Immune surveillance: The immune system can recognize and destroy nascent malignant cells. T cells are believed to play a major role in controlling tumor growth.
T cell-based Immunotherapy

Tumor

HLA class I – TA peptide complex

Tumor Cell lysate

TA

TA derived peptides

Peptide-pulsed APC

Tumor HSP

Activated CTL

CD8+ T cell precursor

CD4+ T cell

Immunization
T cell-based immunotherapy clinical trials: lessons learned

- Immunization strategies have been successful in eliciting tumor antigen-specific CTL in at least a proportion of the immunized patients.

- Disappointing clinical response rates have been obtained.

- A tumor antigen-specific CTL immune response is often not accompanied by a clinical response.
Why does a TA-specific CTL immune response not correlate with clinical response in patients with malignant disease treated with immunotherapy?

- defects in CTL
- resistance of tumor cells to CTL recognition
HLA class I antigen-peptide complex expression is necessary for tumor antigen derived recognition by CTL
How are HLA class I antigen-tumor antigen peptide complexes generated?

Antigen processing and presentation pathway

- DRiPs
- Protein
- Tumor antigen (TA)
  - Ub
  - Proteasome/Immunoproteasome
  - Peptide
  - Cytosolic peptidases
  - Cytoplasm
  - ER (endoplasmic reticulum)
  - Calnexin
  - Heavy chain
  - Calreticulin
  - Tapasin
  - TAP
  - Translocon

- TA-specific CTL
  - TCR
  - Kill
  - No killing
Immunohistochemical staining of formalin fixed, paraffin embedded malignant lesions by HLA class I specific mAb

Heterogeneous expression

Loss of expression in undifferentiated cells

Serial Sections of a Breast Carcinoma Lesion
Different frequency of HLA class I antigen downregulation in different tumor types

- **Monomorphic**
  - HNSCC: 9%
  - Breast: 24%
  - Lung: 27%
  - Colon: 27%
  - Cervical: 29%
  - Prostate: 50%
  - Melanoma: 16%

- **Polymorphic**
  - HNSCC: 43%
  - Breast: 16%
  - Lung: 27%
  - Colon: 27%
  - Cervical: 20%
  - Prostate: 35%
  - Melanoma: 118%
Correlation of LMP2 and tapasin expression with HLA class I antigen expression in primary laryngeal squamous cell carcinoma lesions

- **LMP2**
  - Correlation: $r=0.41$, $p=0.0055$

- **TAP1**
  - Correlation: $r=0.23$, $p=0.12$

- **tapasin**
  - Correlation: $r=0.70$, $p<0.001$

- **calnexin**
  - Correlation: $r=0.25$, $p=0.10$
Association of APM component and HLA class I antigen expression with CD8+ T cell infiltration in primary laryngeal squamous cell carcinoma lesions
Association of HLA class I antigen expression and CD8+ T cell infiltration in primary laryngeal squamous carcinoma lesions with poor survival

![Graph showing cause-specific survival for HLA class I antigen and β₂m.](image-url)

- **HLA class I antigen**
  - Positive vs. negative: \( p = 0.020 \)
  - Positive vs. heterogeneous: \( p = 0.42 \)
  - Heterogeneous vs. negative: \( p = 0.010 \)

- **β₂m**
  - Positive vs. negative: \( p = 0.052 \)
  - Positive vs. heterogeneous: \( p = 0.52 \)
  - Heterogeneous vs. negative: \( p = 0.0017 \)
Restoration by IFN-γ of recognition of SCCHN PCI 13 cells by HLA class I antigen restricted, TA-specific CTL.
Relationship between upregulation of TAP1 and tapasin level and recognition of IFN-γ treated SCCHN cells PCI-13 and SCC4 by HLA class I antigen restricted, TA-specific CTL
Phage display antibody libraries

Immunoglobulin  Semi-synthetic single chain fragment of antibody variable region (scFv)  phage displayed scFv
Panning phage display antibody libraries with HLA class I allele-TA peptide complexes

A. Incubation

B. Binding

C. Wash

D. Elution

E. Amplification

scFv library

HLA class I allele-TA peptide complex

Phage bound HLA class I allele-TA peptide complexes

Eluted phage
Enriched phage displayed scFv recognize purified HLA-A*0201-peptide complexes

- HLA-A2-MART1\textsubscript{27-35} (ELAGIGILTV)
- HLA-A2-HER2/neu\textsubscript{689-697} (RLLQETELV)
- HLA-A2-HER2/neu\textsubscript{369-377} (KIFGSLAFL)
Isolation of unique HLA class I allele-TA peptide complex specific scFv

<table>
<thead>
<tr>
<th>Clone</th>
<th>Heavy chains</th>
<th>Light chains</th>
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<tbody>
<tr>
<td></td>
<td>Family</td>
<td>Segment</td>
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<tr>
<td>HLA-A*0201-MART1&lt;sub&gt;27-35&lt;/sub&gt;-specific scFv</td>
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<tr>
<td>8.3</td>
<td>VH3</td>
<td>DP-45</td>
</tr>
<tr>
<td>24.3</td>
<td>VH3</td>
<td>DP-45</td>
</tr>
<tr>
<td>25.3</td>
<td>VH3</td>
<td>DP-45</td>
</tr>
</tbody>
</table>

| HLA-A*0201-HER2/neu<sub>369-377</sub>-specific scFv |     |     |     |     |     |
| 2.3.5  | VH3    | DP-13*01 | AGPAGAGPWGQ | V<sub>κ</sub>2 | DPK-29*01 | MQSIQLHT |
| 2.4.38 | VH3    | DP-13*01 | AGPAGAGPWGQ | V<sub>λ</sub>3 | DPL-19*01 | NSRDSSGNHPDV |

| HLA-A*0201-HER2/neu<sub>689-697</sub>-specific scFv |     |     |     |     |     |
| 1.3.13 | VH3    | DP-23*01 | ARGEFRTYFPT | V<sub>κ</sub>1 | DPK-39*01 | QQANSFLSST |
scFv 8.3 does not bind to MART1_{27-35} peptide alone
scFv 8.3 binds to a determinant located on the $\alpha_1/\alpha_2$ domains of HLA-A*0201 and MART1$_{27-35}$ peptide.
Can we enhance the sensitivity of HLA class I allele-TA peptide complex specific probes?
Generation of HLA class I allele-TA peptide complex specific scFv tetramers

scFv tetramer

Site specific biotinylation site

Streptavidin

Biotin

Phycoerythrin

scFv tetramer
scFv tetramerization enhances their ability to detect HLA class I allele-TA peptide complexes on target cells.
Heterogeneous HLA-A*0201 surface expression and intracellular MART1 protein expression in human melanoma cells

![Graph showing fold change in HLA-A*0201 and MART1 protein expression with and without IFN-γ](image-url)
Heterogeneous APM component expression in human melanoma cells

Fold change over background

- IFN-γ
+ IFN-γ
Heterogeneous HLA-A*0201-MART1_{27-35} peptide complex expression on human melanoma cells
Lack of correlation between HLA-A*0201, MART1 and HLA-A*0201-MART1<sub>27-35</sub> peptide complex expression
Lack of relationship between HLA-A2 antigen and HLA-A2 antigen-HER2/neu \textsubscript{369-377} peptide complex expression by SCCHN cell lines
Conclusions

• The level of APM components and HLA class I antigens markedly vary in malignant cells

• Measure of the level of APM component and HLA antigen expression provides only limited information about their functional properties

• The level of HLA class I antigen-tumor antigen peptide complexes on tumor cells does not correlate with the level of APM components, HLA class I antigens and tumor antigens

• These results stress the need to measure the level of HLA class I antigen-tumor antigen peptide complexes on tumor cells to characterize their interactions with CTL
HLA class I antigen-peptide complexes mediate recognition of target cells by cytotoxic T lymphocytes (CTL)
Reactivity of scFv 8.3 with peptide pulsed T2 cells is dependant on scFv & MART1\textsubscript{27-35} concentration.

![Graph showing reactivity of scFv 8.3 with peptide pulsed T2 cells depending on scFv & MART1\textsubscript{27-35} concentration. The graph displays the mean fluorescence intensity as a function of peptide concentration (µM) and scFv concentration (nM). The x-axis represents peptide concentration (µM) ranging from 490 to 1, with values 490, 245, 123, 61, 30, 15, 8, 4, 2, 1, and 0. The y-axis represents mean fluorescence intensity ranging from 60. The graph includes a legend indicating T2-MART1\textsubscript{27-35} and T2-HER2/neu\textsubscript{369-377}. The data points are connected by lines showing the concentration range of peptide (µM) from 65 to 3,550 and scFv (nM) from 65 to 3,550.}
scFv 8.3 mimics the reactivity of HLA-A*0201-MART1\textsubscript{27-35}-specific TCR

Antibody

- scFv 8.3 tetramer
- mAb CR11-351
- mAb 9E10
- scFv 2.3.5 tetramer
Enriched phage displayed scFv recognize peptide pulsed T2 cells

- T2-HER2/neu$_{369-377}$
- T2-MART1$_{27-35}$

Antibody
- scFv 8.3
- scFv 2.3.5
- mAb CR11-351
- mAb 9E10 JH1
- scFv JH1

Specificity
- HLA-A2-MART1$_{27-35}$
- HLA-A2-HER2/neu$_{369-377}$
- HLA-A2, -A24, -A28
- c-myc
- Human VEGF
HLA class I allele-TA peptide complex specific scFv tetramers retain their specificity

**Antibody**
- scFv 8.3
- mAb CR11-351
- mAb 9E10
- scFv 2.3.5

**Specificity**
- HLA-A2-MART1\textsubscript{27-35}
- HLA-A2, -A24, -A28
- c-myc
- HLA-A2-HER2/neu\textsubscript{369-377}
Lack of correlation between APM component and HLA-A*0201-MART1\textsubscript{27-35} peptide complex expression