



Leids Universitair
Medisch Centrum

Epigenetic Immune Cell Counting as a Second Tier Test in Newborn Screening for SCID

Maartje Blom, MD
Leiden University Medical Center
Leiden, the Netherlands
m.blom@lumc.nl



FACULTY DISCLOSURE

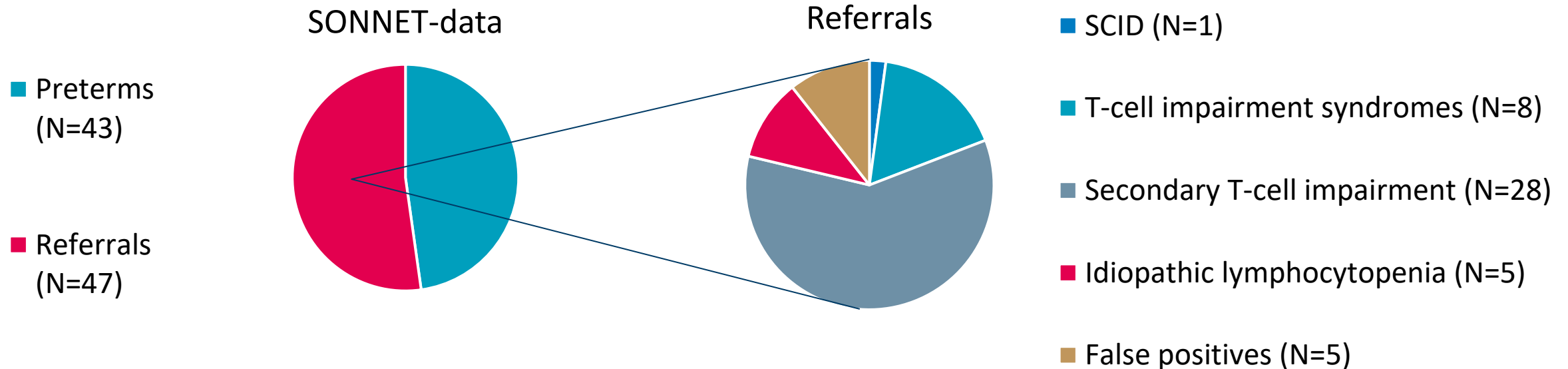
X	No, nothing to disclose
	Yes, please specify



19TH BIENNIAL MEETING OF
**THE EUROPEAN SOCIETY
FOR IMMUNODEFICIENCIES**
ONLINE MEETING | 14-17 OCTOBER 2020

A second tier test in newborn screening for SCID

- Newborn screening for SCID based on T-cell receptor excision circles (TREC) quantification
 - Secondary findings
 - False-positive cases
- SONNET-study (Dutch NBS pilot for SCID): N=140,593 newborns

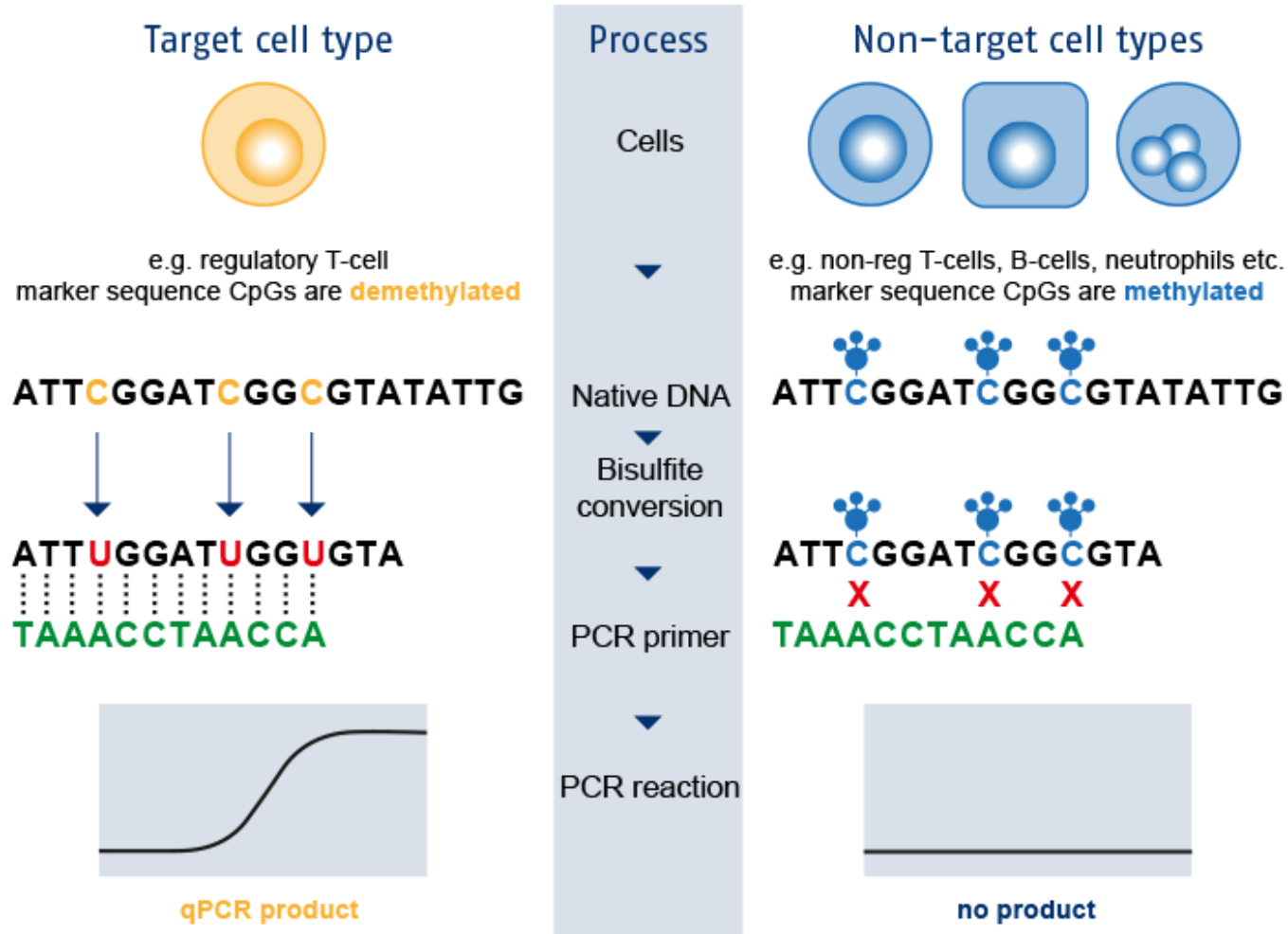


A second tier test in newborn screening for SCID

- High emotional impact of referral procedure for parents (Abstract 220: Friday 16th October - ESID-INGRID- IPOPI)
- Need for a second tier test after TREC analysis
 - Reduce the number of secondary findings and false-positive cases
 - Increase sensitivity for NBS for SCID
- **Epigenetic immune cell counting on dried blood spots**



Epigenetic immune cell counting



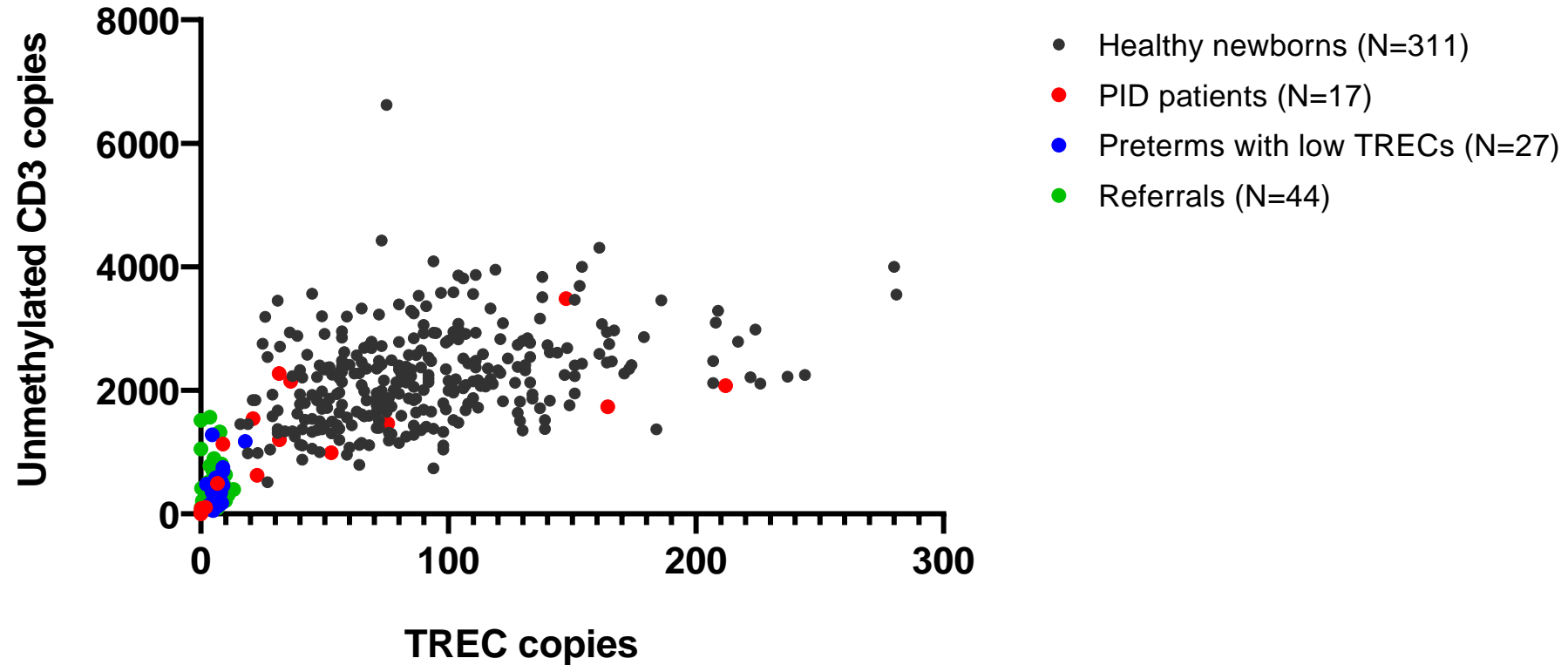
Study population and methods

Original newborn screening cards

- Healthy newborns (N=311)
 - PID patients (N=17)
 - Preterm newborns with low TRECs (N=27)
 - Referrals with low TRECs (N=44)
-
- Protocol: lysis, bisulfite conversion and purification, followed by qPCR amplification and measuring
 - Measuring regulatory T-cells (Tregs), **CD3+ T-cells**, B-cells, NK-cells and granulocytes

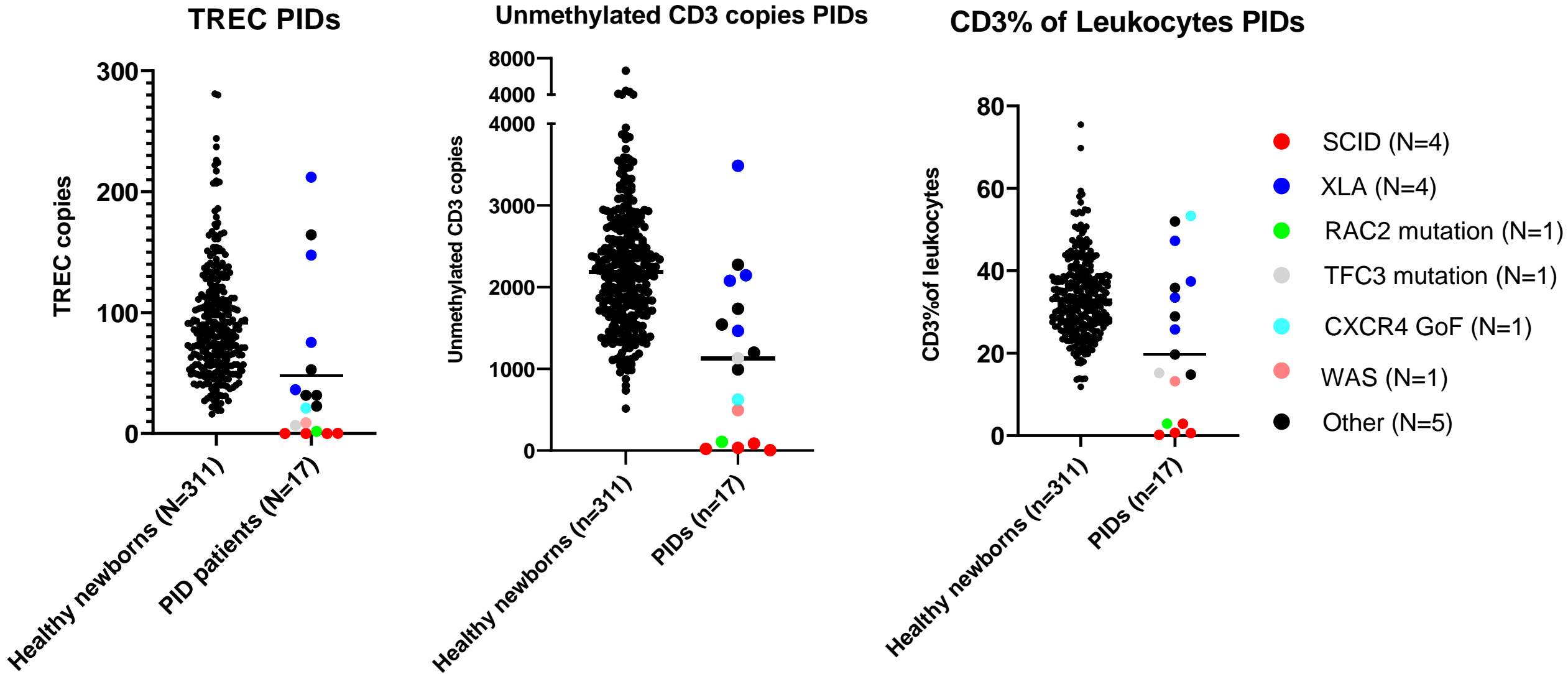


Correlation TRECs and unmethylated CD3 copies

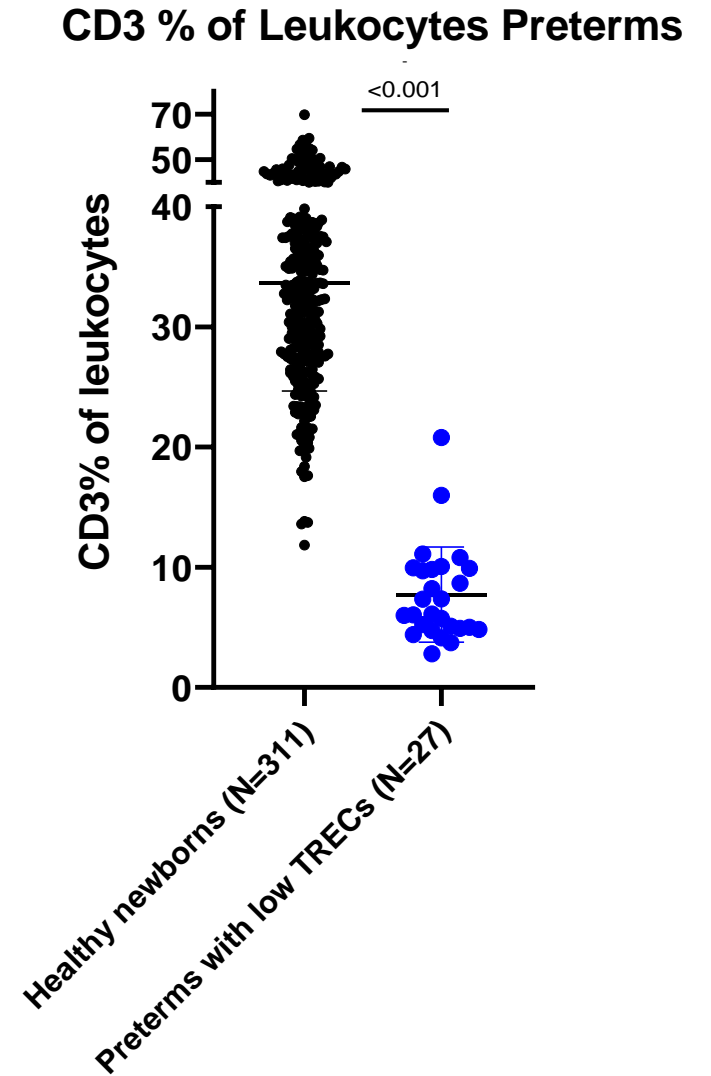
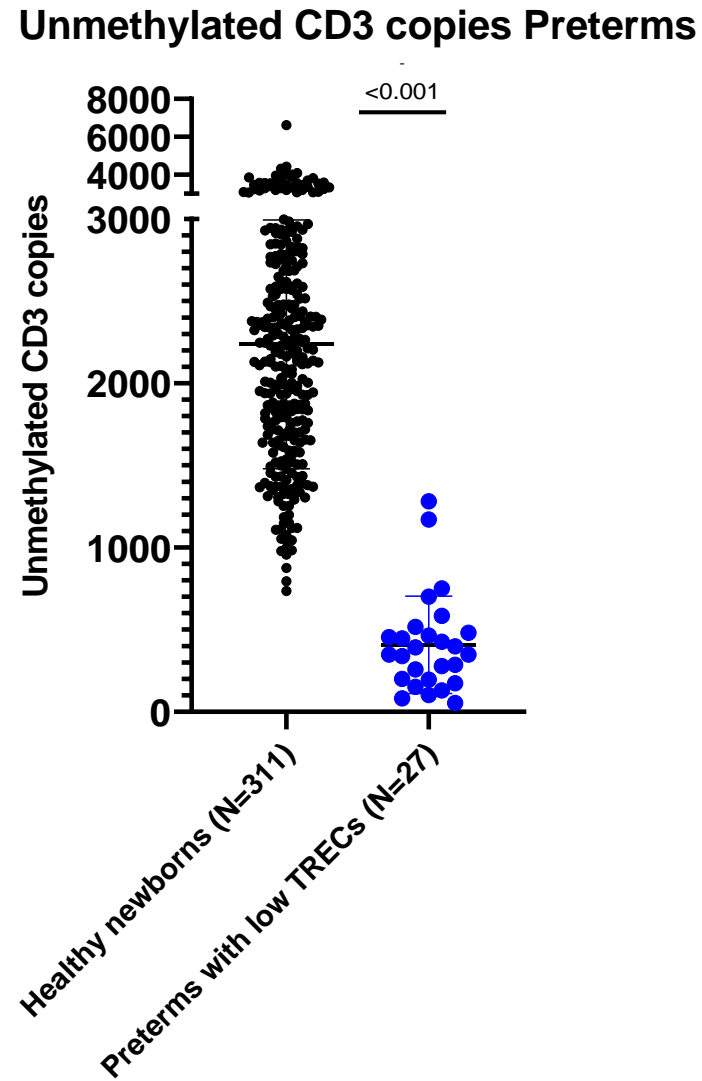
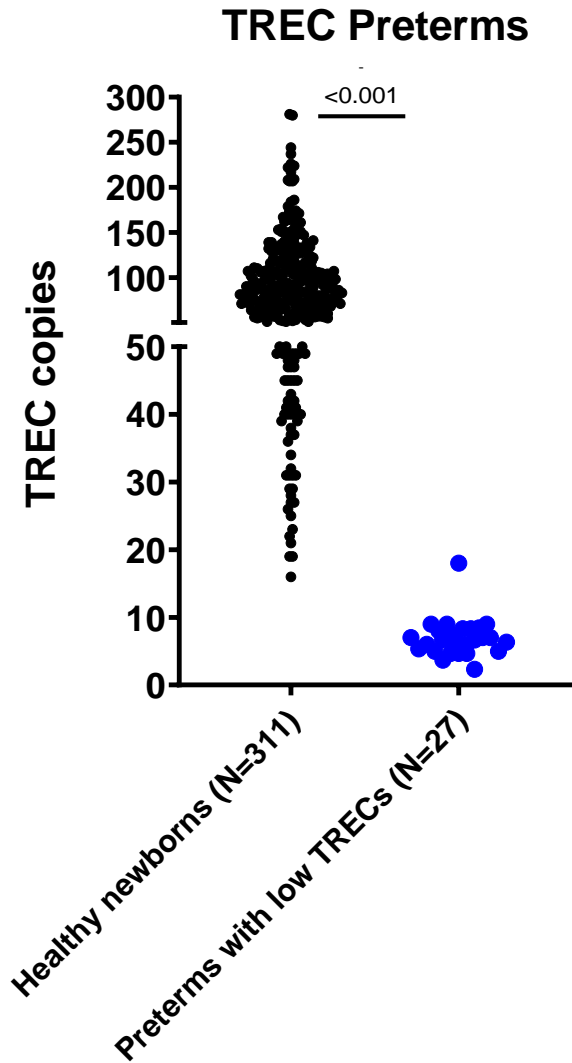


R=0.70 P<0.001

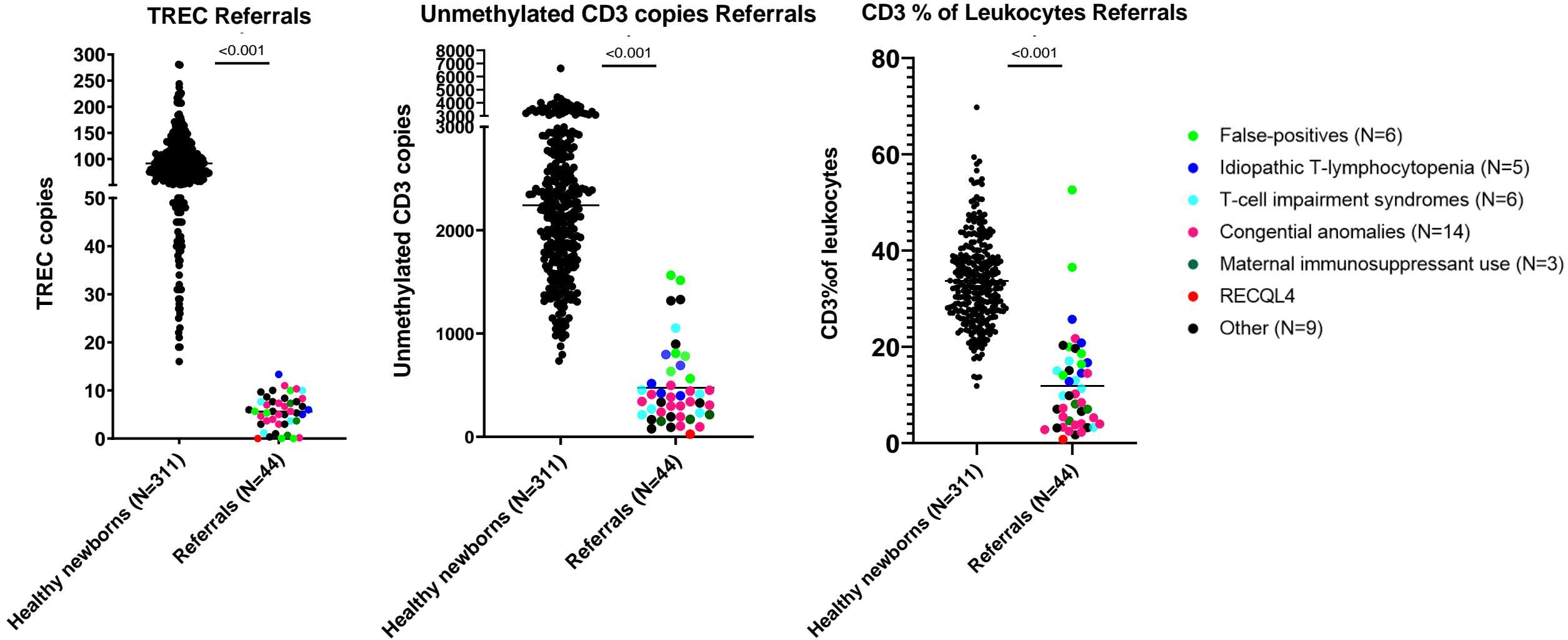
PID patients – TRECs and relative T-cell counts



Preterm newborns – TRECs and relative T-cell counts



Referrals – TRECs and relative T-cell counts

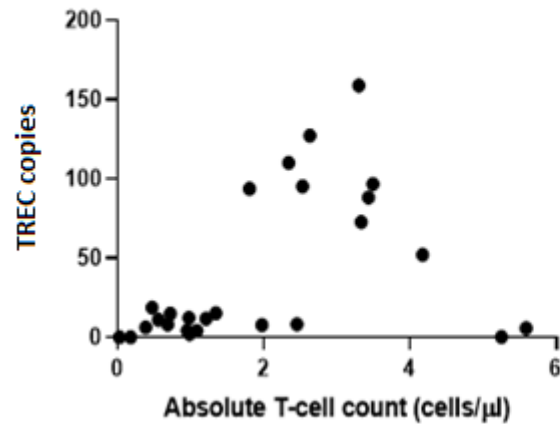


Correlation with absolute T-cell counts

Referred infants (n=26) with cards spotted with peripheral blood used for flow cytometry

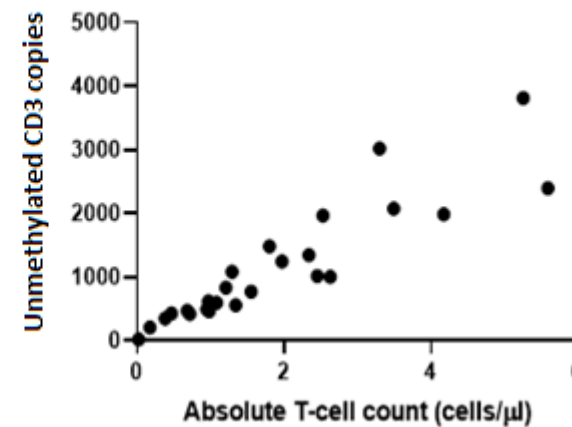
- TREC
- Unmethylated CD3 copy numbers (epigenetic qPCR)
- Absolute T-cell counts (flow cytometry)

A. Correlation TREC and absolute T-cell count



Moderate correlation
 $R=0.45$ $P=0.02$

B. Correlation unmethylated CD3 copies and absolute T-cell counts



Strong correlation
 $R=0.94$ $P<0.001$

Discussion: Epigenetic qPCR as a second tier after TREC analysis

1. Potential to increase positive predictive value and sensitivity of NBS for SCID
 - Reduction in second heel pricks (preterm newborns)
 - Reduction in referrals (false-positive cases)
2. Further development and automatization is required
3. Further evaluation of epigenetic immune cell counting in newborn screening programs
 - Role as a first tier?
 - Newborn screening for XLA, IPEX syndrome and SCN
4. Exploring other potential second tier options (NGS)

Acknowledgements

LUMC, Leiden, the Netherlands



Mirjam van der Burg
Lotte Vissers

RIVM, Bilthoven, the Netherlands



National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Sandra Imholz
Vincent Eding
WONHS-committee

Epimune GmbH, Berlin, Germany



EPIMUNE™
DIAGNOSTICS

Jeannette Werner
Janika Schulze
Christoph Sachsenmaier