Modulating the Tumor Microenvironment to Enhance Antitumor Immunity

Antigen-encoding poxvirus vectors overcome immune escape

Edmund C. Lattime, Ph.D.

The Cancer Institute of New Jersey

Disclosure

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.

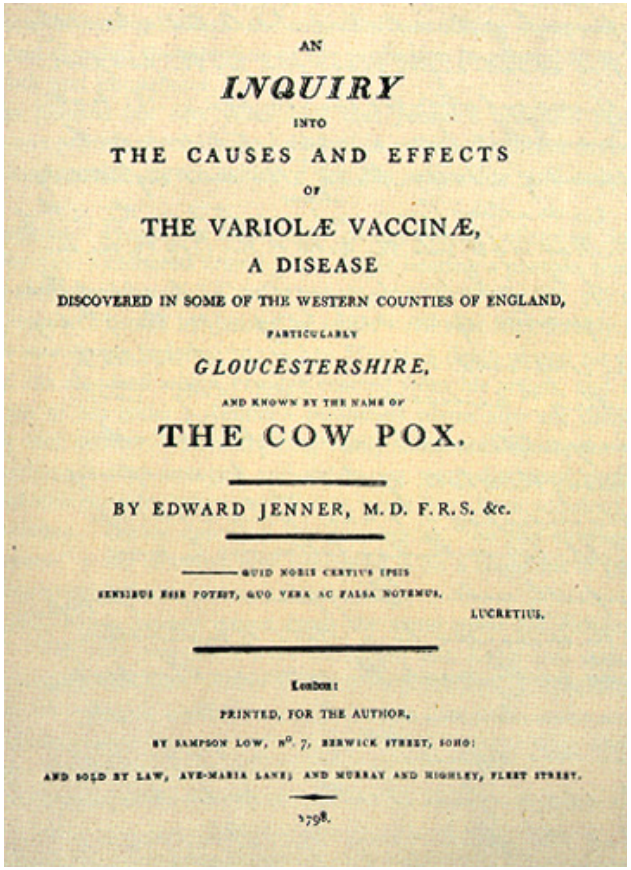
Poxvirus Vector Platform for Immunotherapy

- **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



AN

INQUIRY

INTO

THE CAUSES AND EFFECTS

OF

THE VARIOLÆ VACCINÆ,

A DISEASE

DISCOVERED IN SOME OF THE WESTERN COUNTIES OF ENGLAND,

PARTICULARLY

GLOUCESTERSHIRE,

AND KNOWN BY THE NAME OF

THE COW POX.

BY EDWARD JENNER, M.D. F.R.S. &c.

——— QUID NOBIS CERTIUS IPSIS
SENSIBUS HIBI POTEST, QUO VERA AC FALSA NOTEMUS.

LUCRETIUS.

London:

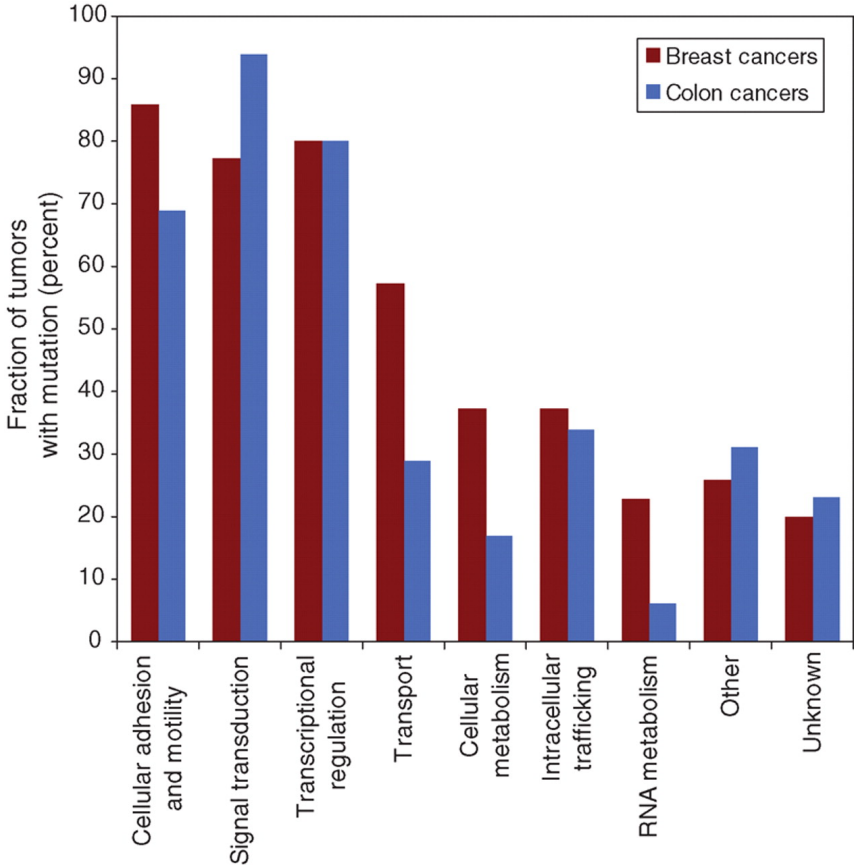
PRINTED, FOR THE AUTHOR,

BY SAMPSON LOW, N°. 7, BERWICK STREET, SOHO:

AND SOLD BY LAW, AVE-MARIA LANE; AND MURRAY AND HIGHLEY, FLEET STREET.

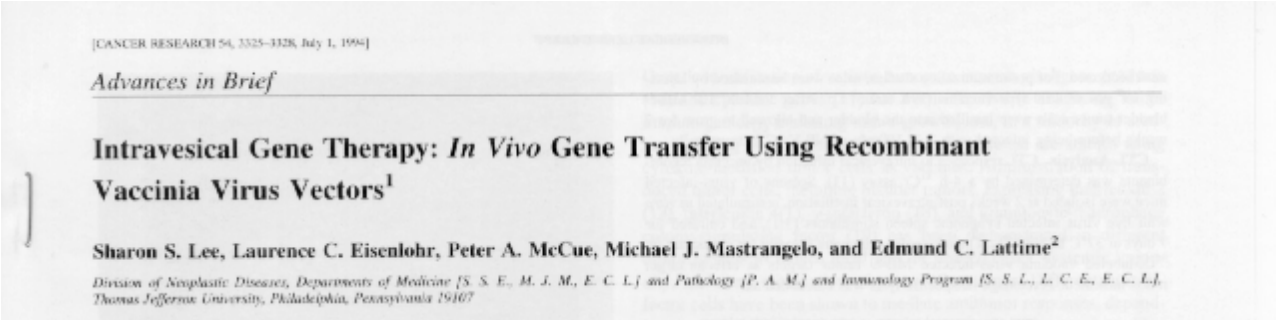
1798.

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

Sjoblom, T. et. Al. Science 314:268, 2006



[CANCER RESEARCH 56, 3325–3328, July 1, 1996]

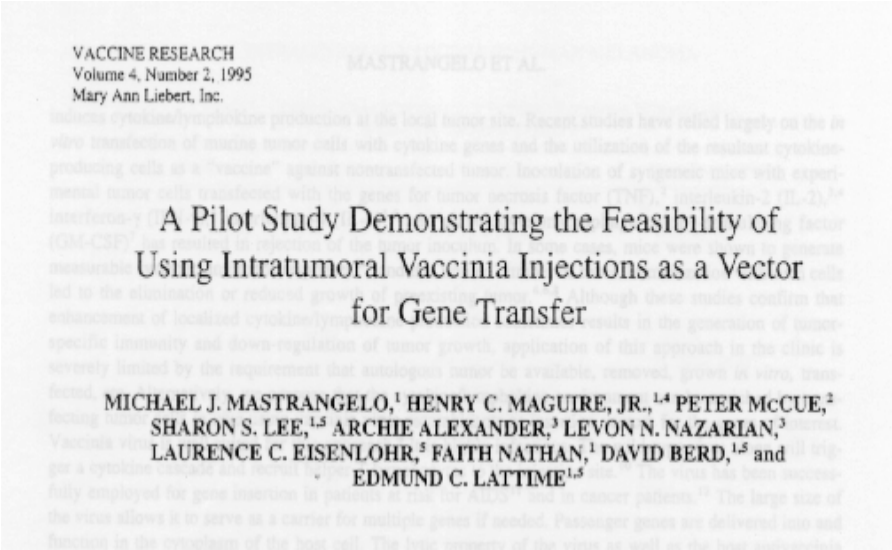
Advances in Brief

Intravesical Gene Therapy: *In Vivo* Gene Transfer Using Recombinant Vaccinia Virus Vectors[1]

Sharon S. Lee, Laurence C. Eisenlohr, Peter A. McCue, Michael J. Mastrangelo, and Edmund C. Lattime[2]

Division of Neoplastic Diseases, Departments of Medicine [S. S. E., M. J. M., E. C. L.] and Pathology [P. A. M.] and Immunology Program [S. S. L., L. C. E., E. C. L.], Thomas Jefferson University, Philadelphia, Pennsylvania 19107

- 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

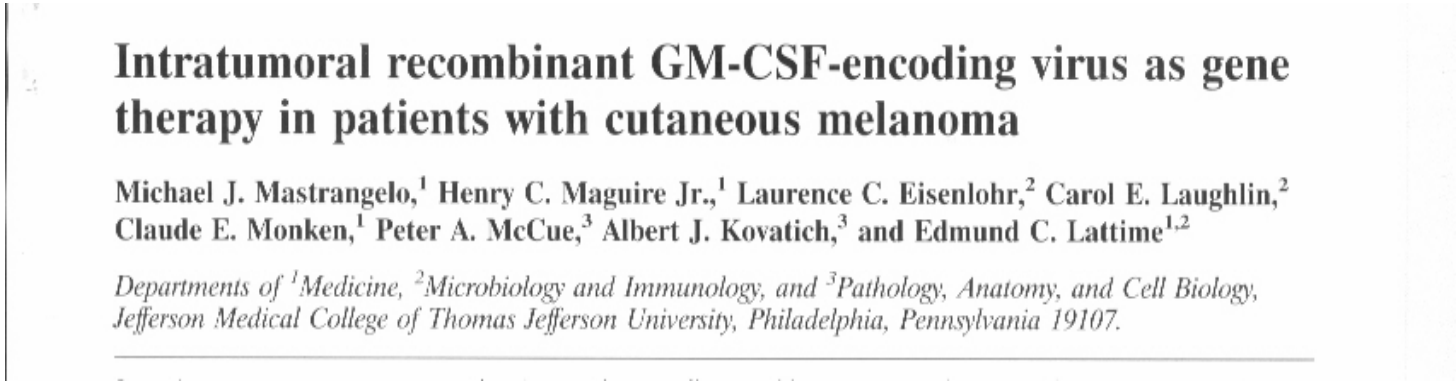


VACCINE RESEARCH
Volume 4, Number 2, 1995
Mary Ann Liebert, Inc.

A Pilot Study Demonstrating the Feasibility of Using Intratumoral Vaccinia Injections as a Vector for Gene Transfer

MICHAEL J. MASTRANGELO,[1] HENRY C. MAGUIRE, JR.,[1,4] PETER McCUE,[2] SHARON S. LEE,[1,5] ARCHIE ALEXANDER,[3] LEVON N. NAZARIAN,[3] LAURENCE C. EISENLOHR,[5] FAITH NATHAN,[1] DAVID BERD,[1,5] and EDMUND C. LATTIME[1,5]

- 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



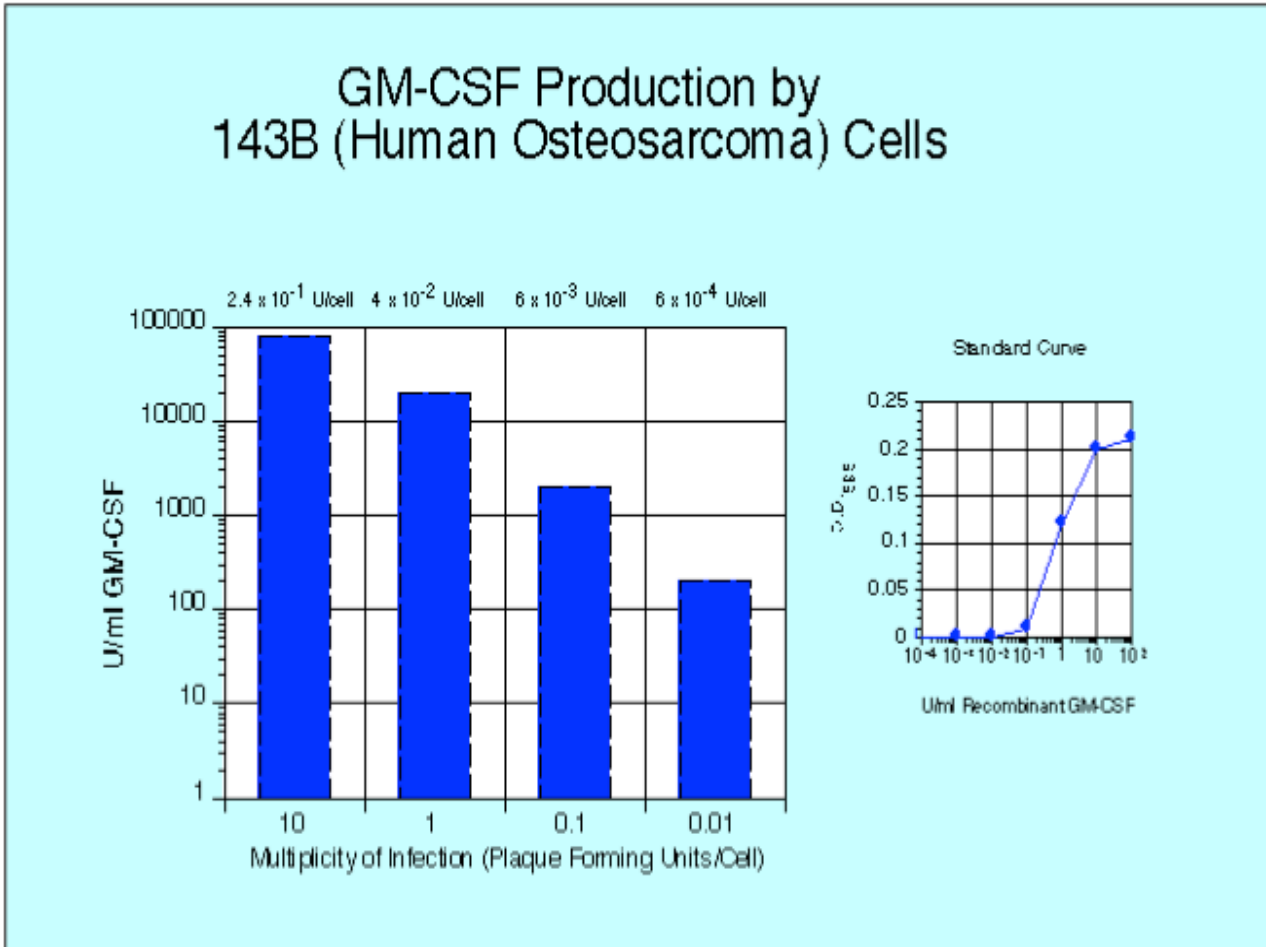
Intratumoral recombinant GM-CSF-encoding virus as gene therapy in patients with cutaneous melanoma

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. Kovatich,[3] and Edmund C. Lattime[1,2]

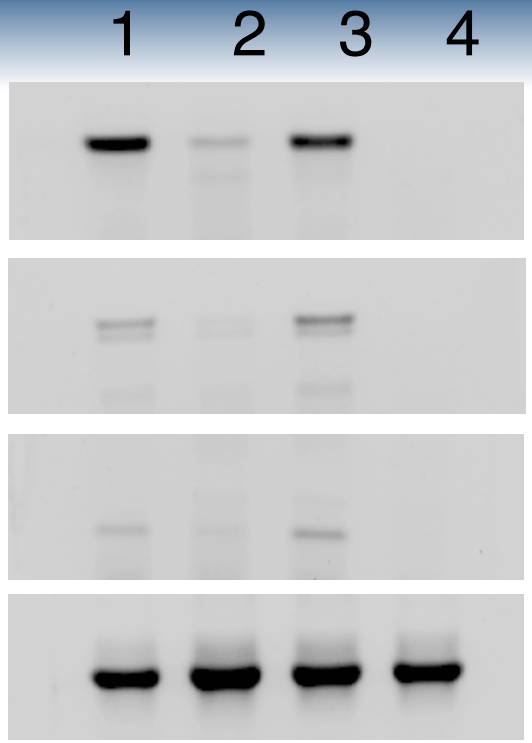
Departments of [1]Medicine, [2]Microbiology and Immunology, and [3]Pathology, Anatomy, and Cell Biology, Jefferson Medical College of Thomas Jefferson University, Philadelphia, Pennsylvania 19107.

- Intralesional 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

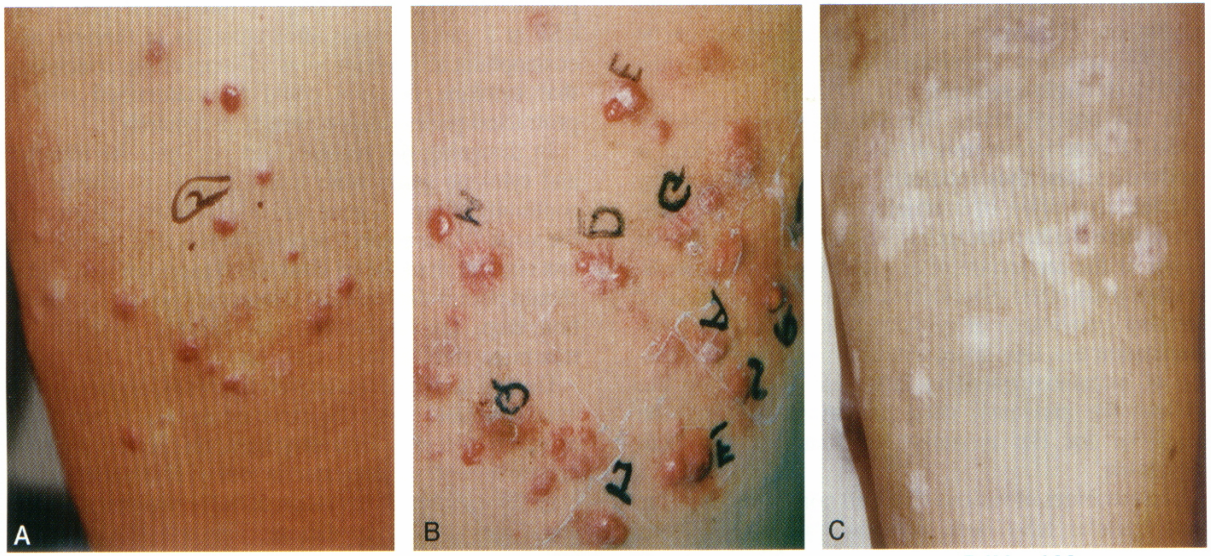


(Patient 3)

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

Intralesional VAC-GMCSF Injected and Local Uninjected Lesions

PATIENT # 3



PRE-TREATMENT DAY +81 DAY +600

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)**.

Intralesional VAC-GMCSF Distant Uninjected Lesions

PATIENT #3
UNTREATED LESION

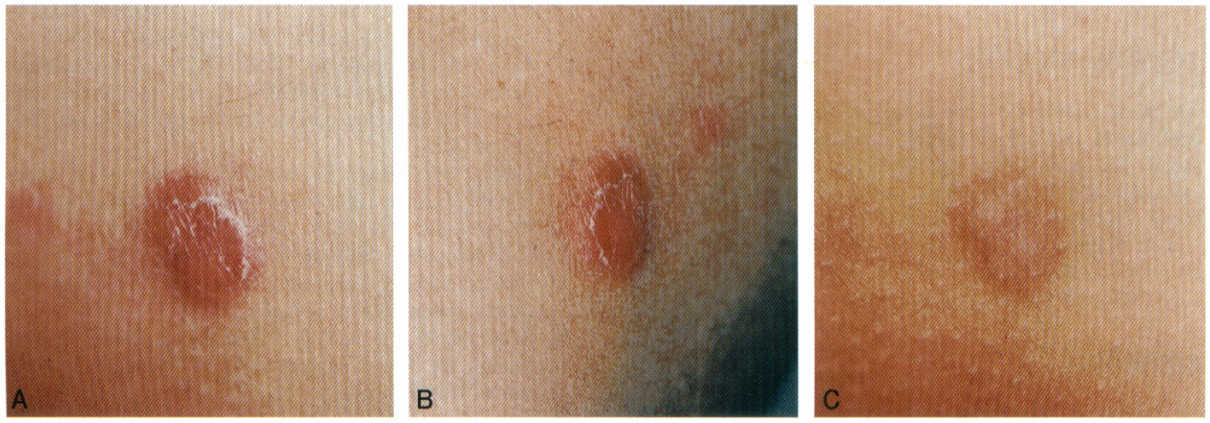


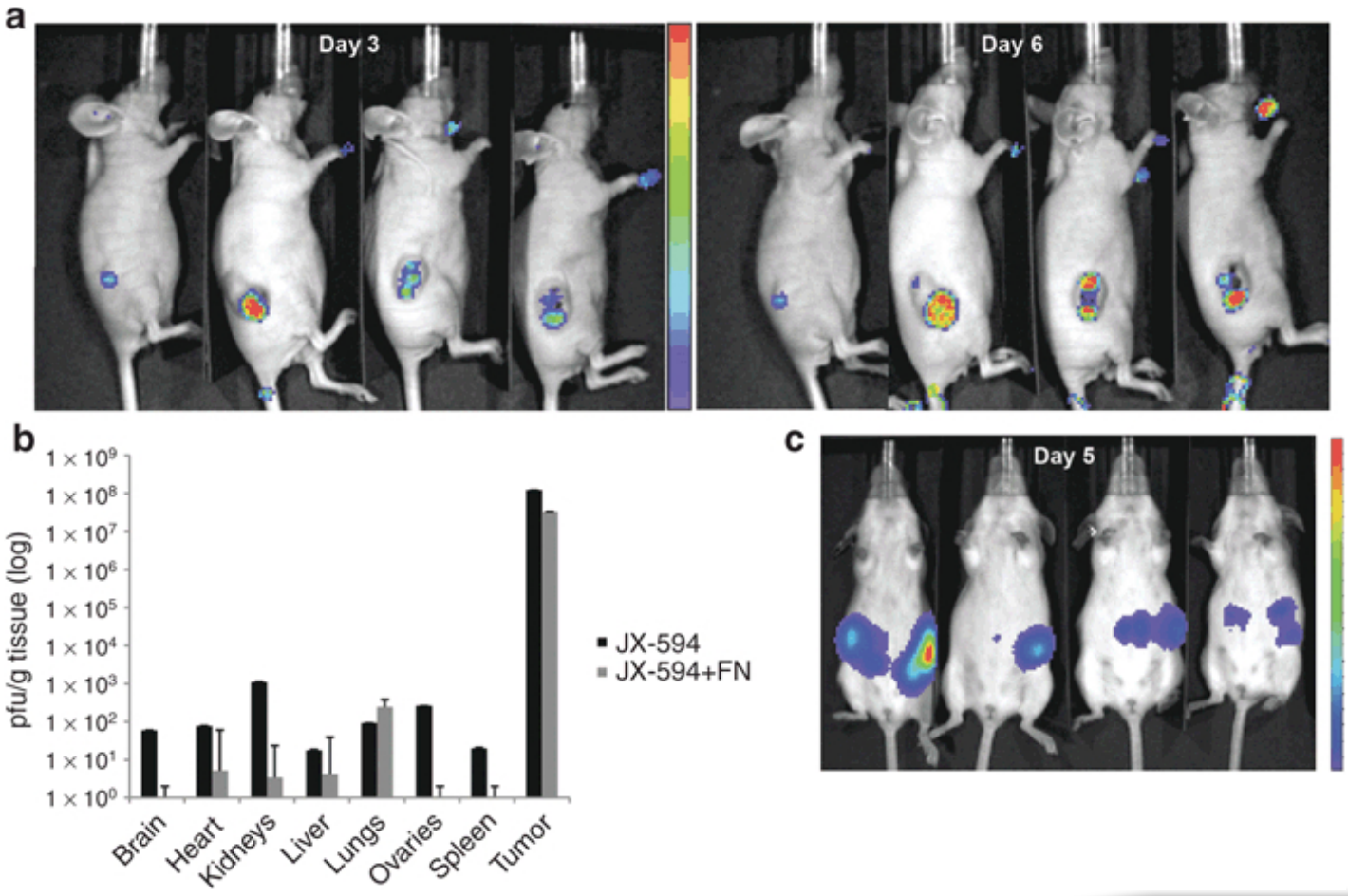
Figure 11. Patient 3. Regression of an untreated signal dermal metastasis located lateral to the knee and upstream from the treated lesions is shown: day 63 **(a)**, day 81 **(b)**, and day 160 **(c)**.

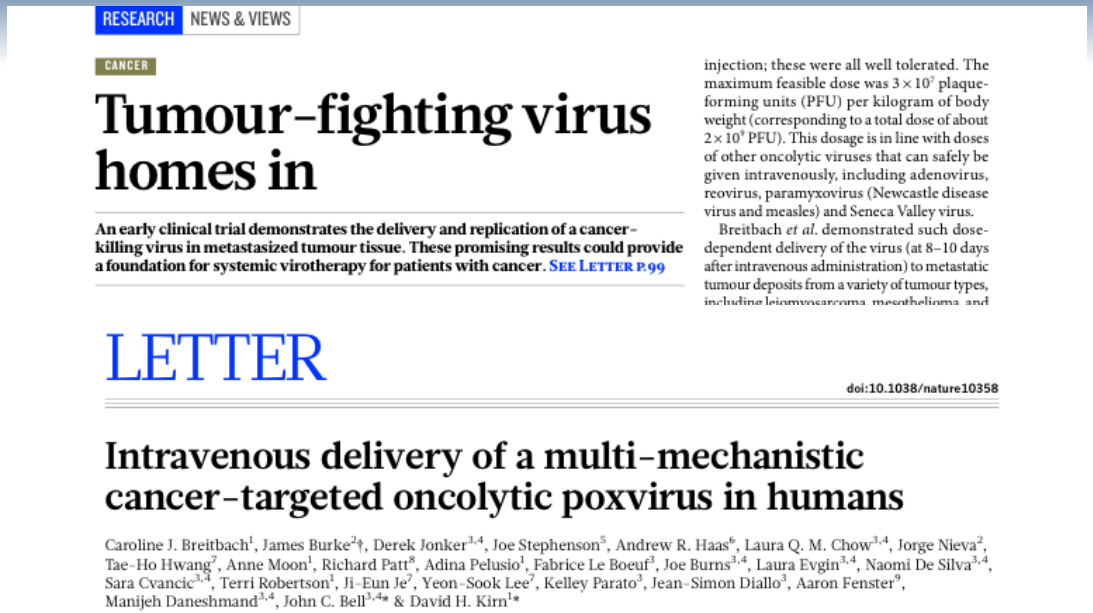
Results: Intralesional VV-GMCSF

- Local infectivity: Occurs consistently at 10^7 PFU per injection
- Reporter gene: 7 of 7 patients made IgG antibodies to β-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 Parato[1], Caroline J Breitbach[2], Fabrice Le Boeuf[1], Jiahu Wang[1,2], Chris Storbeck[1,2], Carolina Ilkow[1], Jean-Simon Diallo[1], Theresa Falls[1], Joseph Burns[1], Vanessa Garcia[1], Femina Kanji[1], Laura Evgin[1,3], Kang Hu[1,2], Francois Paradis[1], Shane Knowles[1], Tae-Ho Hwang[4], Barbara C Vanderhyden[1,5], Rebecca Auer[1,3], David H Kirn[2] and John C Bell[1–3]





RESEARCH NEWS & VIEWS

CANCER

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. SEE LETTER P.99

injection; these were all well tolerated. The maximum feasible dose was 3×10^7 plaque-forming units (PFU) per kilogram of body weight (corresponding to a total dose of about 2×10^9 PFU). This dosage is in line with doses of other oncolytic viruses that can safely be given intravenously, including adenovirus, reovirus, paramyxovirus (Newcastle disease virus and measles) and Seneca Valley virus.

Breitbach *et al.* demonstrated such dose-dependent delivery of the virus (at 8–10 days after intravenous administration) to metastatic tumour deposits from a variety of tumour types, including leiomyosarcoma, mesothelioma, and

LETTER

doi:10.1038/nature10358

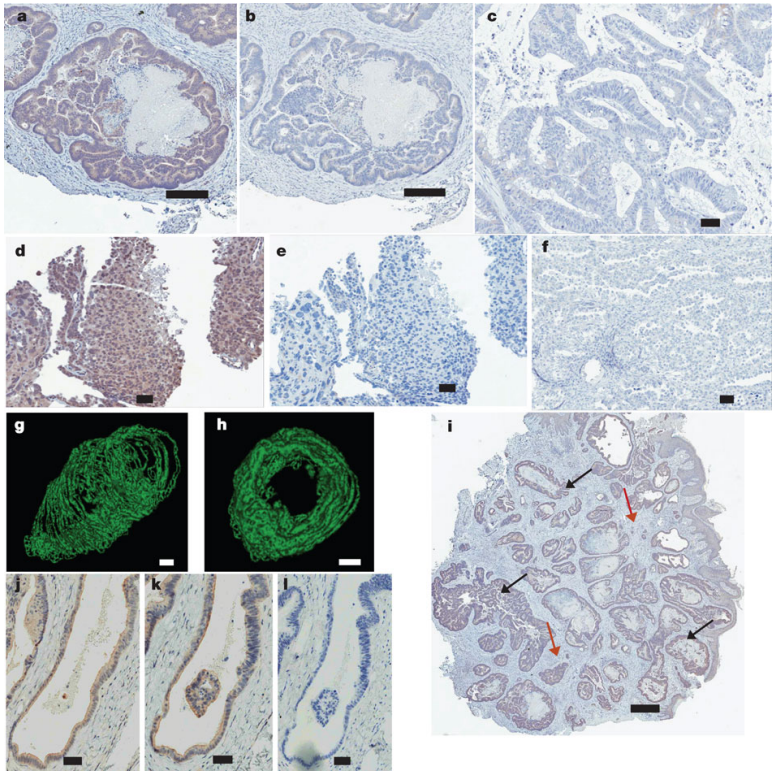
Intravenous delivery of a multi-mechanistic cancer-targeted oncolytic poxvirus in humans

Caroline J. Breitbach[1], James Burke[2]†, Derek Jonker[3,4], Joe Stephenson[5], Andrew R. Haas[6], Laura Q. M. Chow[3,4], Jorge Nieva[2], Tae-Ho Hwang[7], Anne Moon[1], Richard Patt[8], Adina Pelusio[1], Fabrice Le Boeuf[1], Joe Burns[3,4], Laura Evgin[3,4], Naomi De Silva[3,4], Sara Cvancic[3,4], Terri Robertson[1], Ji-Eun Je[7], Yeon-Sook Lee[7], Kelley Parato[1], Jean-Simon Diallo[1], Aaron Fenster[9], Manijeh Daneshmand[3,4], John C. Bell[3,4]* & David H. Kirn[1]*

- 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.



CJ Breitbach *et al*. *Nature* **477**, 99-102 (2011) doi:10.1038/nature10358

nature

0022-5347/01/1664-1291/0
THE JOURNAL OF UROLOGY®

Vol. 166, 1291–1295, October 2001
Printed in U.S.A.

PHASE I STUDY OF INTRAVESICAL VACCINIA VIRUS AS A VECTOR FOR GENE THERAPY OF BLADDER CANCER

LEONARD G. GOMELLA, MICHAEL J. MASTRANGELO, PETER A. McCUE, HENRY C. MAGUIRE, JR., S. GRANT MULHOLLAND* AND EDMUND C. LATTIME†

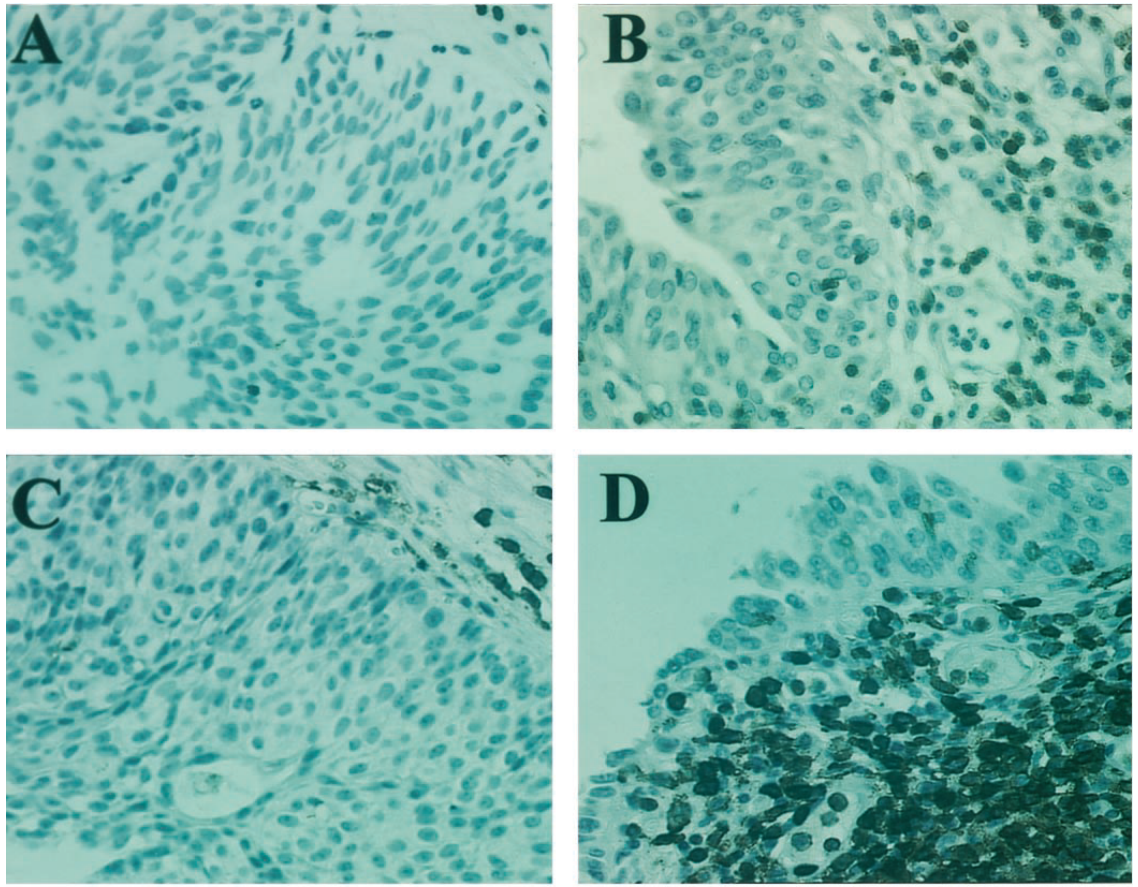
From the Departments of Urology, Pathology and Medicine (Division of Medical Oncology), Jefferson Medical College and the Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania

- 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

Pretreatment

Post treatment
(24 hrs post dose 3)



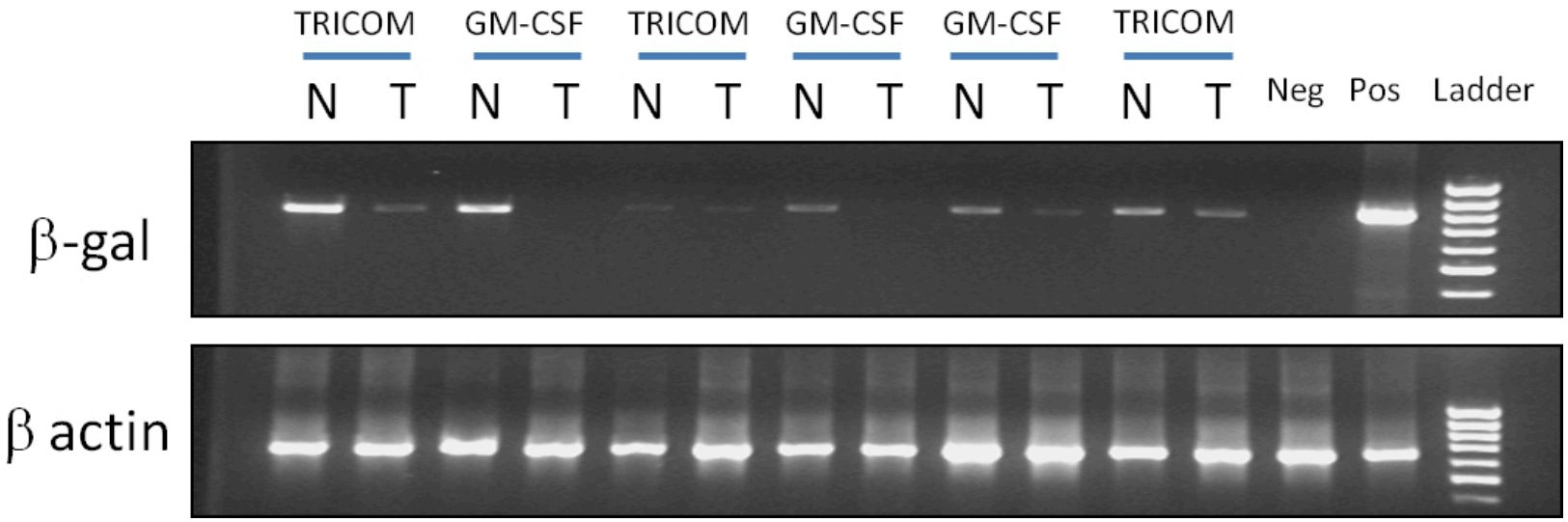
CD3

CD45RO

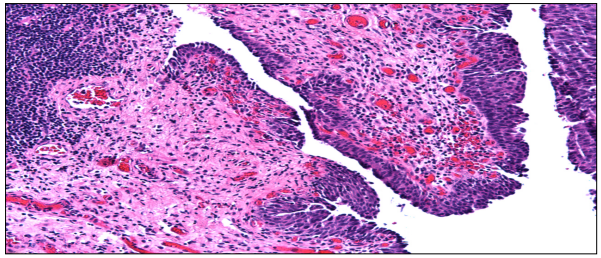
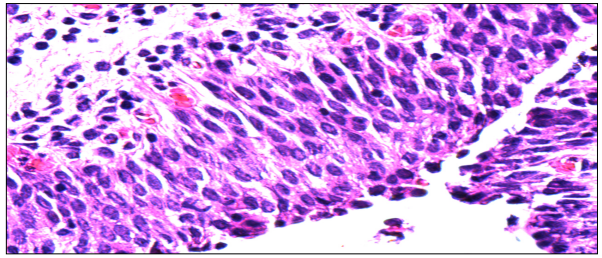
Phase I Study of Intravesical Fowlpox-GMCSF, TRICOM (NCT00072137)

- 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)

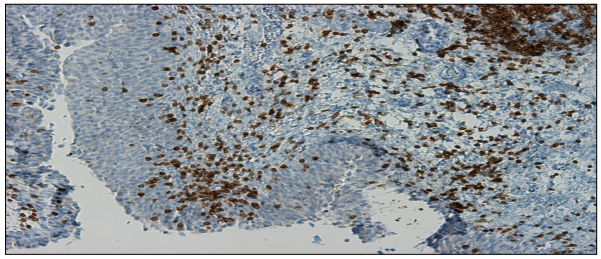
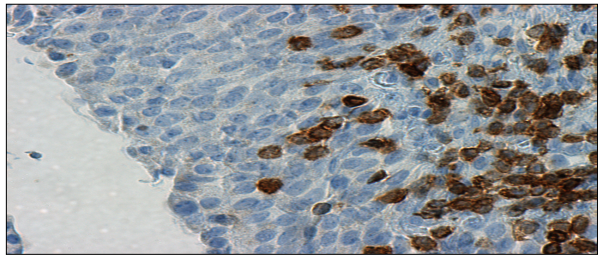
Expression of Lac-Z. gene in the tumor and NAT, post cystectomy, by RT-PCR



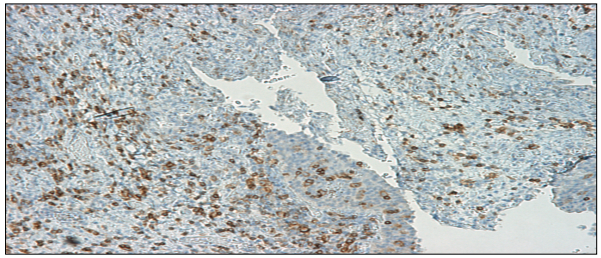
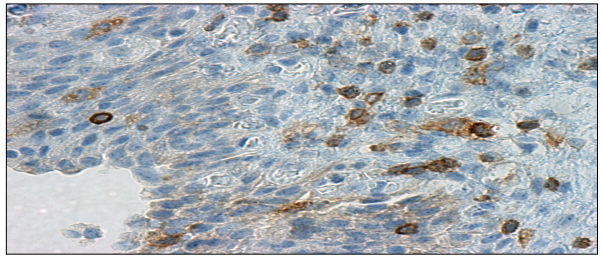
Immunohistochemistry Pt. 3 rF-GMCSF (7 X 10^7 PFU)

H&E

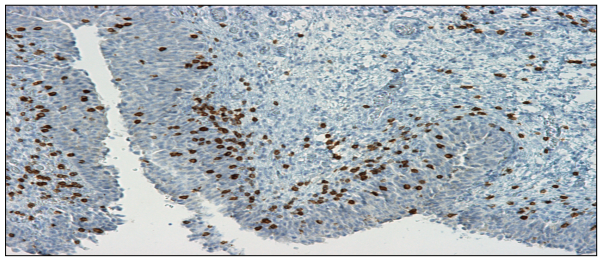



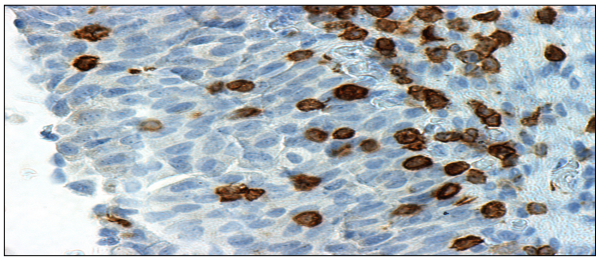
CD3 (T)

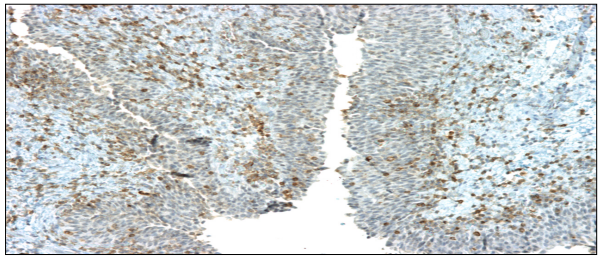
CD4 (T_h)

Immunohistochemistry Pt. 3 rF-GMCSF (7 X 10^7 PFU)

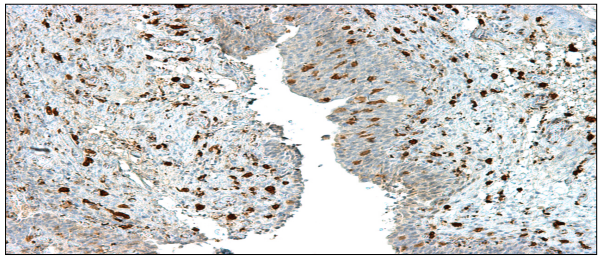


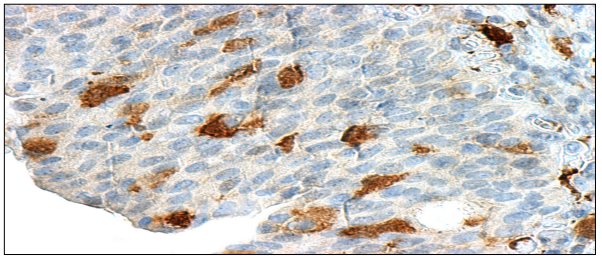


CD8 (T_c)



CD45RO (T_{act})





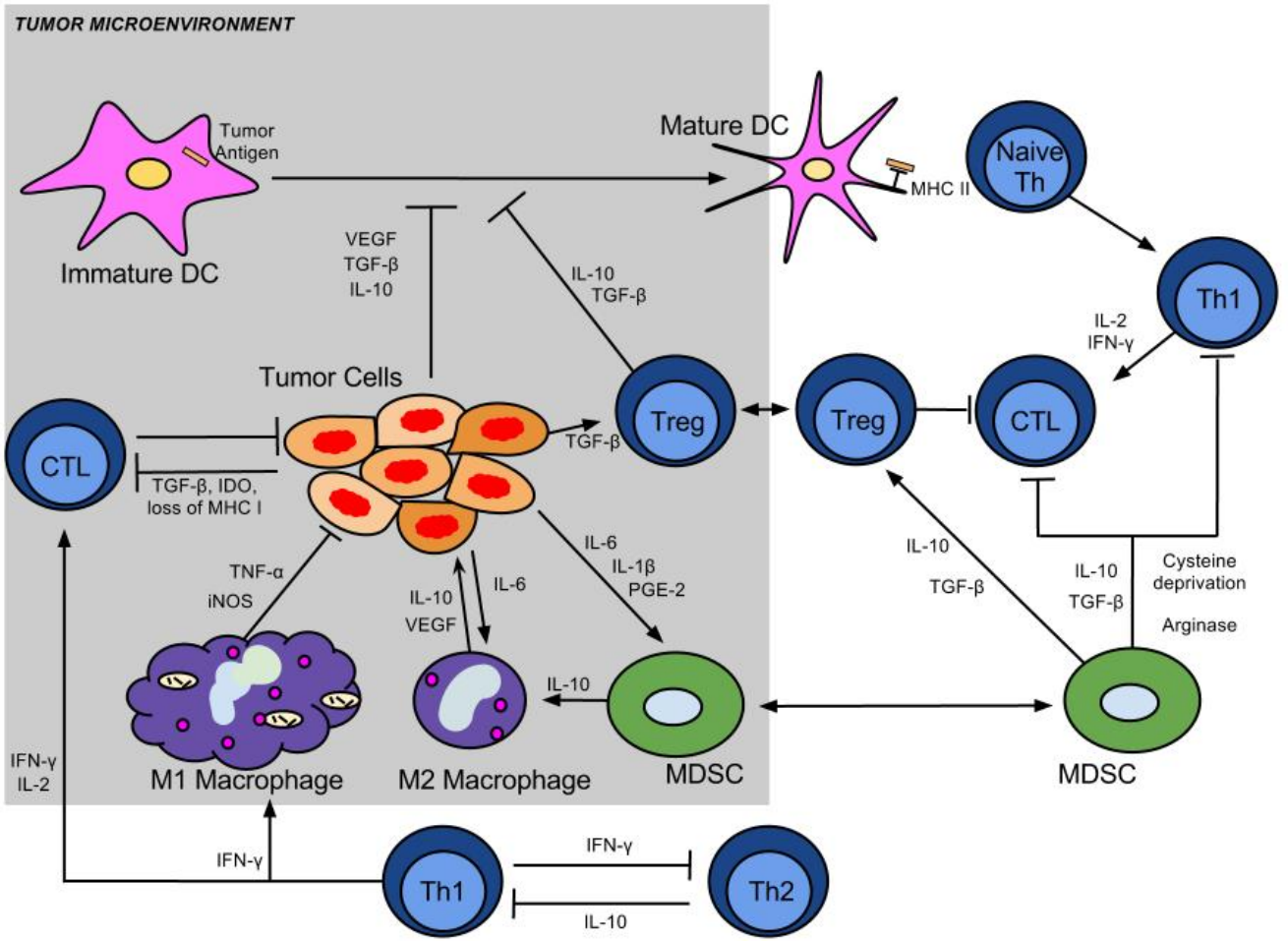
Factor XIII (DC)

Poxvirus Vector Platform for Immunotherapy

- 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”**

Immune escape and the tumor microenvironment



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-γ upregulates Class II)
- Presents soluble antigen to $CD4^+$ T cells

[CANCER RESEARCH 63, ●●●–●●●, October 15, 2003]

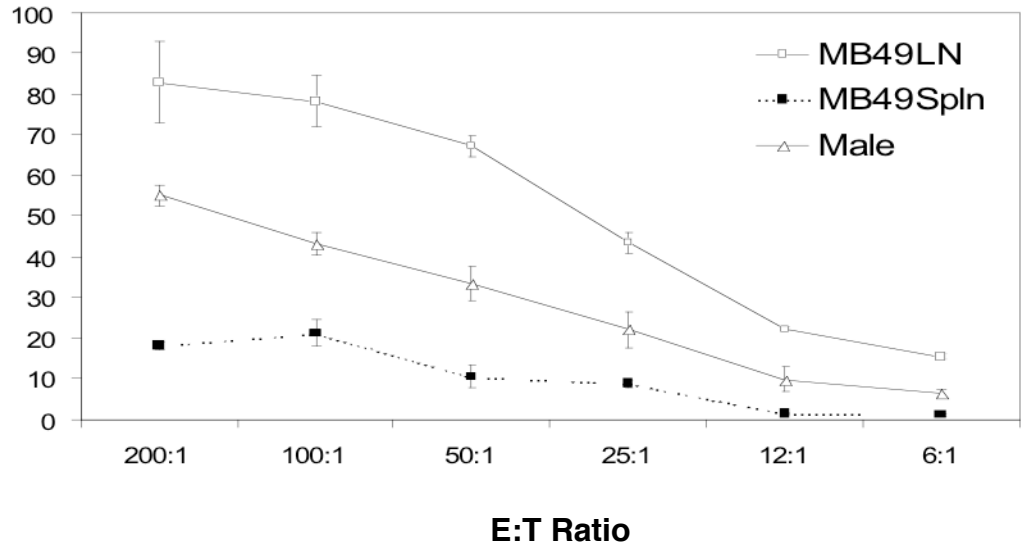
Intratumoral Vaccination with Vaccinia-Expressed Tumor Antigen and Granulocyte Macrophage Colony-Stimulating Factor Overcomes Immunological Ignorance to Tumor Antigen[1]

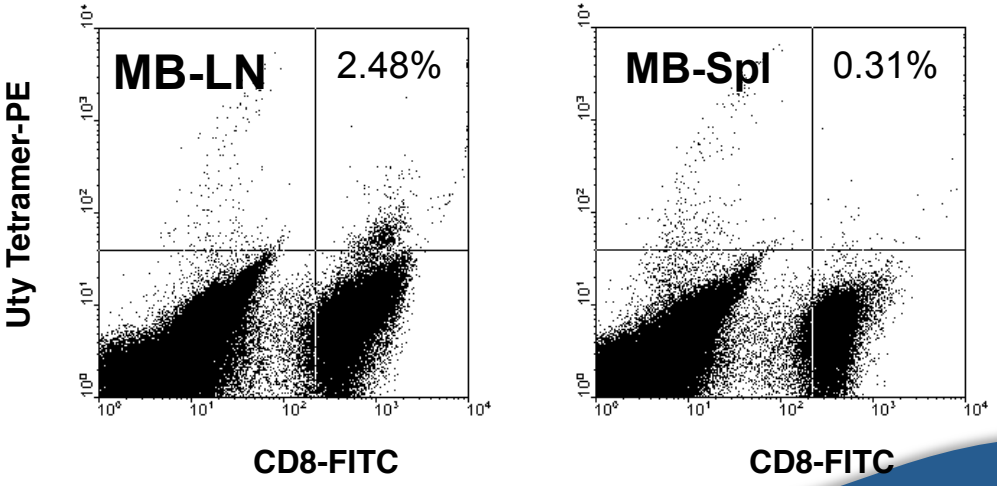
Arvin S. Yang, Claude E. Monken, and Edmund C. Lattime[2]

Departments of Surgery and Molecular Genetics, Microbiology, and Immunology, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School and The Cancer Institute of New Jersey, New Brunswick, New Jersey 08901

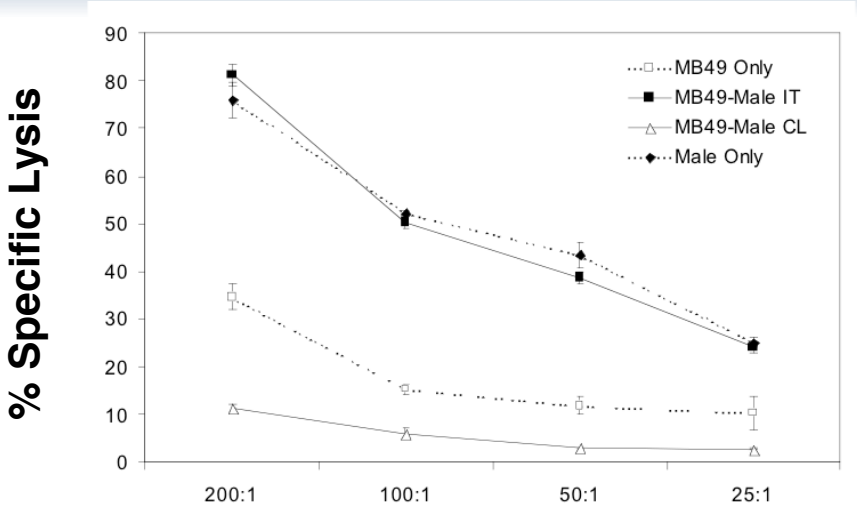
- Develop recombinant vaccinia-HY vaccine vector
- Evaluate vaccine stimulated T cell (CD8) responses
- Assess tumor induced immunity/ignorance
- Evaluate effects of systemic vs intratumor immunization

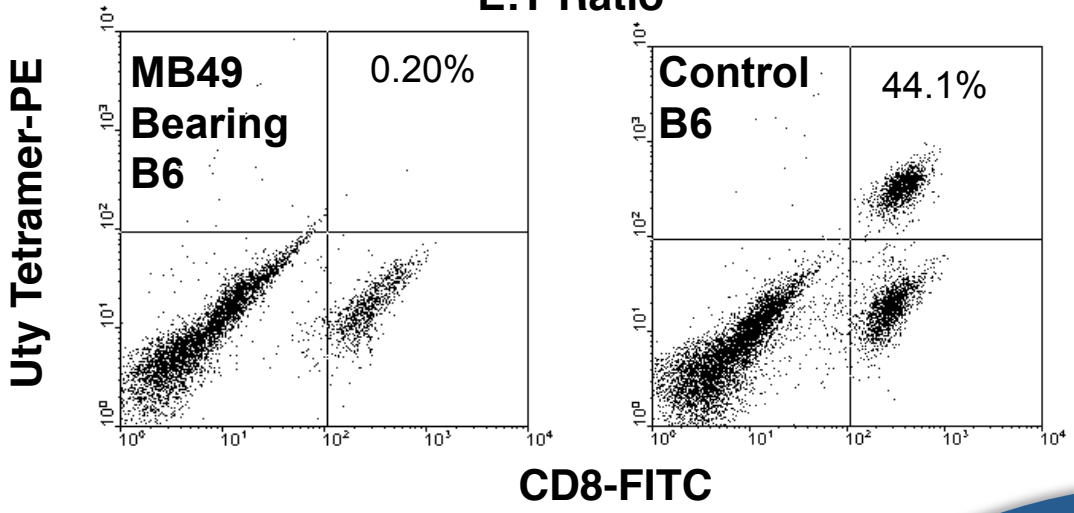
Antitumor Responsiveness in LN (local) but not Splenic (systemic)





MB49 Bearing Mice are Systemically Anergic to HY





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

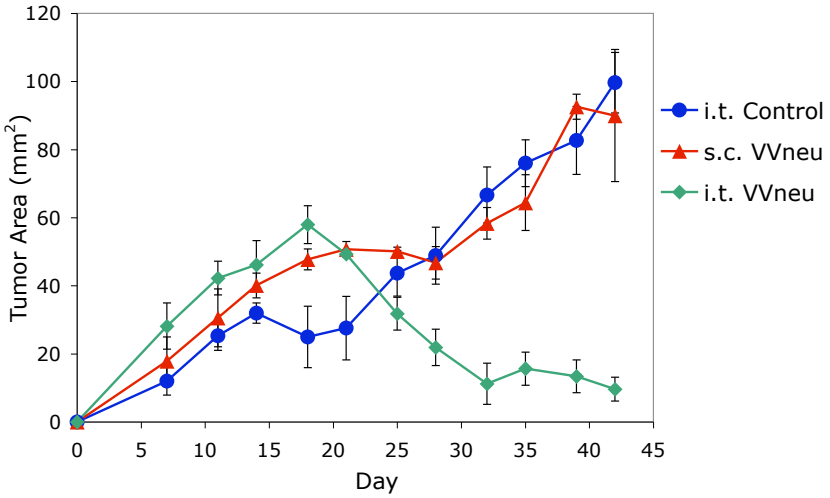
NBT1: orthotopic breast cancer model

- **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 $2x10^6$ NBT1 cells

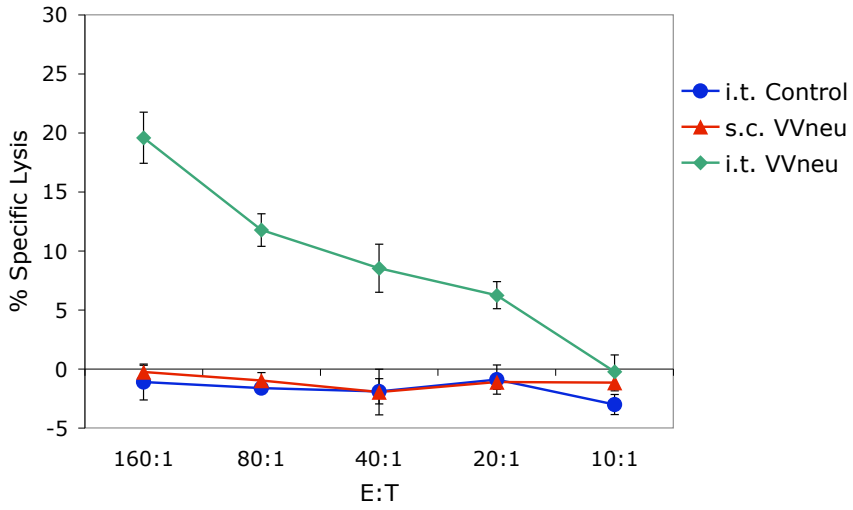
- **VVneu** is a recombinant vaccinia virus expressing rat HER2/neu. **VVGMCSF** is a recombinant vaccinia virus expressing GM-CSF. **VVBGal** is a vaccinia control. Mice received $1x10^6$ pfu dose of each indicated virus ($2x10^6$ pfu total).

Intratumoral but not systemic immunization induces Neu-specific CTL and eliminates tumor

Orthotopic Tumor Growth

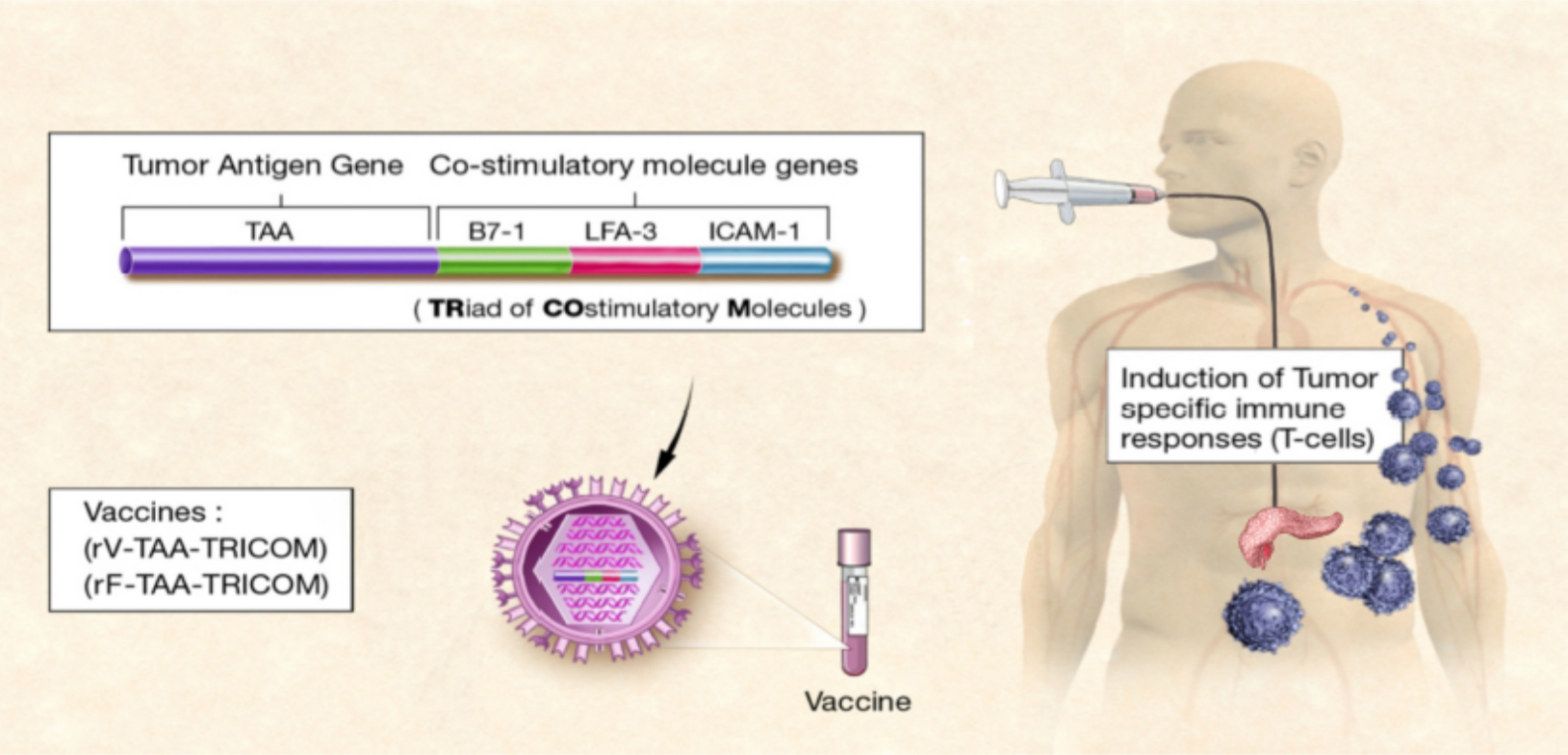


Induction of Systemic CTLs



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

day	schema
1-5	D1 PANVAC-F **IT** via EUS; d2 PANVAC-**V**+GMCSF; D3-5 GMCSF
15-9	D15 PANVAC-F **IT** via EUS; d16 PANVAC-F +GMCSF; D3-5 GMCSF
29-32	D29 PANVAC-F+ D29-32 GMCSF
35	(Pts may start standard of care chemo or chemoRT)
43	D43 PANVAC-F+ D43-46 GMCSF
71	D71 PANVAC-F + D71-74 GMCSF

Clinical results

pt	stage	IT/SC inj	f/u First Chemo	f/u RT	CA19-9	F/U	Progression to met
76/m	T4N0M0	2,4	gem	yes	133	30 mo+	No
78/m	T4N0M0*	2,5	no	no	19	Ex/862	No
70/f	T4N1M1	2,2	Sys-off	unk	218	Off study Ex/159	Metastases
81/f	T4N0M1*	2,5	gem	yes	205	Ex/177	No
74/f	T4N1M1*	2,5	gem	yes	815	Ex/566	No
75/f	T3N1M0	2,2	none	no	544	Ex/35	--
69/f	T3N1M0	2,5	gem	no	12990	15 mo+	No
73/F	T3N1M1	2,5	gem	no	190	Ex/166	Metastases
47/M	T3NxM1	2,5	gem	No	20	10 mo+	Metastases
74/M	T4N1M0	1,2	gem/cap	No	9	Off study 9 mo+	No
45/M	T4N1M0	2,5	gem	none	950	8 mo+	No

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

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