Members of the Committee on Animal Resources (CAR) are concerned about the report prepared by Faculty Senate President Stephen F. Eisenman entitled “Report Concerning the Procurement and Use of Research Antibodies at Northwestern University” that was posted on the Faculty Senate webpage. While members of CAR feel that the overall goal proposed by Professor Eisenman is laudable, the plan he proposes is impractical and fiscally unfeasible. Specifically, Professor Eisenman calls for the establishment of a Northwestern University core facility that will be staffed and charged with preparing thousands of recombinant monoclonal antibodies with the specificities required for research use. The proposal, in large part, is based on flawed assumptions (see below for specifics). To impose this restriction would be detrimental to the research productivity of many laboratories and Northwestern University. Furthermore, the cost to the investigators would be prohibitive, as the University would inevitably pass the costs along to investigators at a rate that would be much higher than from commercial suppliers who have the ability to do this on a larger scale. The recombinant antibody approach would also not be useful for preparation of antibodies against conformational protein epitopes, carbohydrates, or lipids. In addition, monoclonal antibodies tagged with a host of different fluorophores are routinely used for flow cytometry assays to identify cell populations in health and disease - the vast numbers of these antibodies are only practically available to researchers by purchasing these pre-conjugated antibodies from numerous suppliers. It would be impractical to believe that a University facility could prepare the vast array of conjugated antibodies required by researchers, even if they could prepare the parent recombinant antibody. In conclusion, members of CAR feel that this is not an effort that should be undertaken by Northwestern University.

Below, we address the specific premise underlying the claims made by Professor Eisenman.

Claim: “monoclonal antibodies (mAb) produced in live animals (in vivo) should be replaced within three to five years by the procurement and use of recombinant antibodies (rAb) produced in the laboratory (in vitro)”

This statement is based upon the premise that mAbs are currently produced in vivo by growing antibody-secreting hybridomas as ascites or in vitro, after the “initial generation of a small number of hybridoma cells in the spleen.” Unfortunately, no further explanation is offered for the in vitro production method.

Unfortunately, inaccuracies mar the Report:

1. Use of ascites. Here Professor Eisenman correctly states that large quantities of antibodies may be obtained from ascites fluids. He cites a 1997 Office of Laboratory Animal Welfare (OLAW) brochure (reference 9). Dr Eisenman correctly indicates that ascites fluids also contain non-mAb serum antibodies. On May 20, 2015, Dr. Carl Waltenbaugh spoke with Dr Leonore ‘Lee’ Herzenberg. Dr. Leonore Herzenberg and Dr. Leonard Herzenberg were instrumental in inventing and developing the fluorescence-activated cell sorter (FACS, also known as a flow cytometer), and introducing mAb to the biomedical community. In fact, Dr. Leonore Herzenberg coined the term hybridoma in late 1976. For over 50 years, the Herzenberg laboratory at Stanford University has been on the cutting edge of biomedical discovery. When asked about the current use of ascites for mAb production, Dr. Leonore Herzenberg stated that neither they nor industry has used this methodology for years. Virtually all commercial antibodies are produced in bioreactors. And this is a sizable market. Transparencymarketresearch.com “predicts growth for this market to be around USD 60 billion in the year 2012 and is predicted to have a double digit growth of around 15% from the
year 2012 to 2018.” Lastly, mouse mAbs are often ‘humanized’ by recombinant technology for treatment of human disease and cannot contain contaminating unrelated antibodies or proteins.

2. Antibody validation. Jennifer Bordeaux (Eisenman reference 2) states that “antibodies against a synthetic peptide may not work well when a protein is in its native conformation with intact 3-dimensional structure.” Fixed and fresh proteins often have different 3-dimensional structures, meaning that a mAb that binds to a fixed protein may not bind to the protein in its native state and vice versa. Strong and specific binding in vitro does not necessarily predict performance in blood, tissue, or electrophoretically-separated lysates. The argument for validation of recombinant antibodies is identical and would require extensive use of in vivo-derived materials to confirm that they bind to native protein structure. Therefore, wholesale shift to recombinant antibodies, especially when mAb already exist, would necessitate the use of additional human and/or animal tissue.

3. Recombinant antibodies. Recombinant antibodies offer a number of strategies for epitope (antigenic determinant) binding. One strategy isolates epitope-binding single-chain moieties. Biologically, only members of camelidae (camels and lamas) produce single-chain antibodies. Biologic signaling resulting from antibody-epitope interactions often result from conformational changes in the constant part of the immunoglobulin molecule upon binding to an epitope. Immunoglobulin constant regions may be spliced onto epitope-binding recombinant antibody constructs and these are then placed into yeast cells, insect cells, or mammalian cells for production in a bioreactor. Yeast tend to over glycosylate proteins, in ways that mammalian cells do not. Insect cell constructs tend to over produce proteins to near the concentration that would result in crystallization. Basically, only vertebrate organisms respond to antigens by producing antibodies that are selected by biologically significant physiological mechanisms, mechanisms that are not present in yeast, bacteria or viruses.

High affinity recombinant antibodies can readily be produced and identified by high throughput screening methods. But is this the optimal solution? In biologic systems, where antibodies are used to identify tissues, such as circulating cells, antibodies with the highest affinity do not necessarily produce the desired effect. This is because antibody efficacy often depends not only on epitope binding, but on post-translational modifications, such as glycosylation of the non-binding portion of the immunoglobulin molecule (R Jefferis, Trends in Pharmacological Science. 30: 356-362, 2009). Thus, identification of optimal rAb's for biologic systems requires far more testing both in vitro and in vivo, than is seen for biologically mAb. Extensive rAb validation comes at the expense of use of additional animals.

Lastly, on February 21, 2012, the United States Supreme Court denied the patentability of mAb in Janssen v. Abbott. However, because of the nature of their construction, rAb are patentable. The wholesale discontinuance of mAb use at Northwestern University in favor of rAb raises the possible specter of greater expense for commercially available reagents.

Thus, contrary to Professor Eisenman’s report, rAb cannot fully replace conventional antibodies.

4. Core facility for recombinant antibody production at Northwestern University. While this is a wonderful idea, start-up costs are grossly underestimated. A flow cytometer alone costs approximately $250,000. The service contract required to maintain the instrument costs another 10% of the purchase price. Robotics and molecular biology support equipment
along with trained support staff would easily exceed $1 million per year. The establishment of a mAb facility at NU was proposed in the early 1980’s and despite repeated start-up attempts the facility proved to be financially non-viable.

5. **Current polyclonal antibodies.** Professor Eisenman rightfully points out the unethical behavior of two commercial polyclonal antibody producers. There is no justification for how these firms performed. Reports are that one, Rockland, has greatly improved its compliance with the animal welfare act. The other, Santa Cruz, does not appear to have made such efforts. In fact, amongst the immunological community, Santa Cruz antibodies have been dismissed as worthless.

**Action currently being taken by Northwestern University.**
Northwestern University only uses animals in research where there are no alternatives. In fact, the law demands that where a non-animal approach exists, it should be used. The principles of reduction, refinement and replacement of animals in research (the ‘3Rs’) underpin all related work carried out at Northwestern University. The Northwestern University Institutional Animal Care and Use Committee (NU-IACUC) is responsible for ensuring that the number of animals used is minimized and that procedures, care routines, and husbandry are refined and regularly reviewed to maximize animal welfare. Regarding the later, Northwestern University is currently reviewing its purchase of antibodies from companies such as Santa Cruz. The NU-IACUC and CAR were both dismayed to find that Santa Cruz had a history of animal welfare violations. The USDA has pending litigation against Santa Cruz regarding its alleged animal welfare violations and NU has been in communication with Santa Cruz regarding the University’s expectation that Santa Cruz resolve all open noncompliance issues and develop a plan to prevent futures occurrences. Currently, the NU-IACUC is in the process of compiling a list of all purchases of antibodies from Santa Cruz and is contacting each user with the aim of assisting them in finding alternative sources with no such history of welfare violations.

In summary, members of the CAR understand the intent of the report drafted by Professor Eisenman. However, we felt it was imperative to clarify several of the assumptions made in the Report, and to point out the impracticality and financial infeasibility of the solution proposed by Professor Eisenman. Lastly, we applaud the efforts of Northwestern University in ensuring the humane use of animals in research.