Modulating the Tumor Microenvironment to Enhance Antitumor Immunity

Antigen-encoding poxvirus vectors overcome immune escape

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The patented use of Recombinant Vaccinia virus encoding GMCSF has been licensed to Jennerex Biotherapeutics.

As an inventor on the patent, I have a royalty position.
Poxvirus Vector Platform for Immunotherapy

• Localized gene transfer for immune modulation of tumor microenvironment
  – Cytokine gene transfection for cell recruitment
  – Costimulatory molecules for enhanced T activation
  – Modulation of tumor associated suppressive milieu

• Antigen-encoding viral based vaccines
  – Defined tumor antigens (CEA, PSA)
  – Infectivity in tumor microenvironment
  – Cytokine/Costimulatory molecules as “adjuvants”
VACCINIA VIRUS

- Large DNA pox virus
- 25 kb insert without compromise
- Highly infective and lytic in epithelial cells
- Replicates in cytoplasm without chromosomal integration
Multiple Potential Antigens (colon and breast) for Immune Recognition

- 13,023 genes in 11 breast and 11 colorectal tumors
- ~ 90 mutations per tumor
- Subset involved in neoplastic process
- Any could be recognized as tumor antigens presented appropriately

• Vaccinia recombinants infect/transfect a variety of murine tumors in-vitro

• Intravesical vaccinia infects/transfects bladder tumor cells

• Immunity to vaccinia does not prevent tumor infection/transfection
• Intralesional vaccinia virus can be given safely to patients with melanoma

• Vaccinia virus infects human melanoma cells after intralesional injection

• Anti-vaccinia immunity does not prevent tumor infection

• Melanoma cells express viral gene products
Intratumoral recombinant GM-CSF-encoding virus as gene therapy in patients with cutaneous melanoma

Michael J. Mastrangelo,1 Henry C. Maguire Jr.,1 Laurence C. Eisenlohr,2 Carol E. Laughlin,2 Claude E. Monken,1 Peter A. McCue,3 Albert J. Kovatch,3 and Edmund C. Lattime1,2

Departments of 1Medicine, 2Microbiology and Immunology, and 3Pathology, Anatomy, and Cell Biology, Jefferson Medical College of Thomas Jefferson University, Philadelphia, Pennsylvania 19107.

- Intrallesional injection of Vac-GMCSF in recurrent superficial melanoma
- Patients failing all conventional therapy
- Escalating doses of Vac-GMCSF given 2X weekly
Recombinant GM-CSF encoding vaccinia
mRNA in VAC-GMCSF Injected Lesions

1 - 18 hr. biopsy (chronic injections)
2 - 18 hr. biopsy (single injection)
3 - 18 hr. biopsy (single injection)
4 - Uninjected lesion

(Patient 3)
Figure 9. Patient 3, a 32-year-old female with extensive dermal metastases of the left thigh: anterior aspect before treatment (a), on day 81 (b), and on day 600, 150 days posttreatment (c).
Figure 11. Patient 3. Regression of an untreated signal dermal metastasis located lateral to the knee and upstream from the treated lesions is shown: day 63 (a), day 81 (b), and day 160 (c).
Results: Intralesional VV-GMCSF

- Local infectivity: Occurs consistently at $10^7$ PFU per injection
- Reporter gene: 7 of 7 patients made IgG antibodies to $\beta$-gal
- Recombinant GM-CSF gene:
  - VAC-GM-CSF mRNA measured in 18 hr. biopsies early and late in course of treatment
  - Eosinophilia was seen at treatment sites
- Injected lesions regressed in 5/7 patients
- Uninjected lesions regressed in 4/7 patients
The Oncolytic Poxvirus JX-594 Selectively Replicates in and Destroys Cancer Cells Driven by Genetic Pathways Commonly Activated in Cancers

Kelley A Parato1, Caroline J Breitbach2, Fabrice Le Boeuf3, Jiahue Wang1,2, Chris Storbeck1,2, Carolina Ikon1, Jean-Simon Dillo3, Theresa Falls1, Joseph Burns1, Vanessa Garcia1, Fennina Kanji1, Laura Evgen1, Kang Hui1, Francois Paradis1, Shane Knowles1, Tae-Ho Hwang1, Barbara C Vanderhyden1,4, Rebecca Auer1,4, David H Kim2 and John C Bell1,2
Tumour-fighting virus homes in

An early clinical trial demonstrates the delivery and replication of a cancer-killing virus in metastasized tumour tissue. These promising results could provide a foundation for systemic virotherapy for patients with cancer.

LETTER

Intravenous delivery of a multi-mechanistic cancer-targeted oncolytic poxvirus in humans

Caroline J. Bruttach1, James Buric2, Derek Juskevich3,4, Joe Stephenion5, Andrew R. Hau6, Laura Q. M. Chow7,4, Jorge Nieva8, Tae-Ho Hwang9, Anne Moon4, Richard Patt10, Adina Pelusio11, Fabrice Le Boeff12, Joe Burns13, Laura Evgeni14, Naomi De Silva15, Sara Cramb16, Terri Robertson17, Ji-Eun Je17, Yoon-Sook Lee17, Kelley Parato17, Jean-Simon Diallo17, Aaron Favretti17, Manijeh Darzehzad18, John C. Bell19,20 & David H. Klim1

- Vac-GM (JX-594)
  - Cancer selective after i.v. administration
    - In-vitro explants
    - In-vivo Phase I

Nature 477, 1 September, 2011
IHC reveals infection and β-gal expression in a colorectal tumor from Phase I study of intravenous JX-594.


*nature*
• Intravesical Vaccinia can be given without toxicity
• Intravesical Vaccinia infects bladder mucosa
• Intravesical vaccinia results in the recruitment of CD3^+, CD45RO^+ T cells to the bladder mucosa
Intravesical Vaccinia Recruits CD3⁺, CD45RO⁺ T Cells to Bladder Mucosa

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Post treatment (24 hrs post dose 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
</tr>
</tbody>
</table>

**CD3**

**CD45RO**
Phase I Study of Intravesical Fowlpox-GMCSF, TRICOM (NCT00072137)

• Hypothesis
  – GM-CSF will recruit and activate APC
  – TRICOM (LFA3, ICAM-1, B7.1) will allow antigen presentation by tumor cells

• Antigen provided by tumor cell

• Assess the safety of intravesical fowlpox virus encoding GM-CSF and/or TRICOM

• Determining the kinetics of viral infection and gene function

• Determine the host immune response to vector and tumor antigen (epitope spreading)
Expression of Lac-Z. gene in the tumor and NAT, post cystectomy, by RT-PCR
Immunohistochemistry Pt. 3 rF-GMCSF (7 X $10^7$ PFU)

- H&E
- CD3 (T)
- CD4 (T_h)
Immunohistochemistry Pt. 3 rF-GMCSF (7 X $10^7$ PFU)

- CD8 (T$_c$)
- CD45RO (T$_{act}$)
- Factor XIII (DC)
Poxvirus Vector Platform for Immunotherapy

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  - Modulation of tumor associated suppressive milieu

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  - Infectivity in tumor microenvironment
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The MB49 Model

• C57BL/6J male urothelium treated in-vitro with DMBA (Ian Summerhayes)
  
• Grows progressively in male and female B6 intravesically and subcutaneously
  
• Expresses male (HY) antigen complex
  – Male-primed female B6 reject tumor inoculum
  – HY-specific CTL lyse MB49 in $^{51}$Cr assays
  – MHC Class I$^+$, II$^-$ (IFN-$\gamma$ upregulates Class II)

• Presents soluble antigen to CD4$^+$ T cells
• Develop recombinant vaccinia-HY vaccine vector
• Evaluate vaccine stimulated T cell (CD8) responses
• Assess tumor induced immunity/ignorance
• Evaluate effects of systemic vs intratumor immunization
Antitumor Responsiveness in LN (local) but not Splenic (systemic)

![Graph showing E:T Ratio vs. percentages of specific lysis.](image)

- **MB49LN**: 2.48%
- **MB49Spl**: 0.31%

![Flow cytometry plots for CD8-FITC and Uty Tetramer-PE.](image)
MB49 Bearing Mice are Systemically Anergic to HY

- MB49 Bearing Mice have a 0.20% specific lysis, compared to a 44.1% lysis in control B6 mice.

Graphs showing the relationship between E:T Ratio and % Specific Lysis, with different conditions indicating varying levels of lysis.
Suppressive pathway commitment is an early event in MB49

- Naïve mice can be systemically immunized
- Tumor inoculation “sets” suppressive phenotype (before palpable)
- Suppression is tumor specific
  - Induction of T regulatory cells
  - Tumor-bearing mice can be immunized to unrelated antigen
- MB49 stimulates LN (local) but not Splenic (systemic) CTL activity
- MB49-bearing mice are anergic to systemic immunization
- Suppression inhibits Antigen Presentation
NBT1: orthotopic breast cancer model

- **NBT1** is a rat HER2/neu-overexpressing mouse mammary carcinoma derived by our group from FVB/neuT transgenic mice. In experiments involving tumor-bearing mice, female FVB/N (n=5) mice were injected into the mammary fatpad with $2 \times 10^6$ NBT1 cells.

- **VVneu** is a recombinant vaccinia virus expressing rat HER2/neu. **VVGMCSF** is a recombinant vaccinia virus expressing GM-CSF. **VVBGal** is a vaccinia control. Mice received $1 \times 10^6$ pfu dose of each indicated virus ($2 \times 10^6$ pfu total).
Intratumoral but not systemic immunization induces Neu-specific CTL and eliminates tumor

**CTEP-7606:** A Phase 1 Study of Intra-tumoral Injection of Antigen Encoding Pox Virus Vaccine in Patients with Locally Advanced Pancreas Cancer
Intrapancreatic PanVac Phase I Trial (NCTC00669734)
## Schedule of immunizations

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<th>day</th>
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<tr>
<td>1-5</td>
<td>D1 PANVAC-F IT via EUS; d2 PANVAC-V+GMCSF; D3-5 GMCSF</td>
</tr>
<tr>
<td>15-9</td>
<td>D15 PANVAC-F IT via EUS; d16 PANVAC-F +GMCSF; D3-5 GMCSF</td>
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<tr>
<td>29-32</td>
<td>D29 PANVAC-F+ D29-32 GMCSF</td>
</tr>
<tr>
<td>35</td>
<td>(Pts may start standard of care chemo or chemoRT)</td>
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<tr>
<td>43</td>
<td>D43 PANVAC-F+ D43-46 GMCSF</td>
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<tr>
<td>71</td>
<td>D71 PANVAC-F + D71-74 GMCSF</td>
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# Clinical results

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<th>pt</th>
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<th>f/u First Chemo</th>
<th>f/u RT</th>
<th>CA19-9</th>
<th>F/U</th>
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<td>gem</td>
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<td>133</td>
<td>30 mo+</td>
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<td>no</td>
<td>19</td>
<td>Ex/862</td>
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<td>Sys-off</td>
<td>unk</td>
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<td>Off study Ex/159</td>
<td>Metastases</td>
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<td>Ex/177</td>
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<tr>
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<td>815</td>
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<td>544</td>
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<td>190</td>
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<td>Off study 9 mo+</td>
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<td>gem</td>
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<td>950</td>
<td>8 mo+</td>
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</tbody>
</table>
Conclusions (Intrapancreatic Immunization)

• Intrapancreatic injection of 2 doses of Panvac-F together with a series of sc injections of Panvac-V and Panvac-F was well tolerated

• In the first 6 pts, 2 pts were removed due to progressive disease

• While local progression is noted, no patient (10/10) presenting without metastatic disease has developed distant metastases
Poxvirus Vector Platform for Immunotherapy

• Localized gene transfer for immune modulation of tumor microenvironment
  – Poxvirus (Vaccinia and Fowlpox) can be given safely and repeatedly via the intratumoral route.
  – Poxvirus productively infects tumor and non tumor cells following localized administration
  – Infection results in transfection the production of the gene product in-vivo
  – Infection modulates the cellular makeup of the tumor microenvironment

• Tumor antigen-encoding poxvirus
  – Effectively immunizes to tumor antigen
  – The intratumoral route of immunization can overcome significant immune escape associated with both Treg and MDSC phenotypes
Acknowledgements

• Lattime lab:
  – Christiaan deVries
  – Emmanuel Gabriel
  – Amal Mansour
  – Claude Monken
  – Arvin Yang

• Clinical collaborators
  – David August
  – Tamir Ben-Menachem
  – Robert DiPaola
  – Elizabeth Poplin
  – Joe Shih
  – Mark Stein
  – Robert Weiss

• Thomas Jefferson
  – Laurence Eisenlohr
  – Michael Mastrangelo

• National Cancer Institute
  – James Gulley
  – Jeff Schlom
  – Howard Streicher

• Supported by:
  – R01CA42908, R21CA121589
  – CTEP U01CA07031
  – CINJ Shared Resources
    • P30CA72720
  – The CINJ Foundation