VACCINEX

Science in the Service of Medicine

Unique Targets • Novel Mechanisms • New Medicines

SCAN ME

Discovery of High-Affinity Functional Antibodies Specific for CXCR5 and Other Multi-Pass Membrane Proteins

Ernest Smith

Senior Vice President, Research

CSO, Antibody Discovery, Vaccinex

Discovery on Target

Thursday, September 28th, 2023

Forward Looking Statement

To the extent that statements contained in this presentation are not descriptions of historical facts regarding Vaccinex, Inc. (“Vaccinex,” “we,” “us,” or “our”), they are forward-looking statements reflecting management’s current beliefs and expectations. Such statements include, but are not limited to, statements about our the applicability and ability of the “Antigen Virus” application of the ActivMab® platform, plans, expectations and objectives with respect to, the expected timeline for disclosure of results at scientific conferences or through publications, and other statements identified by words such as “believes,” “being,” “may,” “will,” “appears,” “expect,” “continue,” “estimate,” “ongoing,” “potential,” “prevents,” “suggest”, and similar expressions or their negatives (as well as other words and expressions referencing future events, conditions, or circumstances). Forward-looking statements involve substantial risks and uncertainties that could cause the outcome of our research and pre-clinical development programs, clinical development programs, future results, performance, or achievements to differ significantly from those expressed or implied by the forward-looking statements. Such risks and uncertainties include, among others, uncertainties inherent in the execution, cost and completion of preclinical studies and clinical trials, that interim and preliminary data may not be predictive of final results and does not ensure success in later clinical trials, uncertainties related to regulatory approval, risks related to the impact of the COVID-19 pandemic, the possible delisting of our common stock from Nasdaq if we are unable to regain compliance with the Nasdaq listing standards, and other matters that could affect our development plans or the commercial potential of our product candidates. Except as required by law, we assume no obligation to update these forward-looking statements. For a further discussion of these and other factors that could cause future results to differ materially from any forward-looking statement, see the section titled “Risk Factors” in our periodic reports filed with the Securities and Exchange Commission (“SEC”) and the other risks and uncertainties described in the Company’s annual year-end Form 10-K and subsequent filings with the SEC.

VACCINEX

Science in the Service of Medicine

Unique Targets • Novel Mechanisms • New Medicines

Publicly Traded
NASDAQ: VCNX

Employees
40

Established
1997
Rochester, NY

Pepinemab (anti-Semaphorin 4D mAb) Antibody Platform

- **Phase 1b/2 trial in Head and Neck Cancer (combo with Keytruda)**
  - **KEYNOTE-B84**
- **Phase 1b/2 single agent trial in Alzheimer's Disease**
  - **SIGNAL-AD**

Activmab Technology Platform

Pioneering Discovery Solutions for Diseases with High Unmet Needs

CXCL13/CXCR5 Biology

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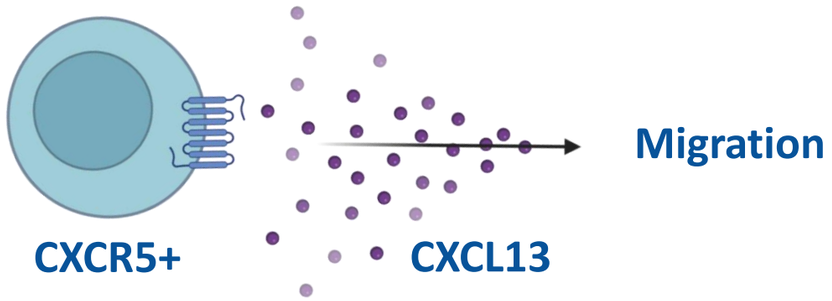
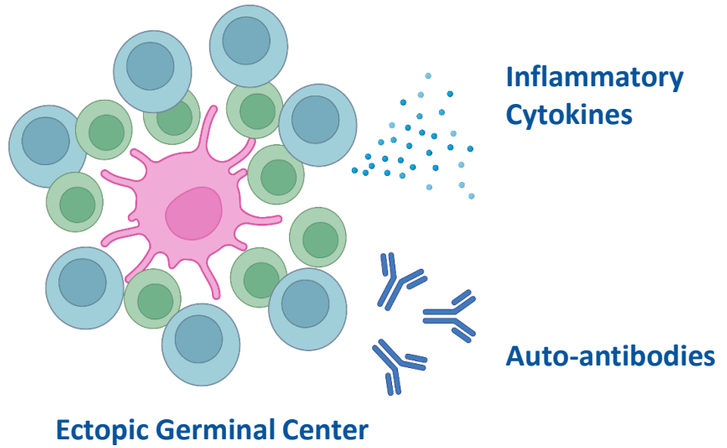
- Chemokines are a family (~40 members) of small proteins that act as chemo-attractants to control immune cell trafficking.
- CXCL13 is expressed in the B cell follicles of secondary lymphoid organs.
- CXCR5 is the sole receptor of CXCL13.
- CXCR5 is highly expressed on mature B cells and subpopulations of CD4+ T cells (ex Tfh).
- CXCL13/CXCR5 axis is important for the organization of secondary lymphoid organs and in the formation of germinal centers and immune responses.
- Ectopic expression of germinal centers and over-expression of CXCL13/CXCR5 has been detected in a number of autoimmune disorders and cancers.





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CXCL13/CXCR5 Disease Involvement

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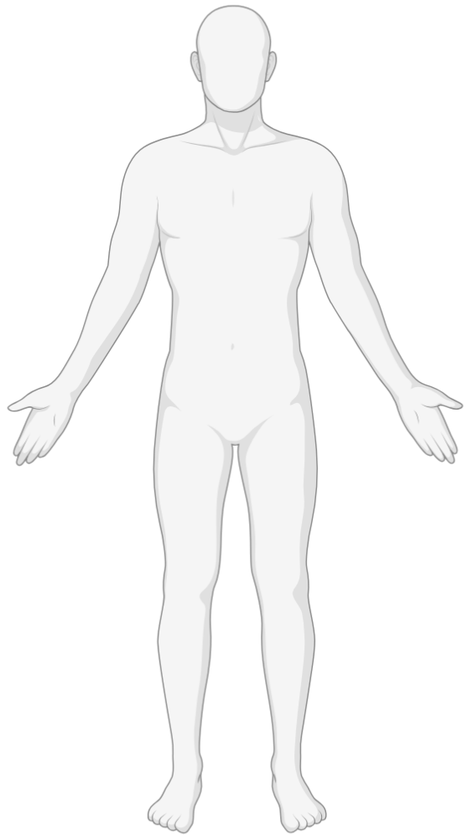
CXCL13/CXCR5 Axis and Human Diseases

Cancer
- Solid tumors
- Hematological malignancies

Autoimmune Diseases
- Rheumatoid arthritis
- Multiple sclerosis
- Systemic lupus erythematosus
- Primary Sjögren's syndrome
- Myasthenia gravis
- Type I diabetes mellitus
- Inflammatory bowel disease
- Primary biliary cholangitis
- Graves' disease
- Bullous pemphigoid
- Psoriasis
- Systemic sclerosis



Infectious Diseases
- Lyme neuroborreliosis
- Neurosyphilis
- HIV infection
- *H. pylori* infection
- Myasthenia gravis
- Hepatitis virus infection
- SARS-CoV-2 infection

Other Diseases
- COPD
- Asthma
- Idiopathic pulmonary fibrosis
- Atherosclerosis
- Giant cell arteritis
- Allograft rejection
- Neuropathic pain

Blockade of CXCL13/CXCR5 axis by a monoclonal antibody could result in reduced ectopic germinal centers and improvement in autoimmune diseases.

CXCR5 expression is maintained by many B cell and T cell tumors and could be targeted by an antibody drug conjugate or CAR T cells.

CXCR5 is a GPCR, which are very difficult to make antibodies against.

Adapted from:
Pan Z, Zhu T, Liu Y and Zhang N (2022) Role of the CXCL13/CXCR5 Axis in Autoimmune Diseases.
Front. Immunol. 13:850998.

Challenges for Antibody Discovery Against CXCR5 and Other Membrane Proteins

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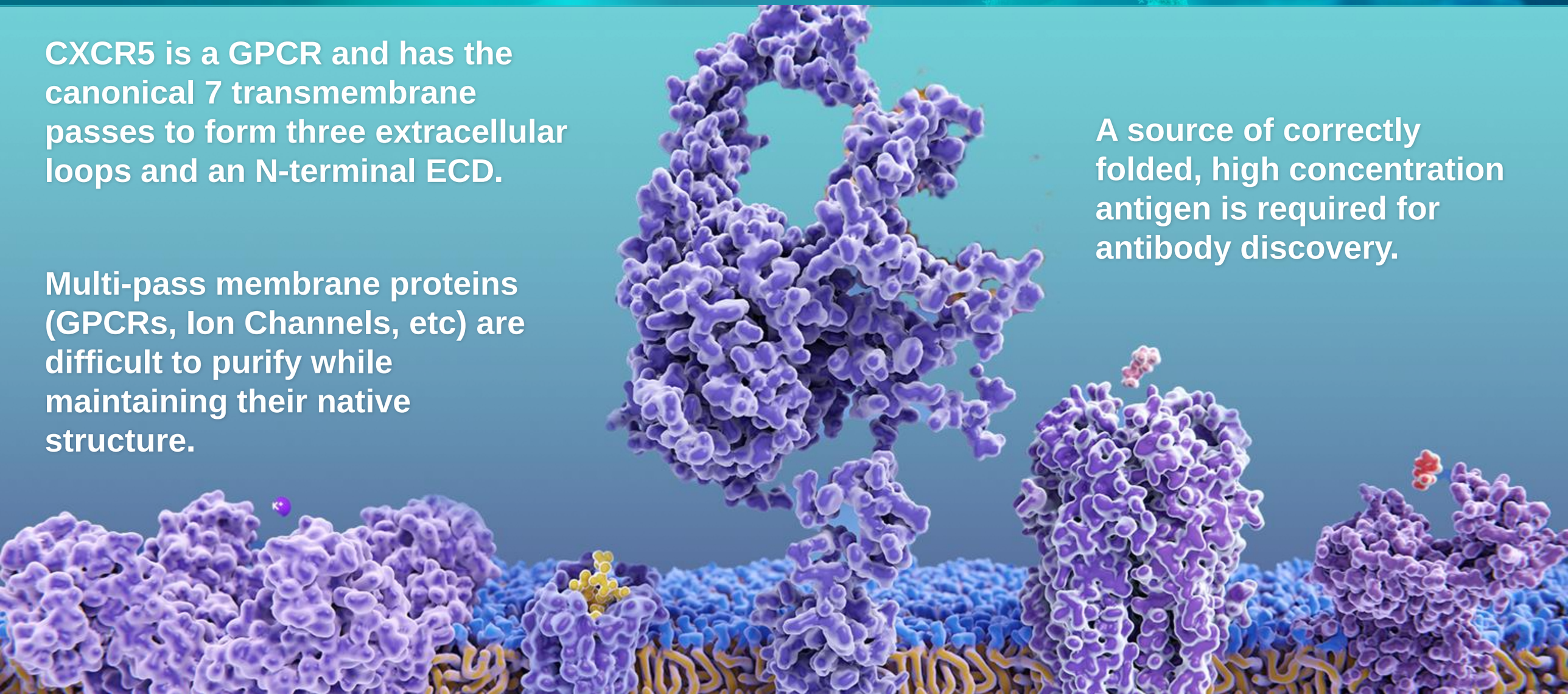
CXCR5 is a GPCR and has the canonical 7 transmembrane passes to form three extracellular loops and an N-terminal ECD.

Multi-pass membrane proteins (GPCRs, Ion Channels, etc) are difficult to purify while maintaining their native structure.



A source of correctly folded, high concentration antigen is required for antibody discovery.

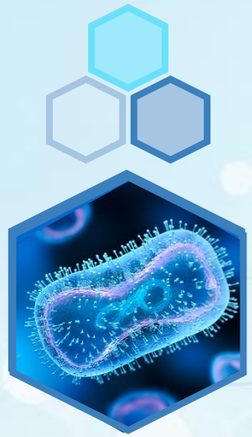
ActivMab® DISCOVERY SOLUTIONS

ActivMAb Technology

ActivMab® provides a suite of technologies utilizing poxvirus to facilitate antibody discovery projects targeting **complex membrane proteins** at every stage of research.

In Vitro Discovery

In Vivo Discovery



Protein Optimization

Target Identification

Recent Publications

ActivMAb Technology

nature communications

Article

https://doi.org/10.1038/s41467-023-37191-8

A *Vaccinia*-based system for directed evolution of GPCRs in mammalian cells

Received: 10 November 2022

Accepted: 6 March 2023

Published online: 30 March 2023

Check for updates

Christoph Klenk[1], Maria Scrivens[2], Anina Niederer[1], Shuying Shi[2], Loretta Mueller[2], Elaine Gersz[2], Maurice Zauderer[2], Ernest S. Smith[2], Ralf Strohner[3] & Andreas Plückthun[1]

Directed evolution in bacterial or yeast display systems has been successfully used to improve stability and expression of G protein-coupled receptors for structural and biophysical studies. Yet, several receptors cannot be tackled in microbial systems due to their complex molecular composition or unfavorable ligand properties. Here, we report an approach to evolve G protein-coupled receptors in mammalian cells. To achieve clonality and uniform expression, we develop a viral transduction system based on *Vaccinia* virus. By rational design of synthetic DNA libraries, we first evolve neurotensin receptor 1 for high stability and expression. Second, we demonstrate that receptors with complex molecular architectures and large ligands, such as the parathyroid hormone 1 receptor, can be readily evolved. Importantly, functional receptor properties can now be evolved in the presence of the mammalian signaling environment, resulting in receptor variants exhibiting increased allosteric coupling between the ligand binding site and the G protein interface. Our approach thus provides insights into the intricate molecular interplay required for GPCR activation.

MABS
2023, VOL. 15, NO. 1, 2249947
https://doi.org/10.1080/19420862.2023.2249947

Taylor & Francis
Taylor & Francis Group

REPORT

OPEN ACCESS Check for updates

Use of poxvirus display to select antibodies specific for complex membrane antigens

Ernest S. Smith, Leslie A. Balch, Maria Scrivens, Shuying Shi, Wei Wang, Caroline D. Harvey, Angelica A. Cornelison, Malgorzata Gil-Moore, Renee A. Kirk, Loretta L. Mueller, Richard L. Hall, Alan P. Howell, Christine A. Reilly, Jessica M. Mayer, Francis G. Murante, Kari A Viggiani, Elaine M. Gersz, Holm Bussler, Madeleine R. Keefe, Elizabeth E. Evans, Mark J. Paris, and Maurice Zauderer

Research, Vaccinex, Inc, Rochester, NY, USA

ABSTRACT

Antibody discovery against complex antigens is limited by the availability of a reproducible pure source of concentrated properly folded antigen. We have developed a technology to enable direct incorporation of membrane proteins such as GPCRs and into the membrane of poxvirus. The protein of interest is correctly folded and expressed in the cell-derived viral membrane and does not require any detergents or refolding before downstream use. The poxvirus is selective in which proteins are incorporated into the viral membrane, making the antigen poxvirus an antigenically cleaner target for in vitro panning. Antigen-expressing virus can be readily purified at scale and used for antibody selection using any in vitro display platform.

ARTICLE HISTORY
Received 15 February 2023
Revised 15 August 2023
Accepted 16 August 2023

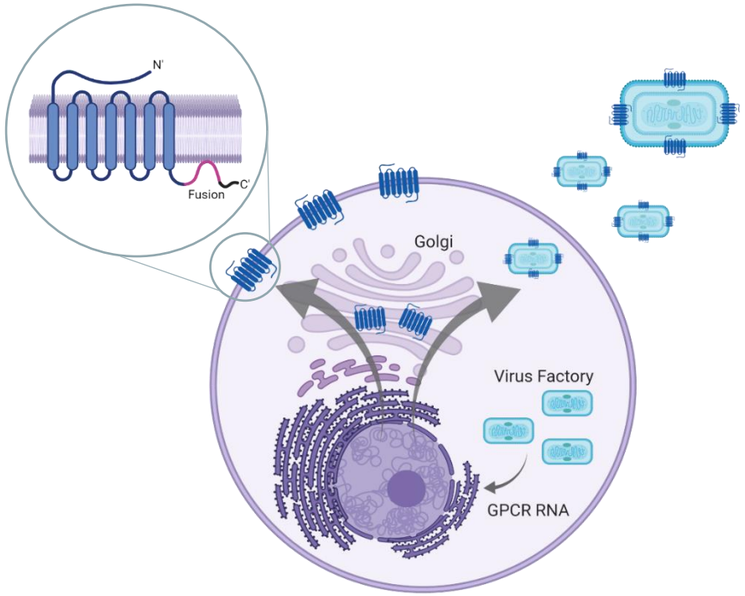
KEYWORDS
Antibody; GPCR; ion channel; membrane; panning; phage; poxvirus

Antigen Virus Is “One Size Fits All”

ActivMAb Technology

Vaccinex’s fusion protein technology provides for *efficient incorporation* of multi-pass membrane proteins into the membrane of poxvirus

- Untagged molecules do not incorporate efficiently
- Retains native conformation and orientation
- Concentration of membrane protein

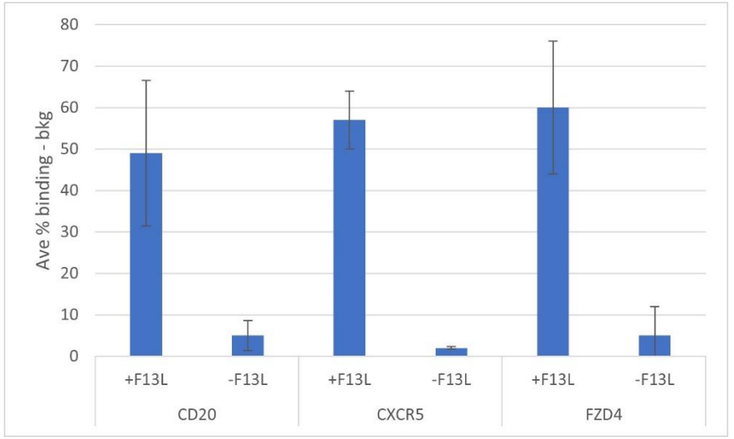


Easy to generate virus expressing antigen

- No purification steps required – harvested by simple centrifugation
- No detergents or refolding
- Essentially **“one size fits all”**

Suitable for antibody selection technologies

- ***Low background*** due to restricted viral membrane complexity
- Available in 2 antigenically distinct BSL1 strains (MVA and FPV)



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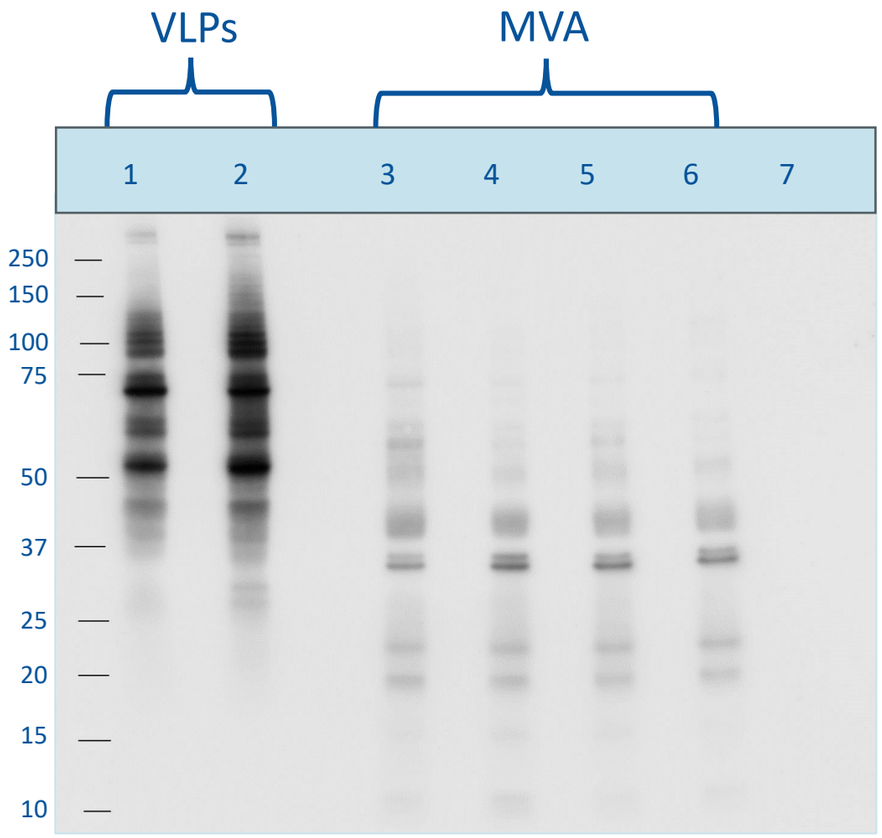
Antigen Virions: VLP vs Poxvirus
Western for surface exposed proteins

ActivMAb Technology



- BCA Assay used to quantitate protein concentration of VLP or MVA.
- Equal total protein concentrations were **surface biotinylated**
- Reduced samples run on 4-12% NuPAGE Bis-Tris Gel
- Equal loading in each lane based on BCA concentration.
- Western blot – Probed with streptavidin-HRP

Lane-	Sample
1	**GPR65** VLP's
2	**CXCR5**-VLP's
3	MVA-**GPR65**
4	MVA-**CXCR5**
5	MVA-**Sema4D**
6	MVA-**CXCR4**
7	MVA-**CXCR4 (not biotinylated)**

Antigen Virions:
Protein folding and function

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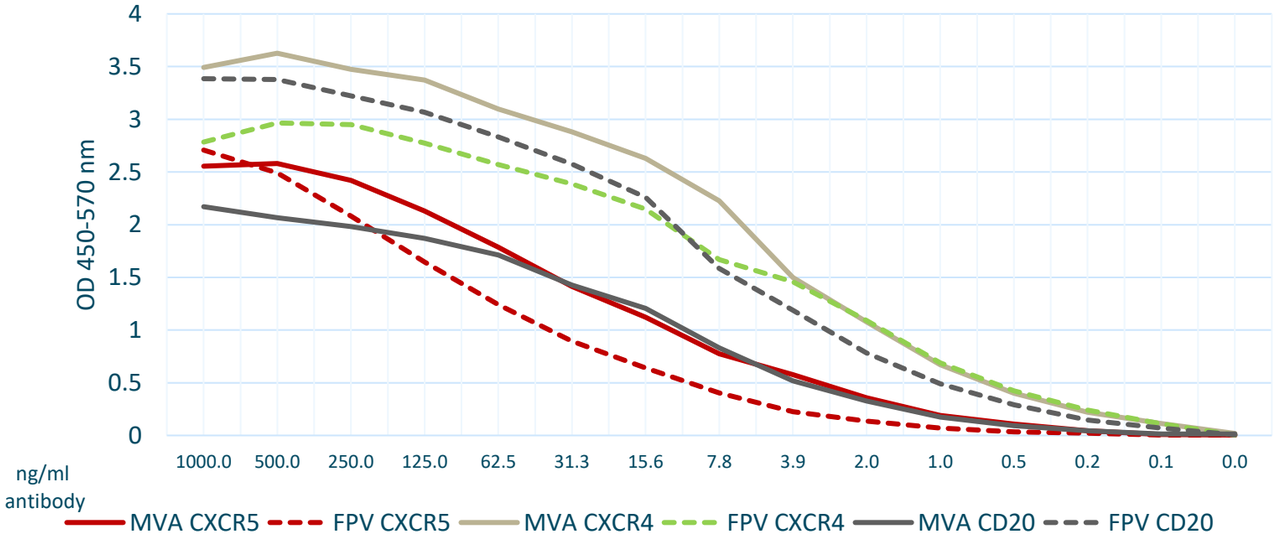
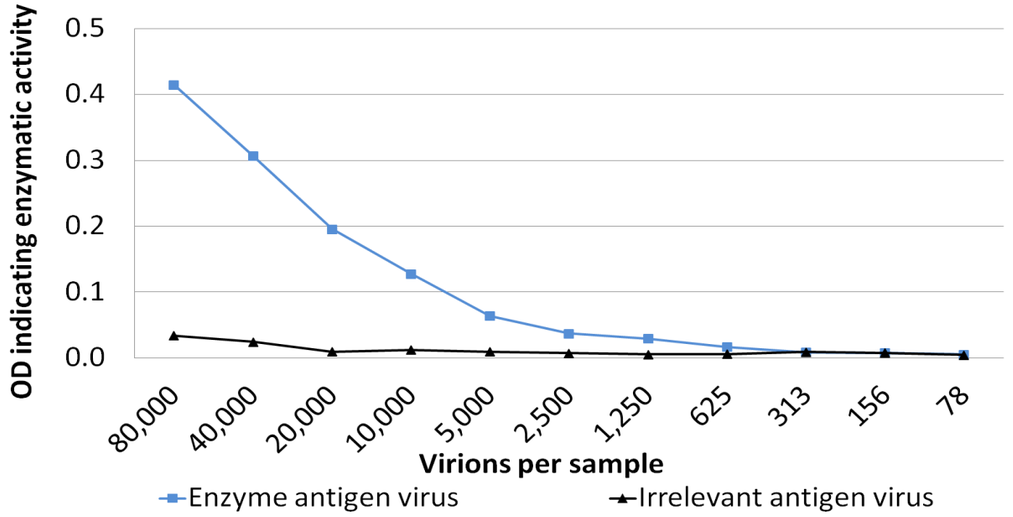
Antigen Virus is bound by conformationally specific antibodies



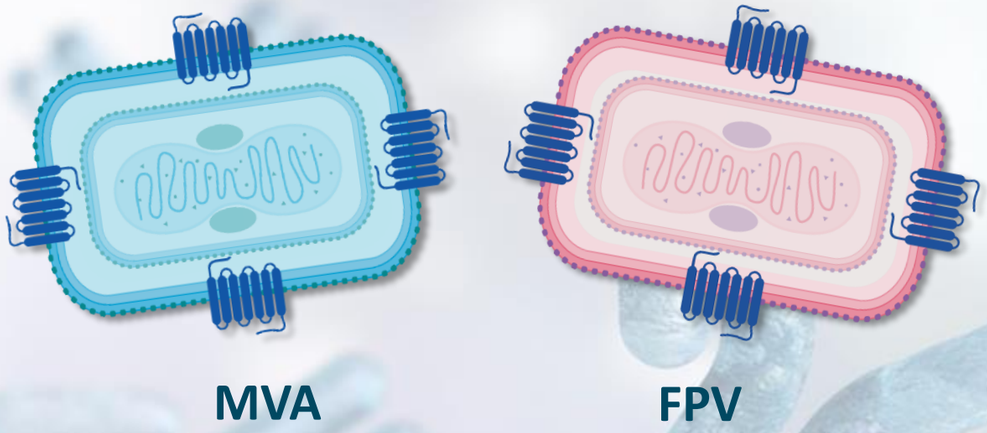
CD39 virus demonstrated enzymatic activity



ActivMab®
Poxvirus Antigen Expression

ActivMAb Technology

Vaccinex offers two antigenically distinct poxviruses compatible with both *in vivo* and *in vitro* antibody discovery methods.



Using our fusion protein technology, our Biosafety Level 1 antigen virions efficiently incorporate multi-pass membrane proteins in their outer membrane and are released into the culture medium for simple purification.

Antigen Virus Checklist:

- ✓ Retains native conformation and orientation
- ✓ No purification steps required – use the virus
- ✓ ***Specific*** viral membrane complexity
- ✓ Concentration of membrane protein
- ✓ Potent immunogens
- ✓ Two antigenically distinct strains
  - ✓ Alternating panning and ex vivo B cell selection
- ✓ Compatible with multiple methodologies including bead coupling, ELISA and FACS

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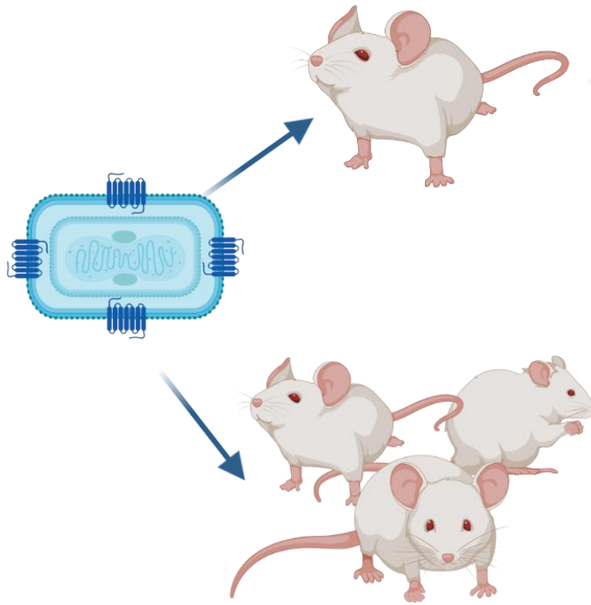
Antibody Discovery:
Mouse Immunization and Process

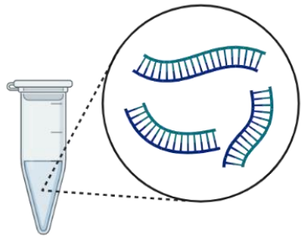
ActivMAb Technology

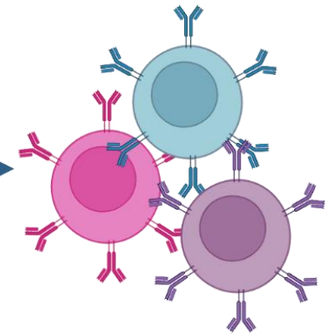
Mouse immunization with Antigen virus



Immunization

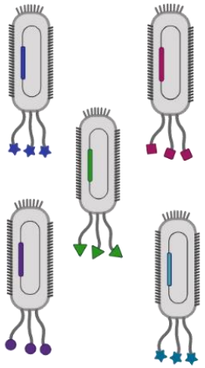
mRNA extraction from Whole spleen

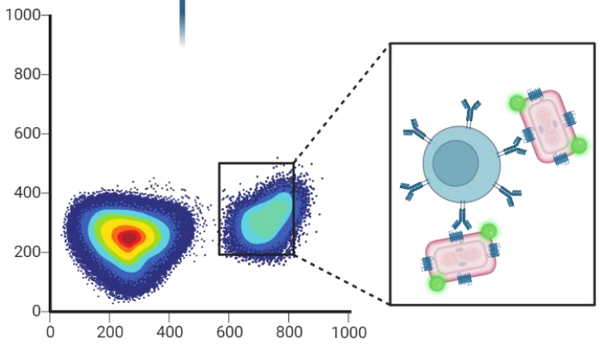




B Cells from spleens
(multiple pooled mice)

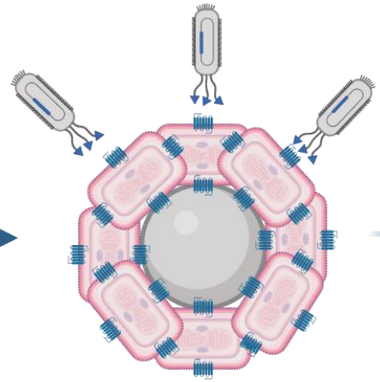
Phage library generation



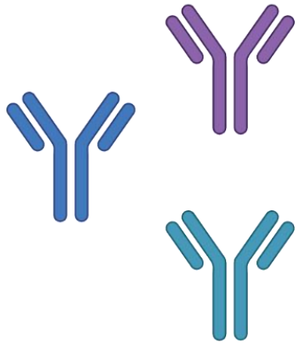


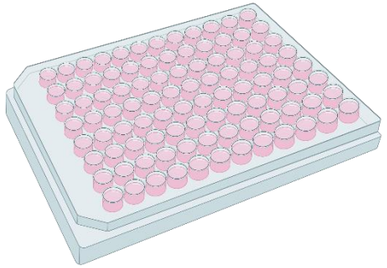
FAC Sorting for Memory B cells
[B220+, IgM-, IgG+]
Bound to Fluorescent labelled Antigen Virus

Phage panning antigen virus Coupled to magnetic beads



Specific anti-antigen antibodies





Single Cell Analysis

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CXCR5 Titers from viral immunization

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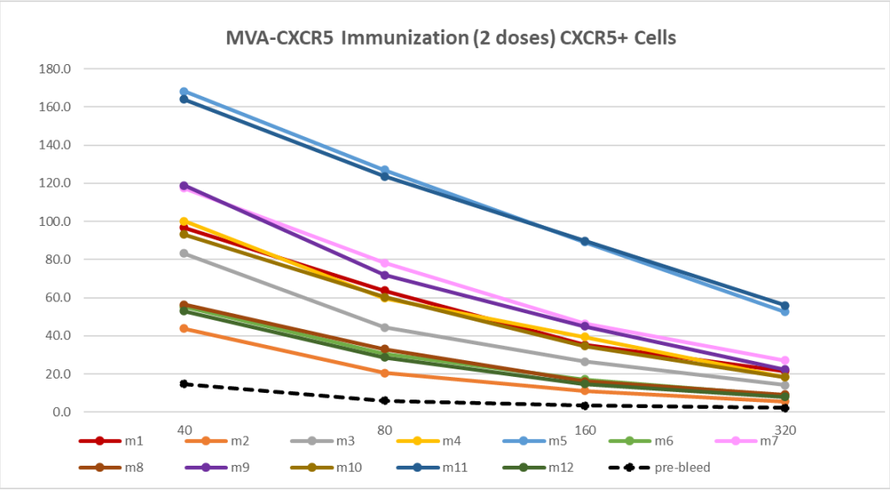
Twelve Balb/c mice were immunized with MVA/CXCR5 for 2 doses.

Four days after the second dose, sera was tested for anti-CXCR5 titer by flow cytometry on CXCR5+ vs CXCR5- cells.

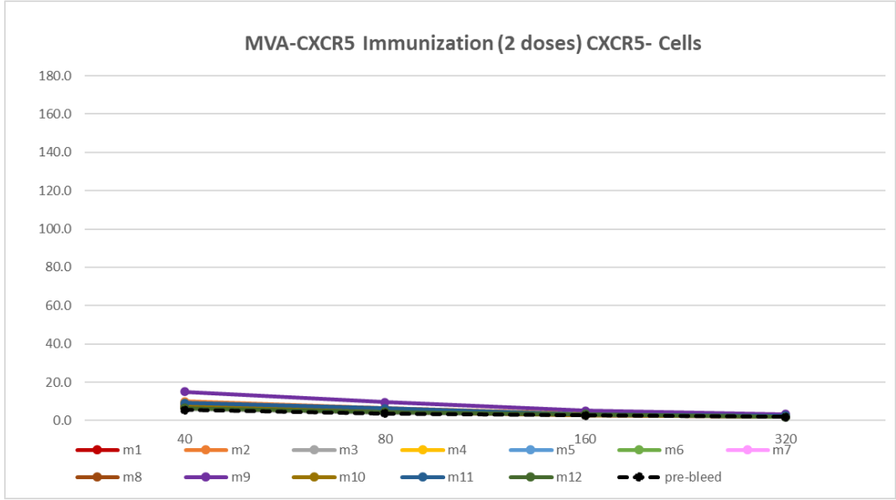
All mice showed some level of anti-CXCR5 titer with MVA immunization.

Background titers were very low for all mice.

CXCR5+ Cells



CXCR5- Cells



Spleens from the top 6 mice were harvested for B cell isolation and sorted for CXCR5 binding B cells using poxvirus labelled with fluorescent beads.

CXCR5 B cell sorting

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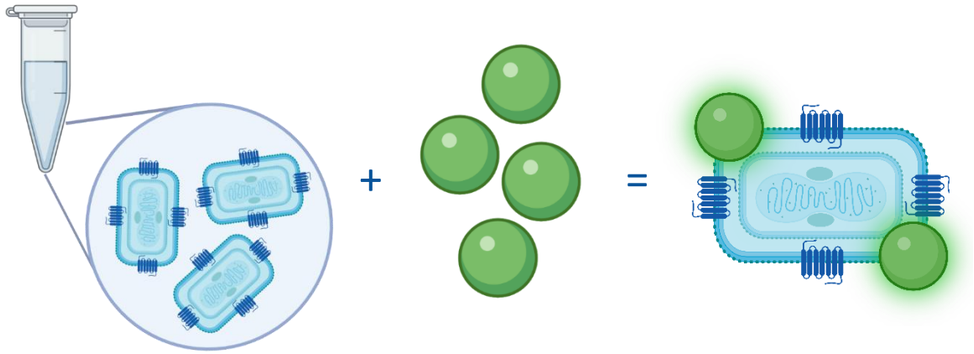
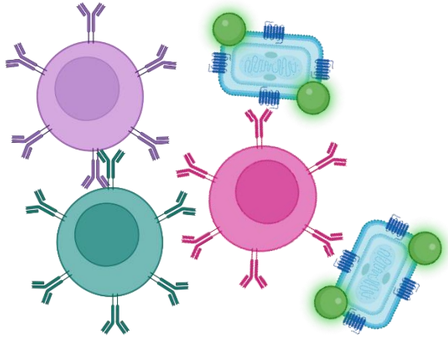
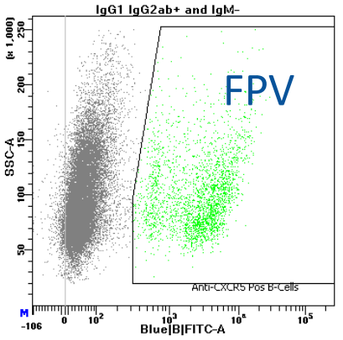
Biotinylated FPV/CXCR5 virus was fluorescently labelled using Streptavidin coated Fluorescent beads



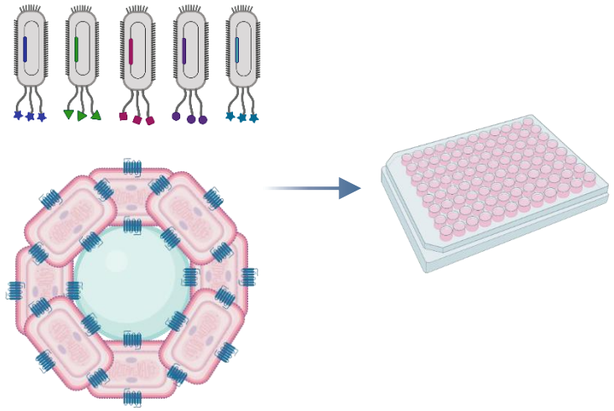
Labelled virions are incubated with isolated memory B cells from 6 mice and sorted for CXCR5 specific binding.





20,000 cells sorted

A phage library of ~1x10^{8} clones was generated and panned on FPV/CXCR5 coated magnetic beads for 3 rounds.



- V genes were extracted from enriched phage while preserving VH-VL pairing
- The V genes were cloned into a mammalian expression vector as secreted IgG

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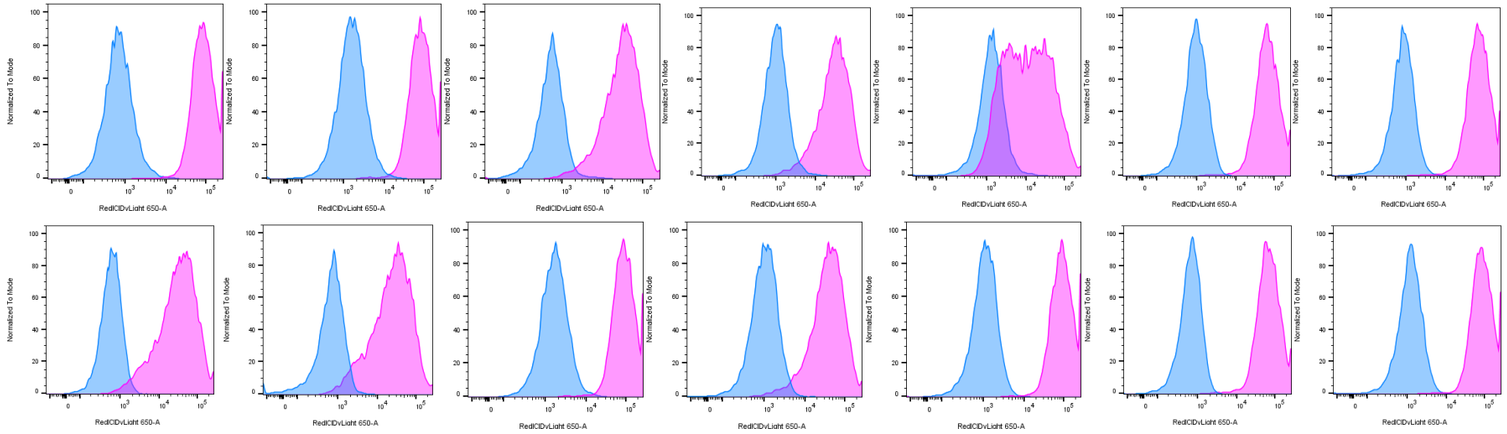
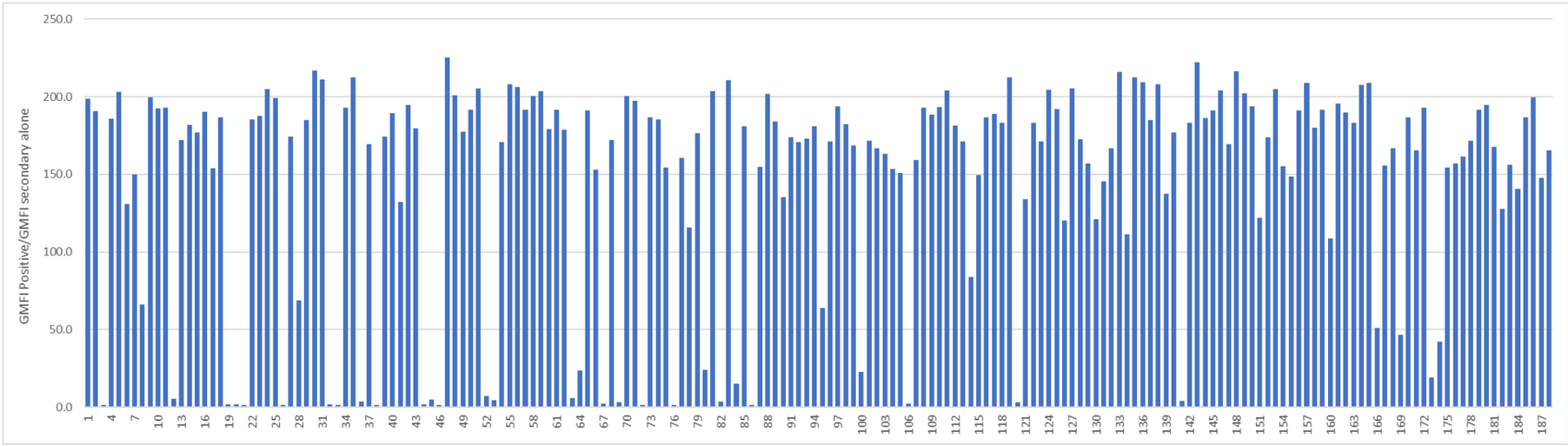
CXCR5 Antibody Discovery

ActivMAb Technology



376 individual clones were screened by transient transfection in CHO cells by flow cytometry and positive binding antibodies were sequenced.



Results:

165 Unique antibodies

18 Unique HCDR3s with many found only once.

Select antibodies were purified and tested for specificity, functionality and affinity

CXCR5 Antibody Characterization: Specificity

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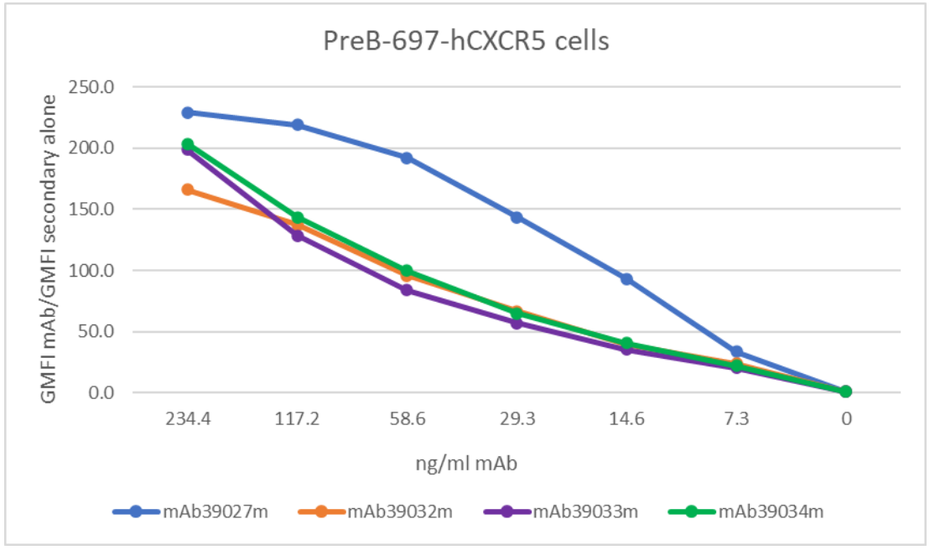
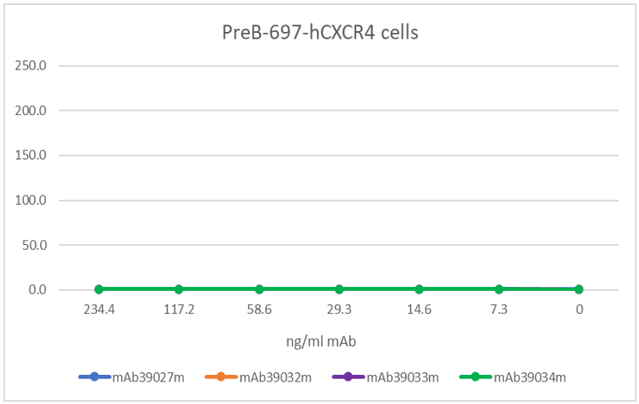
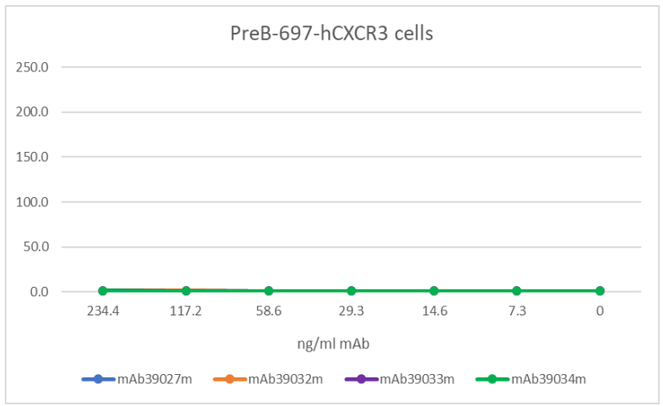
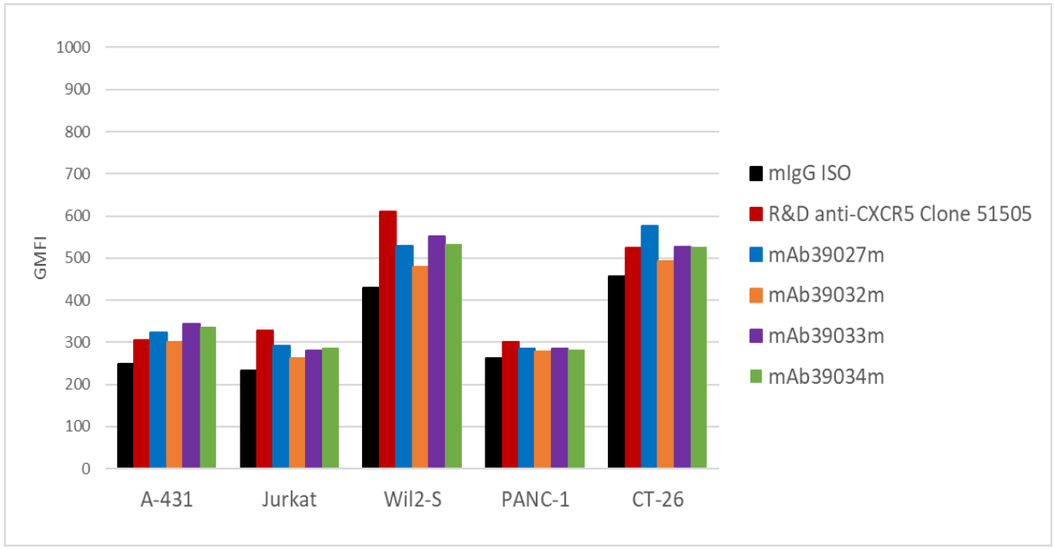
All four antibodies purified showed specificity to CXCR5 and no binding to CXCR4 or CXCR3 by FACS.







Additional testing on a panel of cell lines showed no off-target binding and behaved similar to commercial Anti-CXCR5 control antibody.



Additionally, no off-target binding in specificity predictive ELISA assays

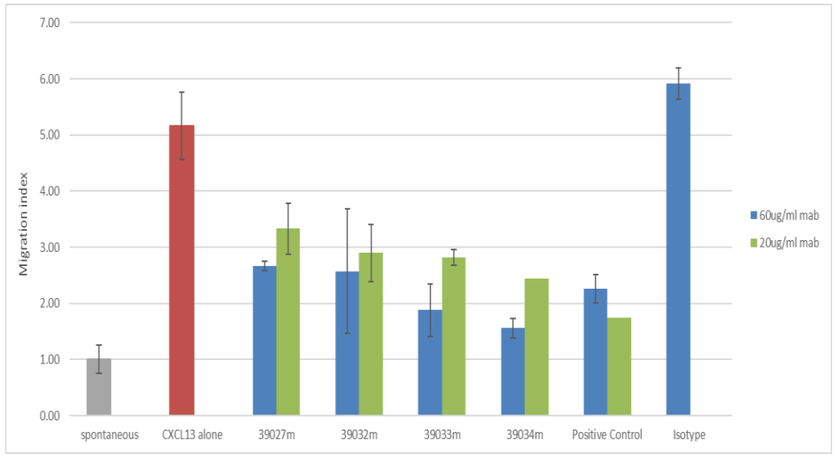
Mabs passed biophysical characterization assays (SEC, CIC, etc)

CXCR5 Antibody Characterization: CXCL13 Blocking

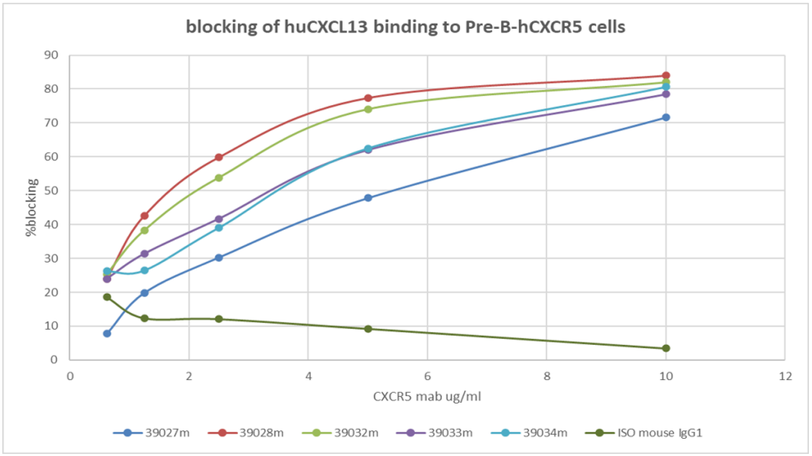
ActivMAb Technology

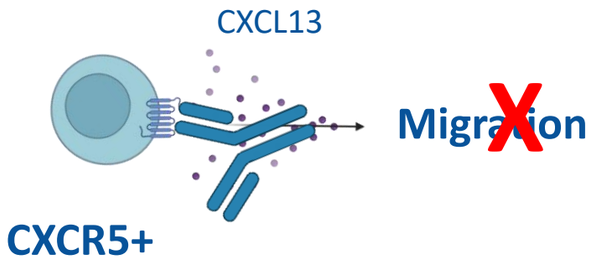
Antibody	Affinity (nM)	% Migration blocking
39027m	0.04	36%
39032m	0.53	44%
39033m	0.34	45%
39034m	0.35	53%

Blocking of CXCL13 Induced Migration



Blocking of CXCL13 Binding





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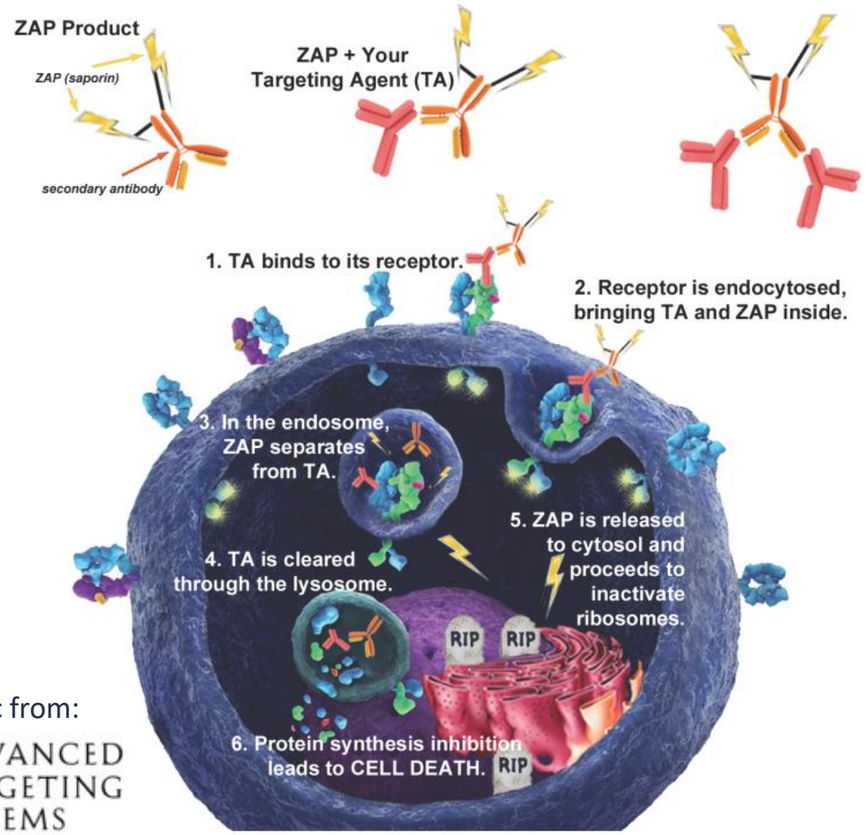
CXCR5 Antibody Characterization: Antibody Internalization

ActivMAb Technology

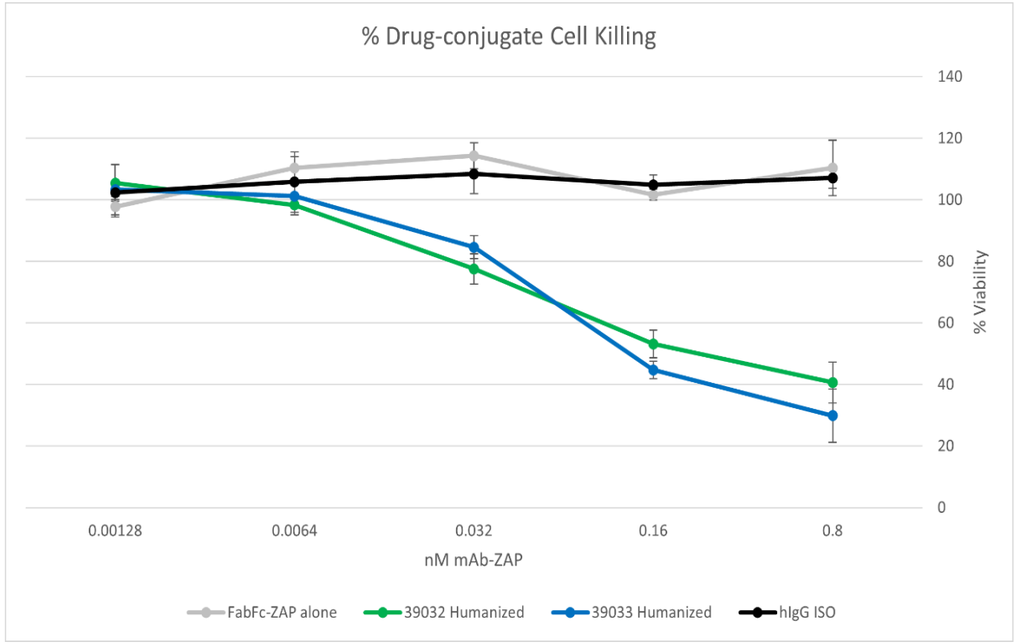
Internalization and ADC potential was evaluated using Fab-ZAP Saporin reagent from Advanced Targeting Systems:



Graphic from:

ADVANCED TARGETING SYSTEMS

Drug-conjugate Cell Killing



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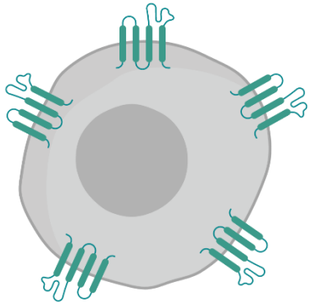
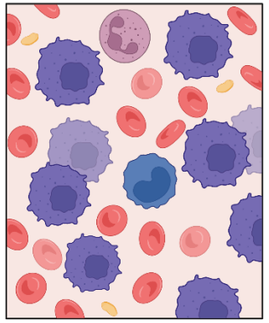
CD20 Antibody Discovery

ActivMAb Technology



CD20 is a tetra-span protein expressed on B cells and well characterized as an antibody drug target.



Anti-CD20 Drug Indications:

- Non-Hodgkin lymphoma
- Chronic lymphocytic leukemia
- Rheumatoid arthritis
- Autoimmune disease

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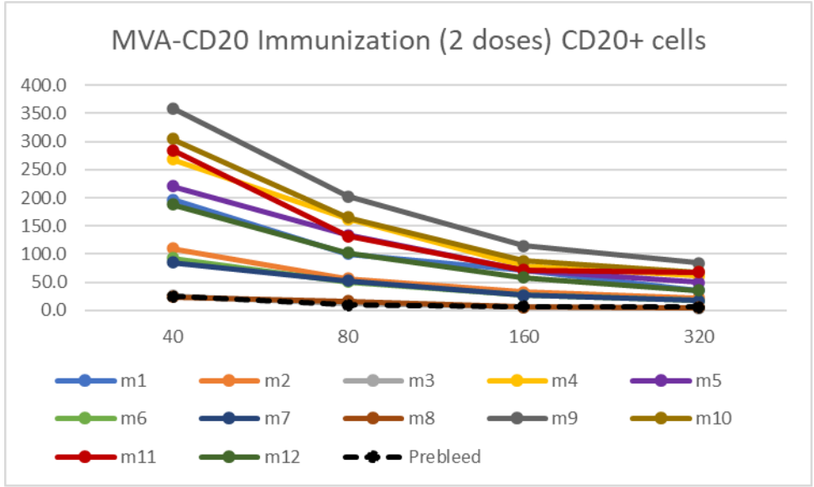
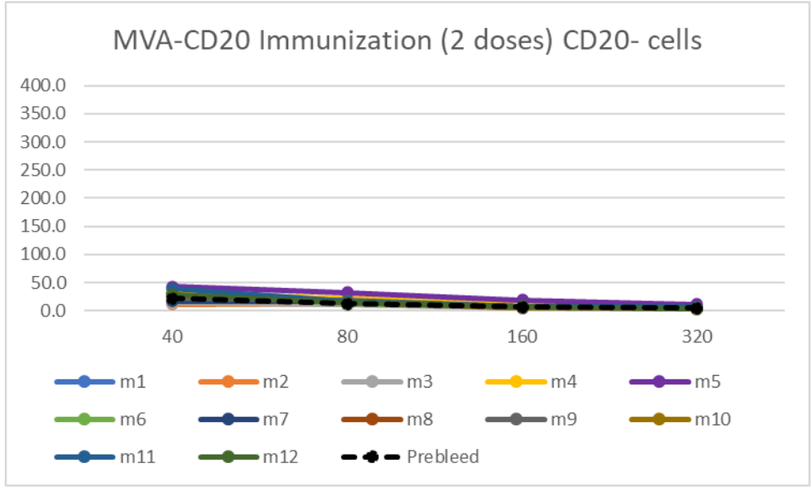
Twelve Balb/c mice were immunized with MVA-CD20 for 2 doses and sera titers were checked by flow cytometry.

The top 6 mice were sacrificed, and their Memory B cells were sorted using FPV/CD20.

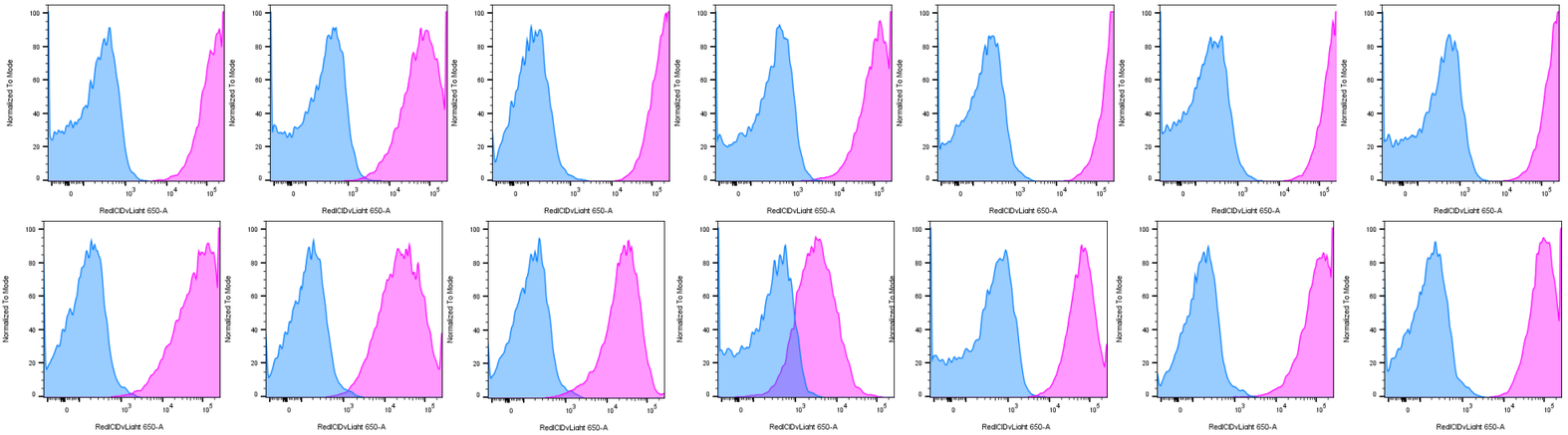
A phage library of ~1x10^8 clones was generated and panned on FPV/CD20 coated magnetic beads for 3 rounds.

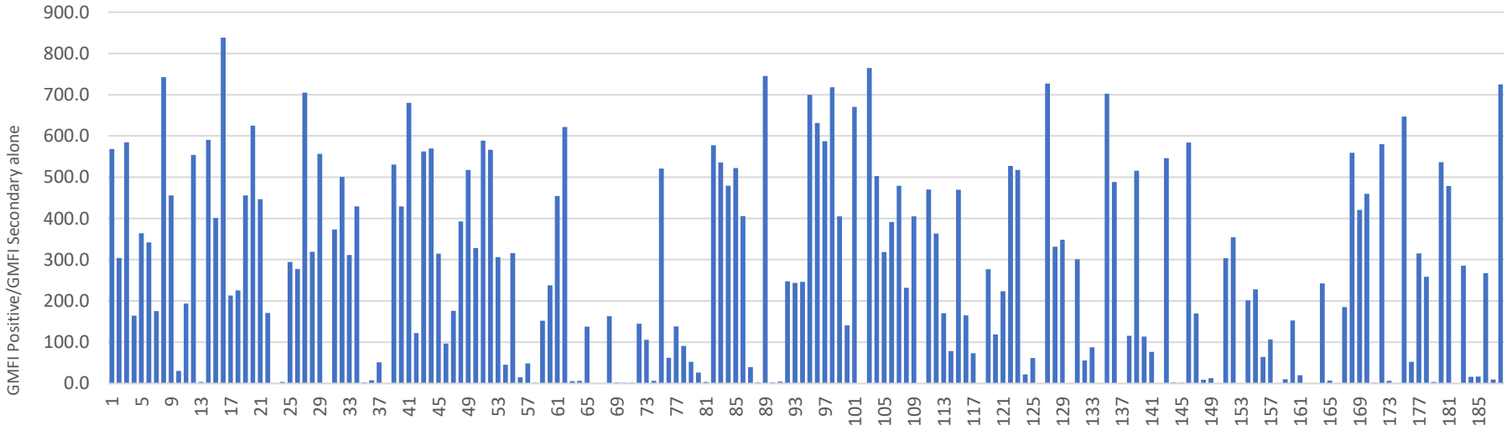




CD20 Antibody Discovery

ActivMAb Technology





188 individual clones were screened by transient transfection in CHO cells and flow cytometry on stable cell lines and sequenced.

Results:

125 Unique antibodies

51 Unique HCDR3s with many found only once.

CD20 Antibody Characterization

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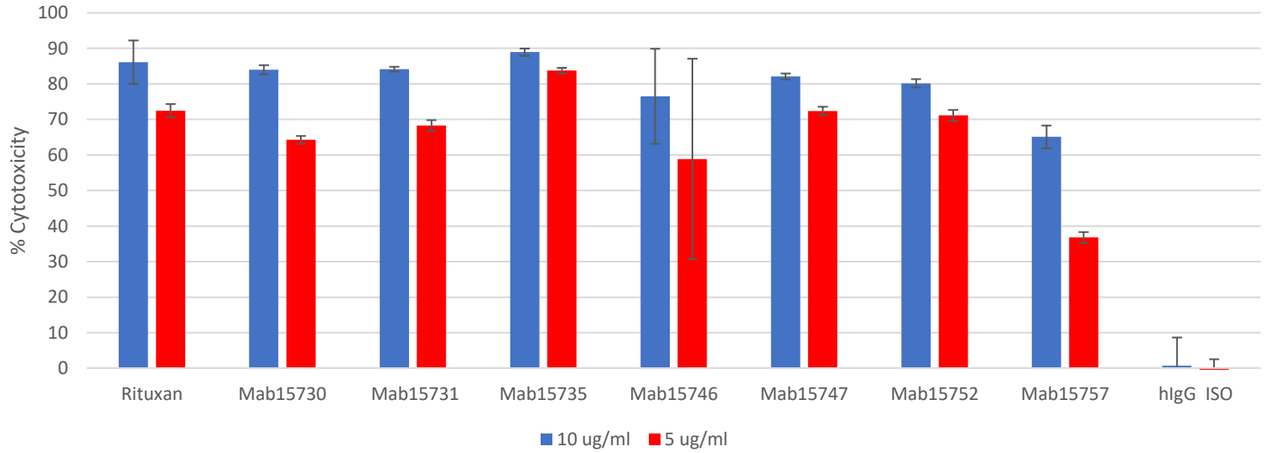
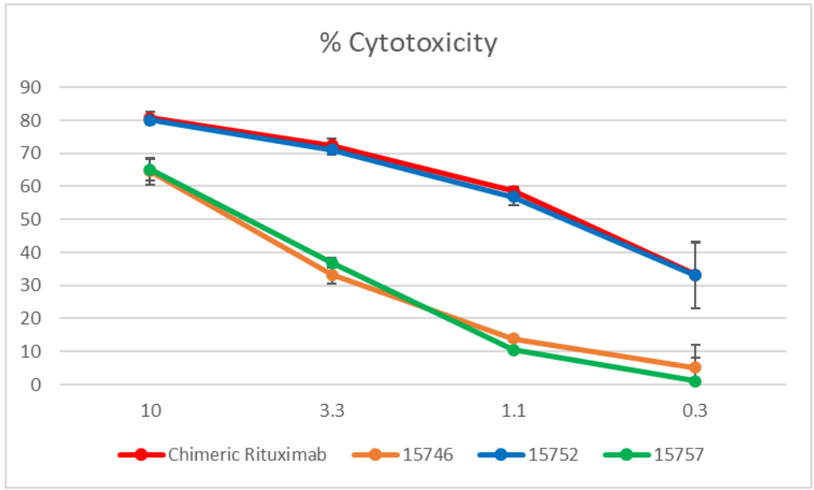
A panel of antibodies were purified and found to be functional in a CDC assay and comparable to Rituxan.



Selected antibodies were further titrated down and one of these primary mAbs appears to have activity equivalent to Rituxan.



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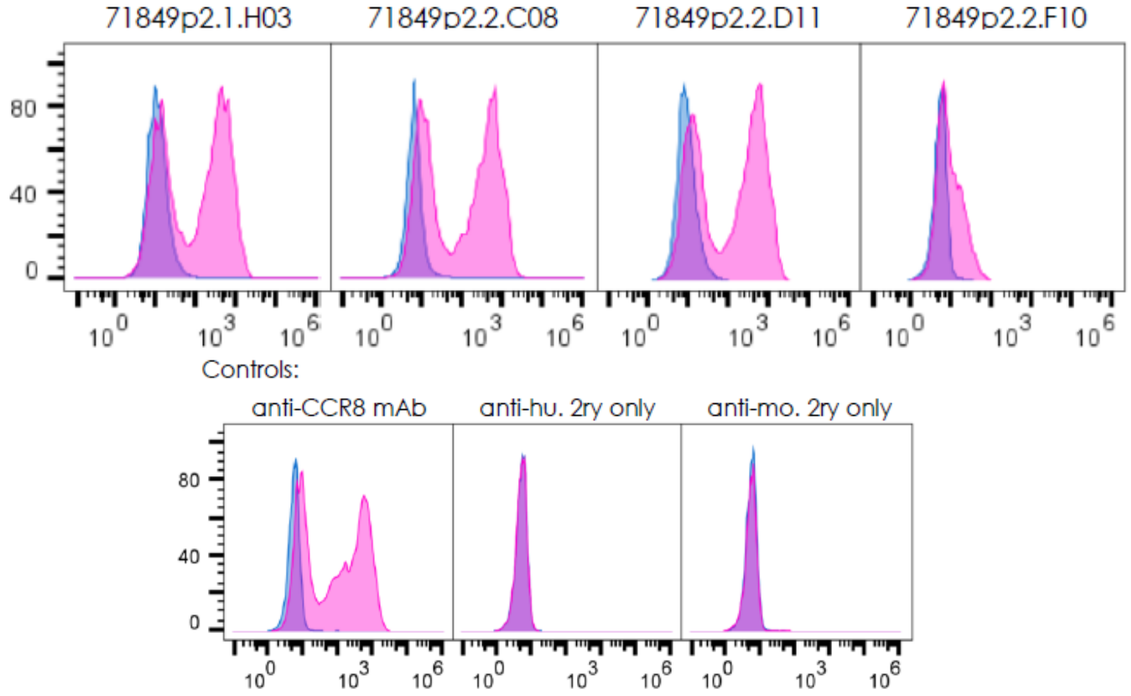
Collaboration with OmniAb: CCR8 & Kv1.3

OmniAb®

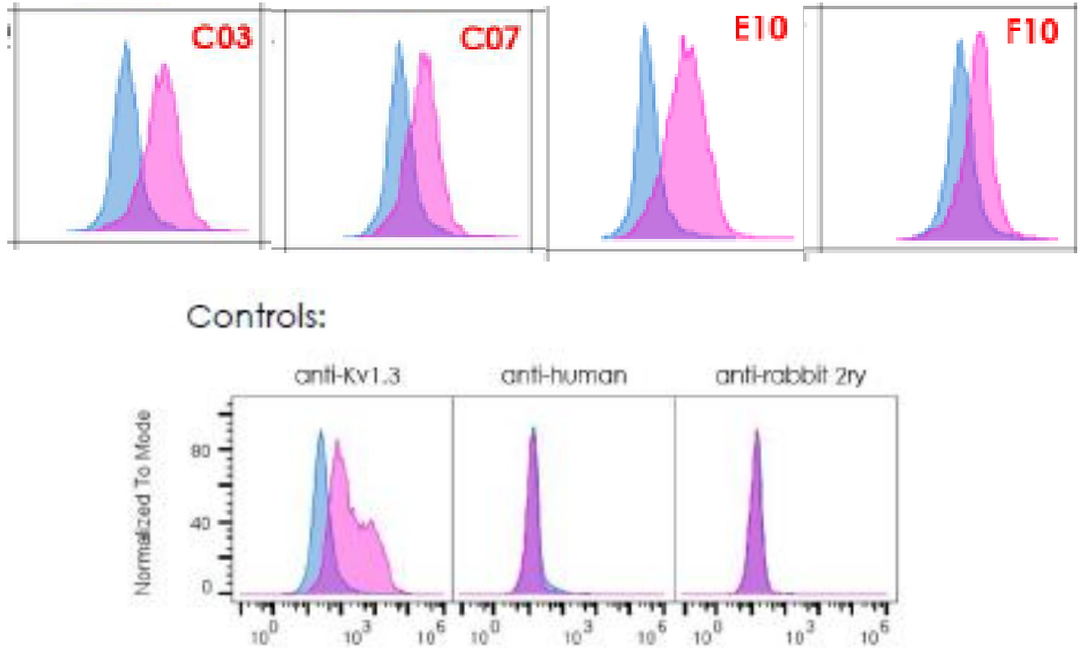
ActivMAb Technology

- OmniAb® performed a GEM (*G*el *E*ncapsulated *M*icroenvironment) assay using splenocytes from chickens previously immunized for CCR8 or Kv1.3 and beads coated with poxvirus expressing the same antigens.
- Antibodies were then tested for specificity by flow cytometry on transiently transfected cells.
- Multiple specific mAbs were discovered for both targets.

CCR8

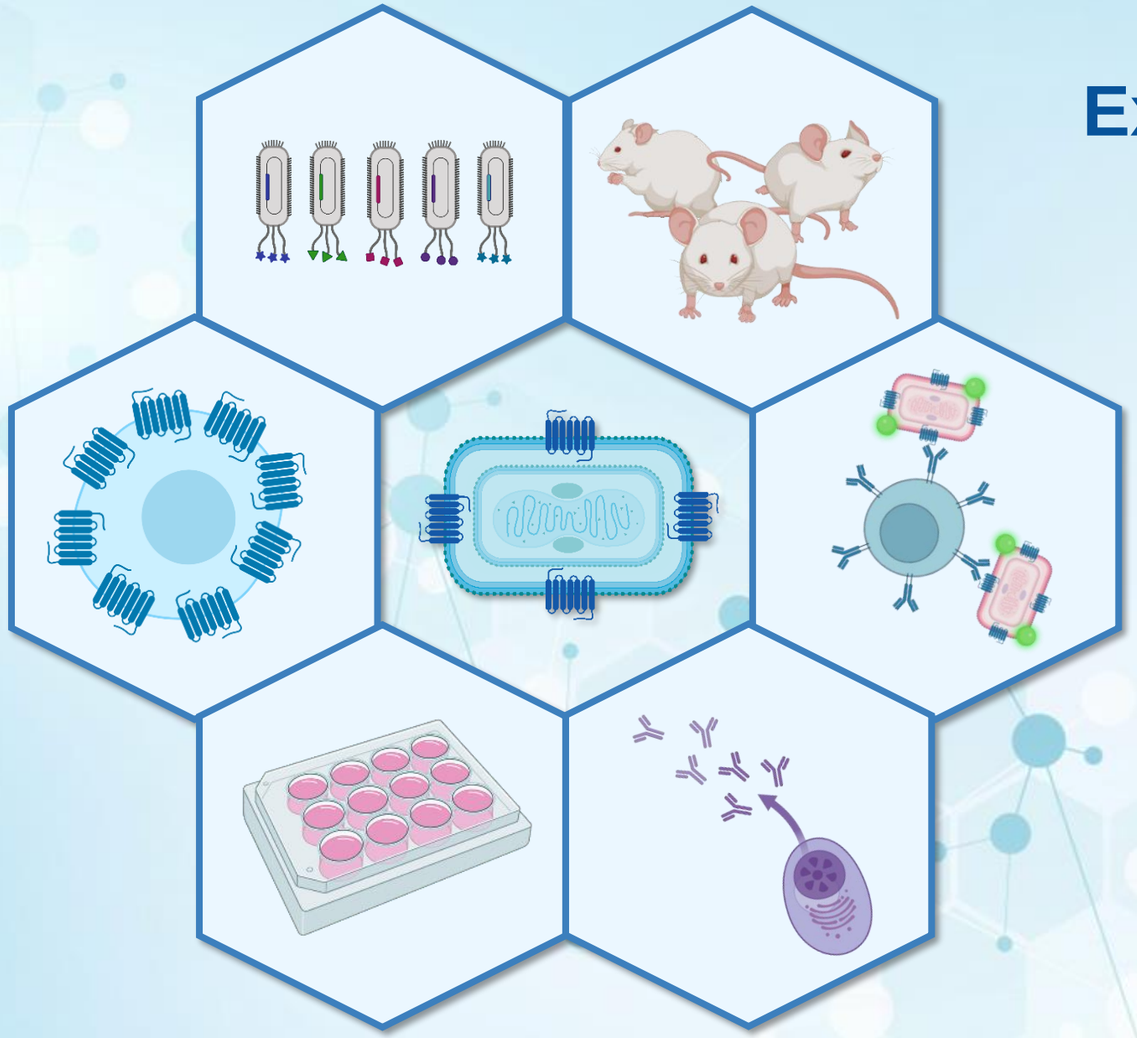


Kv1.3



Research Capabilities

ActivMAb Technology



Expression of ANY membrane protein

- GPCRs, Ion Channels, Multi-spanners, and ECDs
- Native conformation without detergents or refolding

Ideal for Antibody Discovery

- Suitable for phage, yeast or immunization
- Two distinct poxvirus strains for specificity
- Compatible with B cell sorting applications

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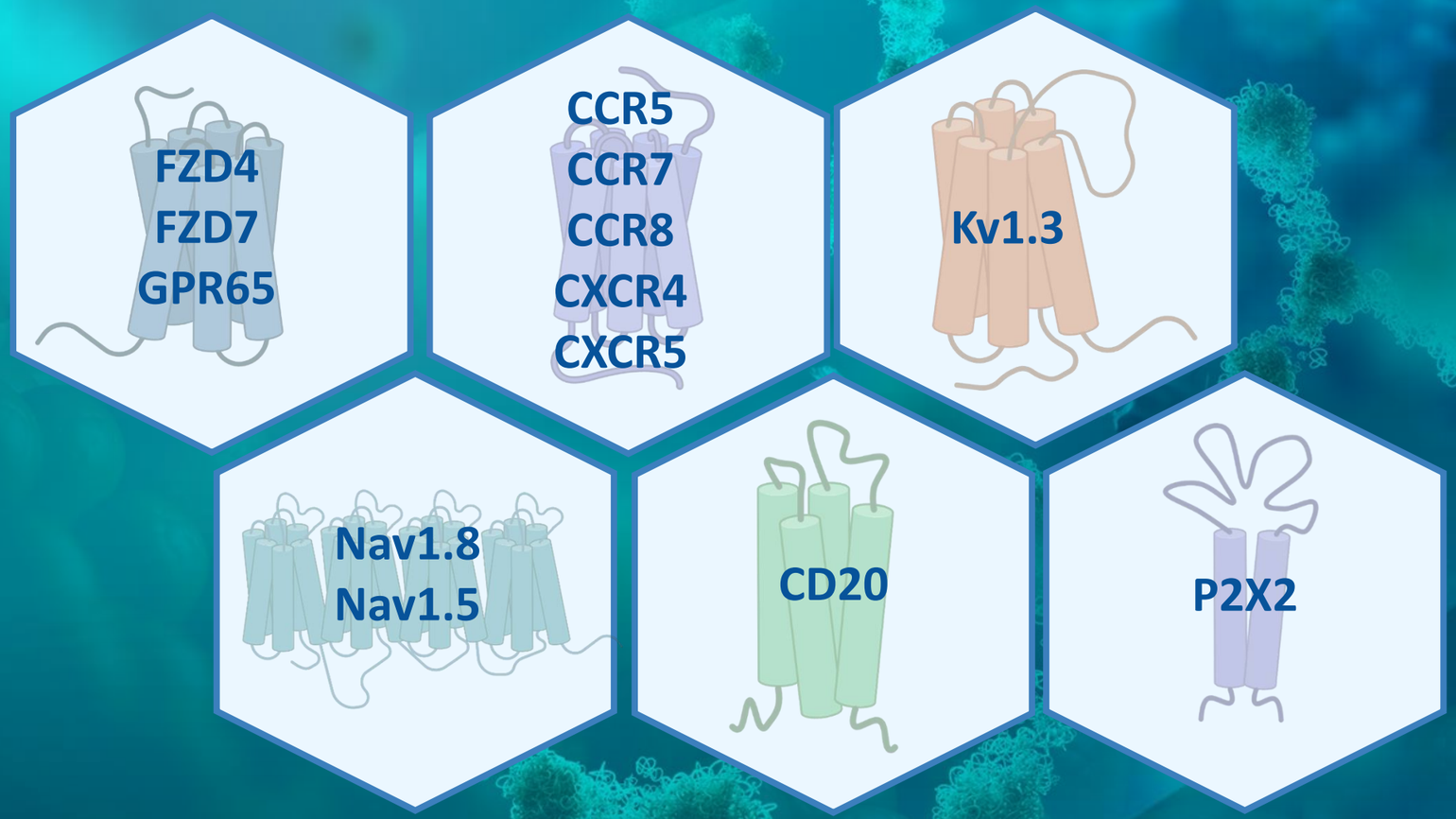
Antibody Discovery Campaigns and Constructs

Antigen	Immun.	mAbs	Function
CCR8	*Partnered Project*		
CXCR5			
CD20			
P2X2			
SEMA4D			
BCMA			
CCR5			
CCR7			
CXCR4			
Kv1.3			
CCR2			
Claudin 18.2			
Nav1.8			
GPR65			



*Vaccinex Announced First Patient Dosed with
Anti-CCR8 Antibody Licensed to Surface Oncology on 1/9/23

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Acknowledgements

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Discovery on Target

SCAN ME

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Poster Presentation:
P089: Discovery of High-Affinity Functional Antibodies Specific for CXCR5 and Other Multi-Pass Membrane Proteins

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