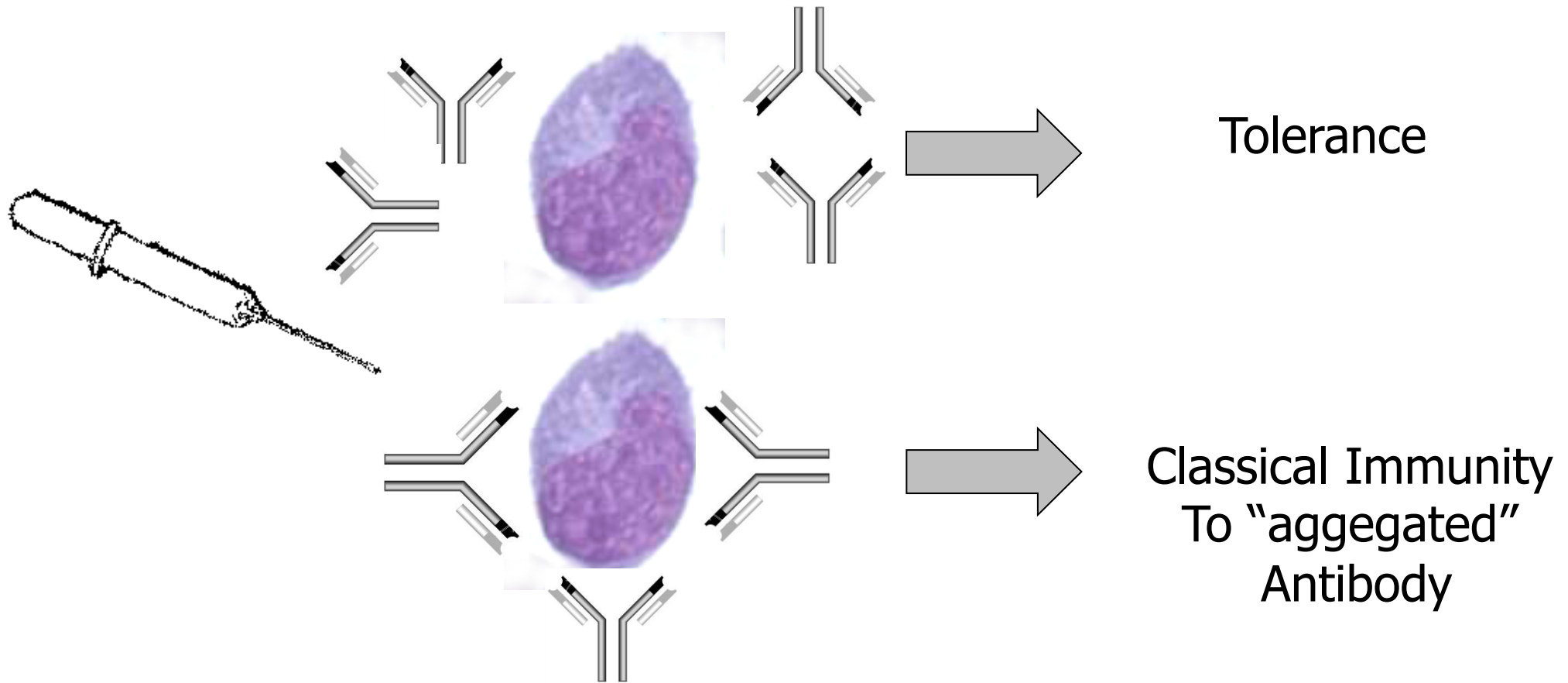


# ANTIBODY IMMUNOGENICITY



Chiller and Weigle, PNAS, 65:551, 1970;  
Benjamin and Waldmann, et al, J Exp Med, 163:1539, 1986)

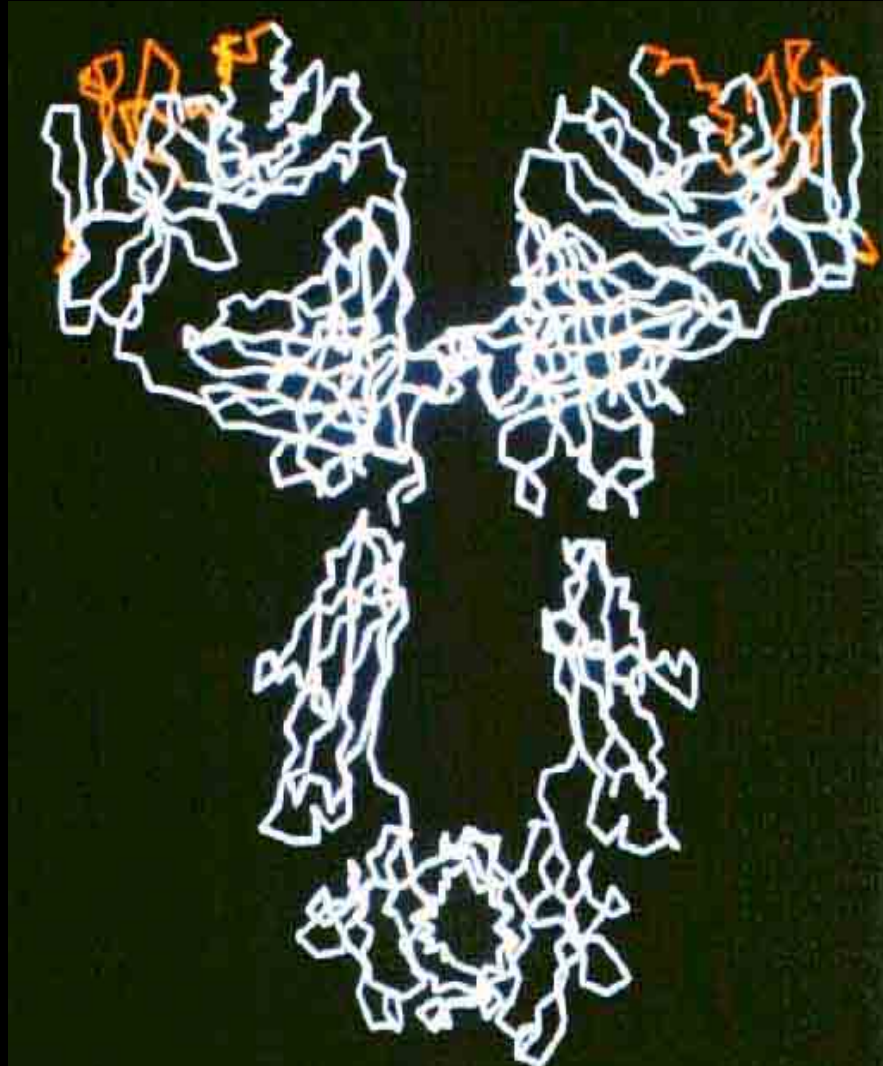
# THE IMMUNOGENICITY PROBLEM WITH THERAPEUTIC PROTEINS

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## Solutions?

1. Avoid alerting the innate immune system to “danger”
2. Prevent antigen processing
3. Reduce epitopes that can be recognised by T-cells
4. Short-term use of anti-inflammatory/ immunosuppressive drugs
5. Pre-tolerisation to the therapeutic protein

# ALEMTUZUMAB/LEMTRADA-THE FIRST HUMANISED ANTIBODY IN CLINICAL USE



Reichmann, L. et al 1988

Reshaping human antibodies for therapy. Nature 332, 323-327.

# Two stage tolerisation

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## **Elimination of the Immunogenicity of Therapeutic Antibodies**

**Lisa K. Gilliland,\* Lousie A. Walsh, Mark R. Frewin, Matt P. Wise, Masahide Tone, Geoff Hale, Demitris Kioussis, and Herman Waldmann**

*The Journal of Immunology, 1999, 162: 3663-3671.*

*In summary.....*

By creating non- (or limited) cell binding mutants, one can reduce or eliminate an anti-globulin immune response

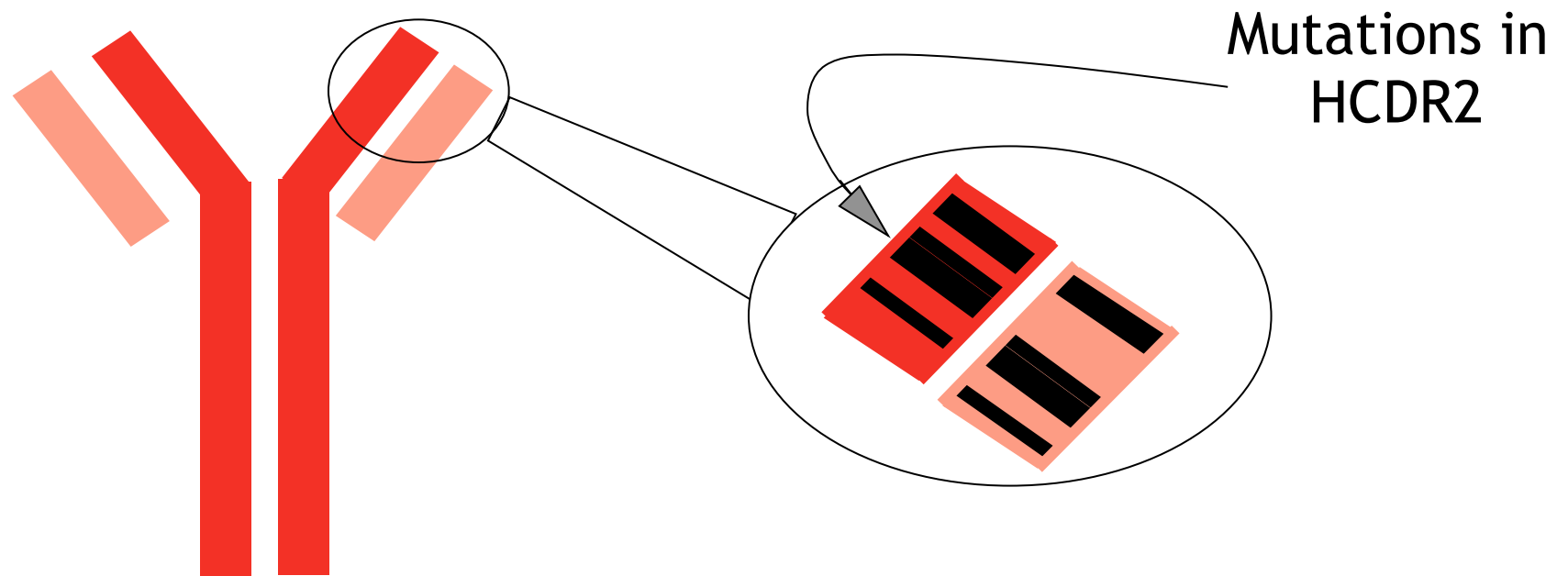
# Classical “Chiller and Weigle”

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- Aggregated immunoglobulins immunize
- Deaggregated immunoglobulins tolerize
- Chiller J.M., Habicht G.S. & Weigle W.O. (1970)  
Cellular sites of immunologic unresponsiveness. *Proc Natl Acad Sci U S A*, 65, 551.

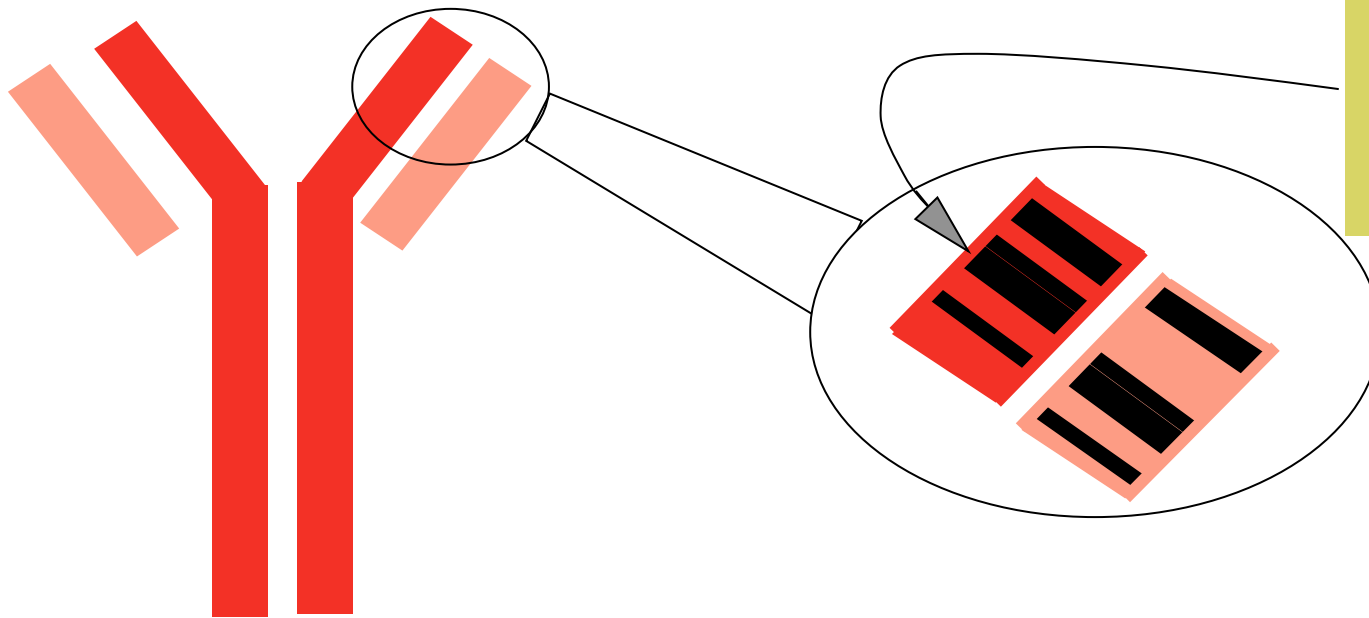
# PREVENTING IMMUNOGENICITY BY PRE-TOLERISING TO A MUTANT FORM

Creating Non-Cell Binding Mutants of ALEMTUZUMAB



*Gilliland et al. The Journal of Immunology, 1999, 162: 3663-3671.*

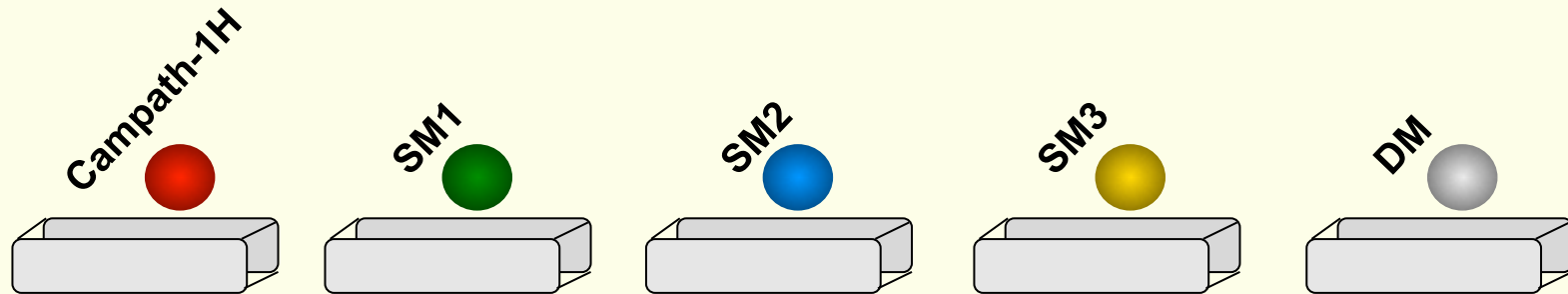
# Creating Non-Cell Binding Mutants



SM1: Lys**52b**Asp  
SM2: Asp**52a**Lys  
SM3: Lys**53**Asp  
DM: SM2 + SM3

# Assessing *Non-Cell Binding* Mutants Against Target *in vitro*

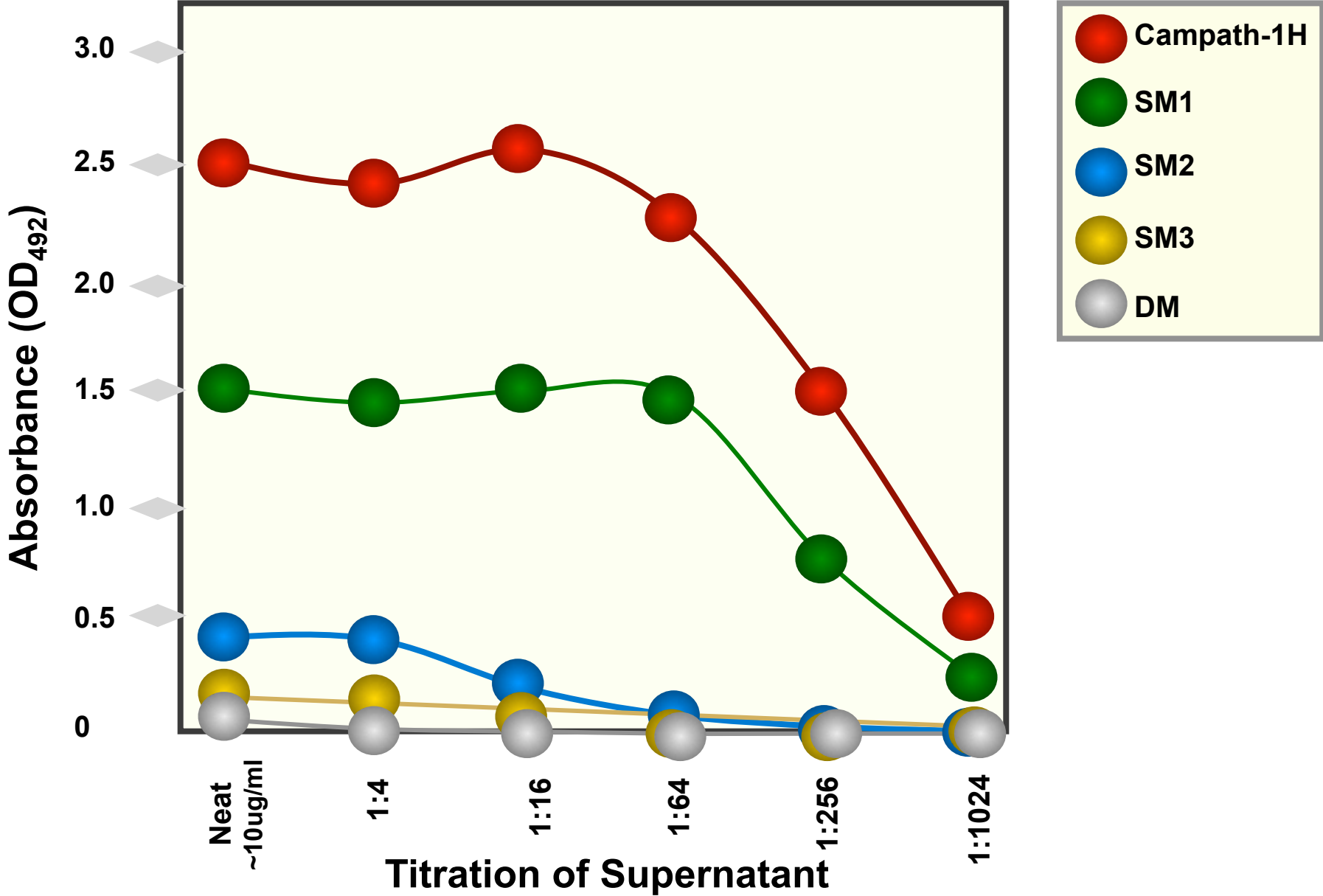
## Production of Antibody Variants



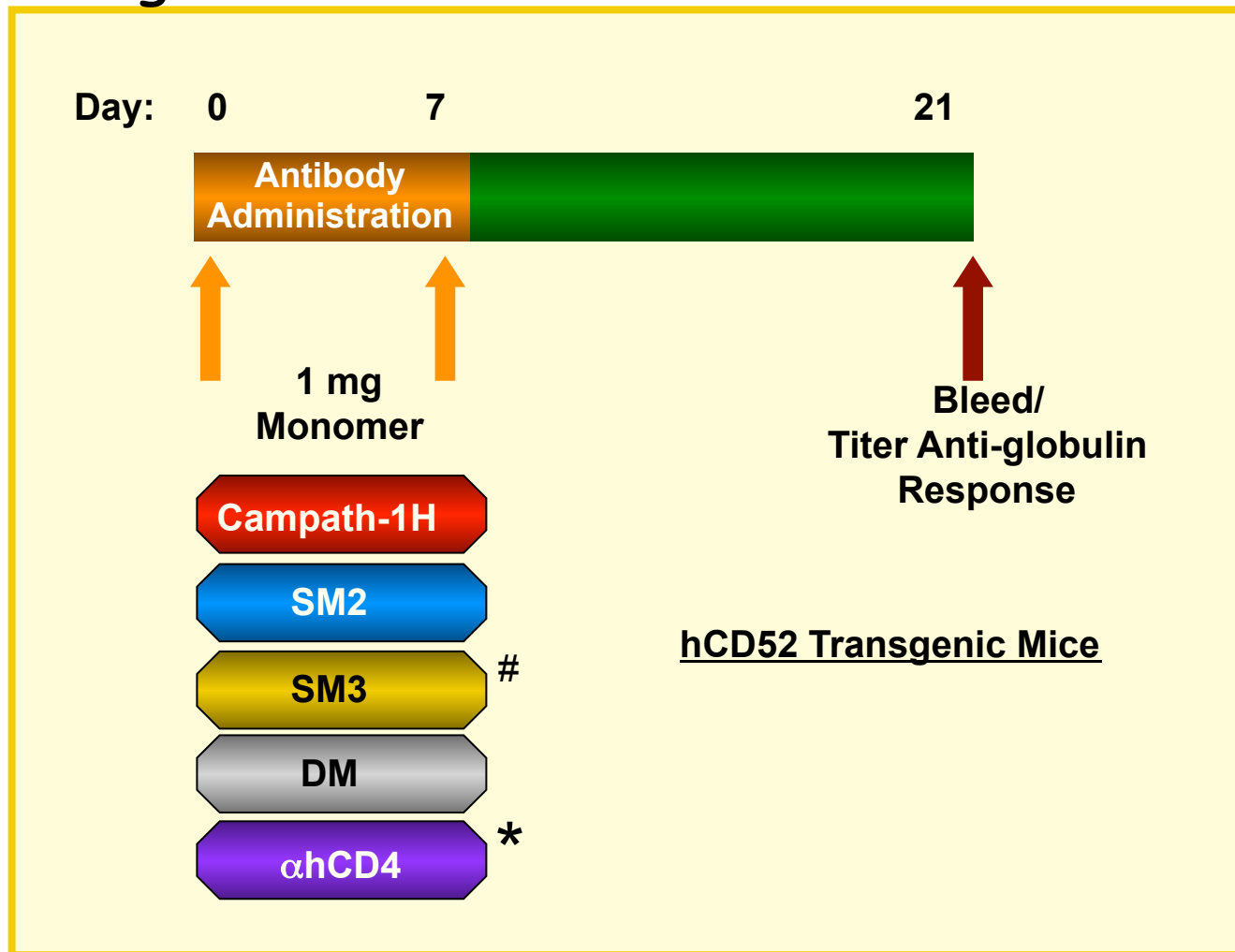
Assess Binding to Ig-CD52  
Fusion Protein  
By ELISA



# Binding of Minimal mutants to rIg-CD52



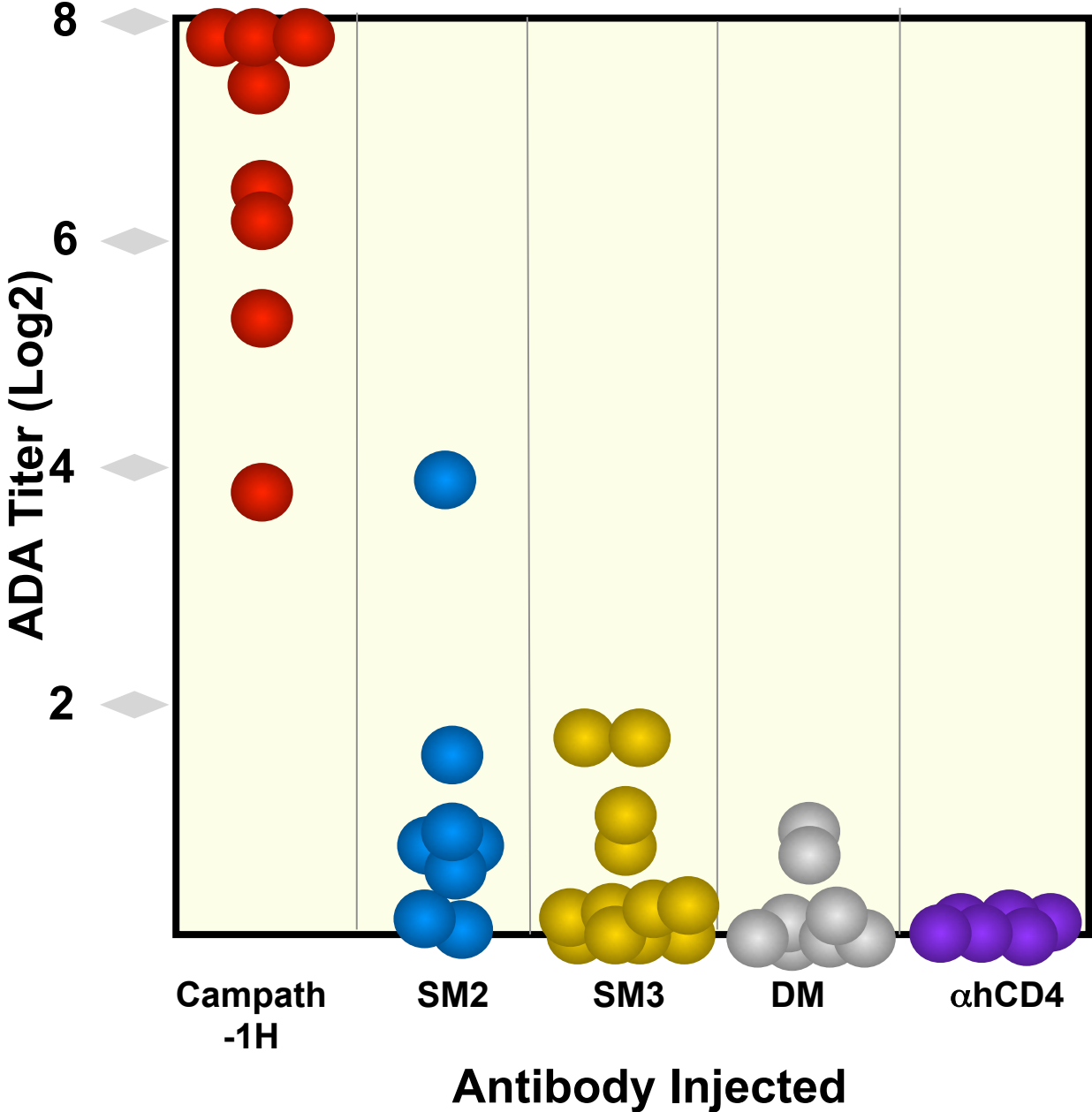
# Assessing immunogenicity of *Non-Cell Binding* Mutants Against Target *in vivo*



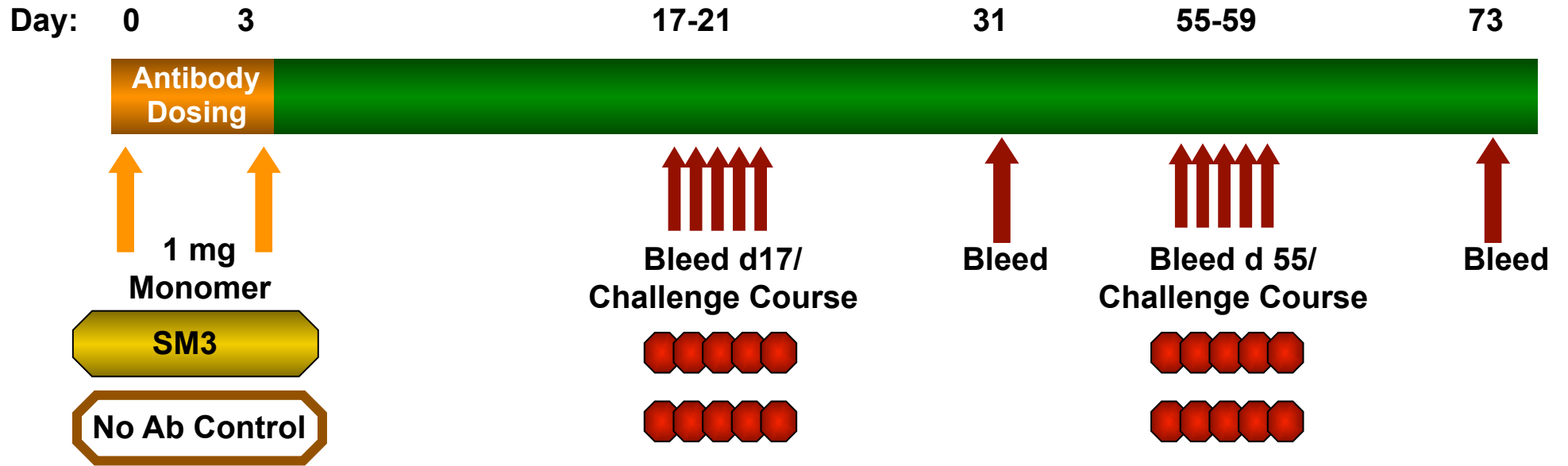
# Was injected at Days 0 and 3

\* Has no target to bind at all -- non-cell binding

# Assessing immunogenicity of *Non-Cell Binding* Mutants Against Target *in vivo*



# Non-Cell Binding Mutants: Tolerization Protocol

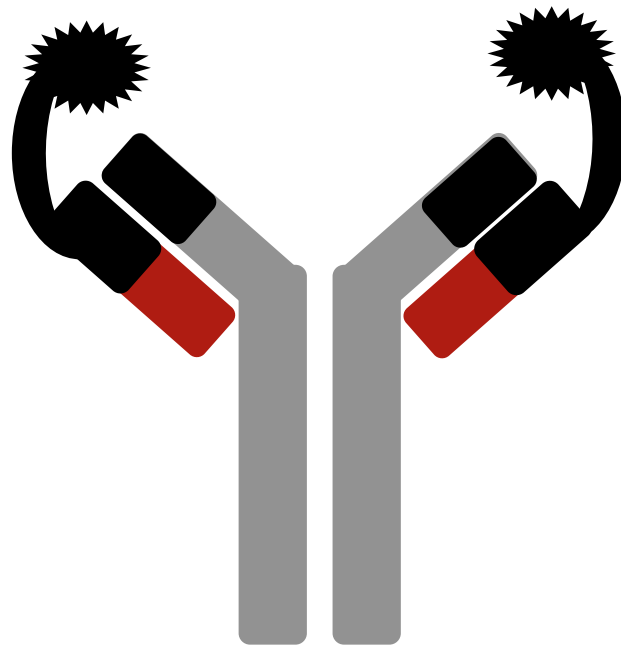


hCD4



# One stage tolerisation

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**Stealth antibodies**

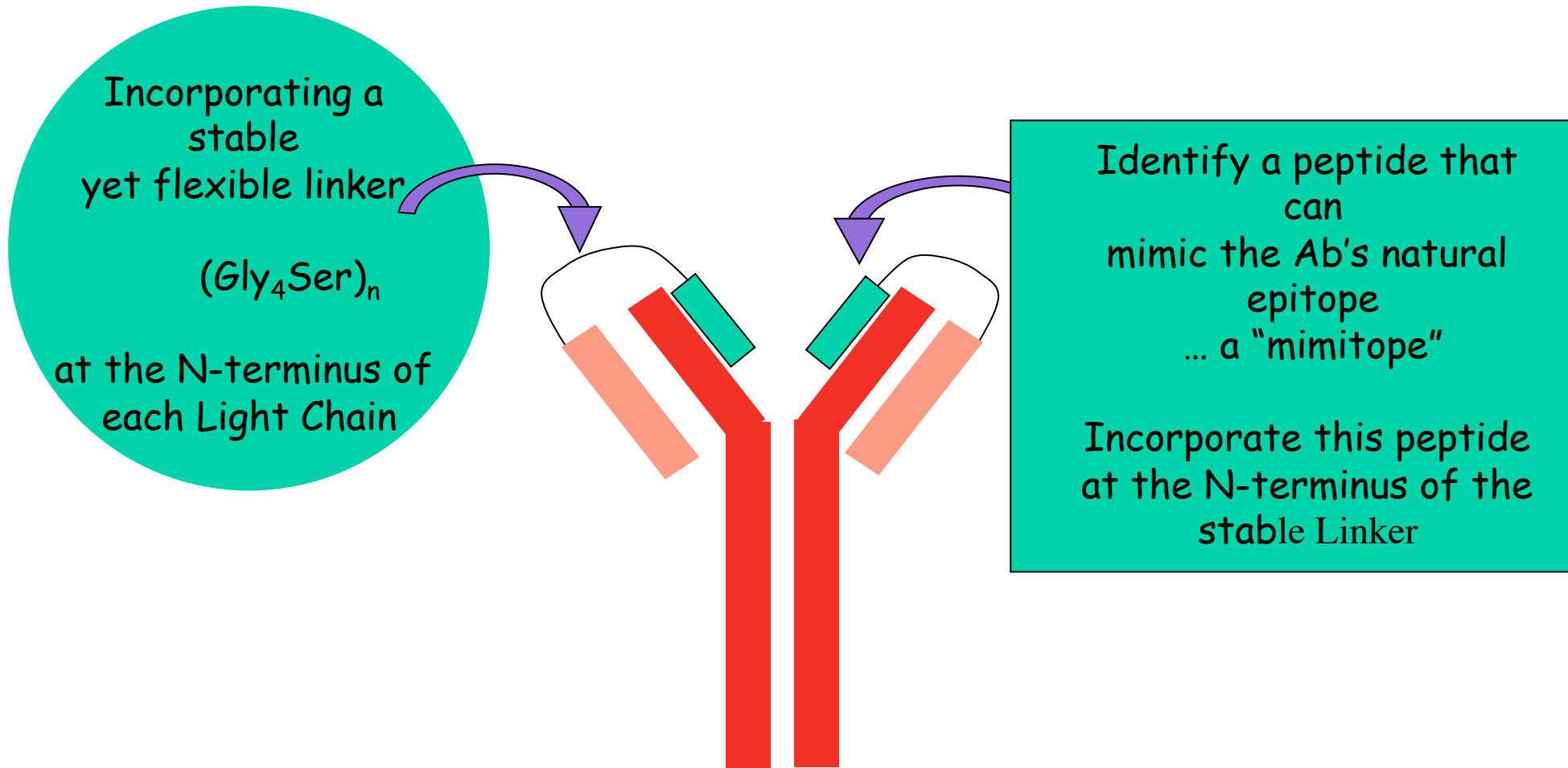
*In general ...*

**The aim of stealth antibody modification is to reduce Ab binding within the host long enough to induce a tolerogenic response.**

**Following the tolerization, the same antibody is able to bind its target and fulfill its therapeutic goal.**

# Principal:-interfere with antigen-binding yet retain efficacy

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The binding of the obstructive element is reversible



# Conclusions

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**Non-cell-binding**

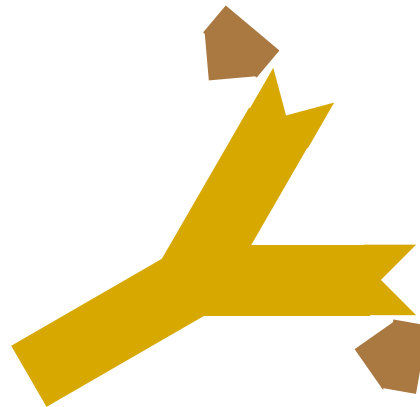


**Not immunogenic**

**Tolerogenic**

*Ratio binding/non-binding of 1:100  
at outset results in tolerance*

**Cell-Binding**



**Tolerance is achieved over  
the initial two weeks**

**Biological activity: Cell depletion**

**Depletion lasts longer:**

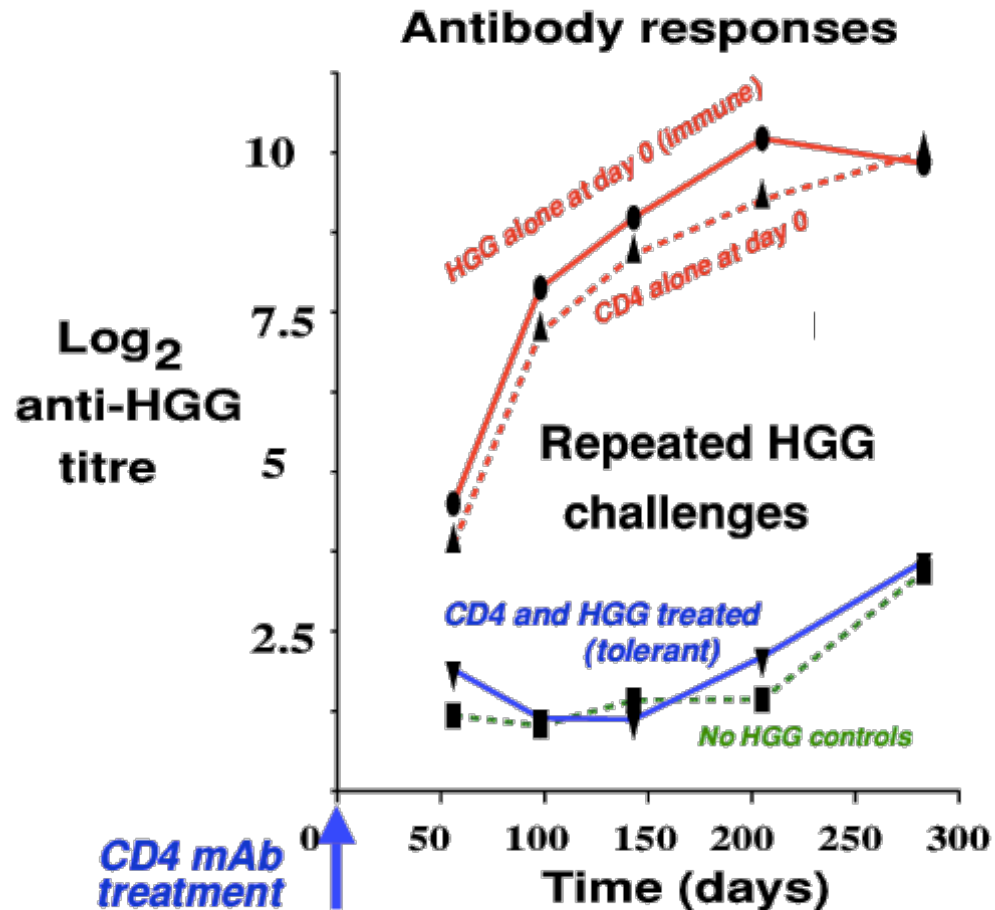
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**No immunogenicity  
Tolerogenic to cell binding form**

**→ No antiglobulins →**

**Longer half-life of  
Therapeutic mAbs**

# Assisted tolerisation:-CD4 mAb treatment induces tolerance to aggregated HGG in mice



Benjamin, R and Waldmann, H. 1986. Induction of Tolerance by monoclonal antibody therapy. Nature, 320, 449-451.

Benjamin et al. 1988. Europ. J.Immunol.18, 1079-1088

# Assisted Tolerance Induction to foreign proteins Anti-CD4 Mab Rx.

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1. Benjamin RJ, Waldmann H. Induction of tolerance by monoclonal antibody therapy. *Nature* 1986, **320**(6061): 449-451.
2. Qin S, Cobbold S, Tighe H, Benjamin R, Waldmann H. CD4 monoclonal antibody pairs for immunosuppression and tolerance induction. *European journal of immunology* 1987, **17**(8): 1159-1165.
3. Benjamin RJ, Qin SX, Wise MP, Cobbold SP, Waldmann H. Mechanisms of monoclonal antibody-facilitated tolerance induction: a possible role for the CD4 (L3T4) and CD11a (LFA-1) molecules in self-non-self discrimination. *European journal of immunology* 1988, **18**(7): 1079-1088.
4. Winsor-Hines D, Merrill C, O'Mahony M, Rao PE, Cobbold SP, Waldmann H, *et al.* Induction of immunological hyporesponsiveness in baboons with a non-depleting CD4 antibody. *Journal of immunology* 2004, **173**(7): 4715-4723.

# Attenuation of undesirable immune responses to therapeutic proteins

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## Gene therapy

McIntosh JH, Cochrane M, Cobbold S, Waldmann H, Nathwani SA, Davidoff AM, et al. Successful attenuation of humoral immunity to viral capsid and transgenic protein following AAV-mediated gene transfer with a non-depleting CD4 antibody and cyclosporine. *Gene therapy* 2012, **19**(1): 78-85.

## Factor VIII.

Oliveira VG, Agua-Doce A, Curotto de Lafaille M, Lafaille JJ, Graca L.

Adjuvant facilitates tolerance induction to factor VIII in hemophilic mice through a Foxp3-independent mechanism that relies on IL-10. *Blood* 2013.

# CONCLUSIONS

- Many and complex reasons for immunogenicity
- Danger signals (innate immune system).
- “Foreignness” (adaptive immune system)
  
- Generic strategies aimed at minimising perception of danger, and limiting T-cell epitopes to prevent priming of T-helper cells.
  
- Pre-tolerisation looks to be a novel and potential useful strategy for (at least) immunoglobulin-based products

# STEALTH VERSIONS OF ANTIBODIES (THE FUTURE)

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1. TO FURTHER REDUCE/REMOVE IMMUNOGENICITY
2. CLEAVABLE LINKERS TO FINESSE PROPER SLOW RELEASE FORMS OF THE THERAPEUTIC ANTIBODY
3. TO PRODUCE OPERATIONAL SELECTIVITY FOR TISSUES (CLEAVABLE LINKERS)