Scientific Validity of Replacements for Animal-Derived Antibodies

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ESAC review of the scientific validity of replacements for animal-derived antibodies

- Working Group meeting on 8-9 November 2018
- Opinion endorsed on 3-5 June 2019

ESAC Core members: Rebecca Clewell (*Chair*); Carl Westmoreland
ESAC Ad-hoc members: Carl Borrebaeck; Andrew Bradbury; Stefan Dübel; Alison Gray; Achim Knappik; Andreas Plückthun
ECVAM: Joao Barroso, Marlies Halder

Expertise covering…

- Antibody generation with animal and non-animal technologies
- Antibody engineering
- Antibody use in many research applications including diagnostics, therapeutics and research
- Academia and industry

Charge question

Review the available proof of the **scientific validity** of antibodies and non-antibody **affinity reagents**, used in **research**, **diagnostics** and **regulatory applications**, **generated using animal-free technologies**

Definitions

Animal-derived antibodies – Produced using methods where animal immunization is required.

- Includes monoclonal and polyclonal antibodies, and immunized recombinant methods.

Nonanimal-derived antibodies – Immunglobulin antibodies derived from in vitro systems without the use animal immunization at any phase of production.

Non-antibody affinity reagents – Non-immunoglobulin scaffolds including aptamers and affimers. May use peptide or oligonucleotide scaffolds.

Recombinant technologies - Recombinant technologies may be used in the production of both animal and non-animal derived antibodies. This term encompasses a wide range of techniques that includes libraries from immunized animals and hybridomas.

Phage display: the technology that revolutionised animal-free antibody production

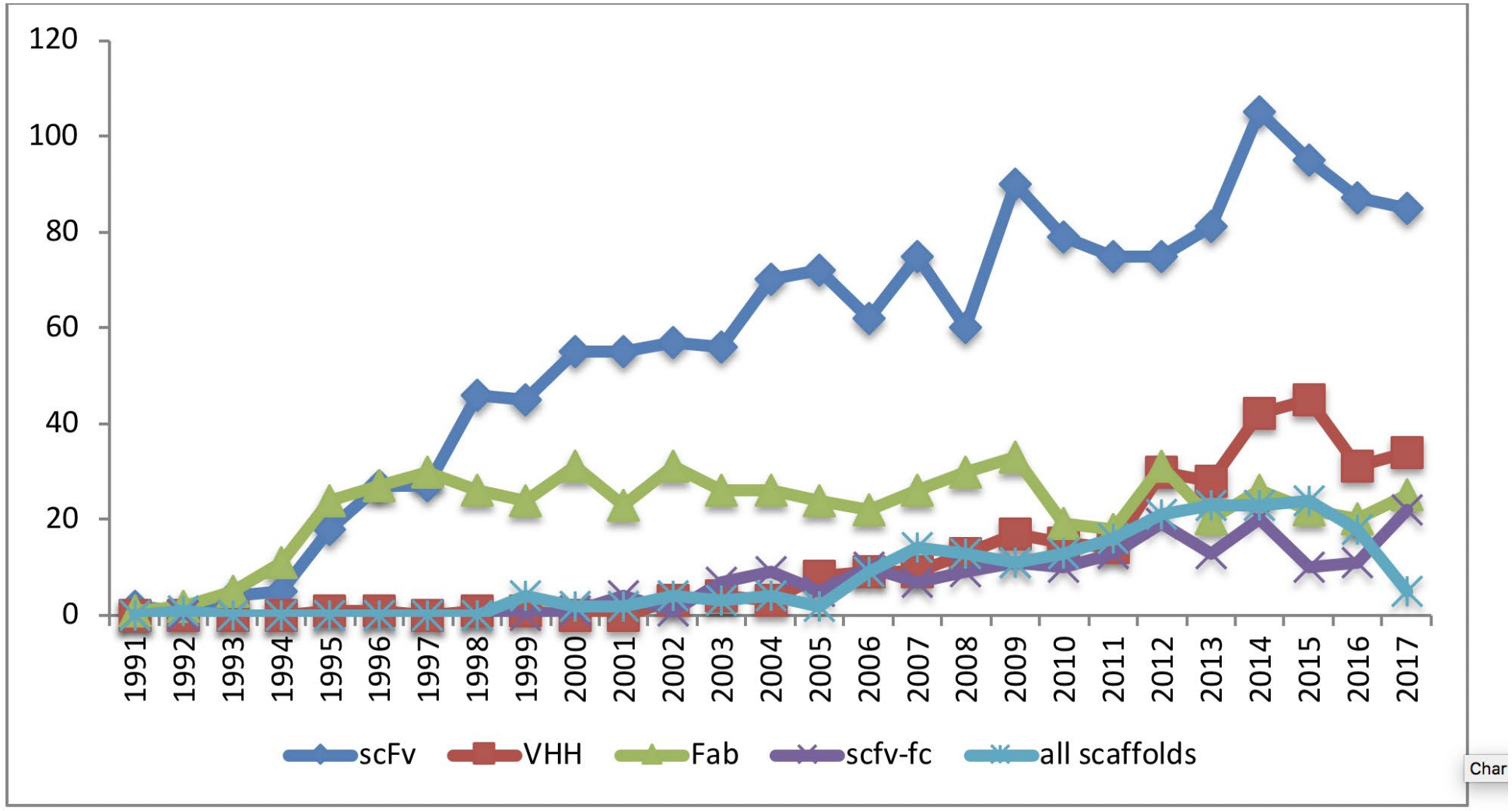
The 2018 Chemistry Laureates

The Royal Swedish Academy of Sciences has decided to award the Nobel Prize in Chemistry 2018 with one half to Frances H. Arnold "for the directed evolution of enzymes" and the other half jointly to George P. Smith and Sir Gregory P. Winter "for the phage display of peptides and antibodies".

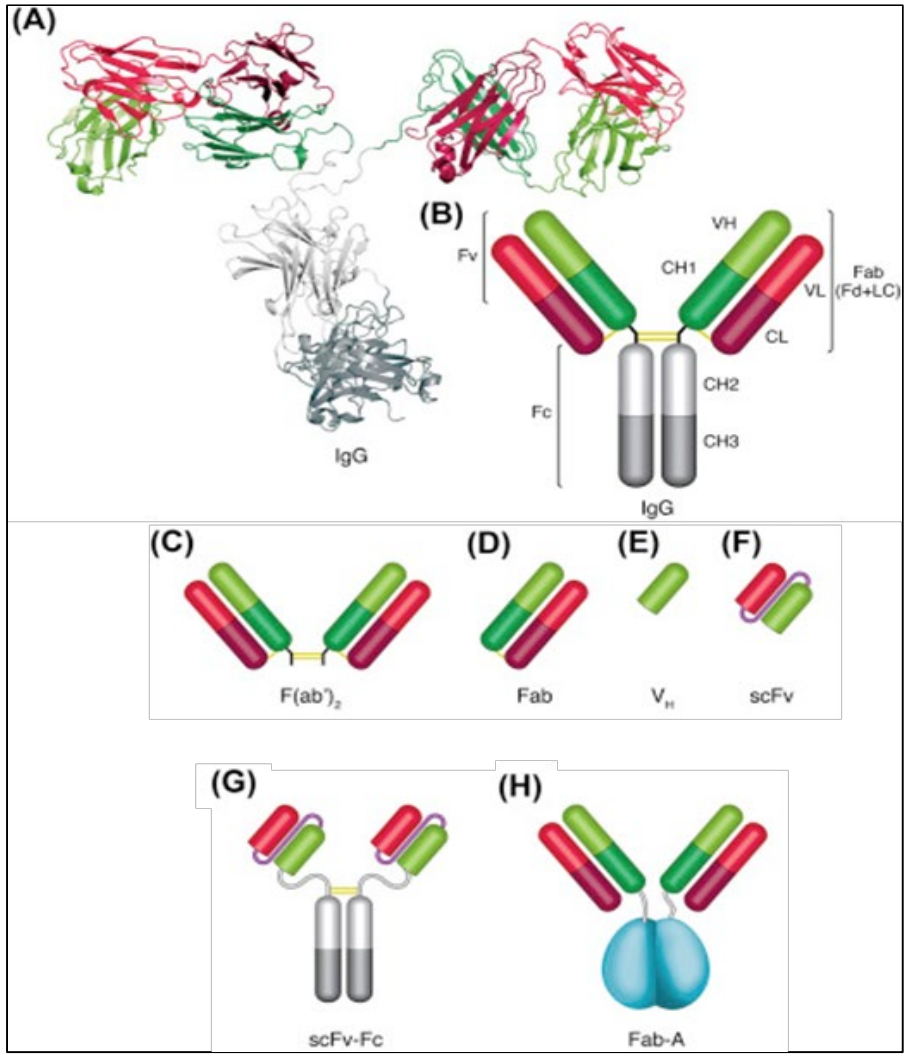
Read the press release

Ill. Niklas Elmehed. © Nobel Media

Current state of nonanimal-derived antibodies



Recombinant antibody formats



(A) (B) Full-length IgG antibody where V=variable,C=constant, H=heavy, L=light, F=fragment, ab=antibody binding. Disulfide bonds are dark yellow. (C) Bivalent F(ab')2
(D) Monovalent Fab.
(E) Single-domain VHH antibody.
(F) Single-chain fragment variable (scFv).
(G) scFv-Fc are scFvs dimerized by the Fc domain.
(H) Fab-A is with the fusion of alkaline phosphatase, that can be directly detected using a colorimetric substrate and is an example of a wide variety of tags that can be directly fused to the antibody fragment, pre- or post-selection.

Principles of action

The design of a non-animal derived antibody universal phage display library and selection of binders adopts the same mechanistic principles of nature that have been relied upon to produce animal derived antibodies thereby mimicking and surpassing the whole animal immune response through the careful control of *in vitro* parameters.

These include:

combinatorial diversification,

selection of antibody-antigen binding partners, and

affinity maturation for improved specificity.

Current applications for nonanimal-derived antibodies - research

- Research Applications
  - ELISA (sandwich)
  - Western blotting
  - IHC
  - Flow cytometry
  - IP / Pull-down
  - Multiplexed assays / Arrays
  - Microfluidic assays
- In vitro diagnostics
  - ELISA (sandwich, competition, direct, indirect, homogeneous)
  - Calibrators and controls
  - Lateral flow
  - Immunohematology, e.g. blood typing
  - Multiplexed assays
- Biopharma analysis
  - PK and immunogenicity testing
- Environmental monitoring, security
- Food testing

Case studies with nonanimal derived antibodies

- Non-animal derived antibodies in clinical use
  - 20/125 human or humanized antibodies in Phase II, III or approved, are human antibodies derived from phage display (Jain et al., 2017)
- hClat
- EU project to demonstrate feasibility of generating the large number of antibodies
- Publications showing favorable comparison to traditional technologies
- Non-animal derived antibodies are successfully used in diagnostic applications
- Catalogue antibodies exist and are similar in price to traditional antibodies

Scope of the Working Group Report

With the goal of supporting current research and diagnostic applications, the working group focused on non-animal derived antibodies, as they:

are relatively mature technologies,

have large bodies of evidence supporting their utility,

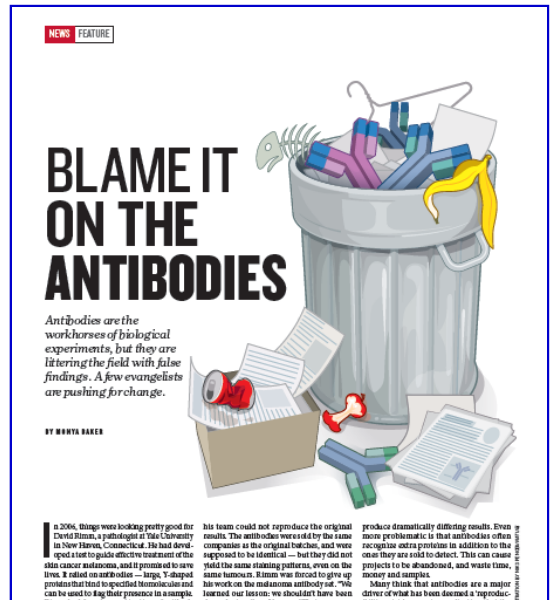
have been used in broad ranging applications, and

have few perceived hurdles to rapid implementation (e.g., cost, patents).

It was noted, however, that there would be value in convening a separate working group to review non-antibody affinity reagents as replacements for animal-derived antibodies.

Advantages of nonanimal-derived antibodies

- Control over affinity selection conditions (e.g. selection under defined biochemical conditions selects only antibodies that are functional at these conditions)
- Free choice of detection system (e.g. fusion to tags, enzymes, etc.)
- Non-animal-derived antibodies are sequence-defined:
  - Polyclonals not sequence-defined
  - Hybridomas only sequence-defined with substantial effort
- Duplicate antibody with identical binding and specificity profiles easily reconstituted
- Unlimited reproducibility of scientific results:
  - Reproducibility of antibodies over time and across labs
  - No batch-to-batch variation
  - No irretrievable loss of clone



NEWS FEATURE

BLAME IT ON THE ANTIBODIES

Antibodies are the workhorses of biological experiments, but they are littering the field with false findings. A few evangelists are pushing for change.

BY MONYA BAKER

In 2006, things were looking pretty good for David Rimm, a pathologist at Yale University in New Haven, Connecticut. He had developed a test to guide effective treatment of the skin cancer melanoma, and it promised to save lives. It relied on antibodies — large, Y-shaped proteins that bind to specified biomolecules and can be used to flag their presence in a sample.

His team could not reproduce the original results. The antibodies were sold by the same companies as the original batches, and were supposed to be identical — but they did not yield the same staining patterns, even on the same tumours. Rimm was forced to give up his work on the melanoma antibody sat. "We learned our lesson: we shouldn't have been

produce dramatically differing results. Even more problematic is that antibodies often recognize extra proteins in addition to the ones they are sold to detect. This can cause projects to be abandoned, and waste time, money and samples.

Many think that antibodies are a major driver of what has been deemed a 'reproduc-

Misconceptions about limitations of nonanimal-derived antibodies

- Perceptions that non-animal-derived antibodies have low affinity due to wrongly comparing avidity of animal-derived antibodies to monovalent affinity of non-animal-derived antibodies
  - After selection, non-animal-derived antibodies similar to rat and mouse monoclonal affinity
  - After affinity maturation, non-animal-derived antibodies similar to rabbit monoclonal affinity
- Few providers perceived as lack of utility, but actually more related to cost/demand (majority focused on therapeutic applications)
- Some of the same limitations as conventional antibodies
  - Antigen specific difficulties: carbohydrates, complex biological samples, etc.
  - *These limitations represent areas that have not been fully explored, not necessarily impossibilities*

ESAC Opinion

1. **Non-animal-derived antibodies are mature reagents generated by a proven technology**
   - No general or systematic disadvantages with respect to affinity, stability/shelf life and specificity
   - Used in approved therapeutic & diagnostic applications
   - Available from catalogues as research reagents and generated as commercial service
   - Thousands of non-animal-derived affinity reagents generated in EU- & NIH-funded programs

ESAC Opinion

2. **Non-animal-derived antibodies offer significant additional scientific benefits**
   - Knowledge of the sequence provides a unique identifier as well as unlimited and sustainable supply, which will improve experimental reproducibility
   - Phage display technology allows the guided selection of essential properties, such as specificity, compatibility to certain assay conditions, cross-reactivities, stability or affinity

ESAC unanimous conclusion

The experts conclude on the scientific evidence that **non-animal-derived antibodies are able to replace animal derived antibodies in the vast majority of applications**. Moreover, well-characterised, recombinant affinity reagents **will improve the reproducibility of science** and positively impact society

EURL ECVAM recommendations

- Awareness raising and dissemination of information: availability, cost, scientific benefits
- Education and training: webinars, e-learning, hands-on training courses
- Project authorisation: use of animals to generate antibodies should systematically be challenged by authorising bodies
- Review of publicly and privately funded projects: in the interest of ethical standards and quality of science, all funding applications should detail any newly generated antibody, and these should be non-animal-derived
- Provision of funding to fully characterise affinity reagents generated in EU- (and US NIH)-funded programs
- Manufacturers/suppliers should replace the animal-derived antibodies available in their catalogues by non-animal derived affinity reagents.
- Academic institutions should co-ordinate efforts to establish non-animal-derived universal recombinant libraries for their research activities

Thanks!

Any questions?