Tumor Treating Fields (TTFields) induced cancer cell death may be immunogenic resulting in enhanced antitumor efficacy when combined with immune-modulating therapy.

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TTFields Distribution in and Around Quiescent and Dividing Cells

A
Field of alternating direction

Oscillating direction of force acting on charge
Oscillating direction of force acting on dipole

uniform electric field leading to dipoles alignment

B
Field of alternating direction

Unidirectional net force acting on dipole during all cycle phases
Unidirectional net force acting on charge during all cycle phases

nonuniform electric field leading to dielectrophoresis

TTFields MOA Summary

NSCLC

Ovarian

Glioma

Modified from Giladi M, Schneiderman RS, Scientific Reports, 2015 and Gera et al., PLoS One. 2015

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TTFields Are Delivered by Optune

- Single-use transducer arrays deliver TTFields through the scalp\(^1\)
  - Arrays deliver TTFields at a low intensity (1-3 V/cm) and intermediate frequency (200 kHz)\(^2\)

EF-14: Secondary Endpoint, OS
Interim Analysis, Per Protocol Population

<table>
<thead>
<tr>
<th></th>
<th>TTFields + TMZ (n=196)</th>
<th>TMZ Alone (n=84)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median OS from randomization, months</td>
<td>20.5</td>
<td>15.6</td>
</tr>
<tr>
<td>95% CI, months</td>
<td>16.6-24.9</td>
<td>12.9-18.5</td>
</tr>
<tr>
<td>Stratified log-rank</td>
<td>P=0.0042</td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>0.666 (0.495-0.898)</td>
<td></td>
</tr>
<tr>
<td>Median OS from diagnosis, months</td>
<td>24.4</td>
<td>19.4</td>
</tr>
<tr>
<td>2-year OS</td>
<td>48%</td>
<td>32%</td>
</tr>
</tbody>
</table>

- In the final analysis (n=609), TTFields + TMZ extended median OS by 4.4 months, consistent with the interim analysis (n=280)

TMZ, temozolomide; CI, confidence interval; HR, hazard ratio; OS, overall survival.
In Vivo Evidence of Immune Stimulation in Response to TTFields Application

**Table 1** Lymphocyte infiltration in lung tumors as revealed by immuno-histochemical staining

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CD4</th>
<th>CD8</th>
<th>CD45</th>
<th>CD19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.0 ± 0.8</td>
<td>0.8 ± 0.5</td>
<td>2.0 ± 0.8</td>
<td>0</td>
</tr>
<tr>
<td>TTFields</td>
<td>3.4 ± 0.9</td>
<td>1.6 ± 0.5</td>
<td>3.6 ± 0.5</td>
<td>0</td>
</tr>
</tbody>
</table>

Immunogenic Cell Death - Sequential Events that Link Tumor-Cell Stress with Activation of Antigen-Presenting Cells

Calreticulin acts on the scavenger receptor (Eat me signal)

Toll-like receptor 4 is responsible for activating the innate immune system.

Zitvogel et al., Nature reviews Immunology 2008
TTFields Application Mediate Cell Surface Exposure of Calreticulin

**LLC-1**
Mouse lung cancer

**MOSE-L**
Mouse ovarian cancer

Giladi et al., AAI 2016
TTFields Induce an Increase in HMGB1 Release and a Reduction in Intracellular ATP Levels

LLC-1
Mouse lung cancer

MOSE-L
Mouse ovarian cancer

Giladi et al., AAI 2016
Sequential Events that Link Tumor-Cell Stress with Activation of Antigen-Presenting Cells

**Immunogenic cell death: the rules**

- **Chemo-therapy**
- **Anthra-cyclins or oxaliplatin**
- **TTFields**

### Direct effects on cancer cells
- **Stress**
  - ER stress
  - Autophagy
  - Cell death
- **Ligands**
  - CRT exposure
  - ATP secretion
  - HMGB1 release

### Indirect effects on immune effectors
- **Receptors**
  - CRT receptor
  - P2Y2
  - P2RX7
  - TLR4
- **Immune effects**
  - Engulfment by DC
  - DC recruitment, IL-1β secretion
  - Tumor antigen processing

### Indirect immune-mediated effects on cancer cells

**References**
- Obeid et al. 2007 Nat Med
- Apetoh et al. 2007 Nat Med
- Ghiringhelli et al. 2009 Nat Med
- Michaud et al. 2011 Science
- Menger et al. 2012 Science Transl Med
Cancer Immunity Cycle

Leukocyte fraction of blood donation acquired from MADA: ample supply of cells from multiple donors (5)

PBMC production &

- CFSE staining to detect proliferation
- Activate cells with PHA Superantigen

Flow cytometry analysis

Stain to:
- Isolate T-cells
- Detect immune activity:
  - Cytokine Secretion
  - Cytotoxic Degranulation
  - Activation/Exhaustion Marker

Incubate in normal conditions/invitro for 3.5 days

Diamant et al., ISCR 2016

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Effect of TTFields on T-cell activation in PBMC culture

- Results indicate activated T cells treated with TTFields exhibit slight/no decrease (sometimes even increase) in IFNγ secretion, PD1 up-regulation, and cD107a surface presentation.

Diamant et al., ISCR 2016

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Cancer Immunity Cycle

1. Release of cancer cell antigens
   - Immunogenic cell death
   - Tolerogenic cell death

2. Cancer antigen presentation
   - TNF-α
   - IL-1
   - IFN-γ
   - CD40L/CD40
   - CDN
   - ATP
   - HMGB1
   - TLR
   - IL-10
   - IL-4
   - IL-13

3. Priming and activation
   - CD28/B7.1
   - CD137/CD137L
   - OX40/OX40L
   - CD27/CD70
   - HVE/M
   - GITR
   - IL-2
   - IL-12
   - CTLA4/B7.1
   - PD-L1/PD-1
   - PD-L1/B7.1
   - Prostaglandins

4. Trafficking of T cells to tumors
   - CX3CL1
   - CXCL9
   - CXCL10
   - CCL5

5. Infiltration of T cells into tumors
   - LFA1/ICAM1
   - Selectins
   - VEGF
   - Endothelin B receptor

6. Recognition of cancer cells by T cells
   - Reduced pMHC on cancer cells

7. Killing of cancer cells
   - IFN-γ
   - T cell granule content

- Stimulatory factors
- Inhibitors

TTFields in Combination with Anti-PD-1 Lead to an Increase in Antigen Presenting Cells Infiltration to the Tumor

Giladi et al., AAI 2016

Confidential - For internal training purposes only. Not to be distributed or used in the field
TTFields in Combination with Anti-PD-1 Lead to Elevated PD-L1 Expression and Reduced Tumor Volume

Giladi et al., AAI 2016
Summary of TTFields effects

- Disrupt normal mitotic spindle assembly leading to mitotic catastrophe
- Lead to abnormal chromosomal segregation
- Induce autophagy
- Activate immunogenic cell death
- Demonstrate enhanced antitumor efficacy when combined with immune-modulating therapy
Cancer Immunity Cycle

Novocure preclinical studies