Disclosure

- **Yale** University patents from our research group
- Licensed to Transimmune AG, development company
- Inventor of Extracorporeal Photochemotherapy
- Licensed to J&J (Therakos), arrangement expired
- Commitment to developing the broad field
<table>
<thead>
<tr>
<th>R Edelson</th>
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Inclusion Criteria: Publication Record/Expertise
Visionary Commitment to ECP’s Scientifically-Driven Evolution
CTCL/Cancer, Transplantation, Autoimmunity, Immunology

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# ACE Consensus Conference

## Represented Institutions

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<tr>
<th>Institution</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Columbia</td>
<td>Northwestern University</td>
</tr>
<tr>
<td>Dartmouth</td>
<td>Stanford University</td>
</tr>
<tr>
<td>Bloodworks (Seattle)</td>
<td>Vanderbilt University</td>
</tr>
<tr>
<td>Harvard University</td>
<td>Washington University</td>
</tr>
<tr>
<td>Hutchinson Cancer Center</td>
<td>University of California (San Diego)</td>
</tr>
<tr>
<td>Levine Cancer Center</td>
<td>University of Pennsylvania</td>
</tr>
<tr>
<td>MD Anderson Cancer Center</td>
<td>University of Washington</td>
</tr>
<tr>
<td>Mayo Clinic</td>
<td>Yale University</td>
</tr>
</tbody>
</table>
Points of Unanimous ACE Agreement

• **MECHANISM**: Control of (DC) T CELL MASTER-SWITCH

• **UP** (immunizing) and **DOWN** (tolerizing) polarization

• Immunotherapy of **ALL IMMUNOGENIC CANCERS**

• Enabling of **HAPLOTYPe-MATCHED** stem cell transplants

• Autoimmune disorders with **KNOWN TARGET ANTIGENS**

• Awareness of risk of **ACCELERATED DISEASE PROGRESSION**
The Right DC - Sorted Antigenic Peptides Being Handed the Winning Lottery Ticket
Single DC – Multiple CD8 T Cell Engagement
Apoptotic Dendritic Cells as Tolerogenic Baton

Kushwah and Hu
J Immunol 185:795-802, 2010

Internalization by Normal DC then Directed to Tolerogenic Mode

Antigen-Specific Foxp3+ Tregs
8-Methoxypsoralen (8-MOP)

Reactive 4’-5’ Bond

Reactive 3’-4’ Bond

UVA Energy

O-CH₃

Covalently Binds to Pyrimidine Bases

Second Independent Reactive Site
Psoralen

Psoralen Containing Plasma

UVA Irradiation
(Maximal Surface-to-Volume Ratio)

Return of Treated Leukocytes
Apoptotic Cell Processing by Immature DC
## ECP Impact on the Monocyte Transcriptome

Summary of Gene Up- and Down-Regulation

<table>
<thead>
<tr>
<th>Monocyte Source</th>
<th>Total</th>
<th>Up-Regulated</th>
<th>Down-Regulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Subjects: N=6</td>
<td>2820</td>
<td>1111</td>
<td>1709</td>
</tr>
<tr>
<td>CTCL: N=3</td>
<td>3178</td>
<td>1956</td>
<td>1222</td>
</tr>
<tr>
<td>GVHD: N=3</td>
<td>3235</td>
<td>2008</td>
<td>1227</td>
</tr>
<tr>
<td>Normal/CTCL/GVHD (shared): N=12</td>
<td>1129</td>
<td>498</td>
<td>631</td>
</tr>
</tbody>
</table>
polystyrene Transimmunization (TI) chamber

75 mm

290±15 μm

25 mm
# ECP Activation of 12 Important Dendritic Cell Genes

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<th>Name/Attributes</th>
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<tr>
<td>DC-LAMP</td>
<td>DC Lysosomal Membrane Glycoprotein/Mature DC</td>
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<tr>
<td>GPNMB</td>
<td>Transmembrane Glycoprotein/T Cell Coinhibitor</td>
</tr>
<tr>
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<td>B7.1/T Cell Co-Stimulatory Molecule</td>
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<td>CD86</td>
<td>B7.2/T Cell Co-Stimulatory Molecule</td>
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<tr>
<td>CD40</td>
<td>APC-Activating Co-Stimulatory Protein</td>
</tr>
<tr>
<td>Decysin</td>
<td>ADAM-Like Protein Marker of DC Maturation</td>
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<tr>
<td>CCR7</td>
<td>DC Lymph Node Homing Molecule</td>
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<tr>
<td>CCL2</td>
<td>MCP1/monocyte chemoattractant protein 1</td>
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<tr>
<td>CXCL5</td>
<td>ENA78/Secondarily Attracts Neutrophils</td>
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<tr>
<td>ICAM1</td>
<td>Intercellular Adhesion Molecule 1/Binds Integrins</td>
</tr>
<tr>
<td>$\alpha_5\beta_1$</td>
<td>Integrin Primary Receptor for Fibronectin</td>
</tr>
<tr>
<td>PLAUR</td>
<td>CD87/Activated Monocyte Marker</td>
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Equal Expression in Clinical and “Mouse-to-Man” Systems

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ECP-Induced Entry of Monocytes into DC Pathway

Percent Cytoplasmic CD83/HLA-DR+

Pre-ECP
ECP – Time 0 hr
ECP – Time 18 hr

Test Subject:  CTCL  GVHD  Normal

Mean
Monocyte Adherence to, and Signaling by, Platelets
Schema of Monocyte-to-DC^p Conversion

Activated Monocyte

Monocyte Transition to DC^p

Activated Platelets

Platelet Integrins αIIbβ3 and α5β1

Platelet P-Selectin

Platelet Membrane Fibronectin

Monocyte Integrin α5β1

BG8

Plastic Surface
Murine Melanoma System

- Yale University Mouse Melanoma (YUMM) model
- Developed by the Bosenberg laboratory
- Operative in approximately 100 laboratories
- C57BL/6J background, BrafV600E, Pten-/- Cdkn2a-/-
- Tamoxifen induced tumors
- YUMM1.7 derived cell line, 310 point mutations
Baseline Immunotherapeutic Protocol

TI treatment of syngeneic mouse tumors

$1 \times 10^5$

tumor

cells s.c.
TI treatment of syngeneic mouse tumors

1 \times 10^5 \text{ tumor cells s.c.} \rightarrow 10 \text{mm}^3 \text{ tumor}
Baseline Immunotherapeutic Protocol

TI treatment of syngeneic mouse tumors

1 x 10^5 tumor cells s.c.

10 mm^3 tumor

blood draw and PBMC isolation
Baseline Immunotherapeutic Protocol

TI treatment of syngeneic mouse tumors

1 \times 10^5 tumor cells s.c. \rightarrow 10\text{mm}^3 tumor

blood draw and PBMC isolation

8-MOP/UVA treated tumor cells
Baseline Immunotherapeutic Protocol

TI treatment of syngeneic mouse tumors

1*10^5 tumor cells s.c. → 10mm^3 tumor → blood draw and PBMC isolation

→ 8-MOP/UVA treated tumor cells

→ platelet-coated chamber
Baseline Immunotherapeutic Protocol

TI treatment of syngeneic mouse tumors

1*10^5 tumor cells s.c. → 10mm^3 tumor

blood draw and PBMC isolation

8-MOP/UVA treated tumor cells

PBMC/tumor overnight co-incubation

platelet-coated chamber
Baseline Immunotherapeutic Protocol

TI treatment of syngeneic mouse tumors

1*10^5 tumor cells s.c. → 10mm^3 tumor → blood draw and PBMC isolation → 8-MOP/UVA treated tumor cells

Treatment: 2x per week, 3 weeks

PBMC/tumor overnight co-incubation → platelet-coated chamber
Response of Mouse Melanoma Growth Control by Experimental ECP
Baseline Anti-Melanoma Immunotherapy System
Uniformly Reproducible Tumor Inhibition

- PBS control (n=346)
- Transimmunization YUMM1.7 (n=111)

![Graph showing tumor volume over time after treatment with PBS control and transimmunization YUMM1.7. The graph indicates a significant reduction in tumor volume for the transimmunization group compared to the PBS control group.](image-url)
UVA/MOP Exposure of Induced APC
Abrogation of Immunoprotection

- PBS control (n=68)
- Transimmunization (n=10)
- TI with PBMC 8-MOP\textsubscript{A} exposure (n=10)

Relative YUMM1.7 tumor volume, %

Days post YUMM1.7 tumor injection

NS

**** ****
Dependency of Immunoprotection on Integrity of Both CD4 and CD8 Populations

relative YUMM1.7 tumor volume, %

- PBS control (n=156)
- Transimmunization (n=38)
- TI mouse CD8 depletion (n=19)
- TI mouse CD4 depletion (n=13)

1st depleting Ab injection

days post YUMM1.7 tumor injection
Splenocyte Transfer of Immunoprotection

- untreated control (n=10)
- TI whole spleen (n=5)
- PBS control whole spleen (n=5)
- naive mouse whole spleen (n=5)

relative YUMM1.7 tumor volume, %

days post YUMM1.7 tumor injection and immune cell transfer

NS
CD3 T Cell Transfer of Immunoprotection

untreated control (n=10)
TI spleen CD3⁺ cells (n=5)
PBS control spleen CD3⁺ cells (n=5)
naive mouse spleen CD3⁺ cells (n=5)

relative YUMM1.7 tumor volume, %

0 5 10 15 20 25 30
days post YUMM1.7 tumor injection and immune cell transfer

NS

** *** **** ****

0 50 100 150
rel. YUMM1.7 tumor volume, %
Monocyte Dependency of Immunoprotection
Efficiency of Monocyte Uptake of Apoptotic YUMM Cells

PBS control (n=84)
Transimmunization (n=39)
TI monocyte depletion a-CD11b (n=15)

days post YUMM1.7 tumor injection

0 5 10 15 20 25 30

NS

****

****
Immunoprotection Requirements
Combination of: Apoptotic YUMM and PBMC
Requirement for Immunoprotection

Platelet Contribution

- PBS control (n=68)
- Transimmunization (n=10)
- TI platelet depletion a-CD41 (n=10)

relative YUMM1.7 tumor volume, %

days post YUMM1.7 tumor injection

NS

**** ****

0 5 10 15 20 25 30
Requirement for Immunoprotection
Passage through Activation Plate

- PBS control (n=44)
- Transimmunization (n=19)
- TI no plate passage (n=5)
Baseline Anti-Colon Carcinoma System
Uniformly Reproducible Tumor Inhibition

PBS control (n=12)
Transimmunization MC38 (n=28)

PBS tumor incidence: 12/12 mice (100%)
TI tumor incidence: 18/28 mice (64%)
Tumor Immunoprotection Specificity
Selectivity for Immunizing Cancer (YUMM)
Tumor Immunoprotection Specificity
Selectivity for Immunizing Cancer (MC38)

- PBS control (n=10)
- Transimmunization MC38 (n=10)
- Control Challenge YUMM1.7 (n=17)

relative MC38 tumor volume, %

days post MC38 tumor injection

NS

****
Reversal of Immunizing Capacity

8-MOP-Truncated DC Negate Immunization Effect, May Actually Enhance Tumor Growth

![Graph showing the growth of tumors over time and statistical significance](image-url)
Summary

- DC induction underlies ECP’s bidirectional immune effects.

- Platelet triggering of monocyte-to-DC maturation is the key to ECP’s mechanism and fundamental to immunity and tolerance.

- ECP’s induction of physiologic DC, capable of initiating potent patient-specific clinical responses, opens many clinical doors.

- ECP’s potent tolerization effect is traceable to apoptotic DC.

- Extracorporeal availability of antigen-specific DC places the T cell master-switch squarely in visionary clinicians’ hands.
Principal Collaborators/Advisors

- Carole Berger
- Michael Girardi
- Douglas Hanlon
- Bob Tigelaar
- Aaron Vassall
- Alessandra Ventura
- Kristin Hoffmann
- Tyler Durazzo
- Marcus Bosenberg
- Olga Sobolev
- Adrian Hayday
- Ira Green

- Tarek Fahmy
- Patrick Han
- Anjelica Gonzalez
- Mark Saltzman
- Charles Janeway
- Kim Bottomly
- Leiping Chen
- Peter Cresswell
- Ira Mellman
- Jordan Pober
- Ralph Steinman
- Shrikant Mane