Host-Tumor Interactions

Primer on Tumor Immunology and Biological therapy
Boston, MA
November 1st 2007

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Department of Transfusion Medicine
Clinical Center/NIH
Preface

There are three Golden rules that apply to the successful treatment of any disease

Unfortunately we do not know any of them

Anonymous Stanford Professor
A paradigm shift: Evidence Based Research: Hypothesis vs Discovery Driven Research

**Hypothesis testing** most efficient when one variable at the time is analyzed

- Mice
  - a) Inbred
  - b) Disease Homogenous
  - c) Controllable Environment

**Relevance testing**

- Men
  - a) Polymorphic
  - b) Disease Heterogeneous
  - c) Environmental Influence

**Hypothesis Generating** more realistic in the clinics

Identify **COMMONALITIES** through a **Global Approach**

Hypothesis generation in humans requires a global approach
Two Models

– Active specific immunization against melanoma with or without IL-2

– Treatment of basal cell cancer with toll receptor agonists (Imiquimod)
Two Models

- Active specific immunization against melanoma with or without IL-2

- Treatment of basal cell cancer with toll receptor agonists (Imiquimod)
The role of immunity in its active form generally referred to as “inflammation” at various stages of carcinogenesis and progression.

Phases of cancer growth

- Immuno surveillance (IFN-γ, T cells, Immune competent host)
- Indolent chronic inflammation (Tumor Associated Macrophages, B cells)
- Immune editing
- Established cancer
- Rapid growth
- Immune escape

Immune Pessimism

Immune Optimism

Immunotherapy (CTL, NK cells, APC antibodies)

Mantovani, Romero, Palucka and Marincola – *The Lancet* – in press
Multidimensionality of tumor/host interactions in the context of T cell aimed immunization

1st dimension = TCR/HLA/epitope interaction
2nd dimension = Importance of co-stimulation
3rd dimension = Localization at tumor site
4th dimension = Evolving nature of immune response and genetic instability of cancer cells
5th dimension = Heterogeneity of the tumor micro-environment
Is CTL precursor frequency a factor for disease control?
In vitro induction of anti-MART-1 (○) and anti-flu (□) CTL in HLA-A*0201 individuals

Melanoma Patients

Normal Controls

Cytotoxic T Lymphocyte precursor frequency (CTLpf):

<table>
<thead>
<tr>
<th></th>
<th>Melanoma</th>
<th>Normal</th>
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<tbody>
<tr>
<td>Melan A</td>
<td>1/5,000</td>
<td>1/100,000</td>
</tr>
<tr>
<td>Flu M1:</td>
<td>1/1,000</td>
<td>1/1,000</td>
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</tbody>
</table>

Marincola et al., J. Immunother 1996
The Systemic Response to Anti-Melanoma Vaccines

Model: g209-2M peptide vaccine ± interleukin-2


No Clinical response
Is the functional status of CTL a factor for disease control?
Multidimensionality of tumor/host interactions in the context of T cell aimed immunization

1st dimension = TCR/HLA/epitope interaction
2nd dimension = Importance of co-stimulation/activation
3rd dimension = Localization at tumor site
4th dimension = Evolving nature of immune response and genetic instability of cancer cells
5th dimension = Heterogeneity of the tumor micro-environment
Quiescent phenotype of tumor-specific CD8+ T cells following immunization

In vitro Ag recall + IL-2

Pre-vaccination

Post-vaccination

Post-vaccination After IVs

Monsurro et al. J Immunol 2002

Monsurro et al. Blood 2004
Monsurro et al. Blood 2004
Step required for the effective implementation of vaccines

Studying the micro-environment

**Excisional Biopsies**
- Good quantity of material to study
- Do not allow serial sampling of same lesion
- Do not allow prospective assessment of natural history of a given lesion

**Fine Needle Aspirates**
- Limited quantity of material to study
- They allow serial sampling of same lesion
- They allow prospective follow up of a given lesion

*Wang and Marincola, Immunol Today 2000*
Multidimensionality of tumor/host interactions in the context of T cell aimed immunization

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Kinetics of IFN-γ and gp-100 expression in melanoma metastases

**Treatment:** gp100 based vaccine

**Question:**
Vaccine-elicited T cell may not localize at tumor site.

**Results:**
Vaccine-elicited T cells
1) localize at tumor site
2) interact with the tumor cells
3) this is not sufficient for tumor rejection

<table>
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<tr>
<th>Site</th>
<th>IFN/CD8</th>
<th>Fold Inc</th>
<th>GP100/Actin</th>
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<td>1474</td>
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*Kammula et al., J. Immunol., 1999*
Multidimensionality of tumor/host interactions in the context of T cell aimed immunization

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5th dimension = Heterogeneity of the tumor microenvironment

Increasing biological complexity
Tumor variability and evolving with time

CR = Complete Response
NR = No Response

$p_2 = 0.003$

Proposed hypothesis of how antigen-specific therapy might affect target antigen expression

- **Effective**
  - Target antigen present
  - Inflammation

- **Not effective**
  - Target antigen absent
  - Primary Effect

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Marincola et al., Trends in Immunology, 2003
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Increasing biological complexity
Identification of two sub-classes of melanoma

Unsupervised clustering of melanoma metastases
Sampled by fine needle aspiration (FNA)

Wang et al. Nature Biotech 2000
Wang et al Cancer Res. 2002
Multidimensionality of tumor/host interactions in the context of T cell aimed immunization

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5\textsuperscript{th} dimension = Heterogeneity of the tumor microenvironment

Increasing biological complexity
Prediction of response to vaccine plus High-Dose Interleukin-2 Therapy

Pre-treatment FNA

Wang et al. Cancer Res, 2002
Genes differentially expressed pre-treatment in immune responsive metastases

Wang et al. Nature Biotech 2000
Wang et al. Cancer Res. 2002

TIA-1
IL-27
IRF-2
What is the effect of therapy?

Prediction of response to vaccine plus High-Dose Interleukin-2 Therapy

Pre-treatment FNA

Post-treatment FNA

Wang et al. Cancer Res, 2002
Genes differentially expressed between pre- vs post-treatment CR

Associated with responsiveness of
- genital warts to Imiquimod
- carcinoid tumors to IFN-α
- CML to IFN-α

Wang et al. Cancer Res. 2002
Mechanism(s) of Action of Systemic High-Dose Interleukin-2 Therapy

Pre-treatment 3 hr after 1st and 4th dose

Panelli et al. Genome Biol, 2002
**FNA and the tumor microenvironment:**
Genes differentially expressed in FNA 3hr post 1 and 4 doses of IL-2

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Expression</th>
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</tr>
<tr>
<td>MHC class II DR beta</td>
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</tr>
<tr>
<td>Grancalcin Ca2+ binding protein</td>
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<tr>
<td>Calgranulin Ca2+ binding protein</td>
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<td>CD62L L-selectin</td>
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<td>V-CAM-1</td>
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<td>CD29 integrin=beta 1 fibronectin receptor</td>
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<td>Keratin 10</td>
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<td>IL-1 R</td>
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<tr>
<td>IL-1 receptor antagonist</td>
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<td>IL-2 R beta chain</td>
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<td>TNF-a induced protein 3</td>
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<tr>
<td>TGFβ receptor</td>
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<tr>
<td>Interferon-γ IEF SSP5111upregulated protein (HSP70)</td>
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<tr>
<td>MxA/interferon induced cellular resistance protein</td>
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</tr>
<tr>
<td>MxB interferon induced cellular resistance protein</td>
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<tr>
<td>(Interferon-a inducible protein IF1-6-16)</td>
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<tr>
<td>Guanylate binding protein 1 interferon inducible</td>
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<tr>
<td>IRF-1 interferon regulatory factor-1</td>
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<tr>
<td>IFN induced 56KDa protein</td>
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<tr>
<td>IFNγ receptor alpha chain</td>
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<td>Nmi=IL-2 and IFN-γ inducible potentiator of STAT</td>
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<tr>
<td>C-C chemokine receptor 1</td>
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<td>GRO-1 (MCP-1)</td>
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<td>MCP-3</td>
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<tr>
<td>MIP-1beta</td>
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<tr>
<td>MIP-1 alpha</td>
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<td>PARC=DC-CK1</td>
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<tr>
<td>Monocyte neutrophils elastase inhibitor</td>
<td></td>
</tr>
<tr>
<td>IL-8 chemokine</td>
<td></td>
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<tr>
<td>Human insulin like growth factor</td>
<td></td>
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<tr>
<td>Plasminigen activator urokinase</td>
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</tbody>
</table>

Panelli et al., Genome Biology, 2002, 3(7):res0035.1-0035.17
Genes associated with immune response during IL-2 therapy

Panelli et al Genome Biol 2002

- TIA-1=nucleolysin cytotoxic granule
- NK4= natural killer cell protein 4
- NKG5=granulysin
- EBI3
- TCR alpha
- DAG kinase
- HLA class II region expressed gene KE4
- MHC class II DR beta
- SERPINB1=Leukocyte elastase inhibitor
- MIP-1 delta
- FGF-13
- STIM1=Stromal interaction molecule 1
- VEGF
- CD62 P selectin
- GALECTIN 1
- GALECTIN 1
- N-Myc
- DAP-1
- 53BP1=p53 binding protein

CR LESSION POST IMMUNIZATION EXPRESSED GENE
SIMILAR EXPRESSION TO NK EXPOSED TO I12
UPREGULATED IN ACUTE REJECTION OF KIDNEY TRANSPLANT (PBMC AND RENAL BIOPSY TISSUE Sorwal, H. Immunol, 2001)
Postulated algorithm of tumor immune responsiveness

Spontaneous release of immune modulators by tumor cells

Antigen-specific immunization

Non-specific immune stimulation = IL-2

Immune stimulation/inflammation

Threshold for tumor regression

Spontaneous regression

Response to therapy

Tumor progression

Marincola et al., Trends in Immunol. 2003
• What happens during tissue-specific immune-mediated rejection?

• Can a constant mechanism be identified that is necessary and sufficient for the resolution of the pathogenic process (viral clearance, tumor rejection)?
Double Blind Vehicle Control Study
To Evaluate Apoptosis in Basal Cell Carcinoma
Treated With Aldara™ (Imiquimod Cream, 5%)
Applied Once or Twice a Day

NNMC Bethesda Dermatology Service
Department of Transfusion Medicine Section of Immunogenetics, NIH
Local applications of the TLR-7 agonist Imiquimod for the treatment of Basal Cell Cancer

Pre-treatment 2-8 days after starting treatment

Panelli et al. Genome Biol - 2007
### Vehicle-controlled study of the effect of Imiquimod on BCC

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Doses Received</th>
<th>EOT → Bx Time Lapse (hrs)</th>
<th>Histology</th>
<th>ΔCD8</th>
<th>ΔCD56</th>
<th>Tumor at EOT</th>
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<tbody>
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<td>P5</td>
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<td>0</td>
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<td>P23</td>
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<td>45</td>
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Mean±SD = 22.0±11.5

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<th>Tumor at EOT</th>
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Mean±SD = 18.2±10.0

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<th>Tumor at EOT</th>
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Mean±SD = 28.3±14.5

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Mean±SD = 39.4±50.2
Log_{10} 2−ΔΔCT (post-pre)

**IFN-γ**

- p = 0.08 (n = 7)
- p = 0.04 (n = 6)
- p = 0.02 (n = 7)
- p = 0.03 (n = 8)

**IFN-α**

- p = 0.43 (n = 7)
- p = 0.04 (n = 7)
- p = 0.06 (n = 6)
- p = 0.64 (n = 4)
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Primary = co-expressed in early and later biopsies
Secondary = expressed in late biopsies only
Imiquimod-specific genes with effector functions: cytokines, cytokine receptors and lytic enzymes

Panelli et al. Genome Biol - 2007
Looking for the immunological constant of rejection (Mantovani et al. *The Lancet* – in press)

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[1] = Panelli et al. Genome Biol - *in press*
Conclusions

• Immune mediated tissue destruction whether with beneficial (cancer/viral disease) or pathological (autoimmunity/allograft rejection) effects follows common pathways; among them:
  – Interferon Stimulated Genes (ISGs) appear to be necessary but not sufficient
  – ISGs represent a mixture of genes predominantly induced not only by IFN-α but also by IFN-γ
  – A third group of genes not directly induced by IFNs seems to be more tightly associated with immune-mediated tissue destruction.
  – These genes represent activation of innate and/or adaptive effector functions although, such effector functions appear to be less represented in the context of HCV clearance than in the context of tumor or allograft rejection.
Acknowledgments

Infectious Disease and Immunogenetics Section, NIH

Ena Wang
Vladia Monsurro’
Kate Lally
Jos Even
Sara Deola
Rosemary Werden
Li Xin

Monica Panelli
Kang-Hun Lee
Simone Mocellin
Dirk Nagorsen
Kina Smith
Jin Ping
Eleonora Arico’

Maria Bettinotti
Regina Norris
Silvia Selleri
Katia Zavaglia
Yvonne Ngalame
Maurizio Provenzano
Sonia Voiculescu

David Stroncek
Harvey Klein

Collaborations

Meenhard Herlyn
Soldano Ferrone
Ralph Freedman
Jay Berzofsky
William Biddison
Ken Parker
David Garboczi
Lance Miller
Edison Liu
Jeffrey Trent
Polly Matzinger
Imiquimod-specific genes with effector functions:
Receptors and associated molecules

- JAK-2
- CD68
- CD64
- HLA-G
- KLRC3
- CD2
- CD59
- CD4
- TNF receptor
- ZAP-70
- T cell immune-regulator 1
- insulin-like growth factor 1 receptor
- CD8
- CD5
- T-cell receptor
- CD3 Zeta
- Minor histocompatibility antigen HA-1
- Cathepsin W
- CD69
Response rate after peptide-based vaccinations against melanoma

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<td>g209-2M in IFA + IL-2 (720,000 IU/Kg TID)</td>
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<tr>
<td>IL-2 alone for treatment of metastatic melanoma</td>
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**Conclusion:**
Enhanced T cell responses induced by vaccination have no independent effect on clinical outcome
What is the mechanism of immune rejection?


Spontaneous anti-cancer resistance is mediated through a rapid infiltration of leukocyte mostly of the innate immunity including natural killer cells, neutrophils, and macrophages that are required for the destruction of cancer through rapid cytolysis
A 23 gauge needle seen from a cell point of view

23 Gauge Needle Inner Diameter = 850μm
Average Cell Diameter = 10-20 μm

Material Obtainable:
1 - 100 x 10^5 cells
(median = 10^6)
.1 to 10 μg total RNA
(median = 2 μg)

High-fidelity mRNA amplification for gene profiling
Wang et al J Trans Med 2006
6646 Genes ratio abs=2
% present = 90

Panelli et al. under review
Log_{10} 2^{-\Delta\Delta CT} (post-pre)

IFN-\gamma

\[ p = 0.03 \]

\[ n = 7 \]

\[ n = 6 \]

\[ n = 7 \]

\[ n = 7 \]

\[ n = 4 \]

\[ n = 8 \]

IFN-\alpha

\[ p = 0.04 \]

\[ n = 7 \]

\[ n = 6 \]

\[ n = 7 \]

\[ n = 8 \]

\[ n = 7 \]

\[ n = 4 \]

\[ n = 8 \]

Panelli et al – under review
Proposed Studies

• Transcriptional comparison of PBMC of individuals with HVC viremia, individuals who cleared the viremia and normal donors

• Comparison of CD8 and CD4 T cell responses between individuals with progressive compared to stable liver disease
Genes commonly up-regulated in Imiquimod-treated BCC and in liver of chimps with resolving hepatitis C virus infection
Supervised analysis of all BCC samples

Pre therapy (Px-PB1)

Post therapy (Px-PB3)

Supervised analysis of all BCC samples

6646 Genes,
RatioTest/Ref abs=2
% present = 90

Imiquimod
Vehicle

Figure 3
Dissecting Imiquimod-associated signatures

360 genes

- IL-2/IL-4/IL-7/IL-9/IL-15 receptor common gamma chain
- CD62L=L-selectin, CASP 1, CD106/vcam, Vimentin, EBI2, IL-6, TNFR2, histone H2B, CD18=Integrin
- cullin 1, Pleckstrin, MHC Class I=HLA-C4
- GM-CSF/IL-5/IL-3 receptor common beta chain
- Nedd4 binding protein 1, CSFRb

MCP3, TNF TGFbR, IFNalpha R, TRAF 1 and 2, disintegrin and metalloproteinase domain 8 HLA-E
TNF alpha protein 2 and 3, CD163, CSF2R
CD5, CD2, Lymphotoxin beta, TCR beta, CD4, CD95 FAS, MHC class I, Granzyme B, NKG5, MHC class II DQ, IL-18, CD27, allograft inflammatory factor 1, CD14, CD69, CD38, Lactotransferrin, Perforin 1, KLRC1, formyl peptide receptor/FPR12, Tapasin/NGF17, Cathepsin W
Role of CD4+CD25<sup>high</sup> regulatory T cells

• CD4+CD25<sup>high</sup> T cells frequency is higher in melanoma and renal cell cancer patients than non tumor bearing individuals, after tumor regression in response to rIL-2 therapy their frequency reverts to normal (Desana CG et al. J Clin Oncol 24: 1169, 2006)

• Foxp3+CD4+CD25+ T cells frequency is higher in HCV infected chimps compared to unaffected chimps (Manigold T et al. Blood 107: 4424, 2006)

• Foxp3+CD4+CD25+ T cells frequency is similar in infected chimps compared to those who cleared the infection (Manigold T et al. Blood 107: 4424, 2006)

Conclusion
The frequency of regulatory T cells is higher in chronic inflammatory conditions and persists after clearing of the pathogenic condition in HCV but not in cancer
What about IFN-α?
Delayed polarization of mononuclear phagocyte transcriptional program by type I interferon isoforms – Stroncek et al. J Transl Med 3: 24, 2005
T0-T2 = 124 differentially expressed genes

T42-T44 = 187 differentially expressed genes

Pre IFN-pep Vacc. (T0,T42)

Post IFN-pep Vacc. (T2, T44)
FNA and the tumor microenvironment: Genes differentially expressed in FNA 3hr post 1 and 4 doses of IL-2

Highest median across experiments: cell surface, adhesion inflammatory proteins

- MHC class II DR alpha
- MHC class II DR beta
- Grancalcin Ca2+ binding protein
- Calgranulin Ca2+ binding protein
- CD62L L-selectin
- CD45
- V-CAM-1
- CD64
- CD29 integrin=beta 1 fibronectin receptor (Fibronectin 1)
- Keratin 10
- IL-1 R
- IL-1 receptor antagonist
- IL-2 R beta chain
- TNF-a induced protein 3
- TGFβ receptor
- Interferon-γ IEF SSP5111 upregulated protein (HSP70)
- MxA/interferon induced cellular resistance protein
- MxB interferon induced cellular resistance protein (Interferon-a inducible protein IF1-6-16)
- Guanylate binding protein 1 interferon inducible
- IRF-1 interferon regulatory factor-1
- IFN induced 56KDa protein
- IFNγ receptor alpha chain
- Nmi=IL-2 and IFN-γ inducible potentiator of STAT
- C-C chemokine receptor 1
- GRO-1 (MCP-1)
- MCP-3
- MIP-1 beta
- MIP-1 alpha
- PARC=DC-CK1
- Monocyte neutrophils elastase inhibitor
- IL-8 chemokine
- Human insulin like growth factor
- Plasminogen activator urokinase

Panelli et al., Genome Biology, 2002, 3(7): res0035.1-0035.17
The Model

Tumor Progression vs. MAA and HLA A2 Expression for patient CC

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Cormier et al., 1999
ImiquimodVehicle
dcb

Before treatment (Px -PB1)

(263 genes)

After treatment (Px -PB3)

(67 genes)

(58 genes)

(23 genes)

Panelli et al. Genome Biol - 2007
Looking for the immunological constant of rejection

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[1] = Panelli et al submitted