Beyond Antibodies

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Phage antibodies: filamentous phage displaying antibody variable domains

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NEW ways of making antibodies have recently been demonstrated using gene technology. Immunoglobulin variable (*V*) genes are amplified from hybridomas or B cells using the polymerase chain reaction, and cloned into expression vectors. Soluble antibody fragments secreted from bacteria are then screened for binding activities (see ref. 1 for review). Screening of *V* genes would, however, be revolutionized if they could be expressed on the surface of bacteriophage. Phage carrying *V* genes that encode binding activities could then be selected directly with antigen. Here we show that complete antibody V domains can be displayed on the surface of fd bacteriophage, that the phage bind specifically to antigen and that rare phage (one in a million) can be isolated after affinity chromatography.

The heavy (V_H) and light (V_L) chain variable (V) domains of the anti-lysozyme antibody D1.3 (ref. 2) associate tightly as an Fv fragment and bind to antigen with a similar affinity to that of the parent antibody[3]. To allow expression of both domains on the same polypeptide, they were joined by a flexible linker $(Gly_4\text{-}Ser)_3$ (ref. 4), and the single-chain Fv fragment (scFv) cloned into an fd phage vector (fdCAT1) at the N-terminal region of the gene III protein (Fig. 1). The gene III protein is normally expressed at the tip of fd phage (about four copies per virion), is responsible for attachment of phage to the bacterial F pilus[5], and has been used to display peptide epitopes at the surface of the phage[6–9]. The antibody–gene III fusion was detected in the recombinant phage (but not in parental fdCAT1 phage) by western blotting[10] of polyacrylamide–SDS gels[11], and probing with antisera against the D1.3 Fv fragment[3] (data not shown).

Binding of phage to lysozyme was then analysed by enzyme-linked immunosorbent assay (ELISA) (Fig. 2). The phage had the same pattern of reactivity as the D1.3 antibody[12], and bound to hen egg-white lysozyme, but not to turkey egg-white lysozyme, human lysozyme or bovine serum albumin. The specificity of the phage is particularly illustrated by the lack of binding to the turkey egg-white lysozyme, which differs from hen egg-white lysozyme by only seven amino acids[12]. The antigen-binding site is therefore displayed on the surface of the phage and retains antigen binding and specificity. We term phage that display antibody variable domains as phage antibodies.

As phage expressing small peptide epitopes can be purified from mixtures of other phage using antisera or antibodies[7,8], we attempted to purify phage antibody (D1.3) using antigen. The phage antibody was mixed with the parental fdCAT1 phage (Fig. 1 legend) and 10^{12} phage passed over a column of lysozyme–Sepharose. Colonies derived from the eluates were analysed by probing with an oligonucleotide that detects only the phage antibody (D1.3) (see Table 1 and Fig. 3). At least a thousandfold enrichment of phage antibody (D1.3) was seen with a single column pass. By growing the enriched phage and passing it down the column again, enrichments of up to a millionfold were seen.

Enrichment was also demonstrated using purely immunological criteria. For example, 10^{12} phage (in a ratio 1 phage antibody (D1.3) in 4×10^6 fdCAT1 phage) was subjected to two rounds of affinity selection, and then 26 colonies grown and the phage assayed for lysozyme binding by ELISA. Five colonies yielded phage with binding activities, and these were shown to encode

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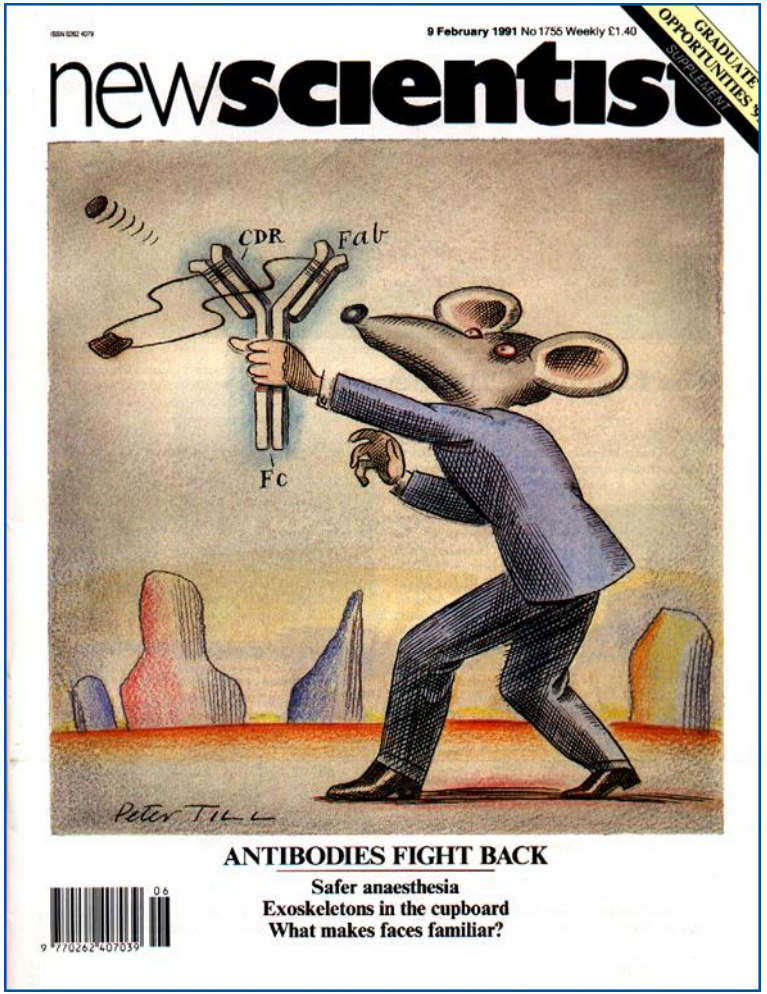
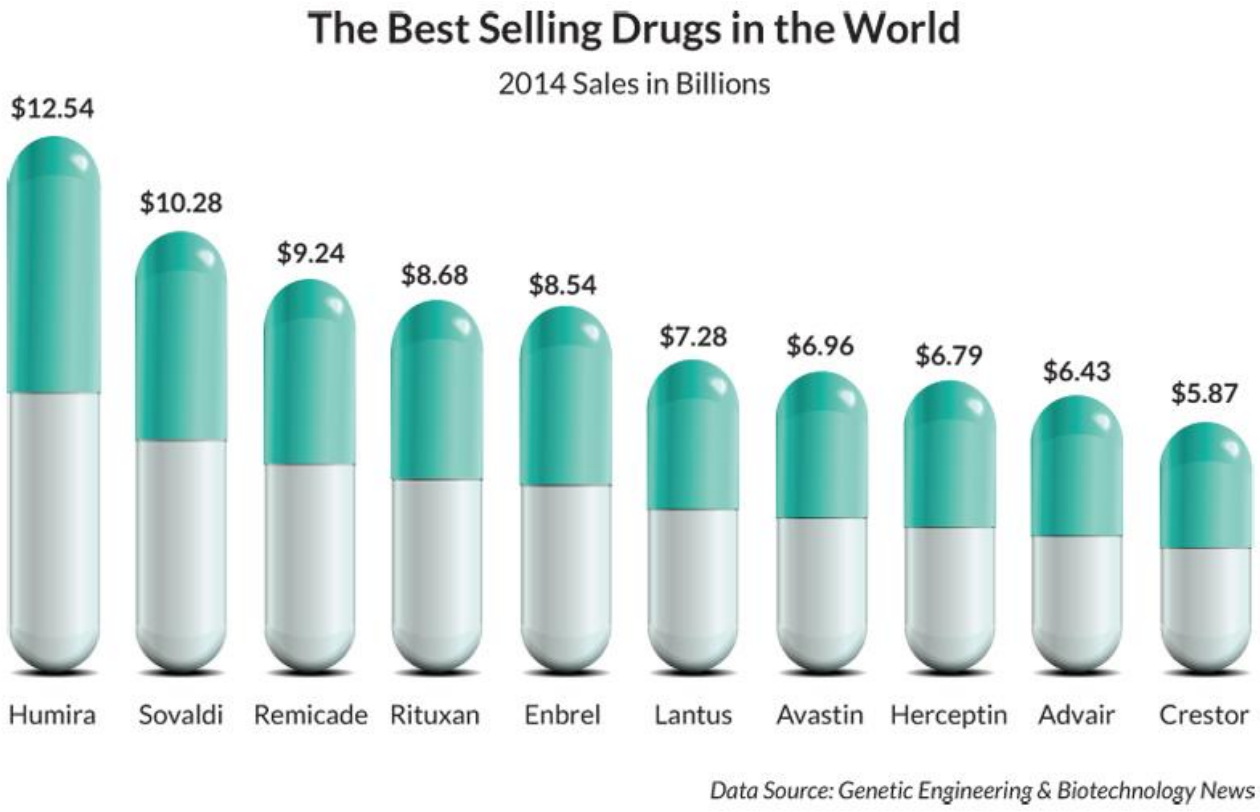
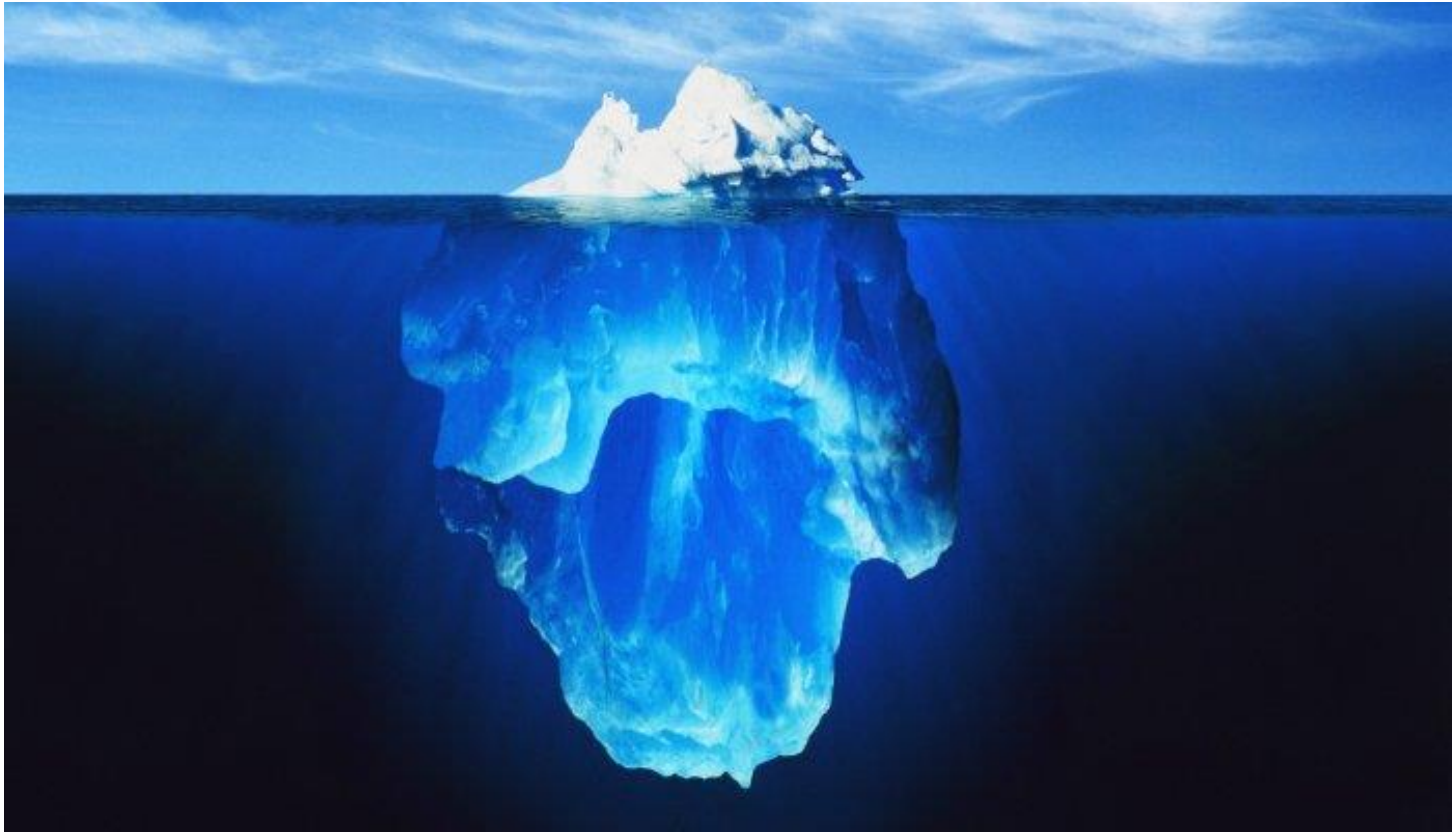
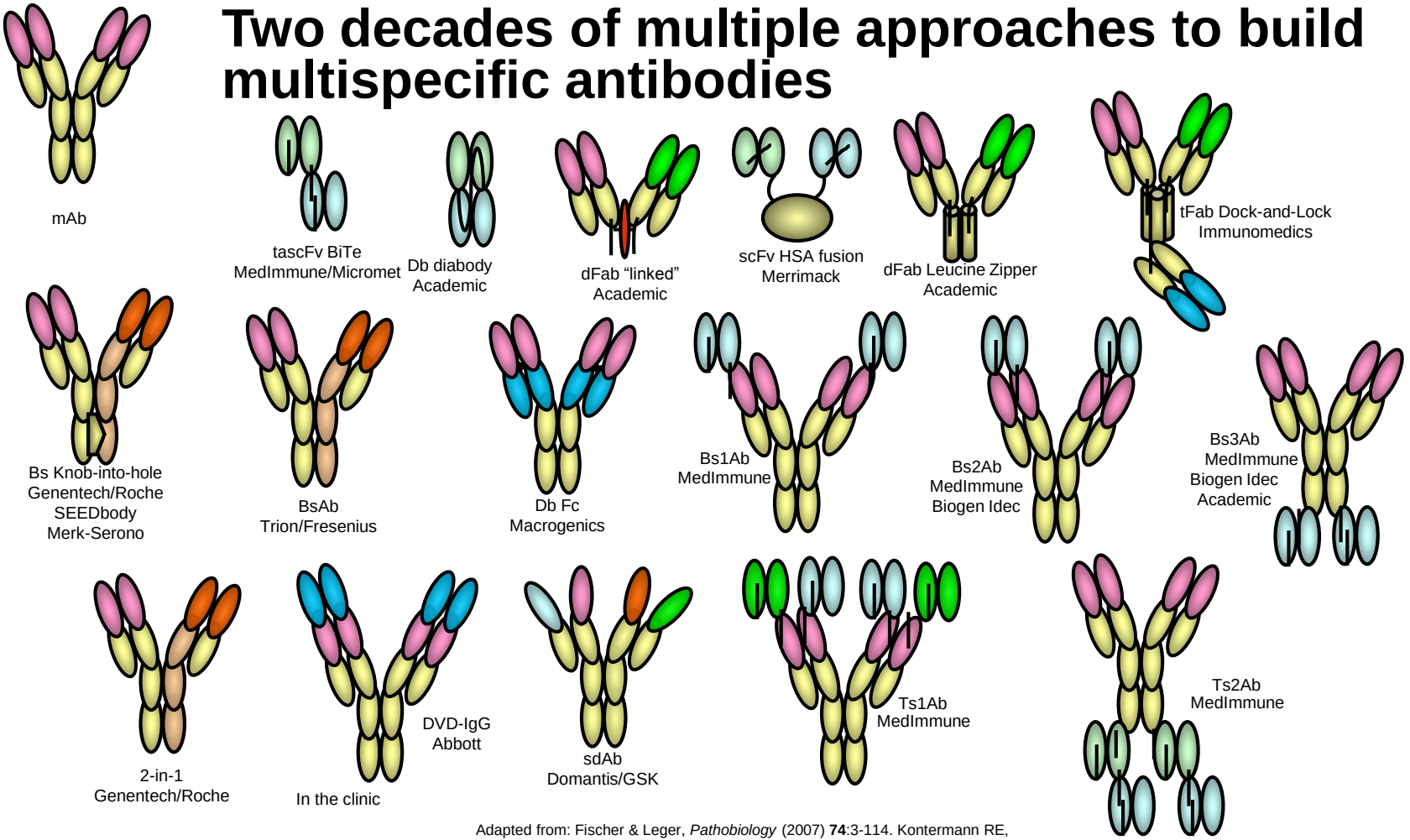



The Best Selling Drugs in the World
2014 Sales in Billions
$12.54
$10.28
$9.24
$8.68
$8.54
$7.28
$6.96
$6.79
$6.43
$5.87
Humira
Sovaldi
Remicade
Rituxan
Enbrel
Lantus
Avastin
Herceptin
Advair
Crestor
Data Source: Genetic Engineering & Biotechnology News

Tip Of The Iceberg?



Two decades of multiple approaches to build multispecific antibodies



Adapted from: Fischer & Leger, *Pathobiology* (2007) **74**:3-114. Kontermann RE, *Current Opinion in Molecular Therapeutics* (2010) **2**:176-183.

What Has Been Solved Over the Past 20 Years?

Human antibodies now routinely generated

Antibody manufacturing has undergone a step change in yield and process consistency

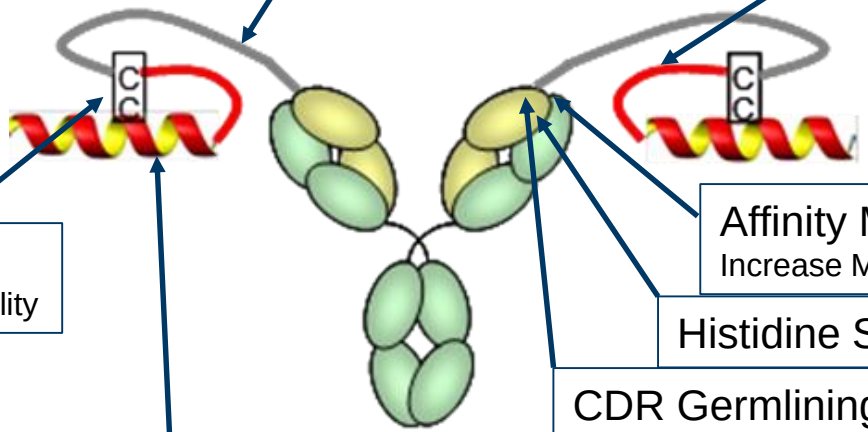
Disease biology has revealed validated targets which have resulted in potent drugs

PK/PD has become well understood as has the assessment of immunogenicity

Antibody formats and engineered effector functions have been developed to different disease settings and mechanisms of action

Cost of treatment has improved

Example – Peptide Mab Construct



Linker Length
Maintain peptide and Mab activity

Peptide C-terminus
Reduce Aggregation and optimise peptide activity

Disulphide
Improve *in vitro* and *in vivo* stability

Affinity Maturation
Increase Mab activity

Histidine Switch

CDR Germlining
Reduce immunogenicity

Peptide point mutation
Improve *in vitro* and *in vivo* stability
Optimise peptide potency

Agenda

- **The rapidly growing pipeline of Antibody Drug Conjugates (ADCs) requires a toolbox approach to problem solving and the safe development of this complex product class**
  - Dave Simpson, Glythera
- **Bispecific Antibodies: New Opportunities for Novel Therapies**
  - Mike Davies, F-Star
- **SoloMERsTM; Site-specific therapeutic biologics for the treatment of inflammatory disease**
  - Caroline Barelle, Elasmogen
- Questions and discussion – (all speakers)