Plasma Immunotherapy of Cancers

Plasma Elicited Immunogenic Cell Death in Tumors for Stimulation of Protective Immune Responses

From Laboratory to the Clinic
Clinical trials for precancerous and cancerous skin lesions
State of Plasma Medicine

TREATMENT OF SUPERFICIAL CONDITIONS

• Prokaryotic Cells more susceptible than Eukaryotic Cells

• Cancer Cells more susceptible than Normal Cells in vitro and in vivo

• Plasma treatment conditions may be altered to achieve different effects e.g., proliferation, differentiation, apoptosis, ICD etc.

Challenge:

➢ Treatment of systemic diseases
➢ Improved specificity of effects

Answer: Treat through plasma triggering of a highly specific, natural, reliable system: The Immune System
Treatment of diseases like Cancers, Inflammatory Bowel Disease, Rheumatoid Arthritis etc. through stimulation of these professional cells.
Names Cancer Immunotherapy “Advance of the Year” (2016)

1. Chemical Immune Stimulators
2. Overcome immune suppression
3. Immune Cell transfusion
4. Chemotherapy/Radiation to increase visibility of cancer cells to the immune system

Plasma is the better choice!
THE SETUP FOR DIRECT PLASMA TREATMENT

- HV Power Supply
- Dielectric Housing
- Copper Electrode
- Quartz Dielectric
- Plasma
- Cells

Voltage
Frequency
Pulse duration
Distance
Time
Can Plasma Boost Macrophage Function?

**Plasma Parameters**

Uniform Nanosecond Pulsed Dielectric Barrier Discharge

- Voltage: 29kV
- Gap Distance: 1mm
- Time: 10 sec
- Frequency: 5, 15, 30, 75Hz
- Dose: 47, 141, 282, 705mJ

**RAW 264.7**

Murine macrophage cell line

**Macrophage Function**

1) Recognition of Foreign Antigens
2) Phagocytosis of Foreign Targets
3) Cytokine Secretion
4) Migration
5) Antigen Presentation to T and B Cells
6) Recruitment of other Immune Cells
7) Cancer Cell Killing

**Macrophage Migration**

Untreated

47mJ : 5Hz

Untreated

141mJ : 15Hz

Plasma stimulates anti-tumor activity in macrophages

**Uniform nspDBD**
- Voltage: 29kV
- Gap Distance: 1mm
- Time: 10sec
- Frequency: 15, 30Hz
- Dose: 141, 282mJ

**THP-1 Cell Treatment**

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Plasma</th>
<th>Macrophage</th>
<th>Plasma Activated Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Cancer Cell Viability**
48 h Post Plasma Treatment of THP-1

- % Cell Loss Normalized to Control
  - M0: 1
  - 141mJ: 2
  - 282mJ: 2

*nspDBD can directly enhance anti-tumor effects of macrophages*
Plasma Stimulates Cytotoxic Macrophages to Kill Cancer Cells by Triggering Release of TNF-alpha

Any role of the immune system in plasma mediated cancer control?


Mouse Survival Post Plasma

Effect of Plasma seems to be “Better” when Immune System is intact

Immune System is Involved in the Development and Control of Cancer

Failure of immune system contributes to the development of cancer. Tumor Immunologists are developing new approaches to address this.
What about the Problem of Low Antigenicity of Tumor Cells!

**Immunogenic Cell Death (ICD)**

- Increase immunogenicity of tumor cells
- Express *damage associated molecular patterns (DAMPs)*[^1]

[Image of a diagram showing the processes of ICD with labeled components: Secreted DAMP and Surface DAMP.

**Secreted DAMP**
- *Adenosine Triphosphate (ATP)*
  - ‘*FIND ME*’ signal to alert APCs of potential danger
  - Recruits APCs to area of danger

**Surface DAMP**
- *Calreticulin (CRT)*
  - ‘*EAT ME*’ signal recognized by APCs
  - Leads to subsequent engulfment

New Immunotherapeutic Approach for Cancers

Development of tumor-specific immune response\[1\]

Secreted DAMPs
Surface DAMPs

Development of Effector Cells
Selective Tumor Cell Death
Development of Memory Cells

Patient’s Own Immune System Destroys Cancer Cells

Cancer Cell Lines
- A549- Human Lung
- CNE-1- Human Radioresistant Nasopharyngeal
- CT26- Mouse Colon

Uniform Nanosecond Pulsed Dielectric Barrier Discharge
- Voltage: 29kV
- Gap Distance: 1mm
- Time: 10sec
- Frequency: 5, 15, 30, 75Hz
- Energy: 50, 100, 300, 700mJ

Plasma + Tumors → Tumor ICD → Anti-tumor Immune Response → Cancer Cell Killing

Surface CRT 24 h Post Treatment

- ‘Eat me’ signal
- ‘Find me’ signal
Can DAMPs from plasma induced ICD stimulate anti-tumor activity in macrophages?

**Macrophage Cell Line**
- THP-1 Human Monocyte Cell Line
- PMA Differentiated into Macrophages

**Uniform nspDBD**
- Voltage: 29kV
- Gap Distance: 1mm
- Time: 10sec
- Frequency: 15, 30Hz
- Dose: 100, 300mJ

<table>
<thead>
<tr>
<th>Cancer Cell Treatment</th>
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<th>Plasma</th>
<th>Macrophage</th>
<th>Plasma Activated Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-) Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>+</td>
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<tr>
<td>3</td>
<td>-</td>
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<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**Cancer Cell Viability- 48 h Post Plasma Treatment**

**nspDBD stimulated DAMPs enhance anti-tumor effects of macrophages**
Contribution of ROS in plasma induced ICD

Plasma generated oxygen species and charges are important for ICD

Air

ROS

Change Intracellular ROS

ICD/DAMPs

N₂

ROS

ICD/DAMPs

O₂

ROS

ICD/DAMPs

Air

Intracellular ROS Scavenger
Contribution of intracellular ROS in plasma induced ICD
Two Pathways of Plasma Stimulation of Immune Function

Direct

Plasma + Immune Cell

Plasma + Tumors → Tumor ICD → Anti-tumor Immune Response → Cancer Cell Killing

Indirect

Plasma + Tumors

Development of Memory Cells → Development of Effector Cells → Selective Tumor Cell Death

Long term Protection

Selective Tumor Cell Death
Plasma induced specific anti-cancer immune response via ICD in an in vivo mouse model

Radiation mediated, specific anti-cancer effects\textsuperscript{[1]}

\textbf{Tumor Cure Rates}

\textbf{Tumor Size Post Rechallenge}

Plasma induced specific anti-cancer immune response via ICD in an in vivo mouse model

**Cohorts (n=7)**
1) Control- No Treatment
2) Negative Control- Vaccine
3) Positive Control- 8Gy X-ray Therapy + Vaccine
4) Low Dose Plasma
5) Low Dose Plasma + Vaccine
6) High Dose Plasma + Vaccine

**Immunological Endpoint:**
GUCY2C specific T-cell response

Tumor challenge (sc, flank) +/− Vaccine

Day 0  Day 7  Day 14  Day 26  ELISpot

Increase in specific T-cell in the spleen would indicate generation of a specific, systemic immune response
Plasma induced specific anti-cancer immune response via ICD in an in vivo mouse model

<table>
<thead>
<tr>
<th></th>
<th>Low Dose Treatment</th>
<th>High Dose Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Voltage</strong></td>
<td>29kV</td>
<td>29kV</td>
</tr>
<tr>
<td><strong>Frequency</strong></td>
<td>250Hz</td>
<td>1000Hz (Day1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>750Hz (Day2-5)</td>
</tr>
<tr>
<td><strong>Treatment Time</strong></td>
<td>10s</td>
<td>10s</td>
</tr>
<tr>
<td><strong>No. of Treatments per Day</strong></td>
<td>5 (1 min break in between)</td>
<td>5 (1 min break in between)</td>
</tr>
<tr>
<td><strong>Number of Days Treated</strong></td>
<td>5d</td>
<td>5d</td>
</tr>
</tbody>
</table>

Day 1

High dose plasma + vaccine augments specific T-cell response

Need to optimize plasma treatment system
Plasma elicits ICD in vivo

Biological Readouts (24, 48, 72 h):

1) Visual assessment of skin damage after treatment
   Little visible damage at all treatment frequencies

2) DAMP Signals (CRT)
   CRT and HMG B1 was observed at 500 and 750Hz 3 days after treatment
Plasma elicits ICD in vivo

Calreticulin

HMG B1

Control  X-ray  Plasma

Dendritic Cell Recruitment

DAPI – Blue, GCC (tumor)- Teal, CRT – Red, HMG B1 – Green, Yellow – CD11c
Plasma elicits ICD in vivo followed by systemic generation of tumor specific T-cells.

**Early effects:** ICD

**Late effects:** T-cells
ICD gold standard experiment

- In vitro ICD
- Immunize Animals
- Challenge
- Tumor growth

*** p<0.0001
Clinical Trial:
Precancerous and Cancerous Skin Tumors

Treated 17 lesions at the following treatment conditions:
20 kV pulse of 20 ns pulse width at 200 Hz, 1-2 minute treatment time

Report at One-Month Follow-up:
- 9 show complete clinical resolution
- 3 show significant improvement
- 5 had minor to no improvement

Report at Four-Month Follow-up:
- One patient - “overall clearing” of skin
- Two patients - fewer new lesions

??? IMMUNE/MEMORY EFFECTS???

NO ADVERSE EFFECTS (IMMEDIATE OR DELAYED) REPORTED
Challenges with Cancer Therapeutics

Radiotherapy
- **Local** delivery of radiation to tumor
- Non-specific treatment = toxicity

Chemotherapy
- Introduce drug **systemically**
- Non-specific treatment = toxicity

Surgeries
- More specific resection of tumor
- Some tumors are in-operable
- Residual cancer cells → recurrence

Cancer immunotherapy
- Could be very specific
- Individualized treatment
  - Patient specific
  - Tumor specific
Clinical Administration of Plasma

**Plasma and Surgery**
- **INVASIVE**
- Conflicting Immune responses

**Plasma Activated Media**
- Deep Tumors
- Storage?
- Plasma Components?

**Non-Invasive Delivery**
- Delivers most plasma components
- Depth of Penetration?


http://www.hasbrochildrenshospital.org/research-and-special-programs.html
Immune Cell Recruitment in the Skin Following Plasma Exposure

Untreated

Plasma Treated

APC (CD163)
Mechanism of Penetration of Plasma Effects

DIRECT PLASMA EFFECTS

INFLUENCE OF CELLS

in vitro ICD

in vivo ICD
Intercellular Signaling Important for Penetration of Plasma Effects

Han, L et al. Advanced Functional Material, 23 (2013)
Plasma Onco-Immunotherapy

Plasma Treatment:
- Tumor ICD
- Anti-tumor Immune Response
- Reduced Tumor Burden
- Rechallenge (Memory Effect)

Plasma Induced ICD
- CRT
- HMGB1

Plasma Stimulated Recruitment
- Dendritic Cells

Effector Cells

Plasma Cancer Vaccination?