Immunotherapy in Lung Cancer
- TLR9 as a therapeutic target -

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The immune system uses different defenses against extra-and intracellular infections. Defenses against intracellular infections (NK cells, killer T cells) kill infected cells. These defenses can be redirected to kill tumor cells.
Toll-Like Receptors (TLRs) recognize pathogen-expressed molecules such as:

- Bacterial lipopeptides
- GPI-anchored proteins (parasites)
- Lipoteichoic acid (gram + bacteria)
- Zymosan (fungi)
- LPS (gram - bacteria)
- Flagellin (motile bacteria)
- dsRNA (viruses)
- ssRNA (viruses)
- CpG DNA (bacterial and viral DNA)
Some TLRs Are on the Cell Surface, to Detect Extracellular Pathogens

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Endosome

- dsRNA (viruses)
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- CpG DNA (bacterial and viral DNA)
Some TLRs Are Inside the Cell, to Detect Intracellular Pathogens

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TLR9 detects unmethylated CpG – common in pathogens, rare in vertebrate DNA.
Immune Effects of Stimulating TLR9

Rapid Induction of Innate Immune Response

- **B Cell**
  - IFN-α's, IFN-β's
  - IP-10, other chemokines
  - IL-10, IL-6, costimulatory factors

- **Plasmacytoid Dendritic Cell**
  - IFN-γ, TRAIL
  - IFN-α's, IFN-β's
  - IP-10, other chemokines

- **NK Cell, Monocyte, PMN**

- **TLR9**
  - PF-3512676

- **Hours**
How Could TLR9 Activation Work in Treating Cancer?

- Activation of anti-tumor Th1-like innate immune responses
  - IFN, chemokines and cytokines
  - Cell based; NK cells, monocytes/macrophages
- Activation of tumor-specific Th1 adaptive immunity
  - CpG activates pDC, mDC in tumor and DLN
  - Mature pDC and mDC present tumor antigens; induce tumor-specific killer T cells

In Vivo Activation of Dendritic Cells
Switching on Anti-Tumor Immunity by in vivo DC Activation Through TLR9

Antigens + PF-676

Immature dendritic cells

Tumor-specific, effector CTLs

Costimulatory Molecules, Th1-like milieu

T cell Tolerance
CpG TLR9 Agonist Monotherapy Induces Tumor Rejection

- 5mm s.c. cervical carcinoma in flank
- Daily CpG nuchal area injections d. 10 to 19
- 60% of mice had complete regression

CpG TLR9 Agonist Monotherapy Works Through Tumor-Specific Killer T Cells (CTLs)

Protective Memory: 100% of Mice Reject Contralateral Tumor Challenge 30d Later

Elimination of CTLs Abrogates CpG Effect

Protective Memory: 100% of Mice Reject Contralateral Tumor Challenge 30d Later

CpG Treatment: MHC and Killer T Cells ↑ In Tumor

CpG Induces Tumor Antigen Presentation (MHC I and II)

And CD8 T Cell Infiltration

PF-3512676 (formerly CPG 7909) Can Treat Cancer As a Monotherapy

• In mice, PF-3512676 can cause T cell-dependent immune rejection of established SC or metastatic tumors

• In humans, PF-3512676 monotherapy has been associated with objective responses (RECIST) in:
  – Metastatic melanoma
  – Cutaneous T cell lymphoma
  – Non-Hodgkin’s lymphoma
  – Renal cell carcinoma
  – Basal cell carcinoma
PF-3512676 As Tumor Vaccine Adjuvant Enhances Killer T cell Response In Melanoma Patients

Rapid and strong human CD8+ T cell responses to vaccination with peptide, IFA, and CpG oligodeoxynucleotide 7909

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Figure 1

Rapid in vivo responses of Melan-A–specific T cells to vaccination (vacc) with low doses of CpG 7909. Melan-A peptides, and IFA. PBMCs were collected before, as well as 7–10 days after, vaccinations 2 and 4, and they were analyzed ex vivo by flow cytometry. (A) Dot plots of PBMCs of patient LAU 627, with percentage of Melan-A–specific cells among CD8+ T cells. (B) After 2 and 4 vaccinations, 6/8 and 8/8 patients, respectively, had significantly increased percentages (i.e., greater than 2-fold) of Melan-A–specific T cells. (C) A control group of 8 patients was similarly treated with Melan-A peptide and IFA but without CpG 7909. After 2 vaccinations, none of the patients had more than 2-fold increased percentages. After 4 vaccinations, 4/8 patients had more than 2-fold increased frequencies, but percentages of Melan-A–specific T cells remained significantly (P < 0.01) lower as compared to those of CpG-vaccinated patients. (D) Fold increase of Melan-A–specific T cells before or after 4 vaccinations in patients vaccinated with or without CpG. Horizontal lines indicate mean values.

Speiser et al., J Clin Invest, 2005

~10X Increase in Tumor-Specific CD8 Killer T Cells in Melanoma Patients
How Can We Get Stronger T cell Responses Against The Tumor?

• Conventional tumor vaccines don’t contain all tumor Ag
• Dendritic cell vaccines are cumbersome & impractical
• Can we make the tumor into a vaccine?
  – Activate DC through TLR9, tricking the immune system into thinking the tumor is an infection?
• The tumor mimics healthy tissue, defends itself against immune rejection (IL-10, VEGF, TGF-b, regulatory T cells, IDO)
• Intact tumor fragments are much more malignant than disrupted, isolated tumor cells (2-3 logs)
• Theory – immune therapy should work better if the tumor is disrupted
PF-3512676 is synergistic with local radiotherapy.

*3/7 cured
Milas, et al., Cancer Research, 2004
What If Disrupt Tumor with Surgery?

1. Inject $10^3$ rhabdomyosarcoma cells IM
2. Resect tumor & DLN d 14
3. Rx: 100µg IP CpG d14, 17, 21, wkly X 4
4. CpG alone has only modest activity against large tumors in mice

Weigel et al, Clin Cancer Res. 2003

Days Post Injection

Proportion Surviving

Surgery + CpG
Surgery
No Surgery
Can TLR9 Stimulation by PF-3512676 Enhance Chemotherapy?

• Theory
  – Chemotherapy:
    • Disrupts tumor stroma
    • “Make space” for T cell response to TAA
    • Suppress regulatory T cells
  – CpG activates pDC in vivo
  – pDC induce T cell response that rejects tumor
• Demonstrated in vaccine model: chemo *increases* Ag-specific T cell response
Metastatic Cancer Models

- **Lewis lung carcinoma**
  - Cells injected IV (B6 mice)
  - Metastasis to lungs

- **Renca renal cell carcinoma**
  - Cells injected beneath kidney capsule on one side (BALB/c)
  - Metastasis to lungs, other kidney and heart

- **Treatment (both models)**
  - Starts day 7
  - PBS or Paclitaxel (36 mg/kg) ± PF 3512676 (SC, 100µg)
Lewis Lung Cancer Model: Increased Efficacy of PF-3512676 + Paclitaxel

- PF-3512676 > PBS (P=0.0006)
- Paclitaxel > PBS (P<0.0001)
- PF-3512676 + Paclitaxel > PF-3512676 or Paclitaxel alone (P<0.0001)
Renca Renal Cancer Model: Increased Efficacy of PF-3512676 + Paclitaxel Requires T Cells

Percent Survival

BALB/c

# Days Post Tumor Induction

PBS

PF-3512676

PF-3512676 + Paclitaxel

Paclitaxel

BALB/c Nude

Percent Survival

0 10 20 30 40 50 60 70 80

0 10 20 30 40 50 60 70 80
Does PF-3512676 + Paclitaxel Induce Tumor-specific CD4/8 T Cells?

**Naïve Mice**
- Inject irradiated renca cells in the hind foot pads
- Remove draining (popliteal) and distal (axillary) LN
- LN cells cultured w/10 U/ml IL-2 for 4 d
- IFN-γ secretion by LN cells assayed by FACS

**Mice Surviving Renca**
Increased IFN-γ Secreting CD8+ and CD4+ Tumor-specific T Cells After PF-3512676 + Chemo

<table>
<thead>
<tr>
<th>Mice</th>
<th>Draining LN (Popliteal)</th>
<th>Distal LN (Axillary)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>0/0.1</td>
<td>0.1/0</td>
</tr>
<tr>
<td>Renca Survivors</td>
<td>18.5/2.0</td>
<td>0.1/0.1</td>
</tr>
</tbody>
</table>

In Vivo DC Activation Through TLR9 Promotes Potent Anti-tumor T Cell Response, *Without A Vaccine*
Treg Depletion Enhances PF-3512676 Like Chemo

Percent Survival

Anti-CD25 Ab

Control (IgG1) Ab

No Ab

# Days Post Tumor Induction

PBS

Paclitaxel (36mg/kg)

PF-3512676 (50µg x 4)

PF-3512676 + Paclitaxel
Summary: Murine Studies Show Synergy of PF-3512676 with Chemo and Other Cancer Therapies

- The TLR9 agonist PF-3512676 targets plasmacytoid DC, induces innate and adaptive anti-tumor immunity
- PF-3512676 has activity as monotherapy
- Activity of PF-3512676 monotherapy vs. large tumors is limited
- PF-3512676 shows synergy when used in combination with:
  - Anti-tumor antibody
  - Vaccines, other immunotherapies (e.g., anti-CTLA4)
  - Radiotherapy
  - Surgery
  - Chemo
- **Chemo inhibits Treg cells, promotes TLR9-induced anti-tumor T cell response, inducing regression of large murine tumors**
- Will PF-3512676 synergize with chemotherapy in humans?
Chemo naïve patients; **prognostic factors were balanced between the arms, except the combo arm had more advanced disease (86% stage IV vs 65% in chemo alone)**
Objective Response Rate
Primary Objective (RECIST Criteria)

**Physician Evaluation**

- PF-3512676 + Chemotherapy: 37% (CR 22%, PR 15%)
- Chemotherapy: 19% (CR 11%, PR 8%)

**Independent Radiology Review**

- PF-3512676 + Chemotherapy: 22% (PR 11%)
- Chemotherapy: 11% (PR 11%)

*p = 0.048
CR = Complete Response; PR = Partial Response*
Immune Effects of Stimulating TLR9

Later Induction of Adaptive Immune Response

- B Cell
- Plasmacytoid Dendritic Cell
- NK Cell, monocyte, PMN
- Antigen-specific T Cells

TLR9
PF-3512676
79% Improvement in Median Overall Survival (2° Objective)

Survival (Months)

Percent Survival

Chemotherapy + PF-3512676  n=74; Censored = 16
Chemotherapy Alone  n=37; Censored = 8

1 Yr Survival Improved from 33% In Chemo Alone to 50% in Combo
Conclusions

- PF-3512676 may be useful in human cancer therapy as:
  - Vaccine adjuvant
  - Therapeutic (combined with chemo, XRT, MAb, surgery, immunotherapy)
  - Phase III trials underway in 1st line advanced NSCLC combos with standard chemo
  - Phase II trials underway in combos with erlotinib, pemetrexed, bevacizumab
- The safety profile of PF-3512676 appears generally good so far
  - Chemotherapy-related toxicity does not appear to be increased by addition of PF-3512676
  - Most common AEs are injxn site rxn, flu-like symptoms
  - >1000 humans exposed to PF-3512676 in Coley and partner trials
  - MTD not reached
  - Longest duration of CpG therapy >3 yr