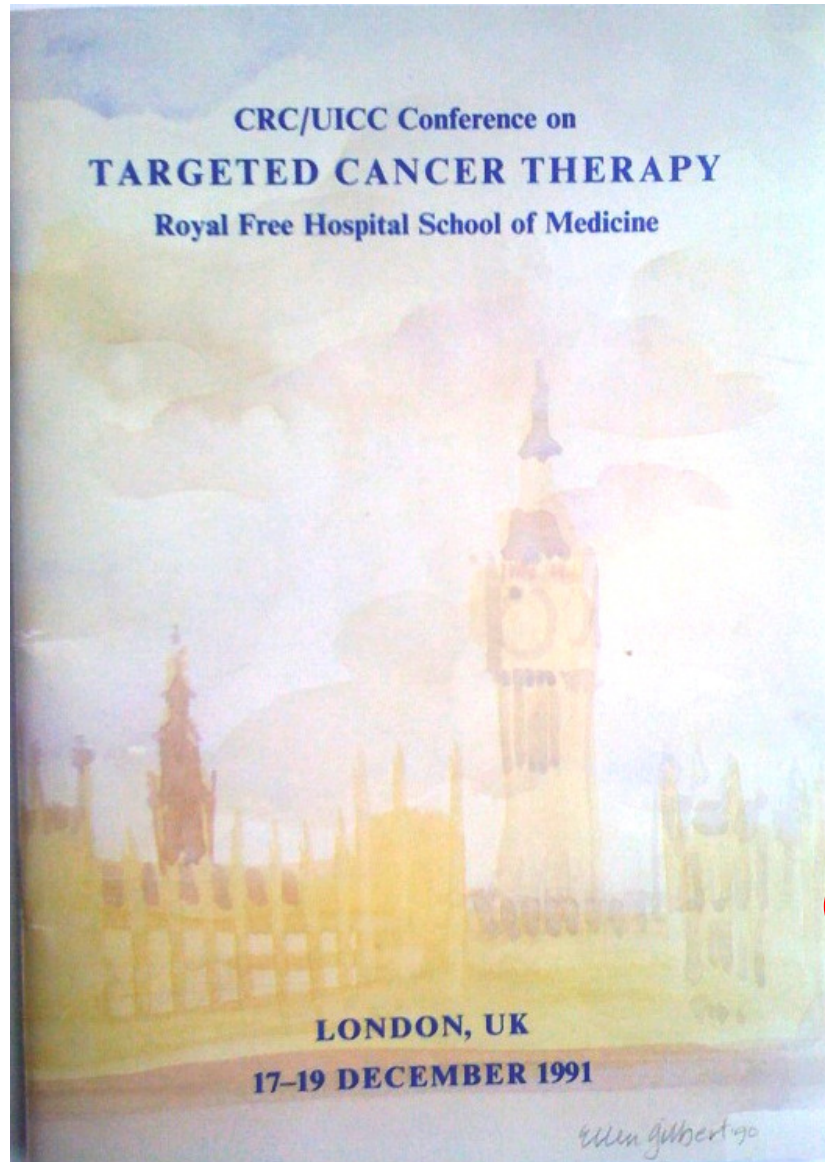


# Phage antibodies for the clinic

Richard Begent and Kerry Chester

UCL Cancer Institute

# That Eureka Moment



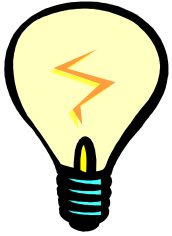
9.10 am Introduction - Professor, R H J Begent

*Session 1. Antibody Engineering*  
Chair: Professor T A Connors

9.15 am D S Secher (Cambridge)  
"Antibody engineering for targeted cancer therapy"

9.40 am J R Adair (Slough)  
"Antibody humanisation for therapy"

10.05 am G Winter (Cambridge)  
"Making antibodies using phage display technology"

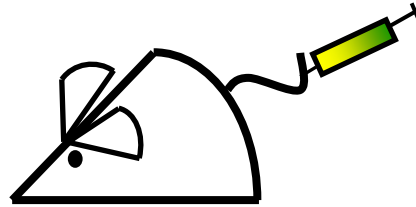


## Phage technology would give unprecedented control of antibody for imaging and therapy

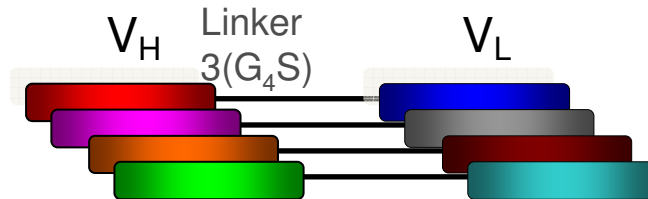
- Access to great diversity
  - optimised for a known target by immunisation
- Selection for optimal performance
  - affinity
  - stability
  - yield
- Access to ScFvs
  - building blocks for imaging & therapeutic agents

# Making an scFv to target CEA

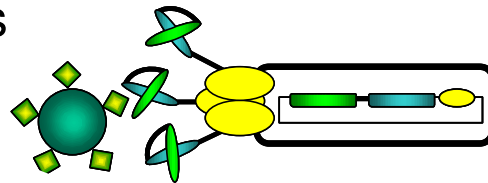
Antibody V regions  
CEA-immunised mouse



PCR & link to  
scFv format

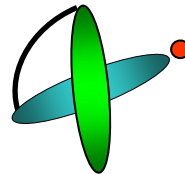


Clone into phage  
library of 10<sup>7</sup> scFvs

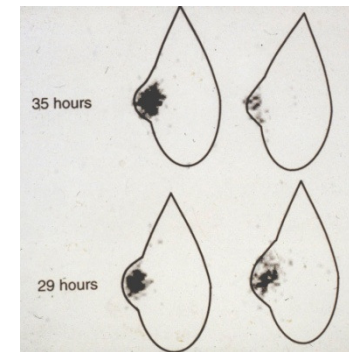


Select the best  
CEA binder (MFE-23)

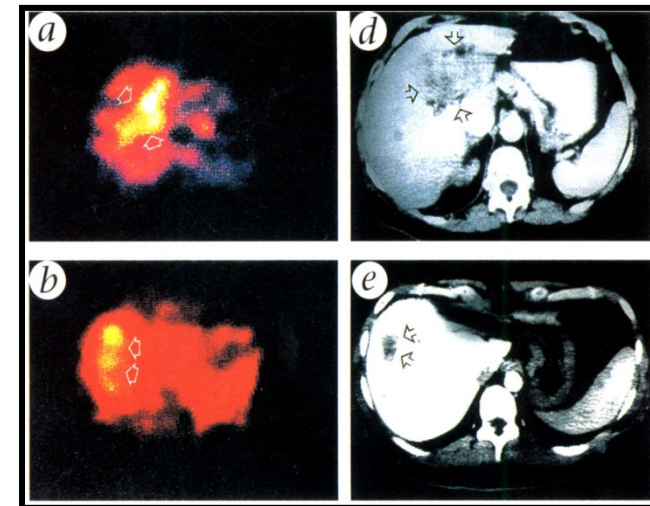
Express MFE-23 as  
soluble scFv purify by  
IMAC via his tag



Localizes to CEA-  
expressing tumours



Mice: *Chester K: Lancet 1994*

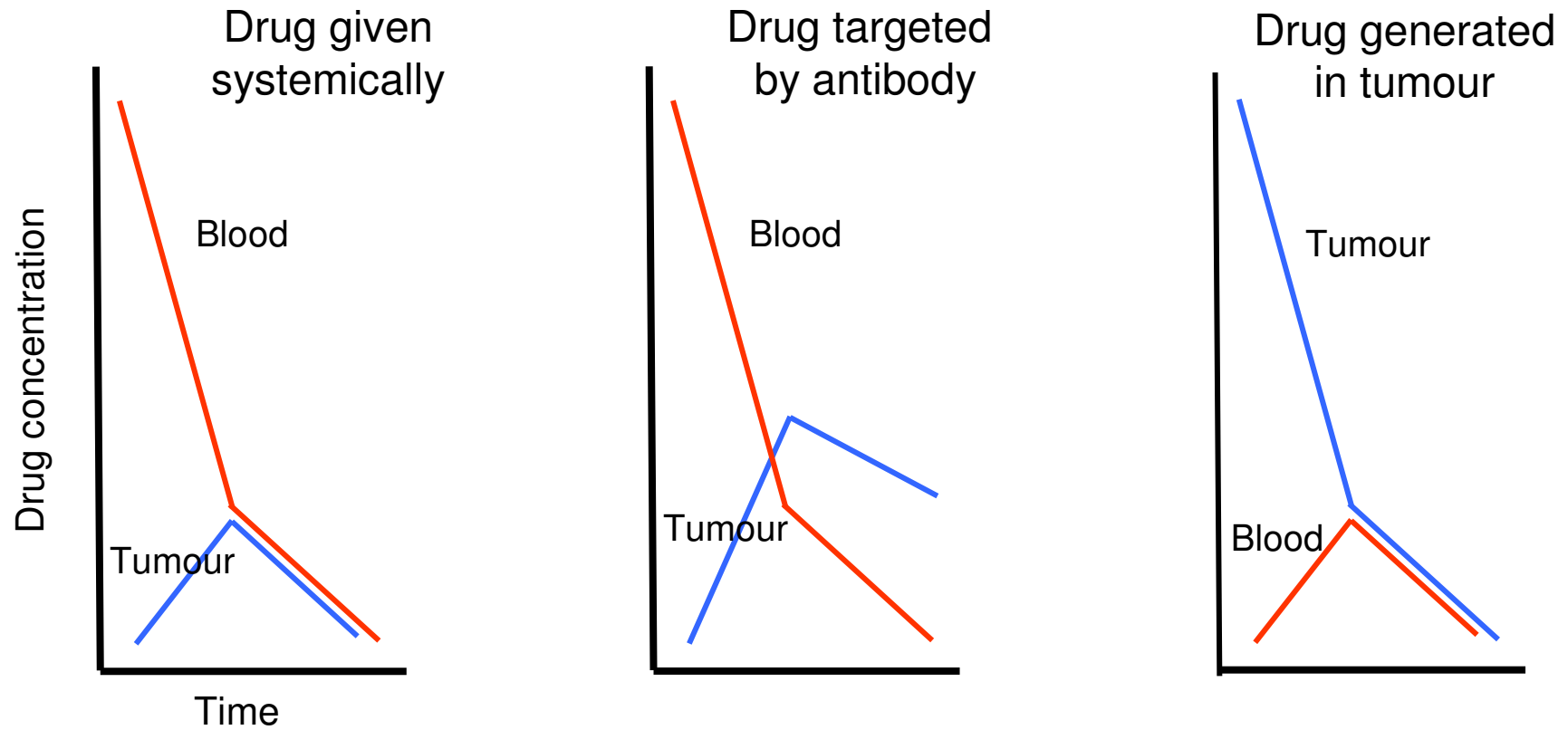


Man: Begent et al: *Nature Medicine 1996*

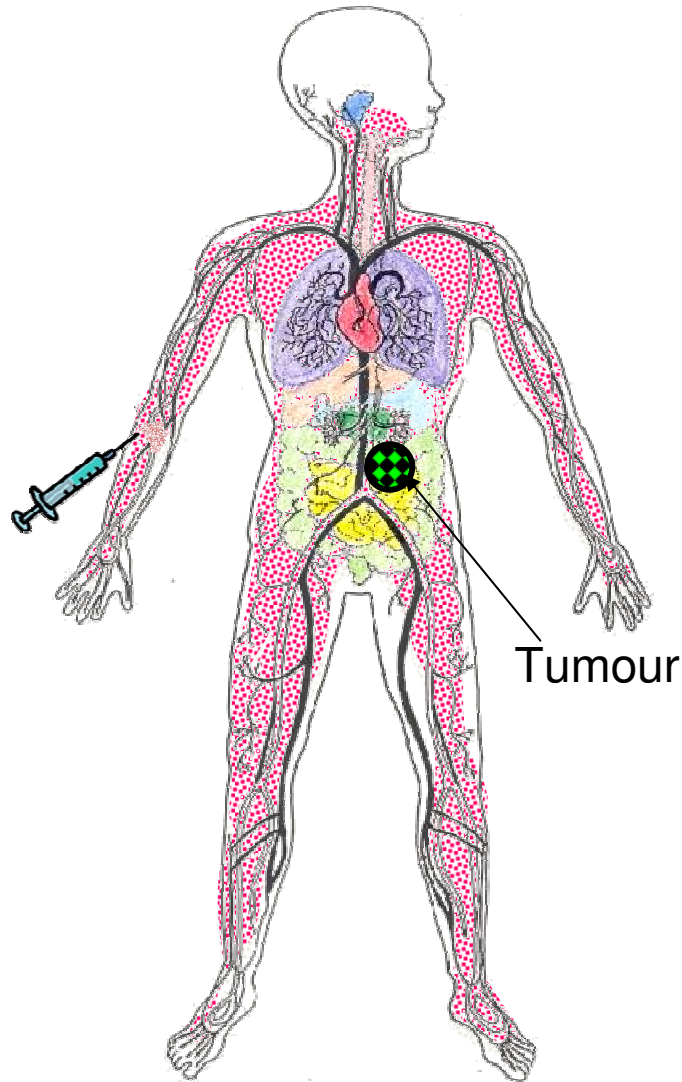
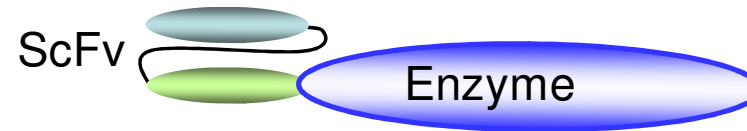
# Conclusion 1

- MFE-23 scFv phage-derived anti-CEA antibody was a valuable and practical tool for clinical cancer imaging
- Appeared suitable for use in fusion proteins for delivery of therapeutics to cancer

# Role of scFv in generating a drug in tumour for cancer therapy



# ADEPT



## 1. Antibody-Enzyme IV

- Localizes in tumor
- Clears from healthy tissue

## 2. Prodrug given IV

- Activated in tumor by targeted enzyme

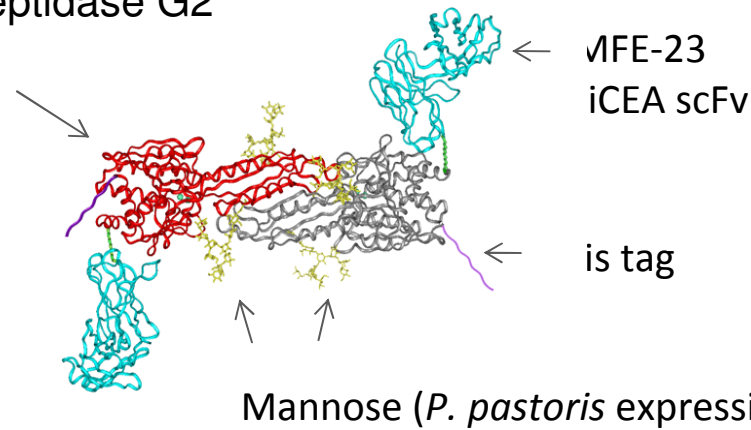
## 3. Tumour kill

- Selective
- Potent: amplification by enzyme
- Bystander effect

# Anti-CEA scFv fused to carboxypeptidase G2

MFECP multifunctional fusion protein

Carboxypeptidase G2

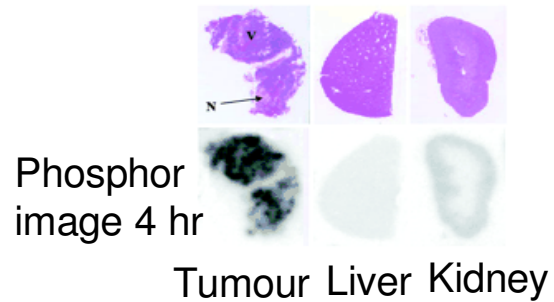


MFECP manufacture (*P. pastoris*)  
in UCL GMP facility

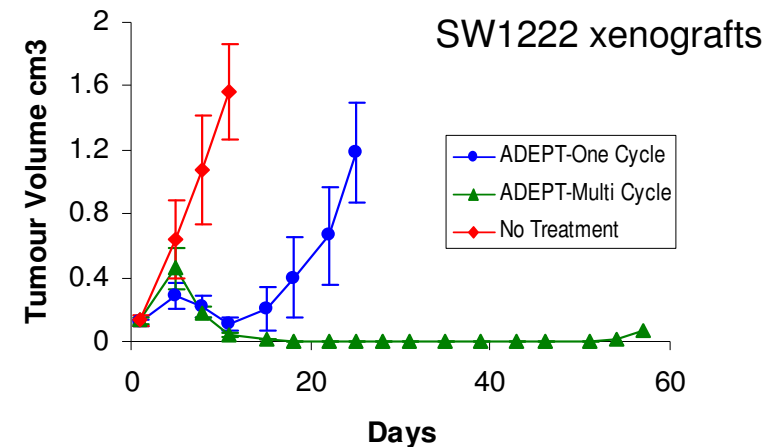
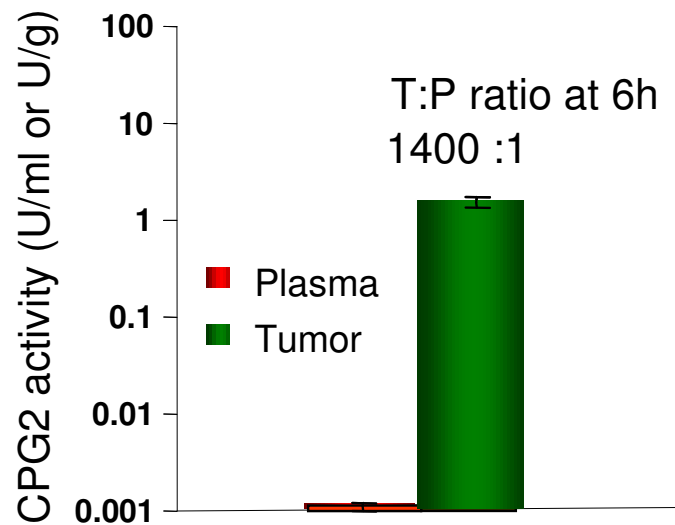
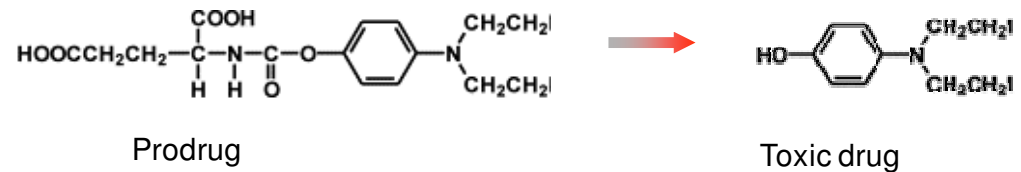


# Components and function

## Biodistribution

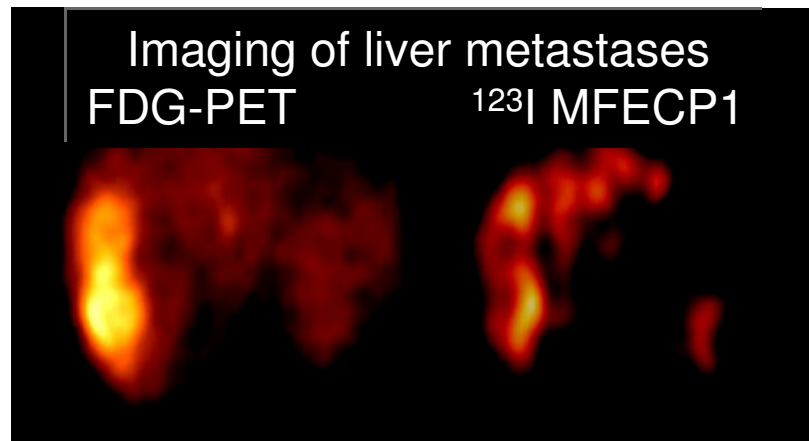
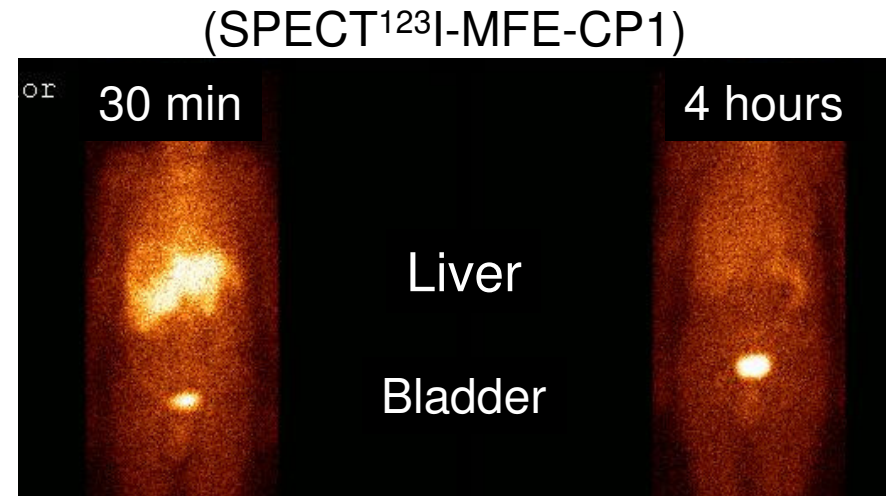
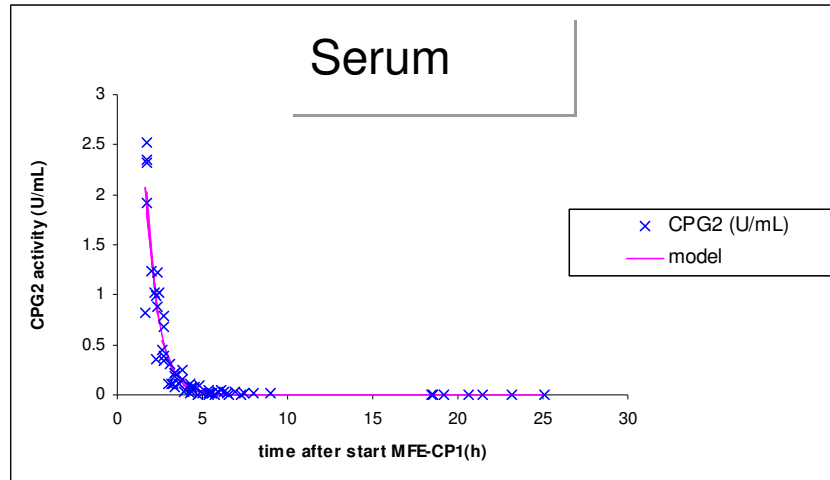


## Therapy



Sharma et al Clin Cancer Res 2005;  
Kogelberg et al Glycobiology 2007

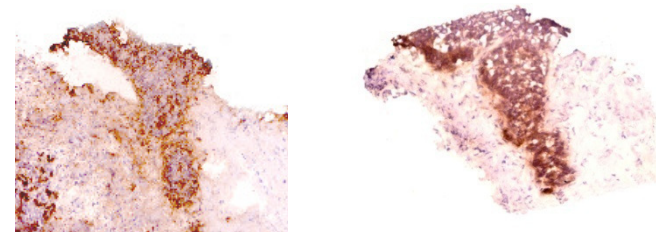
# Enzyme clearance and tumour localization



Median enzyme in tumor:critical normal tissue by SPECT

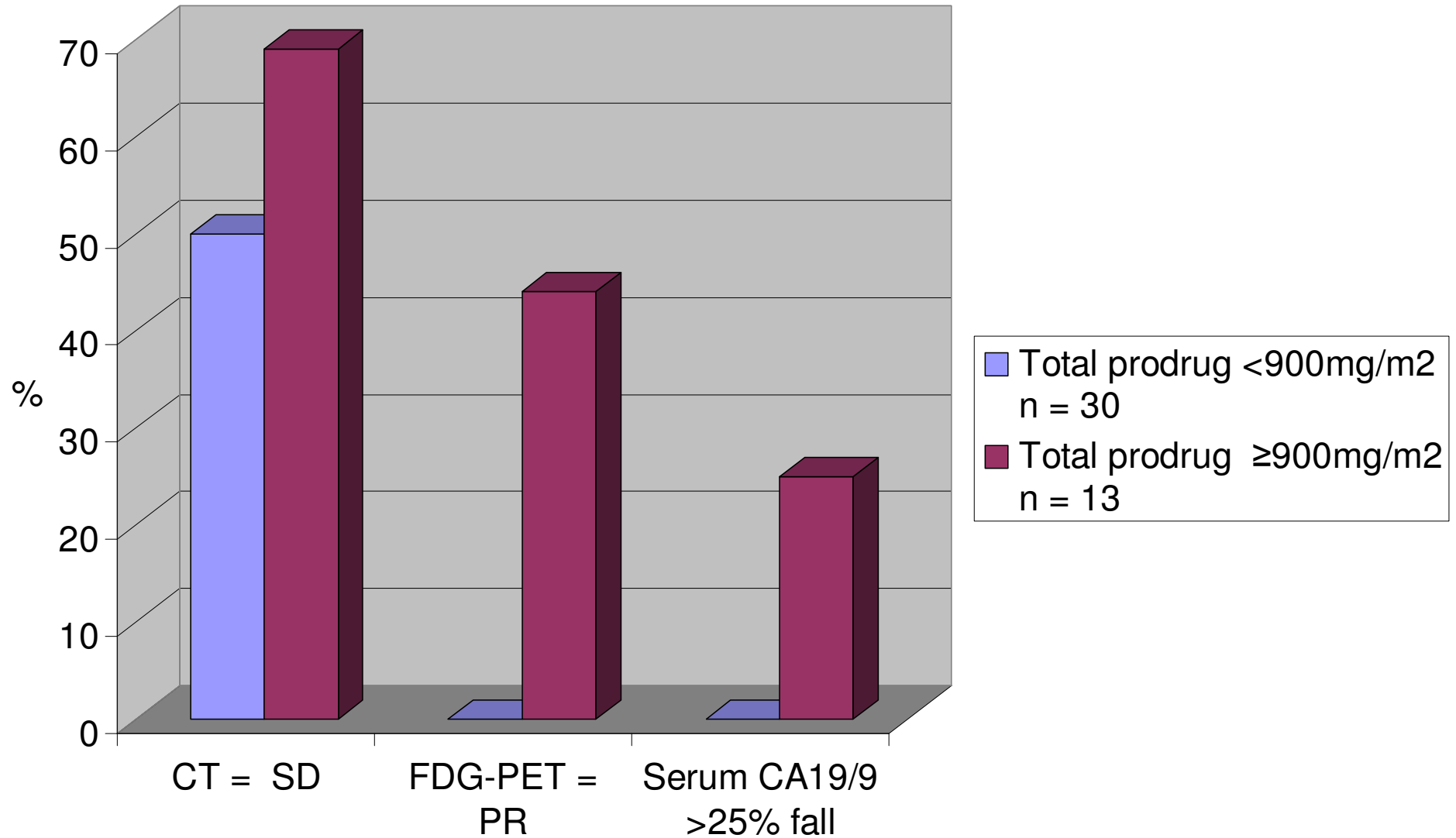
Tumor:blood at 4 hrs	> 40:1 (200u/l)
“ at 22 hrs	> 55:1

Liver biopsy  
Target (CEA)    Enzyme



Mayer A et al Clin Cancer Res 2006

# Efficacy versus total prodrug dose



Significant correlation between total prodrug dose and % change on PET.  
p=0.031, Spearman Rank correlation test.

## Conclusion 2:

### Selective tumour activation of prodrug achieved

- Fusion protein with 4 functions
  - Purified product (his tag)
  - Rapid blood clearance (mannosylation)
  - Localisation in tumour (scFv)
  - Biological function targeted (enzyme)
- Prodrug activated and delivered to target in tumour cell nuclei
- Anti-tumour effect in drug-resistant cancers

## The immunogenicity of the ADEPT enzyme needs to be addressed

	Number of treatments	No of patients positive for anti CPG2 antibody (HACA)
Chemical conjugate of antibody & enzyme <sup>1,2</sup>	1	36/37 (97%)
MFE-CPG2-his fusion protein <sup>3</sup>	1	11/30 (36%)
	2	3/4 (75%)
	3	7/7 (100%)

1, Napier M et al *Clin Cancer Res.* 2000

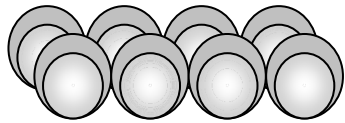
2, Francis R et al *Br J Cancer* 2002

3, Sharma S et al *In preparation*

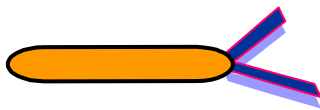
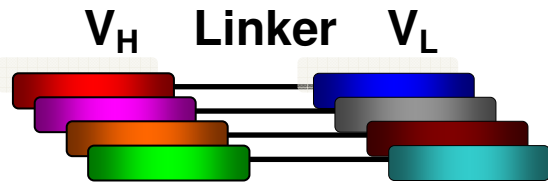
# Dissecting out the elements of the humoral immune response: make scFv 'tool-box'



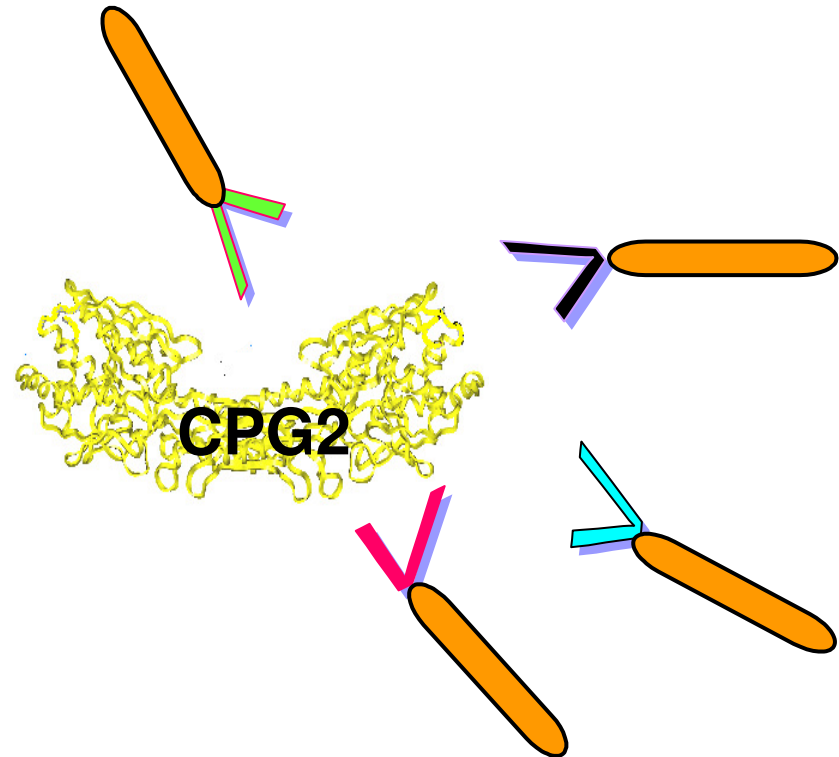
Immunise with CPG2



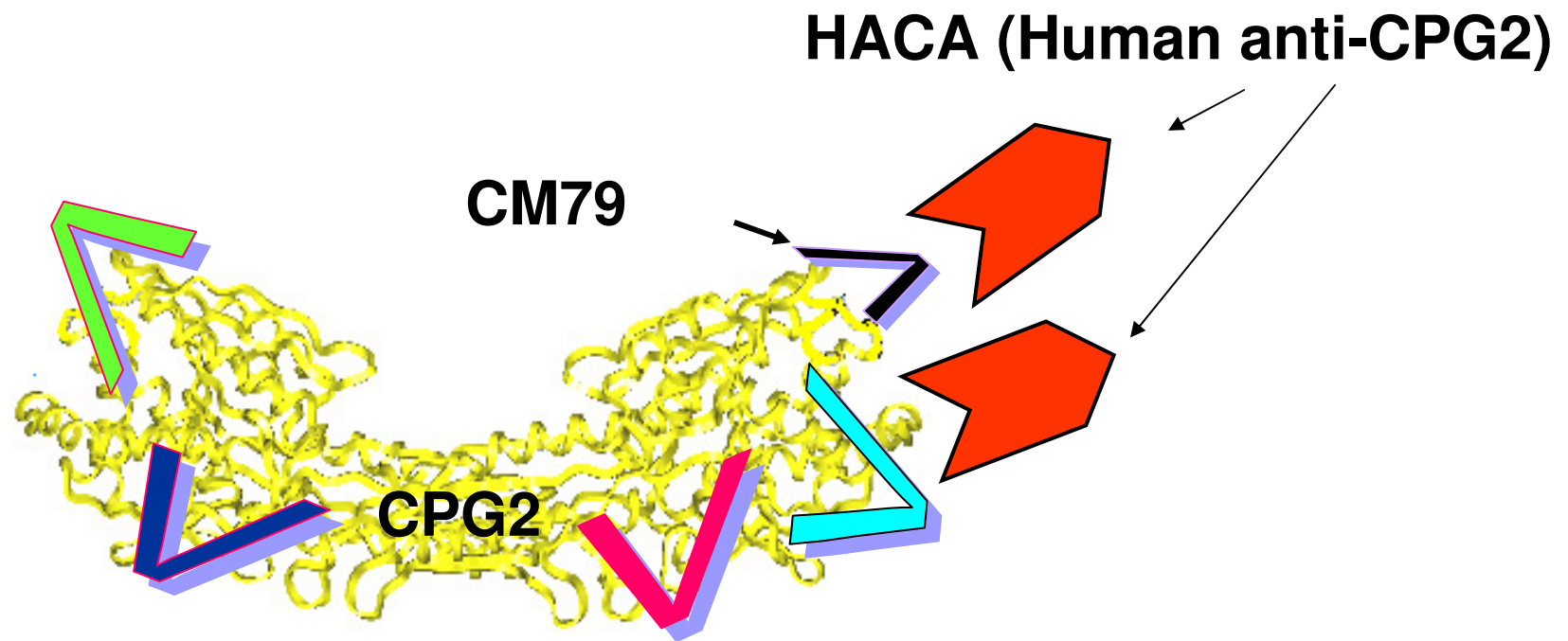
Lymphocytes



Phage scFv library



Select scFv which significantly blocks HACA in ELISA



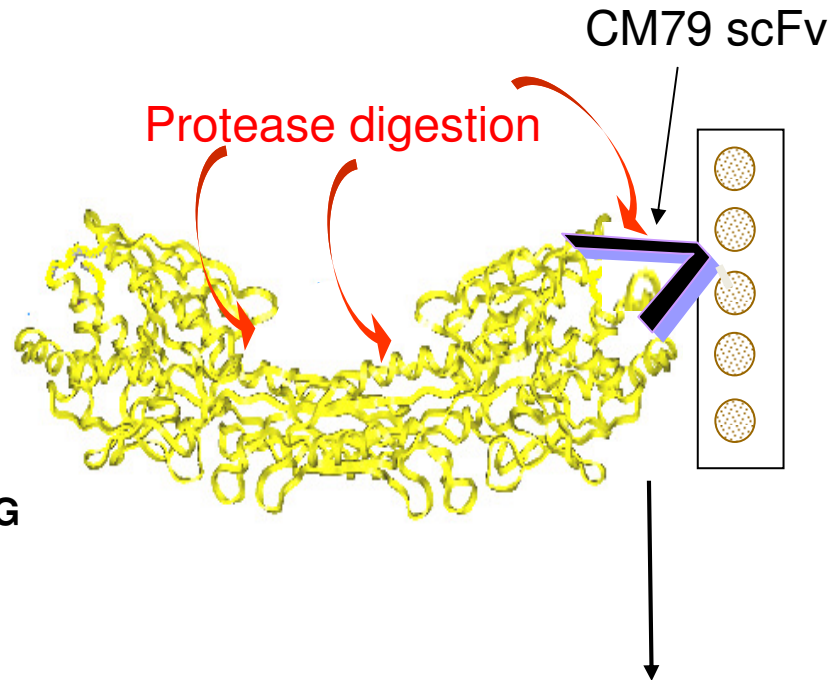
# Define scFv Epitope using SELDI-TOF Mass Spectrometry

Map to CPG2 linear

MRPSIHRTAIAAVLATAFVAGTALAQKRDN  
VLFQAATDEQPAVIKTLKLVNIETGTGDA  
EGIAAAGNFLEAELKNLGFTVTRSKSAGLV  
VGDNIVGKIKGRGGKNLLLMSHMDTVYLKG  
ILAKAPFRVEGDKAYGPGIADDKGGNAVIL  
HTLKLL**KEYGVRDY**GTITVLFNTDEEKGSF  
GSRDLIQEEAKLADYVLSFEPTSAGDEKLS  
LGTSGIAYVQVNITGKASHAGAAPELGVNA  
LVEASDLVLRMTNIDDKAKNLRFNWTIAKA  
GNVSNIIPASATLNADVRYARNEDFDAAMK  
TLEERAQQKKLPEADVKVIVTRGRPAFNAG  
EGGKKLVDKAVAYYKEAGGTLGVEERTGGG  
TDAAYAALSGKPVIESLGLPGFGYHSDKAE  
YVDISAIPRRLYMAARLIMDL**GAGK**



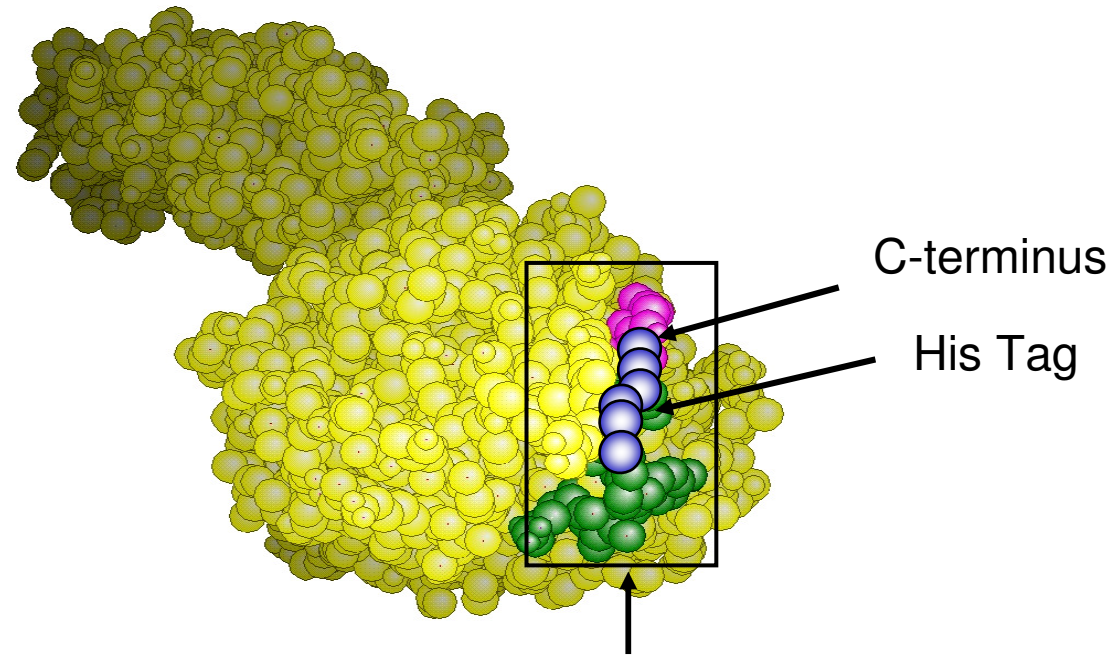
Identification of peptides by mass spectrometry



Laser desorption of scFv-binding peptides



# CM79 epitope shown in green and Magenta



Mutation in either region (R162A and G412A) disrupts conformational epitope:

- **Binding to CM79 99% ↓**
- **Binding of sera of patients immunised with A5CP ↓ (median 16% SD +/-11.2%; p=0.0002)**

## Conclusion 3

Phage technology can be used to dissect the elements of a clinically relevant polyclonal B cell response

# Acknowledgements



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Uzma Qureshi

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CR-UK Drug Development Office

Patients

Nurses

Pharmacy



CANCER RESEARCH UK

EPSRC

