

# A PHASE I, HEALTHY VOLUNTEER EXPLORATORY STUDY TO ASSESS INDUCTION OF APOPTOSIS ASSOCIATED WITH THE THERAKOS™ CELLEX™ AND THE THERAKOS™ UVAR XTS® SYSTEM

CA Burnett\*, PhD, KA Birbeck\*, S. Abhyankar, MD<sup>‡</sup>., Dan Lewis,  
CCRP<sup>‡‡</sup> LJ Dumont, PhD, † Z M Szczepiorkowski, MD, PhD, FCAP †,  
D Parenti, MD\*

\*Therakos, Inc., Raritan, New Jersey, ‡ University of Kansas and the  
University of Kansas Medical Center, Kansas City, KS, ‡ Heartland Institute for  
Clinical and Translational Research, †Dartmouth-Hitchcock Medical Center,  
Lebanon, NH



# Disclosure Statement

---

I am a full time employee of Therakos, Inc. a division of Ortho Clinical Diagnostics, a Johnson & Johnson company.

The topic of my discussion involves commercially available products marketed by Therakos.



# Study Objective

---

- The primary objective of this Phase I study was to assess the degree of apoptosis induced by the mechanical processes used for cell separation and processing of the buffy coat.
  - Sham ECP Procedure to be used in upcoming trials
  - Does the sham induce apoptosis?

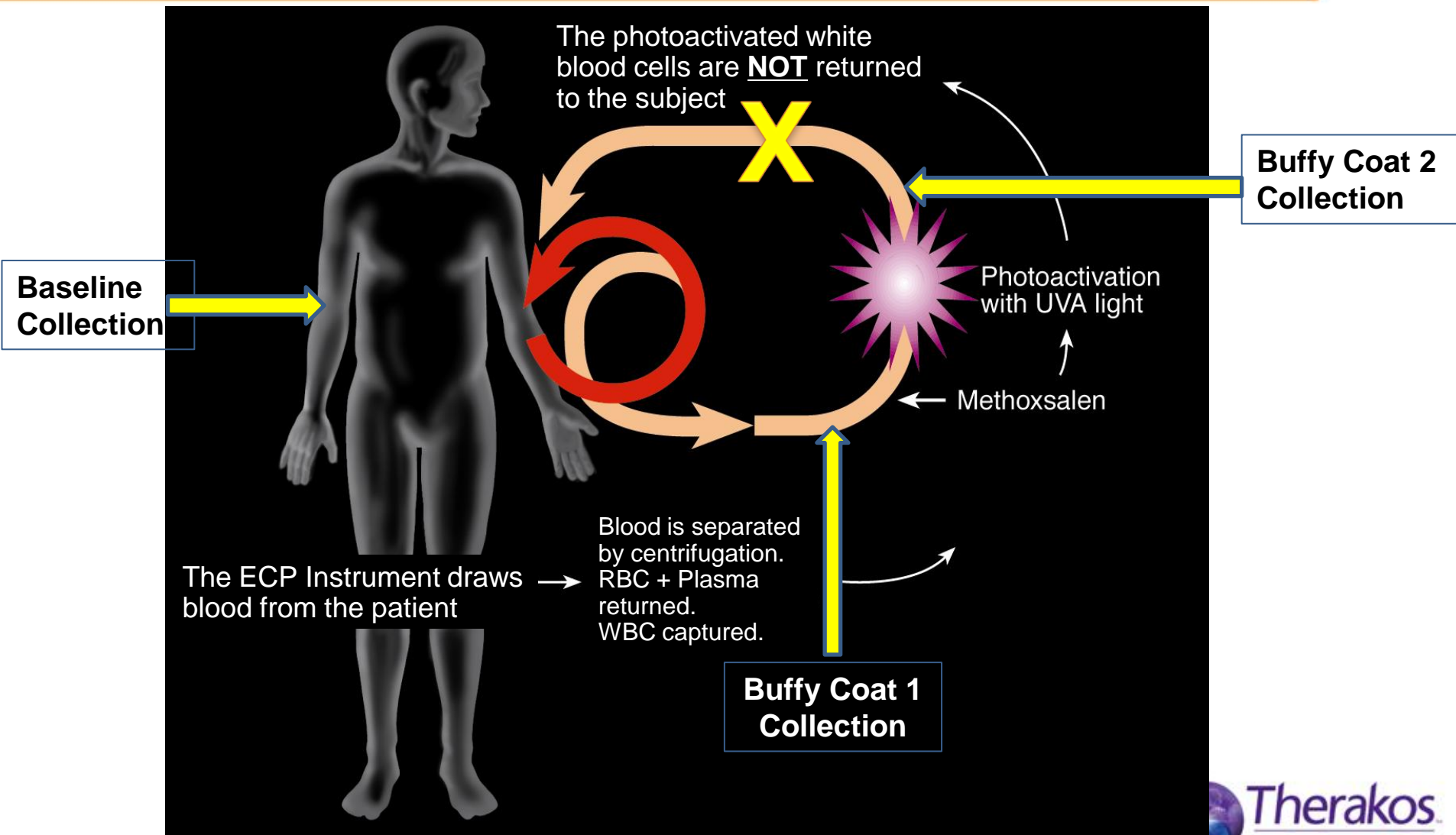


# Study Design

---

- 14 healthy male volunteers at 2 sites:
  - 8 subjects - THERAKOS™ UVAR XTS® system
  - 6 subjects - THERAKOS™ CELLEX™ system
- 3 Samples collected during procedure for analysis:
  - Baseline (Buffy Coat from 20cc Peripheral blood)
  - Buffy Coat 1 (Pre-Treatment)
  - Buffy Coat 2 (Post-Treatment)
- All samples shipped to central lab for analysis of cell death

# Simulated ECP Procedure





# Measuring Cell Death in the Laboratory

---

## Three complementary methods

### **Annexin V Binding / PI Staining –**

Indicator of early events leading up to cell death

Detects changes in plasma membrane

Is able to measure levels of living cells and apoptotic cells (early + late apoptosis)

### **Analysis of Cell Proliferation by BrdU Incorporation –**

Functional assay that measures cell death by observing the ability of cells to become activated in the presence of antigenic stimulus.

### **Analysis of Cell Viability by Trypan Blue –**

Indicator of later events in the process of cell death

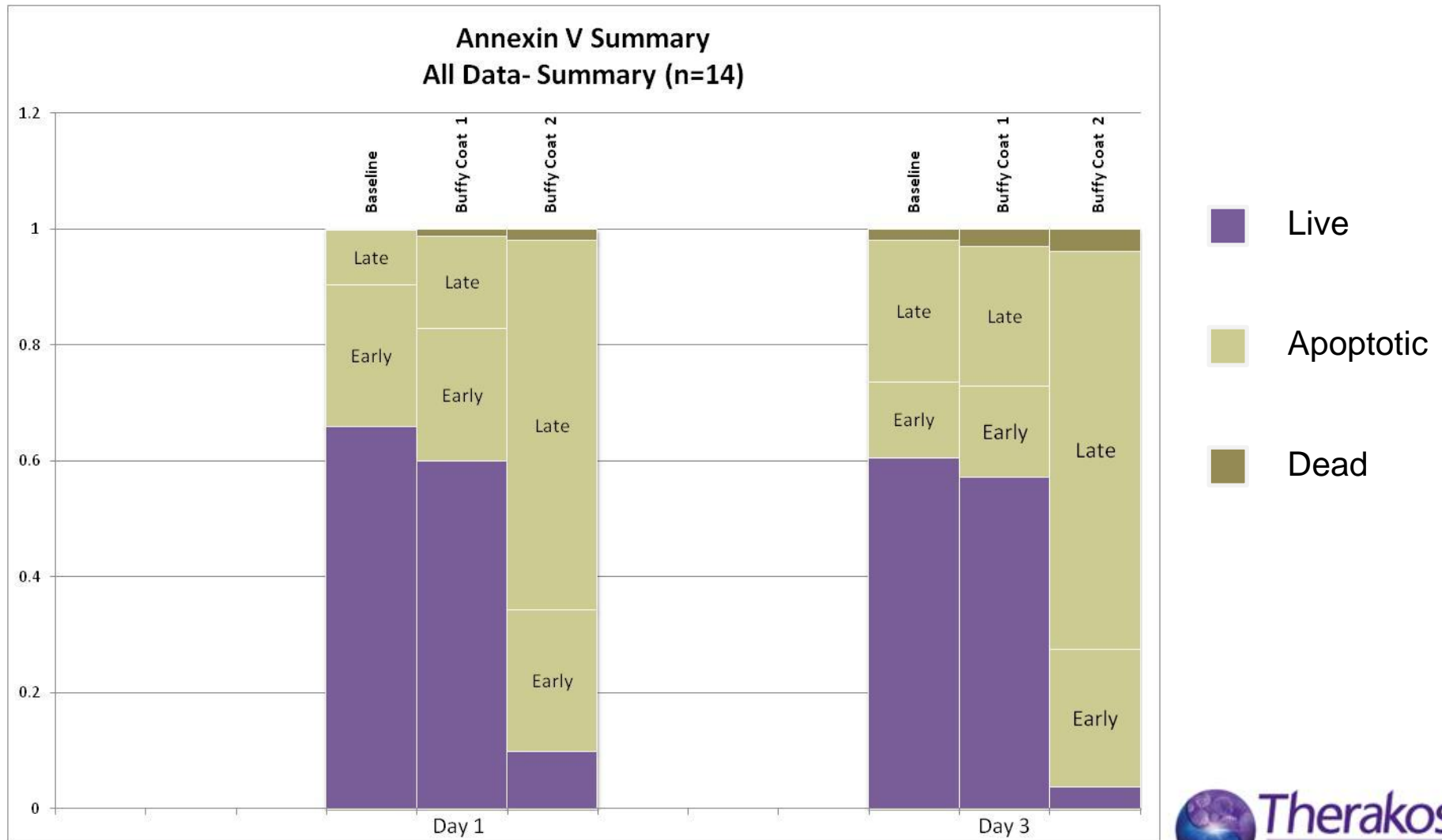
Live or living cells will exclude trypan blue dye.

Measures overall cell death = Apoptotic + Necrotic cell death

(data not presented due to time constraints)

# Results

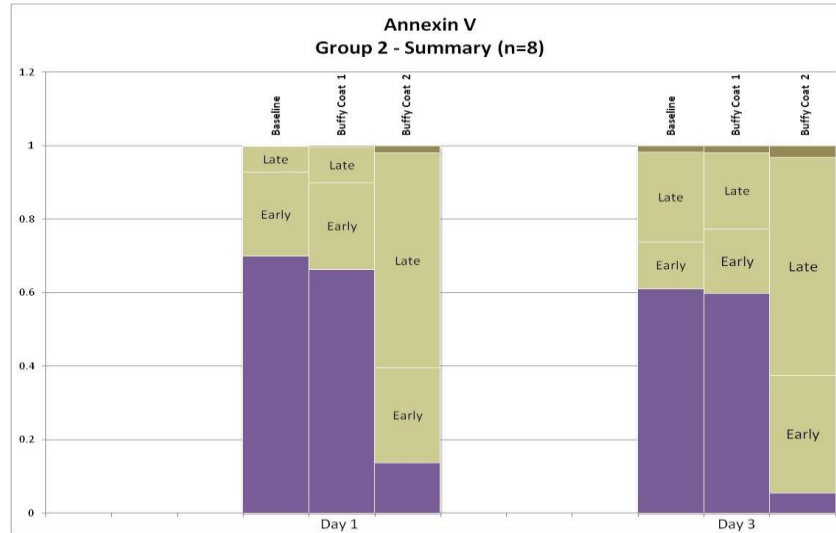
## Apoptosis by Annexin V Analysis - Summary All Subjects



# Results

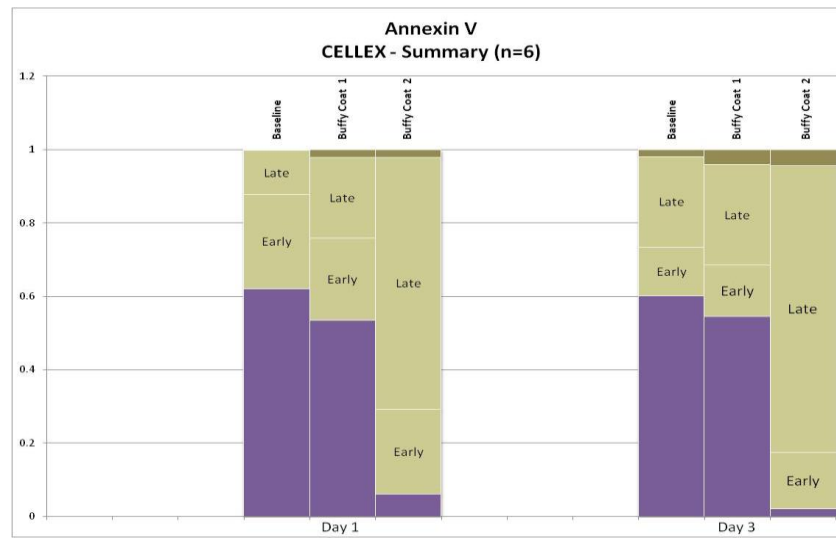
## Apoptosis by Annexin V Analysis - By ECP System

THERAKOS™ UVAR XTS®



Live  
 Apoptotic  
 Dead

THERAKOS™ CELLEX™





# Annexin V Analysis - Results

---

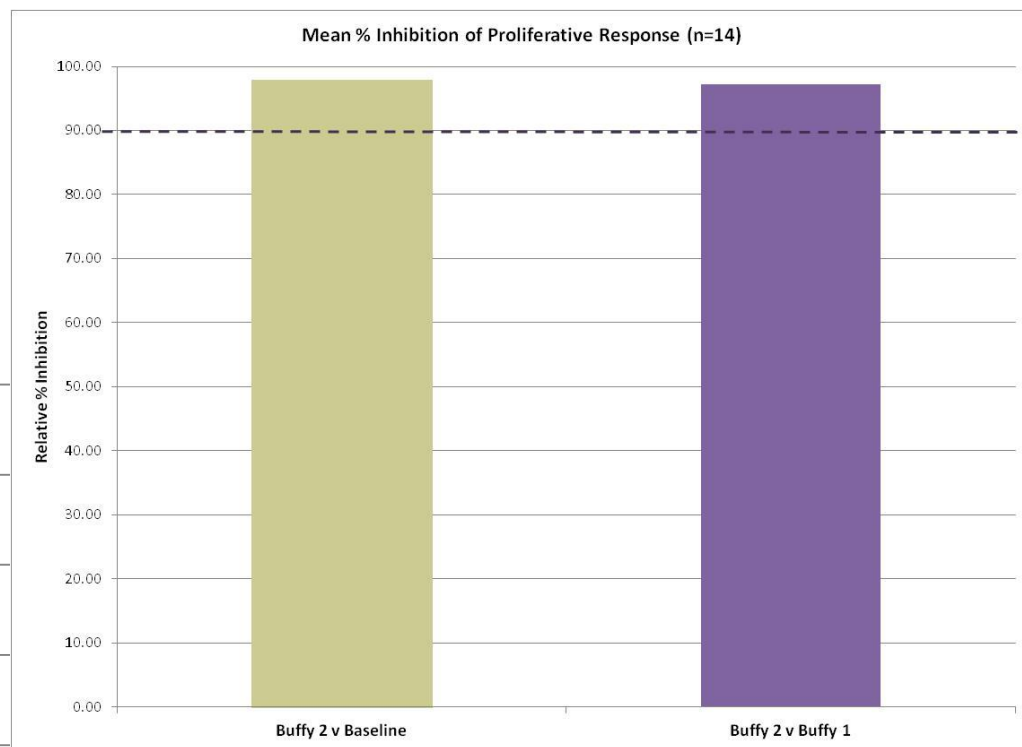
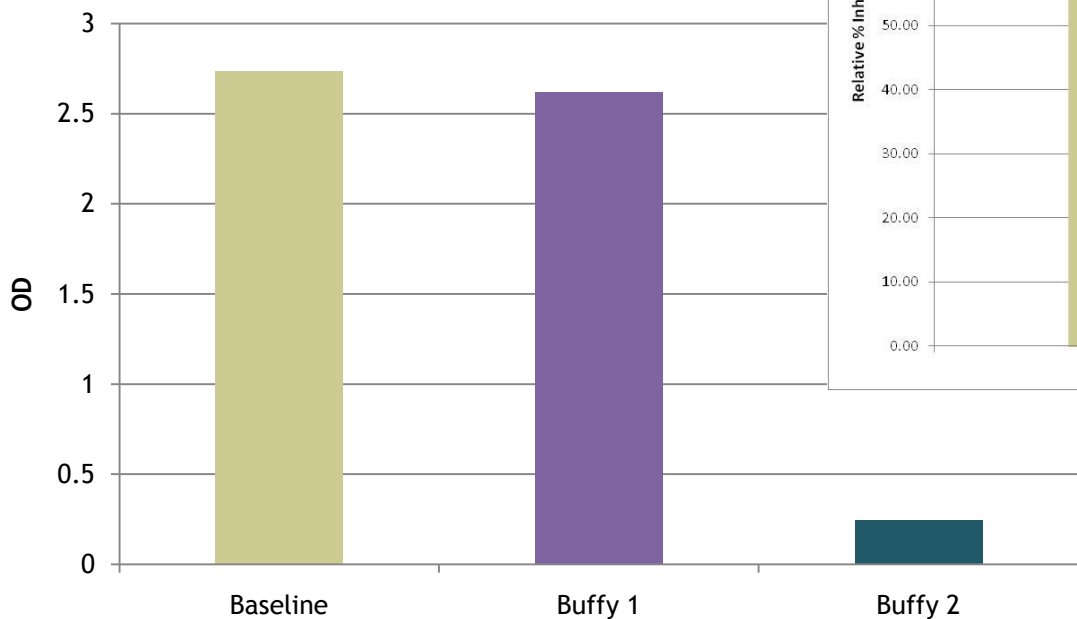
- Annexin V Analysis demonstrated a marked difference between the treated buffy coats (Buffy Coat 2) and the untreated buffy coats (Buffy Coat 1) as well as the Baseline sample.
  - On average, less than 10% viable cells were left after 3 days of culture for treated vs greater than 50% for Buffy 1 and greater than 60% for Baseline
- XTS and CELLEX are equivalent

# Results

## Cell Proliferation by BrdU Incorporation – Proliferative Response

### Example

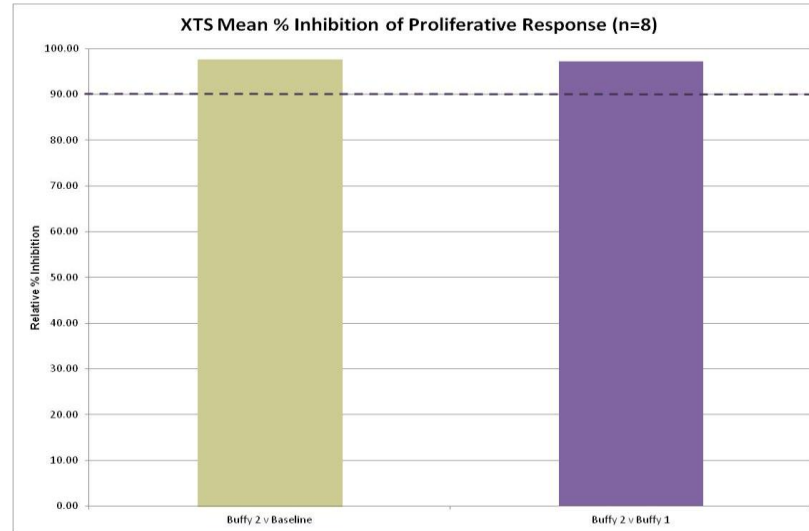
Proliferative Response  
20ug/mL PHA, 72 hr  
Subject 1-03



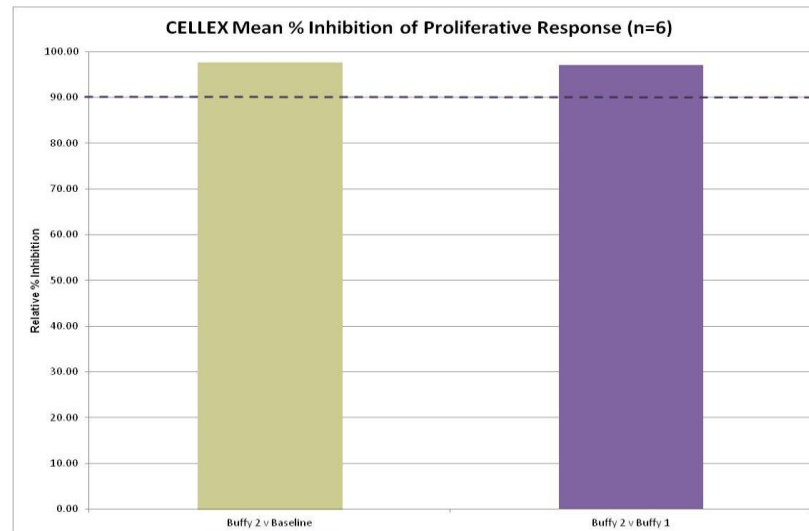
# Results

## Cell Proliferation by BrdU Incorporation - By ECP System

THERAKOS™ UVAR XTS®



THERAKOS™ CELLEX™





# Cell Proliferation by BrdU - Results

---

- Inhibition of Cell Proliferation in response to PHA demonstrated a marked difference between the treated samples (Buffy Coat 2) and the untreated samples (Buffy Coat 1) and Baseline (relative % inhibition greater than 90%).
- XTS and CELLEX are equivalent



# Cell Viability by Trypan Blue - Results

---

- Trypan Blue Analysis also showed a similar difference between the treated buffy coat samples (Buffy Coat 2) and the untreated buffy coats.
- (data not shown)



# Conclusion

---

- The results of this study suggest that the level of apoptosis resulting from mechanical processes alone is negligible as evidenced by 3 complementary assays that demonstrated differences between the Baseline and untreated samples compared to treated samples.
- XTS and CELLEX are equivalent



# Acknowledgements

---

- **Dartmouth-Hitchcock Medical Center - Lebanon, NH**
  - Zbigniew "Ziggy" M. Szczepiorkowski, MD, PhD, FCAP
  - Larry Dumont, MBA, PhD
  - Nancy Dunbar, MD
  - Karen Klinker, MT(ASCP)SBB
  - Louise Herschel, CCRC
  - Caroline Hoffman, RN
  
- **University of Kansas Medical Center - Kansas City, KS**
  - Sunil Abhyankar, MD
  - Omar Aljitawi, MD
  - Joseph McGuirk, DO
  - Siddhartha Ganguly, MD, FACP
  - Dan Lewis, CCRP
  - Dean Merkel, MT (ASCP)
  - Joann Miller, BA, CCRP
  - Anne Hirner, RN