AvantGen Inc.
Your Partner for Antibody Discovery and Optimization

Introduction

AvantGen is a leader in the use of yeast display technology for antibody discovery and optimization. Founded by experts in the creation of antibody discovery and optimization platforms, AvantGen excels in the rapid generation of antibodies for therapeutic, diagnostic and research tool applications.

AvantGen's proprietary technologies include a robust yeast display system, a large natural human antibody database, fully human antibody libraries comprised of over 100 billion antibody clones, an NK cell engager platform and other screening technologies, as well as novel methodologies for generation of rabbit monoclonal antibody clones and VHH nanobodies with remarkably high affinity and specificity, combining the yeast display system and the robust immune response in rabbit and alpaca.

These versatile platforms can be used to discover and optimize antibodies directed at specific disease targets, affinity mature existing antibodies to improve their binding properties and humanize antibodies to render non-human antibodies suitable for human therapeutic applications, as well as generate rabbit monoclonal antibodies for applications that need extremely high specificity, such as antibodies capable of distinguishing point mutations and post-translational modifications for IHC, and anti-idiotype antibodies for PK studies.

To date AvantGen has successfully fulfilled 100s of projects for government, universities, pharmaceutical and biotech companies, facilitated and accelerated their antibody-based therapeutic development through services, partnership collaborations, and technology licensing. In Nov. 2021, a whitepaper AvantGen Technology Review was published. Here we provide a more comprehensive overview of the company's following platforms: 1) NK cell engager and 2) Generation of high performing monoclonal antibodies in rabbit and alpaca.

Anti-CD 16a Antibody Based NK Cell Engager Platform

Natural killer (NK) cells are part of the innate immune system and play a key role in the first line of self-defense against viral infections and transformed, malignant cells both through direct cytotoxic effects and through production of pro-inflammatory cytokines. NK cells express a variety of activating and inhibitory receptors and the overall balance of the two results in either a response to, or tolerance of, the target expressing cells.

Many tumors have low NK cell numbers and anti-tumor activity of NK cells can be adversely influenced by an immunosuppressive tumor microenvironment. This has led to development of strategies to override the negative signaling within the tumor microenvironment and activate NK cell anti-tumor targeting by generating bi-specific or tri-specific antibodies that combine activating NK receptor targeting with tumor targeting. The NK CD16a receptor, a low affinity Fc

receptor, is unique among the activating receptors in that it does not require coordinate activation in combination with other receptors. However, a major impediment to isolating highly specific activating anti-CD16a antibody clones is the high homology, only three residue difference, between the two CD16a allelotypes expressed by NK cells, dendritic cells, and monocytes and the two CD16b allellotypes expressed by granulocytes. Another issue is that a suitable anti-CD16a antibody has to bind to both the F176 and V176 variants of CD16a.

Using our yeast display fully human antibody platform, AvantGen has isolated a panel of clones from their human libraries that fulfill all the requisite criteria of an anti-CD16a antibody that can serve as an NK cell engager. An example of a clone that as a full-length IgG bind with equal high affinity to the extracellular domains of both the CD16a-V176 and CD16a-F176 but not to the extracellular domain of CD16b is shown in Figure 1.

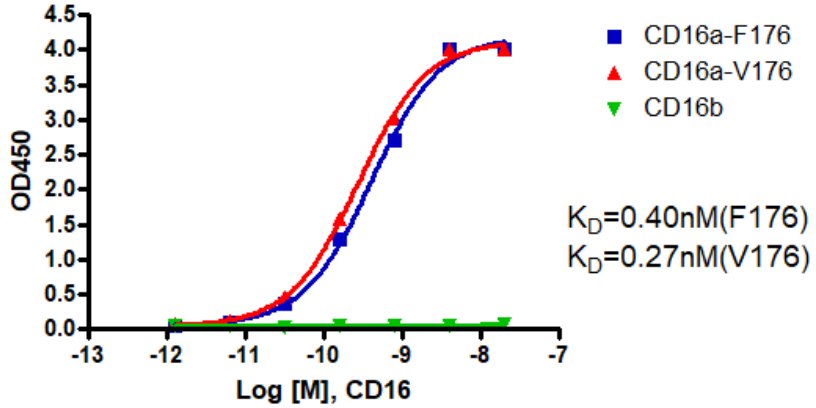


Figure 1. Binding-concentration curve for one of the anti-CD16a clones determined by ELISA. Curve fit and apparent KD value performed using Prism software (GraphPad).

The clones in bispecific formats were tested for their binding activity to neutrophils from donors carrying the NA1 or the NA2 genotype compared to binding by flow cytometry to NK cells. As shown in Figure 2 for one of the clones, it exhibited robust binding to NK cells but no binding to neutrophils.

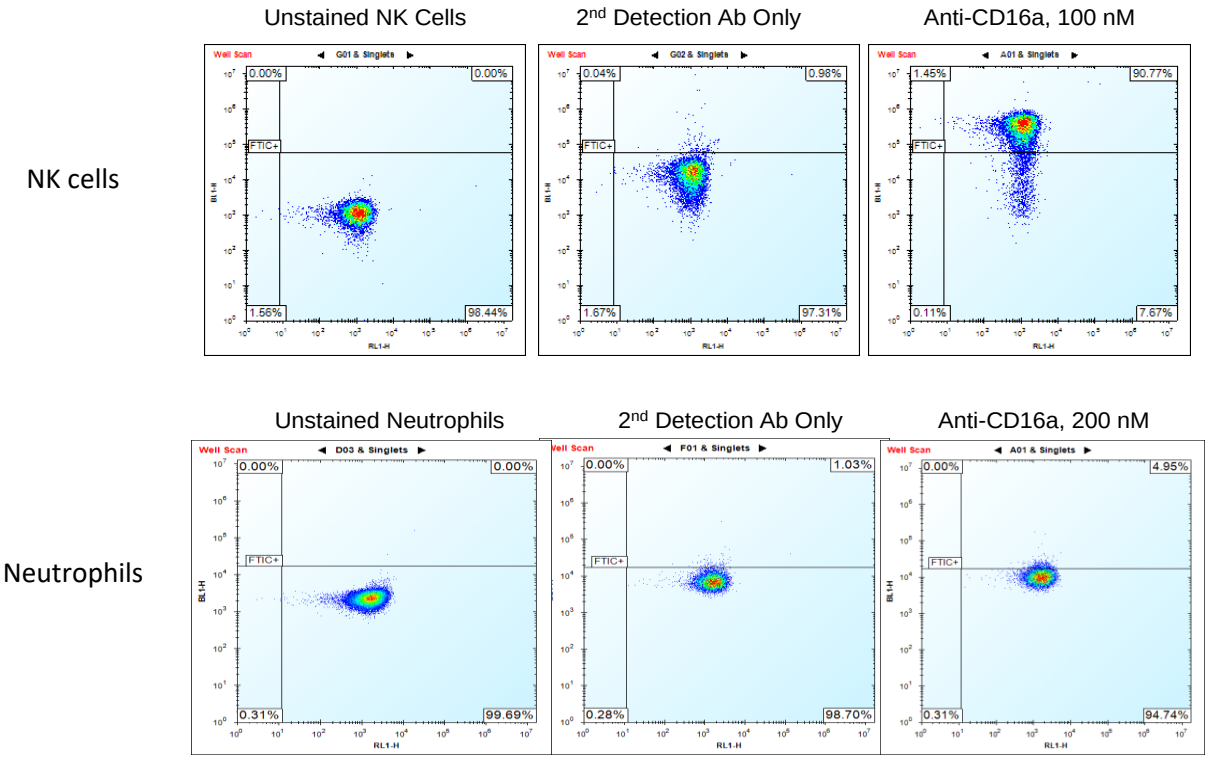


Figure 2. Assessment of relative binding activity of the anti-CD16a clones to NK cells (upper row) and neutrophils (lower row) by flow cytometry.

The clones exhibit robust activation of CD16a expressed by a Jurkat reporter cell line when coupled to an anti-CD19 scFv either in a simple scFv-scFv format (Figure 3) or as an scFv-scFv-IgG format (Figure 4) when the reporter cells are mixed with CD19 expressing Raji target cells compared to non-CD19 expressing MDA-MB-231 breast carcinoma cells. The constructs were purified as monodisperse NK engagers and shown to have EC_{50} values of 0.24 nM for the scFv-scFv construct (Figure 3) and 0.08 nM for the scFv-scFv-IgG4 format (Figure 4).

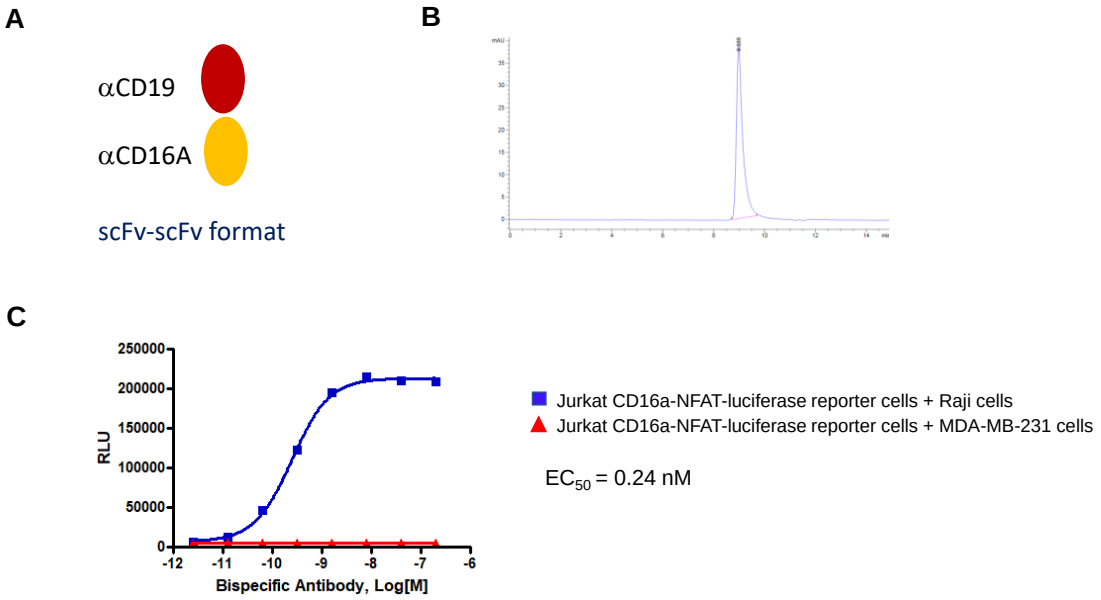


Figure 3. Ability of the scFv-CD19-scFv-CD16a construct shown in panel A to activate Jurkat CD16a reporter cells in the presence of CD19-expressing target Raji cells compared to lack of effect in the presence of non-target expressing MDA-MB-231 cells (panel C). The purity and monodispersity of the purified bi-specific scFv-scFv is shown in panel B.

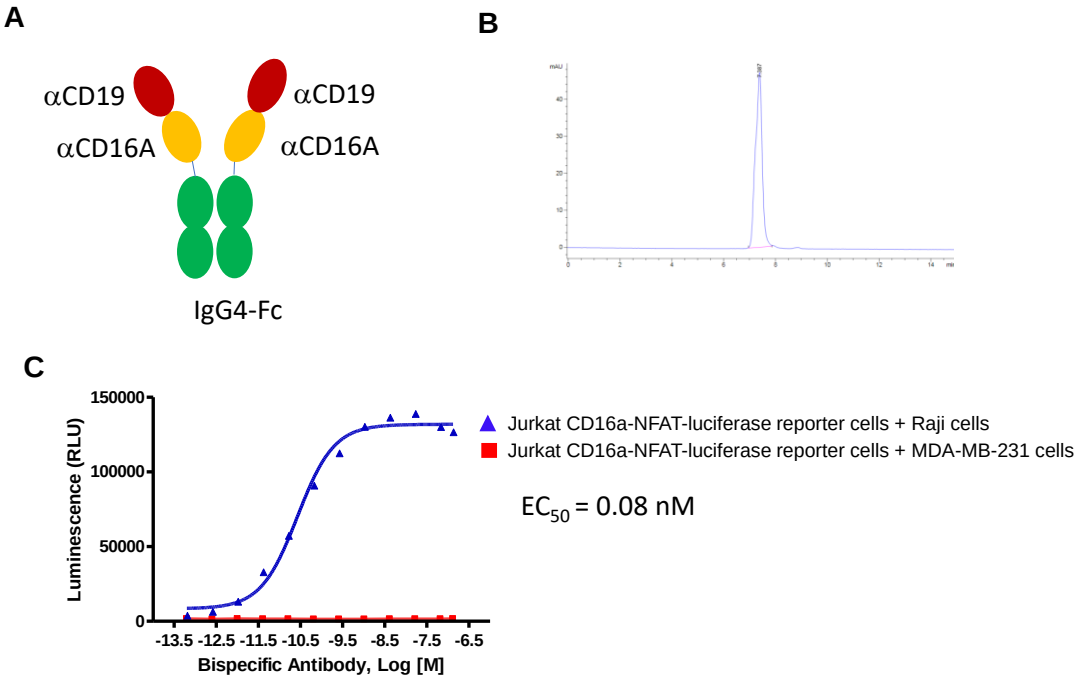
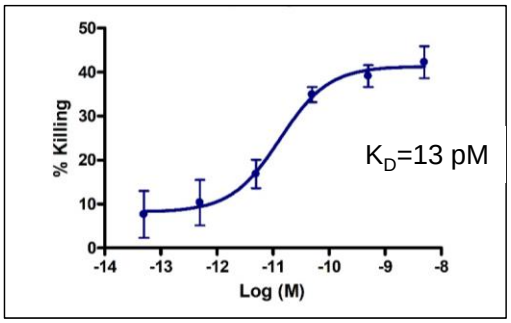


Figure 4. Ability of the scFv-CD19-scFv-CD16a construct as a bivalent IgG4 construct shown in panel A to activate Jurkat CD16a reporter cells in the presence of CD19-expressing target Raji cells compared to lack of effect in the presence of non-target expressing MDA-MB-231 cells (panel C). The purity and monodispersity of the purified bi-specific scFv-scFv is shown in panel B.

Finally, the ability of the anti-CD16a clones in a bivalent scFv-CD19-scFv CD16a-IgG format to induce NK cell mediated ADCC was tested using purified primary human NK cells. NK cells from two donors, one that is F176 homozygous and the other a V176/F176 heterozygous genotype were tested. The NK cell engager was able to induce cell killing of target Raji cells with an EC50 value of 13 and 29 pM, respectively (Figure 5).

A.



B.

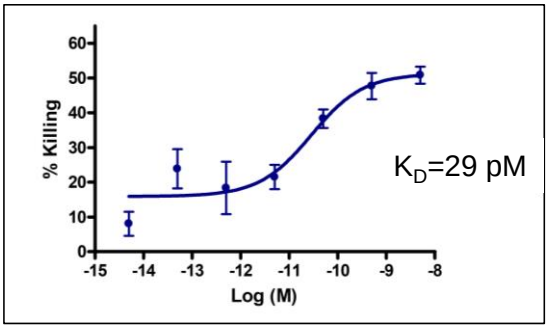


Figure 5. Killing curves of anti-CD16a clone mediated killing of Raji cells with purified primary human NK cells. A. NK cells from a subject with an F176/F176 genotype. B. NK cells from a subject with an F176/V176 genotype. Curve fit and EC50 determination performed using Prism software.

AvantGen offers this NK cell engager platform to couple our potent and specific anti-CD16a clones with clones directed against a client's target antigen of interest. This can either be a clone already on hand, or AvantGen can perform a discovery campaign for the anti-target clone.

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Generation of High-Specificity and High-Affinity Antibodies

I. Rabbit Monoclonal Antibodies

The rabbit immune system generates and affinity-matures antibodies by mechanisms that differ from those of mice and other rodents. Rabbit monoclonal antibody clones normally have 10 to 100-fold higher affinity for antigen than mouse monoclonal antibodies. In addition, the rabbit immune system can generate antibodies that are able to distinguish between very similar molecules with subtle structural variations.

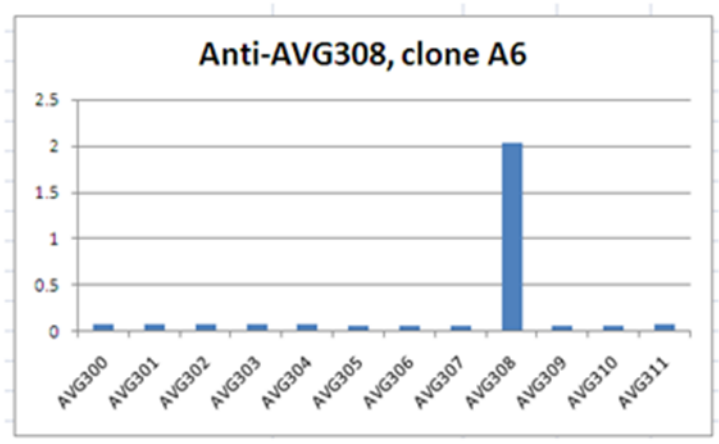
We combine an optimized rabbit immunization protocol and our robust yeast display system to generate a large library of antibodies, a sufficient size that allows us to isolate remarkably high affinity monoclonal antibodies. The yeast display system is uniquely suited for rabbit antibodies, which have an additional disulfide bond in their light chain. This disulfide bond formation is believed to be important for the high affinity and specificity observed for rabbit antibodies. In contrast, phage display normally does not support such modifications.

Our approach overcomes issues associated with rabbit hybridoma-based technology, such as low fusion efficiency, unstable cell lines and low antibody yield. It also greatly shortens the time required to generate monoclonal antibody producing stable cells. Finally, antibodies generated with our technology often exhibit affinities in the range of 0.001-1.0 nM as demonstrated by a recently completed Phase II contract project for the National Cancer Institute (NCI) [HHSN261201300023C].

a) Rabbit Monoclonal Antibodies with High Affinity and Specificity

Examples of monoclonal antibody clones generated for this project with AvantGen's technology are shown below including one example that can specifically distinguish a peptide in its phosphorylated and non-phosphorylated form and two examples of pairs of high affinity clones that can form quantitative ELISAs for NT-ProBNP and polysaccharides from group A Streptococcus (Strep A).

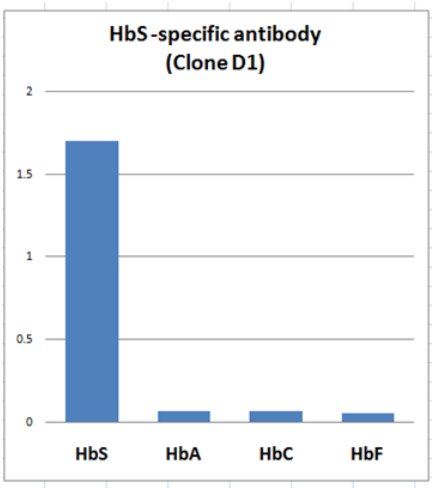
Antigen	Capture Antibody	Affinity (nM)	Detection Antibody	Affinity (nM)
NT-proBNP	A10	0.20	F10	0.03
Strep A	H2	0.05	E10	0.05
AVG300	D4	0.01	LALQAQPVPDELVTK	
AVG301	E11	0.01	DITSDTSGDFR	
AVG308	G11	0.04	**Phospho-peptide specific** VADPDHDHTGFLTEyVATR	
AVG309	H4	0.04	**Phospho-peptide specific** RPHFPQFsYSASGTA	



K_D = 40 pM
AVG308: VADPDHDHTGFLTEyVATR
AVG310: VADPDHDHTGFLTEYVATR
y indicates phosphorylated residue

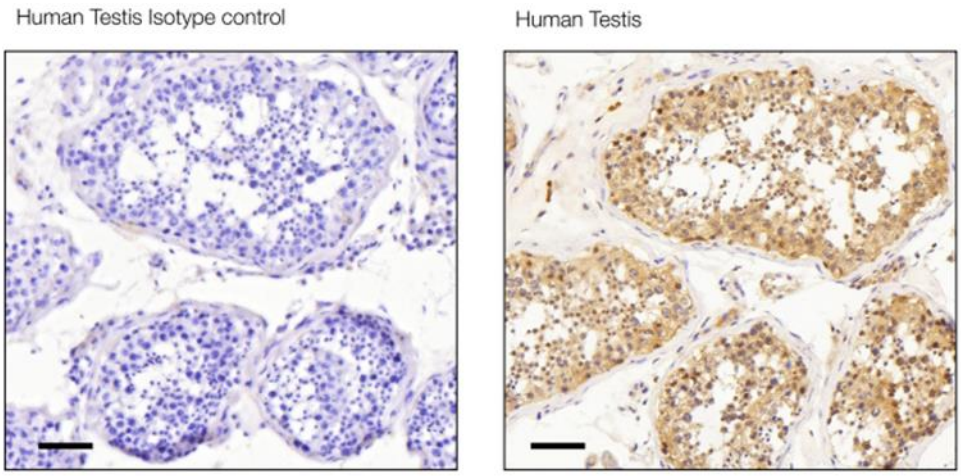
b) Rabbit Monoclonal Antibodies that Distinguish Point Mutations

The Figure below shows an example of a high affinity rabbit clone, D1, that can specifically recognize hemoglobin S, but not adult hemoglobin or hemoglobin C, which differ by only one point mutation.

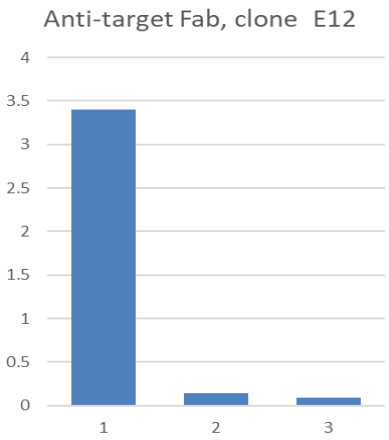


HbS: VHLTPVEKSAV
HbA: VHLTPEEKSAV
HbC: VHLTPKEKSAV
HbF: GHFTEEDKATITS

c) Rabbit Monoclonal Antibodies for IHC and PK Studies (Anti-Idiotype)



Scale bar = 50 mm



1. Target: Human Fab
2. Irrelevant human Fab A
3. Irrelevant human Fab B

II. VHH Nanobody

Applying the same methods as described in the Section of Rabbit Monoclonal Antibodies, AvantGen is able to generate high specificity and affinity VHH Nanobodies in alpaca. Please contact Robin Dong, rdong@AvantGen.com Business Development Director, for further details.

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Summary

- **Proprietary Yeast Display System**
  - Robust and high copy number display of human antibodies in various formats, including Fab, scFab, scFv, etc.
  - Enabled by proprietary peptide display motif fused to the constant region of heavy chain
  - Stable system can be amplified without loss of diversity
  - FACS based techniques allow multiple parameter selections
  - The system also allows antibodies to be secreted to culture media in 96 well plates for ELISA, FACS and functional analysis of individual clones prior to subcloning, sequencing, and purification

- **Large Human Antibody Library and Sequence Database**
  - Over **200 billion clones** (25 sub-libraries in various formats)
  - Rationally designed to mimic diversity in our human antibody database with superior developability
  - Human antibody database compiled from deep sequencing of human antibody repertoires >500 different individuals to inform the library design

- **Antibody Humanization and Optimization**
  - Simultaneous humanization and affinity maturation using epitope guided approach to retain epitope specificity and rationally designed focused library for affinity improvement or
  - CDR grafting with minimum back-mutation to retain affinity

- **Rapid Affinity Maturation and Optimization**
  - Mimics natural sequences observed for antibodies derived from their relevant variable region genes and by length for CDR3 for the heavy chain
  - **10 to 1000-fold affinity improvement** readily achieved

- **Novel Rabbit Monoclonal Antibody and VHH nanobody Discovery**
  - Optimized rabbit and alpaca immunization protocol
  - Generates antibodies with affinities in the low pM range
  - Particularly useful for differentiation between subtle structural variations, such as single point mutation, different cleavage sites, different isoforms, and blocking and non-blocking anti-idiotype antibodies.
  - Well suited for immunodiagnostic, research tool (IHC, FC etc.) and PK purposes.
  - Can be adapted for therapeutic purposes by following discovery with humanization

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	AvantGen Yeast Display	Phage Display	Hybridoma Based
Display System	**novel and uniform**	commonly used, but high biased antibody display	N/A
Rabbit Strains	no constraint	need to use basilar B9 rabbit strain to avoid intra-light chain disulfide bond	no constraint
Library Construction/ Fusion	captures **ALL** of the antibody repertoire	captures only some of the antibody repertoire	inefficient fusion ($1:10^6$), may lose good clones
Library Screening	MACS & FACS for clones with the desired properties from **>100 billion clones**	panning to capture all positive clones, time consuming to identify clones with the desired properties	FACS for clones with desired binding properties but from a very small pool of fused cells
Deliverables	antibody genes, purified antibody, and antibody-expressing mammalian cells	antibody genes, purified antibody, and antibody-expressing mammalian cells	Hybridoma cells, low yield and not very stable
Timeline	~ 5-6 months	~6 months	~8 months

Antibody Attributes	Performance
Affinity	Majority of antibody clones isolated from our libraries exhibits affinity in sub-nM to single digit nM range
Specificity	Specificity can be tailored to be highly specific to the antigen of interest with or without cross reactivity to other homologous proteins or isoforms
Diversity	Typically, a panel of 10s-100s unique antibody clones can be isolated from our libraries against a given antigen
Developability	Antibody is designed to be of high developability
Humanness	Libraries rationally designed to mimic diversity in human antibody database compiled from >500 different individuals
Speed	Ag-specific Ab clones can be isolated in 5-6 weeks and full-length IgG can be produced from mammalian cells in 8-10 weeks

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Examples of Successful Collaborations

Adcentrx and AvantGen Enter a New Partnership with a Three-year, Multi-target Collaboration to Discover Antibodies for Novel Antibody-drug Conjugates

February 14, 2022

SAN DIEGO, Feb. 14, 2022 /PRNewswire/ — Adcentrx Therapeutics ("Adcentrx"), a biotechnology company focused on accelerating breakthroughs in antibody drug conjugate ("ADC") therapeutic development, and AvantGen, a leader in the use of yeast display technology for human antibody discovery and optimization, announced today a three-year, multi-target partnership for the discovery of antibodies to be developed into novel ADC […]

Link: Adcentrx and AvantGen Collaboration

Bloomberg

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Business

AvantGen Announces Licensing of Its Anti-SARS-CoV-2 Antibodies to IGM Biosciences for COVID-19 Therapy Development

January 11, 2021, 3:59 PM PST

BusinessWire
A Berkshire Hathaway Company

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AvantGen and Tanabe Research Laboratories Expand Collaboration for Therapeutic Antibody Discovery

AvantGen to use its proprietary human antibody yeast display platform to generate additional novel cancer therapeutic antibody drug candidates for Tanabe Research Laboratories USA

January 04, 2018 10:00 AM Eastern Standard Time

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AvantGen Services and Business Collaboration Opportunities

AvantGen cordially welcomes the opportunity for the following business collaborations.

- Contracting services using AvantGen's proprietary technologies to rapidly generate and/or optimize antibodies that meet and exceed your design goals.
- Technology transfer package if you are interested in partial or complete internalization of AvantGen's technology platforms non-exclusively.

AvantGen

Proprietary Yeast Display System

Exceptional Results

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