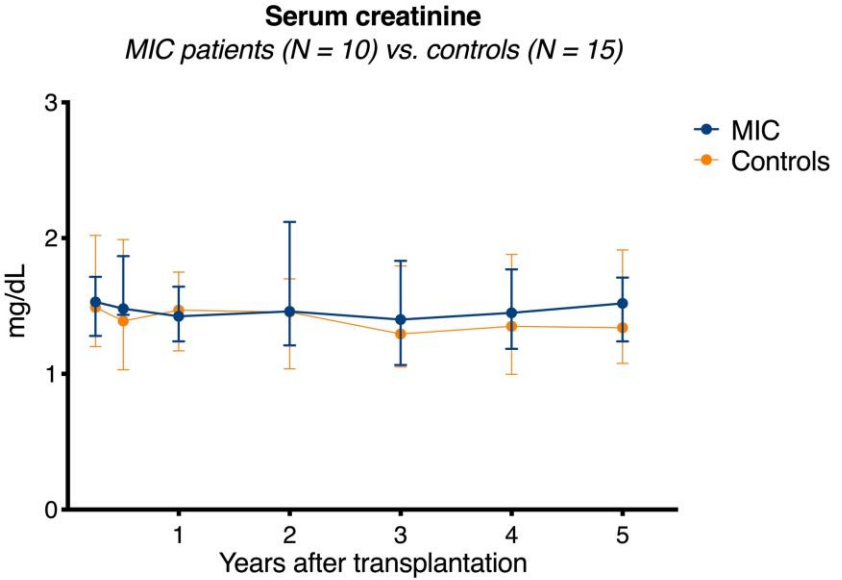
Group C: N = 4 patients (R7, 11, 12, 14) with 1.5 x 10^8 MIC per kg bw on day -7 (LD-CyA, LD-EC-MPS)

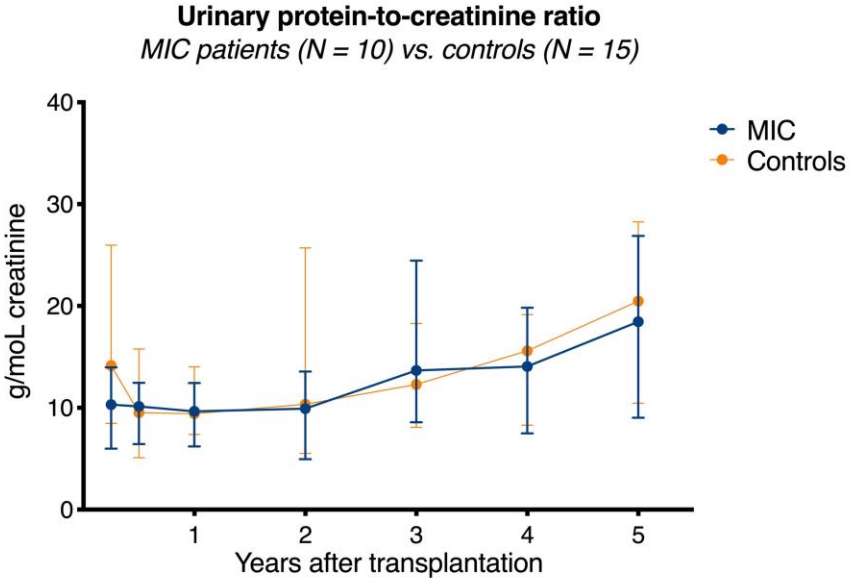
Group A, B: N = 6 patients with 1.5 x 10^6 to 1.5 x 10^8 MIC per kg bw on day -2 (CyA, EC-MPS, steroids)

TOL-1 phase-Ib human study

Donor leukapheresis, GMP manufacturing and infusion into recipient on the same day, kidney transplantation 2 or 7 days later under triple immunosuppression

Morath *J Clin Invest* 2020, Schaier *Front Immunol* 2023, ClinicalTrials.gov Identifier: NCT02560220, EudraCT number: 2014-002086-30

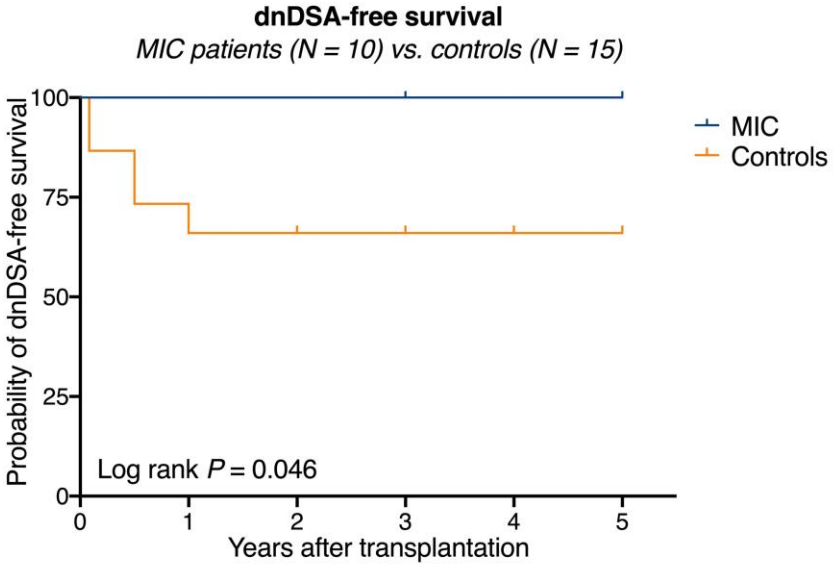


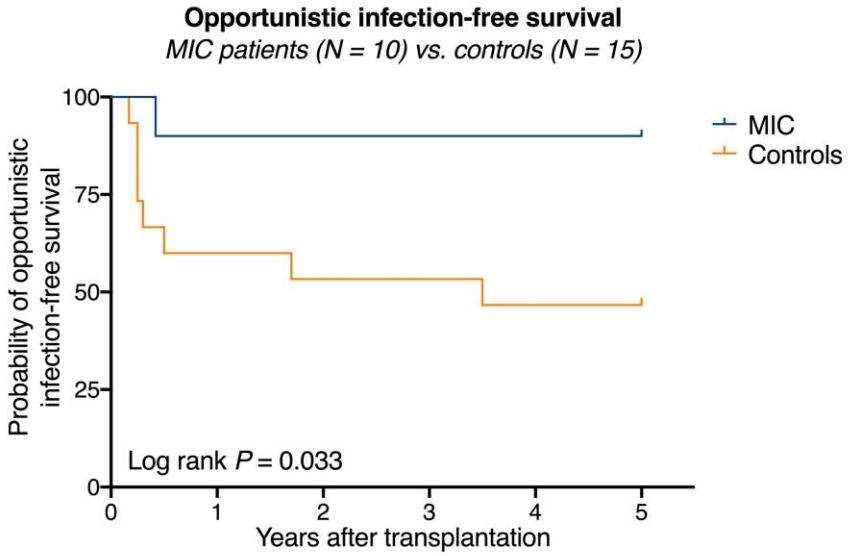


Retrospective analysis of clinical end-points

Excellent kidney graft function and no proteinuria in MIC-treated patients comparable to results in transplanted controls

Schaier *Front Immunol* 2023





Retrospective analysis of clinical end-points

No DSA, no acute rejections, „no“ opportunistic infections, and higher median anti-S1 IgG Index (53 vs. 2, *P* = 0.16) of MIC vs. controls (5-year follow-up)

Schaier *Front Immunol* 2023

Gefördert durch:
Bundesministerium für Wirtschaft und Energie
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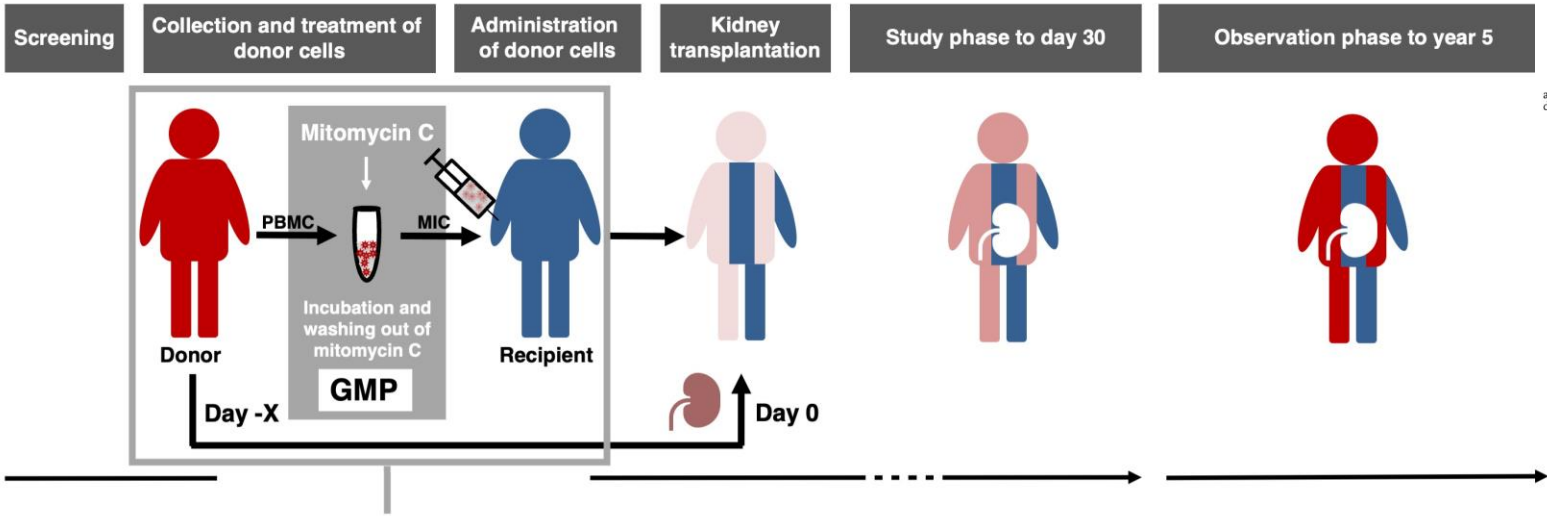

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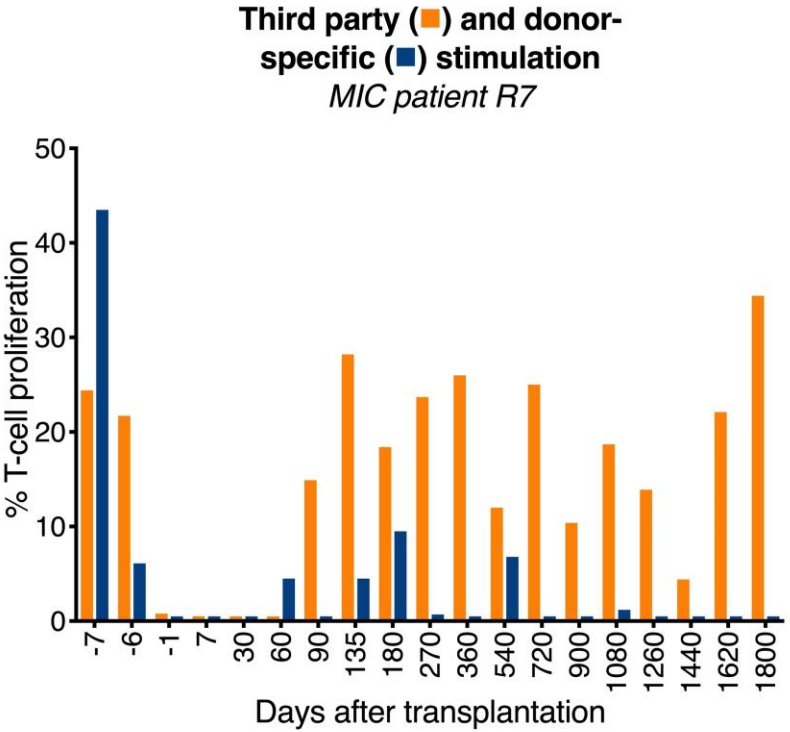
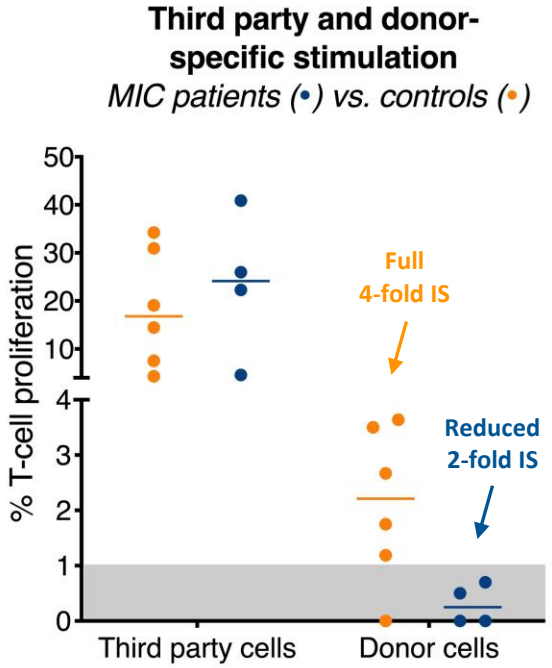
TOL-1 phase-Ib human study

Detailed immunological analysis of 4 group C patients who received the highest MIC cell count of 1.5 x 10^8 MIC per kg bw 7 days prior to surgery.

Morath *J Clin Invest* 2020, Schaier *Front Immunol* 2023, ClinicalTrials.gov Identifier: NCT02560220, EudraCT number: 2014-002086-30

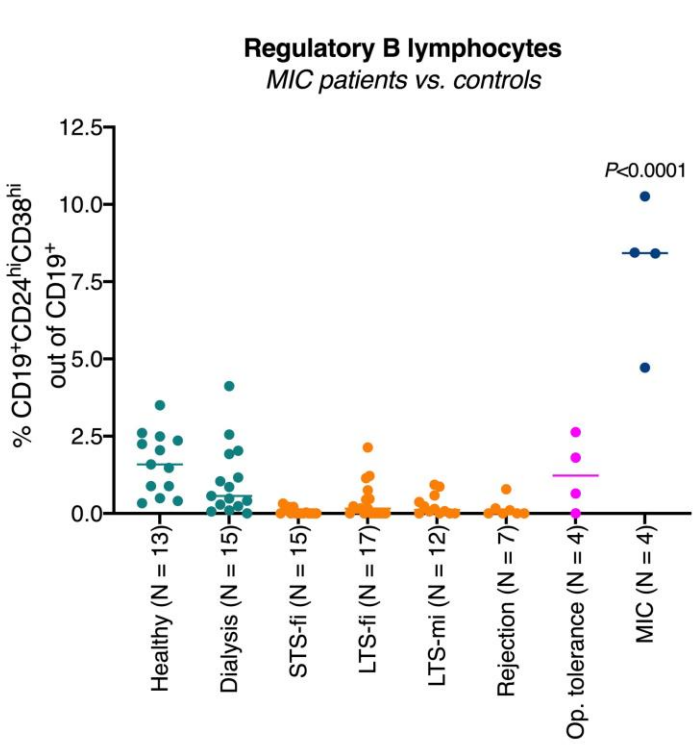


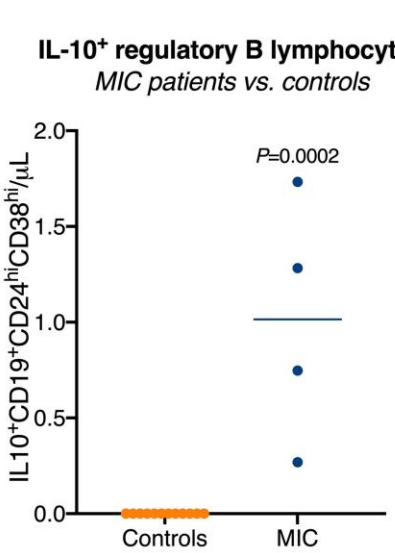


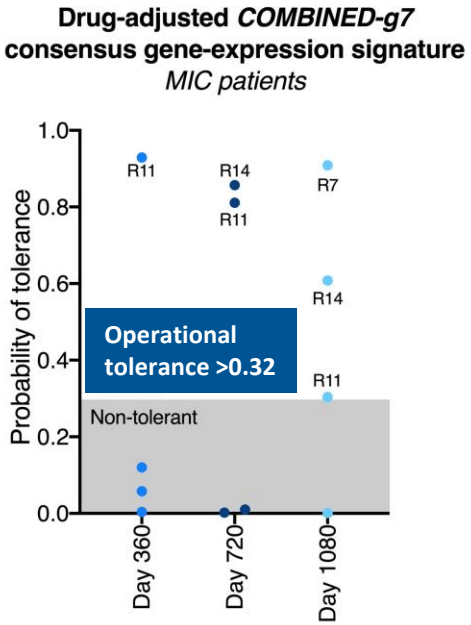
Generation of specific immunosuppression

Reduced *in vitro* lymphocyte reactivity against stimulatory donor blood cells while reactivity against third party cells is preserved

Morath *J Clin Invest* 2020, Morath *J Am Soc Nephrol* 2023



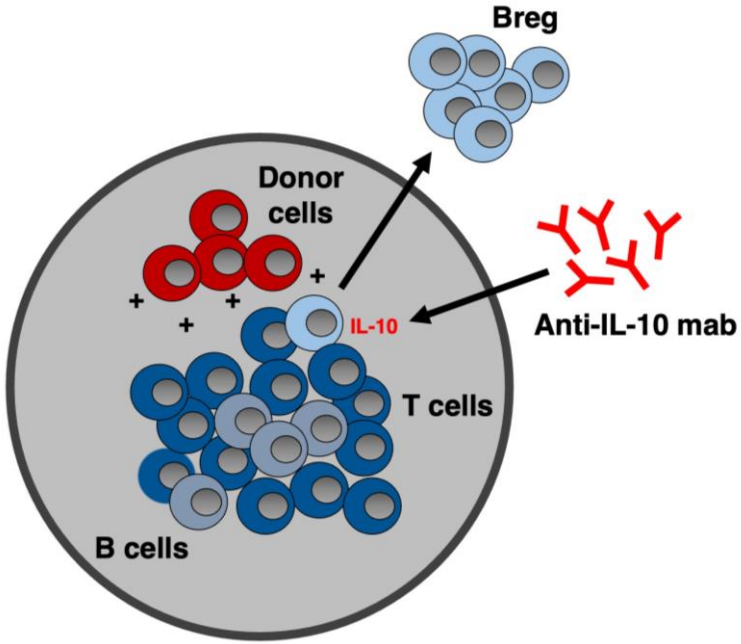


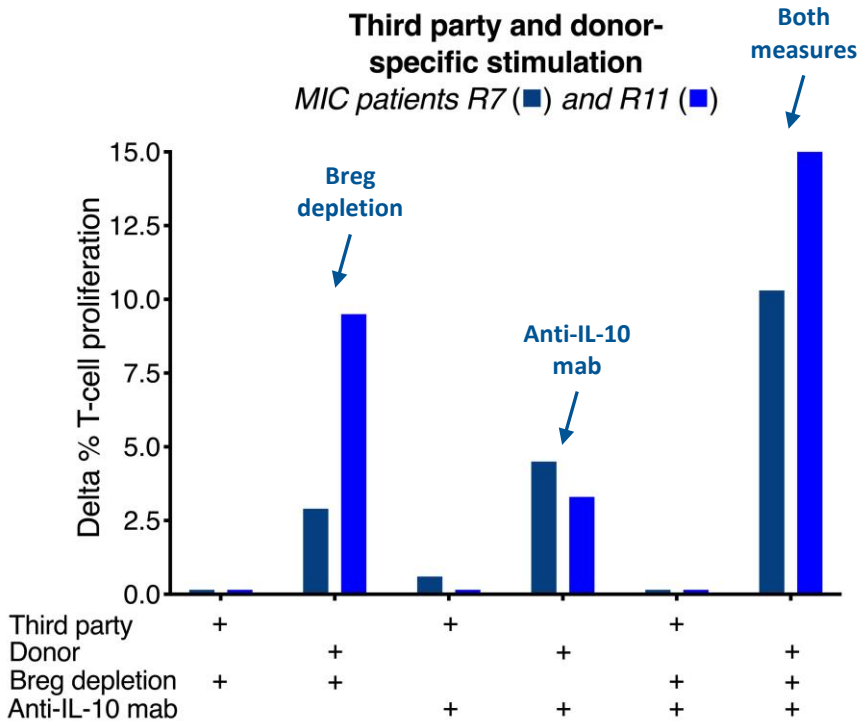


MIC induce an operationally tolerant phenotype

High levels of IL-10 producing regulatory B lymphocytes, evidence of the consensus gene expression signature of operational tolerance.

Morath *J Am Soc Nephrol* 2023

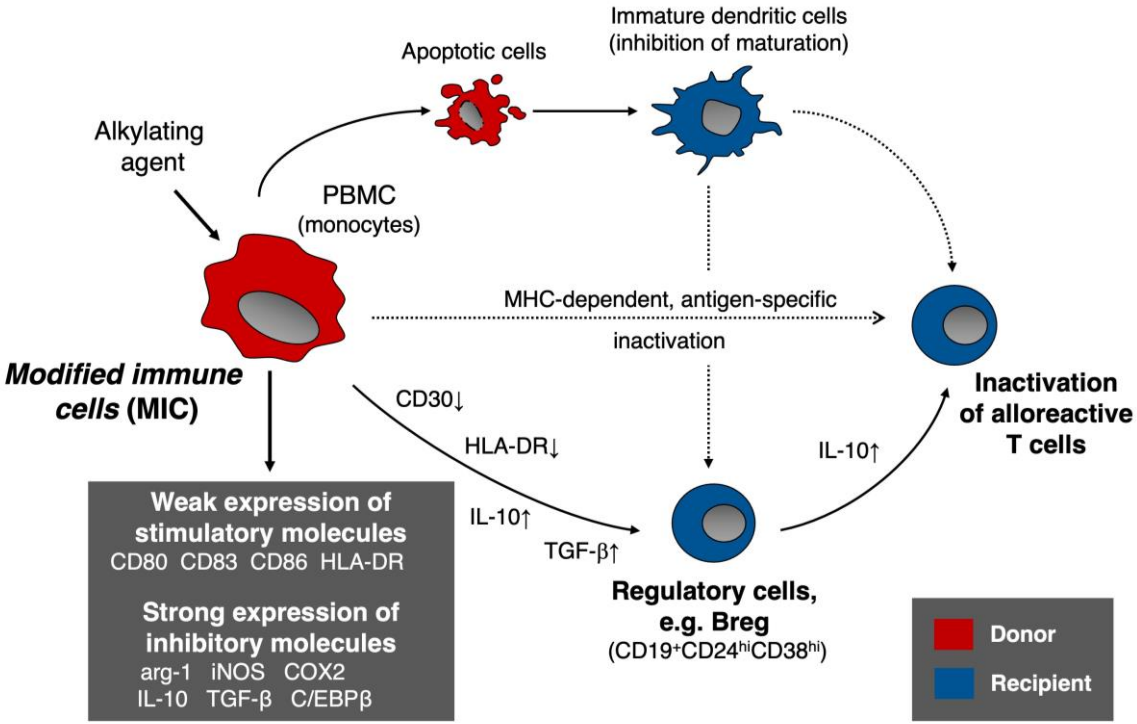




Regulatory B lymphocytes: Key cells of operational tolerance?

Increase in the anti-donor T-cell response after depletion of regulatory B lymphocytes (Breg) and/or addition of anti-IL-10 monoclonal antibody

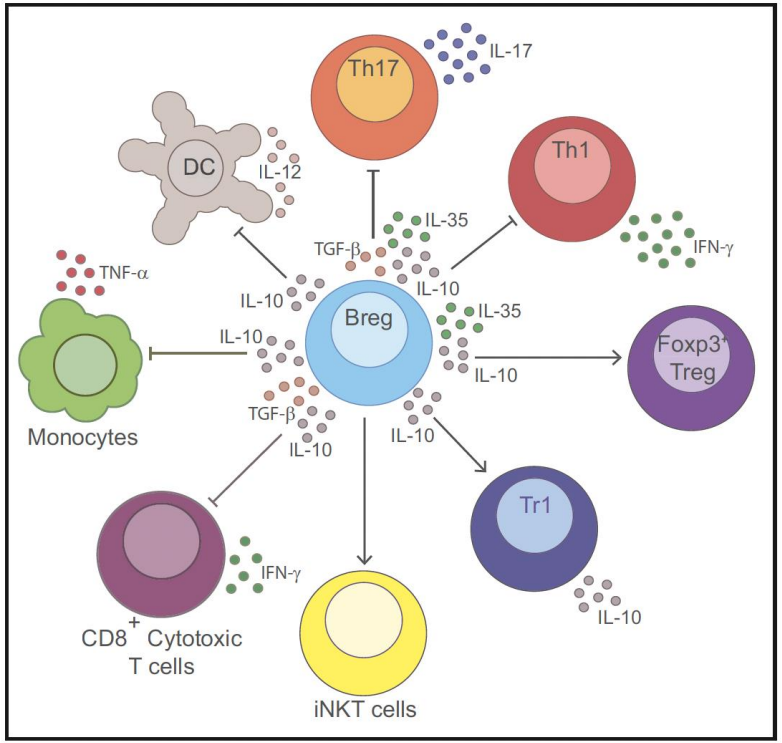
Morath *J Am Soc Nephrol* 2023



How do MIC (= *Modified Immune Cells*) work?

MIC lead to the formation of regulatory cell populations, such as regulatory B lymphocytes (Breg), which in turn inhibit alloreactive T lymphocytes via IL-10.

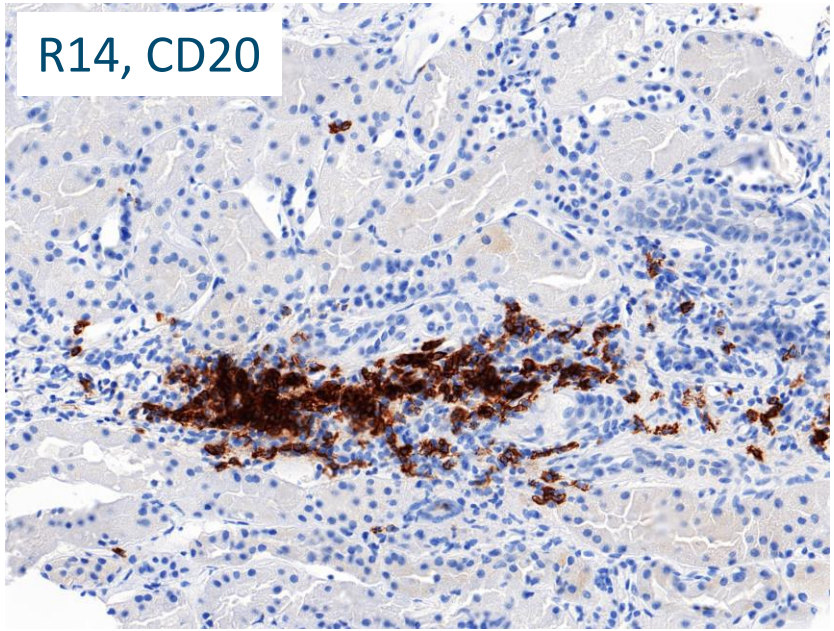
Terness *Proc Natl Acad Sci USA* 2008, Kleist *Clin Sci (Lond)* 2015, Morath *J Clin Invest* 2020, modified from Morath *Pediatr Nephrol* 2018

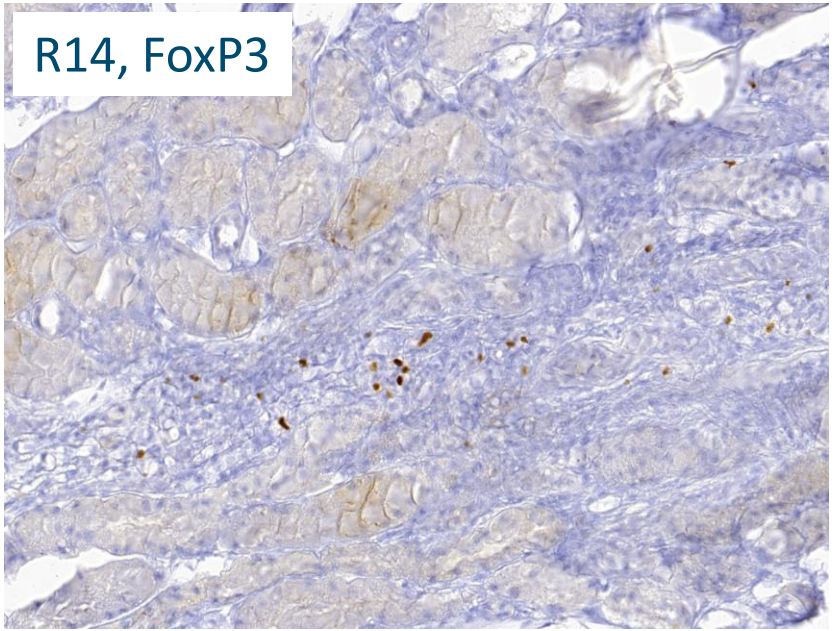


How do regulatory B lymphocytes (Breg) work?

By producing IL-10 and TGF-β, Breg suppress pro-inflammatory lymphocytes and induce Treg differentiation. Cell-cell interactions?

Rosser and Mauri *Immunity* 2015

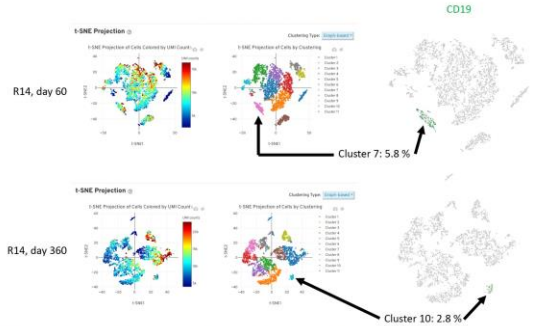




Kidney graft infiltrates in MIC-treated patients

B-cell infiltrates and FoxP3-positive T cells in protocol biopsies, but too few samples to obtain meaningful results

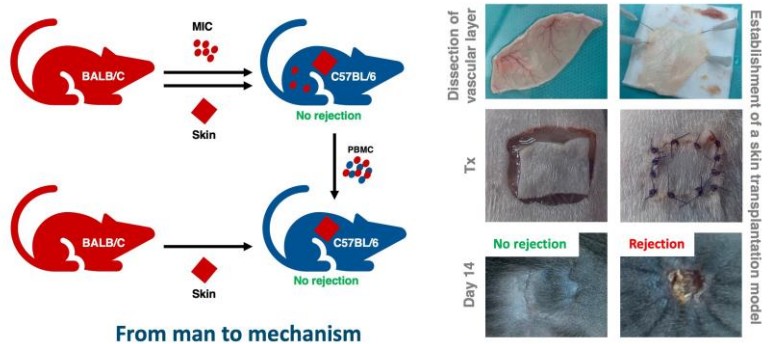
Morath *J Am Soc Nephrol* 2023



How do regulatory B lymphocytes (Breg) work?

Single-cell RNA sequencing to decipher the cellular networks that mediate immunological tolerance in MIC-treated patients

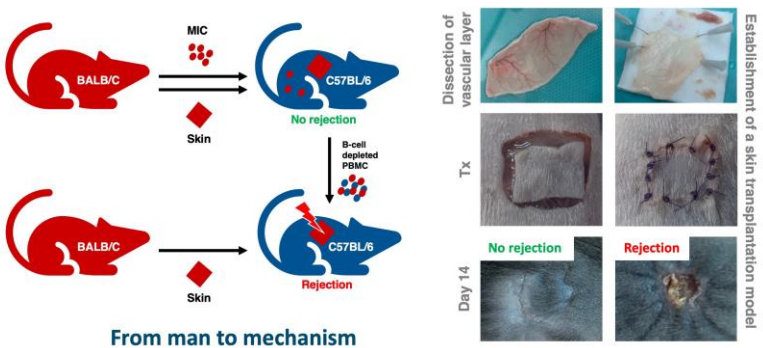
 Collaboration EMBL Heidelberg and Saez-Rodriguez Group, work in progress, Mar 2024



From man to mechanism

Adoptive cell transfer in the BALB/C-to-C57BL/6 skin transplantation model to demonstrate the central role of IL-10-producing regulatory B lymphocytes

 Work in progress, Apr 2024



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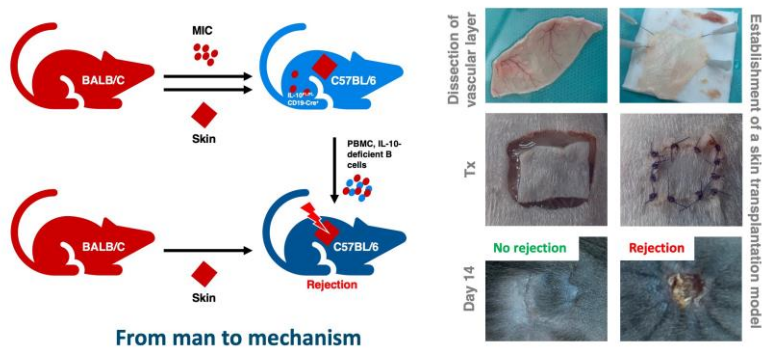

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 Work in progress, Apr 2024

The Journal of Clinical Investigation — COMMENTARY

Moving from transplant as a treatment to transplant as a cure

Sam Kant[1] and Daniel C. Brennan[1,2]

[1]Nephrology Division and [2]Comprehensive Transplant Cent

Research Highlights

Irma Husain, MD[1] and Xunrong Luo, PhD, MD[1]

Phase I Trial of Donor-derived Modified Immune Cell Infusion in Kidney Transplantation

antibodies or donor chimerism or were absent during the 30-day study phase and up to 1 year thereafter. Detailed immunological testing was, however, only performed

Morath C, Schmitt A, Kleist

2364–2376.

Game Changer

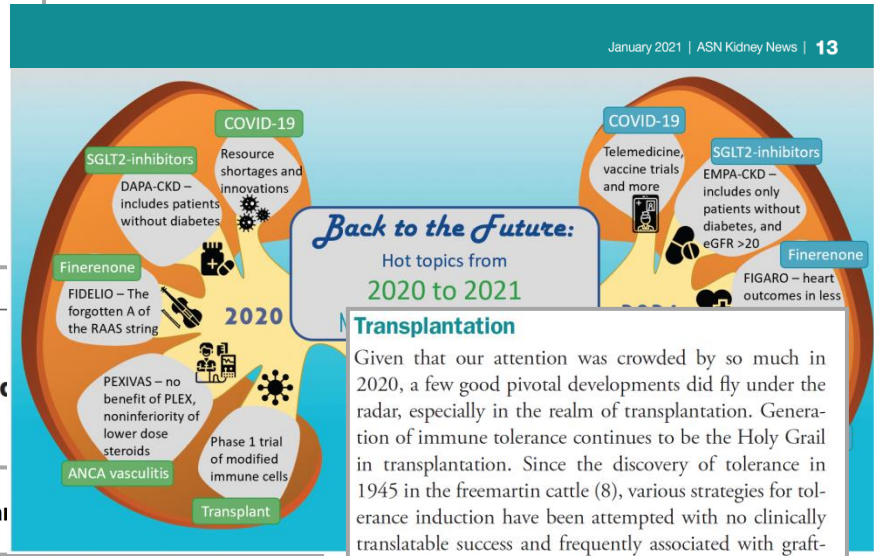
Transplantation Tolerance: Expanded and Selective Roles for B Cells

Cecilia B. Cavazzoni[1] and Peter T. Sage[1]

REVIEW ARTICLE

Regulatory B cells in transpla clinic

Joseph Beckett, Joanna Hester, Fac



Transplantation

Given that our attention was crowded by so much in 2020, a few good pivotal developments did fly under the radar, especially in the realm of transplantation. Generation of immune tolerance continues to be the Holy Grail in transplantation. Since the discovery of tolerance in 1945 in the freemartin cattle (8), various strategies for tolerance induction have been attempted with no clinically translatable success and frequently associated with graft-versus-host disease (GVHD). In 2020, a phase 1 trial (n = 10) was successful in demonstrating not only safety but also efficacy with the injection of modified immune cells (leukapheresis-derived donor monocytes treated with mitomycin C). These cells developed features of immature dendritic cells and resulted in profound suppression of the T cell response, with ensuing development of durable immune tolerance (9). It remains to be seen how phase 2 trials (n = 200) of this strategy will progress, but one thing is for sure: the search for the Holy Grail could be getting closer.

EDITORIAL www.jasn.org

Immune Modulatory Cell Therapy in Kidney Transplantation: Hints of a Durable Mechanism of Action

Matthew D. Griffin,[1] Norberto Perico,[2] and Federica Casiraghi[2]

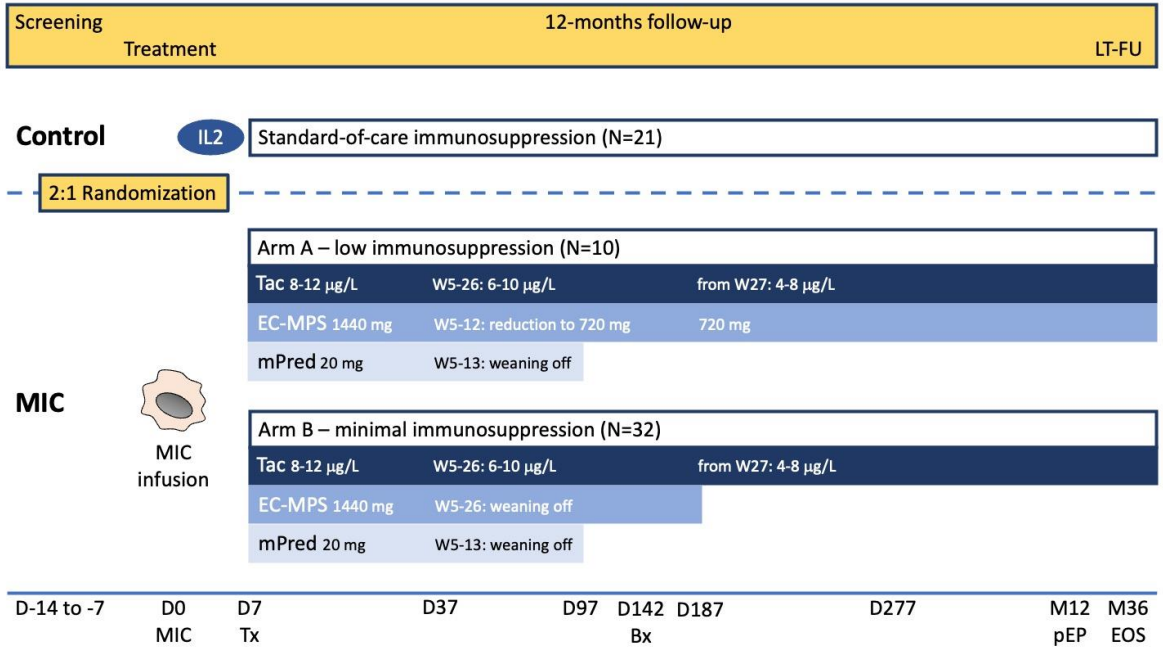
Science

Translation

Science 4 Life
Platz 1
Businessplanphase
TolerogenixX
Venture Cup 2015

TOLEROGENIxX

J Clin Invest 2020, *Transplantation* 2020, *Transpl Int* 2020, *ASN* 2021, *Kidney360* 2021, *Front Immunol* 2021, *J Am Soc Nephrol* 2023



TOL-2 phase-IIb study

Primary endpoint:
Operational tolerance

Key secondary endpoints:
1) Patient-relevant infections
2) BPAR, graft loss, graft dysfunction, or death

PEI SA 08.05.2017
EMEA/H/SA/4197/1/2019/SME/ADT/III
FDA PS005295, CRMTS #12224
Herstellungserlaubnis DE_BW_01_MIA_2020_0118/DE_BW_01_Uniklinik HD_Med Klinik V GMP-Facility
EudraCT number: 2021-000561-33
ClinicalTrials.gov Identifier: NCT05365672

Heidelberg
Stuttgart
M – LMU
Münster
Hamburg
B – Charité
M – TU

Multi-center TOL-2 phase-IIb study

MIC cell therapy plus dual immunosuppressive therapy (arm A) or single therapy (arm B, "*one-pill once-a-day*") compared to SOC

Bundesministerium für Bildung und Forschung
FuE BSFZ
KMU-innovativ

Morath *BMJ open* 2022

ID	Group	Day	S-Crea (mg/dL)	MLC (D / TP, %)	Breg (%)	Immunosuppression	Rejection, HLA-ab
01-R003	Con	367	1.09	5 / 0	11	IL-2, Tac, MPA (1,440), CS	Ø Rej, Ø DSA
01-R013	**MIC**	**367**	**0.97**	**0 / 23**	**28**	**Tac, MPA (720)**	**Ø Rej, Ø DSA**
01-R015	**MIC**	**367**	**0.99**	**1 / 10**	**25**	**Tac, MPA (720), CS***	**Ø Rej, Ø DSA***
01-R017	**MIC**	**367**	**1.11**	**0 / 2**	**33**	**Tac, MPA (720)**	**Ø Rej, Ø DSA**
01-R019	Con	367	3.32	0 / 34	23	IL-2, Tac, MPA (1,000*), CS	Ø Rej, Ø DSA
01-R021	**MIC**	**367**	**1.46**	**0 / 8**	**20**	**Tac, MPA (720)**	**Ø Rej, Ø DSA**
01-R023	**MIC**	**367**	**1.59**	**pending**	**pending**	**Tac, MPA (720)**	**Ø Rej, Ø DSA**
04-R001	**MIC**	**(--)**	**(--)**	**(--)**	**(--)**	**(-- , Center Stuttgart)**	**(--)**
01-R025	Con	277	1.29	8 / 16	3	IL-2, Tac, MPA (1,440), CS	Ø Rej, Ø DSA
01-R027	Con	187	2.04	0 / 6	27	IL-2, Tac, MPA (1,440), CS	Ø Rej, Ø DSA
01-R029	**MIC**	**187**	**1.43**	**0 / 13**	**30**	**Tac, MPA (720)**	**Ø Rej, Ø DSA**
01-R031	**MIC**	**187**	**1.70**	**0 / 6**	**26**	**Tac, MPA (720)**	**Ø Rej, Ø DSA**
01-R035	Con	97	1.12	0 / 1	2	IL-2, Tac, MPA (1,440), CS	Ø Rej, Ø DSA
01-R037	**MIC**	**142**	**2.03**	**pending**	**pending**	**Tac, MPA (720)**	**Ø Rej, Ø DSA**
01-R039	**MIC**	**97**	**1.38**	**pending**	**pending**	**Tac, MPA (720)**	**Ø Rej, Ø DSA**
01-R043	**MIC**	**37**	**2.27**	**2 / 27**	**46**	**Tac, MPA (1,440), CS – weaning**	**Ø Rej, Ø DSA**
04-R003	**MIC**	**(--)**	**(--)**	**(--)**	**(--)**	**(-- , Center Stuttgart)**	**(--)**
04-R005	Con	(--)	(--)	(--)	(--)	(-- , Center Stuttgart)	(--)
01-R045	**MIC**	**37**	**1.34**	**pending**	**pending**	**Tac, MPA (1,440), CS**	**Ø Rej, Ø DSA**
01-R047	Con	7	(--)	(--)	(--)	IL-2, Tac, MPA (1,440), CS	(--)
01-R049	Con	7	(--)	(--)	(--)	IL-2, Tac, MPA (1,440), CS	(--)
01-R051	**MIC**	**randomized**	**(--)**	**(--)**	**(--)**	**(--)**	**(--)**

DSMB approval for treatment arm B

Arm B

Work in progress, Apr 2024

Outlook

- Cellular therapies in clinical trials, e.g. HSCT, (CAR)-Treg
- MIC are replication-incompetent monocytes
- MIC induce an operationally tolerant phenotype (unresponsiveness, Breg, *COMBINED-g7* signature)
- *Back to bench:* Mouse model of skin transplantation
- TOL-2 phase-IIb clinical trial
- Other indications (deceased donor transplantation, transplant rejection, SLE, other autoimmune indications)

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ESF Europäischer Sozialfonds für Deutschland
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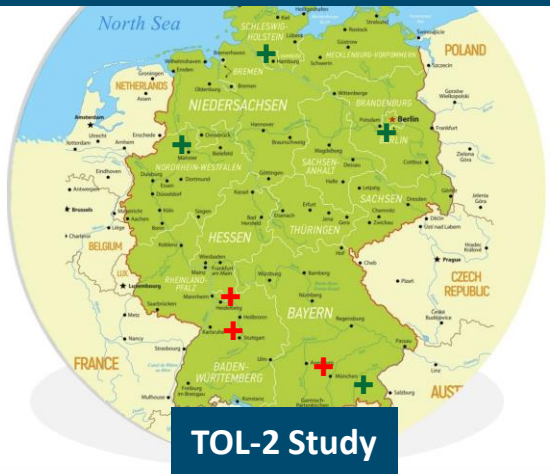
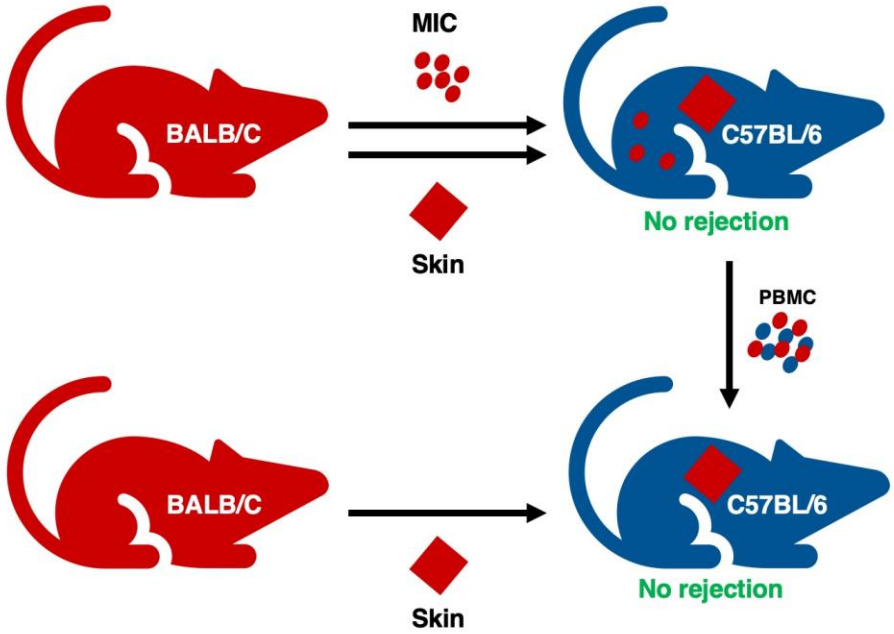
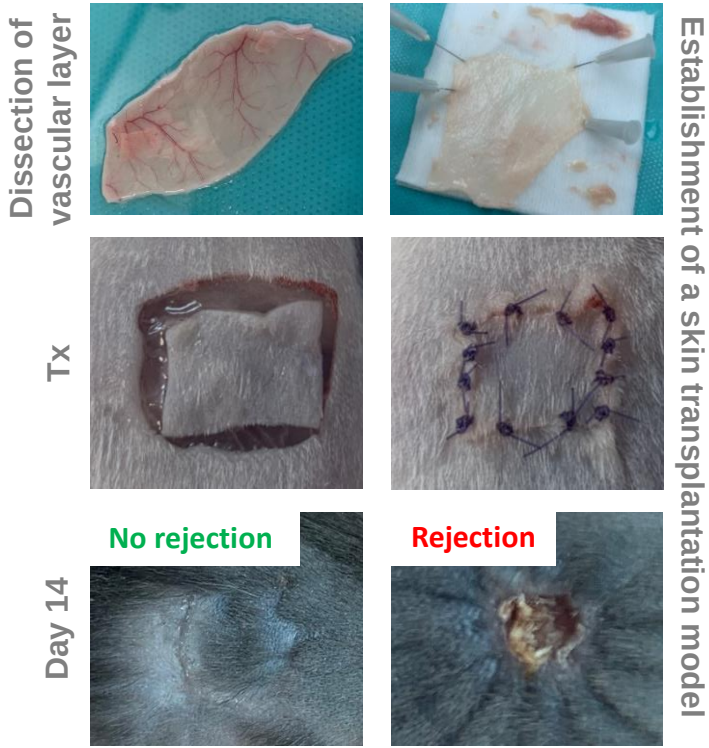


https://www.otto.de/p/muchowow-gemaelde-bunte-deutschlandkarte-1-st-kreis-wanddekoration-forex-rundes-wandbild-wohnzimmer-60x60-cm-506680A1/#variationId=506680AFPDS

TOL-2 Study

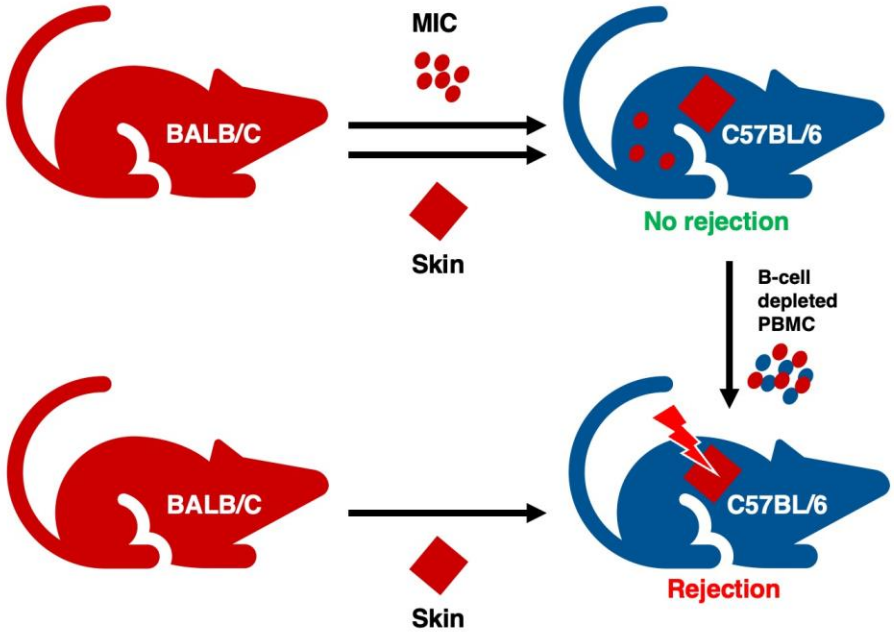
DZIF Deutsches Zentrum für Infektionsforschung
Bundesministerium für Bildung und Forschung
FüE BSFZ 2023

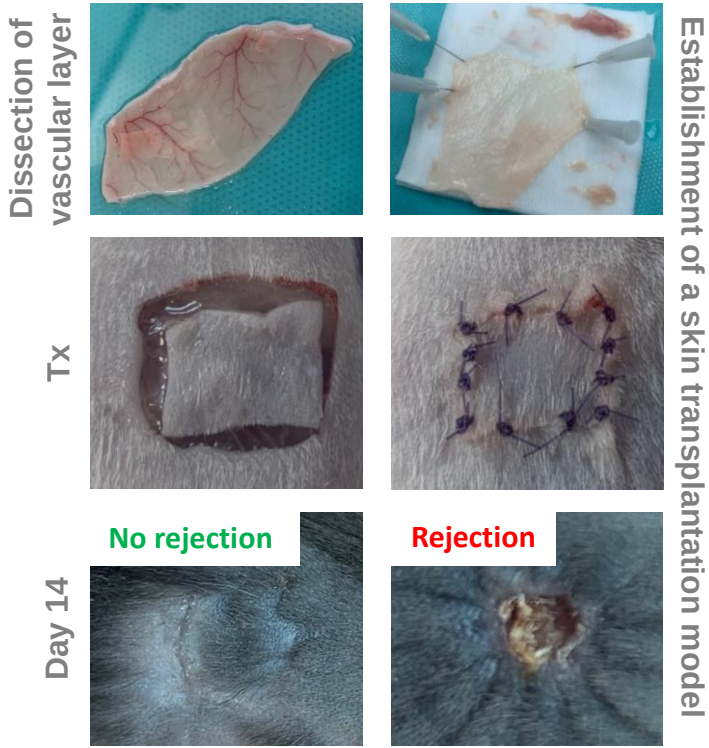




From man to mechanism

Adoptive cell transfer in the BALB/C-to-C57BL/6 skin transplantation model to demonstrate the central role of IL-10-producing regulatory B lymphocytes





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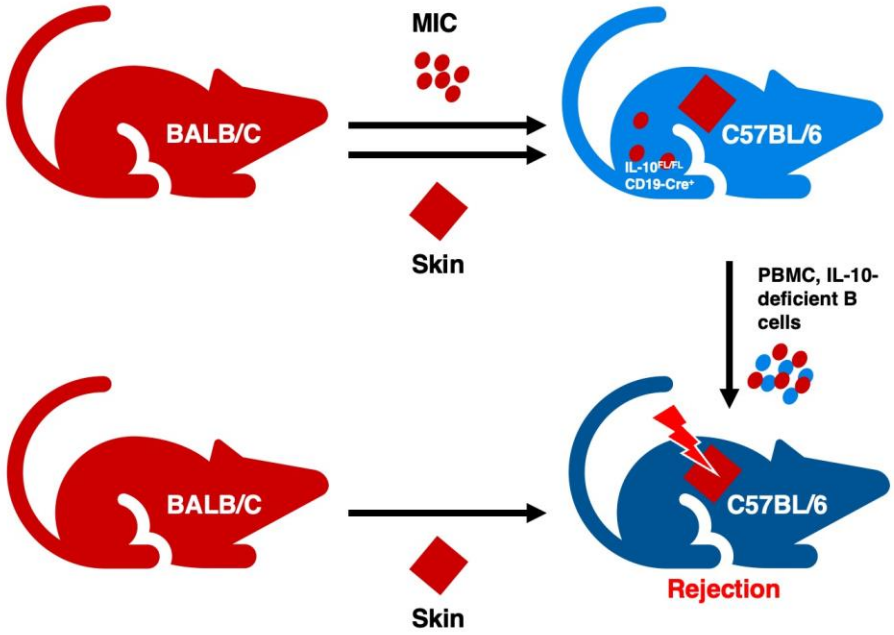

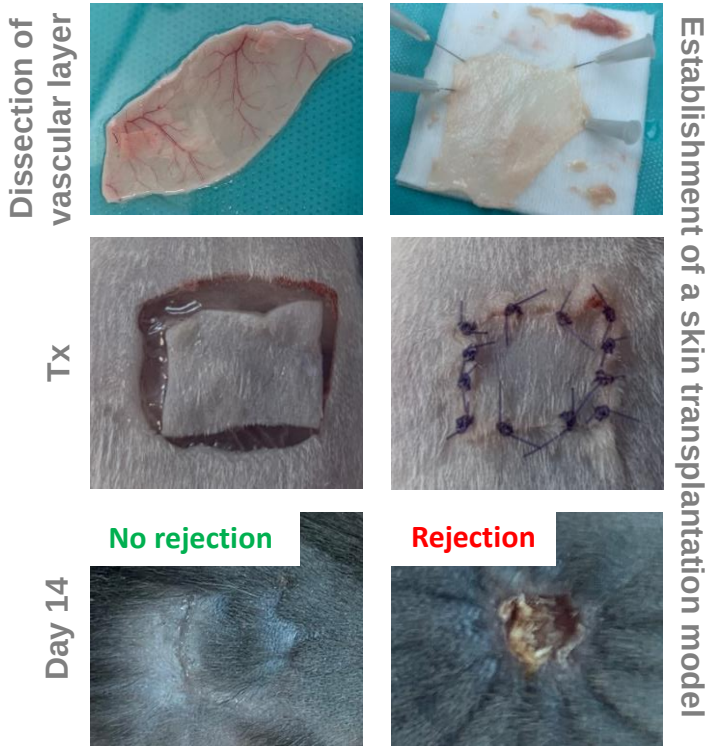

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