



University Medical Center Groningen

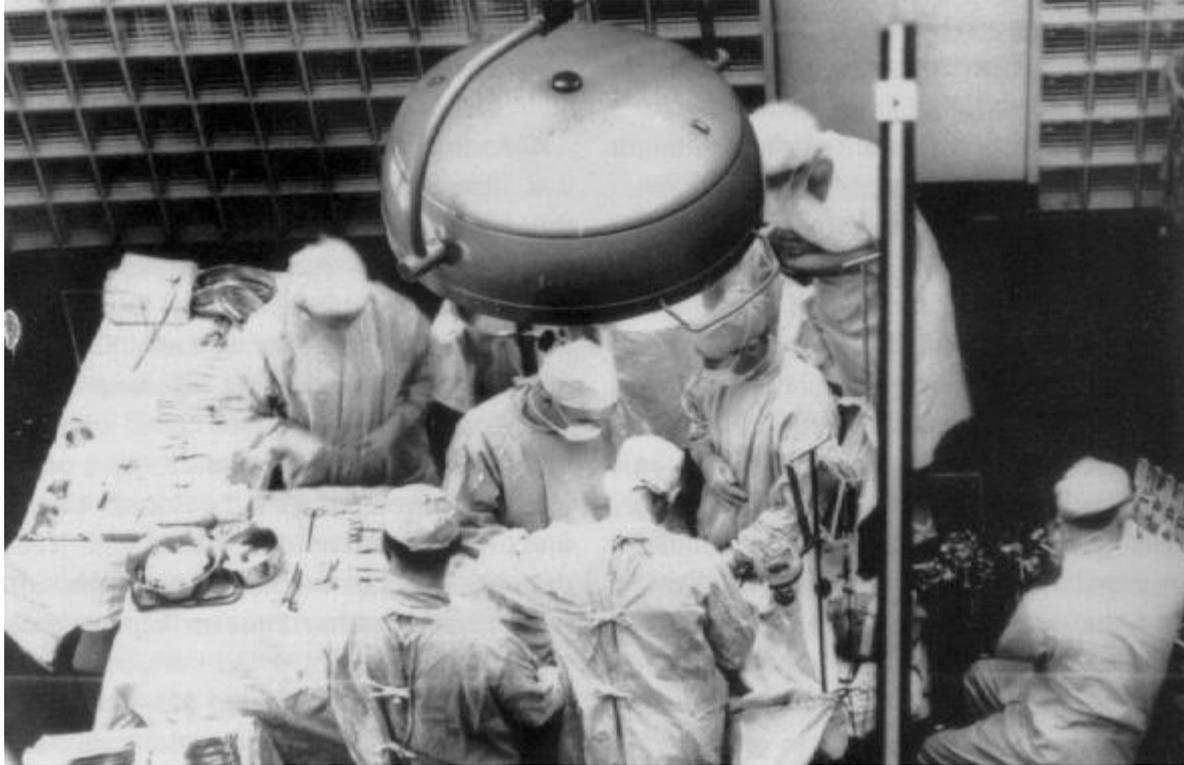


Rejectie en CAR T cellen

Jan-Stephan Sanders

Internist-nefroloog UMCG

Boston 1954



Geschiedenis van transplantatie

1902: first succesful exp. Kidney (dog) Tx (Ulmann, Vienna)

1906: first human kidney Tx (xenograft): Jaboulay

1933: first human kidney allograft (Vororonoy, Ukraine) 1933:
first heterotopic liver Tx (Welch)

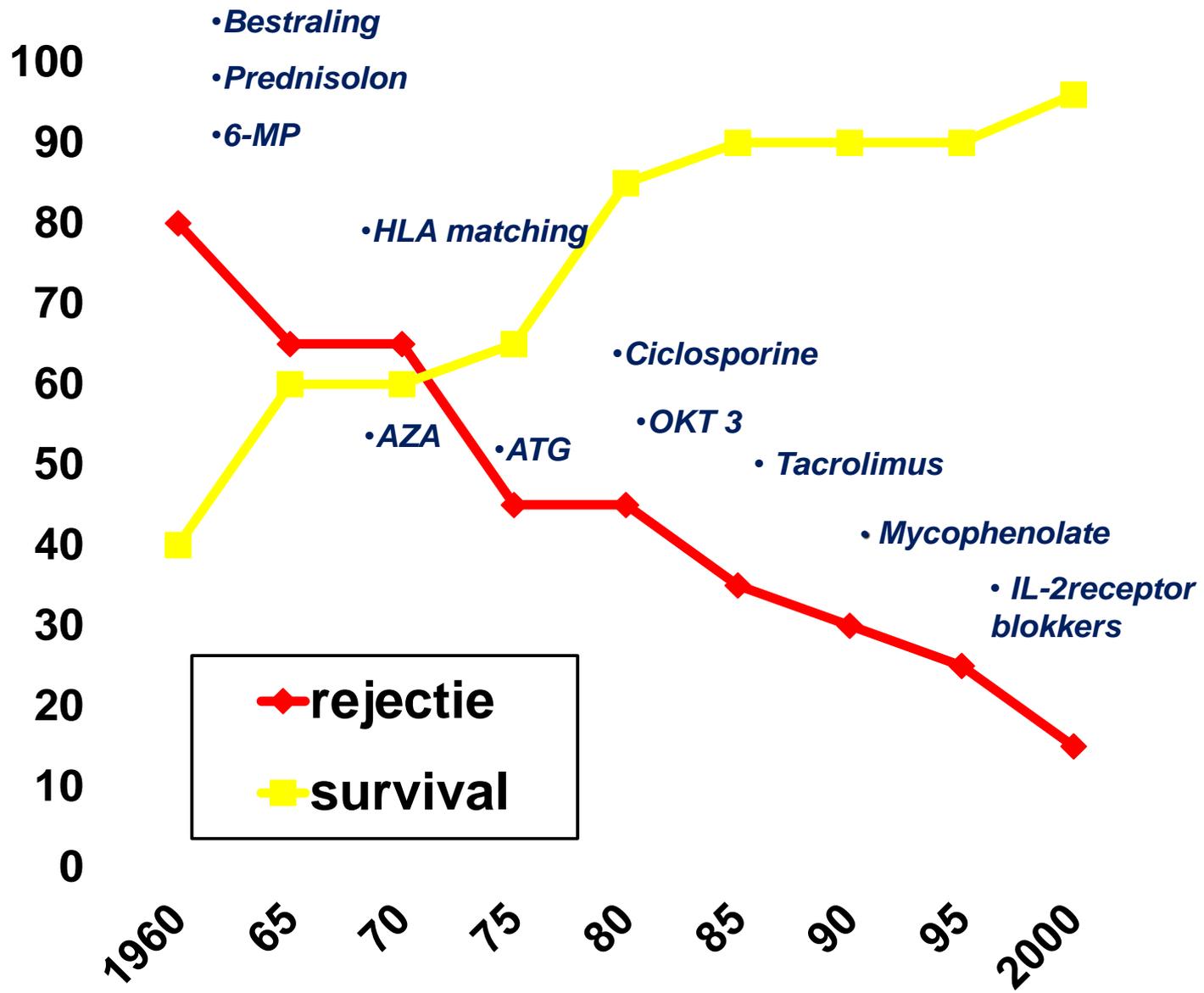
1954: first successful human kidney transplantation (Murray,
Boston)

1959: first orthotopic liver Tx (Starzl; Chicago, Moore; Boston)

1964: first human lung Tx (Hardy)

1967: first heart Tx (Barnard, South Africa)

2022: first pig to human heart xenotransplantation (USA)



•Bestraling

•Prednisolon

•6-MP

•HLA matching

•Ciclosporine

•OKT 3

• Tacrolimus

• Mycophenolate

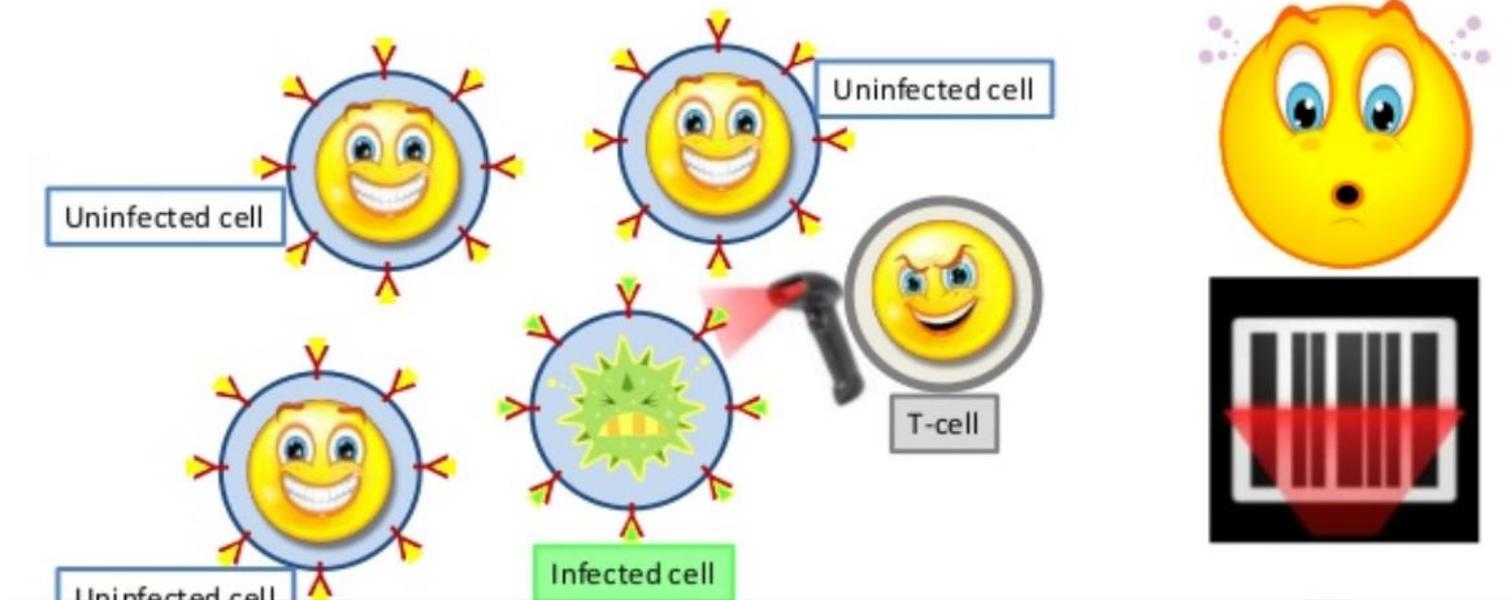
• IL-2receptor blokkers

◆ rejectie

■ survival

HLA

- Human Leukocyte Antigen
- Afweercel leest HLA-barcode op cellen om zelf van niet-zelf te onderscheiden en geïnfecteerde van niet-geïnfecteerde cellen

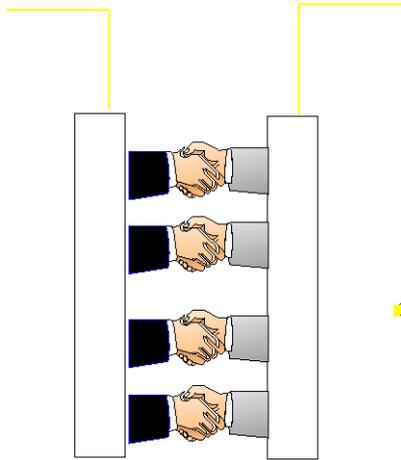


APC

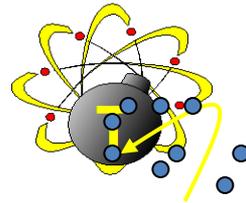
T cel

“antigeen
presenterend”

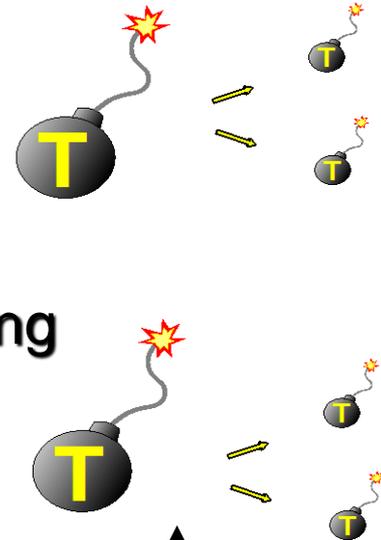
lymfocyt



autocriene loop



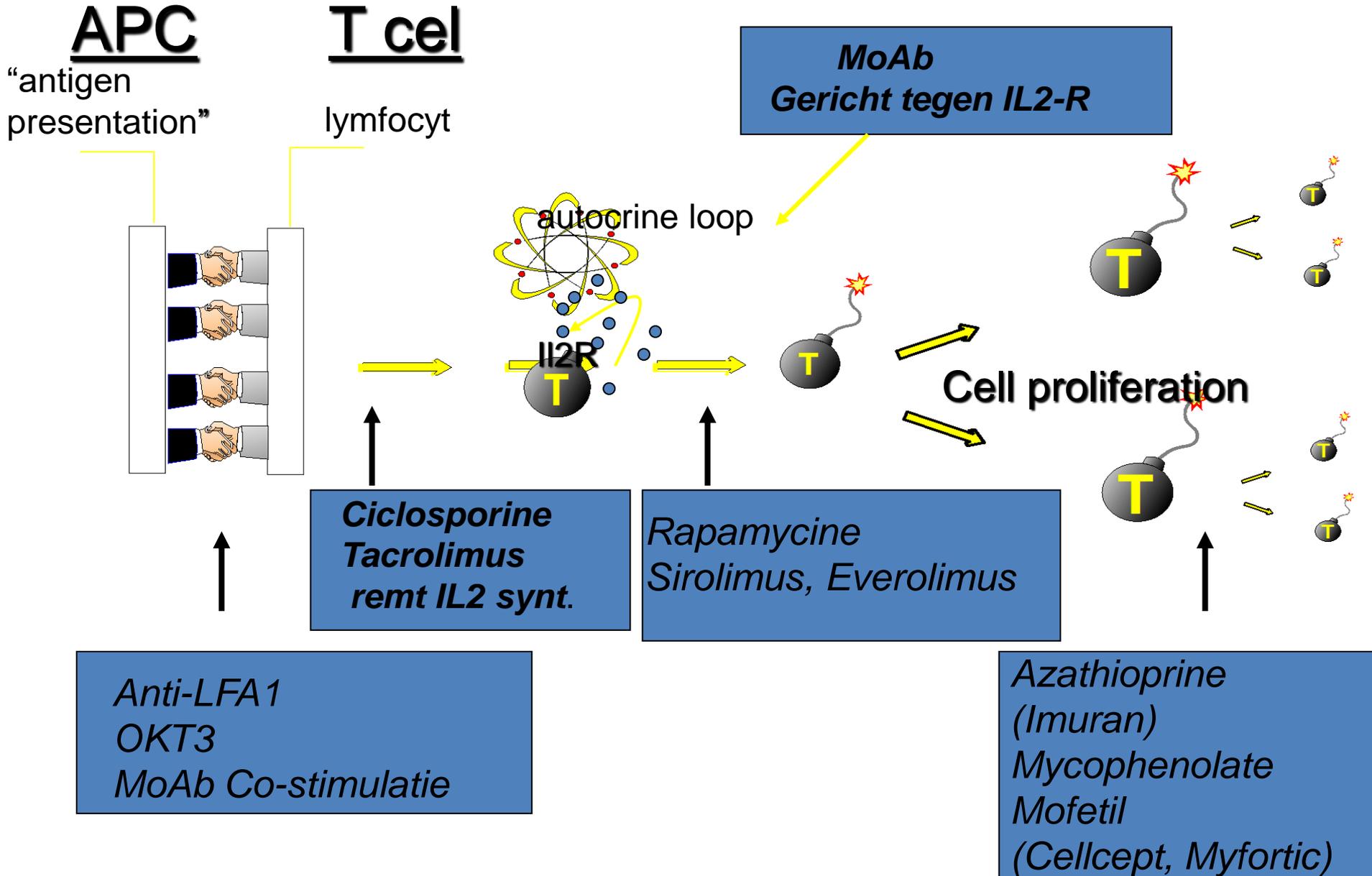
Celdeling



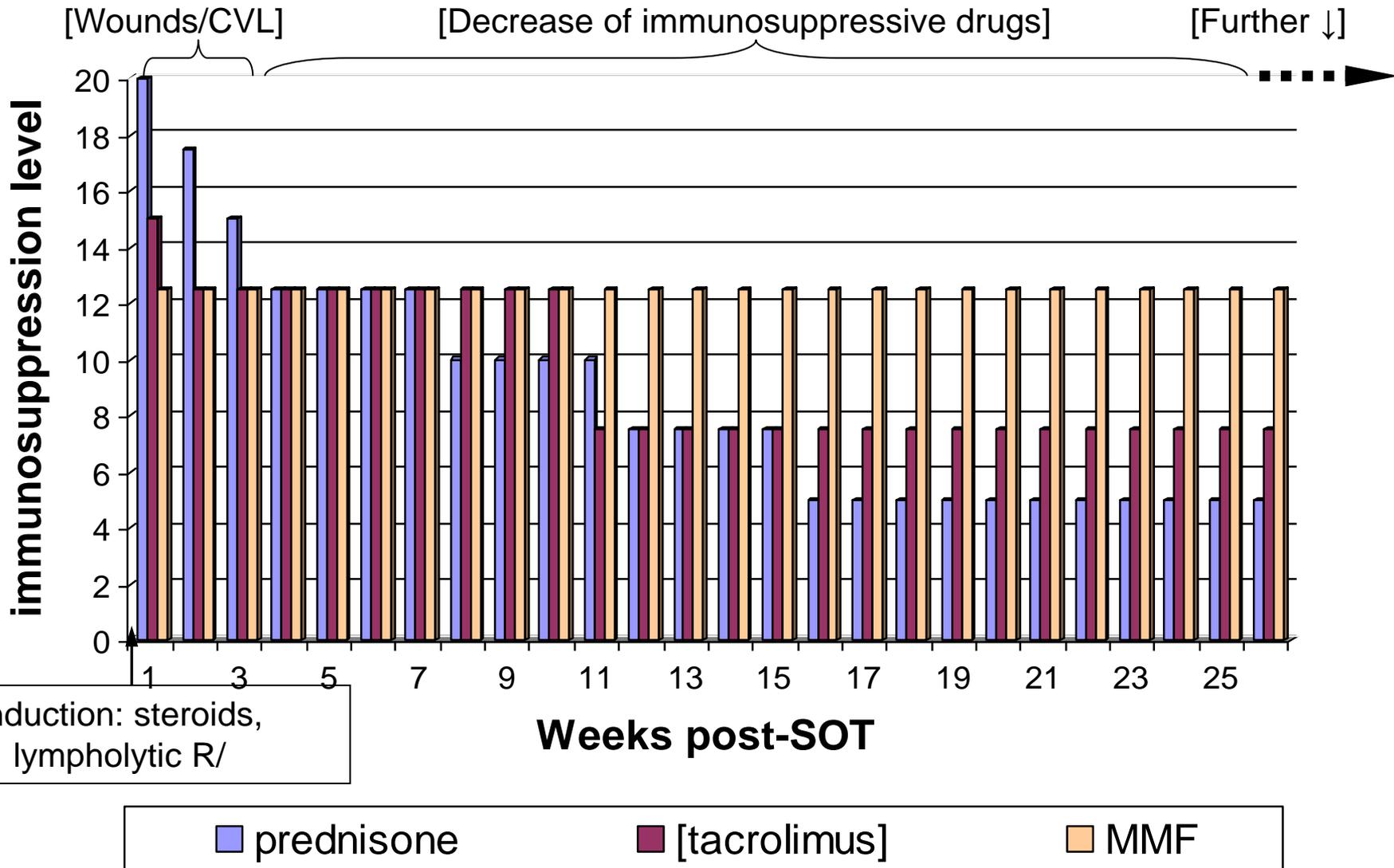
Adhesie
activatie
Herkenning
Co-stimulatie

“clonal proliferatie”

Immuunsuppressie



Immunosuppression after SOT



Doelstellingen

onderhoudsimmuunsuppressie

- Betrouwbare preventie van acute reëctie
- Betrouwbare preventie van chronische (humorale) reëctie
- Minimalisatie nefrotoxiciteit
- Minimalisatie van immuunsuppressieve bijwerkingen
 - Kanker
 - Infecties
- Minimalisatie van metabole bijwerkingen
- Flexibiliteit afhankelijk van patiënt preferenties en individuele bijwerkingen

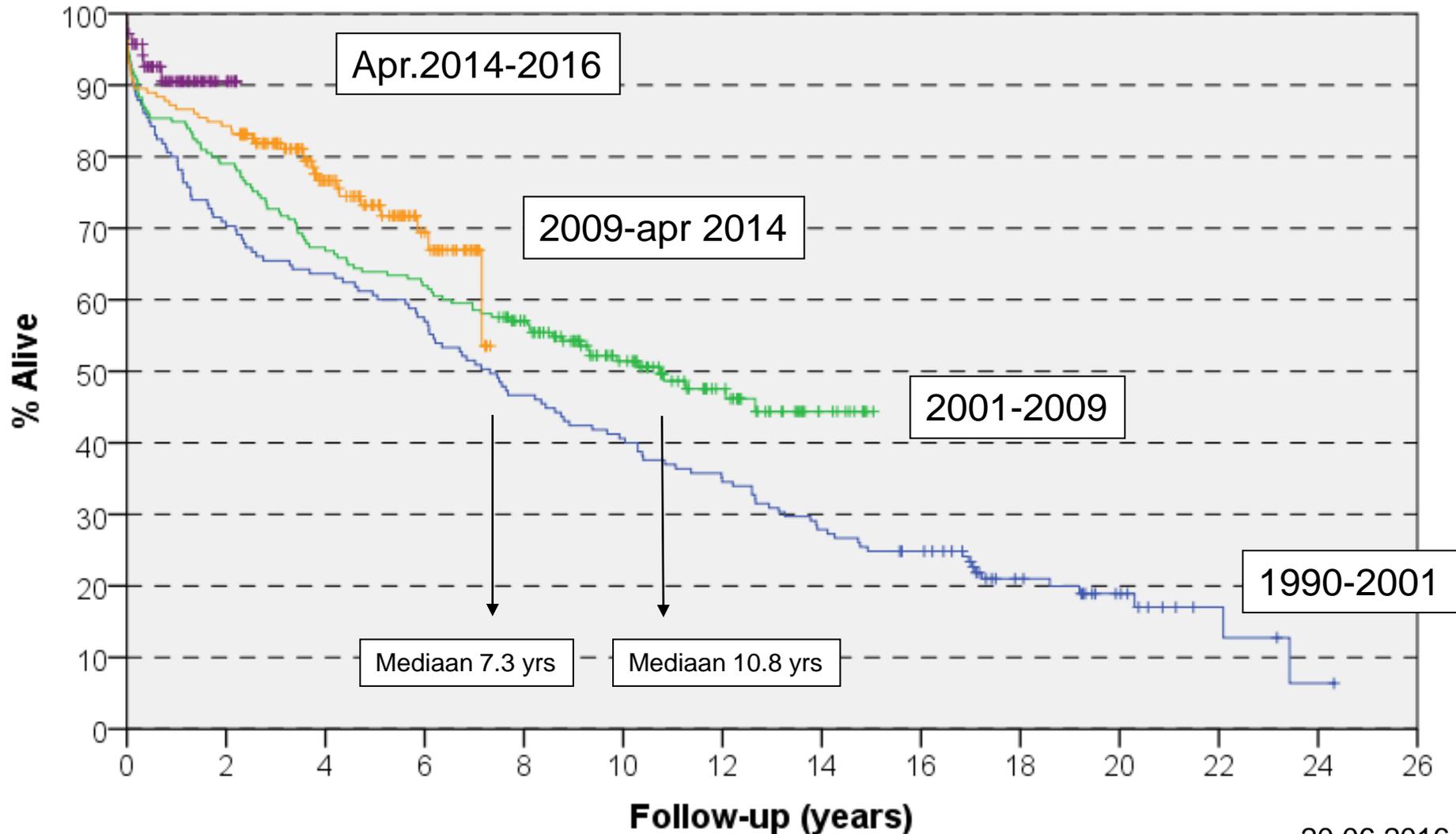
Doelstellingen

onderhoudsimmuunsuppressie

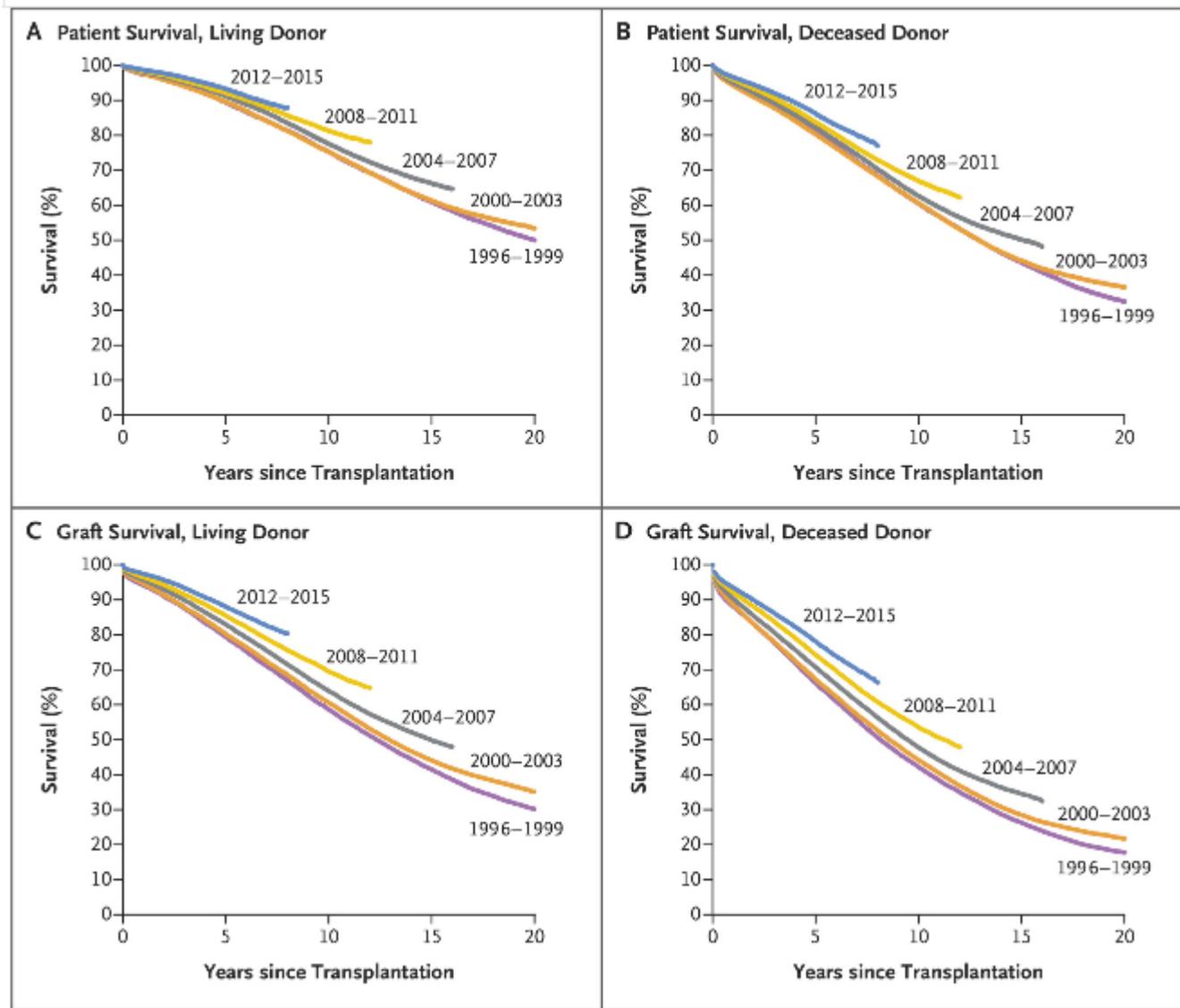
- Betrouwbare preventie van acute resectie
- Betrouwbare preventie van chronische (humorale) resectie
- Minimalisatie nefrotoxiciteit
- Minimalisatie van immuunsuppressive bijwerkingen
 - Kanker
 - Infecties
- Minimalisatie van metabole bijwerkingen
- Flexibiliteit afhankelijk van patiënt preferenties en individuele bijwerkingen
- **Maximale patiënt en transplantatoeverleving**

Longtransplantatie UMC Groningen

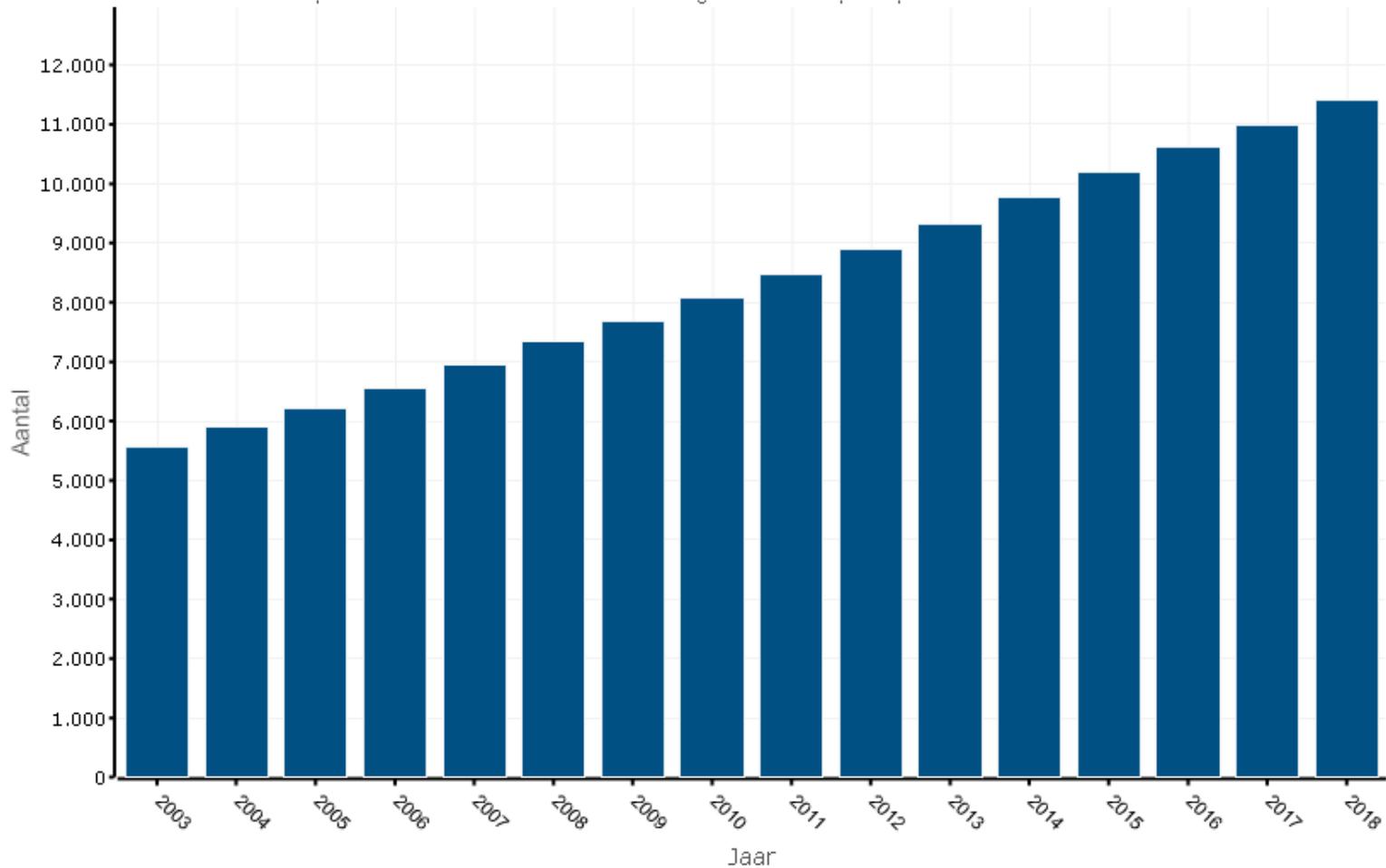
long-, hart-long, long-lever-, kinderlong en re-longtransplantaties



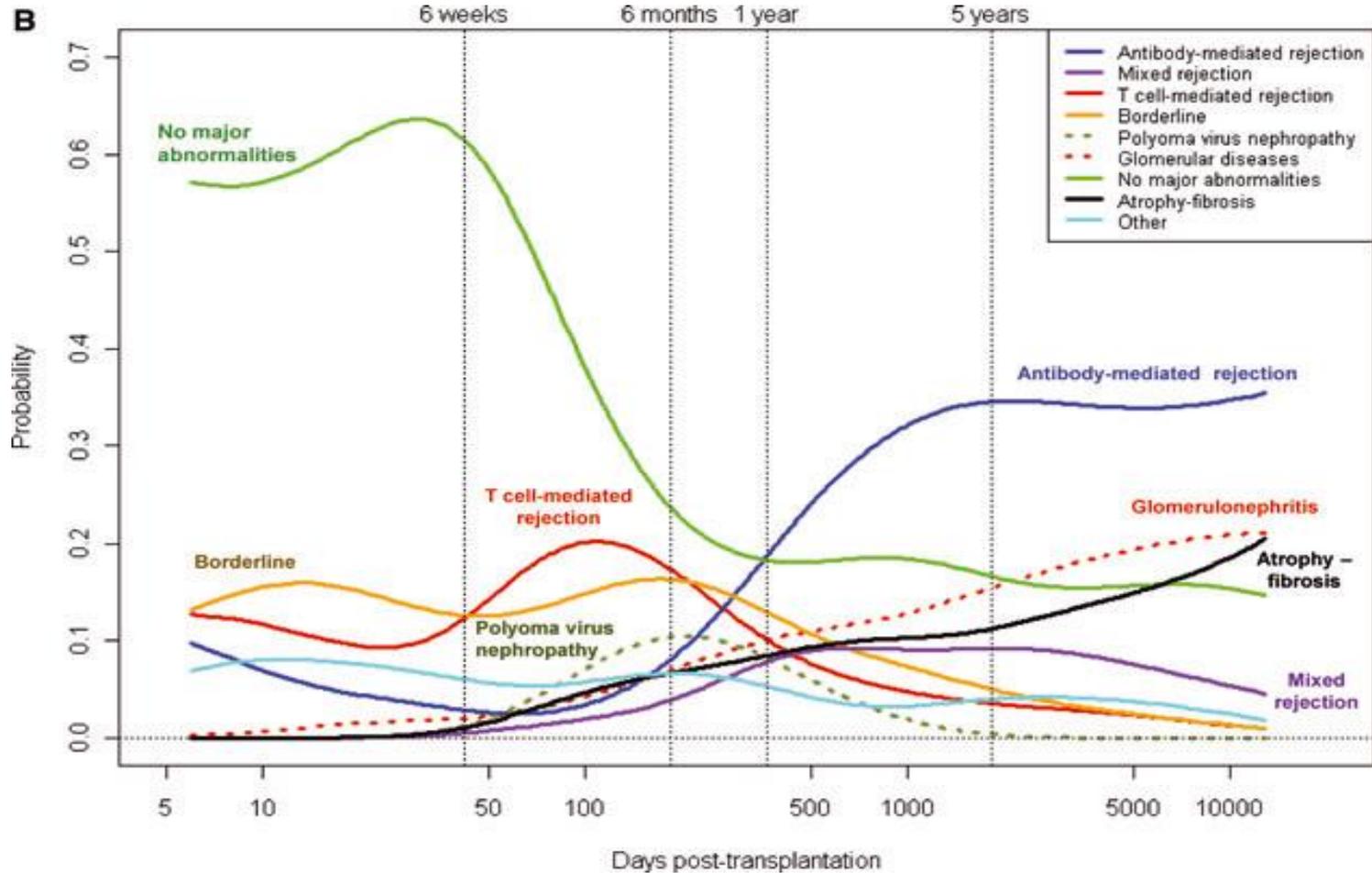
Overleving na niertransplantatie



Patiënten in leven met niertransplantaat



Waarom verliezen we nieren?



Therapietrouw

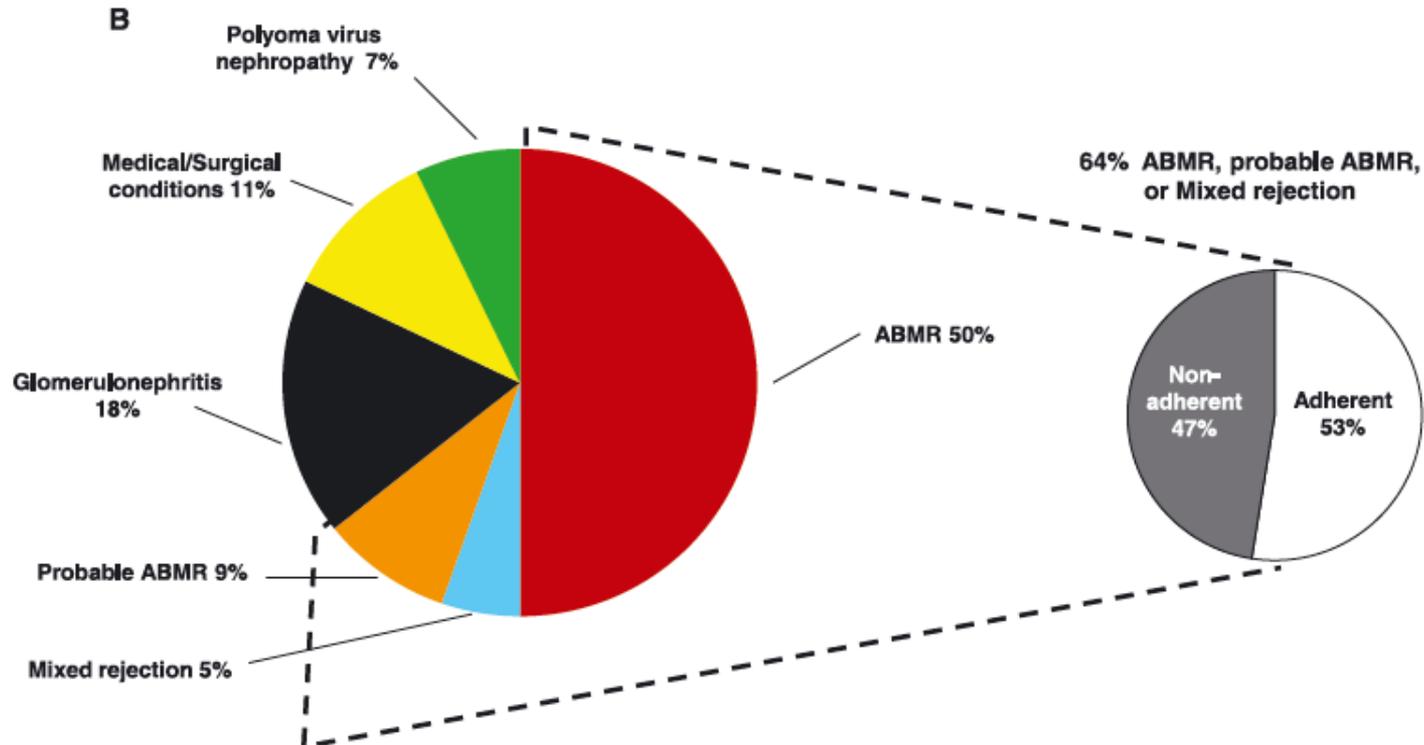
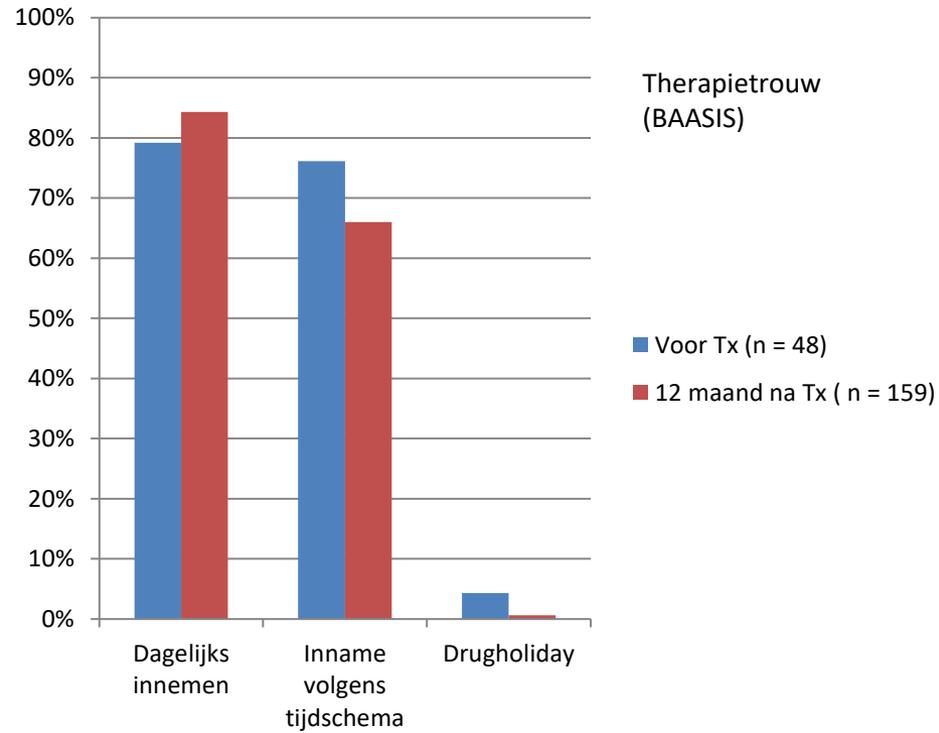
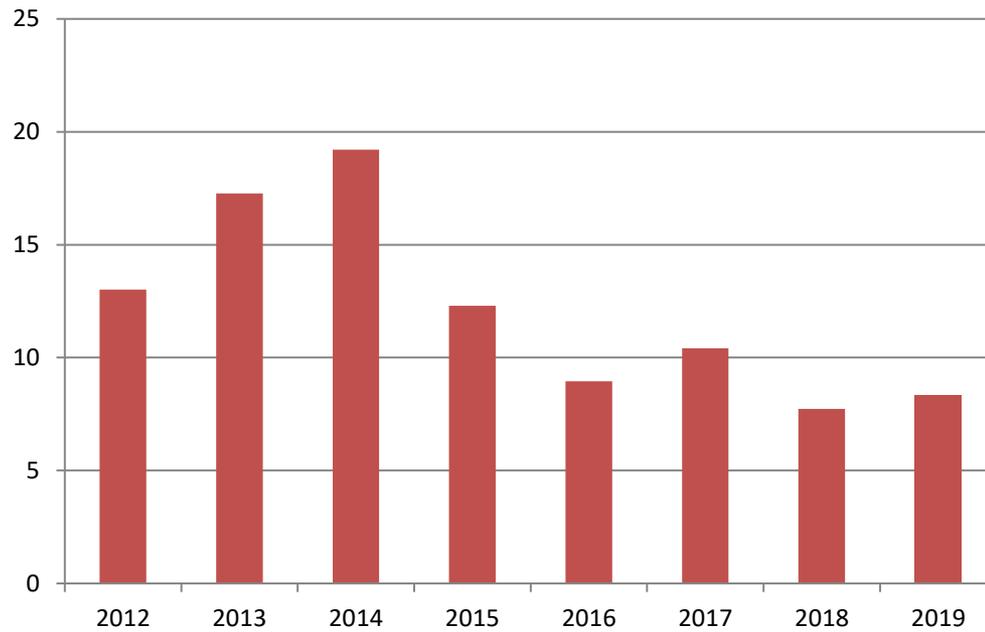


Figure 3: Attributed causes of graft failure in the biopsy-for-cause population. (A) Distribution of the attributed causes of failure (columns) according to the histological diagnosis in the last biopsy available per patient (rows). (B) Distribution of attributed causes of failure. Failures that could not be attributed due to missing clinical information are not represented (n = 4).

Therapietrouw

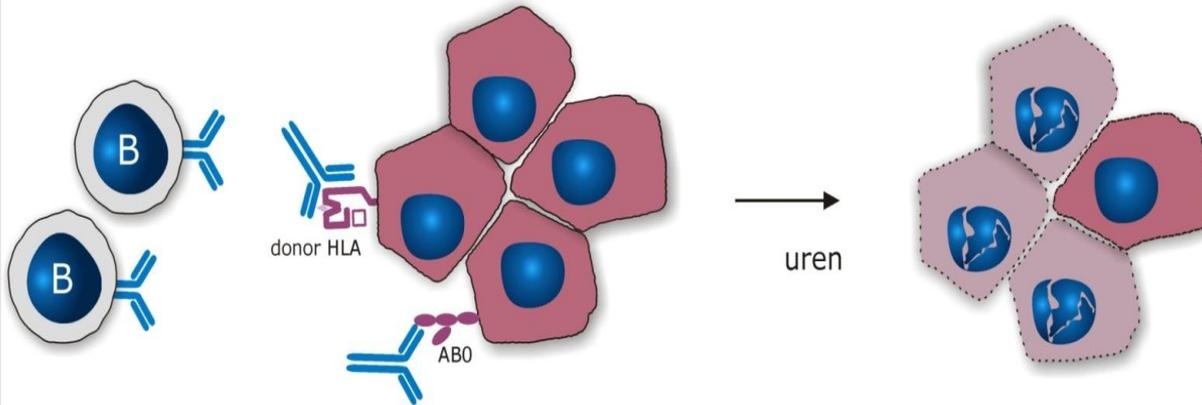


Rejectie eerste jaar na niertransplantatie

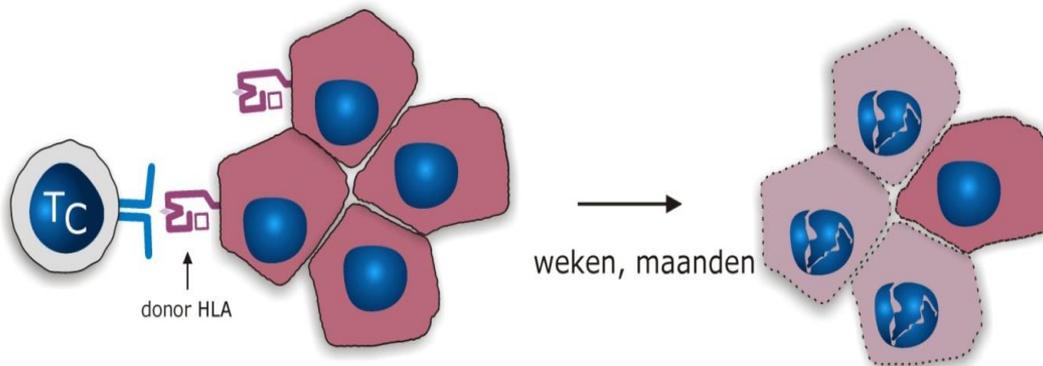


Vroege afstoting

Hyperacute afstoting: anti-HLA, anti-ABO antilichamen

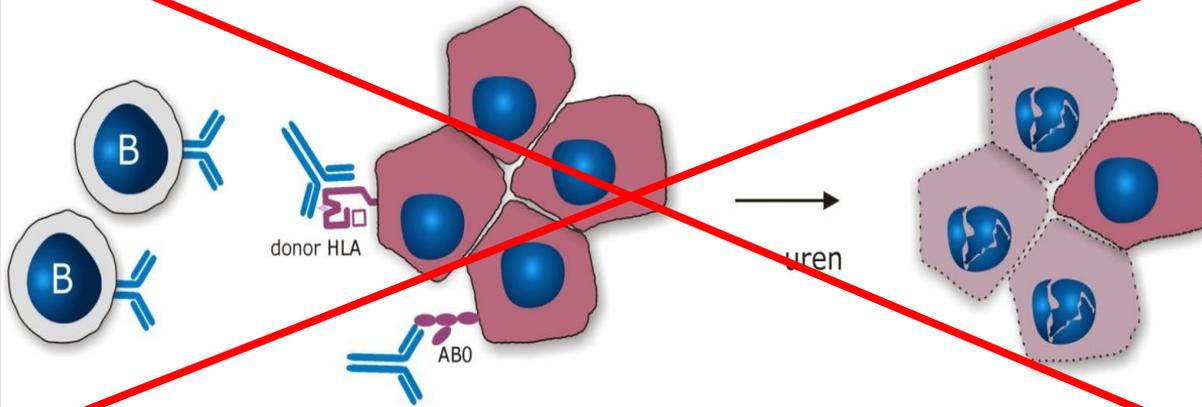


Acute afstoting: anti-HLA, anti-minor T-lymfocyten

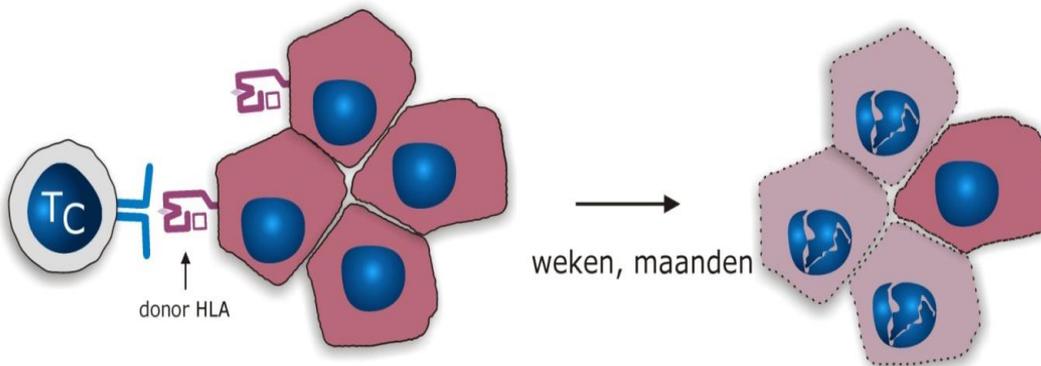


Vroege afstoting

Hyperacute afstoting: anti-HLA, anti-ABO antilichamen



Acute afstoting: anti-HLA, anti-minor T-lymfocyten



Rejectie pathologie
Acute (actieve) rejectie
tubulitis
interstitiele inflammatie
glomerulitis,
peritubulaire capillaritis
arteritis.

Rejectiebehandeling

- Methylprednisolon
- ATG
- Alemtuzumab (anti-CD52)
- Plasmaferese
- Ivlg

ATG

- konijn anti-thymocyte globulin (rATG)
- De “standaard” T cell-depleterende antistof
- Nadelen ->
 - Toegang
 - Cytokine release syndroom
 - Serum-ziekte
 - Langere opname
 - Infecties

Alemtuzumab

- Monoclonaal anti-CD52
- Eenmalige subcutane injectie
- Gelijkwaardig aan ATG in historische case-series minder infusie gerelateerde bijwerkingen en kortere opname
- Nadelen ->
 - Auto-immuun fenomenen (Guillain-Barre)
 - Infecties

Antistof gemedieerde rejectie

- Frequent voorkomend
- Belangrijke reden voor laat transplantaatfalen
- Herkenning?
 - C4d positief: maar waar zijn de antilichamen?
 - Anti-HLA antistoffen
- Humoraal versus cellulair?
- Preventie?/Interventie?

Revised Banff 2017 classification of antibody-mediated rejection in renal allografts

Active ABMR; all 3 criteria must be met for diagnosis* ¶

1. Histologic evidence of acute tissue injury, including one or more of the following:

Microvascular inflammation ($g > 0^{\Delta}$ and/or $ptc > 0$), in the absence of recurrent or de novo glomerulonephritis, although in the presence of

Intimal or transmural arteritis ($v > 0$)[◇]

Acute TMA, in the absence of any other cause

Acute tubular injury, in the absence of any other apparent cause

2. Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following:

Linear C4d staining in ptc (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections)

At least moderate microvascular inflammation ($[g + ptc] \geq 2$)[§]

Increased expression of gene transcripts/classifiers in the biopsy tissue strongly associated with ABMR, if thoroughly validated[‡]

3. Serologic evidence of DSAs (HLA or other antigens). C4d staining or expression of validated transcripts/classifiers as noted above in criteria 2. If HLA antibody testing is negative, is strongly advised whenever criteria 1 and 2 are met.

Revised Banff 2017 classification of antibody-mediated rejection in renal allografts

Active ABMR; all 3 criteria must be met for diagnosis* 1

1. Histologic evidence of acute tissue injury, including one or more of the following:

Microvascular inflammation ($g > 0^A$ and/or $ptc > 0$), in the absence of recurrent or de novo glomerulonephritis, although in the presence of

Intimal or transmural arteritis ($v > 0$)^o

Acute TMA, in the absence of any other cause

Acute tubular injury, in the absence of any other apparent cause

2. Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following:

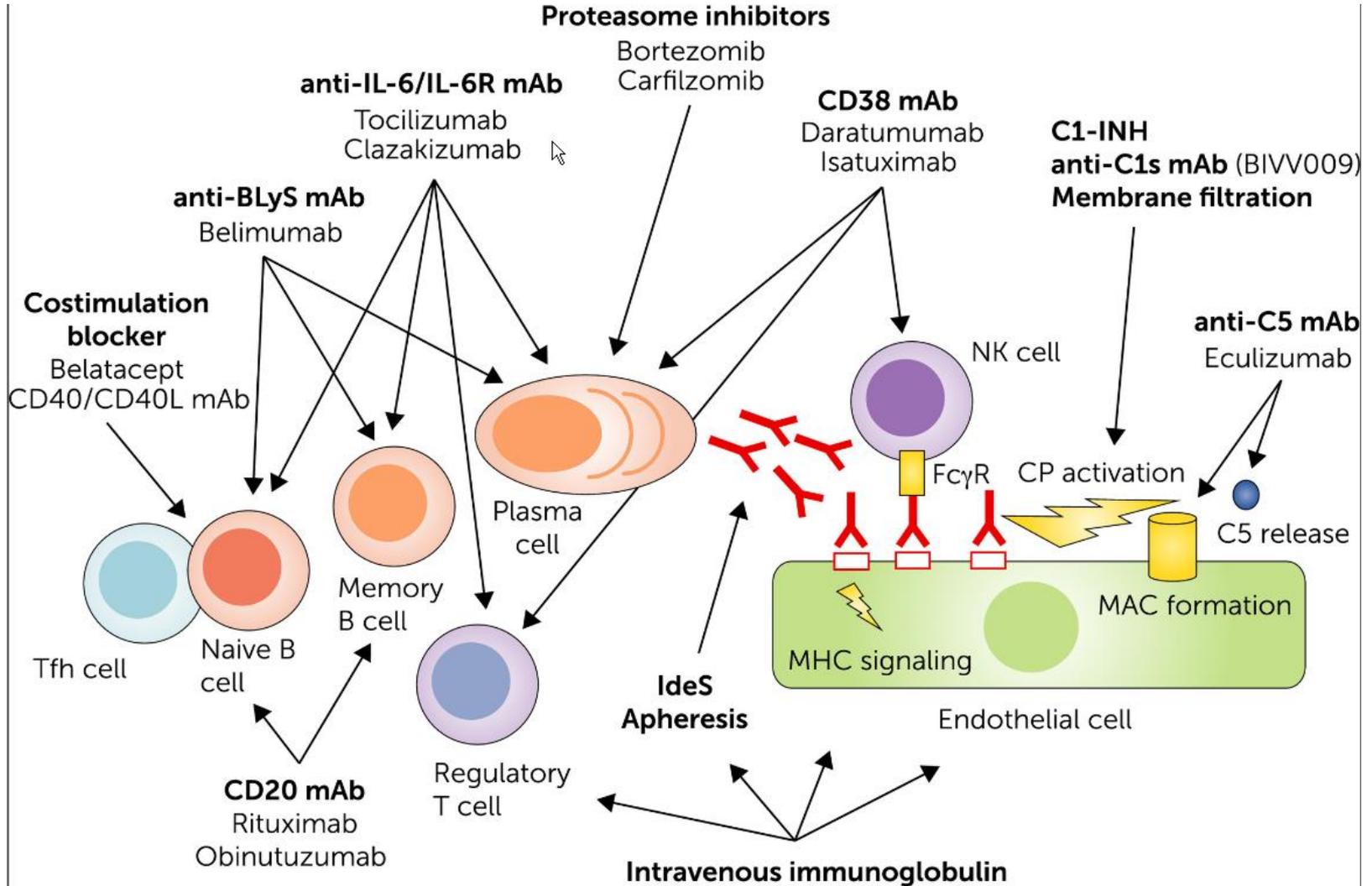
Linear C4d staining in ptc (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections)

At least moderate microvascular inflammation ($[g + ptc] \geq 2$)⁵

Increased expression of gene transcripts/classifiers in the biopsy tissue strongly associated with ABMR, if thoroughly validated^z

3. Serologic evidence of DSAs (HLA or other antigens). C4d staining or expression of validated transcripts/classifiers as noted above in criteria 2. If HLA antibody testing is negative, is strongly advised whenever criteria 1 and 2 are met.

Therapie?

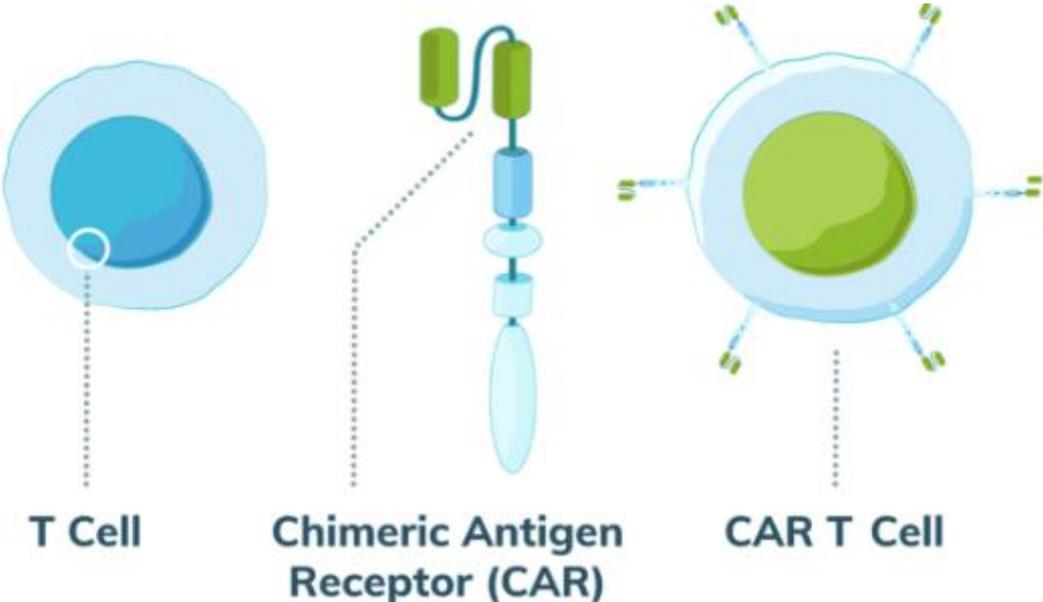


Behandeling humorale rejectie

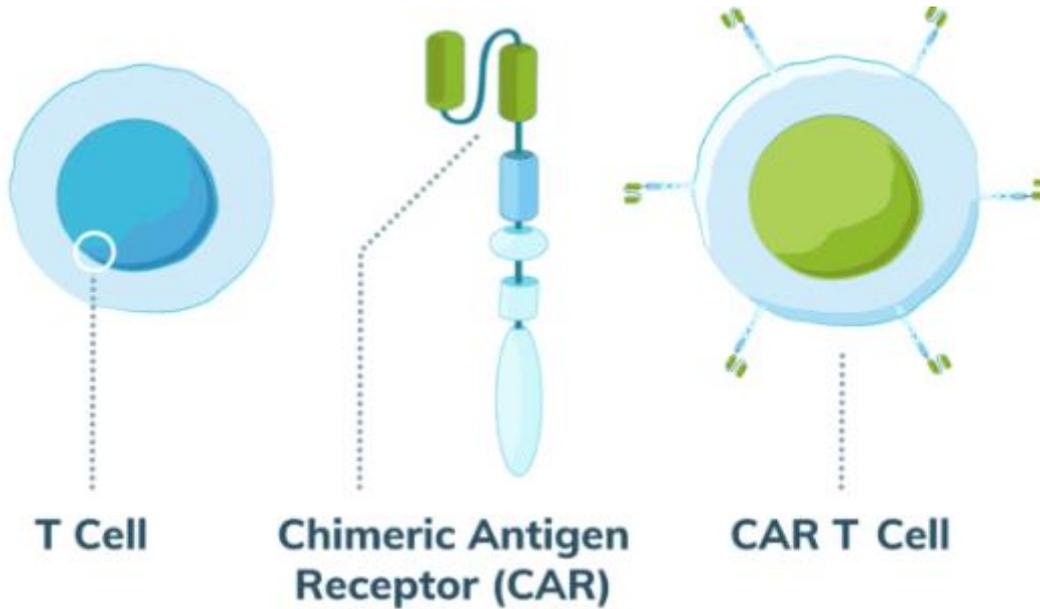
- Methylprednisolon, Ivlg, Plasma-exchange
- IVIG/rituximab, bortezomib en eculizumab – teleurstellend
- complement blockade, C1 esterase inhibitor
- anti-IL6 therapie, tocilizumab?

- Standard of care en goede studies noodzakelijk!

CAR T cel

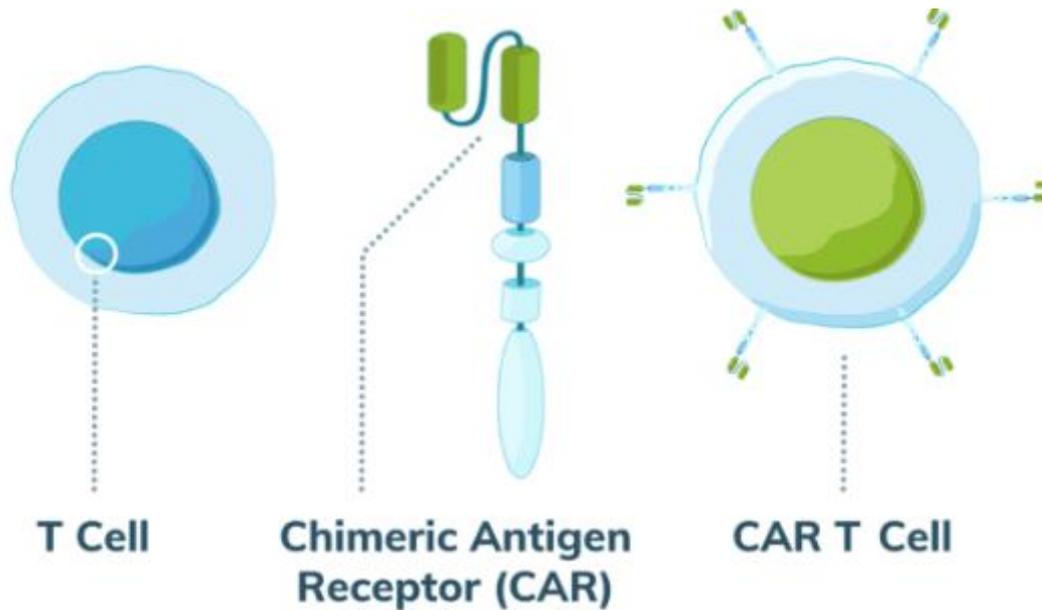


CAR T cel therapie



- Effectief
- Cytokine release syndroom
- Duur

CAR T cel therapie

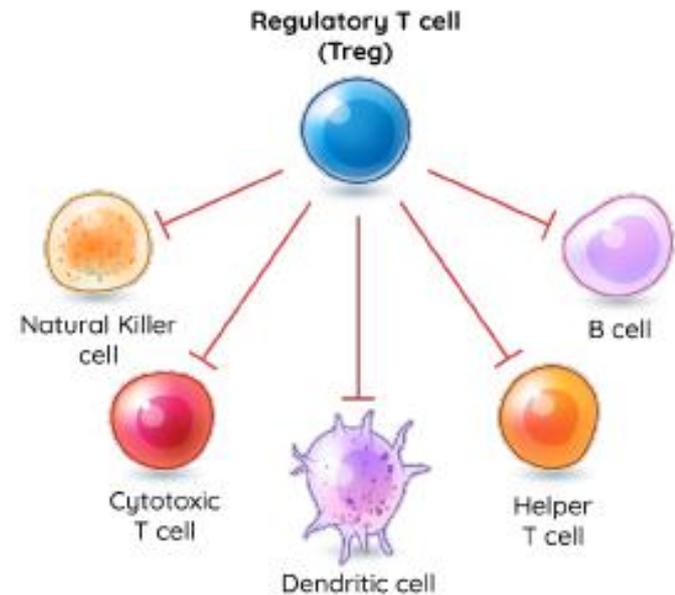


- Effectief
- Cytokine release syndroom
- Duur

DE WEDLOOP NAAR "CARS" IN DE
BEHANDELING VAN KANKER - AAN DE
VOORAVOND VAN NIEUWE THERAPIEËN

T regs

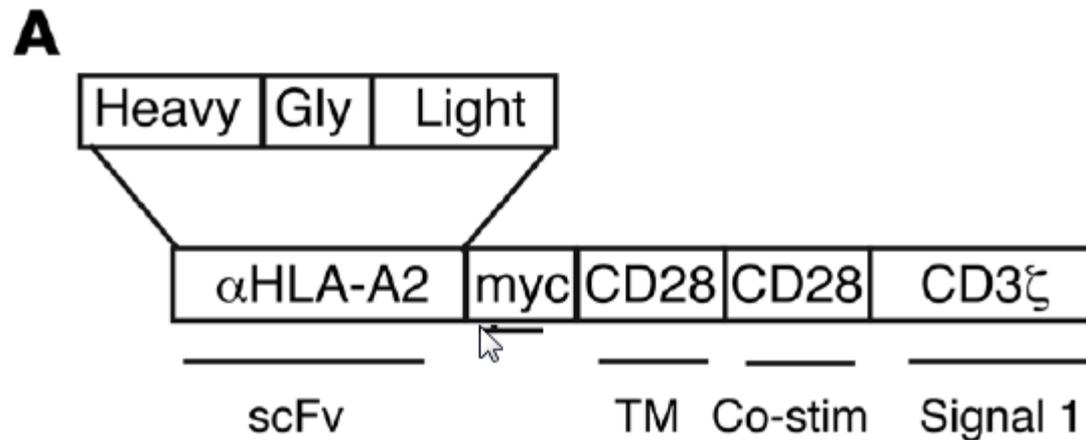
- Gespecialiseerde T cellen die regulerend werken
- 1-2% lymfocyten
- Voorkomen ontsteking en auto-immuunziekte



Alloantigen-specific regulatory T cells generated with a chimeric antigen receptor

Katherine G. MacDonald,¹ Romy E. Hoeppli,¹ Qing Huang,² Jana Gillies,¹ Dan S. Luciani,¹ Paul C. Orban,¹ Raewyn Broady,² and Megan K. Levings¹

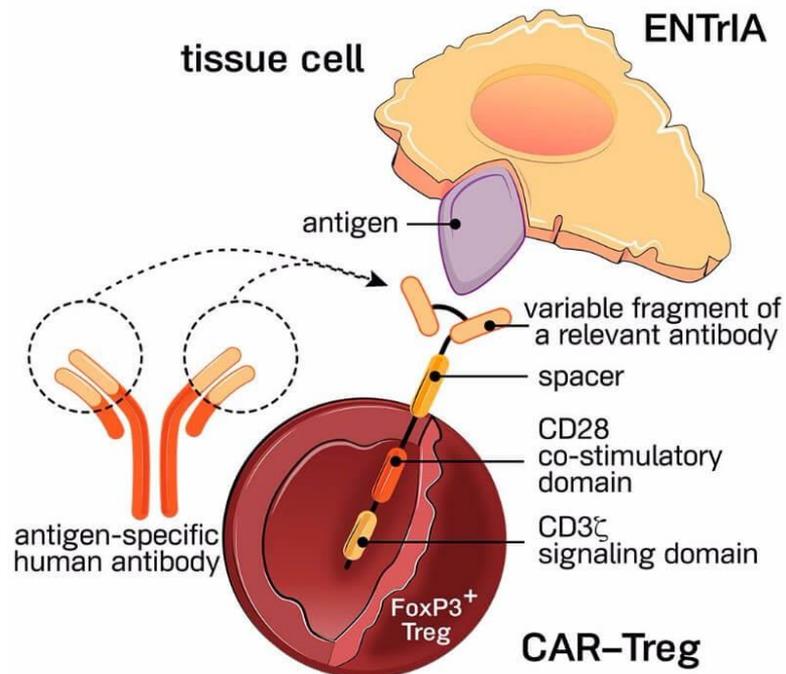
¹Department of Surgery and ²Department of Medicine, University of British Columbia, and Child and Family Research Institute, Vancouver, British Columbia, Canada.



Alloantigen-specific regulatory T cells generated with a chimeric antigen receptor

Katherine G. MacDonald,¹ Romy E. Hoeppli,¹ Qing Huang,² Jana Gillies,¹ Dan S. Luciani,¹ Paul C. Orban,¹ Raewyn Broady,² and Megan K. Levings¹

¹Department of Surgery and ²Department of Medicine, University of British Columbia, and Child and Family Research Institute, Vancouver, British Columbia, Canada.

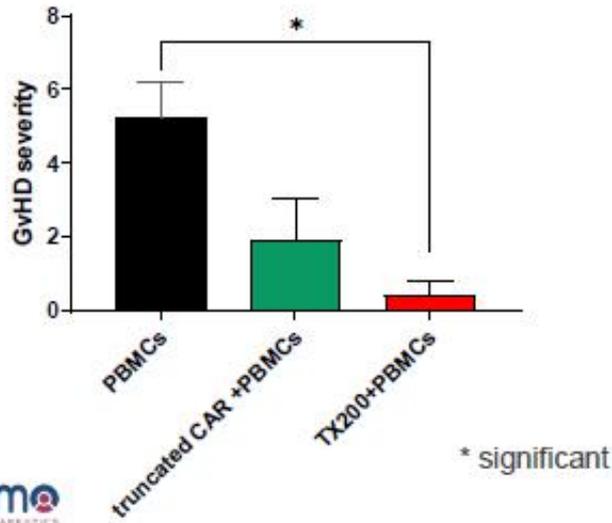


In dierstudies

GvHD model



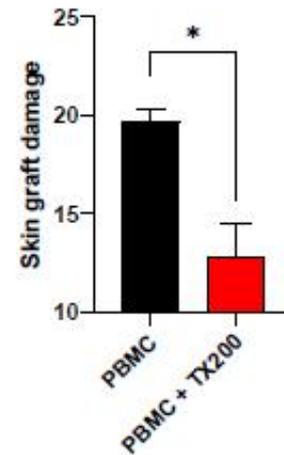
Disease severity



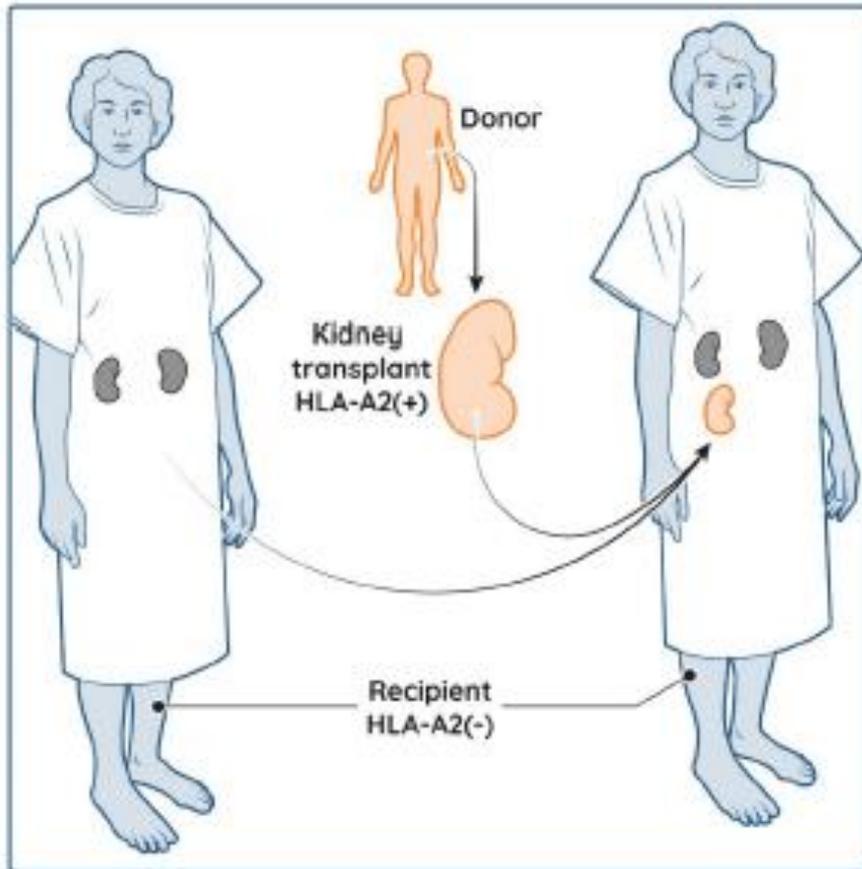
Skin transplant model



Graft Rejection score

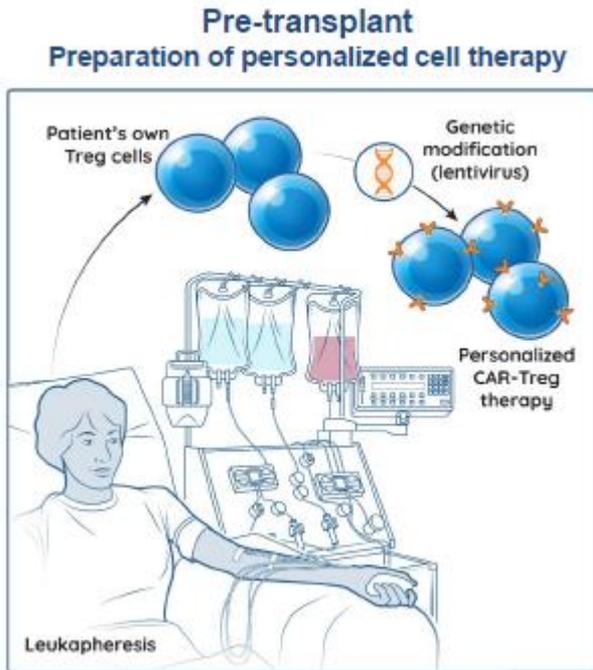


Steadfast studie



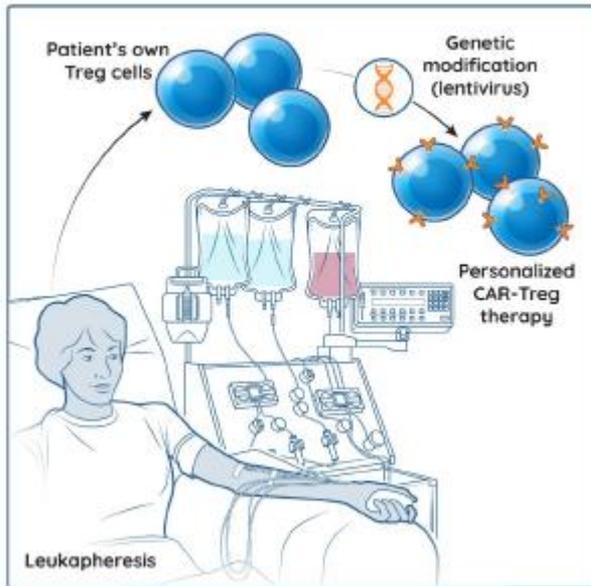
- **Fase 1-2 studie**
- **SAFETY**
- Reguleren het immuunsysteem op gerichte wijze
- Bevorderen immunologische tolerantie naar het niertransplantaat
- Helpen orgaan functie te behouden en voorkomen verlies

Steadfast studie

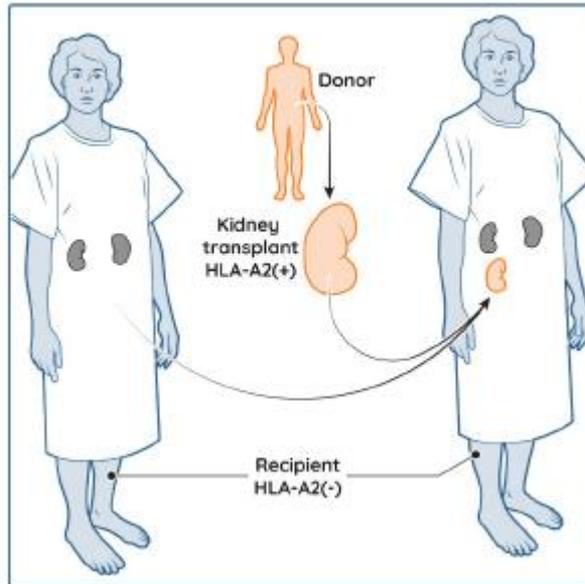


Steadfast studie

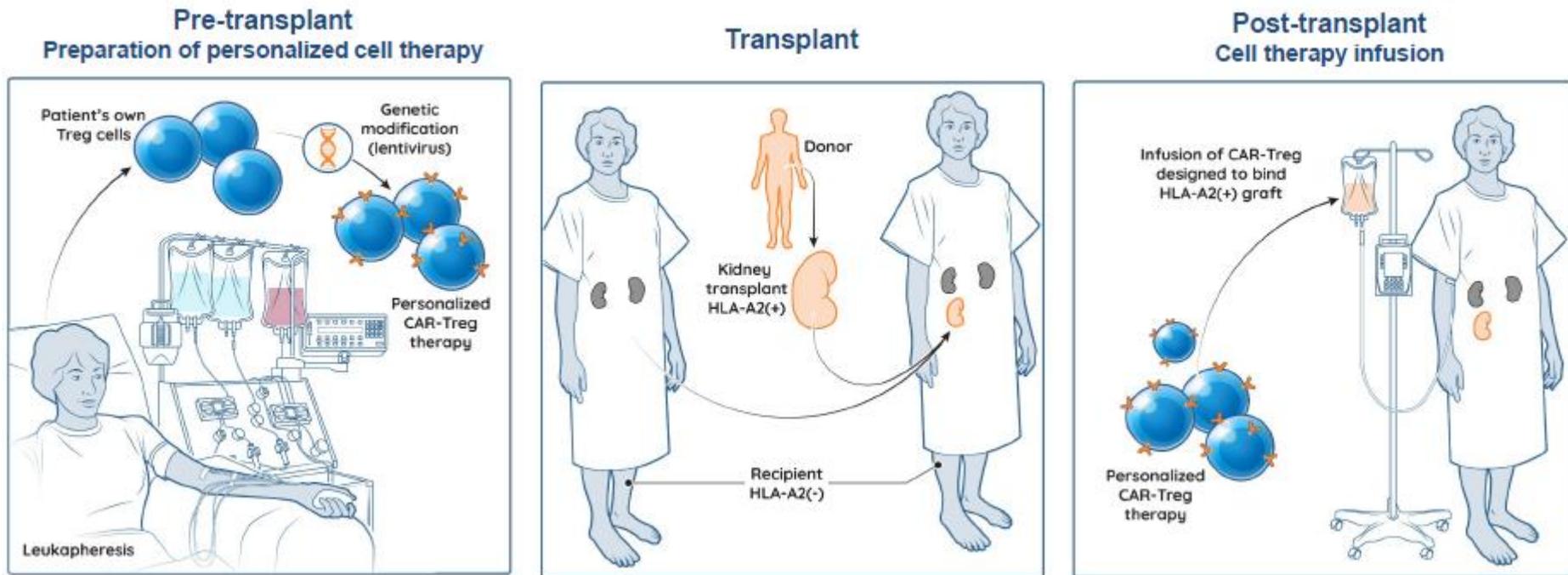
Pre-transplant Preparation of personalized cell therapy



Transplant



Steadfast studie



Multicenter, open label studie
met oplopende dosis

Studie eindpunten

- Primair

Veiligheid in eerste 28 dagen

- Secundair

Effect op niertransplantatie uitkomst

Reductie van immuunsuppressie

Lokalisatie van CAR Tregs in transplantaat

Effect op chronische resectie

Conclusies

- Rejectie

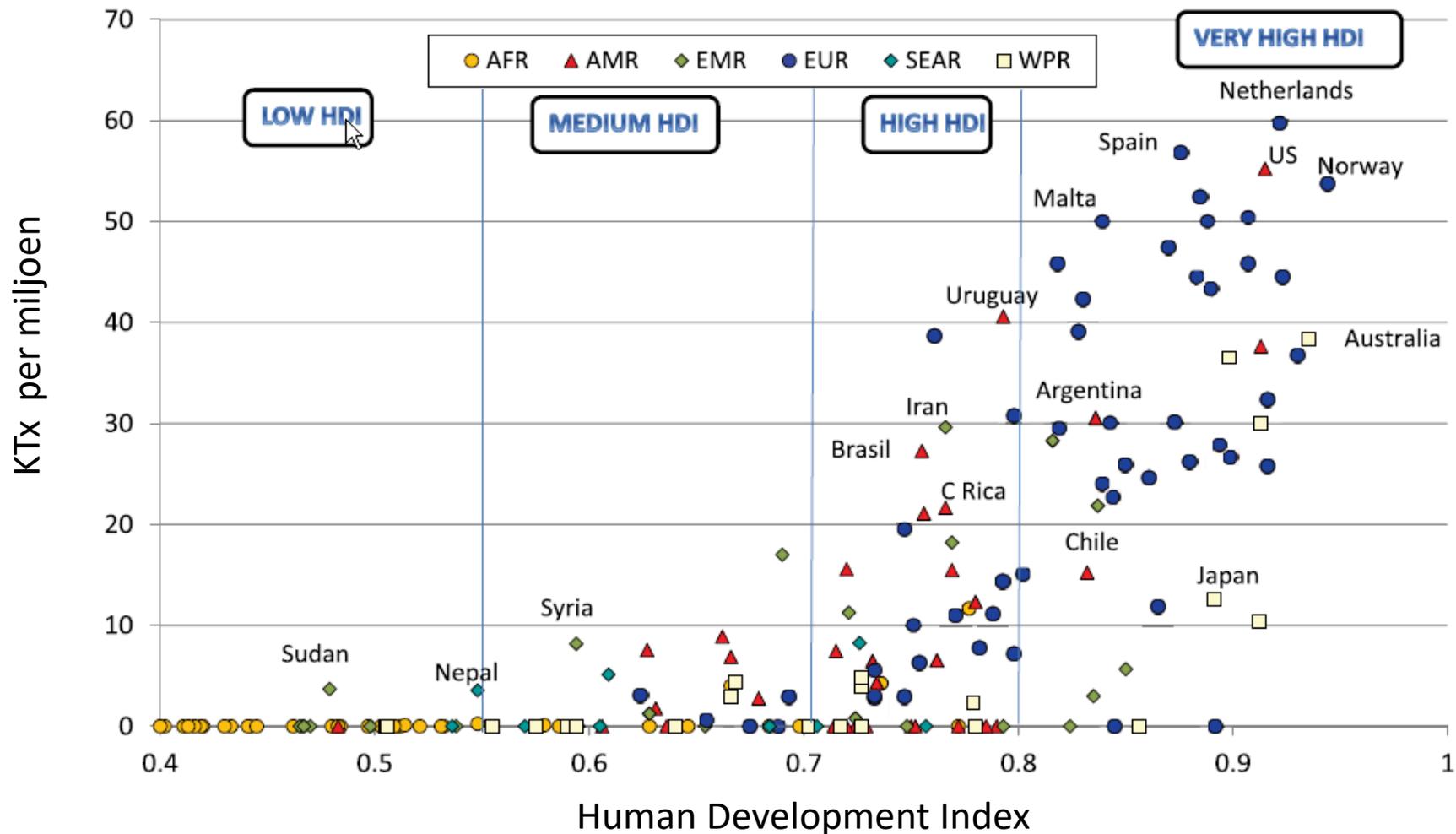
Voorkomen is beter dan genezen

Late chronische afstoting is lastig behandelbaar

- CAR T cellen

Mogelijk veelbelovende nieuwe therapie

Wereldwijde ongelijkheid...





"SIDE-EFFECT?
WHAT SIDE-EFFECT?"



University Medical Center Groningen





HLA antibodies

CDC:

1955

Complement Dependent Cytotoxicity

-cell panel of > 30 individuals

-incubate with patient serum + complement

-analyze specificities by pos./neg. reactions

+/- DTT:

-IgM HLA

-IgG HLA

-auto antibodies

NIH: Nat. Inst. Health
Ting: Alan Ting
TCF: two colour fluoresc
=B-cell specific.

Sera Scr SQ-2007-02

SQ-2007-02- 12

Lab.nr. : 202087

Laboratorium voor Transplantatie Immunologie, UMCC

LAF + +

nr	Naam	-DTT			+DTT			bgr	4	6	A-locus	B-locus	C-locus	DR-locus	DQ-locus					
		NIH	Ting	TCF	NIH	Ting	TCF													
202206	Marco Bijleveld	—	—	—	—	—	—	A+	+	+	2	29(19)	7	44(12)	7		7	8	2	4
202207	P.Bijleveld- Delden	—	—	—	—	—	—	A-	+	+	24(9)	26(10)	51(5)	18	7		11(5)		3	
P1159	Boukje d.Haan-S.	—	—	—	—	—	—	O+	+	+	2	32(19)	51(5)	703	10(3)	14	1	4	5(1)	7(3)
P1132	Erica Konneman-Z.	—	—	—	—	—	—	A+	+	3	31(19)	51(5)	47	6	15	13(6)	14(6)	5(1)	6(1)	
P1998	Hester Balkker	—	—	—	—	—	—	A+	+	3	26(10)	38(16)	35	4	1203	1	12(5)	5(1)	7(3)	
P1827	Elinda Bruin-v. Dyk	—	—	—	—	—	—	A-	+	+	24(9)	68(28)	44(12)	62(15)	9(3)	1601	13(6)	7	6(1)	2
P1337	Gerard Nyenboer	—	—	—	—	—	—	A-	+	+	24(9)	31(19)	44(12)	60(40)	10(3)	5	103	8	5(1)	4
P1996	Guus Kloosterhuis	—	—	—	—	—	—	O+	+	+	29(19)	32(19)	44(12)	35	4	1601	13(6)	7	6(1)	2
P1650	Sicco vd Heide	—	—	—	—	—	—	A-	-	+	3	32(19)	7	64(14)	7	8	15(2)	13(6)	1	
P2245	Arman Berghuis	—	—	—	—	—	—	O+	+	-	29(19)	32(19)	44(12)	58(17)	5	7	11(5)	12(5)	3	
P1024	Rene Houttuin	—	—	—	—	—	—	A+	-	+	2	23(9)	50(21)	60(40)	10(3)	5	13(6)		6(1)	7(3)
P1190	Dicky Idsinga	—	—	—	—	—	—	O+	+	+	24(9)	34(10)	75(15)	27	4	7	15(2)	12(5)	6(1)	7(3)
P1943	Ilby Bouwman	—	—	—	—	—	—	O+	-	+	24(9)	31(19)	35	60(40)	10(3)	4	1	4	5(1)	3
P2011	Bob Jansz	—	—	—	—	—	—	AB+	-	+	11	29(19)	7	18	7		4	10	5(1)	4
P1720	Tanja Mooibroek	—	—	—	—	—	—	O+	+	+	1	2	35	37	4	6	4	10	5(1)	3
P1099	Bert Winter	—	—	—	—	—	—	O-	-	+	1	11	8	56(22)	1	7	17(3)	13(6)	6(1)	2
P1181	Alex Horst	—	—	—	—	—	—	B+	+	-	2	11	77(15)		8		12(5)	9	3	
P263	Rob Fucke	—	—	—	—	—	—	O+	-	+	2	66(10)	7	41	7	17	15(2)	13(6)	6(1)	7(3)
202270	Jager-Meinders.	—	—	—	—	—	—	A+	+	-	24(9)	30(19)	44(12)	13	5	6	13(6)	7	1	2
202279	Boer d.- Bakker	—	—	—	—	—	—	A+	+	+	3	24(9)	37	60(40)	3	6	4	13(6)	1	3
P1366	Joke Koens	—	—	—	—	—	—	A+	+	-	2	26(10)	49(21)	27	1	7	13(6)		1	
P1680	Jeanet Drenth-H.	—	—	—	—	—	—	O+	+	-	30(19)	68(28)	13	57(17)	6		7		2	9(3)
202299	Smit '77	—	—	—	—	—	—	A+	-	+	1	2	8	62(15)	3	7	1	3	1	2
P1808	Hans Schulten	—	—	—	—	—	—	O+	+	-	11	30(19)	51(5)	27	2	16	16(2)	7	5(1)	2
P1473	Corrie t. Meerman	—	—	—	—	—	—	O+	+	+	26(10)	31(19)	39(16)	47	6	12	16(2)		5(1)	
202334	Bozker- Tasci	—	—	—	—	—	—	O+	-	+	3	29(19)	39(16)	35	4		1	16(2)	1	
P1294	Jacobien Noordhoek	—	—	—	—	—	—	B-	+	+	2	29(19)	62(15)	27	2	10(3)	0103	11(5)	5(1)	7(3)
202392	Rook-v. Westenbrugge	—	—	—	—	—	—	O+	-	+	1	25(10)	62(15)	18	3		15(2)	4	1	3
P1057	Nick Scholtens	—	—	—	—	—	—	O+	+	+	2	11	45(12)	27	2	6	1	9	5(1)	2
P1898	Harry Pijper	—	—	—	—	—	—	A+	+	+	1	3	57(17)	55(22)	9(3)	6	3	13(6)	6(1)	2
202438	E.Berkel	—	—	—	—	—	—	A+	+	+	1	3	8	27	2		4	11(5)	3	
P1019	Els vd Boomgaard	—	—	—	—	—	—	A+	-	+	24(9)	26(10)	8	55(22)	9(3)	7	17(3)	4	2	7(3)
202482	Niklaas-B '78	—	—	—	—	—	—	O+	-	+	1	3	8	35	4	7	1	7	5(1)	3
202481	D.Niklaas '03	—	—	—	—	—	—	O+	+	+	1	2	51(5)	8	7		14(6)	7	1	3
202474	H. Ellert '49	—	—	—	—	—	—	A+	-	+	2	25(10)	8	60(40)	3	7	15(2)	3	6(1)	2
202476	F. Ellert '57	—	—	—	—	—	—	A+	-	+	1	3	8	62(15)	3	7	3	13(6)	1	2
P1373	Simon Lems	—	—	—	—	—	—	O+	-	+	1	30(19)	7	8	7		15(2)	14(6)	5(1)	6(1)
P1658	Inge Masolijn	—	—	—	—	—	—	A+	+	+	1	23(9)	8	49(21)	7		4	11(5)	3	3
P1999	J.W. Pomper	—	—	—	—	—	—	A+	-	+	1	3	64(14)	39(16)	5	8	15(2)	13(6)	1	

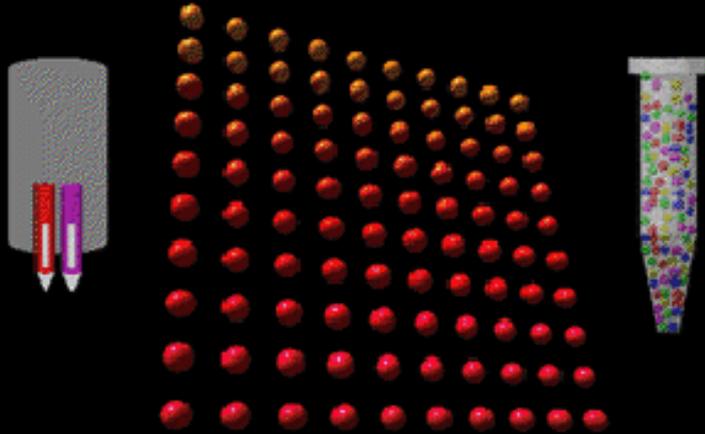
Panel	Reaction	Conclusion
<u>Own cell:</u>		
A1, A3, B7, B8	-	neg.
<u>Random cells:</u>		
A2 , A9 , B7, B8	++	-> anti-A2? anti-A9?
A1, A2 , B7, B8	++	-> anti-A2
A1, A9 , B7, B8	++	-> anti-A9
A1, A3, B7, B15	++	-> anti-B15

HLA antibodies

Elisa: -coat cell-lysate of > 30 individuals
2002-2019 -incubate with patient serum
-incubate with anti-human IgG
-read in Elisa reader pos. / neg.

LSA: -use single HLA-antigen coated beads
2009 -incubate with patient serum
-incubate with anti-human IgG (flu)
-run on Luminex Machine and analyze
2013 -complement binding C1q/C3d

100 Color-codes = 100 Simultaneous Tests



Using this method, over 100 distinct
microsphere sets can be created.



HLA antibodies, techniques

Sensitivity: CDC < ELISA < Luminex

CDC: functional (*in vitro*)
Clinical relevance proven
cross match

Solid phase: only IgG (other conjugate)
cut off?
Clinical relevance?
Complement binding?
DSA monitoring post-tx

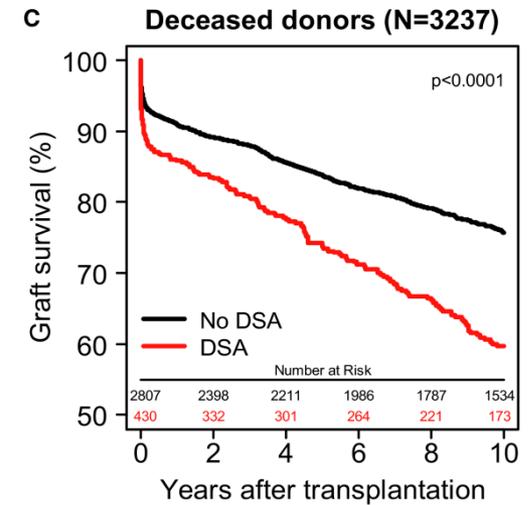
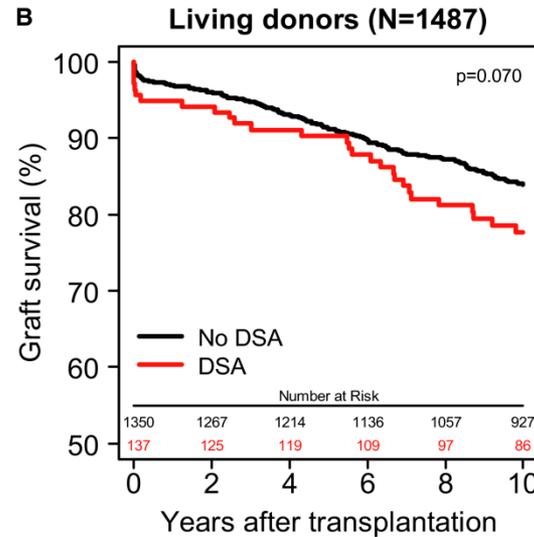
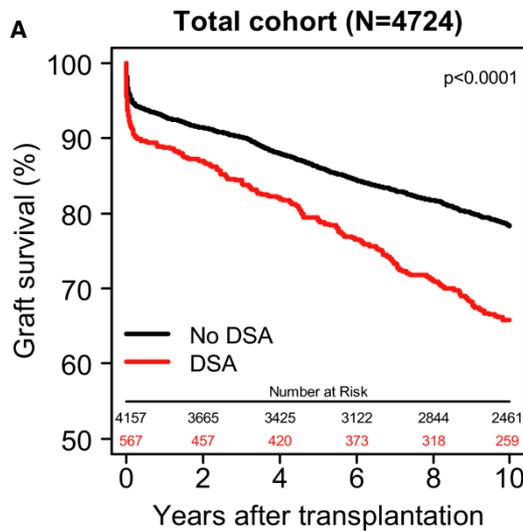
Induction of HLA antibodies:

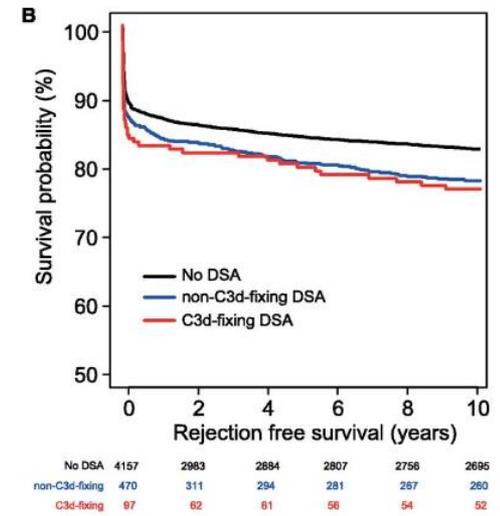
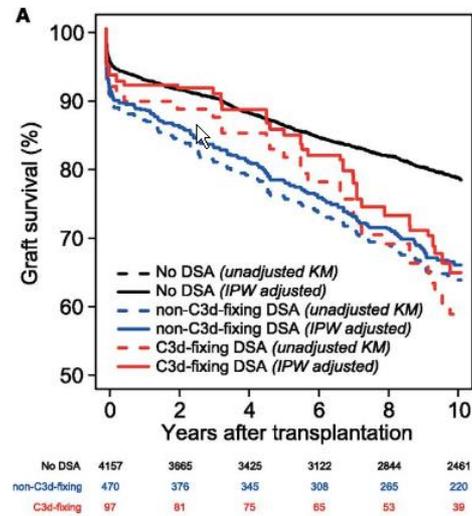
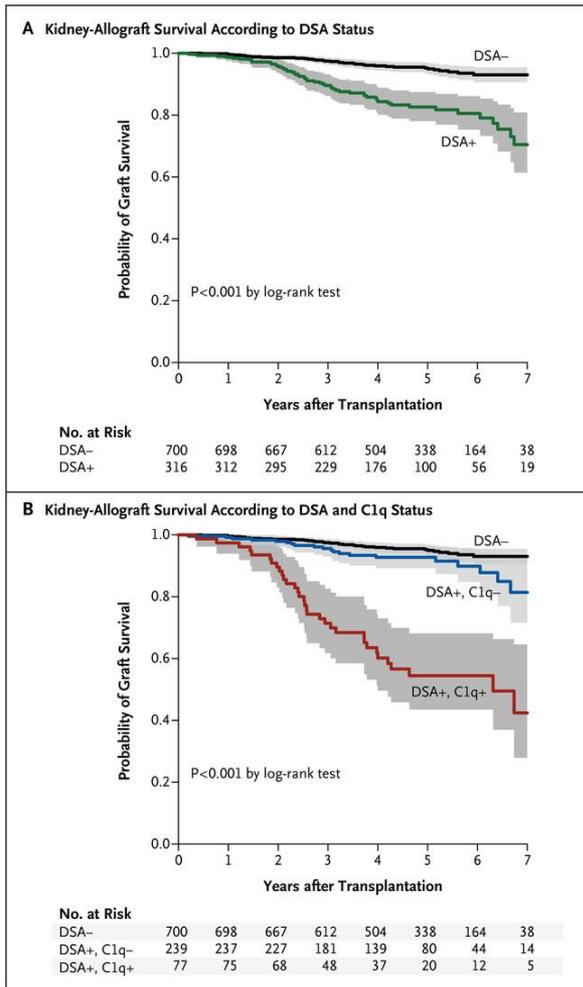
- pregnancy
- transplantation
- blood transfusion
- (rare: “natural” antibodies)

Screening of patients: 4x year

- Donor reactive antibodies are relative contra-indication for transplantation

Differential effects of DSA in living versus deceased donor transplant recipients





Kamburova et al. JASN 2018

Loupy A et al. N Engl J Med 2013

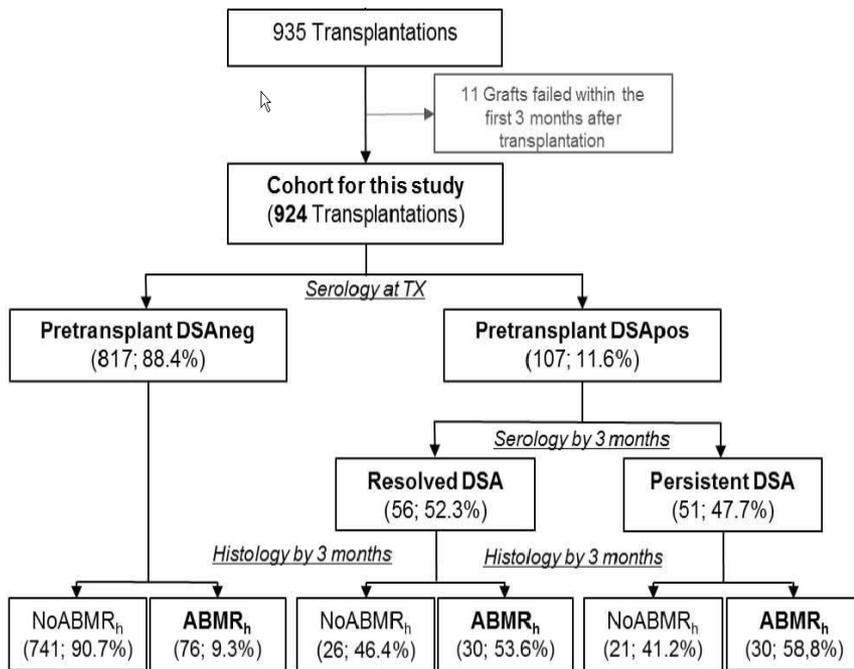


Figure 2. Kaplan-Meier analysis of death-censored graft survival after three months post-transplant (N=924).

(A)

