Antibody Library Display on a Mammalian Virus: Combining the Advantages of Panning and Cell Sorting in One Technology

E. Smith, M. Paris, W. Wang, R. Kirk, A. Moksa, F. Murante, L. Balch, S. Shi, R. Hall, L. Mueller, M.Scrivens, S. Fuhrer, A. Howell, C. Foster, S. Giralico, C. Reilly and M. Zauderer

Vaccinex Inc., Rochester, NY, USA (vaccinex.com)

Abstract

Using a vaccinia virus-based library technology we have developed an antibody discovery platform that enables efficient expression of a library of human antibodies in full-length IgG format on the surface of vaccinia virus; an enveloped mammalian virus. Similar in concept to phage display, but allowing display of full-length IgG synthesized and processed in mammalian cells, conditions are utilized where each vaccinia virion will express a single antibody specificity on its surface. Various panning and magnetic bead based methods have been developed to allow screening of a library of vaccinia-MAb virions and selection of recombinant vaccinia virus encoding specific antibodies. Upon infection of mammalian cells the antibody is not only incorporated into newly produced virus, it is also displayed on the surface of the host cell. In a final purification step, the cells displaying vaccinia encoded antibody can be selected by cell sorting, and the virus encoding the specific antibody readily recovered and analyzed. This technology allows for rapid enrichment of vaccinia-MAb virions in a cell free panning system, and then incorporates a cell based screening assay for isolation of optimal, highly specific antibodies.

This technology has been employed for *de novo* antibody selection, affinity improvement of existing human antibodies, and for the robust conversion of a non-human antibody into a panel of fully human antibodies. In all applications, there is built-in selection for antibodies that are efficiently expressed in mammalian cells.

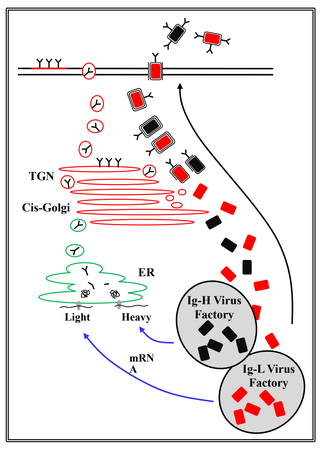
Competitive Advantages

Challenge	Vaccinex Technology Advantage
Efficient selection of fully human, high-affinity, full-length IgG antibodies	• Vaccinex selects IgG antibodies from libraries constructed in a vaccinia virus vector and expressed in mammalian cells with full post-translational modifications. • With Vaccinia Display, virions displaying antibodies are rapidly selected in one or more rounds of binding to magnetic beads in a cell-free system. This is followed by a final cell-based FACS sort of cells infected by highly enriched virus to select specific immunoglobulin H and L pairs.
Affinity improvement of existing human antibodies Conversion of non-human antibodies to fully human	• Selection of multiple antibodies derived from distinct VH and VL germ line genes with different biochemical properties. • Converting mouse antibodies to fully human antibodies with conserved epitope specificity and with similar or improved affinity and functional activity.
Manufacturing	• Intrinsic selection for high expression in mammalian cell lines, easily adaptable to manufacturing.

Introduction

• We have constructed a fusion protein of full length IgG heavy chain with a vaccinia virus membrane protein (VMP) = IgG-VMP

• This fusion protein is expressed both on the surface of the virus AND on the surface of the host cell.



• Antibody Library Display on Vaccinia Virus: Combining the advantages of virus panning and cell sorting in one technology with intrinsic selection for antibodies with good mammalian cell expression.

• Virus panning: Allows for rapid screening of billions of antibody combinations in a cell free selection system

• Vaccinia is a large enough virus that liters of vaccinia virus containing cell culture media can be rapidly concentrated by centrifugation into 1ml for antibody selection.

• Cell Sorting: Allows for tunable affinity selection by adjusting the amount of antigen used to stain the cells and allows for easy confirmation of antibody expression on the cell surface during selection

• Heavy and light chain libraries are derived from naïve or synthetic sources and represent all major germline families (including kappa and lambda constant).

• Heavy chain diversities are on the order of 10^8 and are paired with either pools of germline light or low diversity light libraries to ensure that each heavy pairs with multiple functional light chains.

• The use of germline light creates opportunities for the independent selection of heavy chains with specificity for diverse antigens being assembled in bispecific format.

Vaccinia Virus as an expression host

• Vaccinia virus infects most mammalian cells. Recombinant proteins expressed by vaccinia virus infected cells undergo normal post-translational modifications and trafficking.

• The conventional recombination technology allows for the introduction of *one defined gene* at a time into vaccinia virus. This is sufficient for expression of a single gene product, but does not enable functional cloning of unknown genes from a large library.

• Vaccinex has developed proprietary technology that allows for the creation of large and diverse cDNA libraries in a vaccinia virus-based vector.

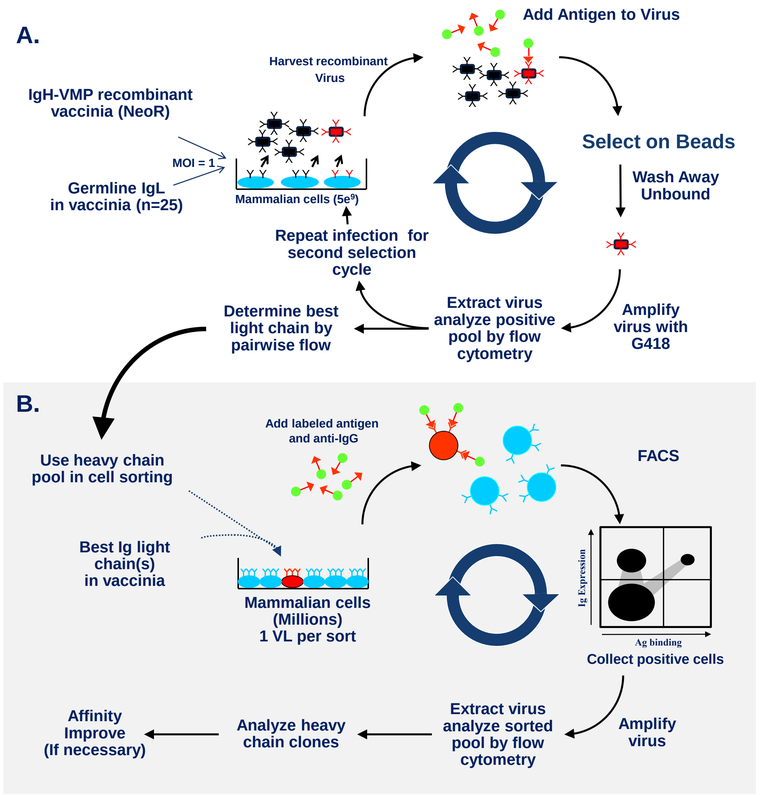
• Hundreds of Millions of different vaccinia recombinants in each library. *Nature Medicine 7:967-972 (2001)*

• Technology for functional cloning in mammalian cells.

• Selection of fully human monoclonal antibodies.

• Efficient identification of immune target antigens.

Vaccinia Display Process Flow



Vaccinia Display. **A.** Mammalian cells are co-infected with a vaccinia library encoding human IgG-VMP (VMP= Vaccinia Membrane Protein) Library and with a cocktail of vaccinia recombinant for germline human light chains. Virus is released from infected cells and concentrated by centrifugation. Virus is then incubated with antigen coated magnetic beads. Unbound virus is washed away, and the recombinant viruses that express a specific antibody are isolated bound to the magnetic beads. The selected virus is rapidly expanded and further enriched by an additional round of selection. **B.** The IgG-VMP fusion protein is also expressed on the cell surface, so in a final step, infected cells can be stained with antigen and binders selected by cell sorting. The selected Ig-H is tested and sorted with individual Light Chains. The specific IgG-VMP can be isolated and characterized.

If necessary, the selected Ig-H can be used to select optimal Ig-L or targeted for mutagenesis

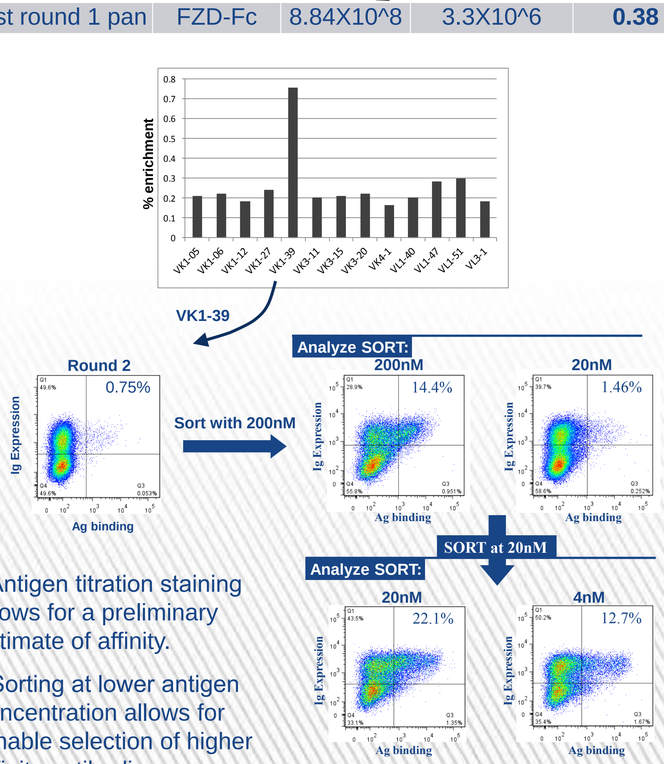
Magnetic Bead-Based Selection

Streptavidin beads
Use biotinylated-antigen and Streptavidin-coated beads
Protein G beads
Use Fc fusion protein
Tosylactivated magnetic beads
No tags required; direct coating of antigen onto the beads

Ex: Selection of FZD4-specific MAbs

• FZD4 used as target antigen for *de novo* selection by vaccinia display.
• Vaccinia fusion protein library with **$1x10^8$ heavy chain** clones was co-infected with a cocktail (n<25) of germline Ig-L and subjected to selection on FZD4-Fc Coated ProG beads and titered.
• Post-panning, bead bound virus was amplified in the presence of G418 to selectively amplify the heavy chains
• In round 2, heavy chain virus from the first cycle of selection was used to co-infect cells along with fresh aliquots of each germline Ig-L.
• Virus from this co-infection was harvested and subjected to selection on FZD4-Fc Coated ProG beads and titered
• Bead bound virus was amplified in the presence of G418 to selectively amplify the heavy chains
• Test for enrichment by flow cytometry

Virus	Selection	Titer Unbound	Titer Bound	% Bound
Positive Control	FZD-Fc	2.3X10^6	4.4X10^5	19
Negative Control	FZD-Fc	2.2X10^6	1400	0.06
Primary Library	FZD-Fc	2.28X10^9	2.1X10^6	**0.09**
Post round 1 pan	FZD-Fc	8.84X10^8	3.3X10^6	**0.38**



• Antigen titration staining allows for a preliminary estimate of affinity.

• Sorting at lower antigen concentration allows for tunable selection of higher affinity antibodies

Characterization of Clones

• The VH genes contained in the sorted virus pools were PCR amplified and cloned into a mammalian expression plasmid containing the constant domain of IgG1.

• 2X96 well plate of clones was sequenced for each paired light chain.

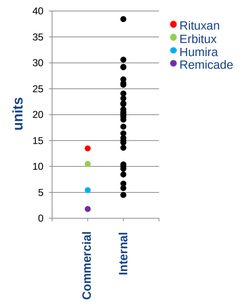
• >120 unique clones were identified that bind FZD4 by ELISA and have affinities better than 100nM

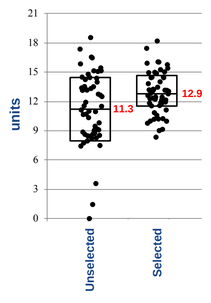
• **About 40 of these clones have affinities of better than 10nM**

  • 30 of these higher affinity clones were identified only 1X, so there are without question many additional specific antibodies that were selected

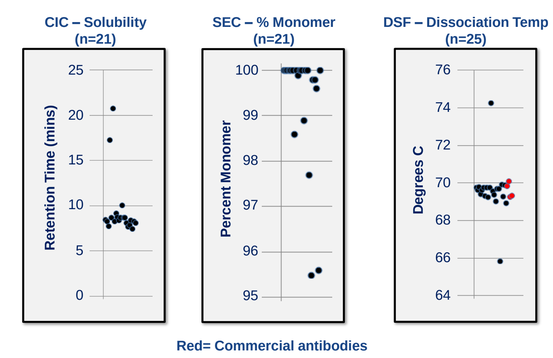
• The Ig-H genes are cloned from recombinant vaccinia virus into mammalian expression plasmids. Antibody is produced as full length soluble IgG1 by transfection of the plasmid along with a mammalian expression plasmid encoding the full length light chain into CHO cells.

Mab Number	Affinity (nM)
mAbC6053	0.003
MAb6750	0.025
MAb6745	0.03
mAbC6061	0.03
mAb6772	0.03
MAb6471	0.05
mAb6768	0.06
mAbC6062	0.08
MAb6476	0.145
mAbC6052	0.231
mAbC6051	0.259
MAb6369	0.84
MAb6727	0.925
MAb6187	1.9
MAb6468	2.175
MAb6071	2.35
MAb6219	3
MAb6223	3.3
MAb6044	3.5



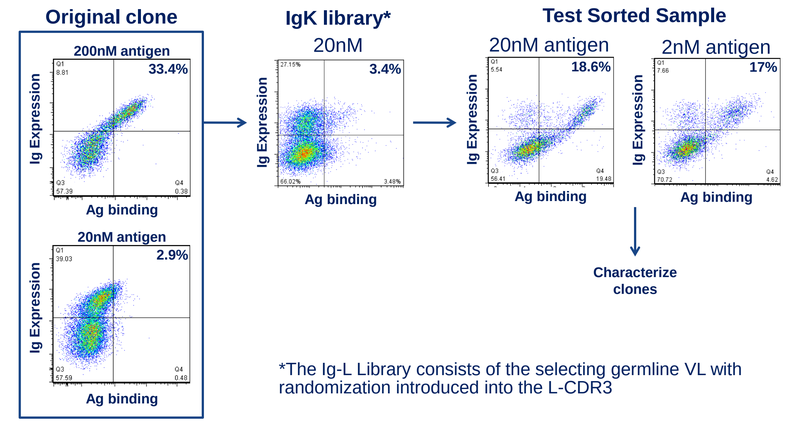


Additional Bioanalytical Characterization of Clones



Red= Commercial antibodies

Affinity Improvement of a FZD4 specific Mab by Selecting Optimal Light Chains



*The Ig-L Library consists of the selecting germline VL with randomization introduced into the L-CDR3

Affinity of Improved Antibodies

Example 1

Mab	Affinity (nM)	Fold
6044 (Parent)	3.5	
MAb6256	0.007	500X
MAb6254	0.01	350X

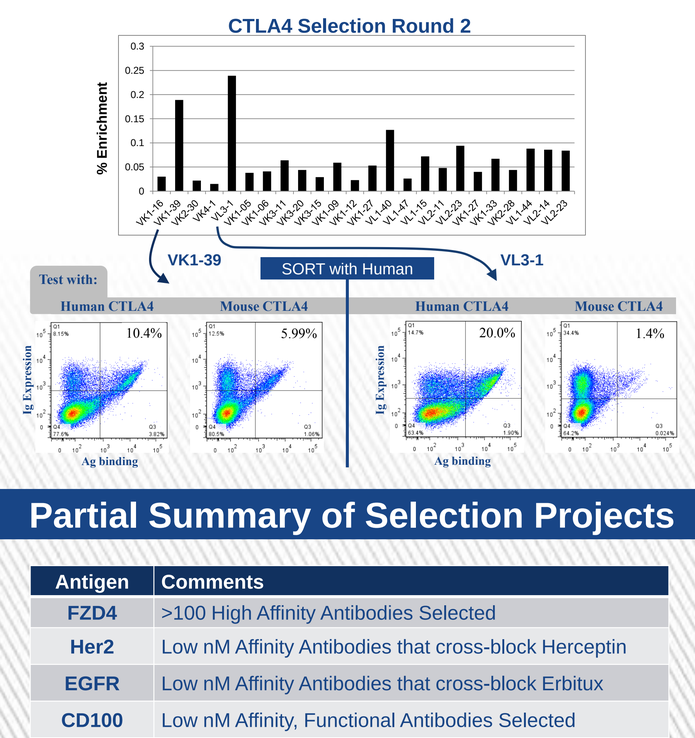
Example 2

Mab	Affinity (nM)	Fold
6071 (Parent)	2.4	
MAb6529	0.08	30X
MAb6522	0.11	22X

Example 3

Mab	Affinity (nM)	Fold
6068 (Parent)	~100	
MAb6529	1.5	67X
MAb6522	1.7	59X

Species cross-reactivity



Partial Summary of Selection Projects

Antigen	Comments
FZD4	>100 High Affinity Antibodies Selected
Her2	Low nM Affinity Antibodies that cross-block Herceptin
EGFR	Low nM Affinity Antibodies that cross-block Erbitux
CD100	Low nM Affinity, Functional Antibodies Selected
CTLA4	Low nM Affinity, Functional Antibodies Selected