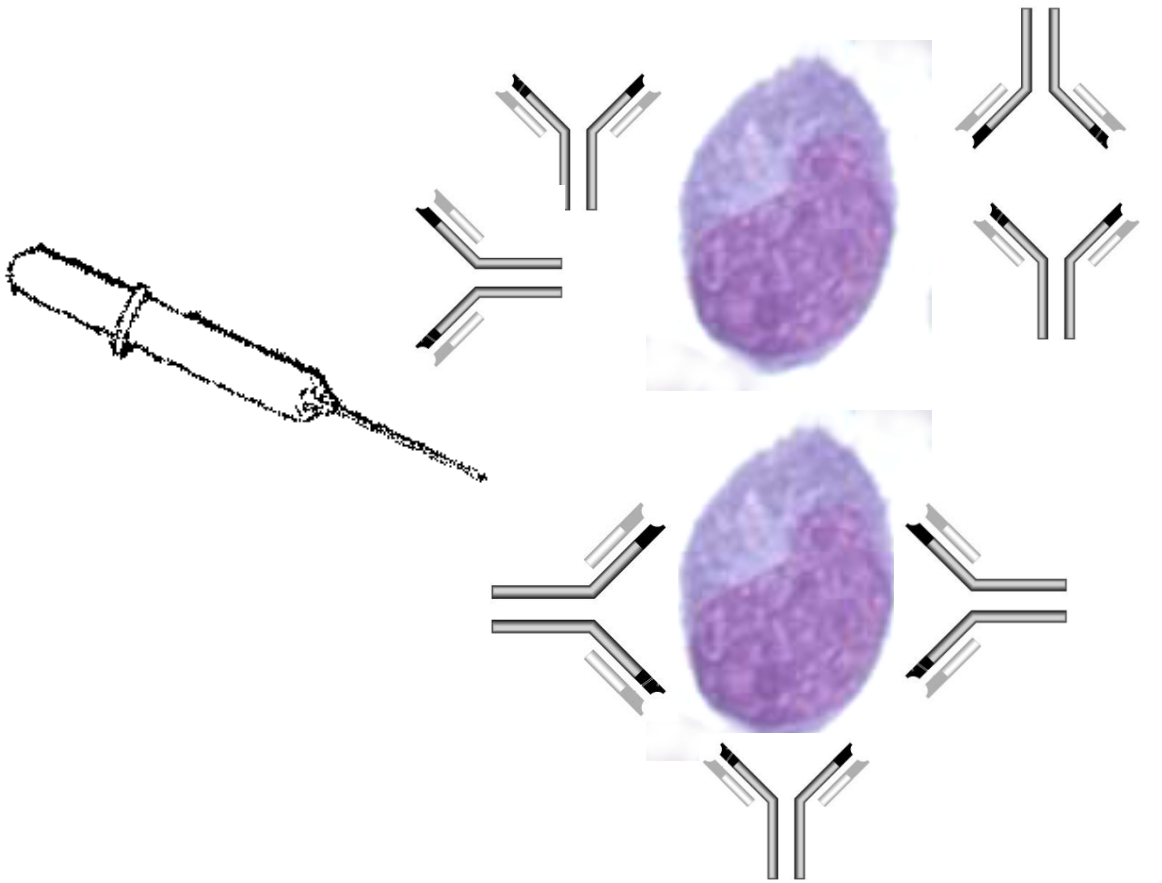
ANTIBODY IMMUNOGENICITY



Tolerance

Classical Immunity
To "aggegated"
Antibody

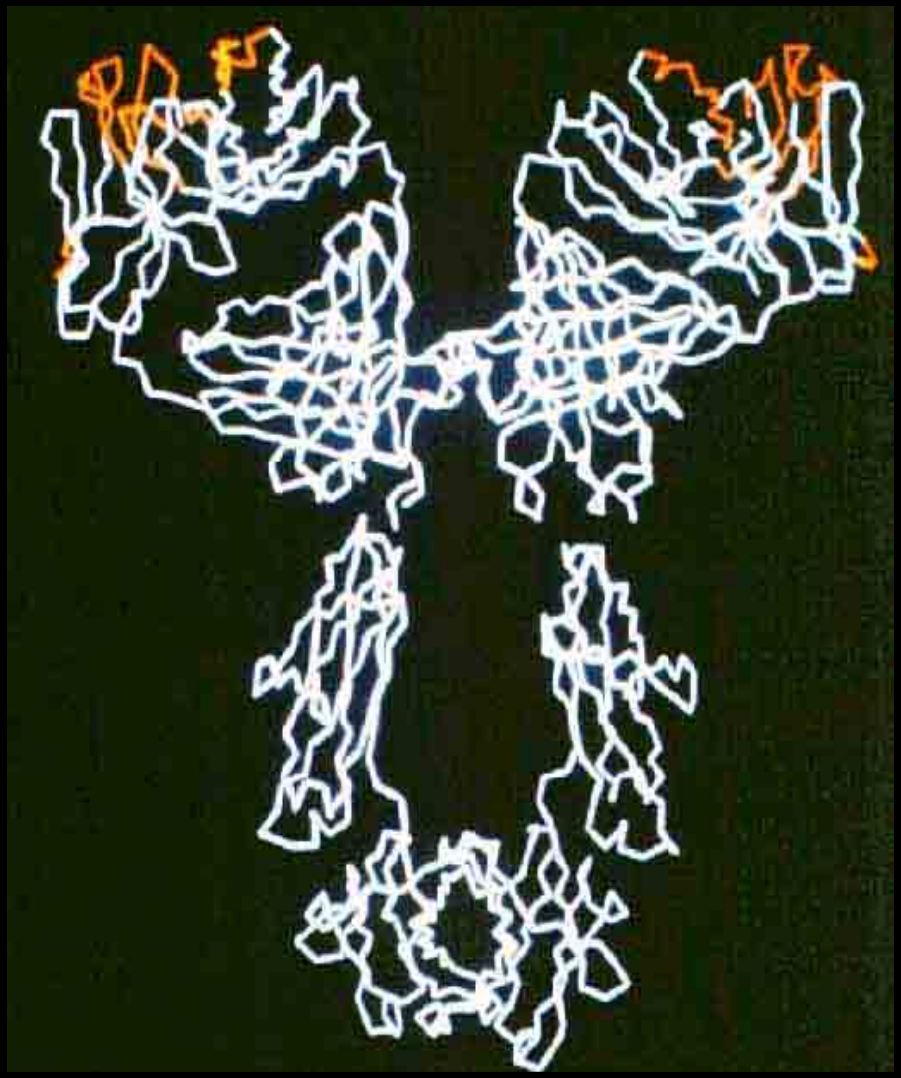
Chiller and Weigle, PNAS, 65:551, 1970;
Benjamin and Waldmann, et al, J Exp Med, 163:1539, 1986)

THE IMMUNOGENICITY PROBLEM WITH THERAPEUTIC PROTEINS

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

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"

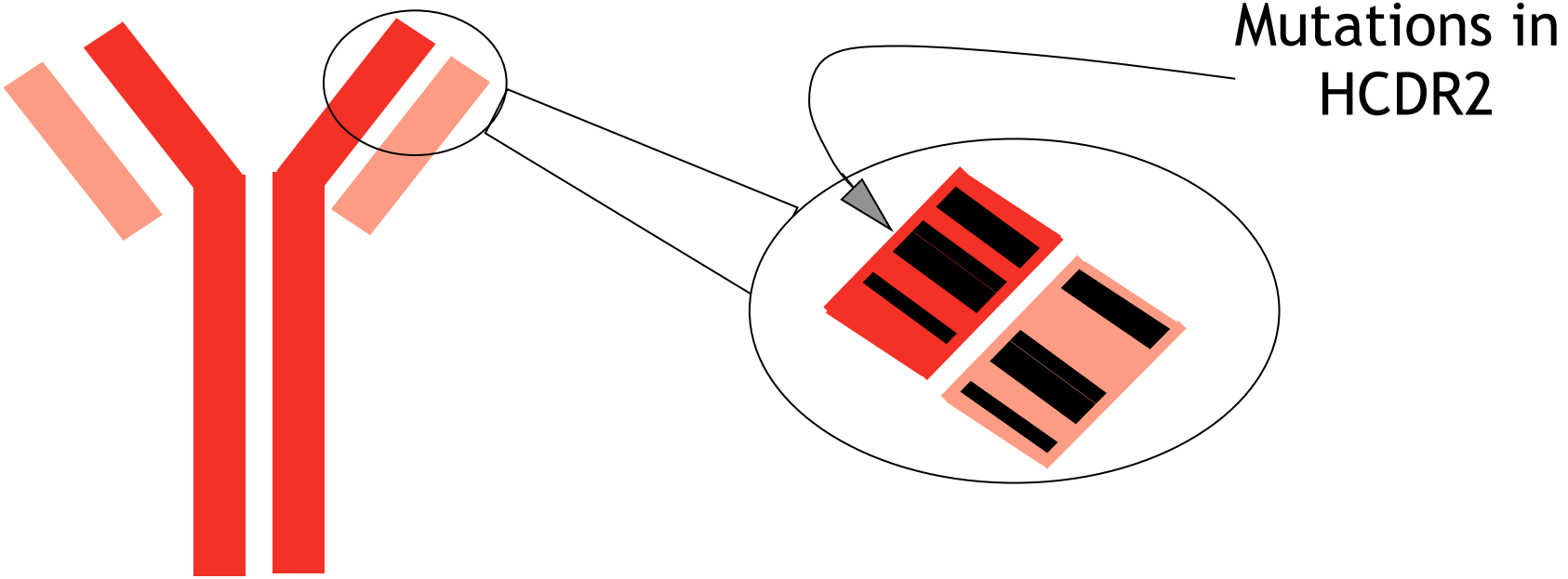
- Aggregated immunoglobulins immunize

- 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.

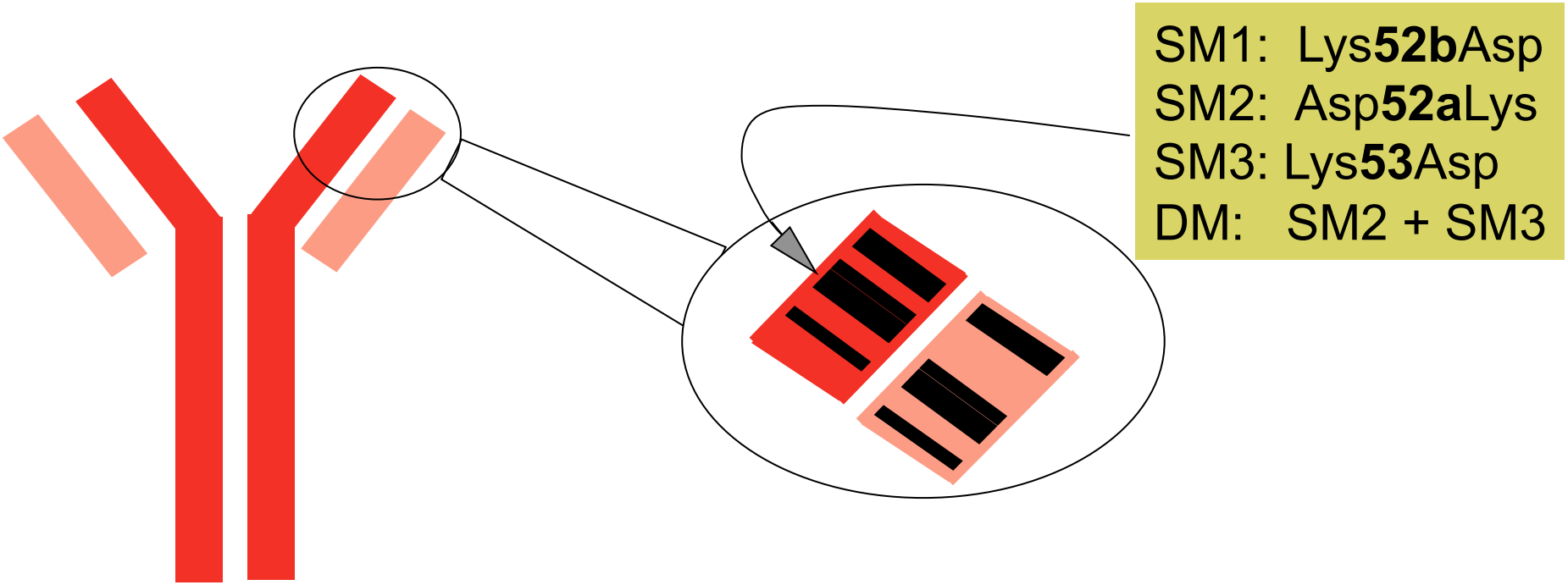
PREVENTING IMMUNOGENICITY BY PRE-TOLERISING TO A MUTANT FORM

Creating Non-Cell Binding Mutants of ALEMTUZUMAB

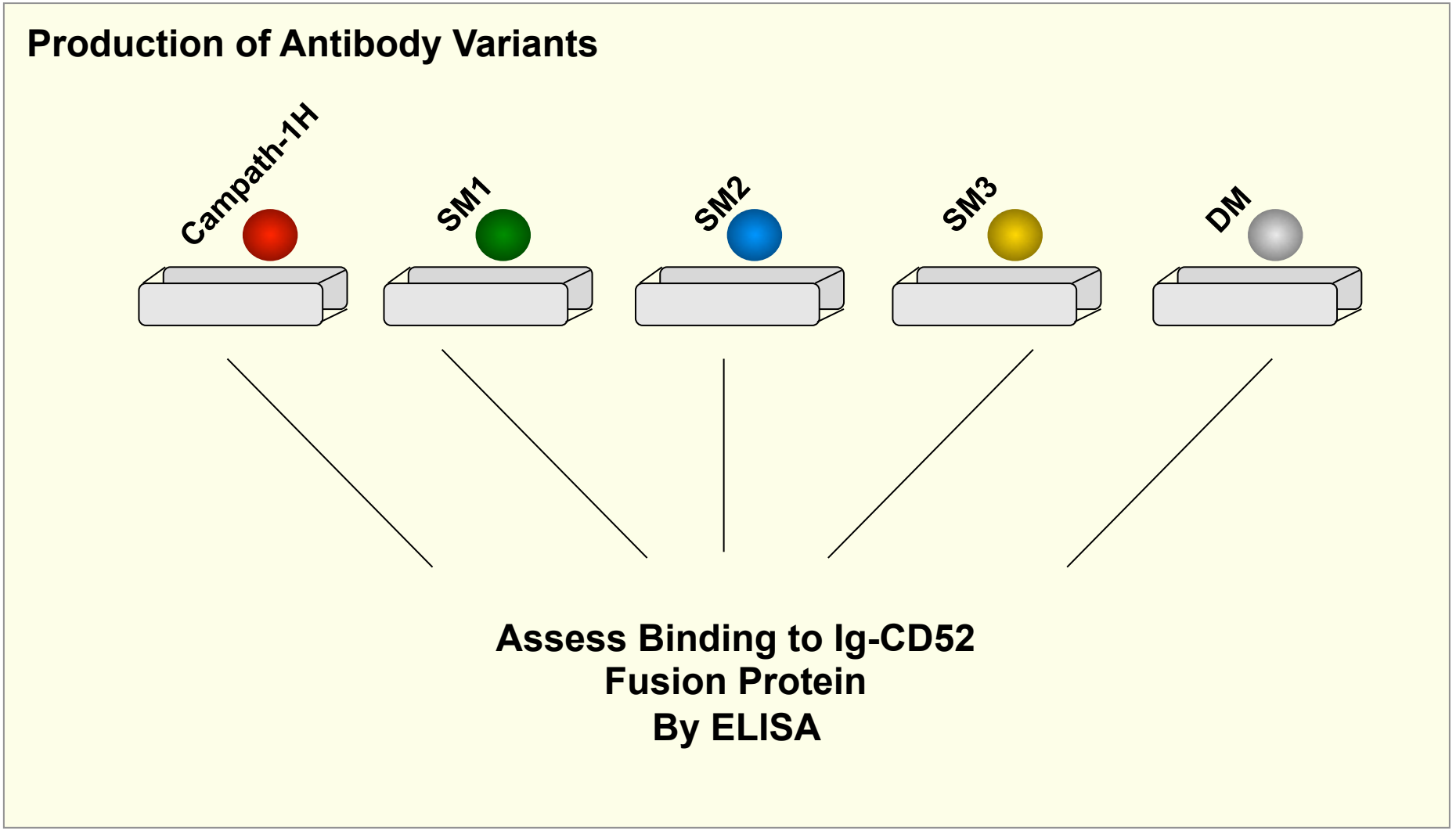


Gilliland et al. The Journal of Immunology, 1999, 162: 3663-3671.

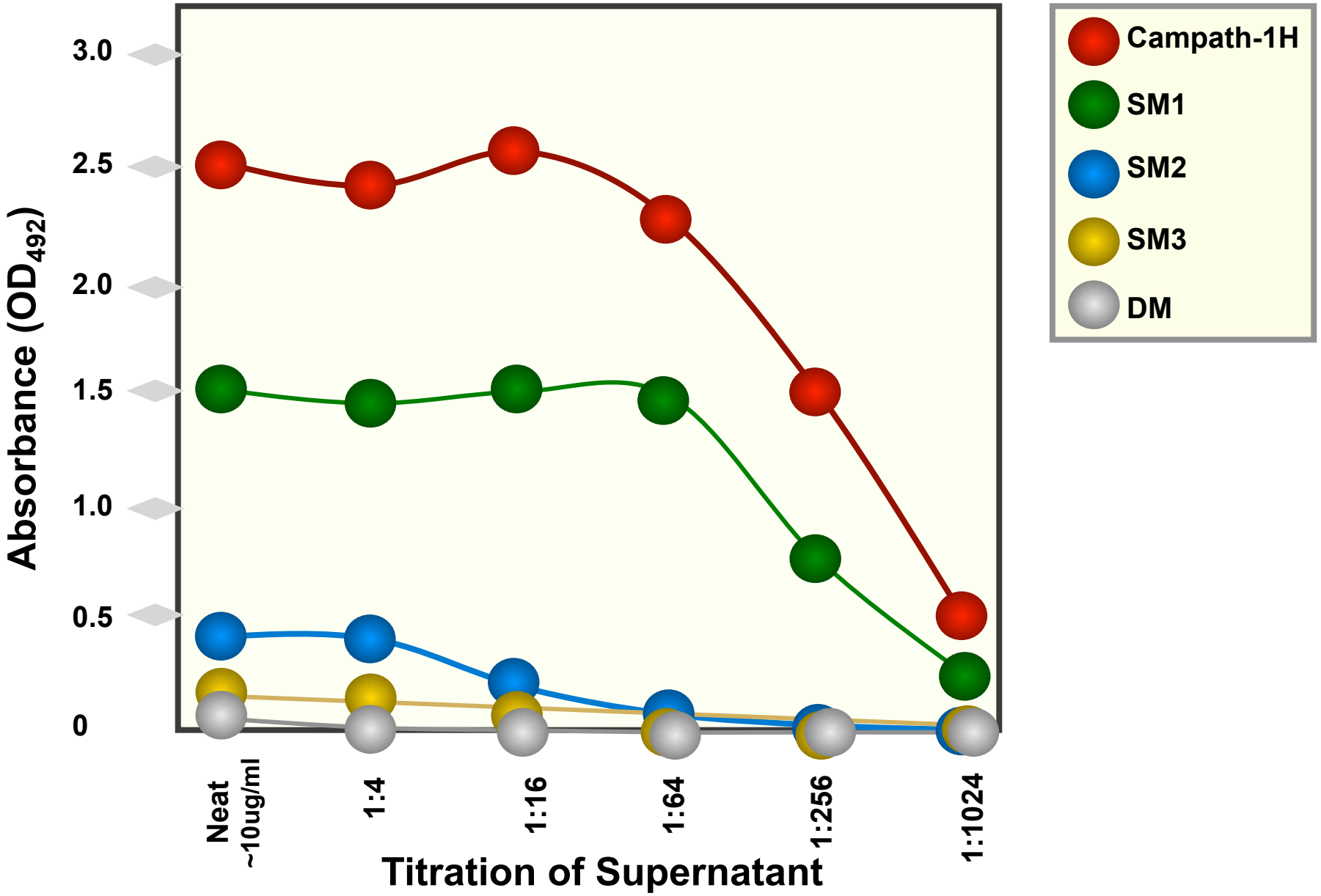
Creating Non-Cell Binding Mutants



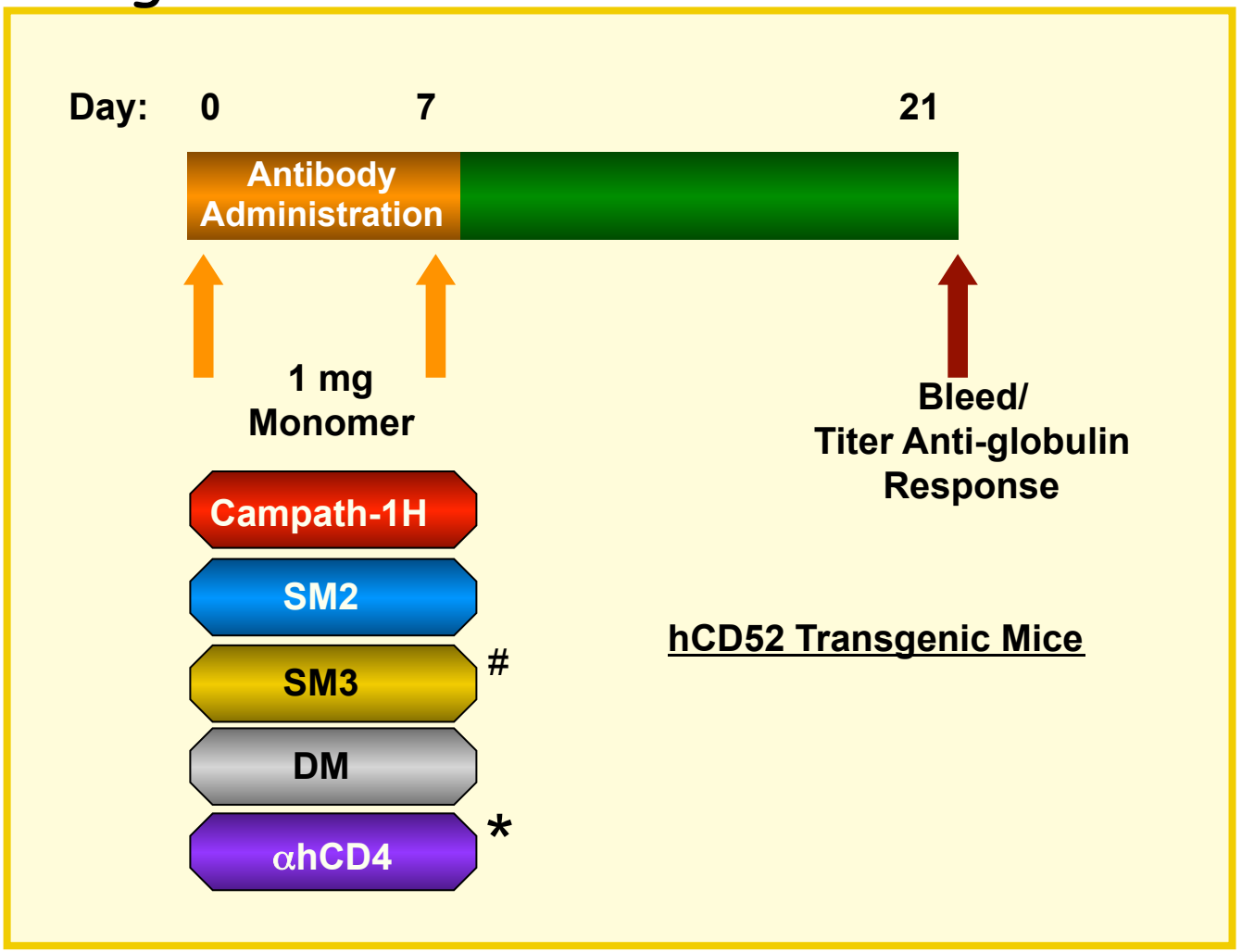
Assessing *Non-Cell Binding* Mutants Against Target ***in vitro***



Binding of Minimal mutants to rIg-CD52



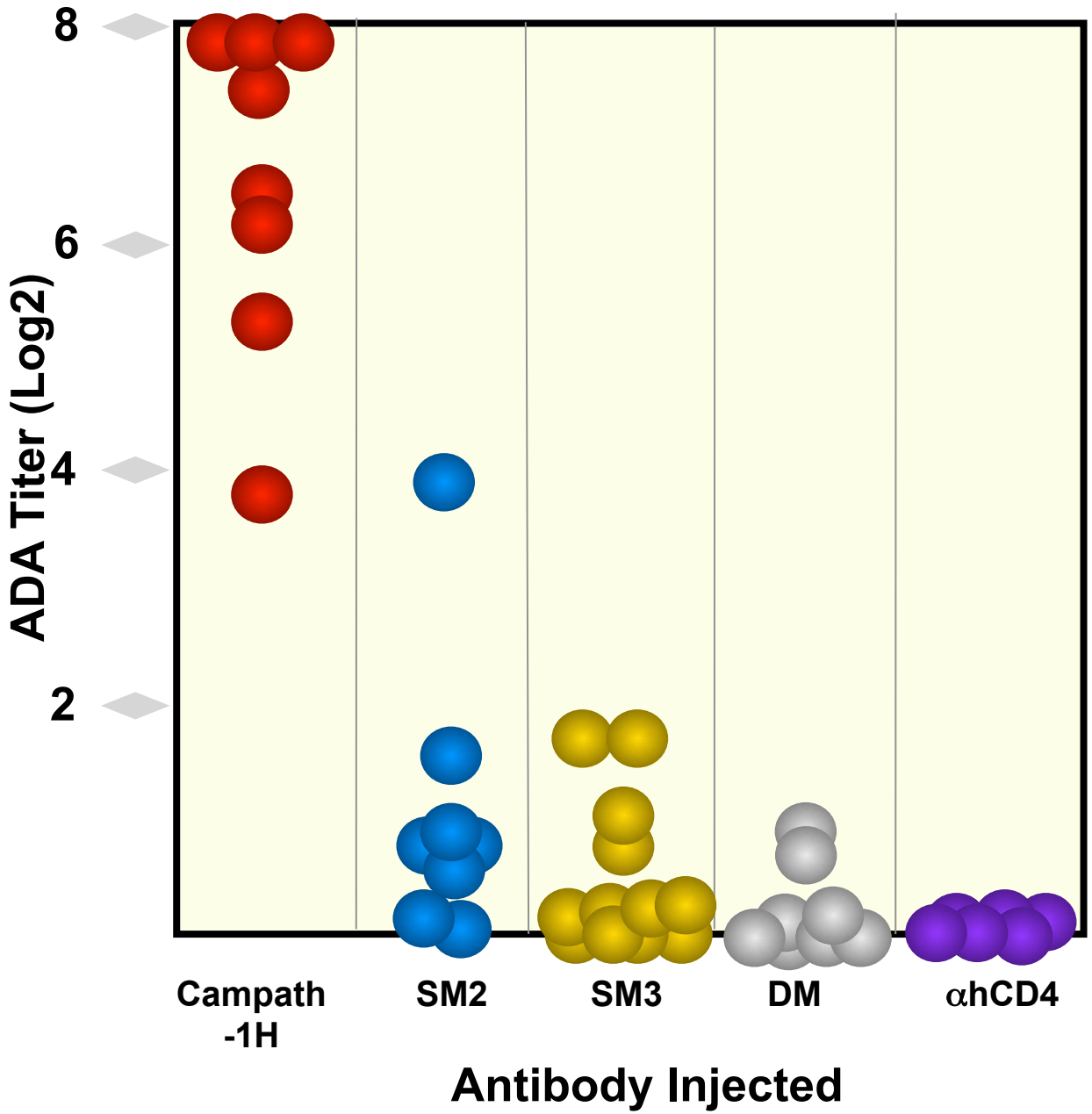
Assessing immunogenicity of *Non-Cell Binding* Mutants Against Target ***in vivo***



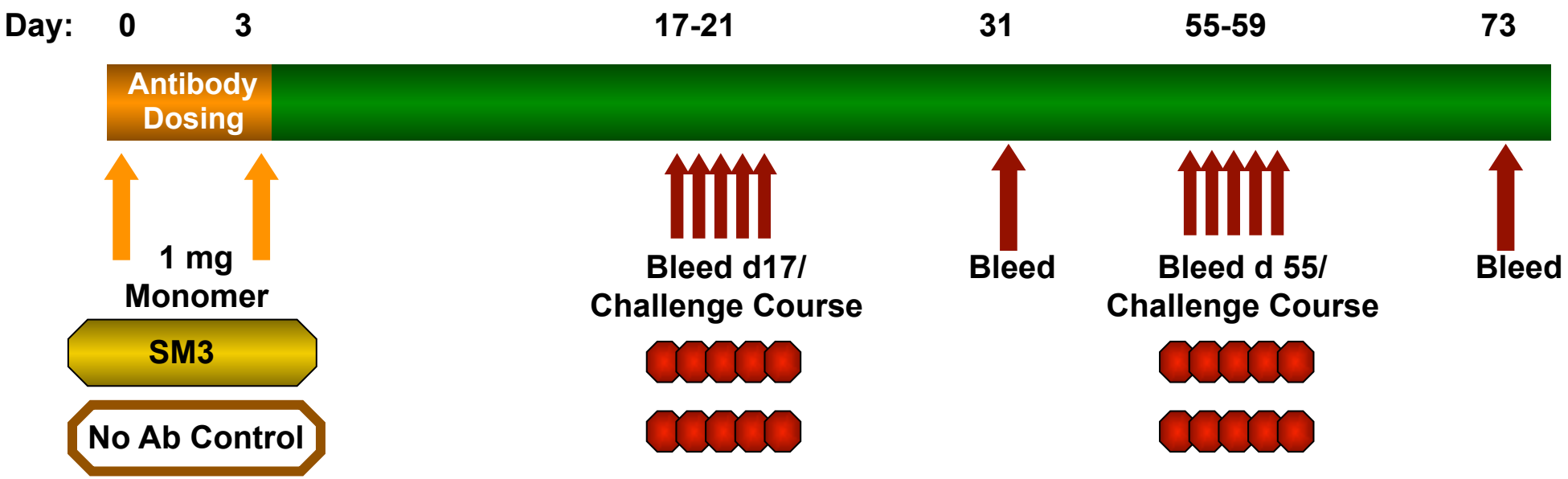
\# Was injected at Days 0 and 3

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

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

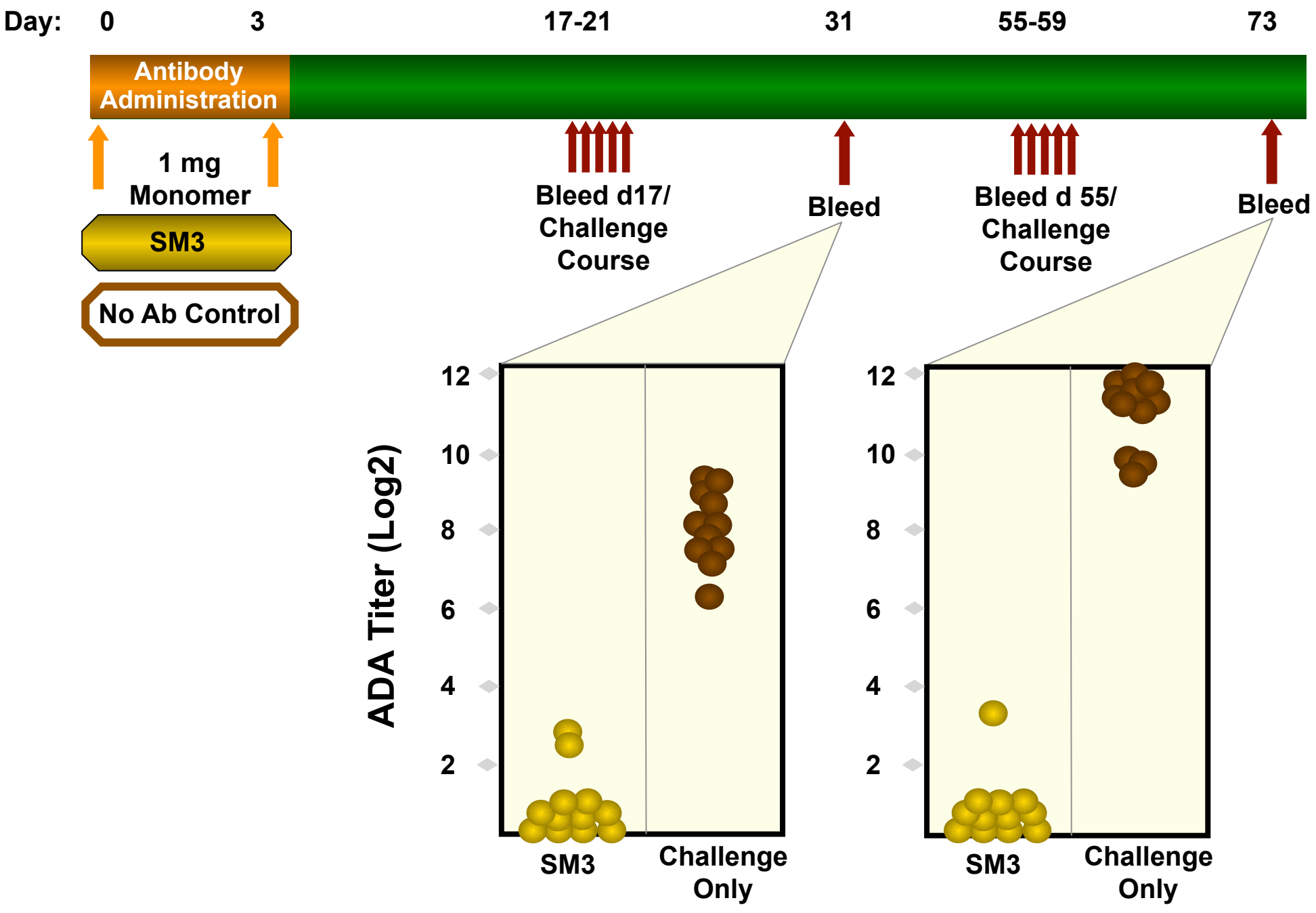


Non-Cell Binding Mutants: Tolerization Protocol

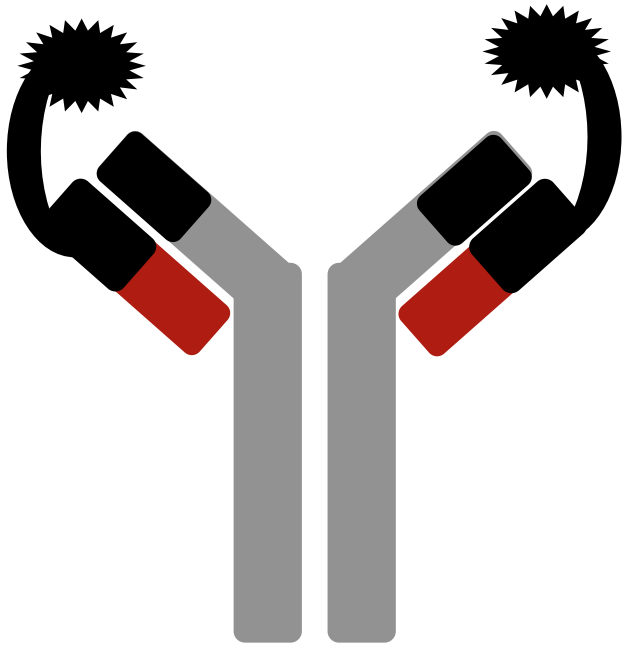


hCD4

Tolerizing with *Non-Cell Binding* Mutants Against Target ***in vivo***



One stage tolerisation



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.

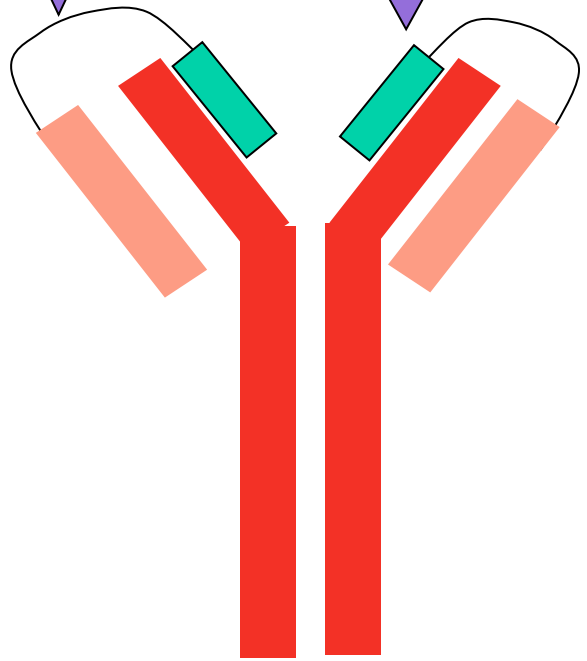
Following the tolerization, the same antibody *is able* to bind it's target and fulfill it's therapeutic goal.

Principal:-interfere with antigen-binding yet retain efficacy

Incorporating a stable yet flexible linker

$(Gly_4Ser)_n$

at the N-terminus of each Light Chain



Identify a peptide that can mimic the Ab's natural epitope … a "mimitope"

Incorporate this peptide at the N-terminus of the stable Linker

The binding of the obstructive element is reversible

Conclusions

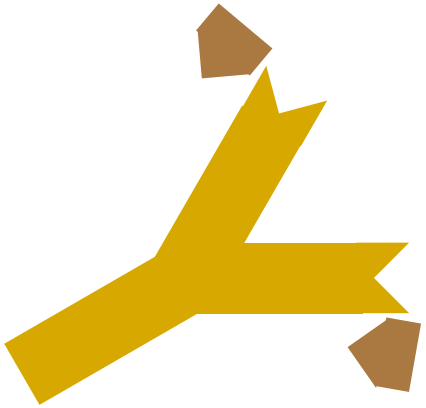
Non-cell-binding

Not immunogenic

Tolerogenic

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

Cell-Binding



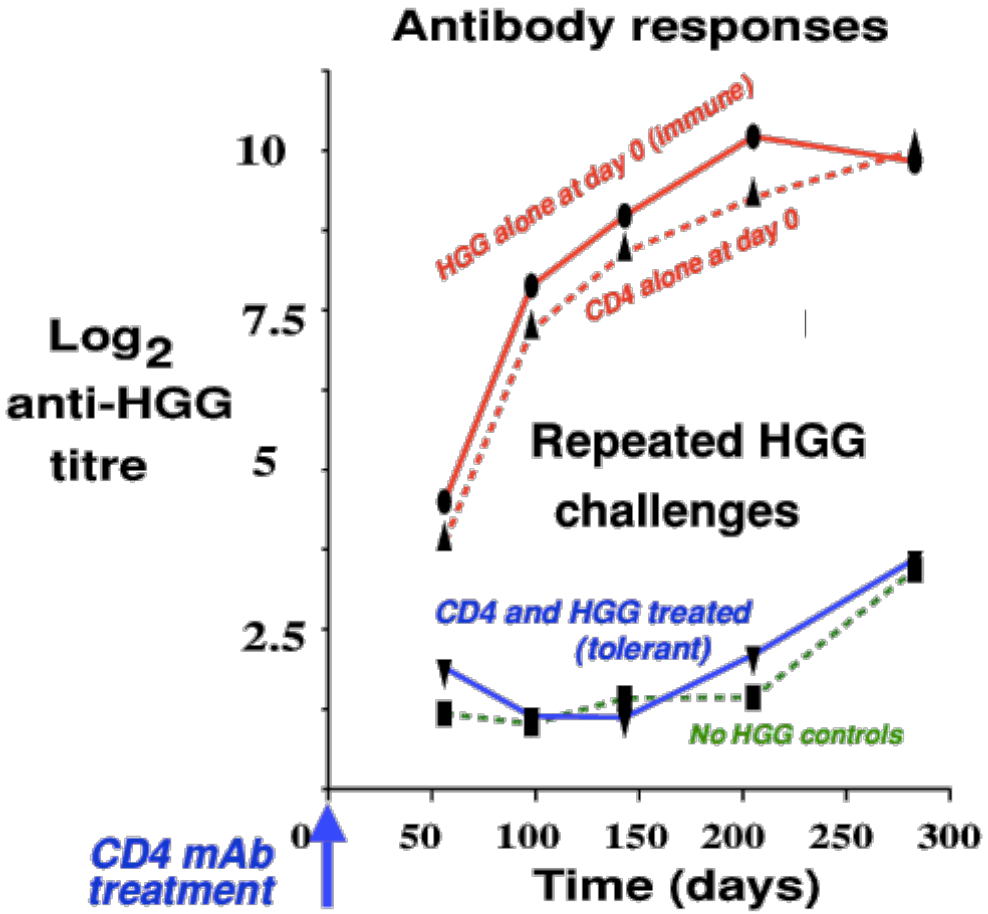
Tolerance is achieved over the initial two weeks

Biological activity: Cell depletion

Depletion lasts longer:

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.

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

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.
- "Foreigness" (adaptive immune system)

- Generic strategies aimed at minimising perception fo 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)

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)