Integration of Recombinant Antibodies

The following Consensus Building Paper presents key topics described in the following sources: comments from pre-workshop online discussions made on protocols.io; feedback from the Workshop Steering Committee; and relevant publications. All content not referenced is derived from online discussions. Source material as of August 30, 2016.

The purpose of this paper is to provide a starting off point for the dialogues and consensus-building process at the *Antibody Validation: Standards, Policies, and Practices* Workshop. The conclusions and preliminary recommendations are not final but are meant to serve as a basis for further discussion.

Existing antibody production methods have considerable limitations that affect their variability over time (Bradbury & Pluckthun, 2015a; Bradbury & Pluckthun, 2015c). Polyclonal antibodies vary in composition and functionality between batches despite the use of the same immunogen. In addition, although less variable, hybridoma cell lines suffer from variability caused by genetic drift, loss of antibody genes, and cell line maintenance. Antibodies are the only widely-used reagents in molecular biology that are undefined at the molecular level and hard to confirm by users. In contrast, recombinant binding reagents provide a solution for the challenge of experimental variability (Bradbury & Pluckthun, 2015a). Purification of antibodies derived from cell lines expressing recombinant antibody genes could result in greater product consistency. Moreover, the sequenced gene immortalizes the antibody and secures its existence beyond the life of the cell line or the company producing it.

Recombinant antibodies come with unique challenges, including the release of sequence information as a transparent and universal reference. The concepts, perspectives, and preliminary recommendations described herein about recombinant antibodies are derived from the pre-workshop online dialogues and published literature. The recommendations are intended to promote thoughtful consideration of and highlight efforts needed to drive broad use of recombinant antibodies in research, the potential for and barriers to open-access sequence information, and the legal environment surrounding recombinant antibodies. This summary paper will serve as a basis for building consensus at the *Antibody Validation: Standards, Policies, and Practices* Workshop.

Benefits of Recombinant Antibodies, Focus on Reproducibility | Recombinant reagents must be validated, as with all binding reagents, for sensitivity, specificity, and selectivity, and thus still require the development of antibody validation standards (Bradbury & Pluckthun, 2015b; Freedman, 2015). However, commercial availability of recombinant antibodies addresses some of the considerable challenges of product consistency and reproducibility of non-recombinant antibodies in several ways:

- By allowing for targeted design and optimization, recombinant reagents are customizable and can have superior specificity allowing recognition of minor changes in target molecules;
- By enabling the production of humanized reagents;
- By defining antibodies based on their molecular identity and functionality, ensuring reagent consistency, reproducibility over time, and improved information-sharing practices;
- By reducing the need for routine and repeated validation (Bradbury & Pluckthun, 2015b).
- By opening up areas of research that require access to the gene, such as intracellular expression, chimeric antigen receptors, and cell surface expression.

Several considerations influence the net benefit of and the economics surrounding broad use of recombinant antibodies:

- Guidelines, ideally developed and promoted jointly by key stakeholders, should provide guidance on whether and how antibody sequence and function should be considered to maximize high-quality antibody reagents, despite the availability of sequence-similar reagents. The cost of developing recombinant reagents (approximated at $40K) is higher than polyclonal and monoclonal antibodies (Weller, 2016). Thus, the generation of sequenced recombinant antibodies across entire catalogs may not be financially justifiable if products are not routinely ordered. However, funding provided by a variety of sources could help off-set this challenge;
- Polyclonal and monoclonal antibodies are widely used (Baker, 2015), but many companies provide recombinant antibodies in catalogs now. A focus on only recombinants may result in antibody catalogs shrinking to the most frequently used recombinant reagents, ultimately limiting the diversity of antibodies available commercially. If non-recombinant reagents are validated in a transparent manner with caveats understood, limiting their availability may hinder scientific advancement. Nonetheless, a transition should be encouraged since the problem of immortality, or questionable identity of different antibodies cannot be solved with monoclonal technology for fundamental reasons.

Comparison of Epitope and Sequence Information

Many companies keep epitope sequences or detailed antigen information as proprietary (Bordeaux et al, 2010; Weller, 2016). Antigen identity is important for researchers to be able to analyze validation data and the suitability of an antibody in their intended experimental use, while epitope sequence resolves issues with reproducibility and reagent traceability. Thus, sequence and epitope identity solve distinct problems surrounding antibody research

Figure 1 Describes the similarities and differences of the release of information on epitope identity as a comparison to the release of sequence information for recombinant antibodies.

Transparency in Sequence Information | The release of sequence information provides a transparent and universal reference system that may offer the following benefits:

- Open-access sequence information allows identification of reagents by researchers over time, enhancing reproducibility by enabling replication of studies with the same reagent;
- Knowledge of the antibody sequence allows researchers to develop new tools and reagents (e.g., chimeric antigen receptors, intracellularly or cell surface expressed antibodies, etc.) that advance scientific research;
- Accessible sequence information ensures that an antibody can be made even if the cell line is lost or altered or the producer goes out of business or stops making the reagent;
- If sequence information is unavailable, producers may offer antibodies with minimal or trivial sequence differences that do not affect function. Functionally indistinguishable reagents that are not linked via sequence similarity may limit the potential analysis of validation data and customer feedback.

Openly available antibody sequences present a challenge to antibody producers and suppliers, who risk losing competitive advantage if sequence information is accessible (Polakiewicz, 2015). Proposed challenges of open-access sequence information are listed below:

- Sequence information may not be valuable to researchers if traditional antibodies are thoroughly validated and traceable throughout their product life cycle even though they cannot be immortalized;
- Sequence data would require curation and quality assurance, increasing the overall cost of recombinant antibodies. However, bioinformatics can greatly streamline this process;
- Antibody sequences are held as trade secrets because their market value cannot justify the cost of patent acquisition and enforcement processes as part of intellectual property rights. Intellectual property issues present the greatest obstacle for open-access sequence information for recombinant antibodies. The costs associated with patenting every recombinant antibody within a catalog far exceed what is feasible for the industry, and as of now other means of protection (e.g. trademarks, copyrights) have not yet been adapted successfully. Figure 2 briefly summarizes recent cases involving the legality of patenting molecular tools.

Examples of Patent Issues Associated with Other Molecular Tools.

Issues surrounding the legality of patenting human genes were made prominent by the Myriad case, which involved the use of human BRCA1 gene in diagnostic tests. In 2013, the Supreme Court of the United States ruled that human genes were ineligible for patents, overturning 4,300 existing patents (2012). However, as part of this discussion, the Court ruled that recombinant DNA modified in a lab setting may be eligible, potentially leaving room for intellectual property rights of recombinant antibody sequences.

Patent applications that relate to therapeutic antibodies are defined by either the antigen or target, hybridoma, sequence, or functionality (Storz, 2011; Webber, 2006). Intellectual property rights surrounding therapeutic antibodies have shifted focus to antibody structure and variable regions, as opposed to functional claims, based on a 2011 ruling (2011). Although antibody patents had previously been exempt from the USPTO Written Description Guidelines, this ruling meant that new antibody patents would likely not be granted without more detailed characterization. The market value of research antibodies cannot compete with therapeutics, a factor limiting the feasibility of patenting antibodies for use in research (Polakiewicz, 2015; Weller, 2016).

Figure 2 Describes examples of patent issues associated with other molecular tools. These examples may be helpful with considering intellectual property issues associated with recombinant antibodies.

Next Steps and Potential Solutions | Several innovative solutions have been suggested to address some of the limitations of existing market pressures and financial considerations associated with wide use of recombinant antibodies:

- For broad inclusion into the antibody market, characterization and sequencing of existing monoclonal antibodies will help ensure that previously-used, high-quality reagents are retained;
- Increased use of methods that determine antibody sequences (e.g., antibody sequence display, two-hybrid methods, B-cell antibody characterization) will expedite the selection and production of reagents (Bradbury & Pluckthun, 2015a; Bradbury & Pluckthun, 2015c);
- An independent, trusted third party could hold sequence information and release accession numbers, effectively maintaining consistency throughout the market, protecting the sequence information, and preventing redundancy;
- A ready-to-use pipeline for generating and validating recombinant antibodies would allow companies to invest only in products that are in demand. Additionally, the cost could be adjusted to account for need;
- Other affinity reagents, including protein scaffolds and nucleic acid-based reagents, further solve some of the biological limitations of antibodies. These reagents are subject to the same issues as recombinant antibodies, including access to sequence information and the need for validation (Bradbury & Pluckthun, 2015a; Bradbury & Pluckthun, 2015c).

Recommendations for Discussion | Preliminary recommendations supporting the broad availability of sequenced recombinant antibodies based on online dialogues and literature include:

- Recombinant antibodies should be introduced more broadly into the antibody market, but not at the expense of other available reagents that are widely used.
- The release of sequence information can maintain continuity in antibody production and promote reproducibility. However, the costs associated with patenting reagents to protect intellectual property and cover up-front costs make the release of sequence information problematic. However, less expensive ways of protection (e.g., trademarks, copyrights) may alleviate this challenge. The proposal of a third-party in assigning sequence accession numbers could meet this goal without compromising revenue.
- Additional funding should be provided by various sources to support the generation of recombinant antibodies, ultimately promoting the technology further.
- In addition, support could be provided to examine different antibody production models – i.e., a "catalog model" versus a "production just-in-time" model – enabled by recombinant antibodies. (Bradbury & Pluckthun, 2015a).
- Consideration of economic factors and market forces is important for implementing recommendations on recombinant antibodies.

Concluding Remarks | The integration of new technologies into the market and the broad availability of recombinant antibodies address key issues surrounding antibody-based research. Recombinant antibodies could be defined by both identity and functionality, allowing for reduced validation requirements over time and increased quality, reproducibility, immortality, and consistency of affinity reagents. Consideration of the economic factors driving recombinant antibody availability and release of sequence information will be critical. A balance between scientific need and economic cost must be met to improve reproducibility through production of recombinant antibodies and release of sequence information.

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