Early Epigenetic Immune Cell Quantification Following αβ T-cell/CD19 B-cell Depleted Haploidentical Stem Cell Transplant Correlates with CD4+ T cell Recovery at Day +100?

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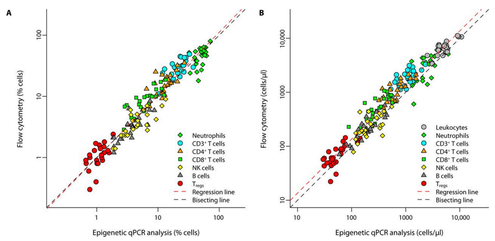
Background

- Patients with delayed immune reconstitution after HSCT are at risk for infection and relapse[1]
- Immune cell quantification by flow cytometry requires large blood volumes and high lymphocyte numbers and suffers from insufficient standardization[2]
- DNA methylation-based quantitative PCR is a novel technology to provide relative and absolute immune cell counts from very low volumes of fresh, frozen, and dried blood spots with high sensitivity[2]
- It has been shown that epigenetic qPCR cell quantification correlates highly with flow cytometry in healthy subjects and individuals with HIV infection[2]



Objective

- To determine if epigenetic qPCR is suitable to detect immune cell reconstitution earlier than flow cytometry
- To this end we tested if epigenetic qPCR at days 15, 30 and 60 correlates with flow cytometry at day 100 post transplant

Methods

- Patients were consented at LPCH Stanford and blood was collected at 4 timepoints post-HSCT: days 11-19, 30-40, 53-67, and 75-125.
- Flow cytometry values were obtained between days 75-125.
- 70µL per sample of frozen blood was subjected to epigenetic qPCR analysis as described[2]

Results

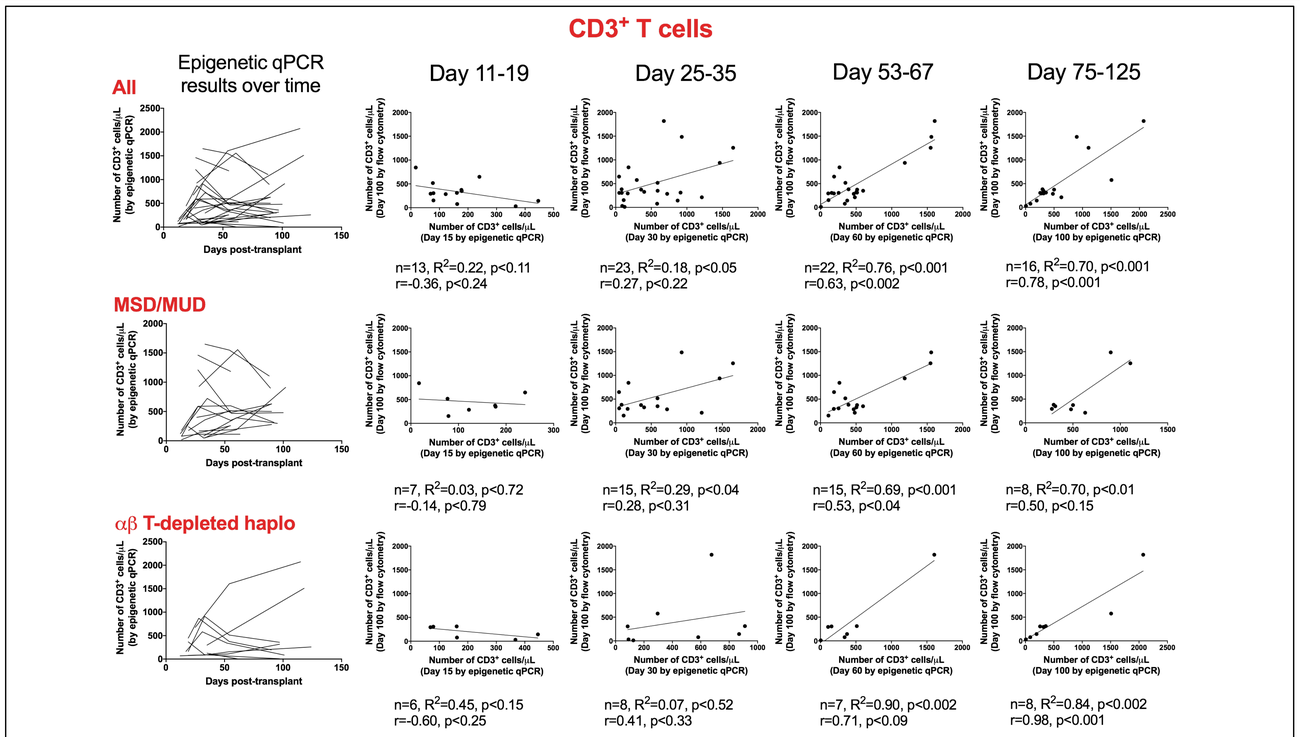


Fig. 1. Correlation of CD3+ T cell epigenetic qPCR quantification at indicated timepoints with CD3+ T cell flow cytometry quantification at day +100. Far left column shows epigenetic qPCR results over time for each patient. Remaining plots show linear regression (top row below each plot) and correlation (bottom row below each plot).

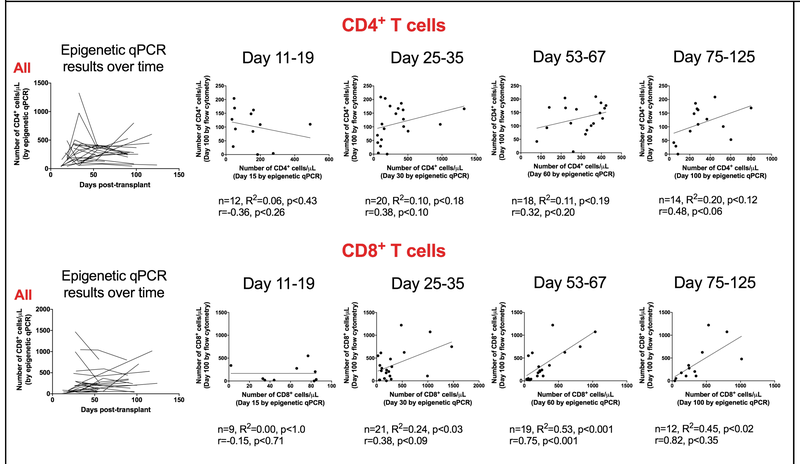


Fig. 2. Correlation of epigenetic qPCR CD4+ T cell and CD8+ T cell quantification at four timepoints post-transplant with flow cytometry-based quantification at day +100. Results are shown as for Fig 1.

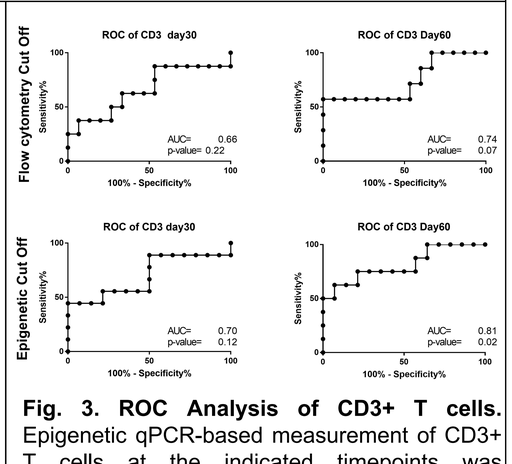


Fig. 3. ROC Analysis of CD3+ T cells. Epigenetic qPCR-based measurement of CD3+ T cells at the indicated timepoints was compared to day 100 measurement by flow cytometry (top row) or by epigenetic qPCR (bottom row) using a 500 cells/µL cutoff.

Limitations

- Our limited sample size precludes robust statistical analysis of the ability of epigenetic qPCR to predict immune reconstitution (including lack of training and validation sets for ROC analysis)

Conclusions

- Epigenetic qPCR can measure peripheral blood immune cell subsets at very low cell numbers, such as after HSCT
- Within this limited cohort, the correlation of epigenetic qPCR-based immune cell counts to day 100 flow cytometry-based counts increases as day 100 is approached, with strong correlation noted for CD3+ T cells at the two later timepoints
- Despite high sensitivity of the assay, the earliest time point tested should be optimized using a larger amount of blood (ie. 150µL rather than 70µL)
- Increasing our sample size will enable more robust analysis of the predictive ability of epigenetic qPCR on immune reconstitution

References

[1]Bertaina A et al. *Blood* 2014 Jul 31;124(5):822-6

[2]Baron U et al. *Sci Transl Med* 2018 Aug 1;10(452):eaan3508.

Disclosures: No relationships to disclose for MM, GB, UL, RP, KW, JC, RA, MGR, RB, and AB. JS and CS disclose salary from Epimune GmbH (JS—Senior Research Scientist, CS—Vice President Business Development): No conflict of interest is reported for this experimental work that may or may not yield a commercial product as no additional compensation in excess of salary would be received.

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