Therapeutic Potential of a Humanized Antibody for the Treatment of Venezuelan Equine Encephalitis Virus Infection

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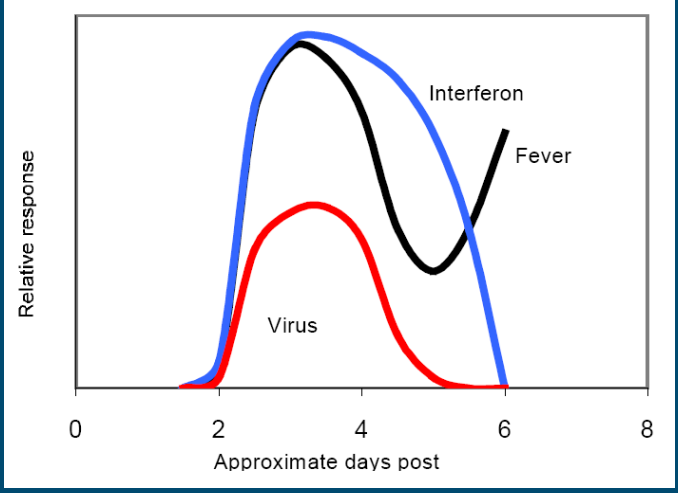
Venezuelan Equine Encephalitis Virus

- Positive strand RNA virus (~11.5Kb)
- Structural proteins translated as a polyprotein
  - 26S mRNA
- Responsible for large outbreaks
  - 1995; 100,000 human cases in Colombia and Venezuela, 300 fatal encephalitis cases
  - 1961-2004, Panama; 6% fatality rate in cases of serotype ID

Venezuelan Equine Encephalitis Virus

- VEEV causes an acute, febrile illness
  - Prostration usually 2-6 days post-infection
  - encephalitis in ~1-5% of cases



- *chills, high fever (38-40.5^{o}C), headache, malaise, photophobia, sore throat, myalgia, vomiting, conjunctival infection, muscle tenderness*

CNS involvement:

- *seizures, ataxia, paralysis, coma*
- *epilepsy, amnesia, mental retardation, hydroencephaly*

VEEV as a potential biological weapon

- Transmissible by the aerosol route
- Low infectious dose
- History of weaponisation
- No licensed vaccines or anti-virals

Antibodies to E2 are protective

Phillpotts RJ *et al*, 2002, Vaccine

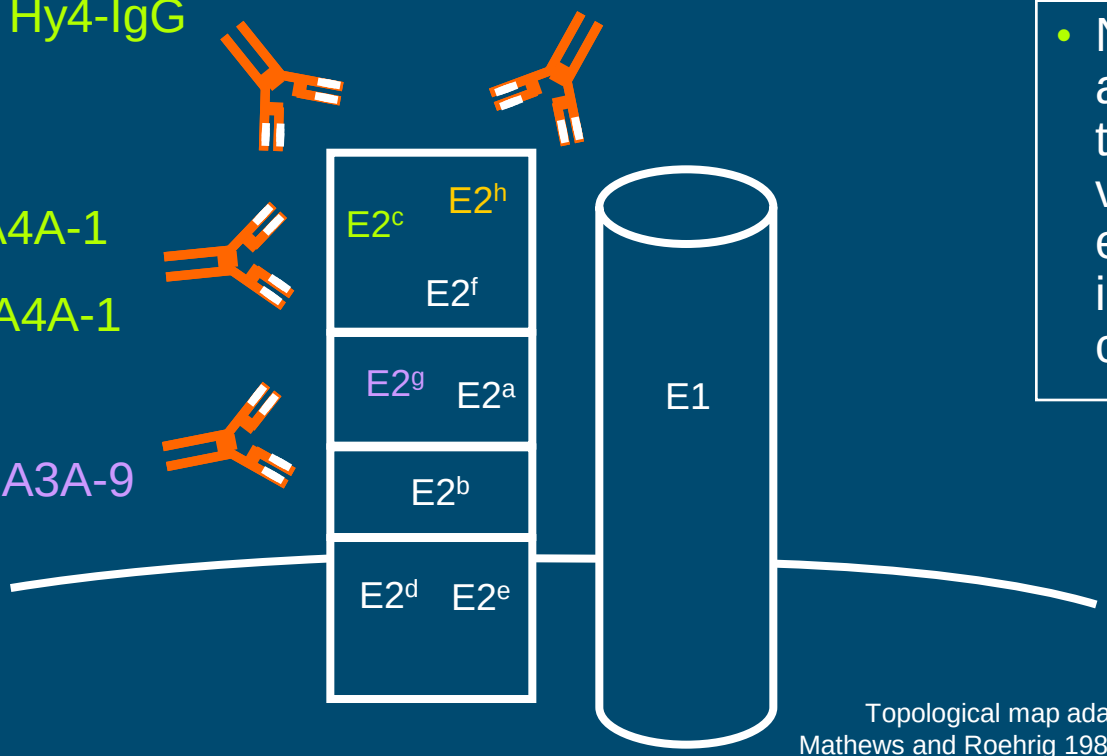
Phillpotts RJ 2006, Virus Res

Hunt AR *et al*, 2006, JGV

3B4C-4

Hy4-IgG

1A3B-7



Hu WG *et al*, 2007, Vaccine

1A4A-1

h1A4A-1

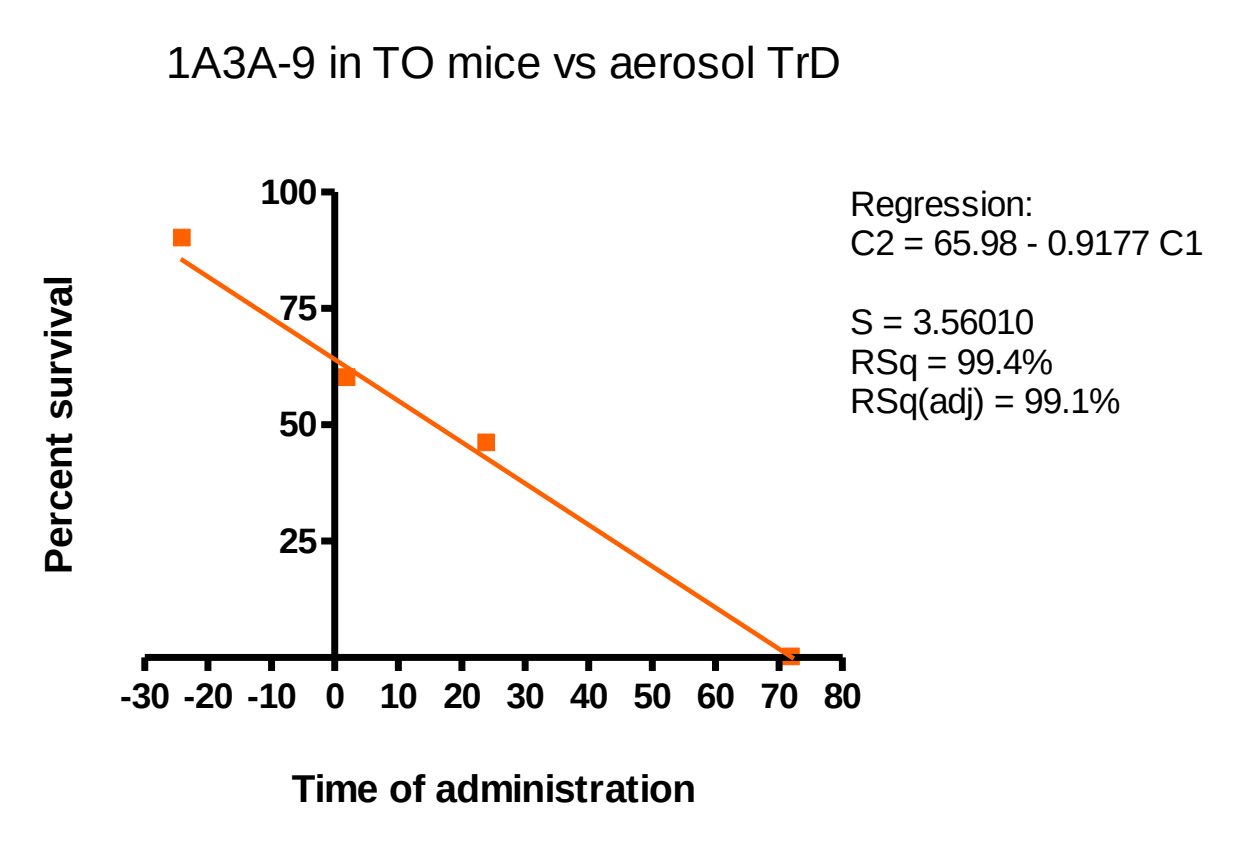
Phillpotts RJ *et al*, 2002, Vaccine

1A3A-9

- Neutralising antibody specific to VEEV prevents virus growth, cell entry and recruits immune cells to clear the virus

Topological map adapted from:
Mathews and Roehrig 1982 J Immunol and
Roehrig and Mathews 1985 Virology

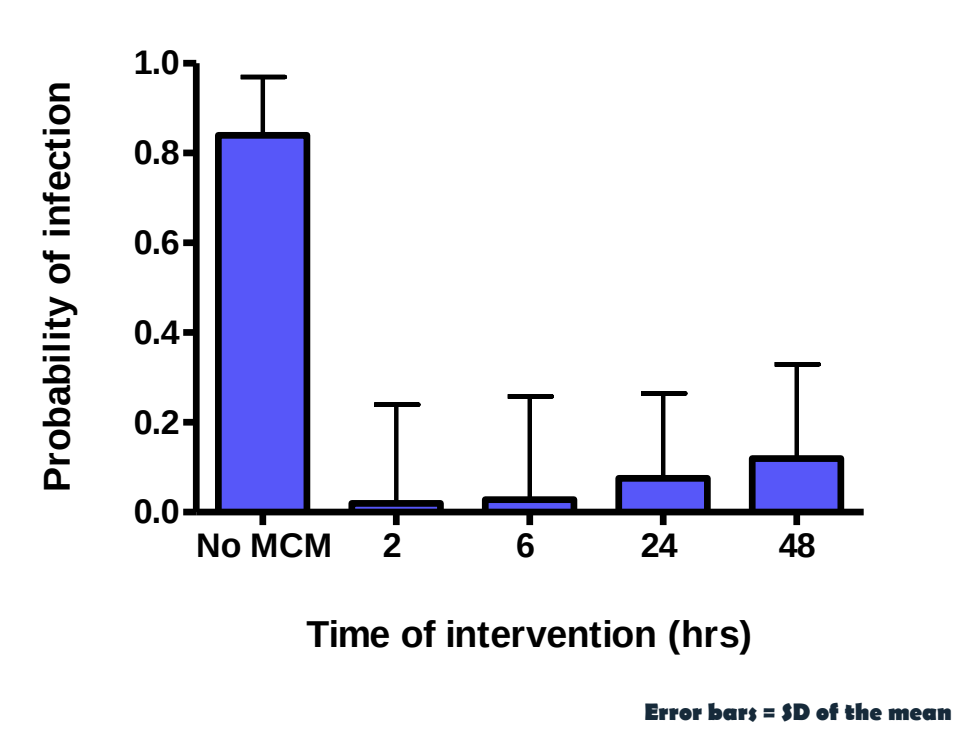
Time of antibody administration



Data taken from Phillpotts RJ *et al*, 2002, Vaccine

Benefit of antibody therapy to the military

- Model military impact of antibodies utilising
  - Agreed representative scenarios
  - Historical meteorology
  - Verified and validated HPAC modelling toolset
  - Sample over uncertainty in the input values

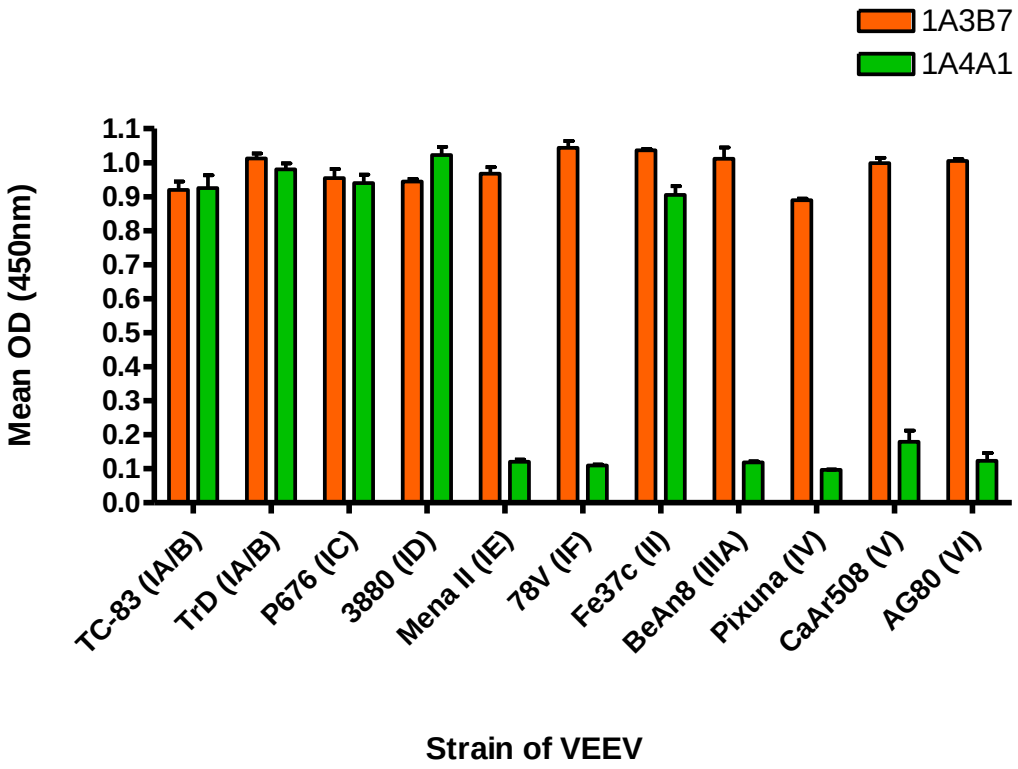


VEEV has a number of serotypes

THE VENEZUELAN EQUINE ENCEPHALOMYELITIS COMPLEX

Subtype	Variety	Prototype Strain	Origin	Cycle	Disease in Horse	Disease in Man
I	A/B	Trinidad donkey	Donkey (Trinidad)[1]	Epizootic	+	+
	C	P-676	Horse (Venezuela)[2]	Epizootic	+	+
	D	3880	Human (Panama)[3]	Enzootic	–	+
	E	Mena II	Human (Panama)[1]	Enzootic	–	+
	F	78V-3531	Mosquito (Brazil)[4]	Enzootic	–	?
II (Everglades)		Fe3-7c	Mosquito (Florida)[5]	Enzootic	–	+
III (Mucambo)	A	Mucambo (BeAn8)	Monkey (Brazil)[6]	Enzootic	–	+
	B	Tonate (CaAn410-D)	Bird (French Guiana)[7]	Enzootic	–	+
	C	71D-1252	Mosquitoes (Peru)[8]	Enzootic	–	?
IV (Pixuna)		Pixuna (BeAn356445)	Mosquito (Brazil)[6]	Enzootic	–	?
V (Cabassou)		Cabassou	Mosquito (French Guiana)[7]	Enzootic	–	?
VI		AG80-663	Mosquito (Argentina)[9]	Enzootic	–	+

Monoclonal antibody 1A3B7 is cross-reactive and cross-protective



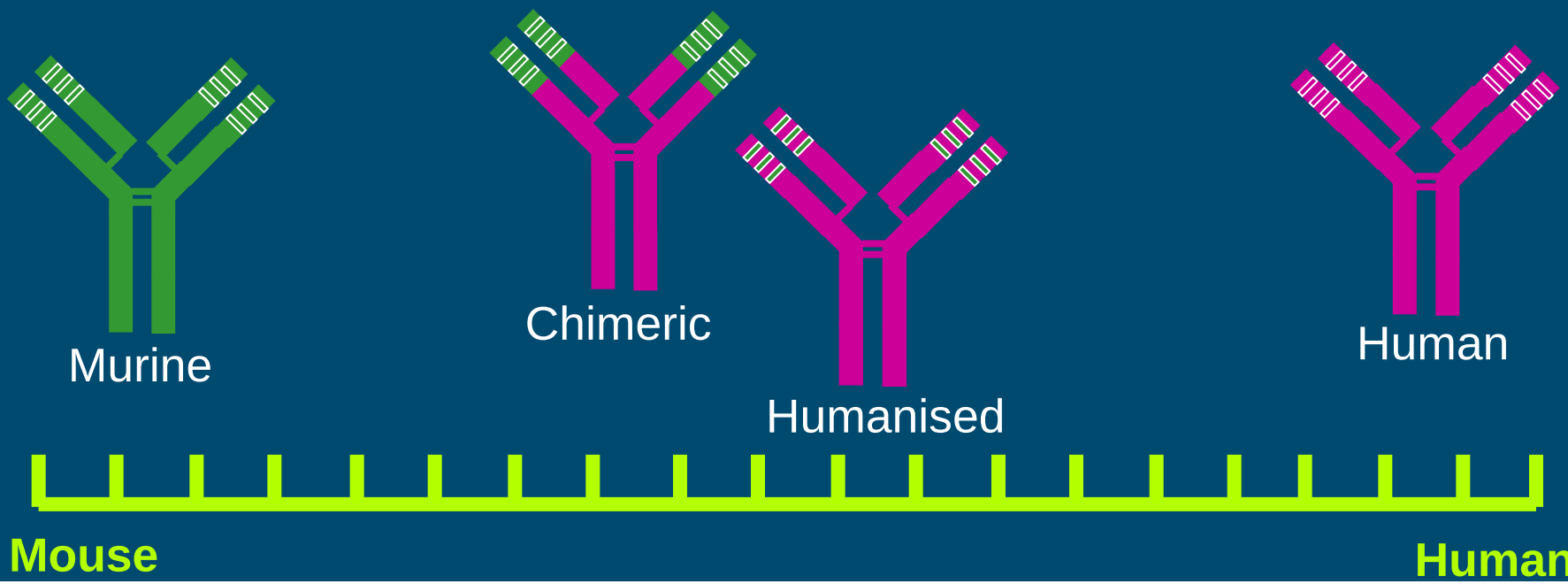
Virus (serogroup) 100 pfu s.c	Survivors/total	
	PBS	1A3B7 100 µg/ml i.p
TrD (IA/B)	0/10	9/10**
P676 (IC)	0/5	4/5*
3880 (ID)	0/5	4/5*
Mena II (IE)	0/5	5/5**
Fe37c (II)	0/5	5/5*
Mucambo (IIIA)	4/10	10/10*

***p<0.05; **p<0.01**

R.J. Phillpotts. Virus Research 120 (2006) 107–112

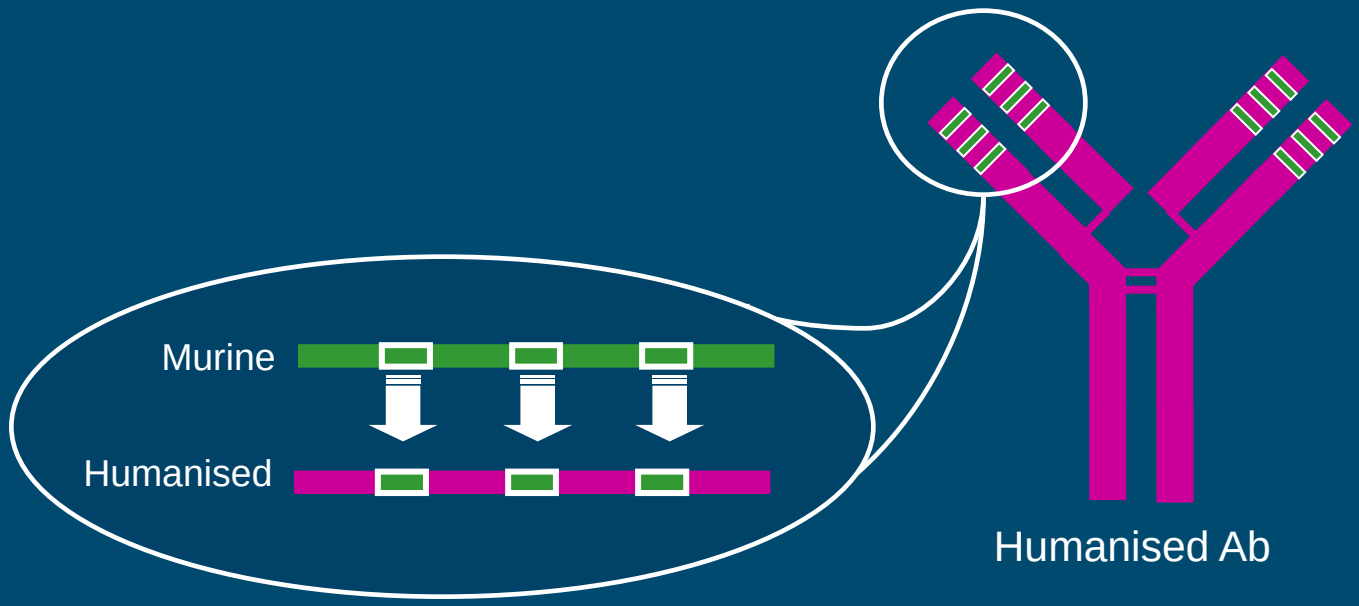
Humanisation strategies

- Murine antibodies may induce an anti-antibody response
  - Clearance of antibody
  - Adverse reactions

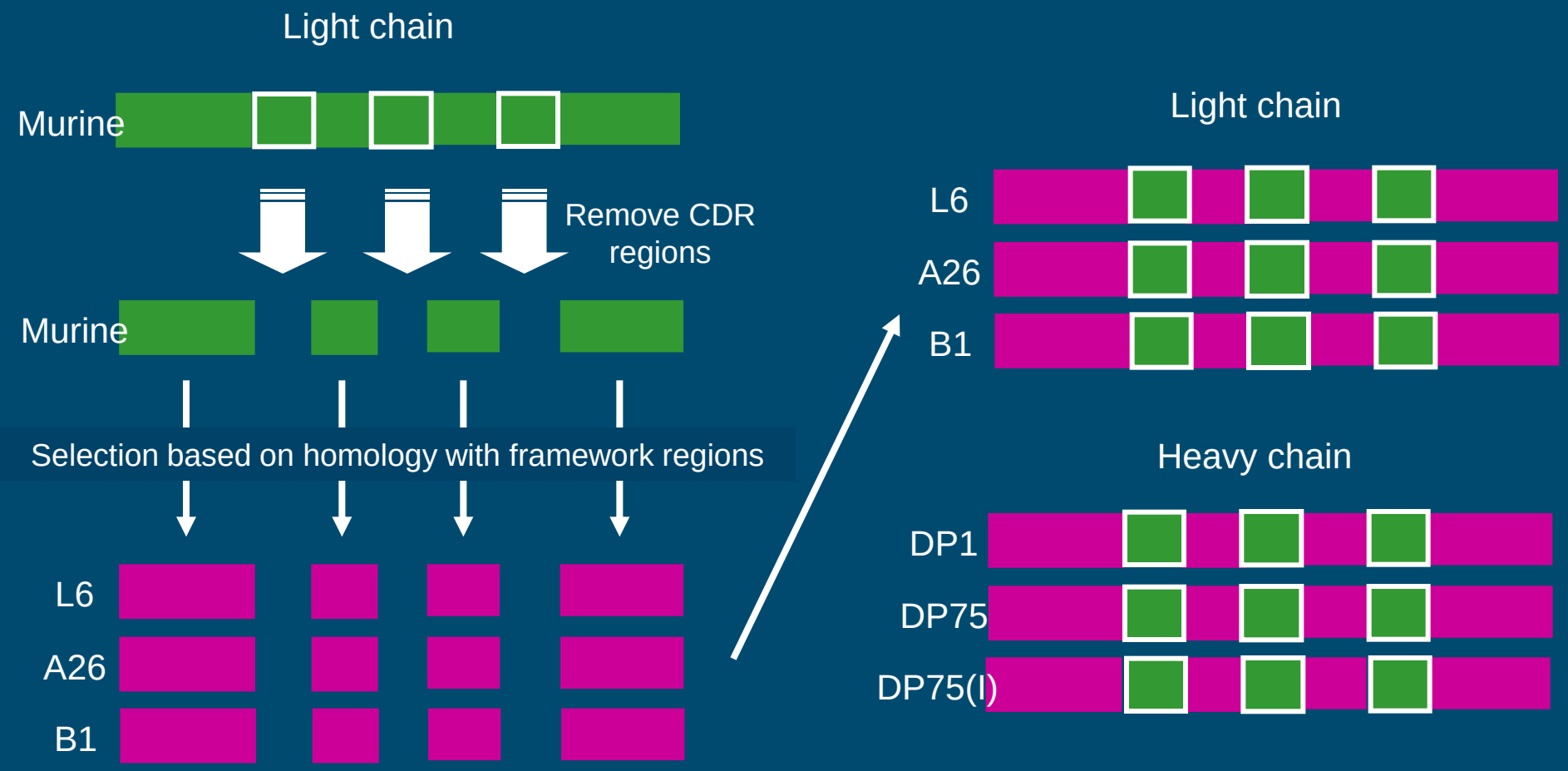


Towards human use

- Murine antibodies may induce an anti-antibody response
  - Clearance of antibody
  - Adverse reactions



Selection of framework regions

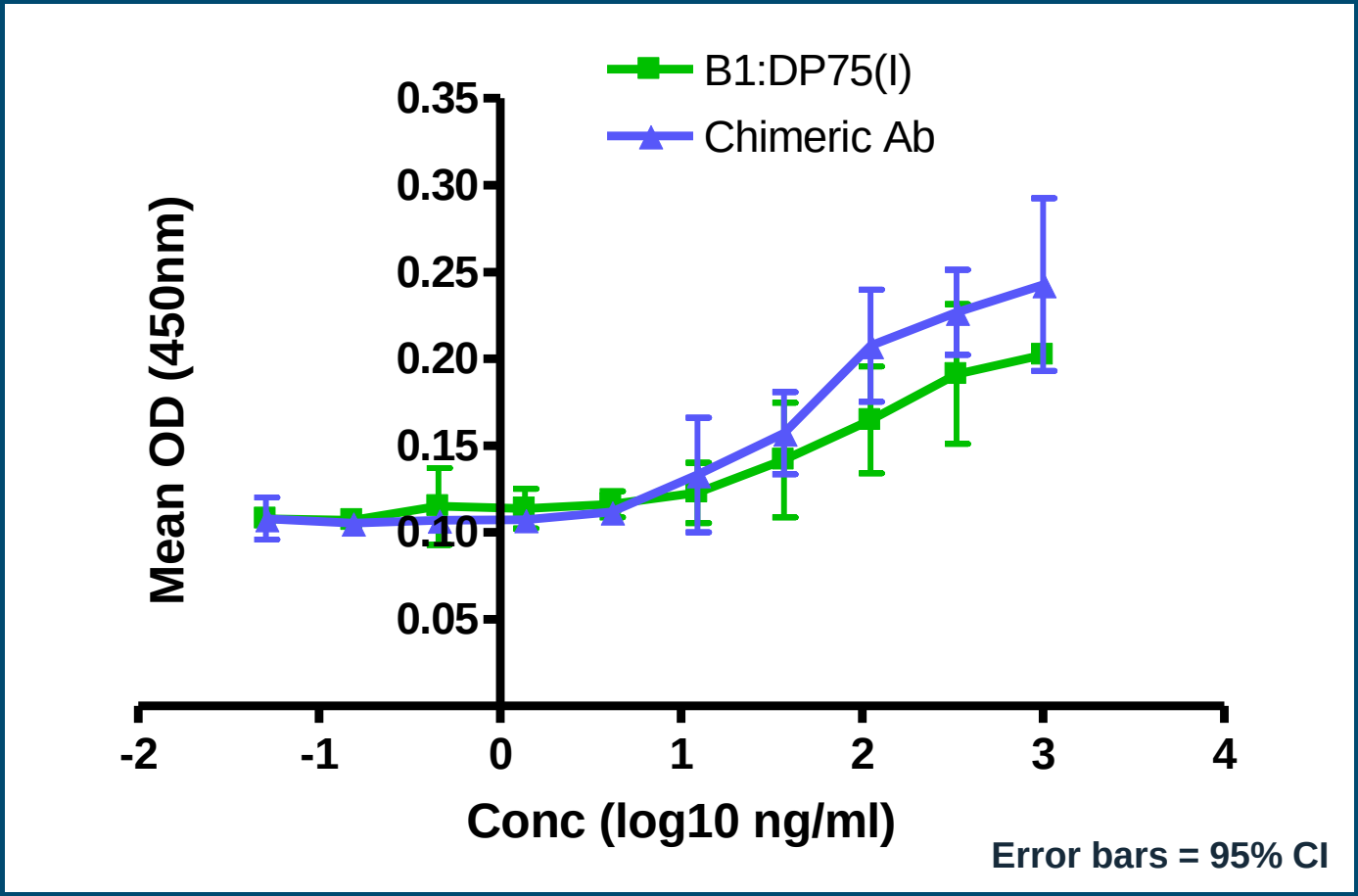


Affinity of humanised monoclonal antibodies

V_L \ V_H	DP1	DP75	DP75(I)
L6	X	X	X
A26	X	X	X
B1	X	X	+

- Binding of monoclonal antibodies to inactivated VEEV antigen

Affinity of humanised monoclonal antibody

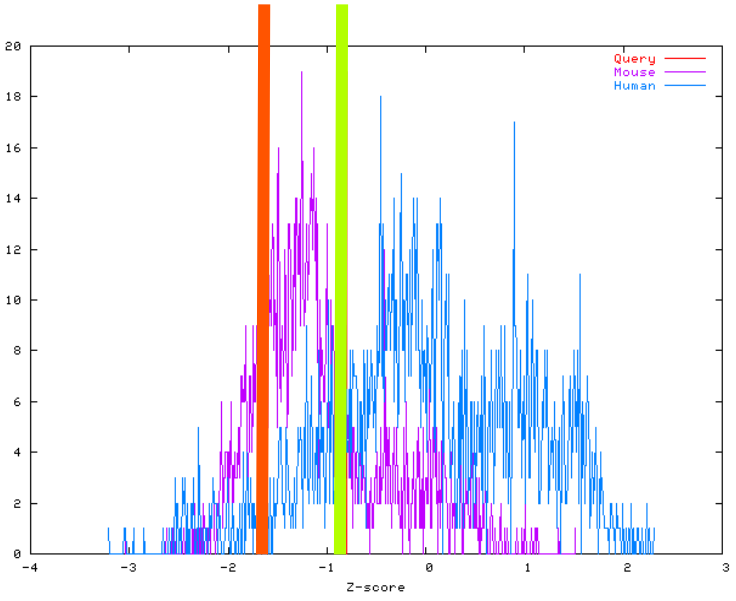


- Binding of monoclonal antibodies to inactivated VEEV antigen

Humanised H+L chains are more human-like

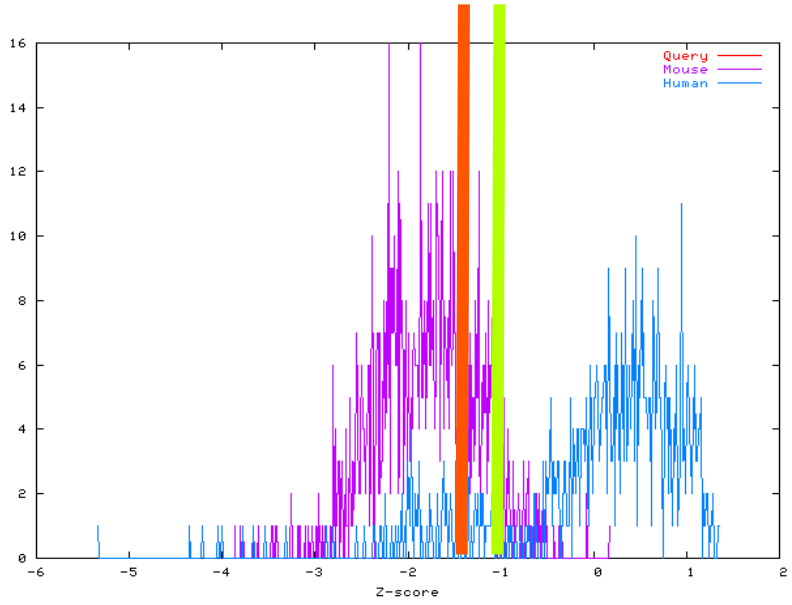
- Humanness score (Z score) - measure of typicality within the human repertoire
- Can assign an antibody as above or below the mean

Before After



Heavy chain

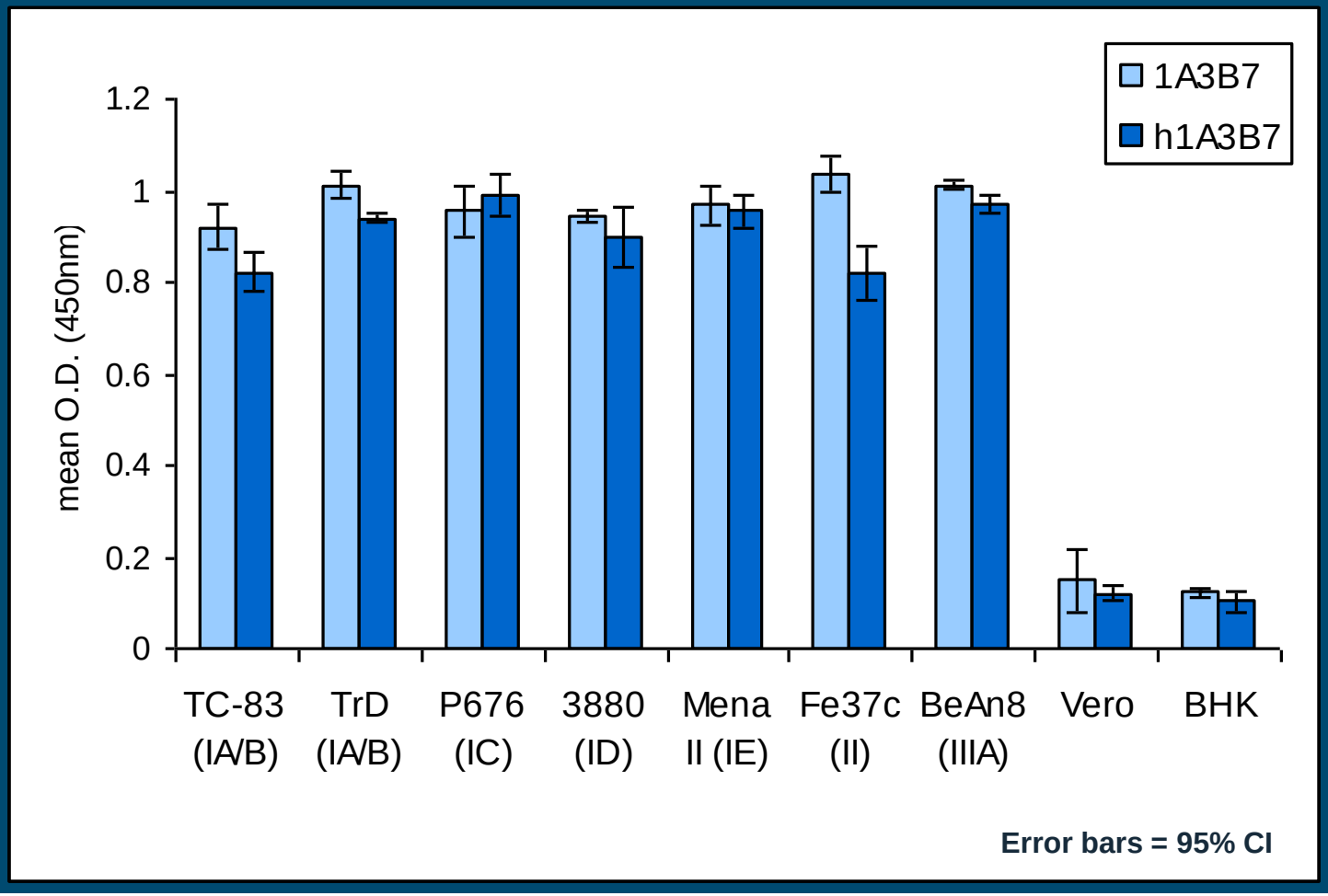
Before After



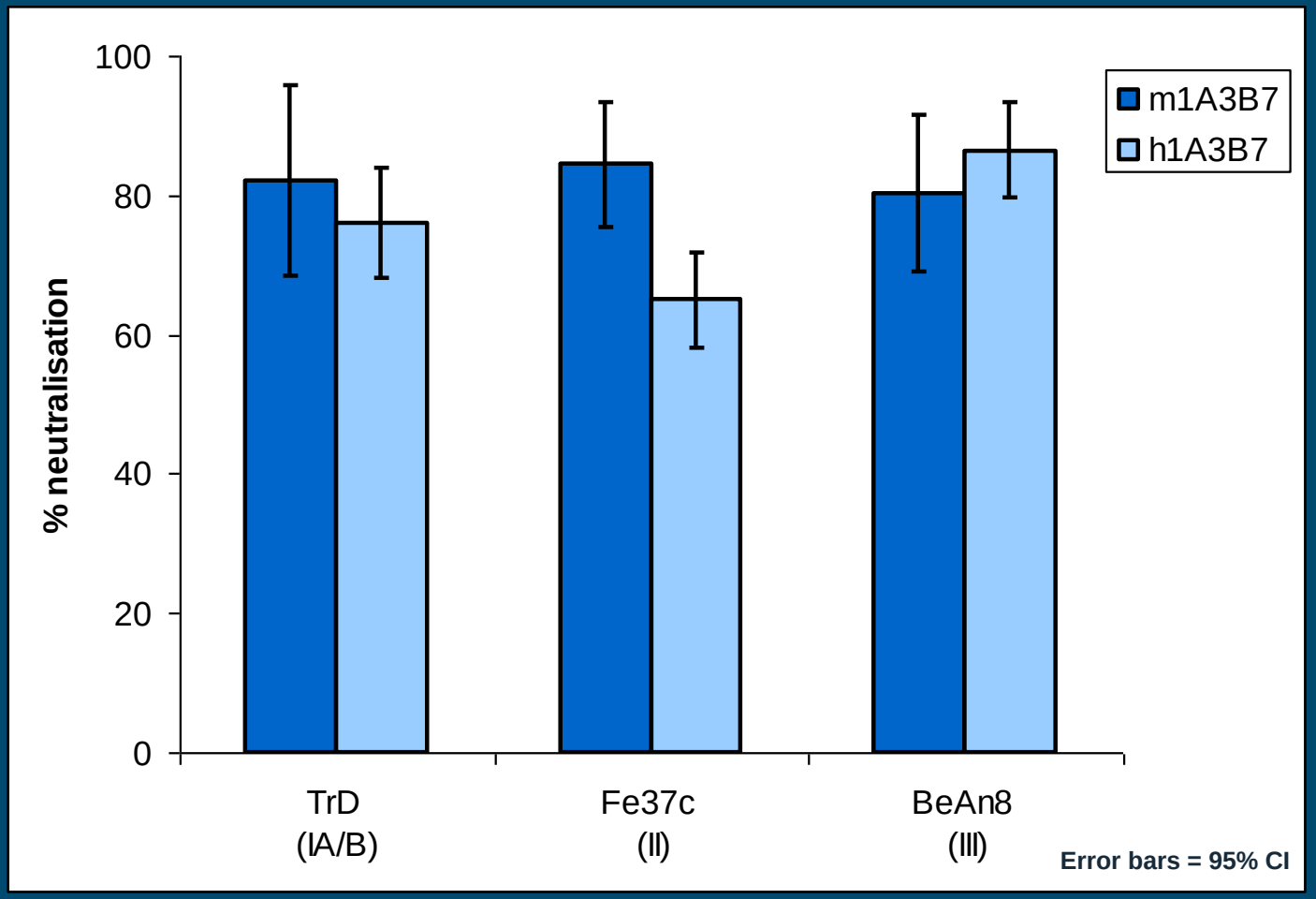
Light chain

	Light chain	Heavy chain
Mouse	1402	1562
Human	1695	1847

Humanised antibody retains broad reactivity against strains known to be pathogenic for humans

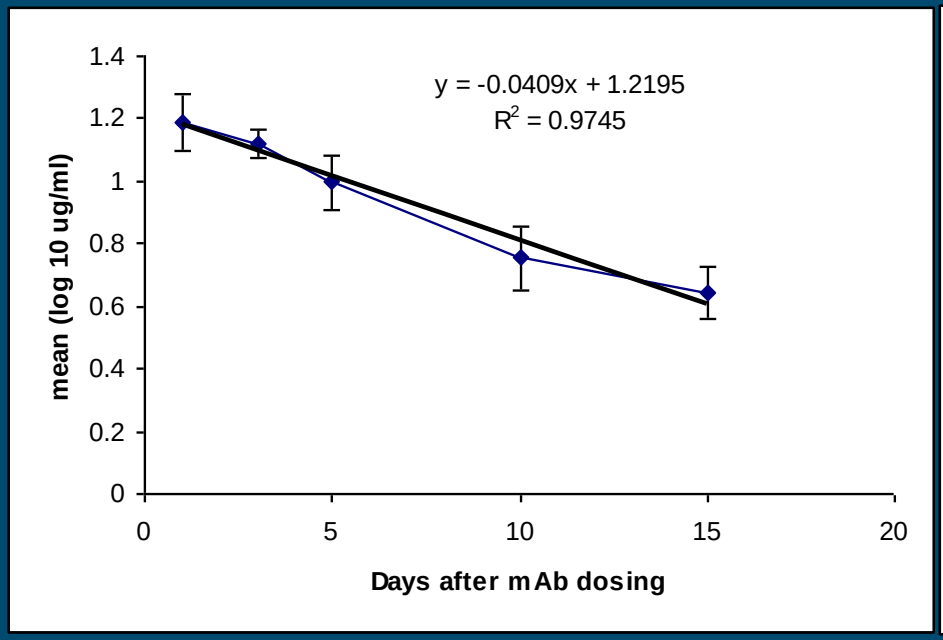


Humanised antibody can neutralise virus

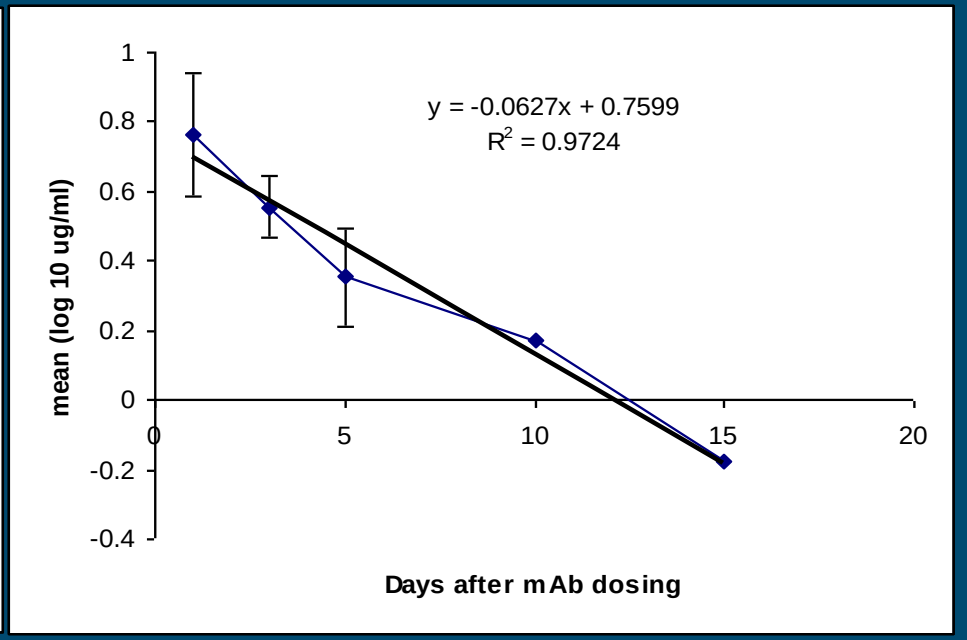


Pharmacokinetic data

1A3B7



h1A3B7



- Half-life:
  - 1A3B7 22.5 days
  - h1A3B7 7.3 days

Mouse challenge model for VEEV

Balb/c mouse

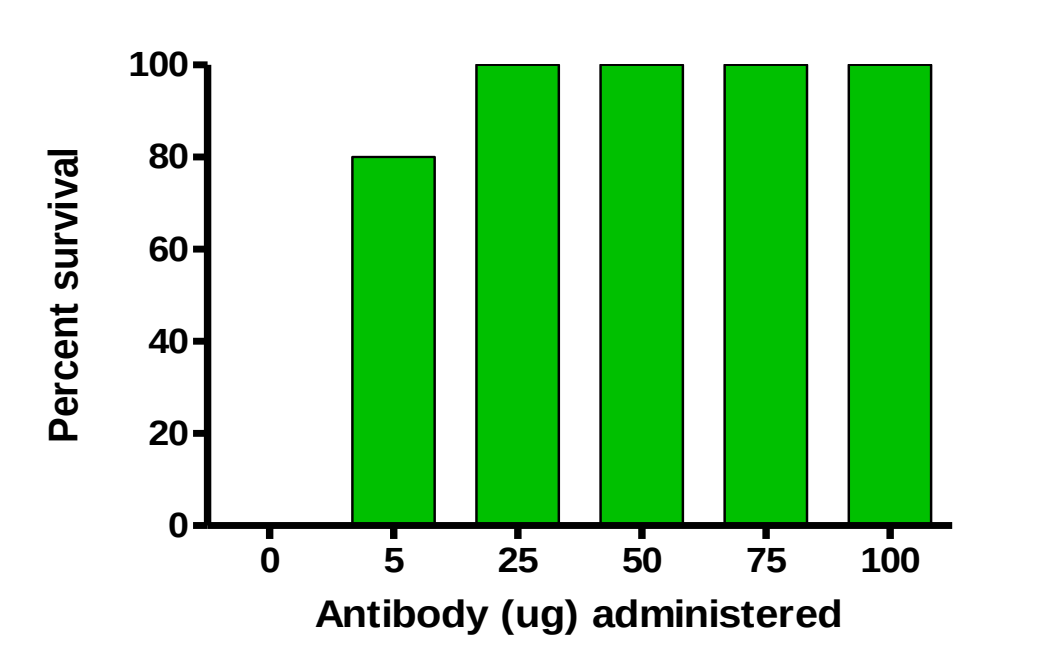
	Mouse	Human
ID_{50}	1-30 pfu	~10 pfu
Clinical signs	Extraneural stage leading to CNS stage	Extraneural stage leading to CNS stage in ~15% of cases
Time to clinical signs	4-7 days	1-5 days
Fatal encephalitis	**100%**	**1-5%**
Pathogenesis	Invasion of the brain via the olfactory system; invasion faster by aerosol route than peripheral routes	Believed that invasion of the brain via the olfactory system is similar to mouse model
Determinants of immunity	Primarily mediated by antibody – passive transfer has protected against peripheral and aerosol challenge.	Serum neutralising antibody correlates with protection

Variables:

- Virus strain
- Mouse strain
- Route of infection

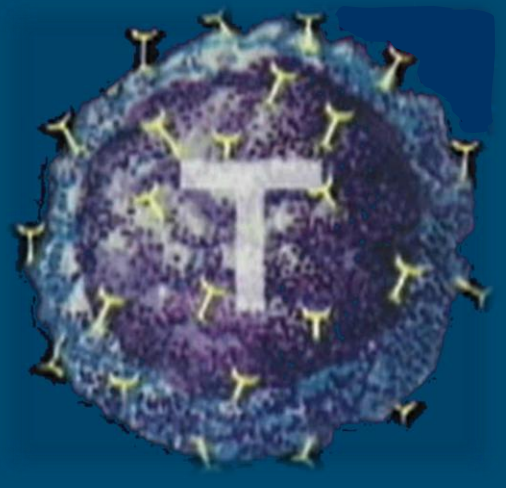
Protection against lethal virus challenge

- Mouse model of disease, antibody given 24 hours prior to challenge
- Injected VEEV challenge (100_{LD50})

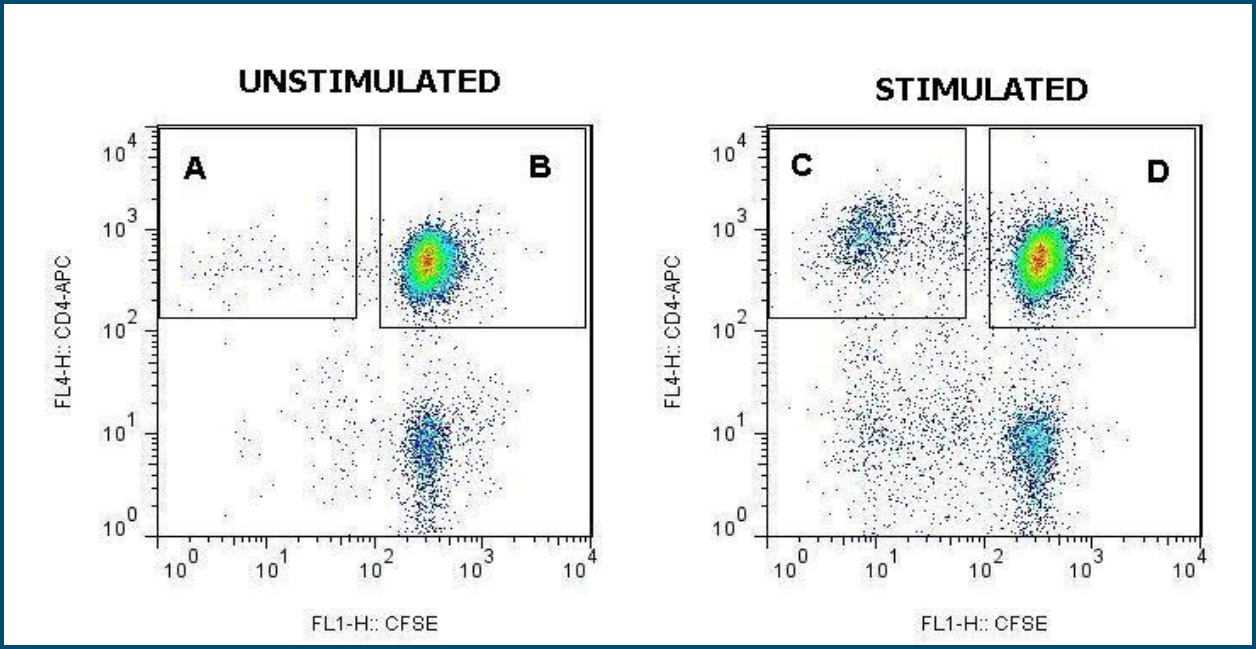


Immunogenicity of h1A3B7

- T-cell proliferation assessed with naïve donors
  - Minimum 40 donors, representative of global HLA class-II profiles
  - CD8 depleted
- Proliferation measured by decrease in fluorescence
  - Cells preloaded with CFSE dye
  - In cell division each daughter cell has 50% less signal than the parent
- Controls
  - PPD (memory)
  - KLH (naïve)
  - TT peptide and HA peptide



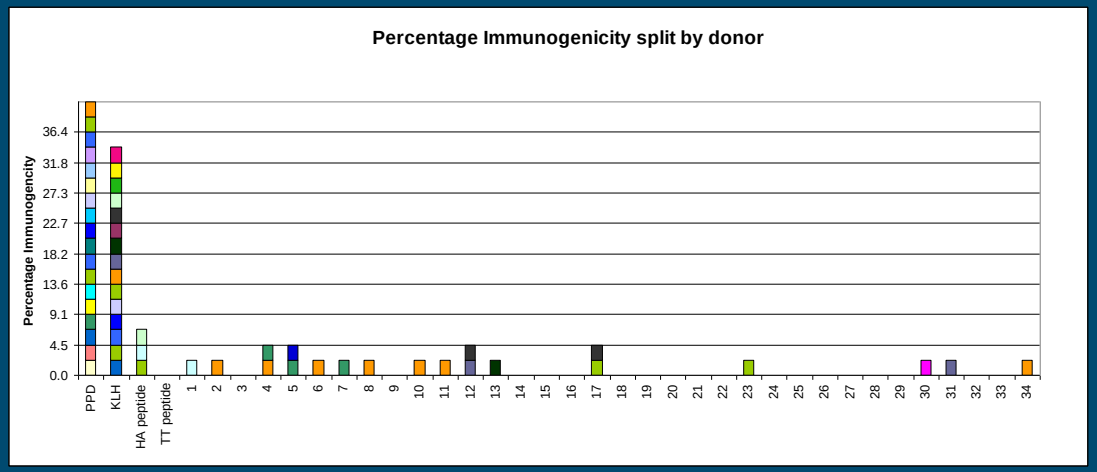
Measuring T –cell proliferation with CFSE

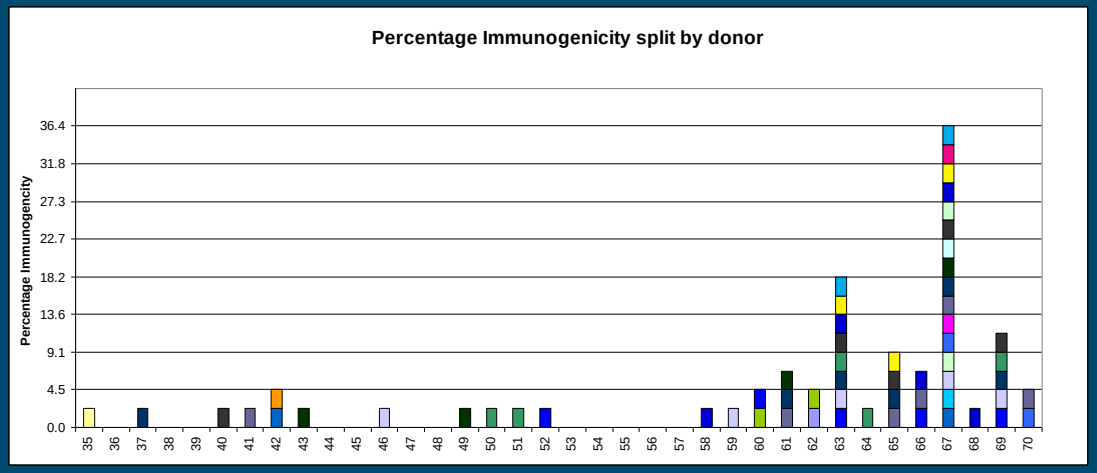


$$CDI = \frac{C / (C + D)}{A / (A + B)}$$

- Proliferation is measured in sextuplicate: the highest and lowest values are discarded from analysis

Immunogenicity of Chimeric 1A3B7



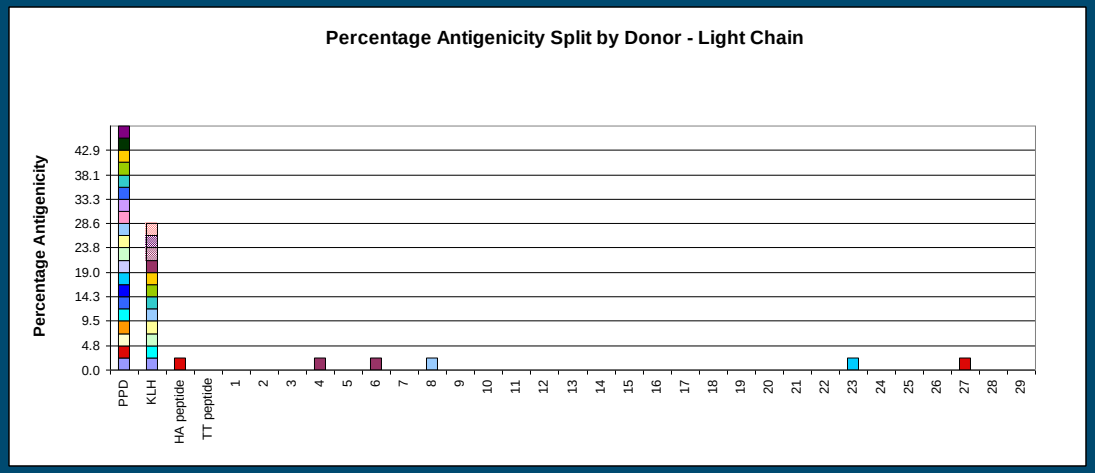


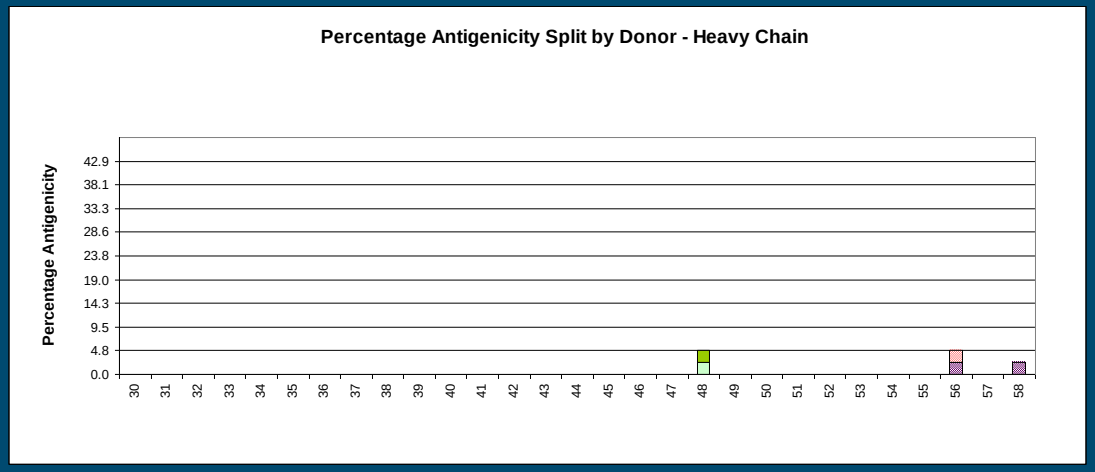
Graphs show NUMBER of responding donors as 'percentage immunogenicity'

CDI>2, SD cut-off = 2

Each coloured segment represents one responding donor

Immunogenicity of h1A3B7





Graphs show NUMBER of responding donors as 'percentage immunogenicity'

CDI>2, SD cut-off = 2

Each coloured segment represents one responding donor

Future work

- Production of antibody
  - Yields are low
- Assess ability of antibody to offer protection against challenge with aerosolised VEEV
- Understand protection offered at different times of administration relative to challenge
- Further assess suitability for humans

Summary

- A monoclonal antibody is effective in treating VEEV
- The molecule is derived from a mouse
  - it may cause adverse reactions in humans
- To reduce this potential, we have produced a panel of ‘humanised’ antibodies
- One antibody has been identified that is biologically active, and is able to offer protection against lethal VEEV challenge in a small animal model of disease
- Has the potential to be a useful therapy not just for military population but also lab’ workers and during outbreaks

Stuart Perkins
Lyn O'Brien
Sarah Goodchild
Amanda Phelps

Christopher Logue
Oliver Lanning
Bob Phillpotts

Victoria Lawson
Jeremy Fry

John Stevens

Andrew Martin
Luke Goodsell

Monoclonal antibodies courtesy of John Roehrig

Questions