Immunotherapy with Antibody Targeted MHC class I/peptide complexes: Results of In Vivo Tumour Cell Killing and Therapeutic Vaccination

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T cell targets and responses in health, viral infections and cancer

- HLA + normal peptide
- HLA + viral peptide
- HLA + ‘cancer peptide’

- T cell recognising HLA + viral peptide [Can reach 20% of all T cells]
- T cell recognising HLA + ‘cancer peptide’ [Usually less than 0.1% of all T cells]
HLA class I molecules and CD20/B9E9

- Recombinant HLA class I/peptide monomers are simple, robust and cheap to make
- HLA tetramers, 4 monomers joined to streptavidin via biotin, are used widely for enumerating epitope specific T cells

**CD20 and B9E9 sfvSA**
- CD20 ~ 60,000 copies on each B cell
- B9E9 sfvSA tetravalent single chain antibody/streptavidin fusion protein
- High avidity and minimal antibody internalisation
- Already used in RIT of NHL with radio-biotin
HLA System 1
The use of anti-viral T cells to kill cancer cells using 2-Step Targeting of HLA class I complexes

Biotinylated HLA-A2/viral peptide monomer
anti-CD20/SA Tetravalent mAb
CD20/TAA
Targeting of HLA class I complexes to cancer cells in vitro

Dose/response of HLA concentration analysed by 4 hr Cr release with clone 25 CTL to HLA-A2/M1 E:T 5:1
In vivo activity of targeted HLA-A2/BMLF1 complexes

- Tumour protection assay in SCID mice (4 mice per group)
  - Day 1 $1 \times 10^7$ IP of an anti-BMLF1 (EBV antigen) CTL line.
  - Day 1 $1 \times 10^6$ Daudi cells targeted ex vivo, with B9E9 scFvSA and HLA-A2/M1 at a separate IP site
  - Day 43 mice sacrificed and tumours measured
**HLA-A2/BMLF1 results of in vivo experiment**

<table>
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<tr>
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<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
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<tr>
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<td>Anti-BMLF1 CTL</td>
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Savage et al 2002 IJC
Tumour targeting with HLA class I complexes
Optimal Disease Characteristics

- Well defined non-internalising tumour antigen recognised by a monoclonal antibody
- Tumour cells readily accessible in blood or Lymph Nodes
- Tumour cells sensitive to T cell mediated lysis
- Tumour vasculature endothelium could also be a target
- Upregulated anti-viral T cell activity would be a bonus!
Tumour targeting with HLA class I complexes

CLL Disease Characteristics

- CLL is a chronic malignancy of B cells
- Tumour cells are found in the blood and Lymph Nodes
- B Cells are very sensitive to T cell mediated lysis
- In CLL greatly elevated levels of CMV specific T cells are frequently found!
- These CMV specific T cells are effector phenotype +ve
  - High levels of perforin and granzyme
CMV specific CTLs in health and CLL

The T cells are of the effector phenotype
CMV T cell specific lysis of CLL cells

CMV specific T cells can kill CLL cells in vitro either Pulsed with the CMV pp65 Peptide or coated with HLA-A2/p65 complexes

E:T 4:1 4hr assay

HLA/CMV was fixed at 100 ng ml-1
Tumour targeting with MHC complexes in mice using endogenous murine T cells

• Mice immunised with OVA peptide
• Immunised mice ~ 2% of T cells OVA specific
• Murine B16 melanoma cell line transfected with human CD20
• B16HuCD20 cells coated with H2/Ova or H2/Hsv complexes via CD20-B9E9sfvSA
• In vitro and in vivo killing experiments
In Vitro killing of B16Hu20 melanoma cells using antibody targeted MHC complexes using Ova immunized mouse splenocytes 4hr Cr release assay
In Vivo Tumour Protection Assay:
OT-1 Ovalbumin immune mice injected IV with 1x $10^5$ B16-HuCD20 melanoma cells targeted with either H2-Kb-Hsv or H2-Kb-Ova MHC complexes.

<table>
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<tr>
<th>Mouse</th>
<th>Number of metastases visible</th>
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<tr>
<td>H2-Kb-Hsv</td>
<td>173</td>
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<tr>
<td>H2-Kb-Hsv</td>
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<td>H2-Kb-Ova</td>
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<tr>
<td>H2-Kb-Ova</td>
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Summary

• We have demonstrated the effective killing of MHC targeted tumour cells by virus specific CTLs in vitro and in vivo

• The system should be amenable for human use
  – B9E9 sfvSA has been used in RIT for NHL
  – HLA class I complexes already circulate without toxicity
  – Virus specific CTLs are present in all patients and in CLL CMV specific CTLs are greatly expanded

• Issues for clinical studies
  • Choice of target CLL or other tumours
  • Targeting tumours or tumour blood vessels
  • Potential immunogenicity of streptavidin
  • Stability of HLA complexes should be enhanced by use of single chain trimers
  • Standard obstacles in clinical trials!
The use of antibody targeted HLA complexes as Cancer and HIV Vaccines

HLA class I/peptide complexes on Antigen Presenting Cell.

Differing approaches to the expansion of CTLs
HLA System 2
Expansion of peptide specific CTL responses by antibody targeted HLA class I peptide complexes

- Biotinylated HLA-A2/peptide monomer
- CD20/SA Tetravalent mAb
- CD20

B cell

CTL

CTL

CTL

CTL
Expression of B9E9 sfvScSA targeted HLA-A2/M1 complexes on HLA class I –ve B cells.
Detected with FITC-W6/32
In Vitro CMV specific T Cell Expansion using B cells targeted with HLA class I/peptide complexes

Unstimulated Donor 1 PBMCs

Autologous Dendritic cells + NLV peptide

B cells + HLA-A*0201/NLV (Experiment 1)

HLA-A*0201/NLV tetramer

Irrelevant tetramer

B cells + HLA-A*0201/NLV (Experiment 2) (Experiment 3)

HLA-A*0201/NLV tetramer

CD8

0.03

6.08

30.6

0.17

9.21

15.5
Tetramer and Elispot enumeration of HLA-A2/HIV specific CTLs expanded in vitro using the antibody-MHC system
In vivo CTL expansion using B cells targeted with MHC class I complexes

• Model system
  – Female C57 mice previously primed with male spleen cells to produce response to H2/Uty
• Responses measured by tetramer analysis
• Experiment 1 Daudi cells targeted with H2/Uty complexes
  – $10^7$ B cells given IV
• Experiment 2 Murine (huCD20 +ve) B cells targeted with H2/Uty
  – $10^7$ spleen cells given IV
In vivo expansion of CTLs using Daudi B cells targeted with MHC class I/peptide complexes

A

Day -2

Day 30

Day 37

B

10^7 H2/uty coated B cells injected IV
In vivo expansion of CTLs using murine hu-CD20 B cells targeted with MHC class I/peptide complexes

Vaccinated Mice

10^7 H2/uty coated B cells injected IV

- < 0.1%
- 2.0%
- 1.2%
- 1.9%
- 0.25%
- 8.8%

Negative controls

- < 0.1%
- < 0.1%
- < 0.1%

Positive controls

Injected with spleen cells

- 2.1%
- 8.8%
The expansion of peptide specific CTL responses by antibody targeted MHC class I peptide complexes

Summary

• Effective specific CTL expansion can be obtained in vitro with B cell bound MHC/peptide complexes
• Preliminary data suggests that B cell bound HLA complexes have similar T cell expanding power as conventional dendritic cells
• To date CTL responses to Influenza virus, CMV, EBV, Melan A, WT-1, HIV, KS and H2/Uty have been demonstrated
• In Vivo results with B cell bound MHC complexes shows that the system can produce significant and long lasting CTL expansion
• B cells bound MHC class I complexes are relatively simple to use, are amenable to clinical application and should be cheap to manufacture
• The system offers the potential for antigen presentation and CTL expansion on a large scale in vivo
• Clinical studies in HIV, CMV and melanoma should be performed
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