

**De basis, van DNA via RNA naar
whole genome sequencing,
exome sequencing en
genenpanels
Lessons learned from primary
immunodeficiency**

Virgil Dalm

Internist-klinisch immunoloog

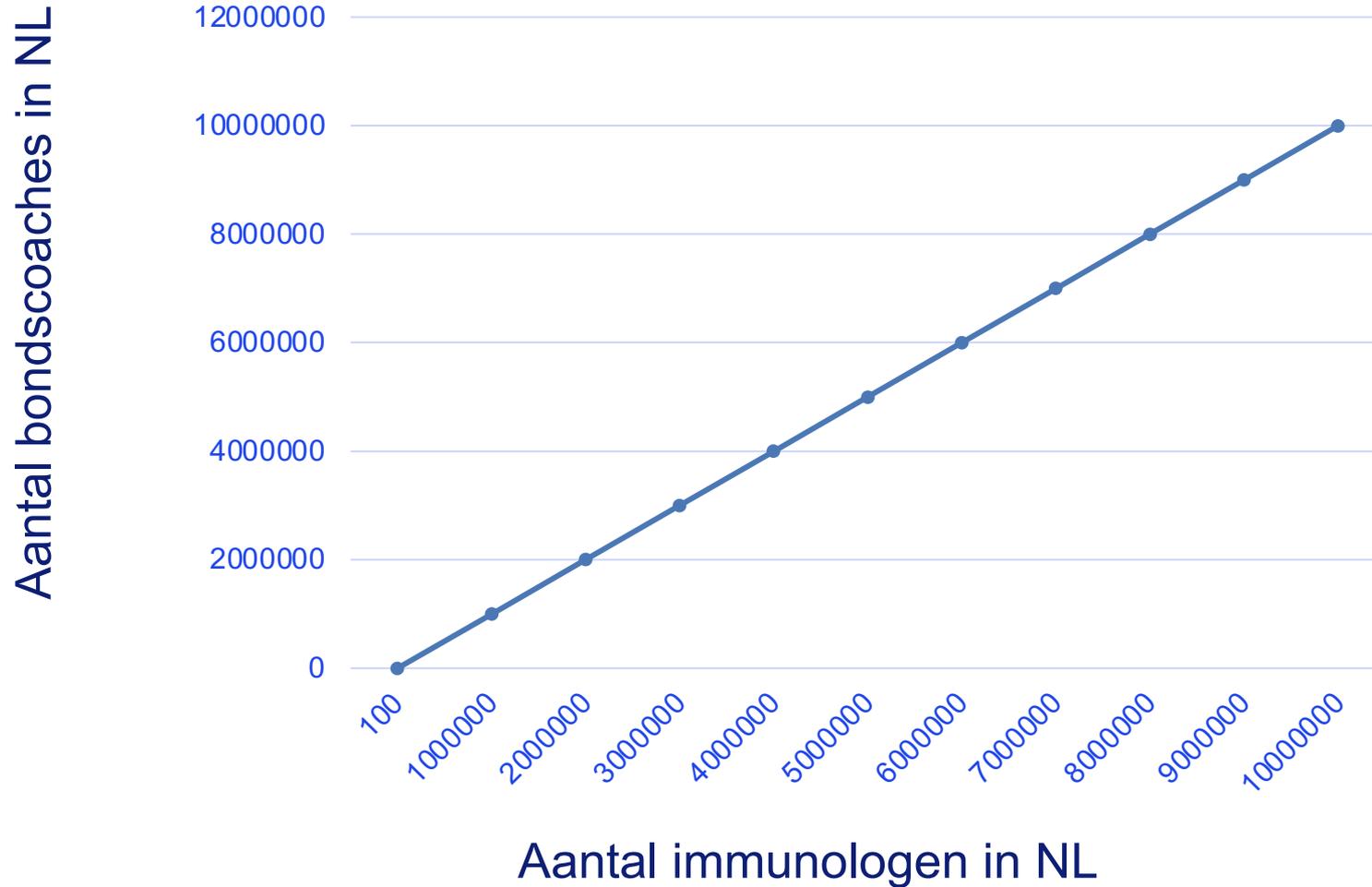
Afdeling Inwendige Geneeskunde

Afdeling Immunologie

Erasmus MC

- **Het immuunsysteem**
- **Primaire immuundeficiëntie (aangeboren afweerstoornis)**
- **Nieuwe diagnostiek**
- **Gerichte en gepersonaliseerde behandeling**
- **Toekomst**

Immunologie in 2021



Bescherming tegen micro-organismen

- Bacterien
- Virussen
- Schimmels
- Parasieten



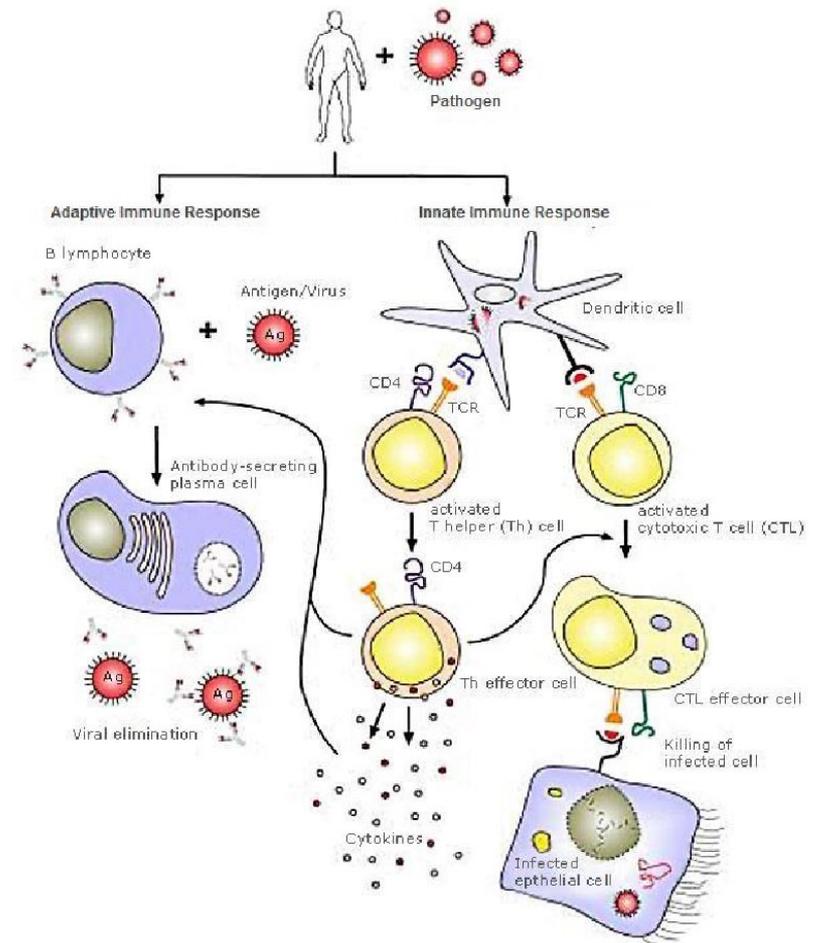
Het immuunsysteem : de basis

Witte bloedcellen
T-lymfocyten
B-lymfocyten
Granulocyten
Macrofagen

Eiwitten
Complement

Antistoffen / antilichamen

Cytokinen

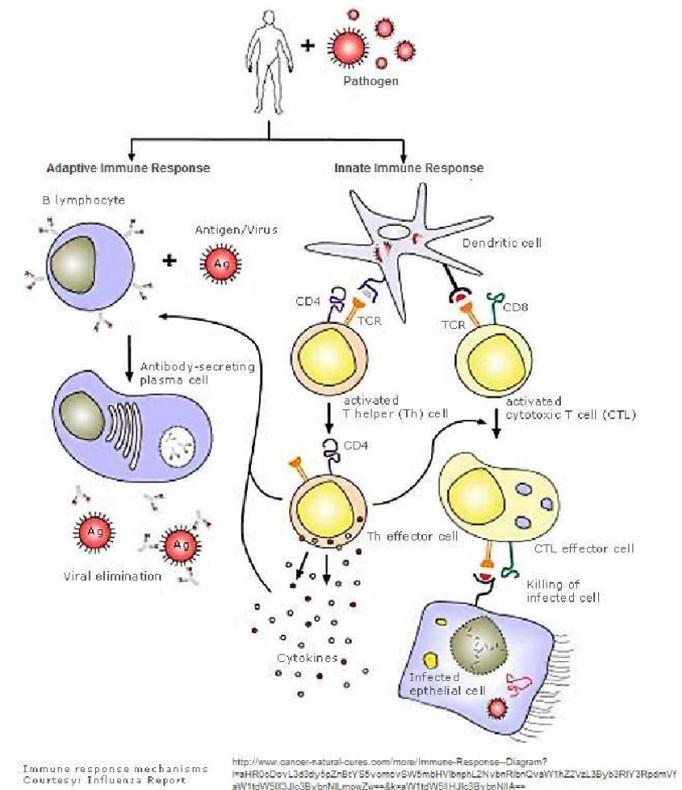


Immune response mechanisms
Courtesy: Influenza Report

<http://www.cancer-natural-remedies.com/more/immune-Response-Diagram?fbclid=IwAR0sDovL3d3dy5pZnBtYS5vcmoVSW5mbHY7bnphL2Nvb3RlbnQvYV1hZ2ZvL3B5b3RlY3RpdmVfYW1tdW5k3Jic3BvbnNlmpaZw==&k=aW1tdW5k3Jic3BvbnNlA==>

Het perfecte huwelijk

- Samenwerking / Ondersteuning
- Overlap in functie
- Herkenning “eigen” en “niet-eigen”
- Verwijderen van binnendringers
- In staat te leren
- Tolerant voor wat ongevaarlijk is



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Immuunsysteem in balans



Geen infecties

Geen auto-immuunziekte

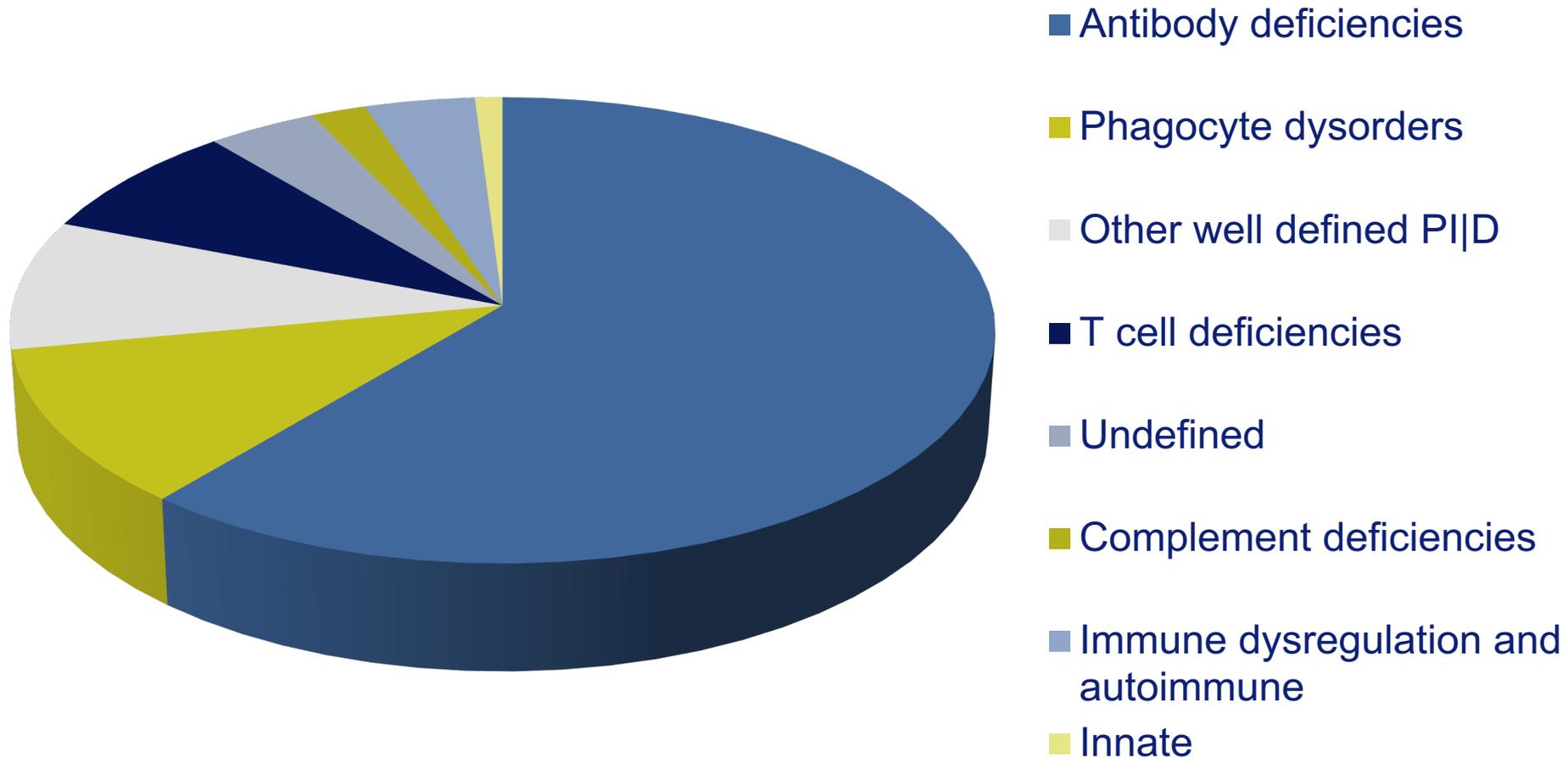
Immuunsysteem in balans



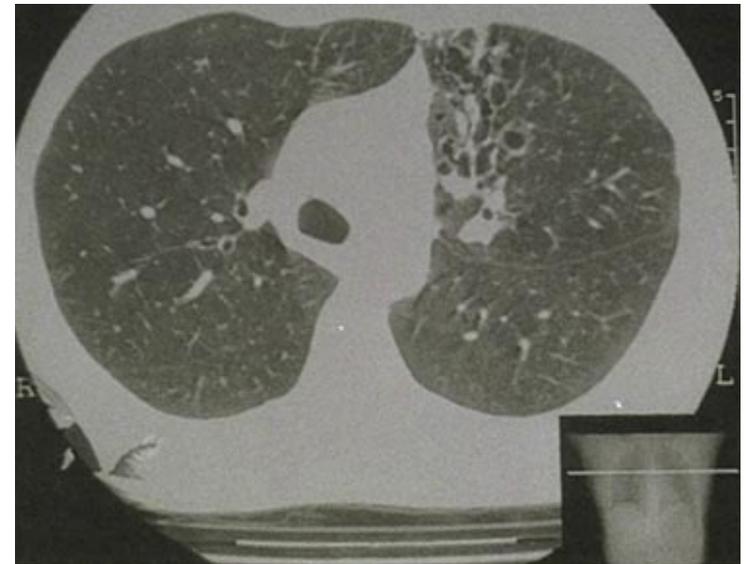
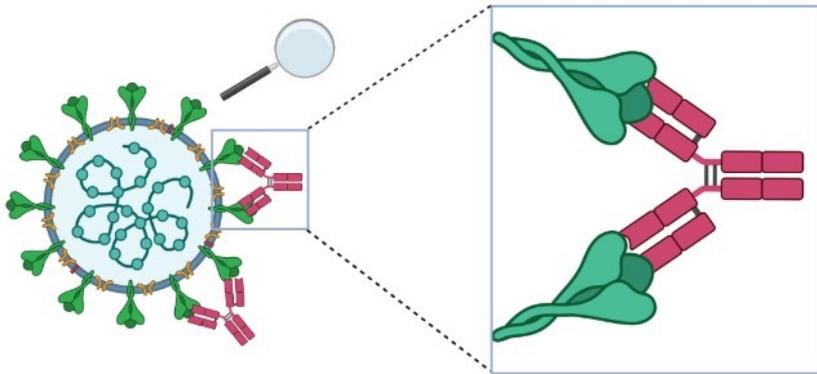
Overmatige infecties

- **Heterogene aandoeningen**
- **Afwijkingen in verschillende delen van immuunsysteem**
- **Kliniek afhankelijk van betrokken deel**
- **Gekenmerkt door terugkerende en ernstige infecties**
- **Inborns errors of immunity (IEI)**

Primaire immuundeficienties in Nederland



- Luchtweginfecties / maagdarminfecties
- Orgaanschade
- Kanker



- **Antibiotica**
bij infectie of onderhoud
- **Antistof toediening (immuunglobuline suppletie)**



J Clin Immunol (2018) 38:96–128
<https://doi.org/10.1007/s10875-017-0464-9>



ORIGINAL ARTICLE

International Union of Immunological Societies: 2017 Primary Immunodeficiency Diseases Committee Report on Inborn Errors of Immunity

Capucine Picard^{1,2} · H. Bobby Gaspar³ · Waleed Al-Herz⁴ · Aziz Bousfiha⁵ · Jean-Laurent Casanova^{6,7,8,9} · Talal Chatila¹⁰ · Yanick J. Crow^{11,12} · Charlotte Cunningham-Rundles¹³ · Amos Etzioni¹⁴ · Jose Luis Franco¹⁵ · Steven M. Holland¹⁶ · Christoph Klein¹⁷ · Tomohiro Morio¹⁸ · Hans D. Ochs¹⁹ · Eric Oksenhendler²⁰ · Jennifer Puck²¹ · Mimi L. K. Tang^{22,23,24} · Stuart G. Tangye^{25,26} · Troy R. Torgerson¹⁹ · Kathleen E. Sullivan²⁷

- Inzicht in pathogenese
- Voorspelling van complicaties / prognose
- Counseling familieleden
- Targets for therapy ?

Monogenetische afwijkingen in PID

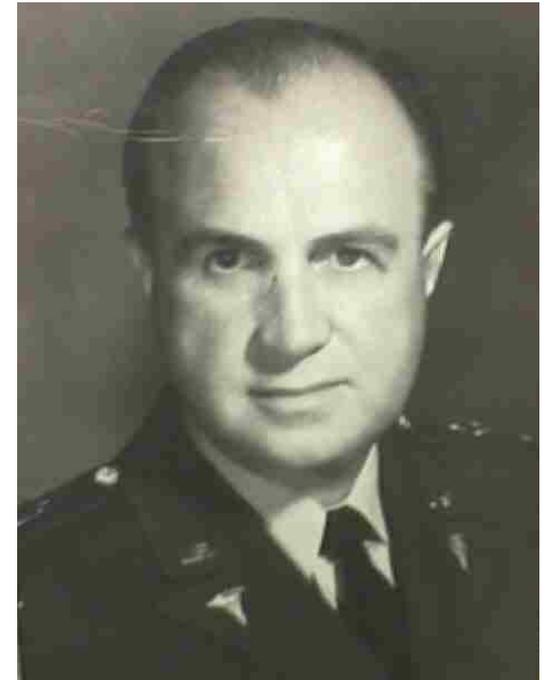
Beschrijving van een jongen met 19 episoden van sepsis in 4.5 jaar

Isolatie van Pneumococcus in 10 gevallen

X-linked agammaglobulinemie

Genetische variant in BTK gen

Afwezigheid van antistoffen



Accuraat in detectie puntmutaties, intronische mutaties, en sommige deleties en duplicaties

Bij verdenking op een monogenetische afwijking

Evaluatie familieleden van patient

Journal of Clinical Immunology (2021) 41:666–679

<https://doi.org/10.1007/s10875-021-00980-1>

ORIGINAL ARTICLE



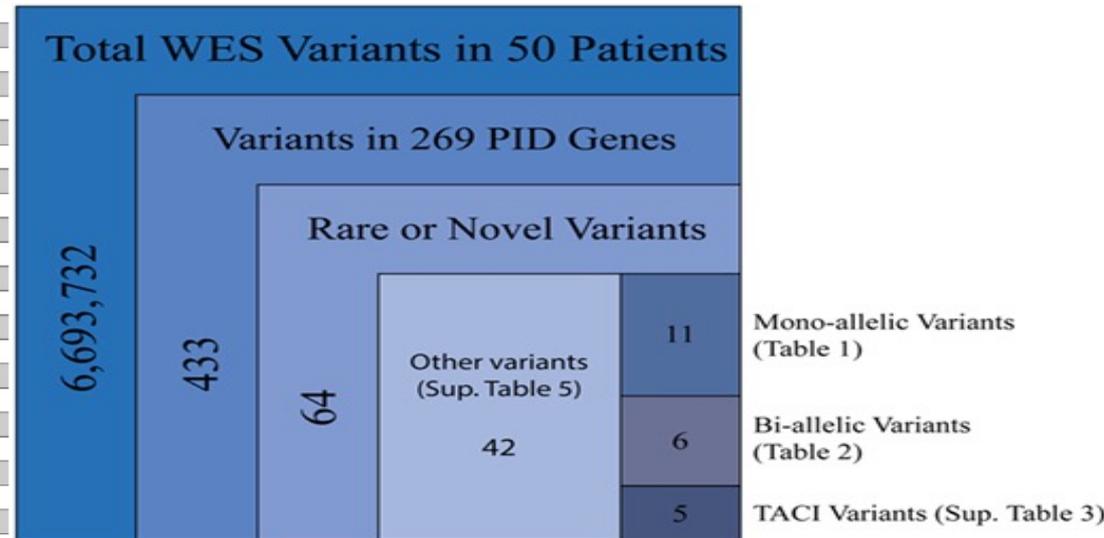
The Ever-Increasing Array of Novel Inborn Errors of Immunity: an Interim Update by the IUIS Committee

Stuart G. Tangye^{1,2}  • Waleed Al-Herz³ • Aziz Bousfiha⁴ • Charlotte Cunningham-Rundles⁵ • Jose Luis Franco⁶ • Steven M Holland⁷ • Christoph Klein⁸ • Tomohiro Morio⁹ • Eric Oksenhendler¹⁰ • Capucine Picard^{11,12} • Anne Puel^{13,14} • Jennifer Puck¹⁵ • Mikko R. J. Seppänen¹⁶ • Raz Somech¹⁷ • Helen C Su⁷ • Kathleen E. Sullivan¹⁸ • Troy R. Torgerson¹⁹ • Isabelle Meyts²⁰

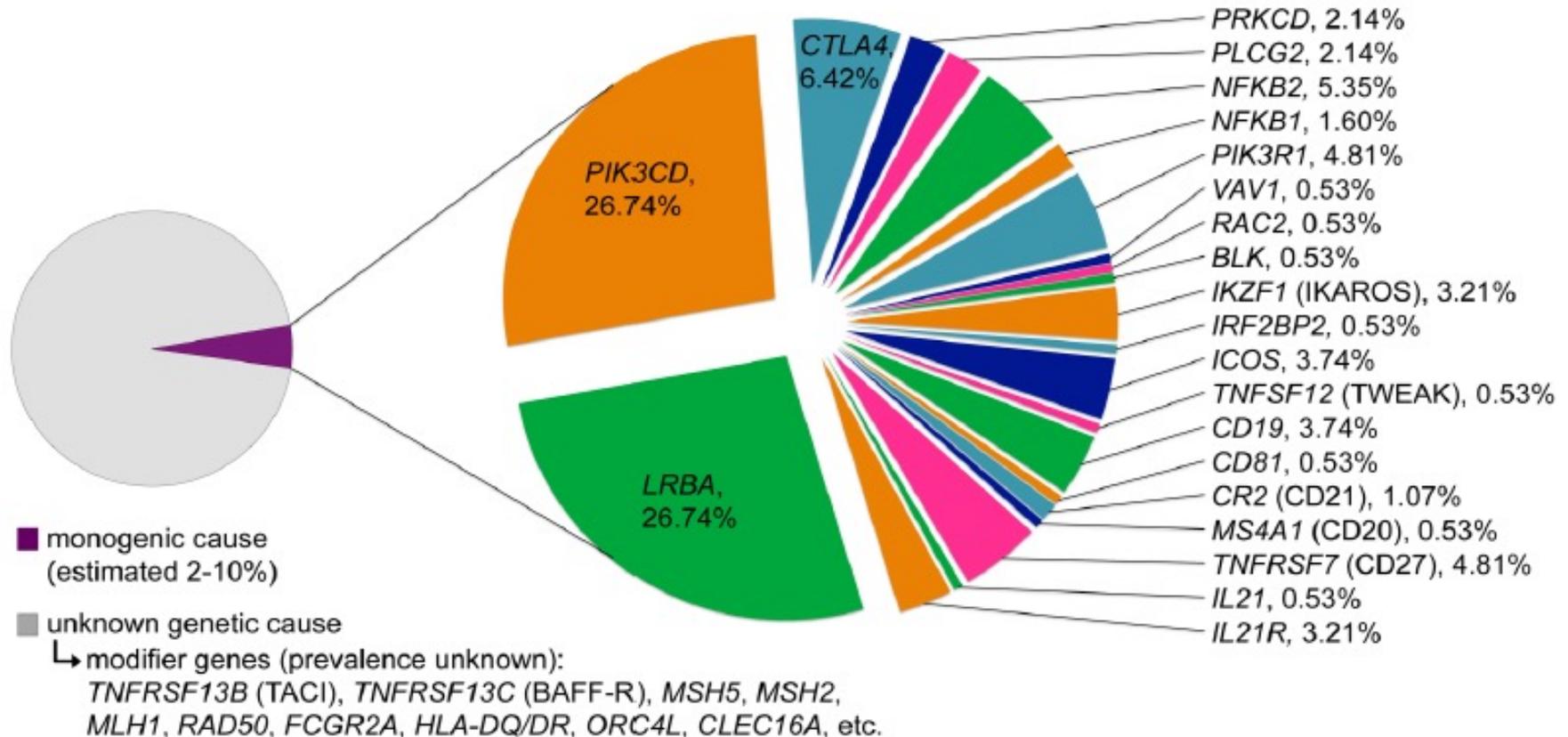
Gene panel analysis / genenpanels

Supplemental Table 1. 269 Gene Screen Panel

ACP5	CD27	ELANE	IL7R	NCF2	RNASEH2A	TLR3
ADA	CD3D	ERBB2IP	INO80	NCF4	RNASEH2C	TMC6
ADAR	CD3E	FADD	IRAK4	NFAT5	RNASEL	TMC8
AICDA	CD3G	FAS	IRF3	NFKB1	RNF168	TMEM173
AIRE	CD40	FASLG	IRF4	NFKB2	RNF31	TNFRSF13B
AK2	CD40LG	FCN3	IRF7	NHEJ1	RORC	TNFRSF13C
AP3B1	CD46	FERMT3	IRF8	NHP2	RPSA	TNFRSF1A
AP3D1	CD59	FOXP1	ISG15	NLRC4	RTEL1	TNFRSF4
APOL1	CD70	FOXP3	ITCH	NLRP12	SAMHD1	TNFSF12
ATM	CD79A	G6PC3	ITGAX	NLRP3	SBDS	TPP2
B2M	CD79B	G6PD	ITGB2	NOD2	SERPING1	TRAC
BCL10	CD81	GATA2	ITK	NOP10	SH2D1A	TRAF3
BLM	CD8A	GFI1	JAGN1	NRAS	SLC11A1	TRAF3IP2
BLNK	CEBPE	GINS1	JAK3	ORAI1	SLC35C1	TREX1
BLOC1S6	CFB	HAX1	KRAS	PCNA	SMARCAL1	TTC37
BTK	CFD	IFIH1	LAMTOR2	PGM3	SP110	TTC7A
C1QA	CFH	IFNGR1	LCK	PIK3CD	SPINK5	TYK2
C1QB	CFHR1	IFNGR2	LIG1	PIK3R1	STAT1	UNC119
C1QC	CFI	IGHM	LIG4	PLCG2	STAT2	UNC13D
C1R	CFP	IGKC	LIPA	PMS2	STAT3	UNC93B1
C1S	CIB1	IGLL1	LPIN2	PNP	STAT5B	UNG
C2	CIITA	IKBKB	LRBA	POLE	STIM1	USB1
C3	CLPB	IKBKG	LYST	PRF1	STK4	VPS45
C4A	COLEC11	IKZF1	MAGT1	PRKCD	STX11	WAS
C4B	COPA	IL10	MALT1	PRKDC	STXBP2	WIPF1
C5	CORO1A	IL10RA	MAP3K14	PSMB8	TADA2A	XIAP
C6	CR2	IL10RB	MASP1	PSTPIP1	TAP1	XRCC4
C7	CSF2RA	IL12B	MASP2	PTPN6	TAP2	ZAP70
C8A	CSF2RB	IL12RB1	MBL2	PTPRC	TAPBP	ZBTB24
C8B	CTLA4	IL12RB2	MCM4	RAB27A	TBK1	
C8G	CTPS1	IL17F	MEFV	RAC1	TBX1	
C9	CXCR4	IL17RA	MKL1	RAC2	TTC37	
CARD11	CYBA	IL17RC	MOGS	RAG1	TCF3	
CARD14	CYBB	IL18	MPO	RAG2	TECR	
CARD9	DCLRE1B	IL1RN	MRE11A	RBCK1	TERC	
CASP10	DCLRE1C	IL21	MSN	RFX5	TERT	
CASP8	DKC1	IL21R	MVK	RFXANK	TGFBR1	
CCBE1	DNMT3B	IL2RA	MYD88	RFXAP	TGFBR2	
CD19	DOCK2	IL2RG	NBN	RHOH	TICAM1	
CD247	DOCK8	IL36RN	NCF1	RMRP	TINF2	



Monogenetische defecten in antistofdeficienties



Whole exome versus whole genome sequencing

Whole exome sequencing

Circa 1.5% van humaan genoom voor eiwitten

Bekende en nieuwe mutaties

In trio

Whole genome sequencing

Volledige genoom (intronische mutaties, splice site mutaties, regulatoire, non-exon mutaties)

Rol van genetica in antistof deficiënties



Patienten met antistof deficiënties



APDS



CTLA4



X-MAID



CD27



LRBA

Patienten met antistof deficiënties



APDS

Man, 38 jaar oud

Medische geschiedenis

Terugkerende longontstekingen, ooronstekingen en bijholteontstekingen

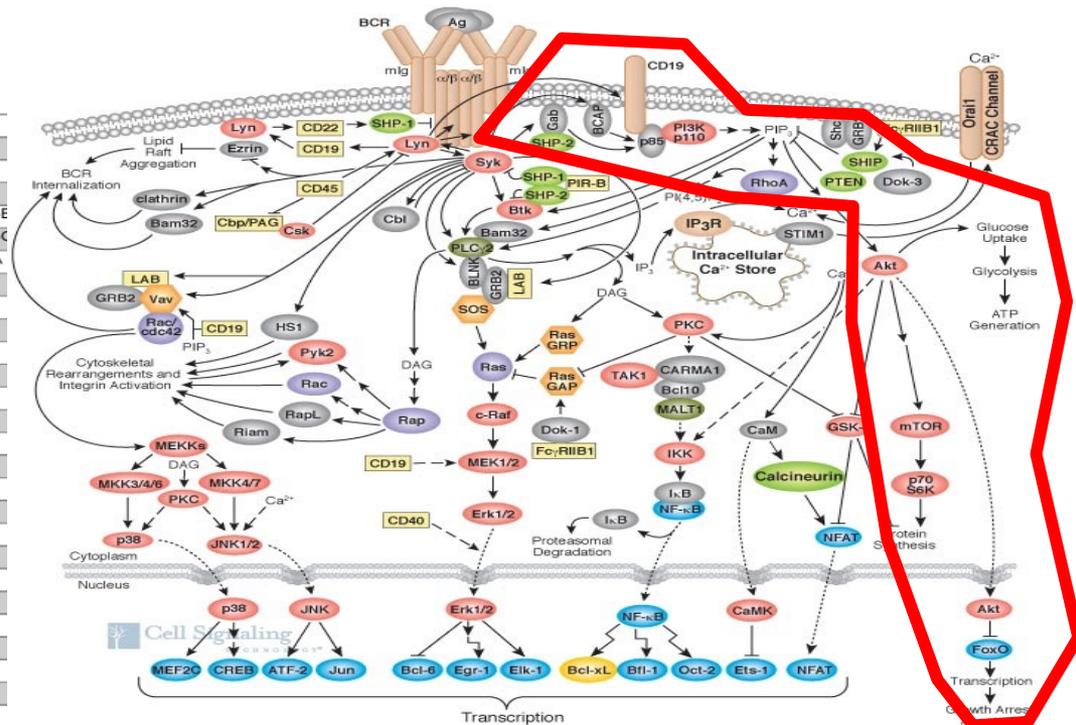
- 0: Bilaterale mastoïdectomie, paracentese
- 3: Linkszijdige mastoïdectomie
- 9: Rechtszijdige mastoïdectomie
- 9: Operatie aan binnenoor beiderzijds, plaatsing gehoortoestellen
- 15: Bilaterale infundibulotomie
- 20: Stage IA non-Hodgkin lymfoom rechter hals, waarvoor operatie en lokale radiotherapie

Behandeling met antibiotica en antistof suppletie

Mutatie in phosphoinositide 3-kinase p110 δ (PI3K δ) gen

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C2	CIITA	IKBKB	LRBA	POLE	STIM1	USB1
C3	CLPB	IKBKG	LYST	PRF1	STK4	VPS45
C4A	COLEC11	IKZF1	MAGT1	PRKCD	STX11	WAS
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C5	CORO1A	IL10RA	MAP3K14	PSMB8	TADA2A	XIAP
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C8A	CSF2RB	IL12RB1	MBL2	PTPRC	TAPBP	ZBTB24
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Verandert dit de behandeling?

› J Allergy Clin Immunol. 2017 Mar;139(3):1046-1049. doi: 10.1016/j.jaci.2016.09.040.
Epub 2016 Nov 12.

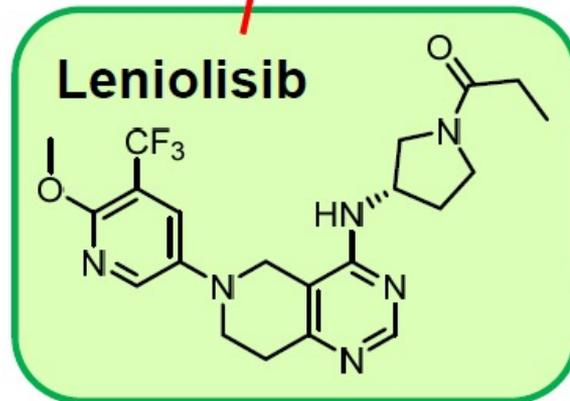
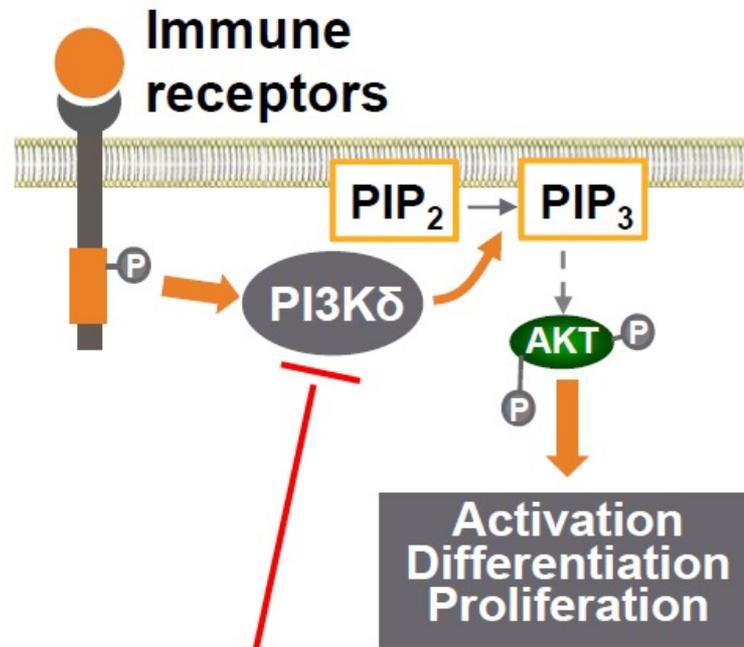
Hematopoietic stem cell transplant in patients with activated PI3K delta syndrome

Zohreh Nademi ¹, Mary A Slatter ², Christopher C Dvorak ³, Benedicte Neven ⁴, Alain Fischer ⁴, Felipe Suarez ⁵, Claire Booth ⁶, Kanchan Rao ⁷, Alexandra Laberko ⁸, Julia Rodina ⁸, Yves Bertrand ⁹, Sylwia Kołtan ¹⁰, Robert Dębski ¹⁰, Terence Flood ¹¹, Mario Abinun ², Andrew R Gennerly ², Sophie Hambleton ², Stephan Ehl ¹², Andrew J Cant ²

Affiliations + expand

PMID: 27847301 DOI: 10.1016/j.jaci.2016.09.040

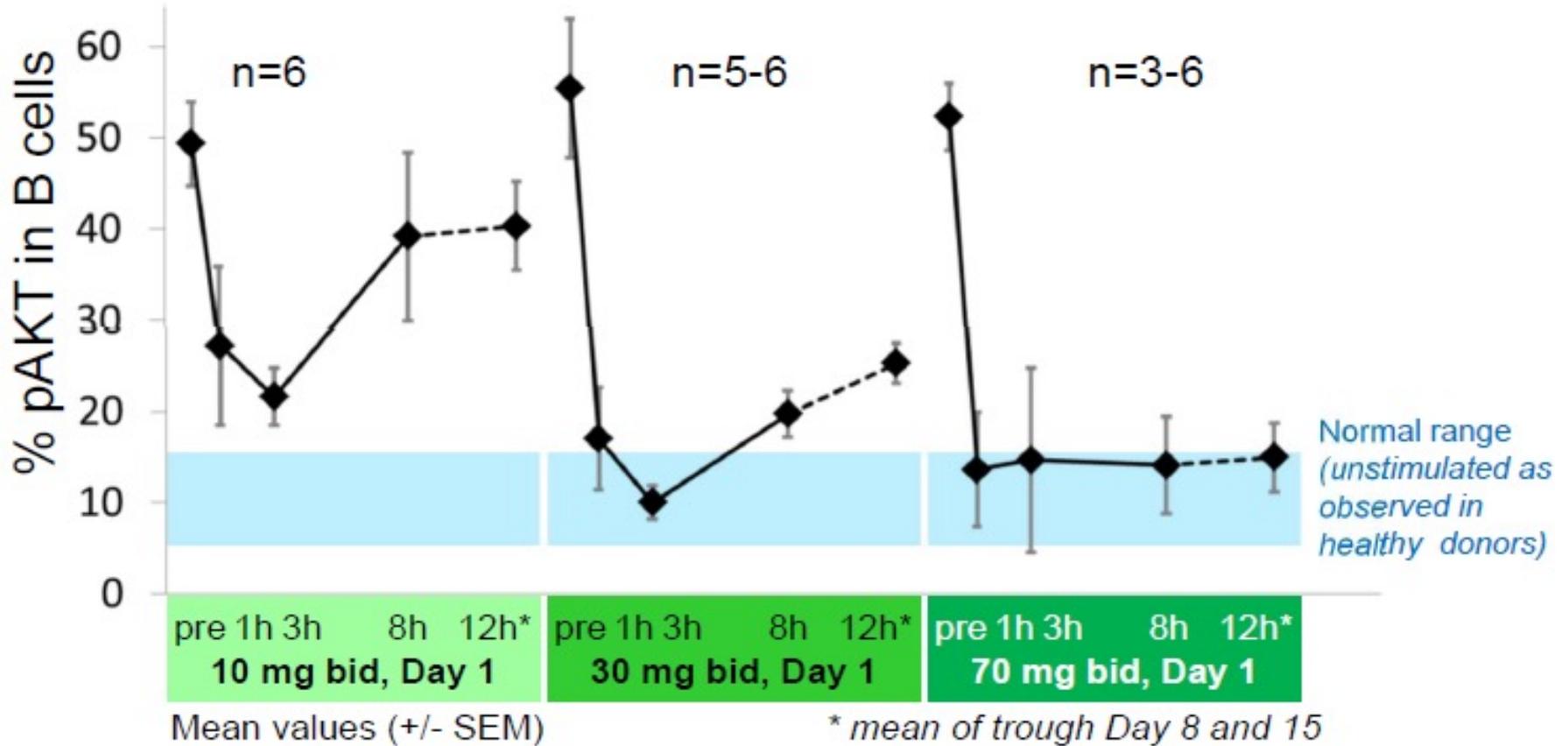
PI3K δ : target for therapy : leniolisib?



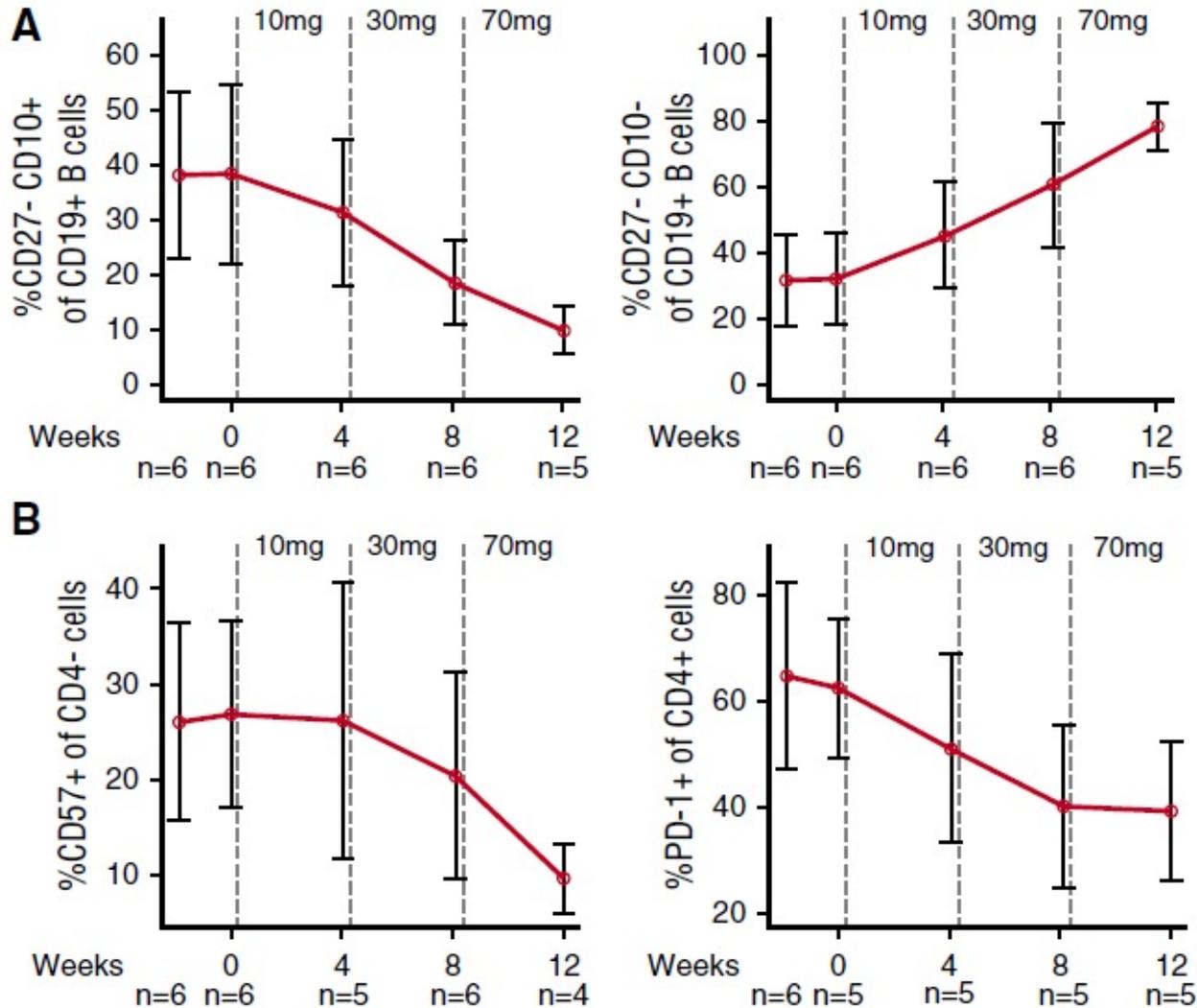
Leniolisib in APDS

Patient ID	#1	#2	#3	#4	#5	#6
Gender	M	F	F	M	M	M
Age of onset/ enrollment (years) ^a	0.5/17	2/24	2/16	6/20	0/25	0/31
Mutation	E525K	E1021K	E1021K	E1021K	E1021K	E1021K
Cytopenias	No	Thrombocytope nia, Neutropenia	No	Lymphopenia, Neutropenia, Anemia	No	Thrombocytopenia
Pulmonary Problems	Bronchiectasis , asthma	No	Asthma, recurrent bronchitis	Chronic sinusitis airway disease, bronchiectasis	Recurrent infections, bronchiectasi s, COPD	Chronic sinusitis, bronchial wall thickening
Lymphoma	No	19 y/o, Stage IV Large B-cell Lymphoma, chemotherapy ^c	No	No	11 y/o, Stage III Hodgkin Lymphoma, chemo ^d - and radiotherapy	20 y/o, Stage IA, non-Hodgkin Lymphoma, partial parotidectomy and local radiotherapy
Infection history	CMV/EBV	No	EBV	No	No	EBV
IgG replacement therapy	SCIG	IVIG	SCIG	None	IVIG	IVIG
Previous treatment with mTOR inhibitor ^b	Yes	Yes	No	No	Yes	No

Leniolisib remt PI3Kd-pAKT pathway activiteit



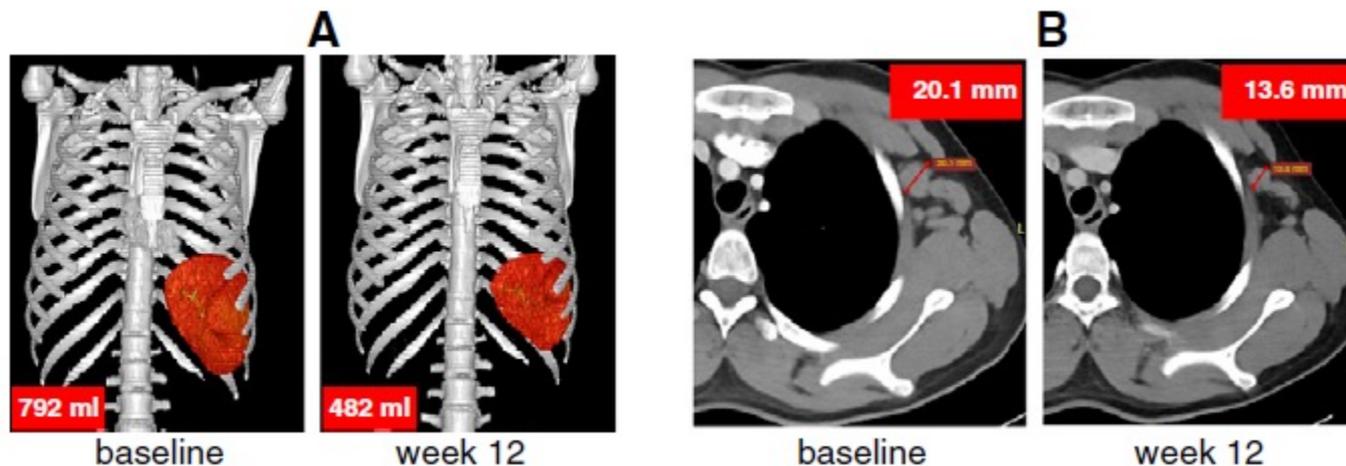
Effecten van leniolisib op witte bloedcellen



Effect van leniolisib op lymfadenopathie

Table 2. Spleen volumes, lymph node size, and liver volumes

	Spleen 3D volume			Lymph node SPD			Liver 3D volume*		
	Baseline, mm ³	End of study, mm ³	ΔBaseline, %	Baseline, mm ²	End of study, mm ²	ΔBaseline, %	Baseline, mm ³	End of study, mm ³	ΔBaseline, %
Patient 1	978 783	719 915	-26	2453	1194	-51	1 849 588	1 650 823	-11
Patient 2	792 042	482 188	-39	1926	1681	-13	1 438 041	1 413 010	-2
Patient 3	385 832	166 402	-57	1235	427	-65	1 349 165	1 442 528	7
Patient 4	782 213	500 841	-36	748	503	-33	1 398 929	1 332 235	-5
Patient 5	419 843	254 634	-39	258	133	-48	1 396 210	1 359 013	-3
Patient 6	721 831	451 208	-37	858	589	-31	1 817 801	1 904 876	5
Mean ± SD			-39 ± 10			-40 ± 19			-1 ± 6



“Herstel” van lymfocyten subsets

Toename energie

Toename inspanningsmogelijkheden

Minder infecties

Afname in lymfadenopathie, afname lever en miltomvang

In 3 patienten behandeling met antistoffen gestaakt

In 3 patienten behandeling afgebouwd

De toekomst : Gene-targeting versus gene-editing

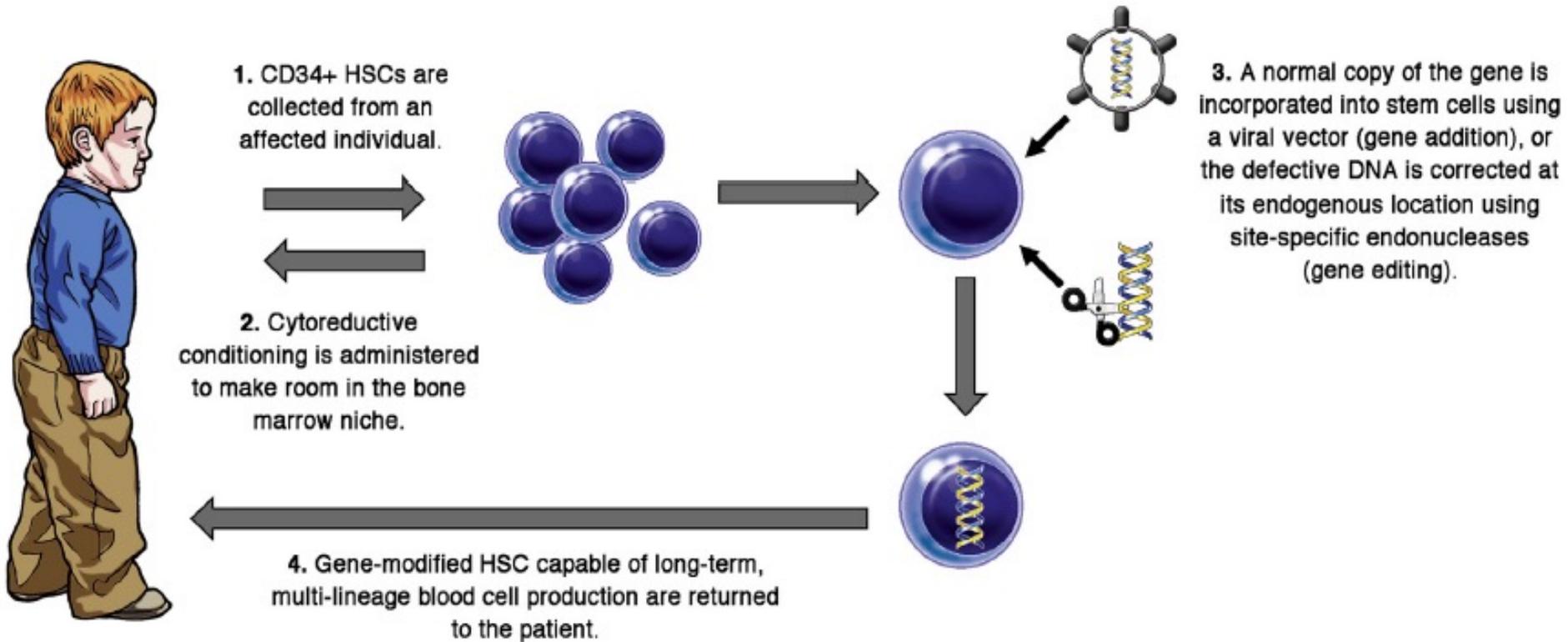
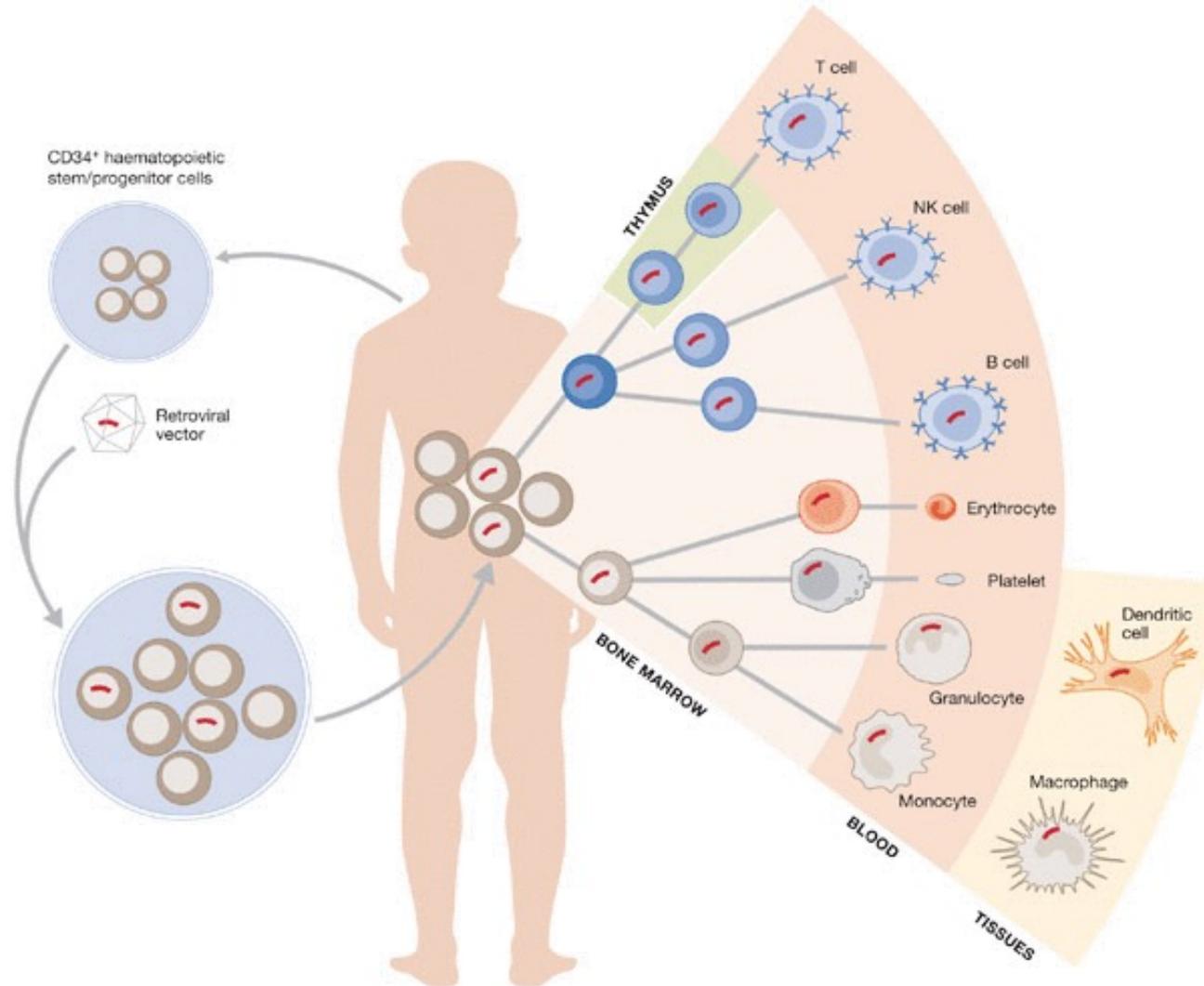


FIG 1. Clinical schema of gene therapy for PIDs.

Gen therapie in ADA-SCID : Strimvelis



Toename genetische diagnostiek in PID

Verschillende benaderingen

Betere en personalized behandeling

Gericht op voorkomen van complicaties

Richting gentherapie?