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"RBC Storage Effect on Coagulation, Microparticles and Microchimerism in Critically Ill Patients."

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14. ABSTRACT The study aim is to investigate specific mechanisms of adverse effects related to RBC storage age in critically ill patients. To date we have enrolled 130 patients at the three clinical sites in Canada. Samples have been collected, processed, and shipped from Canada to the repository at Blood Systems Research Institute (BSRI) in San Francisco, California. The repository consists of plasma, PBMCs and whole blood samples. The testing of patient samples for coagulation parameters, microparticles and microchimerism has begun. Results from these tests will be correlated with clinical outcomes and study groups upon study completion.					
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Adverse Effects of RBC Storage in Critically Ill Patients

INTRODUCTION

Critically ill patients are specifically at risk of adverse effects resulting from the use of RBCs of increased storage age. A large multicenter randomized controlled trial in 30 Canadian centers of 2500 critically ill patients called the Age of Blood Evaluation (ABLE) trial is underway. In this trial of critically ill patients, which includes patients with traumatic injuries, study groups will be randomized to either RBCs of < 8 days storage time or standard RBC storage time. The primary outcome of this trial is 90 day mortality. Secondary outcomes include severity of multiple organ dysfunction syndrome, serious thrombotic events and nosocomial infections, and ICU and hospital length of stay. Prospective clinical studies investigating the mechanisms and clinical outcomes associated with increased or decreased RBC storage age in critically ill patients including traumatic injury have not been performed. The ABLE study presents a unique and probably one-time opportunity to investigate mechanisms in the context of clinical outcomes for well-characterized study groups. Our ancillary study is designed to determine specific mechanisms of adverse effects related to the RBC storage age in transfused critically ill patients enrolled in the ABLE study. Specifically we will determine if the RBC unit storage time affects patient's immune function, inflammation, coagulation, microparticle concentrations and microchimerism.

Hypotheses

- 1.) Increased storage time of transfused RBC units will affect both inflammation and coagulation factors in critically ill patients and these parameters will be positively associated with measured clinical endpoints including increased morbidity (sepsis, serious thrombotic events, multi-organ failure) and mortality.

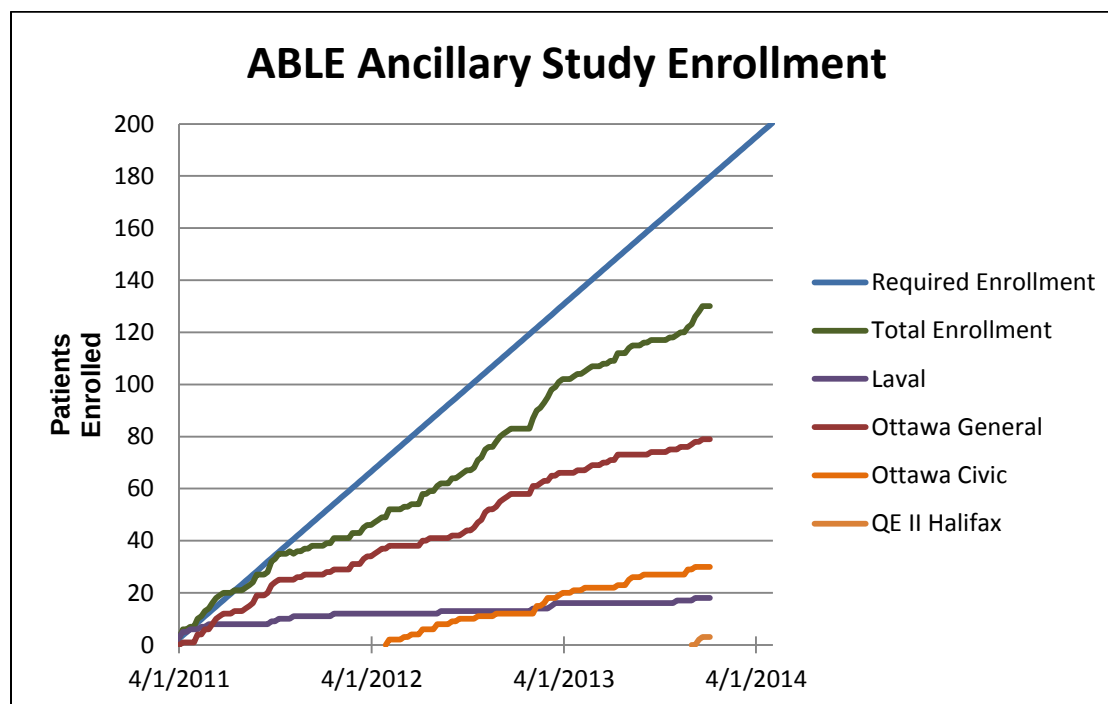
Aims

- 1.) To determine how RBC unit storage time affects inflammation and coagulation in critically ill patients, how these effects change over time after transfusion and if these parameters correlate with clinical outcomes.
 - 1a. Measure the levels of pro-and anti-inflammatory cytokines and coagulation factors in serum from transfused subjects longitudinally using multiplex assays (high and standard sensitivity).
 - 1b. Quantify levels of markers associated with cardiovascular disease including cellular adhesion molecules and growth factors using multiplex bead-based assays.
 - 1c. Correlate patterns of cytokine and inflammatory marker secretion and measures of coagulation with receipt of blood stored for short vs. long periods.
 - 1d. Correlate patterns of cytokine and inflammatory marker secretion and coagulation with all clinical outcomes.
- 2.) To develop a patient sample repository for future analysis of additional effects of RBC storage age in critically ill patients.

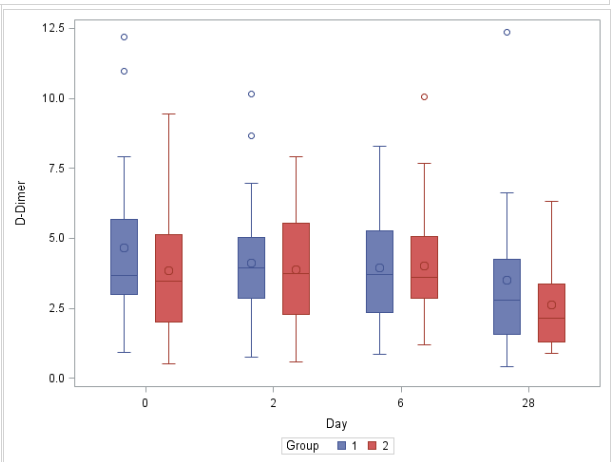
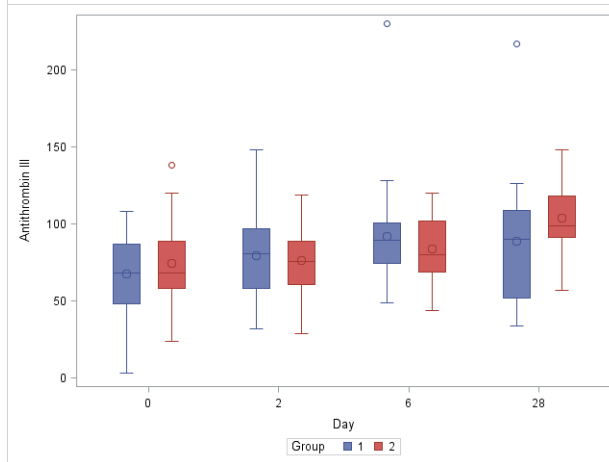
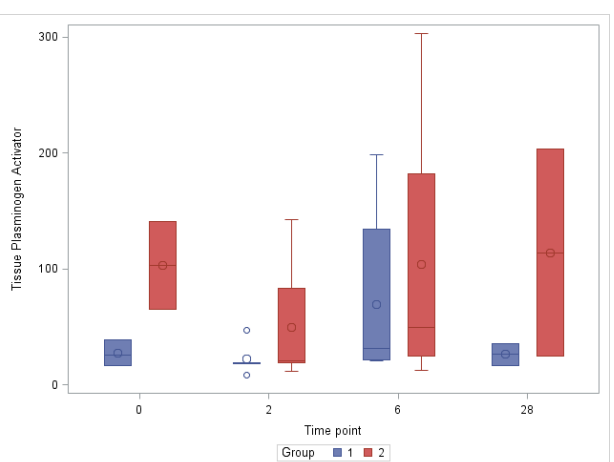
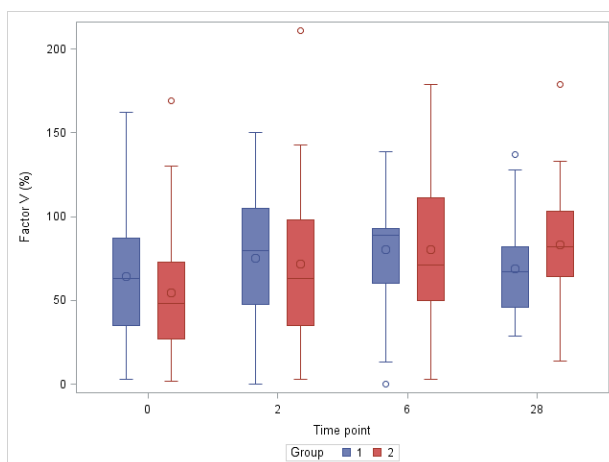
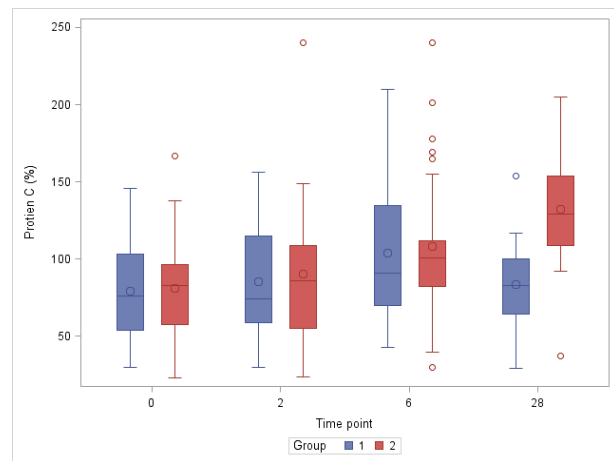
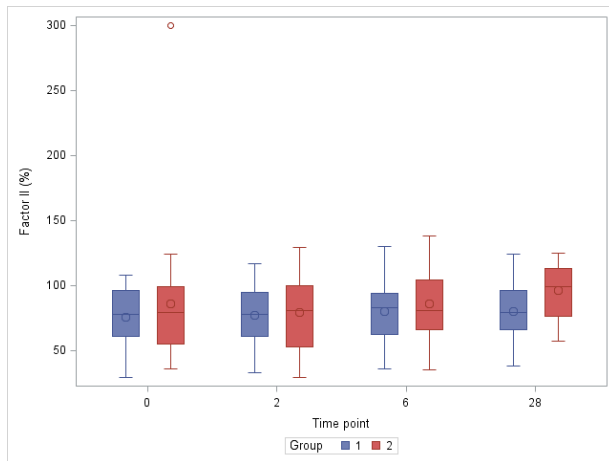
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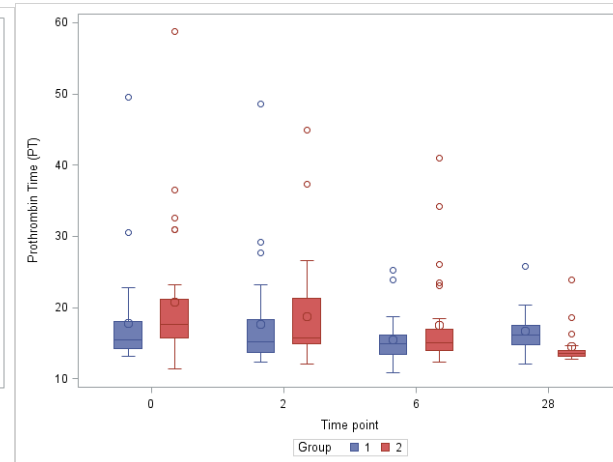
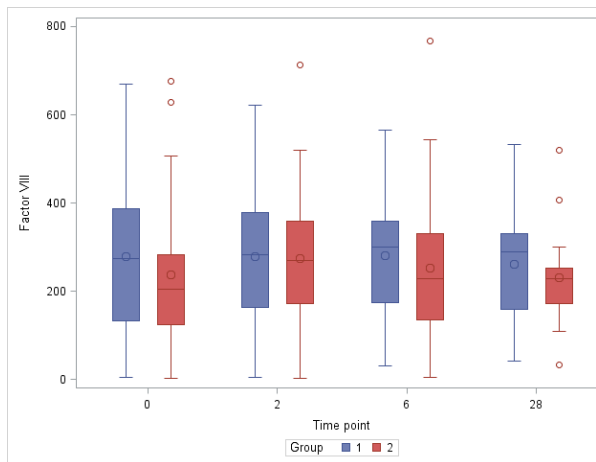
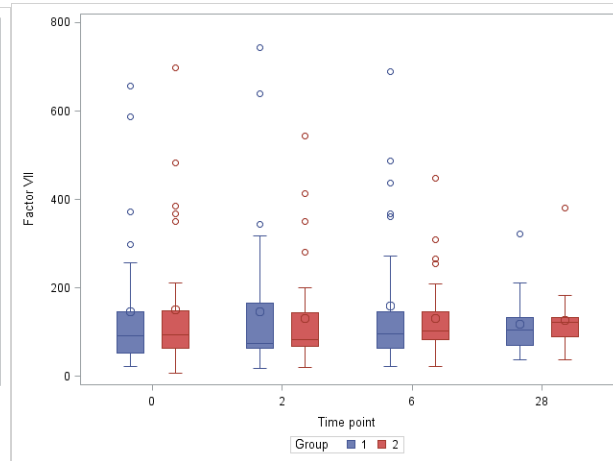
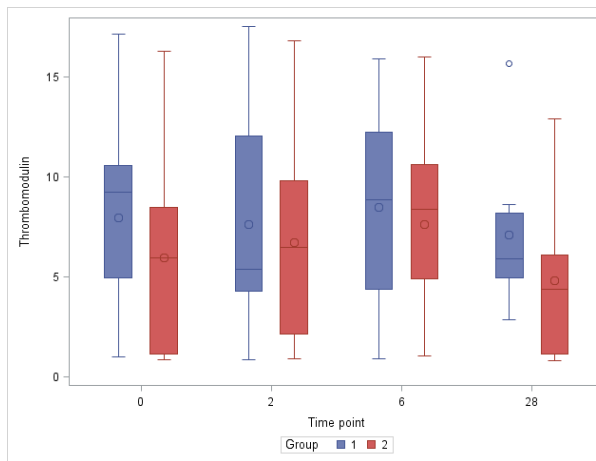
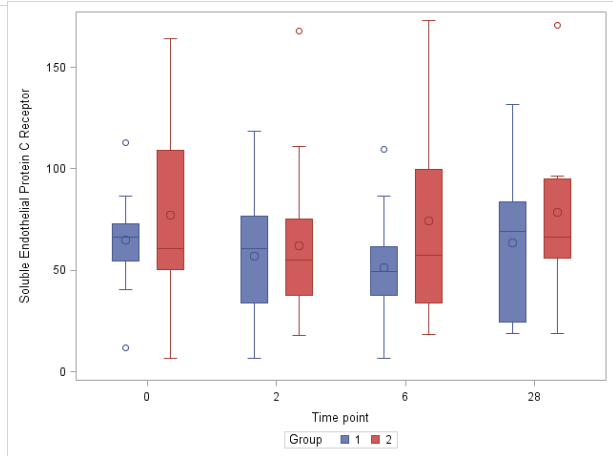
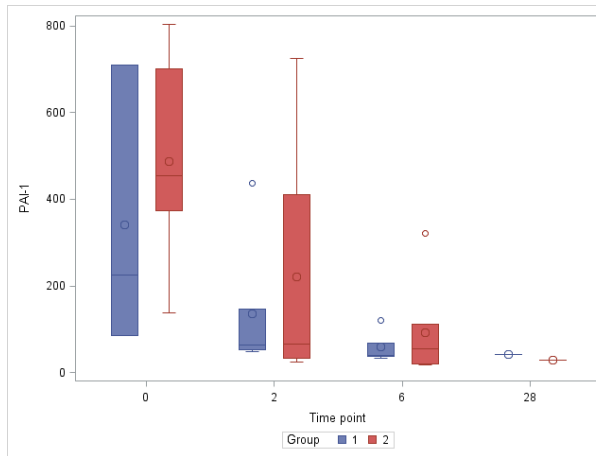
Since the last annual report, we have continued to enroll patients and collect samples at all sites. As of J December 26, 2013, we have enrolled a total of 130 patients of 200 needed in this study. We have recruited 1 new site and are in the process of recruiting 2 additional sites in order to meet the study enrollment goals. The ABLE study is probably going to be extended past 4/1/2014. This will also increase our ability to recruit our target of 200 patients.

Figure 1 below shows total enrollment versus expected enrollment (as of December 26, 2013). Graph also shows actual enrollment for each of the sites.



Evaluable samples have been collected, processed and shipped from the clinical sites to BSRI. These samples are being stored at BSRI. Cytokine analysis will be performed in batches in order to avoid variability of test results due to testing procedures and/or reagents used. We have completed coagulation testing for 65 of patients (see Fig. 2 for representative markers). The results are categorized as Group 1 and 2, since we are still blinded to the treatment groups of the parent RCT. This information along with correlation with clinical outcomes will be available once the study is completed. For most parameters in the samples tested, there seems to be a similarity in change over time between the two study groups. Further analysis on significance of the differences seen here is currently underway.





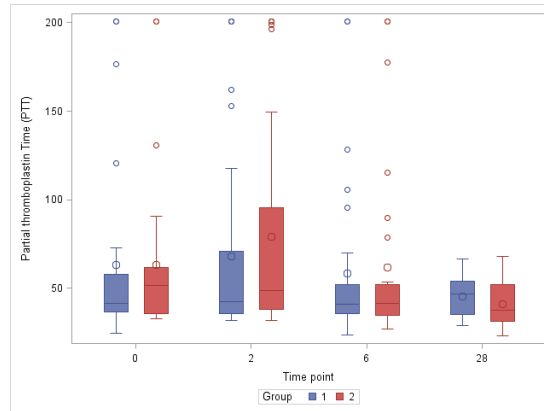


Fig.2 Coagulation parameters tested for 65 subjects for Day 0, 2, 6 and 28. Patients either received fresh blood or standard unit (which group is which is unknown).

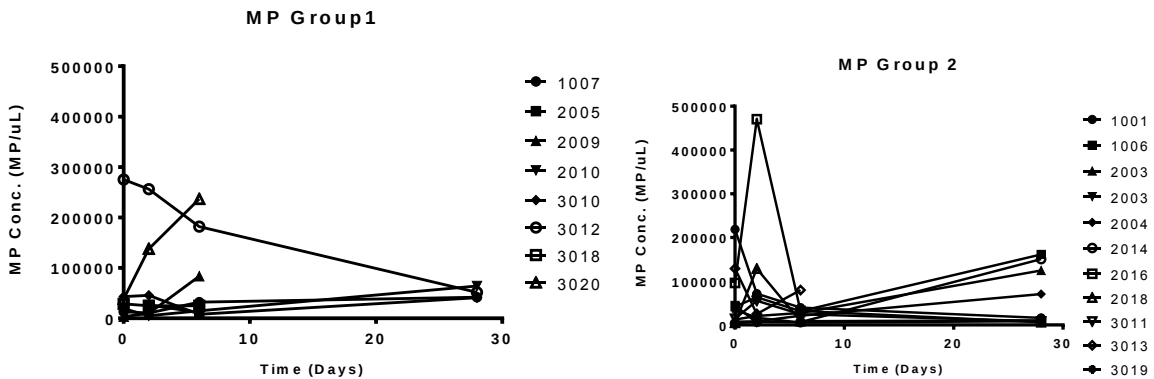


Fig. 3 Microparticle counts of the first 19 subjects tested. Patients in Groups 1 and 2 received either fresh blood (< 8 days storage time) or standard issue/oldest in inventory. Samples were tested at Days 0, 2, 6, and 28. Control MPs were tested using MPs pooled from 3 normal donors. Preliminary results show early elevated MPs in some subjects, with late elevations in others compared to controls. Changes appear to be more pronounced in Group 2 than Group 1.

To date, 99 patients for the Treg assay, 91 patients for the Th17 assay, and 95 patients for the proliferation assay have been tested. For some samples a patient/time point was tested but its results were omitted from the analyses for quality control reasons due to low cell number; these will be repeated once initial testing is finished.

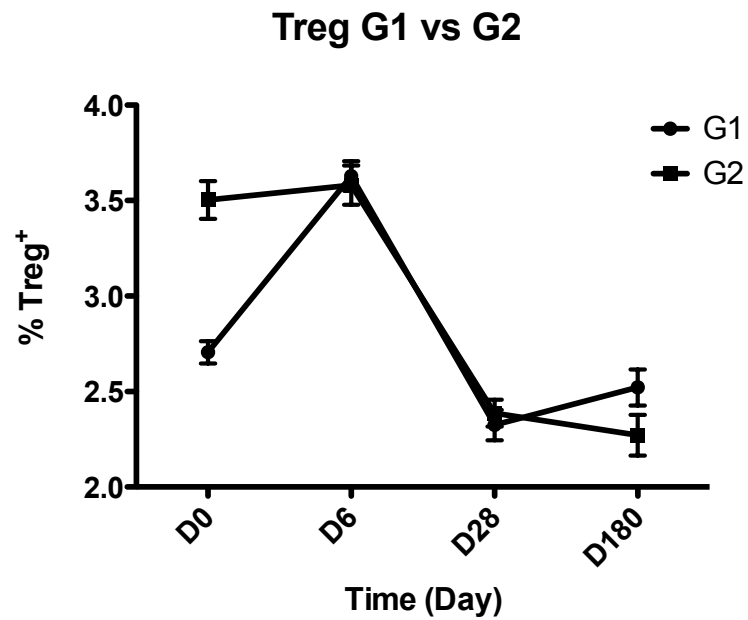


Fig. 4 Evolution of Treg markers. Patients in Groups 1 and 2 received either fresh blood (< 8 days storage time) or standard issue/oldest in inventory. Samples were tested at Days 0, 6, 28, and 180. Results demonstrate reduction in Treg markers in both groups over time. Please note that these do not include results for all samples tested due to reasons stated above.

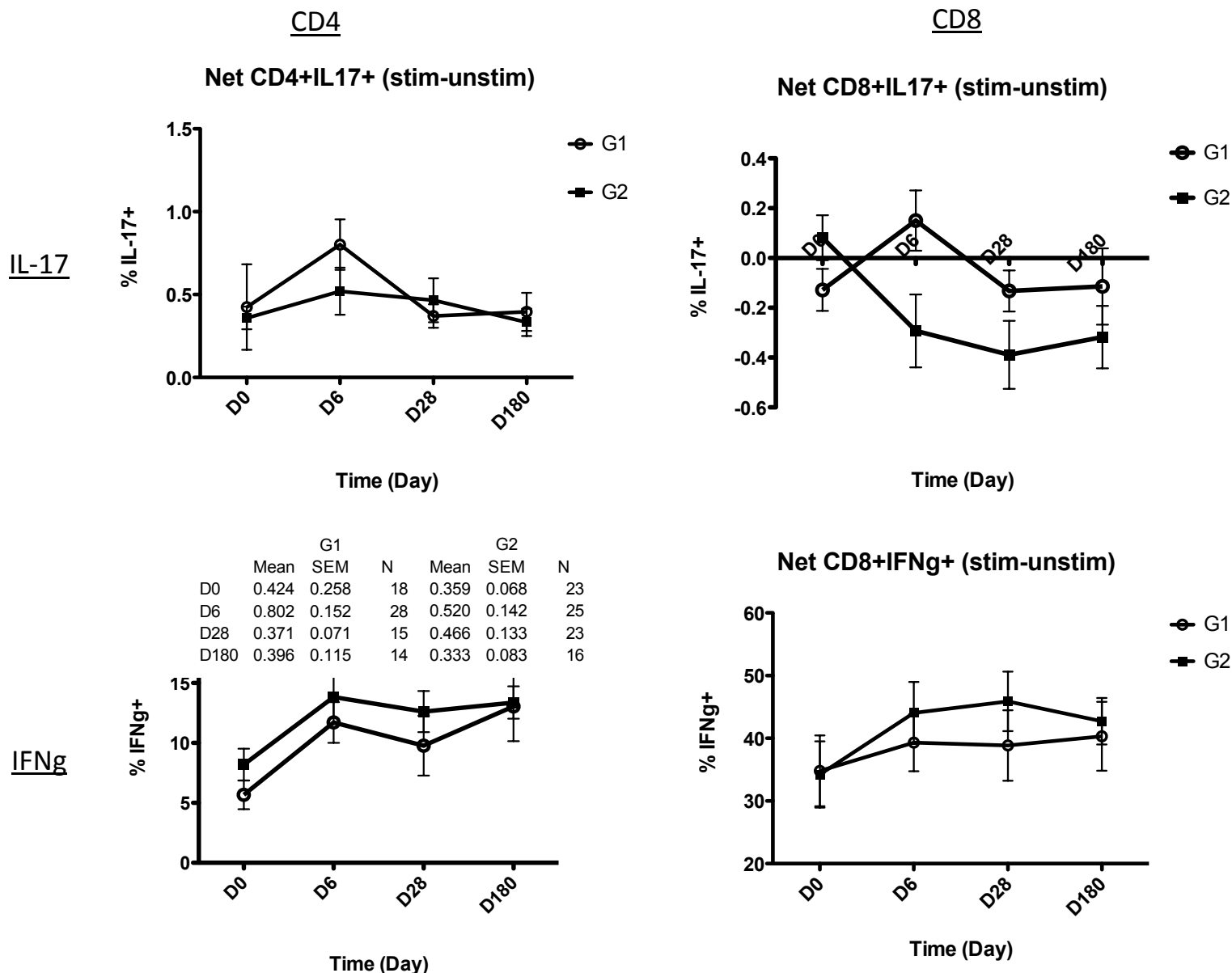


Fig. 5 Evolution of Th17 markers. Patients in Groups 1 and 2 received either fresh blood (< 8 days storage time) or standard blood (mean 21 days storage time). Samples were tested at Days 0, 6, 28, and 180. Please note that these do not include results for all samples tested due to reasons stated above.

The Molecular Transfusion Core Lab was sent samples from 103 transfused subjects. Each subject has at least one follow up sample collected at Day 28 or Day 180 post-transfusion. The HLA-DR and InDel types of each subject were determined using (pre-transfusion) Day 0 samples in order to identify the subjects' type prior to transfusion and to identify which alleles or polymorphisms will be informative. The follow-

up post-transfusion samples were then probed for presence of microchimeric DNA or donor derived DNA using informative alleles or polymorphisms. Typing using the HLA-DR and InDel panels is completed for all Day 0 samples. No microchimerism was detected in all Day 28 and 180 samples when amplified for InDel polymorphisms. We are currently in the process of determining whether microchimeric DNA is present in the last 8 subjects using HLA-DR informative alleles.

KEY RESEARCH ACCOMPLISHMENTS

- We continue to enroll patients and collect samples at the clinical sites in Canada
- The samples are being processed, shipped and stored.
- To date, 130 out of 200 patients have been enrolled.
- We have completed coagulation testing for most parameters for samples of 65 patients. The last batch of samples for 35 patients is currently being analyzed.
- We are in the process of completing microchimerism testing for 63 patients.
- We have completed microparticle testing on all samples from 19 patients.
- To date, 99 patients for the Treg assay, 91 patients for the Th17 assay, and 95 patients for the CFSE assay have been tested.

REPORTABLE OUTCOMES

We have continued building a repository of plasma, PBMCs and whole blood samples. Coagulation, micro particle, microchimerism and immune function testing on study samples is underway.

CONCLUSION

The ABLE ancillary study has continued to enroll patients and collect samples. To improve our enrollment rate 1 additional site has been added and 2 more are about to begin enrolling patients. Testing of samples collected will accelerate in the coming year as we accrue enough to test for each of the study aims.

REFERENCES

None

APPENDICES

None