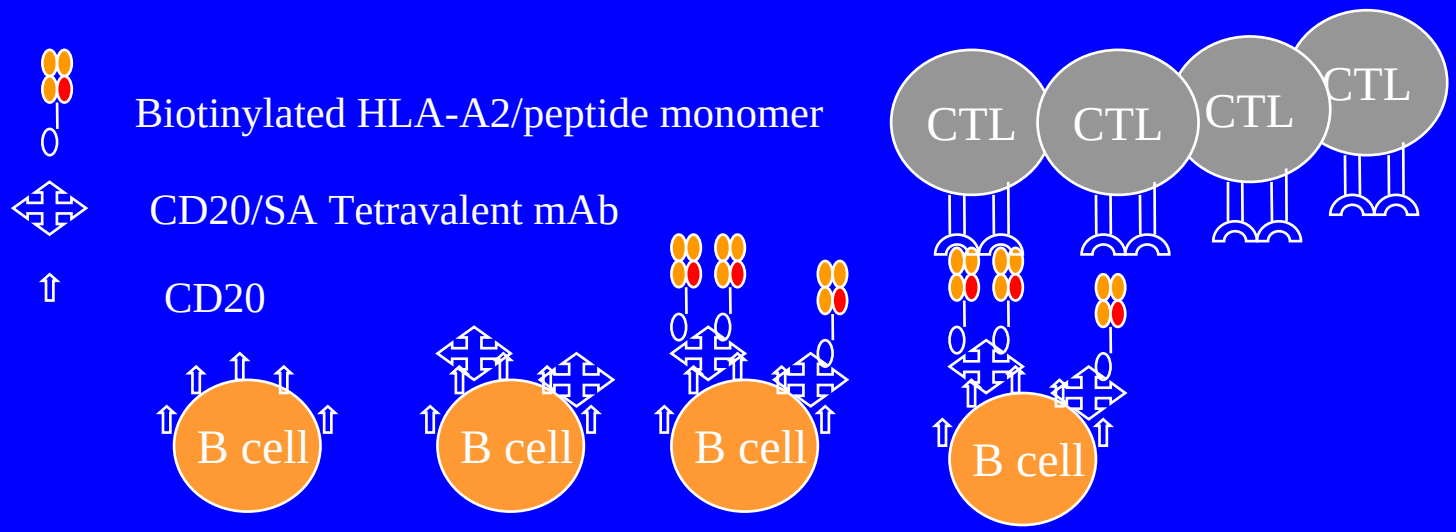
Immunotherapy with Antibody Targeted MHC class I/peptide complexes: Results of In Vivo Tumour Cell Killing and Therapeutic Vaccination

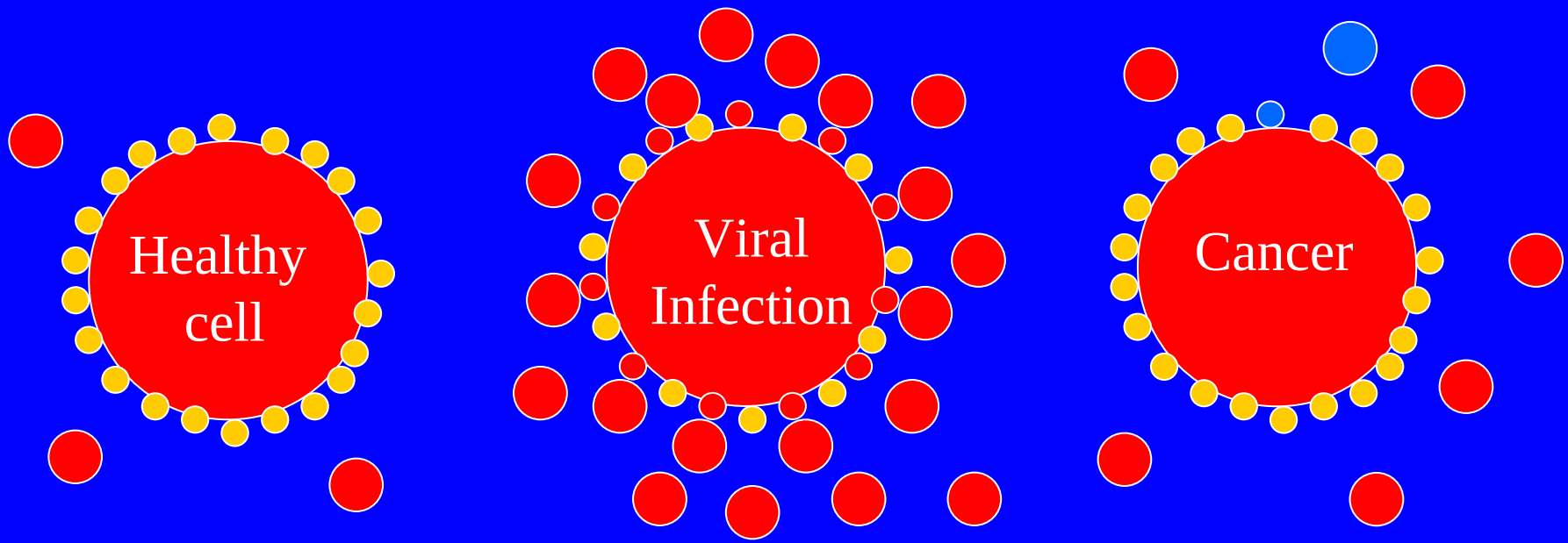
Dr Philip Savage
PhD FRCP
Charing Cross Hospital
London



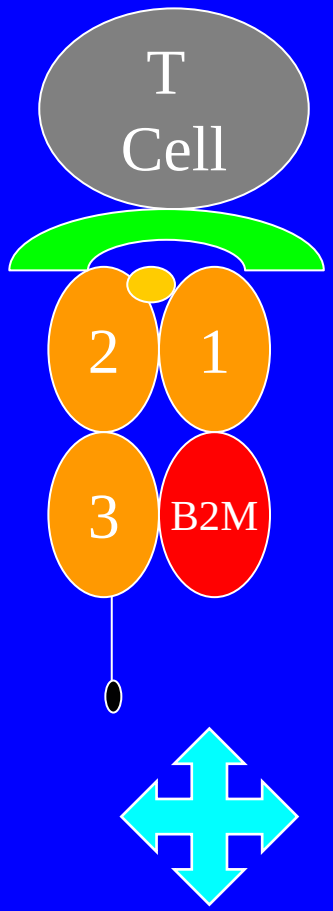
T cell targets and responses in health, viral infections and cancer

- 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]



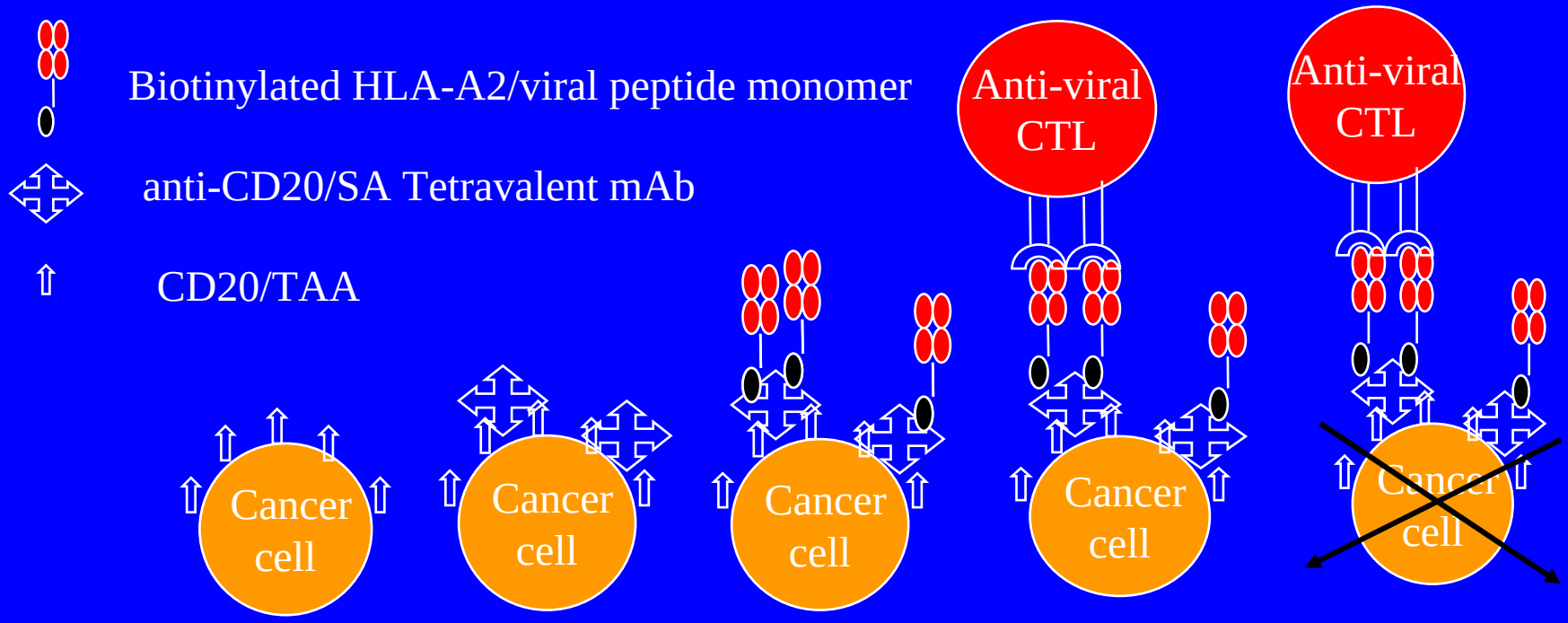
HLA class I molecules and CD20/B9E9



- 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

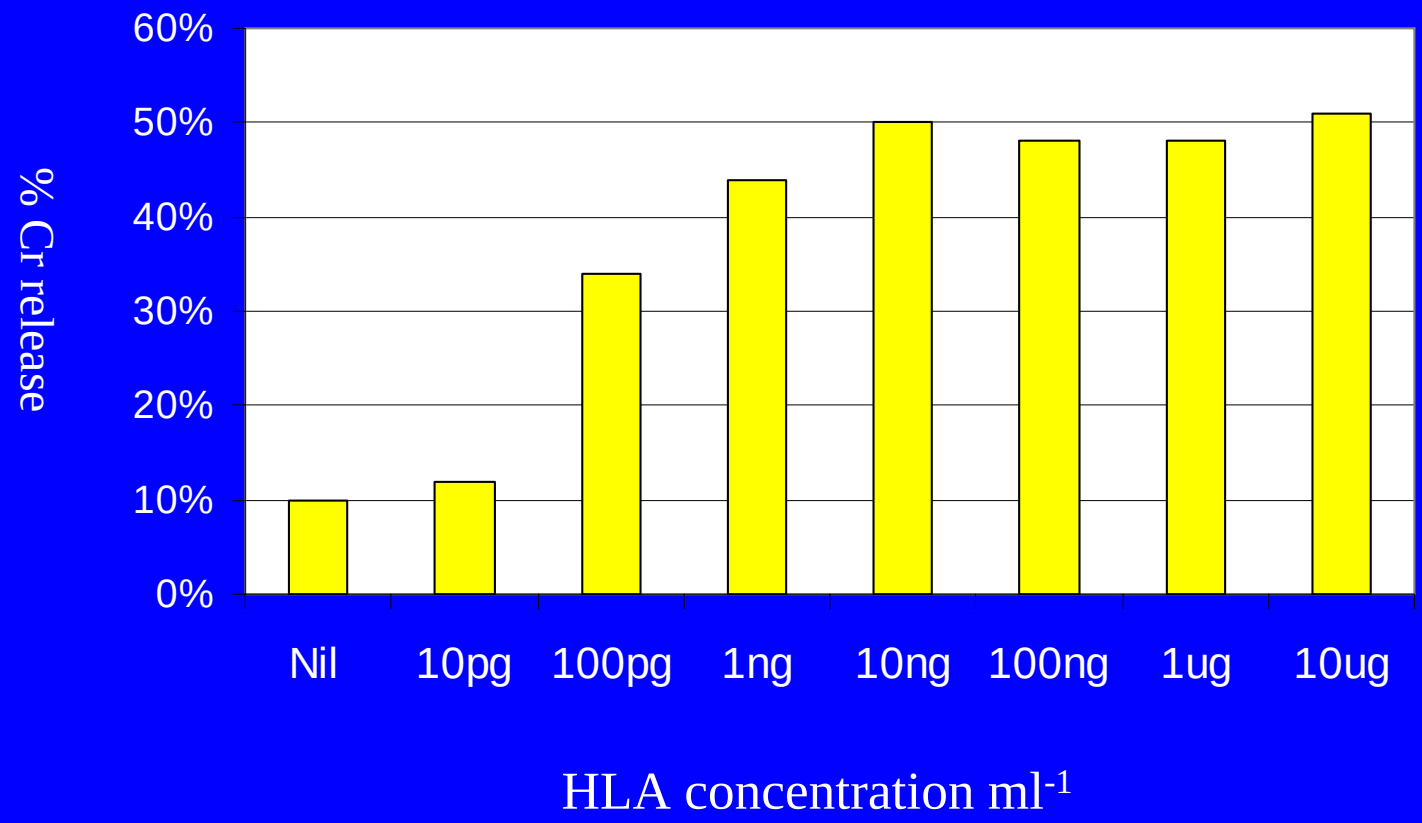
- **CD20 and B9E9 sfvSA**
- CD20 ~ 60,000 copies on each B cell
- B9E9 sfvSA tetravalent single chain antibody/streptavidin fusion protein
- High avidity and minimal antibody internalisation
- Already used in RIT of NHL with radio-biotin

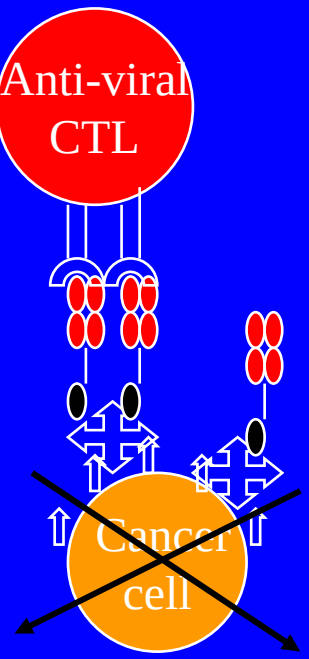
HLA System 1
The use of anti-viral T cells to kill cancer cells using 2-Step Targeting of HLA class I complexes



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 $1x10^7$ IP of an anti-BMLF1 (EBV antigen) CTL line.
  - Day 1 $1x10^6$ 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

Group A Anti-BMLF1 CTL Targeted Daudi	Group B No CTL Targeted Daudi	Group C Anti-BMLF1 CTL Native Daudi	Group D No CTL Native Daudi
Tumour 1.05g	Tumour 2.58g	Tumour 3.95g	Tumour 2.94g
No Tumour	Tumour 1.75g	Tumour 6.3g	Tumour 4.99g
No Tumour	Tumour 2.01g	Tumour 3.68g	Tumour 3.64g
No Tumour	Tumour 3.01g	Tumour 2.36g	Tumour 2.61g

Savage et al 2002 IJC

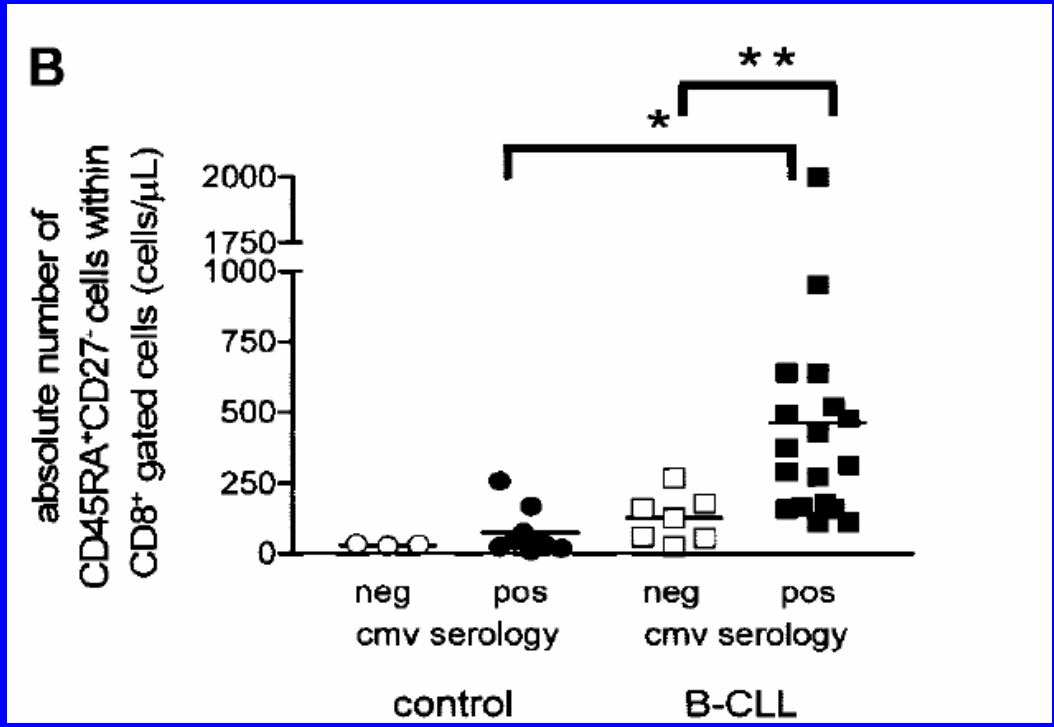
Tumour targeting with HLA class I complexes
Optimal Disease Characteristics

- 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

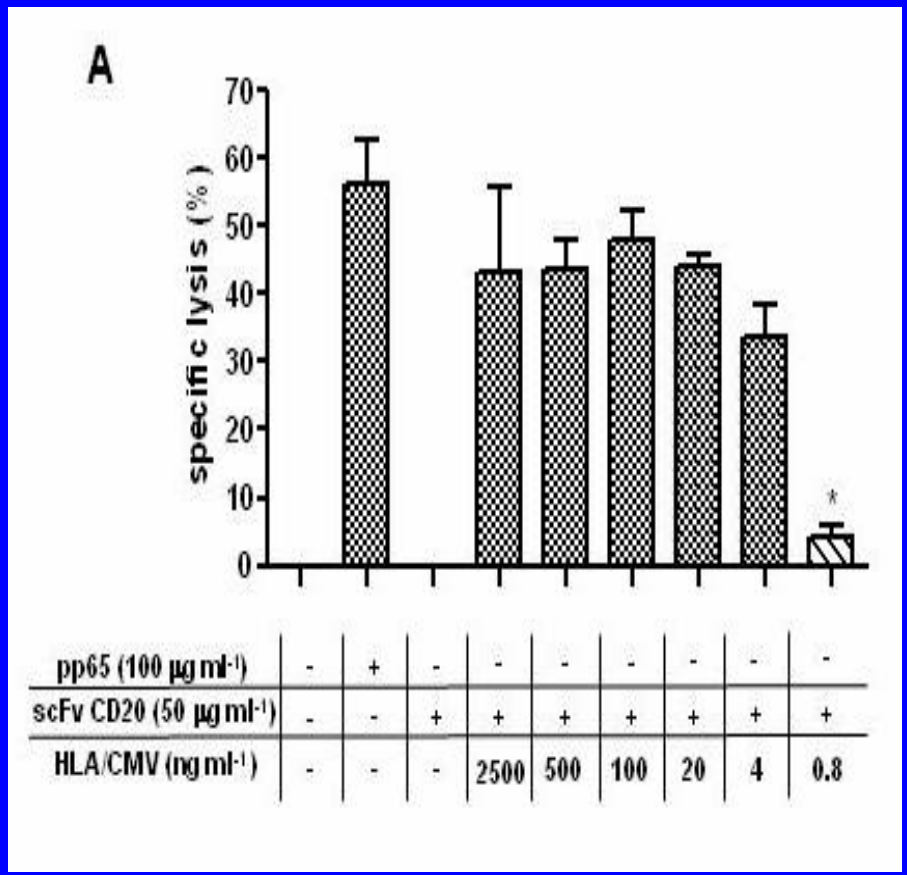
- CLL is a chronic malignancy of B cells
- Tumour cells are found in the blood and Lymph Nodes
- B Cells are very sensitive to T cell mediated lysis
- In CLL greatly elevated levels of CMV specific T cells are frequently found!
- These CMV specific T cells are effector phenotype +ve
  - High levels of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

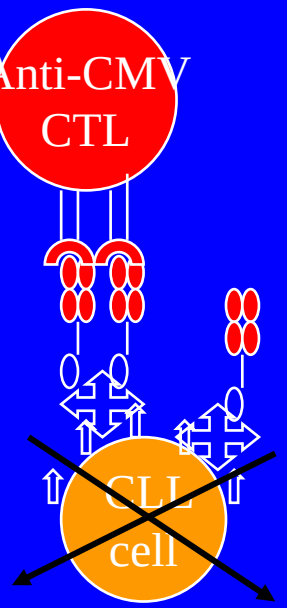
CMV specific CTLs in health and CLL



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

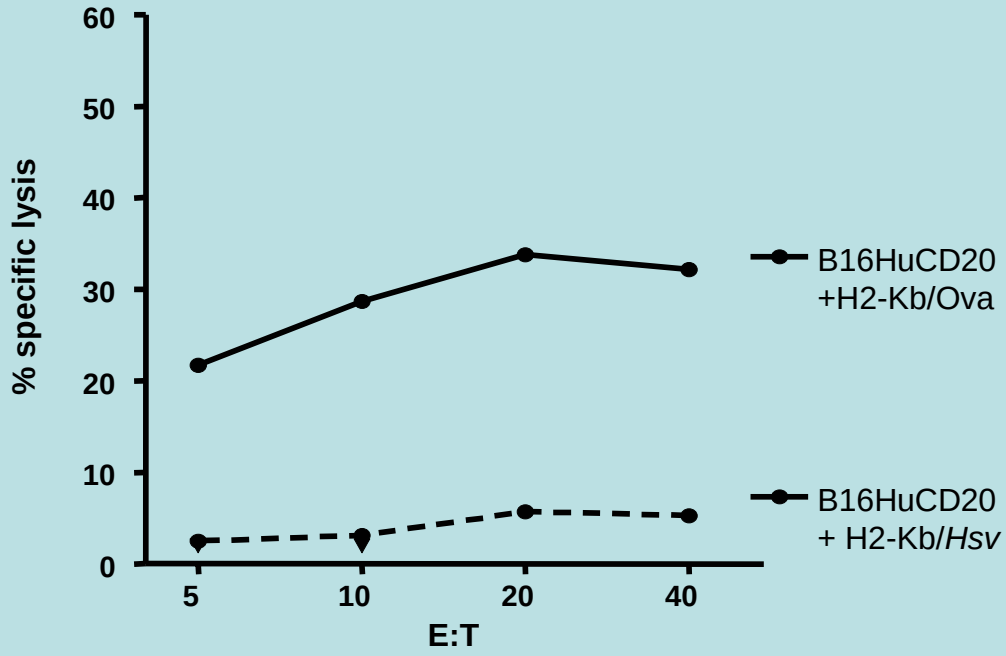
E:T 4:1 4hr assay

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

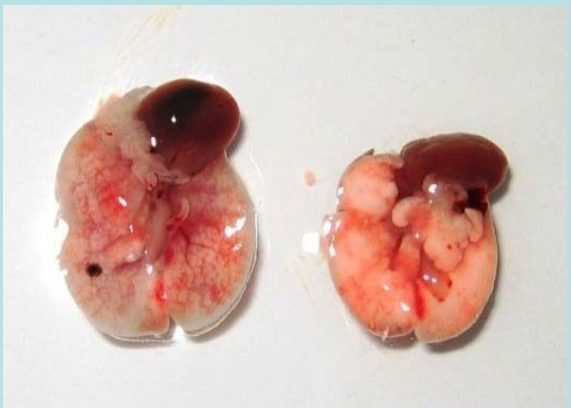
In Vitro killing of B16Hu20 melanoma cells using antibody targeted MHC complexes using Ova immunized mouse splenocytes
4hr Cr release assay



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.

OT1 recipient mouse injected with 1x10^5 B16-huCD20 cells IV, coated with anti-CD20 – H2-Kb-Hsv



OT1 recipient mouse injected with 1x10^5 B16-huCD20 cells IV, coated with anti-CD20 – H2-Kb-Ova

Tumours counted after 28 days

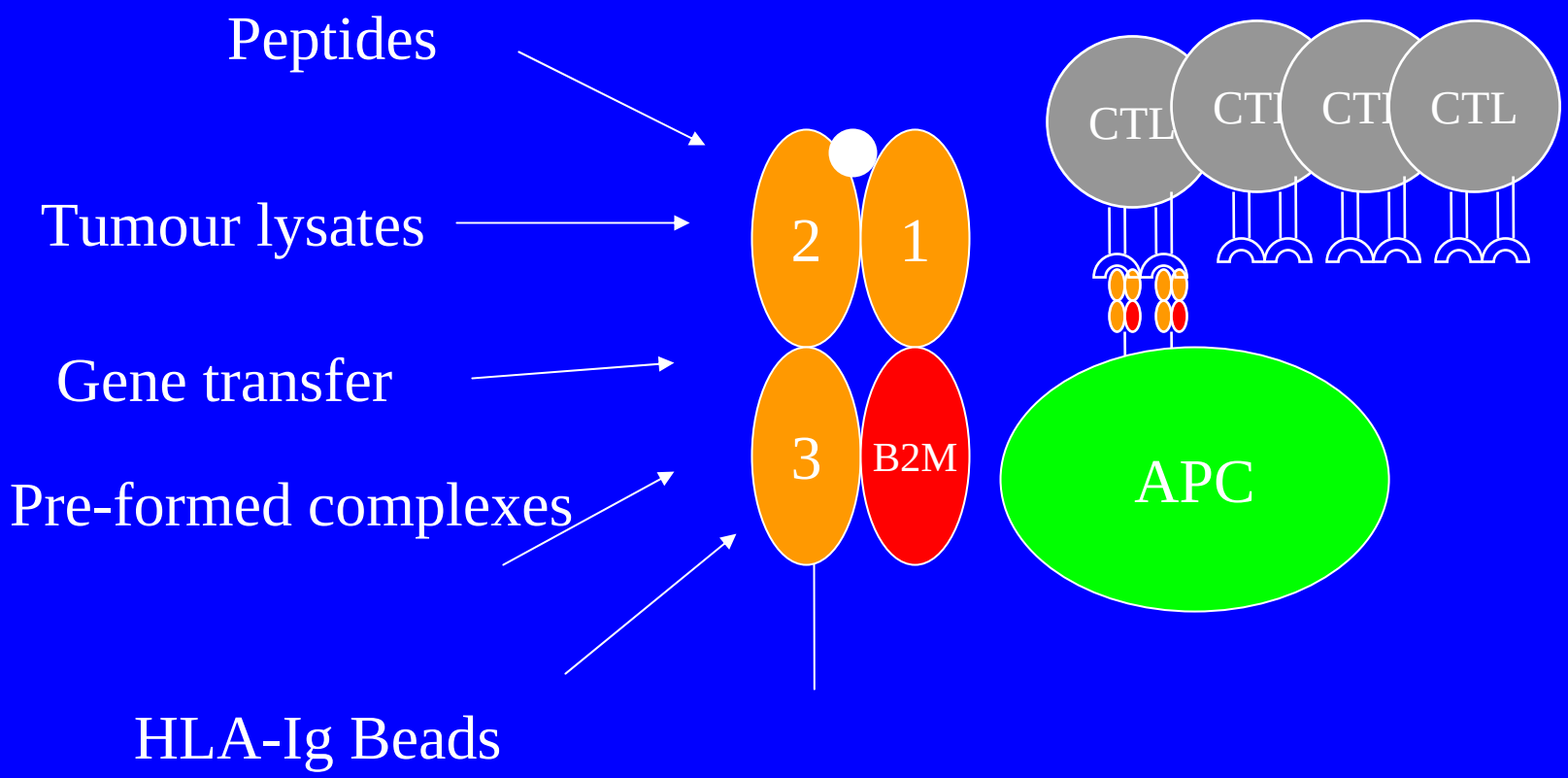
Mouse	Number of metastases visible
H2-Kb-Hsv	173
H2-Kb-Hsv	87
H2-Kb-Ova	1
H2-Kb-Ova	1

Tumour targeting with MHC class I complexes
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

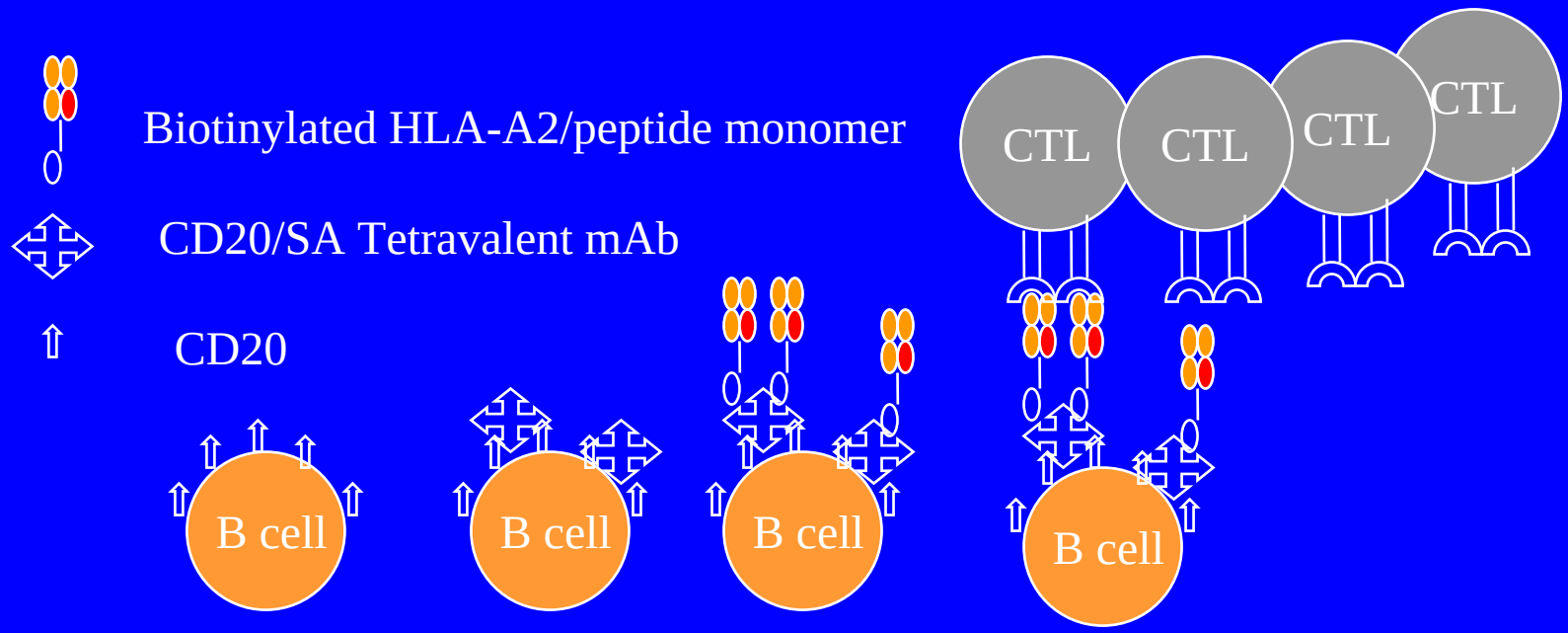
HLA class I/peptide complexes on Antigen Presenting Cell.



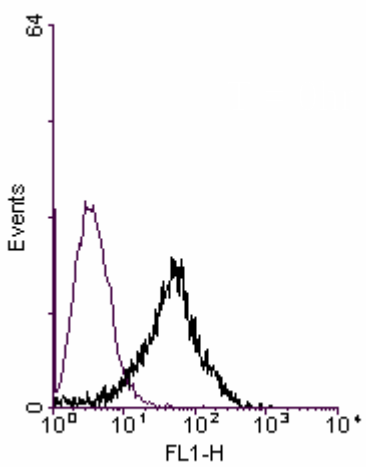
Differing approaches to the expansion of CTLs

HLA System 2

Expansion of peptide specific CTL responses by antibody targeted HLA class I peptide complexes



Expression of B9E9 sfvScSA targeted HLA-A2/M1 complexes on HLA class I –ve B cells. Detected with FITC-W6/32



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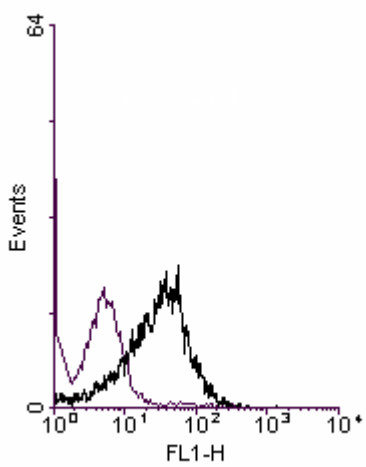

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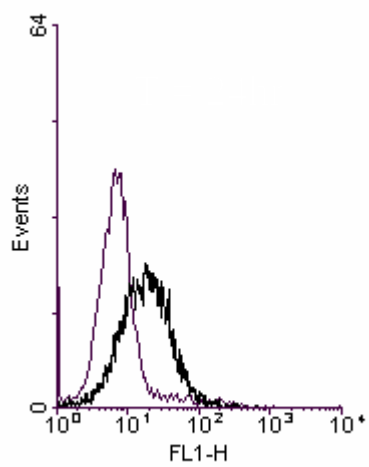

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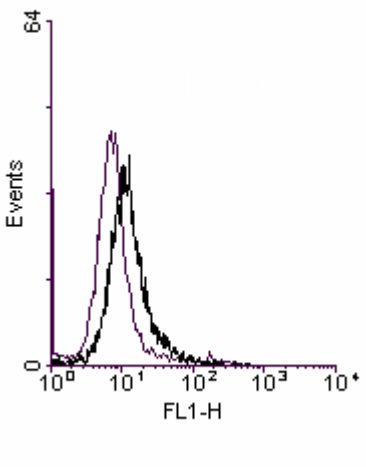

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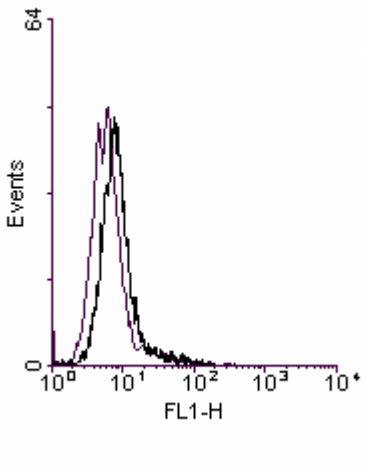
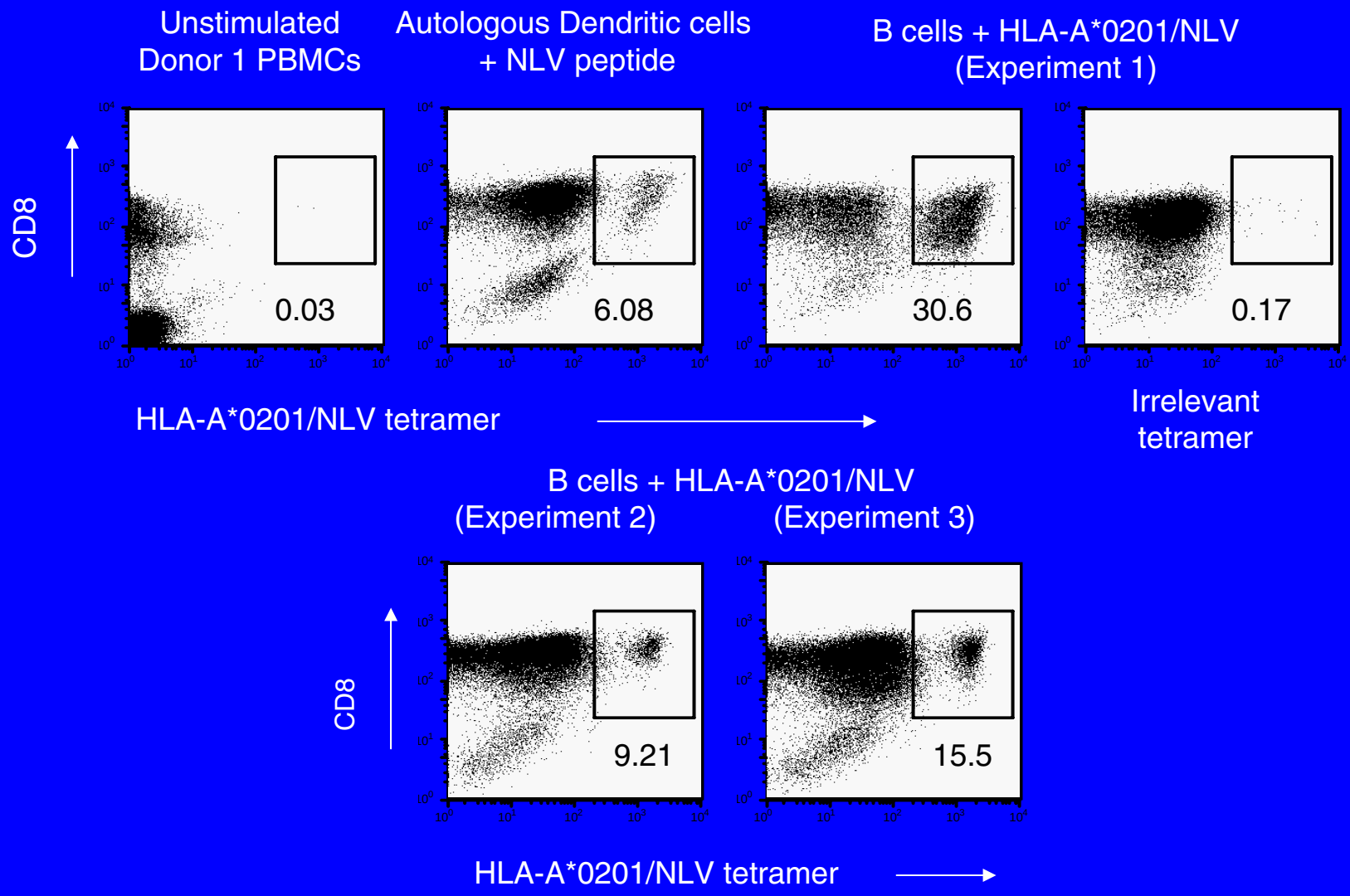
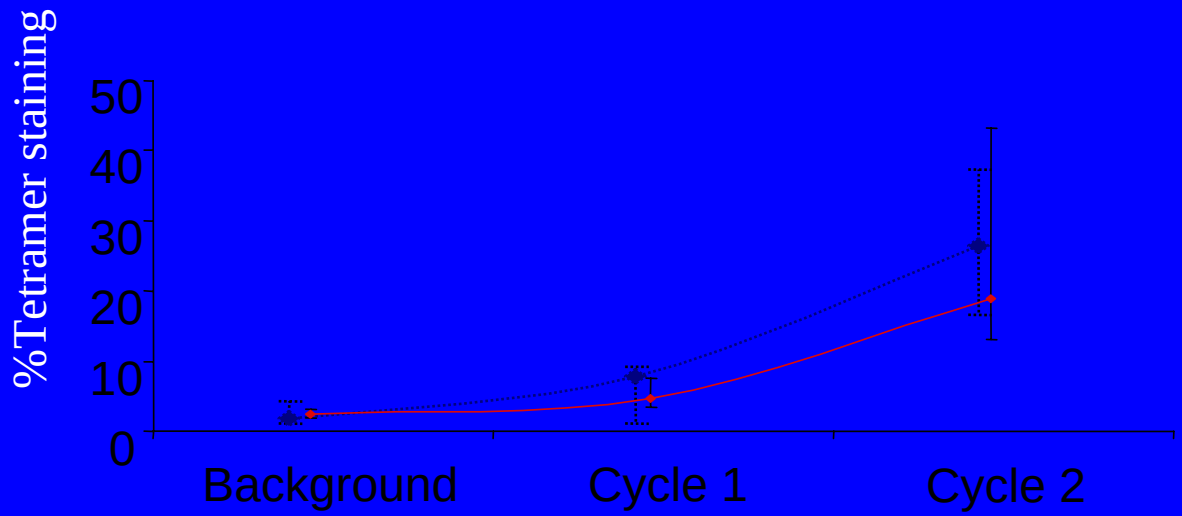


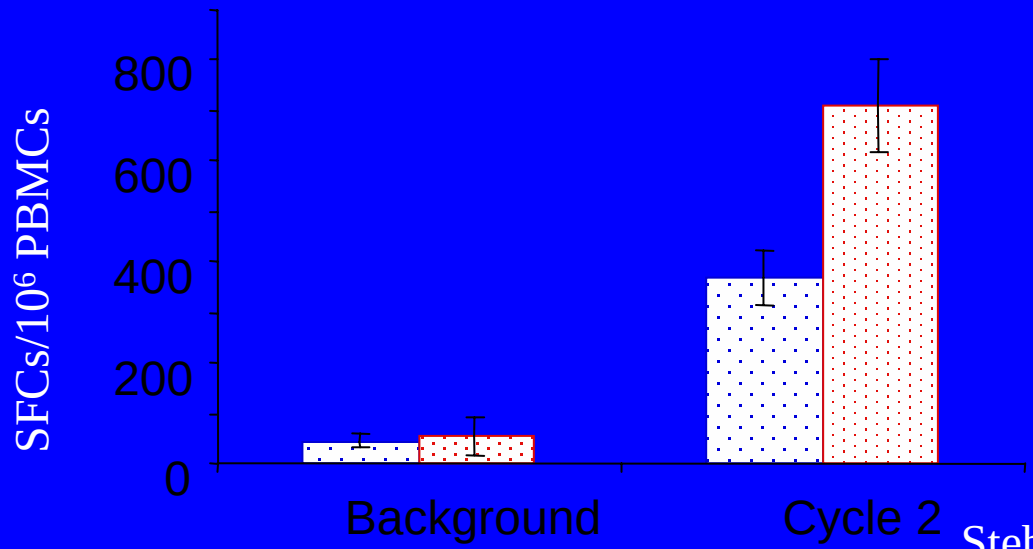
72hr

In Vitro CMV specific T Cell Expansion using B cells targeted with HLA class I/peptide complexes



Tetramer and Elispot enumeration of HLA-A2/HIV specific CTLs expanded in vitro using the antibody-MHC system



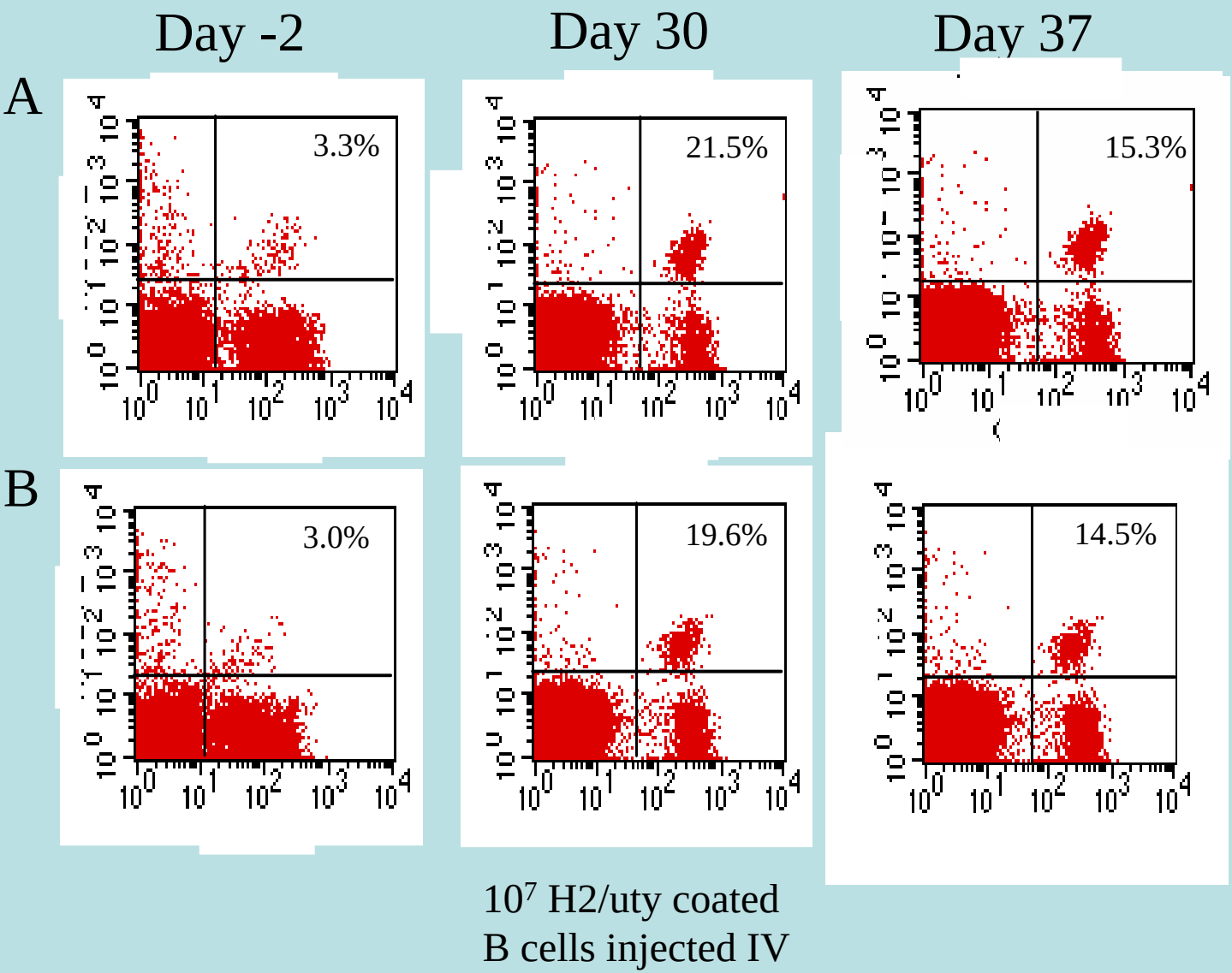


Stebbing et al 2004 Blood

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



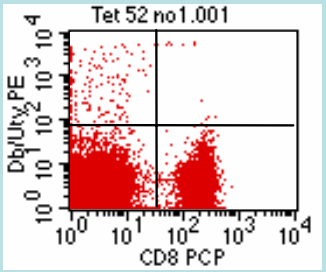
10^7 H2/uty coated
B cells injected IV

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

Negative controls

Positive controls
Injected with spleen cells



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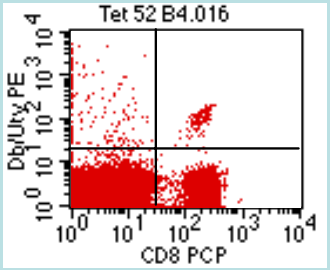

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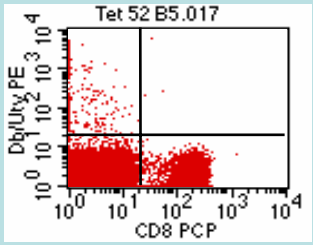

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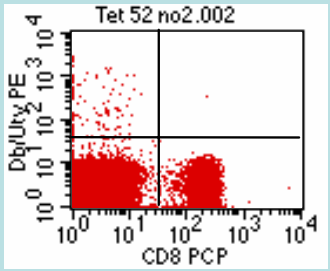

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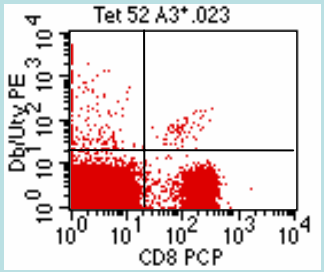

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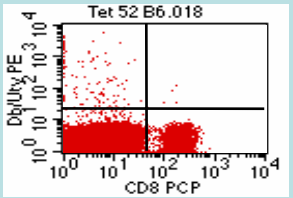

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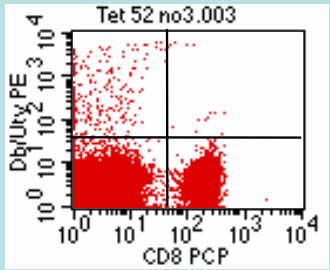

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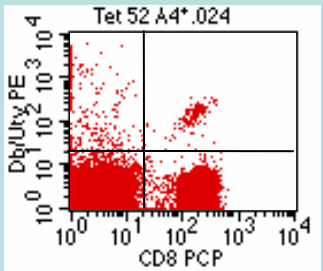

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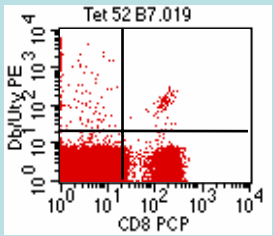

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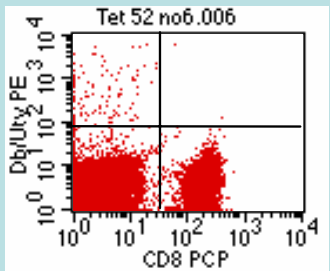

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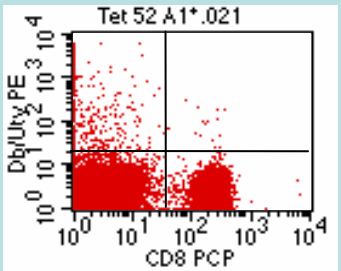

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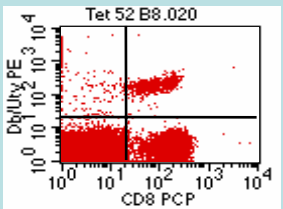

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The expansion of peptide specific CTL responses by antibody targeted MHC class I peptide complexes Summary

- 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

Acknowledgements

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