Comprehensive Epigenetic Immune Cell Monitoring in Diverse Clinical Applications

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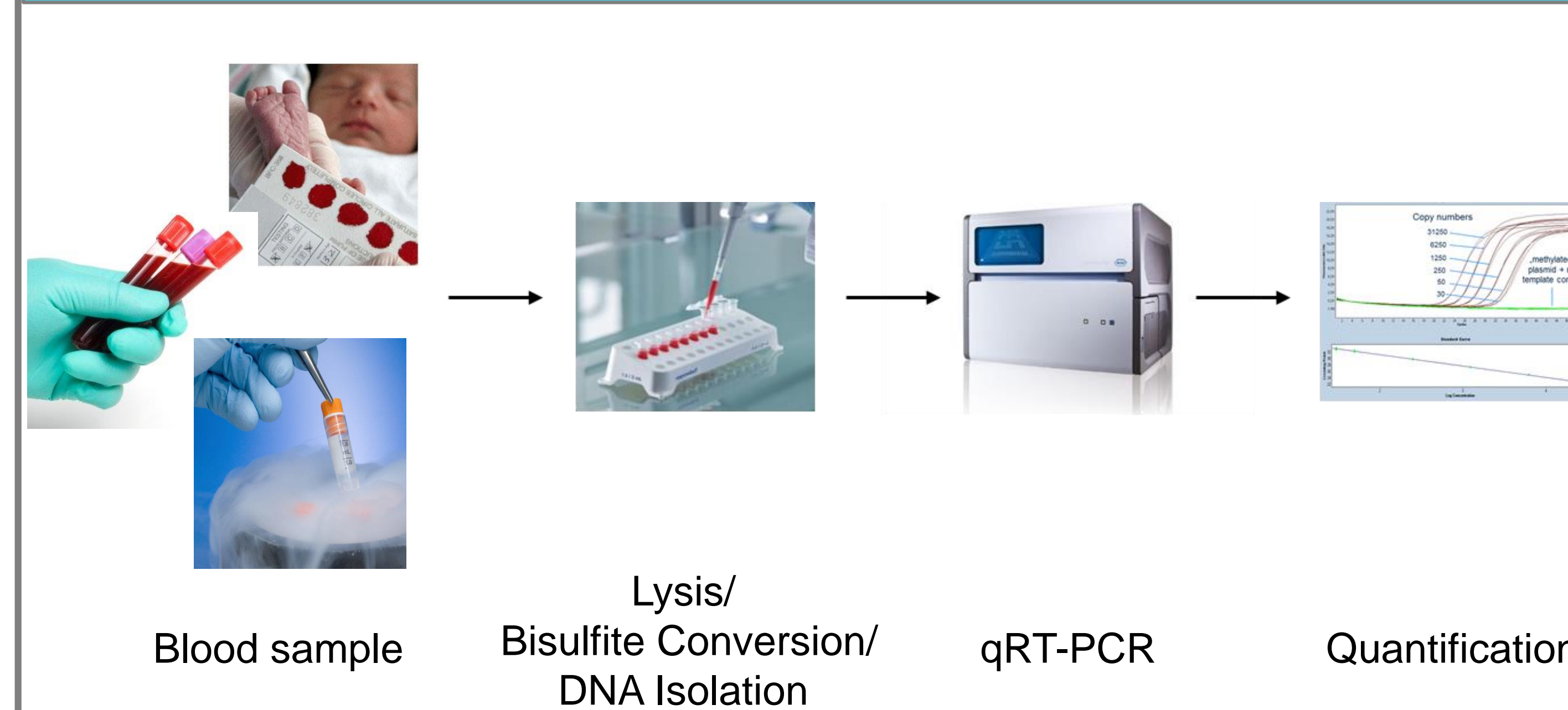
Introduction

DNA-methylation analysis provides a unique approach to molecular immune cell quantification. This method yields identical results to flow cytometry from blood samples of healthy donors and allows precise quantification of cell subsets such as Tregs that are challenging to quantify. This allows immune cell profiling from small amounts of fresh and archived samples – e.g. whole blood, dried blood spots and tissue.

We established epigenetic immune cell quantification for CD3+, CD4+, CD8+ T-cells, FOXP3+ Tregs, B-, NK-cells, neutrophils and monocytes.
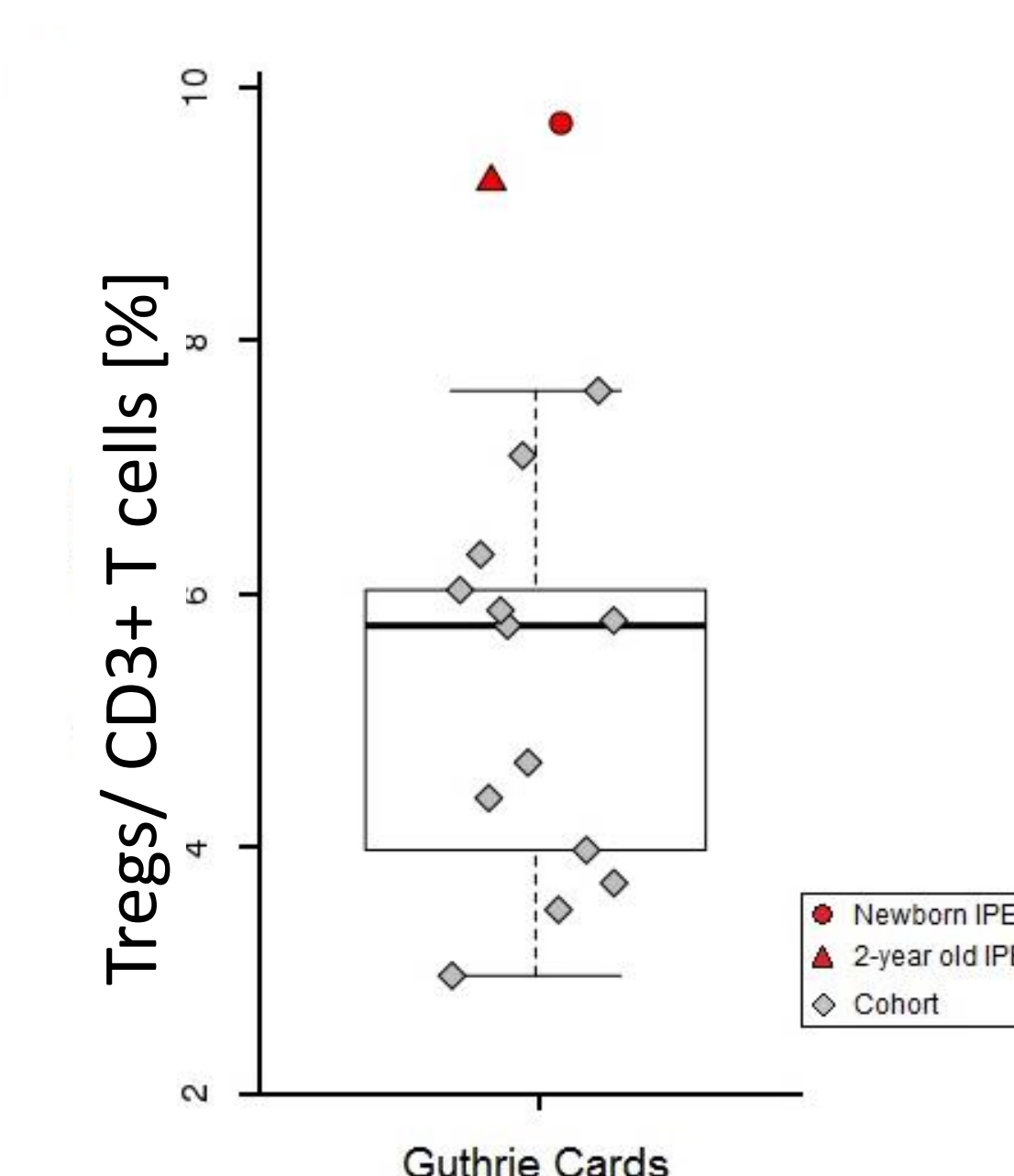
Here we demonstrate:
1. Equivalence of the epigenetic approach with flow cytometry (Fig. 3)
2. Identification of dysregulation of FOXP3+ Tregs in patients with Primary Immune Regulatory Disorders (PIRD) (Fig. 4)
3. Identification of PIRD patients on dried blood spot cards at newborn screening (Fig. 5)
4. Earlier detection of immune cell reconstituion after hematopoietic stem cell transplantation than using flow cytometry (Fig. 6)

Method: DNA-Methylation based Real-Time PCR Assays for the Quantification of Different Immune Cell Types

Figure 1: Identification of demethylated regions specific for individual immune cell types. A) TSDR within the FOXP3 gene is specifically demethylated in regulatory T cells. B) Development of a marker panel for various immune cells. [1]

Figure 2: Development of demethylation-specific real-time PCR assays for the quantification of different immune cells. Methylation pattern is translated into the DNA sequence by bisulfite conversion. Primers and probes match only converted, demethylated target sequence. In parallel, housekeeping gene GAPDH is analyzed. [1,3]

Workflow for Epigenetic Immune Cell Quantification

Blood sample → Lysis/ Bisulfite Conversion/ DNA Isolation → qRT-PCR → Quantification

Comparison of Immune Cell Quantification by Flow Cytometry and Epigenetic qPCR

Figure 3: Equivalence of the epigenetic approach with flow cytometry.

Epigenetic qPCR on blood samples from male patients with various primary immune regulatory disorders or immune deficiencies
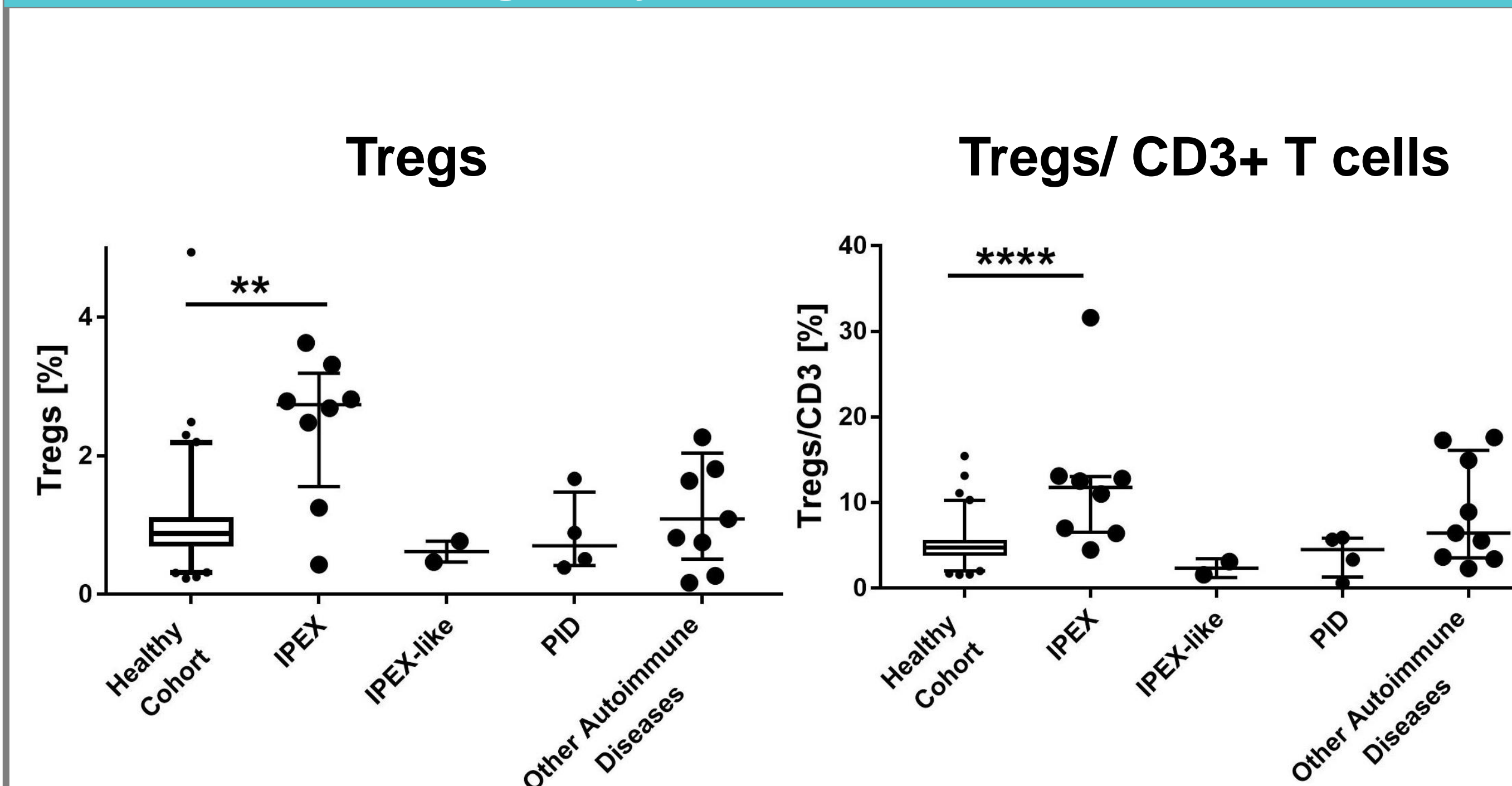
Figure 4: Tregs and Tregs/ CD3+ T cell ratio was analyzed in male IPEX, IPEX-like and PID patients and compared with a healthy cohort (n=404) and a disease control cohort including patients with other autoimmune diseases.
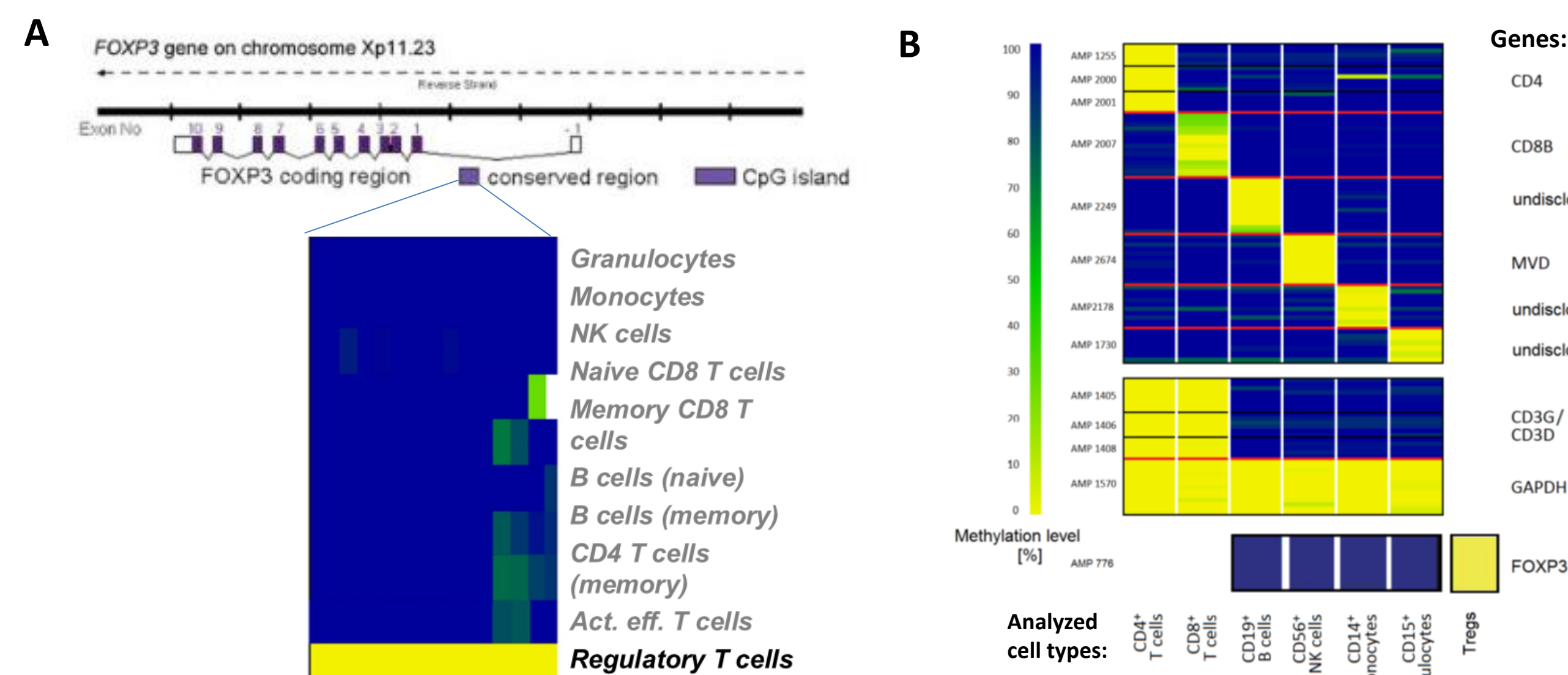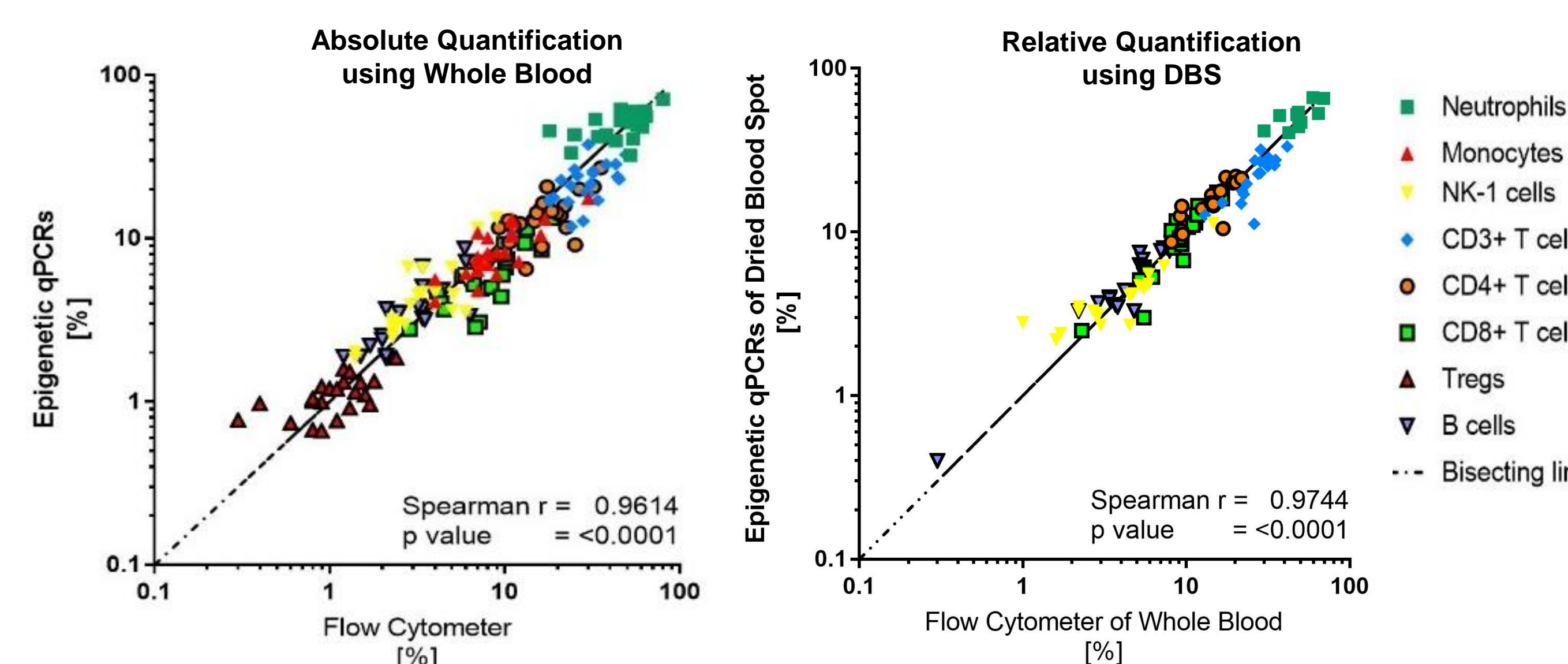
Epigenetic qPCR on DBS from newborns with IPEX

Figure 5: DBS from healthy controls and newborns with IPEX were subjected to epigenetic qPCR for quantification of regulatory T cells within CD3+ T cells. The two IPEX patients showed increased ratio of Tregs to CD3+ T cells. This finding opens the possibility to identify IPEX patients already at birth using epigenetic qPCR in newborn screening (Baron et al., Sci. Transl. Med. 2018). [1]

Highly Sensitive qPCRs are suitable for Early Immune Cell Quantification of HSCT patients

Immune cell types	Flow Cytometry First valid test result (days post Tx)	Epigenetic analysis First valid test result (days post Tx)	P value
Leukocytes	6 (6-12)	9 (5-11)	0.9047
CD3+ T cells	29 (20-40)	19 (13-20)	0.0011
CD3+CD4+ T cells	29 (20-40)	19 (13-20)	0.0003
CD3+CD8+ T cells	29 (20-40)	19 (14-26)	0.0014
CD19+ B cells	47 (34-72)	20 (19-30)	<0.0001
CD56dimCD16+ NK cells	29 (20-40)	19 (17-25)	0.0009

Figure 6: Whole blood samples of 21 pediatric HSCT recipients were sent to clinical laboratory and analyzed in parallel with epigenetic qPCRs. The median day after transplantation (median; interquartile range) was calculated where the respective technology provided first valid immune cell counts.

Conclusion

Epigenetic immune cell quantification offers substantial benefits for broad immune cell profiling where a fresh blood sample in sufficient quality and quantity is difficult or impossible to obtain.

The results demonstrate suitability of epigenetic immune method to quantify highly specialized subpopulations such as Tregs where established methods lack standardization

We also show that epigenetic immune cell profiling is more sensitive than flow cytometry in detecting immune cell reconstitution in HSCT patients. This supports application of epigenetic immune cell profiling in routine patient monitoring using DBS sampling in a near patient setting without the need for phlebotomy at dedicated facilities.

References

[1] **Baron et a**l., Epigenetic immune cell counting in human blood samples for immunodiagnostics. Sci. Transl. Med. 2018 Aug; 10 –pp1-11

[2] **Barzaghi et al.,** Demethylation analysis of the FOXP3 locus shows quantitative defects of regulatory T cells in IPEX-like syndrome. J Autoimmun. 2012 Feb;38(1):49-58

[3] **Wieczorek et al.,** Quantitative DNA methylation analysis of FOXP3 as a new method for counting regulatory T cells in peripheral blood and solid tissue. Cancer Res. 2009 Jan 15;69(2):599-608.