i.Mune NEO – A New Test For Epigenetic Immune Cell Quantification in Newborns

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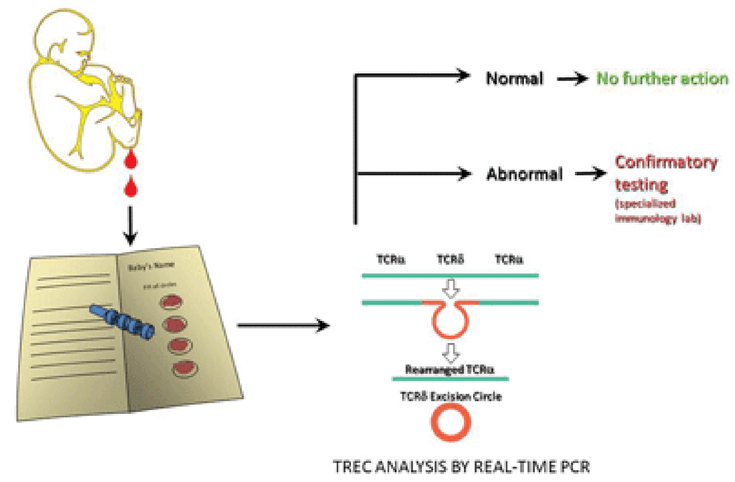
Introduction

Inborn Errors of Immunity (IEI) are characterized by quantitative dysregulation of immune cells already at birth. More than 400 IEIs have been described that affect almost all components of the immune system.

Current newborn screening for IEI is limited to quantification of T-cells using the **T-Cell Receptor Excision Circle (TREC)** test and to B-cells using the **Kappa Receptor Excision Circle (KREC)** test. Both tests produce a fair number of non-actionable screening findings and false-positive results.

Also, expanded screening for quantitative abnormalities of more immune cells has the potential to significantly improve early detection of more IEIs in newborns than currently possible.



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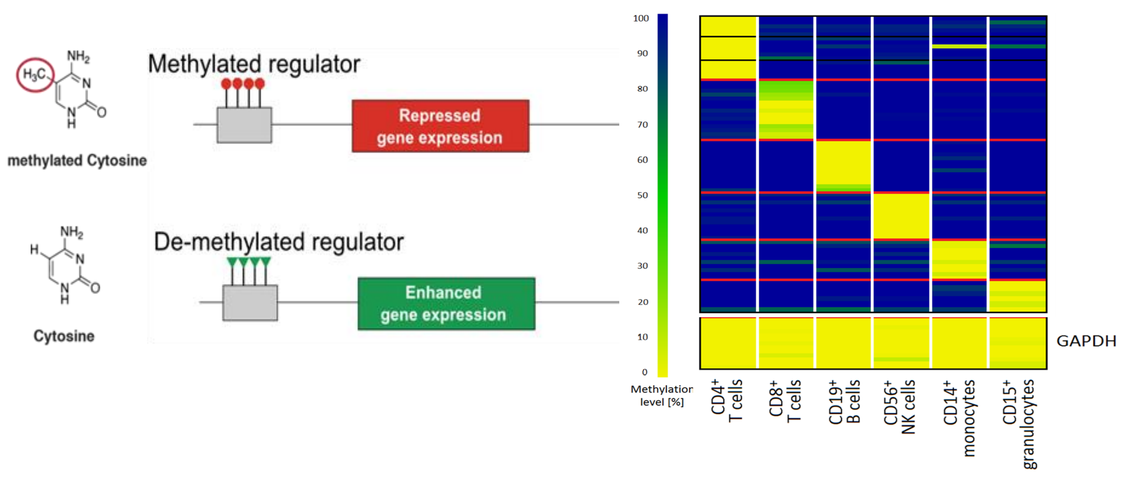


Approach

We developed real-time PCR based **epigenetic immune cell quantification** from a drop of dried blood.

This offers **confirmatory testing** possibilities to existing screening methods as well as the potential to **expand newborn screening** to a variety of other IEIs.

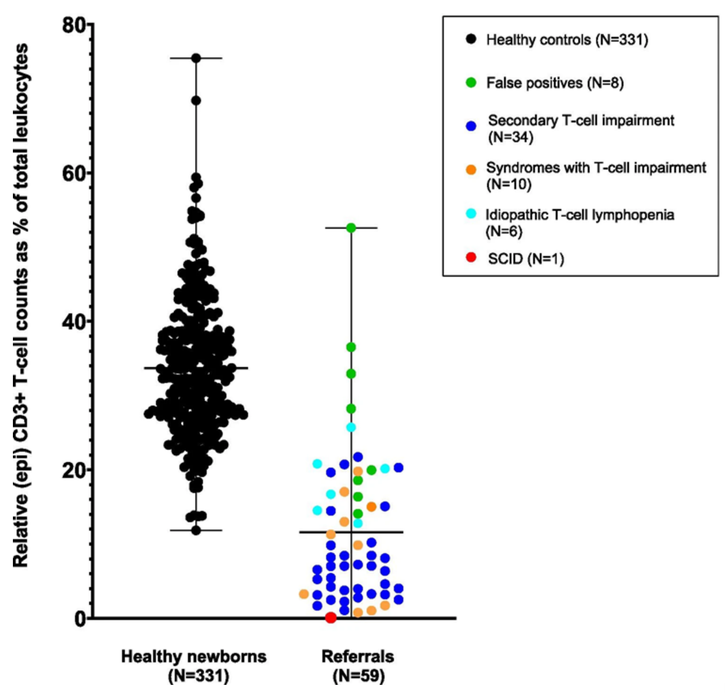
Results

Successful identification of SCID and XLA cases in newborn DBS samples

Disease Description				TREC/KREC Newborn Screening			Epigenetic qPCR Analysis			
Identifier	Classification	Gene Defect	Loss of Function Type	TREC[1] Positive [yes/no]	KREC[2] Positive [yes/no]	Screening Classification	(CD3 G/D, GAPDH)[3] Conspicious [yes/no]	(MVD, GAPDH)[3] Conspicious [yes/no]	(LRP5, GAPDH)[3] Conspicious [yes/no]	Screening Classification
1	SCID	*ADA*	amorph	yes	yes	correctly identified	yes	yes	yes	correctly identified
2	SCID	*ADA*	amorph	no	yes	correctly identified	yes	yes	yes	correctly identified
3	DO-SCID[4]	*ADA*	hypomorph	no	yes	correctly identified	no	yes	yes	correctly identified
4	DO-SCID[4]	*ADA*	hypomorph	no	yes	correctly identified	yes	yes	yes	correctly identified
5	SCID	*AK2*	amorph	yes	no	correctly identified	yes	yes	yes	correctly identified
6	SCID	*AK2*	amorph	yes	yes	correctly identified	yes	yes	no	correctly identified
7	SCID	*Artemis*	amorph	yes	yes	correctly identified	yes	yes	yes	correctly identified
8	SCID	*CD3D*	amorph	yes	no	correctly identified	yes	yes	no	correctly identified
9	**SCID w ME[5]**	***IL2RG***	**amoprh**	**yes**	**no**	**correctly identified**	**no**	**no**	**no**	**not identified**
10	SCID	*IL2RG*	amorph	yes	no	correctly identified	yes	yes	yes	correctly identified
11	SCID	*IL7RA*	amorph	yes	no	correctly identified	yes	no	no	correctly identified
12	SCID	*IL7RA*	amorph	yes	no	correctly identified	yes	yes	yes	correctly identified
13	SCID	*IL7RA*	amorph	yes	no	correctly identified	yes	yes	yes	correctly identified
14	**DO-SCID[4]**	***JAK3***	**hypomorph**	**no**	**no**	**not identified**	**yes**	**yes**	**yes**	**correctly identified**
15	SCID	*PNP*	amorph	yes	yes	correctly identified	yes	yes	yes	correctly identified
16	SCID	*PNP*	amorph	yes	yes	correctly identified	yes	yes	yes	correctly identified
17	SCID	*RAG1*	hypomorph	yes	yes	correctly identified	yes	yes	no	correctly identified
18	SCID	*RAG1*	amorph	yes	yes	correctly identified	no	yes	yes	correctly identified
19	SCID	*RAG2*	amorph	yes	yes	correctly identified	yes	no	yes	correctly identified
20	XLA	*BTK*	amorph	no	yes	correctly identified	yes	no	yes	correctly identified
21	XLA	*BTK*	amorph	no	yes	correctly identified	no	no	yes	correctly identified
22	XLA	*BTK*	amorph	no	yes	correctly identified	no	no	yes	correctly identified
23	XLA	*BTK*	amorph	no	yes	correctly identified	yes	yes	yes	correctly identified
24	**XLA**	***BTK***	**hypomorph**	**no**	**no**	**not identified**	**no**	**yes**	**yes**	**correctly identified**

Baron et al., 2018

Reduction of false-positive TREC screening referrals

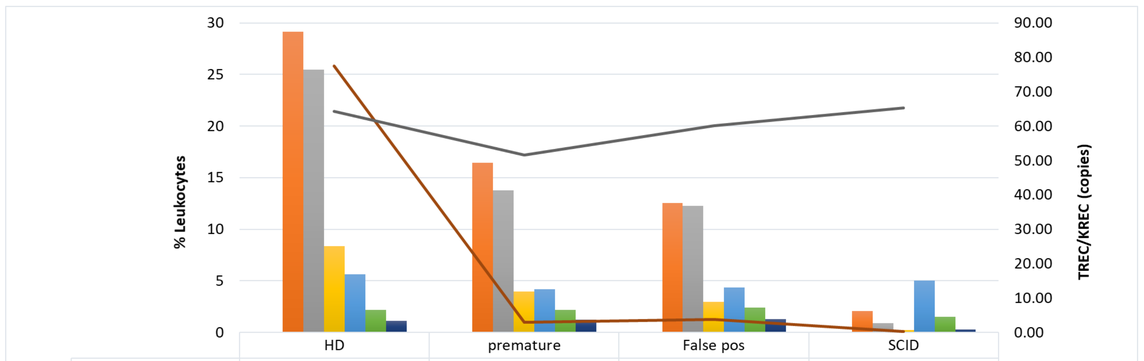


Blom et al., 2021

i.Mune NEO - a real-time PCR based test for immune cell quantification in dried blood spot samples from newborns

	i.Mune NEO	*i.Mune NEO LITE*	*i.Mune NEO CD3*
T lymphocytes (CD3+)	✓	✓	✓
Memory Helper T lymphocytes (CD3+CD4+CD45RO+)	✓	✓	✓
B lymphocytes (CD19+)	✓	✓	
Natural Killer lymphocytes (CD16+CD56dim)	✓	✓	
Helper T lymphocytes (CD3+CD4+)	✓		
Cytotoxic T lymphocytes (CD3+CD8+)	✓		

Ongoing evaluation of **i.Mune NEO** in a cohort of newborn screening samples (Karolinska University Hospital)



	HD	premature	False pos	SCID
Count of Sample	56	4	17	4
CD3 T-cells	29.17	16.47	12.56	2.08
CD4 T-cells	25.47	13.77	12.24	0.91
CD8 T-cells	8.37	3.96	2.95	0.20
B-cells	5.63	4.17	4.33	5.00
NK cells	2.16	2.17	2.39	1.50
memory CD4 T-cells	1.15	1.25	1.27	0.29
TREC	77.42	3.00	3.85	0.25
KREC	64.23	51.50	60.18	65.25

Lundbäck (unpublished)

Summary & Outlook

i.Mune NEO is a novel epigenetic immune cell quantification test allowing confirmatory testing of TREC- and KREC positive screening cases. It also is suitable for screening applications (i.Mune NEO LITE, NEO CD3). Going forward, addition of other epigenetic immune cell markers will allow expanded screening for additional IEI – e.g. Severe Congenital Neutropenia (SCN), Immune Dysregulatory Disorders, NK deficiencies and others (see also: Blom et al., Int. J. Neonatal Screen. 2021, 7, 74)