Vaccine sites as sinks and graveyards for tumor-specific T cells

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The following relationships exist related to this presentation:

No Relationships to Disclose
gp100 peptide vaccine + IL-2 has activity in metastatic melanoma

Stage IV and locally advanced stage III melanoma patients

High-dose IL-2 +/- gp100 in IFA

<table>
<thead>
<tr>
<th></th>
<th>IL-2+gp100/IFA</th>
<th>IL-2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall response rate</td>
<td>22.1%</td>
<td>9.7%</td>
<td>0.022</td>
</tr>
<tr>
<td>Progression free survival</td>
<td>2.9 months</td>
<td>1.6 months</td>
<td>0.010</td>
</tr>
<tr>
<td>Median overall survival</td>
<td>17.6 months</td>
<td>12.8 months</td>
<td>0.096</td>
</tr>
</tbody>
</table>

ASCO 2009 (Schwartzentruber & Hwu)
Reports on detrimental properties of vaccination with peptide/IFA

- Aichele et al., JEM 1995: s.c. inj. → Immunity; i.p. inj. → Tolerance
- Toes et al., PNAS 1996: Systemic peptide presentation → Tolerance
- Bijker et al., EJI 2008: Ag presentation by non-prof. APC → Tolerance

Rosenberg et al.: “The in vivo generation of gp100 reactive T cells was significantly less in patients receiving the olive compared with the beef IFA”

Slingluff et al.: “Responses to HLA-A1, A2, and DR associated peptides were largely preserved, but trended lower for some HLA-A3 associated peptides.”
gp100 peptide/IFA primes T cells ...
gp100 peptide/IFA primes T cells ... then induces tolerance

![Graph showing CD8+/Thy1.1+ (% of CD8+) vs. Days After Treatment with different groups: no vaccine + VSV.OVA, no vaccine + VSV.gp100, gp100/IFA + VSV.OVA, gp100/IFA + VSV.gp100. The x-axis represents Days After Treatment, ranging from 0 to 100, and the y-axis represents CD8+/Thy1.1+ (% of CD8+), ranging from 0 to 60. Each group is represented by a different colored line with data points and error bars. The graph shows a peak at 40 days for gp100/IFA + VSV.gp100, followed by a decrease.]
gp100 peptide/IFA primes T cells ... then induces tolerance

[Graph showing CD8+ T cell activation over time with different treatment groups.]

Similar results with OVA
CD8+ endogenous repertoire
CD8+ OT-I
CD4+ OT-II
gp100 peptide/IFA induces dominant tolerance
gp100 peptide/IFA induces dominant tolerance

![Graph showing the effect of different vaccinations on pmel-1 T cells (% of CD8+) over days after vaccination.]

- **gp100/IFA**
- **VSV.gp100**
- **VSV.gp100 + gp100/IFA (day 0)**
- **no vaccine**

Vaccinate
gp100 peptide/IFA induces dominant tolerance
Where are the T cells?

gp100/IFA s.c. + eLuc-transduced pmel-1 T cells i.v.

Rabinovich et al., PNAS 2008
Where are the T cells?

gp100/IFA s.c. + \textit{eLuc}-transduced pmel-1 T cells i.v.

Rabinovich \textit{et al.}, PNAS 2008
Where are the T cells?

gp100/IFA s.c. + eLuc-transduced pmel-1 T cells i.v.

Rabinovich et al., PNAS 2008
Vaccinate site is a sink for T cells

\[ \text{gp100/IFA} + \text{IL-2} \]

\[ \text{H}_2\text{O/IFA} + \text{IL-2} \]
Antigen-rich vaccine depots persist

30 days post vaccination
Antigen-rich vaccine depots persist

30 days post vaccination

IFN-γ

CD8

H₂O/IFA

gp100/IFA

vaccine depot

dLN

dLN

H₂O/IFA

gp100/IFA

spleen

pmel-1 T cells (% of CD8+ cells)

days after vaccination

pmel-1 T cells (% of CD8+)

days after vaccination

gp100/IFA on day 0 + pmel-1 on day 0

gp100/IFA on day 0 + pmel-1 on day 96
Persistent vaccine induces antigen-driven T cell tolerance: graveyard
Persistent vaccine induces antigen-driven T cell tolerance: graveyard
Limiting T cell interaction time with the vaccine depot

- 28 days
- gp100/IFA
- H2O/IFA
- 48 hr
- transfer pmel-1 effectors from DLN
Limiting T cell interaction time with the vaccine depot

- gp100/IFA
- H2O/IFA

48 hr

transfer pmel-1 effectors from DLN

28 days

measure pmel-1 response
Limiting T cell interaction time with the vaccine depot prevents tolerance
Vaccinating without IFA

![Graph showing pmel-1 T cells (% of CD8+) over days after treatment with no vacc. and gp100/IFA treatments, with a virus boost indicated.](image-url)
Vaccinating without IFA

No priming, some tolerance

![Graph showing pmel-1 T cells (% of CD8+) over days after treatment with different vaccine conditions: no vacc., gp100/IFA, gp100/PBS. The graph includes a virus boost marker.]
Vaccinating without IFA

Water-based vaccines require an adjuvant

Melief et al.
Schoenberger et al.
Noelle et al.
Kedl et al.
and many others
aCD40/imiq/IL-2 combo overcomes tolerance

![Graph showing pmel-1 T cells (% of CD8+) over time after vaccination. The graph compares gp100/IFA + aCD40/imiq/IL-2 and gp100/PBS + aCD40/imiq/IL-2 treatments.](attachment:graph.png)
aCD40/imiq/IL-2 combo overcomes tolerance but not vaccine homing
Water-based vaccines permit T cell accumulation in tumor

T cells at:

- gp100/IFA
  - aCD40/imiq/IL-2
- gp100/PBS
  - aCD40/imiq/IL-2
Water-based vaccines permit T cell accumulation in tumor

GP100/IFA
aCD40/imiq/IL-2

GP100/PBS
aCD40/imiq/IL-2

T cells at:
- tumor site
- vaccine site

T cells in ratio pmel-1 in tumor/vaccine

T cells in absolute pmel-1 in tumor

Photons per sec in tumor (x10^3)
Water-based vaccines permit T cell accumulation in tumor

T cells at:
- gp100/IFA
- gp100/PBS

Tumor vaccine site

Ratio pmel-1 in:
- gp100/IFA + aCD40/imiq/IL-2
- gp100/PBS + aCD40/imiq/IL-2

Absolute pmel-1 in tumor:
- gp100/IFA + aCD40/imiq/IL-2
- gp100/PBS + aCD40/imiq/IL-2

 photons per sec in tumor (x10^3)
Therapy with long-lived vs. short-lived vaccine

- no treatment
- gp100/IFA + aCD40/imiq/IL-2
- gp100/PBS + aCD40/imiq/IL-2

pmel-1 T cells (% of CD8+)

Days after Vaccination

vaccinate
tumor injection + boost
Therapy with long-lived vs. short-lived vaccine

- No treatment
- gp100/IFA + aCD40/imiq/IL-2
- gp100/PBS + aCD40/imiq/IL-2

Tumor size (mm²) vs. Days After Tumor Challenge

- PBS 10/10 alive
- IFA 4/10 alive
- No 0/10 alive

pmel-1 T cells (% of CD8+)

- Vaccinate
- Days after Vaccination
- Tumor injection + boost

Days After Tumor Challenge
Therapy with long-lived vs. short-lived vaccine

- no treatment
- gp100/IFA + aCD40/imiq/IL-2
- gp100/PBS + aCD40/imiq/IL-2

pmel-1 T cells (% of CD8+)

Days after Vaccination
- vaccinate
- tumor injection + boost

Tumor size (mm²)

Days After Tumor Challenge

Vaccine efficacy:
- PBS 10/10 alive
- IFA 4/10 alive
- No 0/10 alive
Therapy with long-lived vs. short-lived vaccine

If I was a MOUSE, I’d be CURED!
Working Model

**Tumor**
- Low [antigen]
- Low MHC-I
- Abnormal endothelium
- Stromal Barrier
- Immunosuppression

**Vaccine site**
- High [antigen]
- Normal MHC-I
- Normal endothelium
- No stromal Barrier
- No Immunosuppression?

brief: priming

chronic: priming → tolerance + sink
Conclusions

• Oil-based/long-lived vaccine formulations:
  ▪ activate T cells, eventually tolerize
  ▪ tolerance can be overcome by additional adjuvants
  ▪ sequester T cells at vaccine site
  ▪ limit T cell accumulation in tumor
Conclusions

- **Oil-based/long-lived vaccine formulations:**
  - activate T cells, eventually tolerize
  - tolerance can be overcome by additional adjuvants
  - sequester T cells at vaccine site
  - limit T cell accumulation in tumor

- **Water-based/short-lived vaccine formulations:**
  - require additional adjuvants to activate T cells
  - do not sequester T cells at vaccine site
  - allow T cell accumulation in tumor
  - may have greater therapeutic efficacy than long-lived formulations
Conclusions

• Oil-based/long-lived vaccine formulations:
  ▪ activate T cells, eventually tolerize
  ▪ tolerance can be overcome by additional adjuvants
  ▪ sequester T cells at vaccine site
  ▪ limit T cell accumulation in tumor

• Water-based/short-lived vaccine formulations:
  ▪ require additional adjuvants to activate T cells
  ▪ do not sequester T cells at vaccine site
  ▪ allow T cell accumulation in tumor
  ▪ may have greater therapeutic efficacy than long-lived formulations

Long-lived vaccines can induce sub-optimal anti-tumor immunity
Short-lived peptide vaccine formulations deserve consideration
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Not high-zone tolerance

pmel-1 t cells (% of CD8+)

hgp100_{25-33} dose

- 0 µg
- 0.01 µg
- 0.1 µg
- 1 µg
- 10 µg
- 100 µg
- 200 µg

no priming \rightarrow no tolerance

priming \rightarrow tolerance

gp100/IFA

virus boost

days after peptide vaccination
gp100 peptide/IFA vaccine: uncertainty about impact of IFA variants

Different Adjuvanticity of Incomplete Freund’s Adjuvant Derived From Beef or Vegetable Components in Melanoma Patients Immunized With a Peptide Vaccine

Rosenberg et al.: “The in vivo generation of gp100 reactive T cells was **significantly less** in patients receiving the olive compared with the beef IFA”

Immunogenicity for CD8$^+$ and CD4$^+$ T Cells of 2 Formulations of an Incomplete Freund’s Adjuvant for Multi peptide Melanoma Vaccines

Slingluff et al.: “Responses to HLA-A1, A2, and DR associated peptides were **largely preserved**, but trended lower for some HLA-A3 associated peptides.”
aCD40/imiq/IL-2 combo overcomes tolerance but not vaccine homing
Therapy with long-lived vs. short-lived vaccine

If I was a MOUSE, I’d be CURED!
Adjuvants do not prevent tolerance
Persistent vaccine induces antigen-driven T cell tolerance