

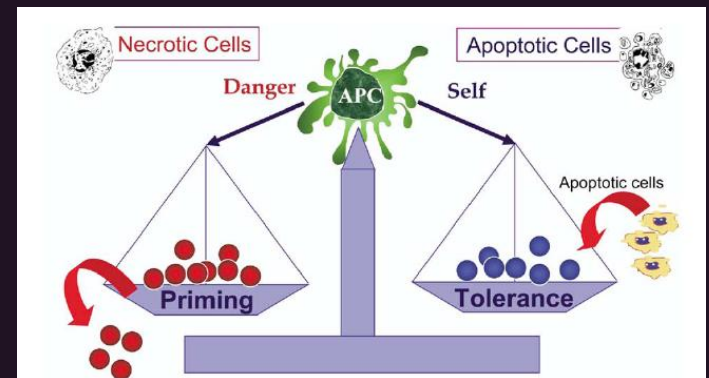
Offline Photopheresis Effectively Results in Apoptosis and Inhibition of Lymphocyte Proliferation

K. Radwanski, C. Heber, K. Min
Fenwal Inc., Lake Zurich, IL USA



Extracorporeal Photochemotherapy (Photopheresis)

- Leukopheresis-based immunomodulatory therapy
 - MNC are collected, exposed to 8-methoxypsoralen (8-MOP), irradiated with ultraviolet-A (UVA) *ex vivo*, and re-infused into the patient
- Treatment of
 - CTCL (FDA approved indication)
 - aGVHD and cGVHD
 - Allograft rejection
- Mechanism of action
 - Still under investigation
 - Leading theory- immune tolerance



Biology of Blood and Marrow Transplantation 12:7-12 (2006)

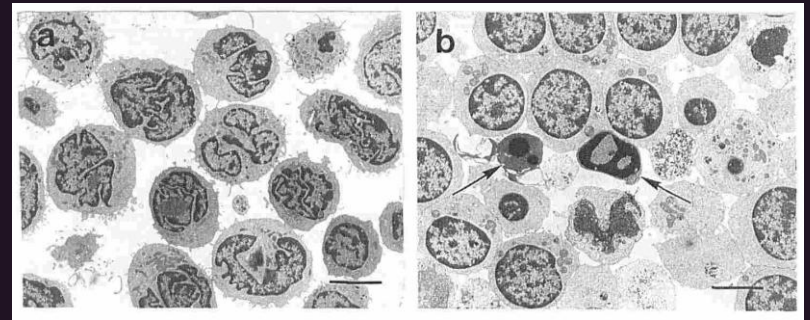
Online and Offline Photopheresis

- Online- dedicated apheresis device
- Offline- 3-step procedure
 - MNC collection
 - Manual 8-MOP injection
 - UV irradiation with light box
- Pros
 - Low cost
 - Low ECV
 - Collect & treat larger cell dose
- Cons
 - Additional traceability
 - Added quality control to validate system components



Techniques for Cell Evaluation Post Photopheresis

- Apoptosis
 - DNA fragmentation
 - Gel electrophoresis
 - TUNEL
 - Cell characteristics
 - Microscopy
 - Light scatter
 - Annexin V binding



Yoo et al. J Invest Dermatol 1996; 107: 235-42

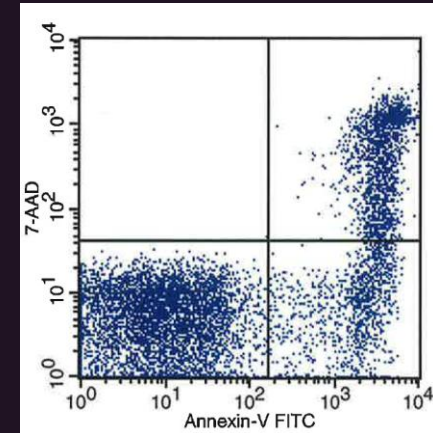
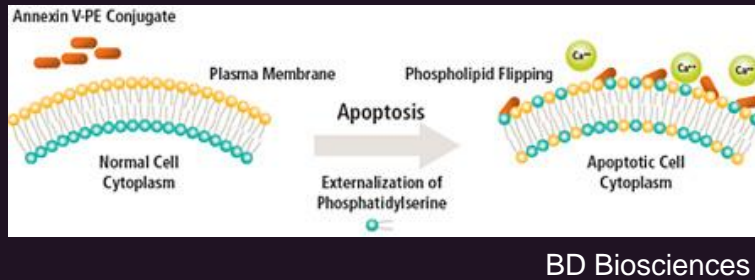
- Proliferation
 - ^3H thymidine
 - CFSE (5,6-carboxy fluorescein diacetate succinimidyl ester)

Most characterizations have been performed on online or culture dish treated cells

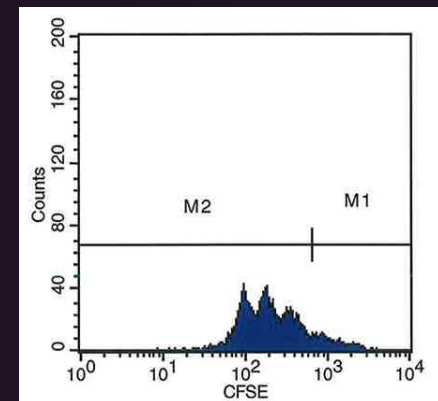
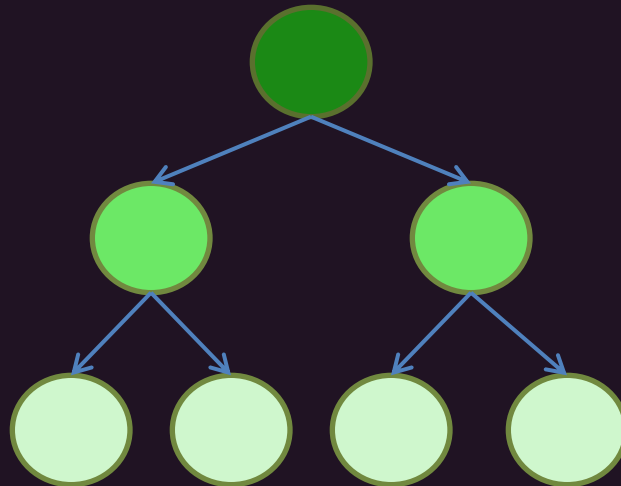
Objective

Utilize both the lymphocyte apoptosis and proliferation assays to examine the in vitro effectiveness of offline photopheresis

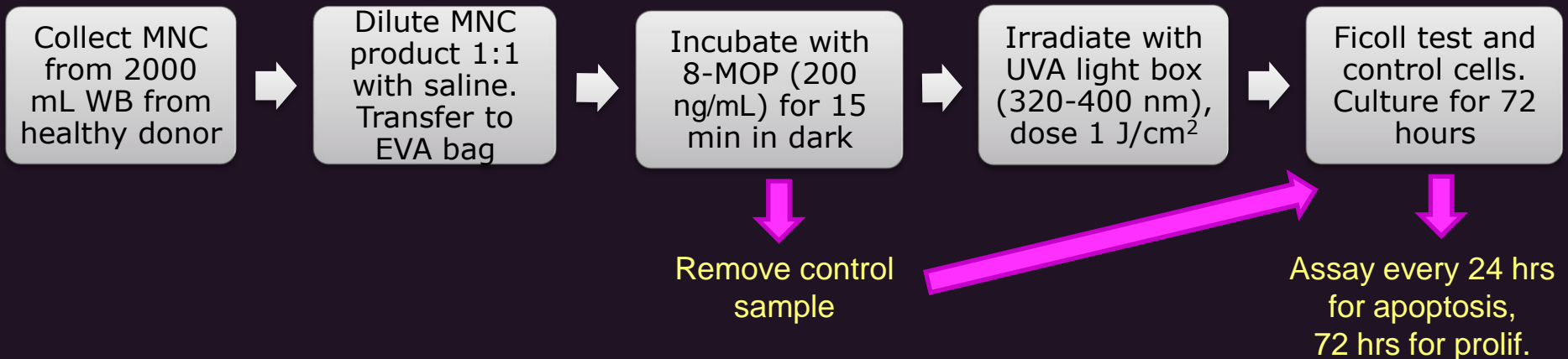
Annexin V binding



CFSE labeling



Methods

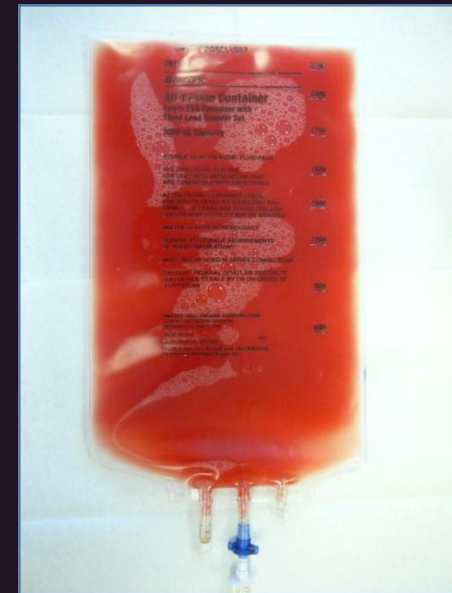


- n = 13 procedures
- Apoptosis measured as % of CD45+/Annexin V+ cells in lymphocyte FCS/SSC gate
- CFSE labeling post stimulation with polyhemagglutinin-A*



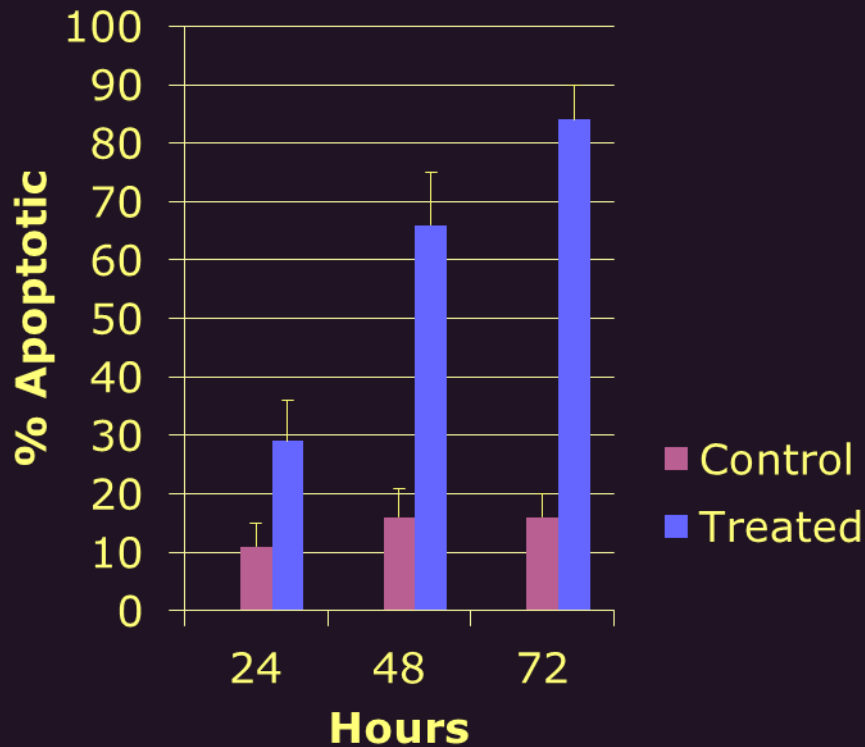
MNC Product Characteristics

Parameter	MNC Product, pre-dilution n = 13
Volume (mL)	184 ± 4
Hct (%)	1.7 ± 0.4
TNC (x10 ⁹)	3.2 ± 0.9
Lymph (x10 ⁹)	2.6 ± 0.8
Lymph Collection Efficiency (%)	62 ± 16
Plt (x 10 ¹¹)	0.4 ± 0.1



MNC product post saline
dilution (~1 % Hct)

Lymphocyte Apoptosis Results



Apoptosis Post Online ECP (Annexin V method)

- 25% Difference between treated and untreated cells after 20-24 hours ^{1,2}
- 45% Difference after 48 hours²

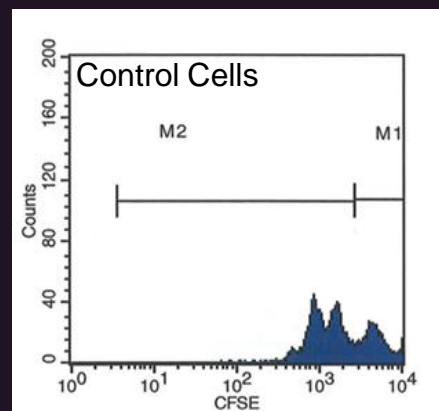
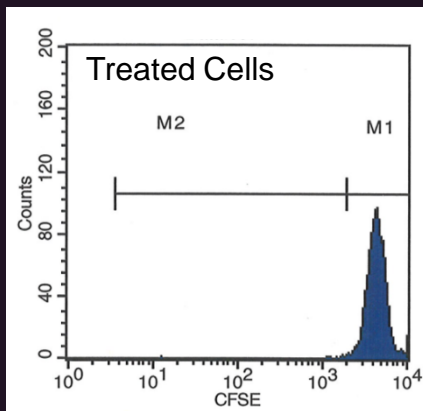
1. Gerber A, et al. *Dermatology* 2000;201:111-117

2. Bladon J, Taylor PC. *British Journal of Dermatology* 2002;146:59-68.

Lymphocyte Proliferation Results

$$\% \text{ Proliferation} = \frac{M2}{M1+M2}$$

$$\% \text{ Inhibition of Proliferation} = \frac{\% \text{ Proliferation}_{\text{control}} - \% \text{ Proliferation}_{\text{treated}}}{\% \text{ Proliferation}_{\text{control}}}$$



Parameter	t = 72 hours	
% Proliferation	Test	3.0 ± 2.1
	Control	72.4 ± 15.1
% Inhibition of Proliferation	96 ± 3 (90 - 98)	

Lower threshold of > 90 % inhibition suggested by Evrard et al.¹

Alert threshold of < 70 % inhibition requires further investigation ²

¹ Evrard B, et al. Trans APh Sci 2010;42:11-19

² Jacob MC, et al. Trans APh Sci 2003;28:63-70

Conclusions

- In vitro criteria for effective photopheresis treatment have been met and are comparable to online ECP results
- Apoptosis and proliferation assays can be used in conjunction to assess effectiveness of photopheresis treatment