Micro 204
Tumor Immunology

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Immunological Surveillance
Ehrlich, Burnet & Thomas

Paul Ehrlich (1909) Concept of cancer immunosurveillance. Predicted that cancer would occur at “incredible frequency” if host defenses did not prevent the outgrowth of continuously arising cancer cells

Lewis Thomas (1957) “primary function of cellular immunity….is to protect from neoplastic disease”

Macfarland Burnet (1957) “It is by no means inconceivable that small accumulations of tumour cells may develop and because of their possession of new antigenic potentialities provide an effective immunological reaction with regression of this tumor and no clinical hint of its existence”
Evidence for immune surveillance in humans

Increased incidence of EBV+ B cell lymphomas in transplant patients treated with immunosuppressive drugs

Increased incidence of Kaposi’s sarcoma & EBV+ B cell lymphomas in AIDS patients

Gastric cancer associated with *H. pylori* infection

Cervical cancer caused by human papillomavirus

Liver cancer caused by hepatitis B & C
Immunosurveillance & “Immunoediting”

Tumor Antigens - definition & discovery

Passive Immunotherapy
   anti-tumor CTL
   anti-tumor antibodies
   inhibitory receptor blockade

Active Immunotherapy
   Vaccination

Tumor Evasion Strategies
Anti-Tumor Effector Mechanisms

CD4\(^+\) T cells, CD8\(^+\) CTL, and NK cells
- direct cytotoxicity or ADCC (NK cells) via perforin & granzymes and/or TNF family members
- cytokine release (e.g., TNF, IFN\(\gamma\), GM-CSF) leading to:
  a) lysis of tumor cells
  b) disruption of angiogenesis
  c) recruitment and activation of DC, macrophages, & granulocytes

B cells
- production of tumor-specific antibodies leading to:
  a) complement-mediated killing
  b) ADCC
  c) antibody-mediated apoptosis by disrupting oncogenic signals

Macrophages
- killing via ADDC
- killing via production of cytokines such as TNF
- killing via production of toxic oxygen or nitrogen intermediates
Immunotherapy Strategies

Cytokine infusions (e.g. IFNa, IL-2)

Induction of inflammation (e.g. CpG)

Tumor-targeted antibodies (e.g., Herceptin)

Adoptive transfer of tumor-specific T cells

Donor lymphocyte infusions after BMT/HSCT
  (allogeneic bone marrow or hematopoietic stem cell transplant)

Vaccination

Innate System

“Passive” Adaptive System

“Active” Adaptive System
Immune Surveillance - Revival

IFN-γ and lymphocytes prevent primary tumour development and shape tumour immunogenicity

Vijay Shankaran*, Hiroaki Ikeda*, Allen T. Bruce*, J. Michael White*, Paul E. Swanson*, Lloyd J. Old† & Robert D. Schreiber†

Nature 410:1107, 2001
Increased Incidence of MCA-Induced Tumors Detected In Mice With Well-Defined Genetic Immunodeficiencies


SubQ MCA Injection

80–160 days

Lack T and B cells

Lack IFNγR or signaling capacity

Wild Type (normal mice)
Spontaneous tumors in wild-type and immunodeficient mice
T cells control latent tumors

Tumors arising in immunodeficient mice are more immunogenic than tumors arising in wild-type mice

Assayed by transplanting tumors into wild-type or immunodeficient mice
Tumor Elimination - Equilibrium - Escape

Schreiber et al. Immunity 2004
Tumor-infiltrating lymphocytes correlation with survival in ovarian cancer patients

Zhang et al. NEJM 348:203, 2003
Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome

Jérôme Galon, Anne Costes, Fatima Sanchez-Cabo, Amos Kirilovsky, Bernhard Mlecnik, Christine Lagorce-Pagès, Marie Tosolini, Matthieu Camus, Anne Berger, Philippe Wind, Franck Zinzindohoué, Patrick Bruneval, Paul-Henri Cugnenc, Zlatko Trajanoski, Wolf-Herman Fridman, Franck Pagès
Melan-A/MART-1-specific CD8$^+$ T cells in lymph nodes of melanoma patients

Romero et al. *J. Exp. Med.*, Volume 188(9), 1998 1641-1650
Tumor Antigens
Discovery & Definition
Experimental Demonstration of Antigen-specific Tumor Immunity

Mouse with chemical carcinogen-induced tumor

- Resect tumor
- Isolate CD8+ T cells

Transplant tumor cells into original tumor-bearing mouse
- No tumor growth

Transplant tumor cells into syngeneic mouse
- Tumor growth

Adaptively transfer T cells into recipient of tumor transplant
- Eradication of tumor
Immunize mouse with irradiated tumor cells

- **Irradiated tumor cells**

  - **Inject viable cells of the same tumor**
    - Response to unique tumor rejection antigens eliminates tumor
  - **Inject viable cells of a different tumor**
    - Response to irradiated tumor will not eliminate unrelated tumors of a different cell type

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Fig 14.10 © 2001 Garland Science
Tumor Antigens

Tumor-specific antigens
  – Expressed by tumors ONLY

Tumor-associated antigens
  – Preferentially expressed by tumors

Oncofetal antigen
  – Expressed by tumors in adult, but also expressed by fetal (not adult) tissues
### Types of Tumor Antigens Recognized by T cells

<table>
<thead>
<tr>
<th>Normal host cell displaying multiple MHC-associated self antigens</th>
<th>Not recognized by T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tumor cells</strong></td>
<td></td>
</tr>
<tr>
<td>Mutated self protein</td>
<td>CD8+ CTL</td>
</tr>
<tr>
<td>Over-expressed or aberrantly expressed self protein</td>
<td>CD8+ CTL</td>
</tr>
<tr>
<td>Oncogenic virus</td>
<td>Virus antigen-specific CD8+ CTL</td>
</tr>
</tbody>
</table>
Normal cell presents self peptides bound to MHC molecules

A point mutation in a self protein allows binding of a new peptide to MHC molecules

A point mutation in a self peptide creates a new epitope for recognition by T cells
### Potential tumor rejection antigens have a variety of origins

<table>
<thead>
<tr>
<th>Class of antigen</th>
<th>Antigen</th>
<th>Nature of antigen</th>
<th>Tumor type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor-specific mutated oncogene or tumor-suppressor</td>
<td>Cyclin-dependent kinase 4</td>
<td>Cell-cycle regulator</td>
<td>Melanoma</td>
</tr>
<tr>
<td></td>
<td>β-Catenin</td>
<td>Relay in signal transduction pathway</td>
<td>Melanoma</td>
</tr>
<tr>
<td></td>
<td>Caspase-8</td>
<td>Regulator of apoptosis</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>Germ cell</td>
<td>MAGE-1 MAGE-3</td>
<td>Normal testicular proteins</td>
<td>Melanoma Breast Glioma</td>
</tr>
<tr>
<td>Differentiation</td>
<td>Tyrosinase</td>
<td>Enzyme in pathway of melanin synthesis</td>
<td>Melanoma</td>
</tr>
<tr>
<td></td>
<td>Surface Ig</td>
<td>Specific antibody after gene rearrangements in B-cell clone</td>
<td>Lymphoma</td>
</tr>
<tr>
<td>Abnormal gene expression</td>
<td>HER-2/Neu</td>
<td>Receptor tyrosine kinase</td>
<td>Breast Ovary</td>
</tr>
<tr>
<td>Abnormal post-translational modification</td>
<td>MUC-1</td>
<td>Underglycosylated mucin</td>
<td>Breast Pancreas</td>
</tr>
<tr>
<td>Oncoviral protein</td>
<td>HPV type 16, E6 and E7 proteins</td>
<td>Viral transforming gene products</td>
<td>Cervical carcinoma</td>
</tr>
</tbody>
</table>

Fig 14.11 © 2001 Garland Science
Epitope Landscape in Breast and Colorectal Cancer

Neil H. Segal,¹,² D. Williams Parsons,⁴ Karl S. Peggs,²,³ Victor Velculescu,⁴ Ken W. Kinzler,⁴ Bert Vogelstein,⁴ and James P. Allison²,³

to identify candidate tumor antigens. Analysis of 1,152 peptides containing missense mutations previously identified in breast and colorectal cancer revealed that individual cancers accumulate on average ~10 and ~7 novel and unique HLA-A*0201 epitopes, respectively, including genes implicated in the neoplastic process. These data suggest
<table>
<thead>
<tr>
<th>Virus</th>
<th>Associated tumors</th>
<th>Areas of high incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA viruses</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Papillomavirus (many distinct strains) | Warts (benign)  
Carcinoma of uterine cervix | Worldwide  
Worldwide |
| Hepatitis B virus             | Liver cancer (hepatocellular carcinoma)                | Southeast Asia  
Tropical Africa |
| Epstein–Barr virus            | Burkitt's lymphoma (cancer of B lymphocytes)  
Nasopharyngeal carcinoma  
B-cell lymphoproliferative disease | West Africa  
Papua New Guinea  
Southern China  
Greenland (Inuit)  
Immunosuppressed or immunodeficient patients |
| RNA viruses                   |                                                        |                                                        |
| Human T-cell leukemia virus type I (HTLV-1)  
Human immunodeficiency virus (HIV-1) | Adult T-cell leukemia/lymphoma  
Kaposi's sarcoma | Japan (Kyushu)  
West Indies  
Central Africa |

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Use of Human Tumor Ag-Specific Cloned CTL for Identification of Tumor Antigens

Generation of tumor-specific CTL clones

A

Melanoma
Purify mononuclear cells from tumor site

Surgically resect tumor

Tumor cells

Patient's mononuclear cells

Coculture mononuclear cells and melanoma cells

Isolate and clone activated CD8+ CTLs

Melanoma cell line
Identification of tumor antigens recognized by tumor-specific CTLs

Tumor cDNA library → Coculture with CTL clone → TNFα Production

Melanoma cell line → Transfect into class I MHC+ target cell line → Isolate transfected DNA and sequence

Gene encoding tumor antigen recognized by melanoma-specific CTL
Serological identification of tumor antigens - Serex

Some cancer patients have antibodies reactive with their own tumor

Use patients’ sera to expression clone the tumor antigens

Surprizingly, many of the antisera recognized the same tumor-associated antigens that detected by CTL
Passive Immunotherapy

Anti-tumor monoclonal antibodies

(a billion dollar business)
Antibody-dependent cellular cytotoxicity
FDA-approved therapeutic monoclonal antibodies for cancer

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Brand name</th>
<th>Approval date</th>
<th>Type</th>
<th>Target</th>
<th>Approved treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alemtuzumab</td>
<td>Campath</td>
<td>2001</td>
<td>humanized</td>
<td>CD52</td>
<td>Chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>Avastin</td>
<td>2004</td>
<td>humanized</td>
<td>vascular endothelial growth factor</td>
<td>colorectal cancer</td>
</tr>
<tr>
<td>Brentuximab vedotin</td>
<td>Adcetris</td>
<td>2011</td>
<td>chimeric</td>
<td>CD30</td>
<td>Hodgkin lymphoma, Anaplastic large-cell lymphoma</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>Erbitux</td>
<td>2004</td>
<td>chimeric</td>
<td>epidermal growth factor receptor</td>
<td>colorectal cancer</td>
</tr>
<tr>
<td>Gemtuzumab ozogamicin</td>
<td>Mylotarg</td>
<td>2000</td>
<td>humanized</td>
<td>CD33</td>
<td>acute myelogenous leukemia (with calicheamicin)</td>
</tr>
<tr>
<td>Ibritumomab tiuxetan</td>
<td>Zevalin</td>
<td>2002</td>
<td>murine</td>
<td>CD20</td>
<td>non-Hodgkin lymphoma (with yttrium-90 or indium-111)</td>
</tr>
<tr>
<td>Panitumumab</td>
<td>Vectibix</td>
<td>2006</td>
<td>human</td>
<td>epidermal growth factor receptor</td>
<td>colorectal cancer</td>
</tr>
<tr>
<td>Rituximab</td>
<td>Rituxan, Mabthera</td>
<td>1997</td>
<td>chimeric</td>
<td>CD20</td>
<td>non-Hodgkin lymphoma</td>
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<tr>
<td>Trastuzumab</td>
<td>Herceptin</td>
<td>1998</td>
<td>humanized</td>
<td>ErbB2</td>
<td>breast cancer</td>
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<tr>
<td>Ipilimumab</td>
<td>Yervoy</td>
<td>2011</td>
<td>human</td>
<td>CTLA-4</td>
<td>Melanoma</td>
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</table>
Rituxan Pivotal Trial: Treatment of Patients With Relapsed B Lymphoma

Rituxan® 375 mg/m² (IV)

Monitoring every 3 months x2 years

<table>
<thead>
<tr>
<th>Weeks</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>8</th>
<th>16</th>
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</thead>
</table>

<table>
<thead>
<tr>
<th>Evaluable Patients</th>
<th>166</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Response</td>
<td>80 (48%)</td>
</tr>
<tr>
<td>Complete Response</td>
<td>10 (6%)</td>
</tr>
<tr>
<td>Partial Response</td>
<td>70 (42%)</td>
</tr>
</tbody>
</table>

CD16 (FcγRIII) mediates Herceptin and Rituxan mediate human tumor elimination in nude mice

*Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets*

Raphael A. Clynes¹, Terri L. Towers¹, Leonard G. Presta² & Jeffrey V. Ravetch¹
**Fig 14.17 © 2001 Garland Science**

- **Tumor-specific antibody**
  - Antibodies bind to the tumor cell
  - NK cells with Fc receptors (CD16) are activated to kill the tumor cells

- **Tumor-specific antibody conjugated to toxin**
  - Antibody-toxin conjugates bind to the tumor cell
  - Conjugates are internalized, killing the cell

- **Tumor-specific antibody conjugated to radionuclide**
  - Radioactive antibody binds to the tumor cell
  - Radiation kills the tumor cell and neighboring tumor cells
Passive Immunotherapy

Inhibitory Receptor Blockade with Monoclonal Antibodies

(e.g. CTLA-4, PD-1)
CTLA-4 Blockade Enhances Tumor-Specific Immune Responses

- Attenuated Proliferation
- Unrestrained Proliferation

Tumor

GM-CSF vaccine
Peptide-pulsed DCs
Chemotherapy
Irradiation
Anti-angiogenesis
Hormone therapy
Surgical reduction
Skin and hair de-pigmentation by treatment of B16 melanoma with anti-CTLA-4 and GM-CSF-producing vaccines

challenge

vaccination

rejected B16-F10 lung metastases
CTLA-4 Blockade:
Anti-tumor immunity, Autoimmunity

The good news....

The bad news....

Phan et al. PNAS (2003) 100:8372
Kaplan-Meier Analysis of Survival

Comparison | HR  | p-value |
------------|-----|---------|
Arms A vs. C | 0.68 | 0.0004  |
Arms B vs. C | 0.66 | 0.0026  |

Survival Rate

<table>
<thead>
<tr>
<th>Survival Rate</th>
<th>Ipi + gp100 (403 patients)</th>
<th>Ipi + pbo (137 patients)</th>
<th>gp100 + pbo (136 patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 year</td>
<td>44%</td>
<td>46%</td>
<td>25%</td>
</tr>
<tr>
<td>2 years</td>
<td>22%</td>
<td>24%</td>
<td>14%</td>
</tr>
</tbody>
</table>
Passive Immunotherapy

Adoptive transfer
of antigen-specific CTL
Adoptive T cell therapy using antigen-specific CD8⁺ T cell clones for the treatment of patients with metastatic melanoma: In vivo persistence, migration, and antitumor effect of transferred T cells

C. Yee*,†, J. A. Thompson*, D. Byrd*, S. R. Riddell*, P. Roche‡, E. Celis‡, and P. D. Greenberg*

IL-2 Dose:
(mU/m²/day)

Day 0    Day 14    Day 35    Day 56

IL-2 (0.5)  IL-2 (1.0)  IL-2 (2.0)

Infusion#:
T cell Dose:

CTL #1  3.3 x 10⁹/m²
CTL #2  3.3 x 10⁹/m²
CTL #3  3.3 x 10⁹/m²
CTL #4  3.3 x 10⁹/m²

PNAS 99:16168, 2002
Adoptive T cell therapy

Transduce autologous T cells with chimeric receptor fusing anti-CD19 antibody to transmembrane and cytoplasmic domain of CD3ζ and 4-1BB.
Active Immunotherapy

Vaccination
First vaccine to prevent human cancer!

vaccine for papilloma virus for cervical cancer!

San Francisco Chronicle

Cervical cancer vaccine due soon
Federal panel urges it go to all girls, 11-12, not a must for school

Erin Allday, Chronicle Staff Writer
Friday, June 30, 2006

Panel's suggestions

The federal Advisory Committee on Immunization Practices recommended:

-- That the cervical cancer vaccine routinely be given to all 11- and 12-year-old girls.

-- That girls and women ages 13 to 26 receive the vaccine, regardless of whether they are sexually active.

-- That physicians have the option of giving the vaccine to girls as young as 9.
Successful Active Vaccination against Virus-induced Cancers

Vaccine to feline leukemia virus for cats

Vaccine to herpes virus (Marek’s virus) in chickens

Vaccine to hepatitis B in humans to prevent liver carcinoma

Vaccination to HPV prevents cervical cancer
Active Immunization - Tumor cells or antigens

- Tumor cells or extracts (Melacin)
- Tumor peptide + adjuvant vaccine
- Tumor peptide loaded on dendritic cell
- Tumor antigen cDNA vaccination
- Tumor antigen in recombinant virus
- Feeding dendritic cells dead tumors
- Feeding dendritic cells tumor RNA
Excise tumor or use tumor cell line

Isolate dendritic cells

Mix killed tumor cells or proteins extracted from tumor cells with dendritic cells from patient

Return dendritic cells and tumor antigens to patient
Tumor cell transfected with GM-CSF gene → ↑Dendritic cells → CTL activation → Tumor destruction

Dendritic cell presents tumor antigen

GM-CSF gene

GM-CSF

Tumor cell

Dendritic cell

CTL-P
FDA NEWS RELEASE

For Immediate Release: April 29, 2010
Media Inquiries: Shelly Burgess, 301-796-4651, shelly.burgess@fda.hhs.gov
Consumer Inquiries: 888-INFO-FDA, OCOD@fda.hhs.gov

FDA Approves a Cellular Immunotherapy for Men with Advanced Prostate Cancer

Provenge
load autologous DC with prostatic acid phosphatase
inject into prostate cancer patient

Summary of Overall Survival Analysis Results

<table>
<thead>
<tr>
<th></th>
<th>Sipuleucel-T Median Survival</th>
<th>Placebo Median Survival</th>
<th>Sipuleucel-T vs. placebo Hazard Ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D9902B (N=512)</td>
<td>25.8</td>
<td>21.7</td>
<td>0.775 (0.614, 0.979)</td>
<td>0.032</td>
</tr>
<tr>
<td>D9901 (N=127)</td>
<td>25.0</td>
<td>21.4</td>
<td>0.586 (0.388, 0.884)</td>
<td>0.010</td>
</tr>
<tr>
<td>D9902A (N=98)</td>
<td>19.0</td>
<td>15.7</td>
<td>0.786 (0.484, 1.278)</td>
<td>0.331</td>
</tr>
<tr>
<td>Integrated Studies (N=737)</td>
<td>25.4</td>
<td>21.5</td>
<td>0.734 (0.612, 0.881)</td>
<td>0.0009</td>
</tr>
</tbody>
</table>

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1. Hazard Ratio (HR), confidence interval (CI), and p-value estimated according to the primary analysis methods.
2. HR, CI, and p-value estimated based on unadjusted Cox model and log rank test as presented in the individual clinical trial report. The analysis methods for overall survival were not pre-specified.
3. HR, CI, and p-value estimated based on Cox model with treatment as independent variable, stratified by study.
4. Based on a Kaplan-Meier estimate (in months).

No difference between the two study arms in time to objective disease progression, progression free survival, time to clinical progression, or time to prostate-specific antigen (PSA) doubling time was observed in any of the Phase 3 studies. The reason for the dissociation between overall survival and these other outcome measures is unclear. However, overall survival is the most reliably measured and clinically meaningful of these endpoints.
Mechanisms of Tumor Escape from Immune Responses

- Loss of MHC or TAP
- Loss of co-stimulatory molecules
- Antigenic variation
- Secretion of immunosuppressive factors
  - E.g. TGF-b, IL-10
- T cells don’t penetrate solid tumors
- Exhaustion of T cells
- T regulatory cells suppress anti-tumor responses
Loss of Class I MHC Expression in a Prostate Carcinoma
NKG2D on NK cells, γδ T cells, and CD8+ T cells detect NKG2D ligands on abnormal cells
Suppressor T cells and tumors
Cutting edge: Regulatory T cells from lung cancer patients directly inhibit autologous T cell proliferation.

Woo EY, Yeh H, Chu CS, Schlienger K, Carroll RG, Riley JL, Kaiser LR, June CH.

Active suppression by T regulatory cells plays an important role in the down-regulation of T cell responses to foreign and self-Ags. Thus far, the potential role of CD4(+)CD25(+) T cells in human tumors has not been reported. In this work we show that lung tumors contain large numbers of these cells and that they have constitutive high-level expression of CD152 (CTLA-4). Furthermore, the CD4(+)CD25(+) T cells mediate potent inhibition of autologous T cell proliferation. Finally, regulatory T cells from patient tumors failed to inhibit the proliferation of allogeneic T cells. Together these results suggest that the CD4(+)CD25(+) T cells found in lung tumors selectively inhibit the host immune response and therefore could contribute to the progression of lung cancer.

J Immunol 2002 May 1;168(9):4272-6
Therapeutic strategies to overcome tumor evasion

Loss of MHC
  - Type I interferon

Secretion of immunosuppressive factors
  – Neutralizing TGF-b, IL-10

Exhaustion of T cells
  – Treat with IL-15 or block PD-1

T regulatory cells suppress anti-tumor responses
  – Deplete Treg
In Vivo depletion of CD25$^+$ T cells facilitates tumor rejection

TGF-β blockade allows tumor rejection

Figure 1. Blockade of TGF-β signaling in T cells renders mice resistant to tumor challenge.

Gorelik & Flavell Nat Med 7:1118, 2001
Full Disclosure
Inflammation promotes cancer!

Immune Enhancement of Skin Carcinogenesis by CD4⁺ T Cells

Dylan Daniel¹, Nicole Meyer-Morse¹, Emily K. Bergsland², Kerstin Dehne³, Lisa M. Coussens³ and Douglas Hanahan¹

Article
IKKβ Couples Hepatocyte Death to Cytokine-Driven Compensatory Proliferation that Promotes Chemical Hepatocarcinogenesis
Shin Maeda¹,² Hideaki Kamata¹,² Jun-Li Luo¹,² Hyam Leffert², and Michael Karin¹,²,*

Article
De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent
Karin E. de Visser¹, Lidiya V. Korets¹, and Lisa M. Coussens¹,²,³,*
Stuff to Remember

• Tumor express antigens that can be recognized by the immune system

• T cells recognizing tumors can be detected in cancer patients…but they are inefficient

• Tumors attempt to evade the immune system by loss of antigens and secretion of suppressive factors

• Vaccination against tumor antigens is possible..but is difficult because many of these antigens are perceived as ‘self’

• mAbs against tumor-associated Ags can be effective