# Discovery of High Affinity Antibodies Specific for CXCR5, P2X7 and Other Multi-pass Membrane Receptors

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VACCINEX ActivMAB Technology

## Abstract

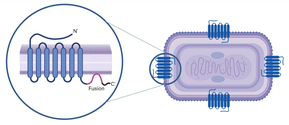
G-coupled Protein Receptors (GPCRs) and Ion Channels present challenges to traditional antibody discovery methods due to their intrinsic reliance on the cell membrane to maintain their native structure. To overcome these challenges, Vaccinex has developed a fusion protein technology to enable the direct incorporation of multi-pass membrane proteins into the membrane of two antigenically distinct poxviruses. 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. Antigen expressing virus can be readily purified and used for antibody selection using any in vitro display platform where alternating between the two strains eliminates selection of anti-viral antibodies. In addition, due to adjuvant properties of the virus, antigen virions induce strong anti-antigen responses following in vivo immunization.

Spleens taken from multiple immunized mice are pooled and used to generate a phage library for antibody discovery with timelines much faster than traditional hybridoma generation. Here we describe selection for functional antibodies against the GPCR CXCR5, the Ion channel P2X7, and the tetra-spanner Claudin18.2 illustrating the effectiveness of our technology for these important therapeutic targets.

## Technology Introduction

Vaccinex fusion protein technology provides for efficient incorporation of multi-pass membrane proteins into the poxvirus membrane. This system ensures native conformations for antibody discovery both *in vitro* and *in vivo*.

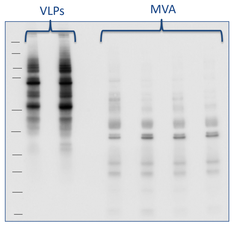
The fusion protein is intra-viral and can be fused with fluorescent proteins like GFP for antigen tracking.




Antigens are produced in two antigenically distinct, highly attenuated BSL1 poxviruses to **eliminate anti-viral** background. Additionally, the poxvirus membrane has limited protein complexity with four known proteins incorporated.

Mammalian Poxvirus (MVA)

Avian Poxvirus (FPV)

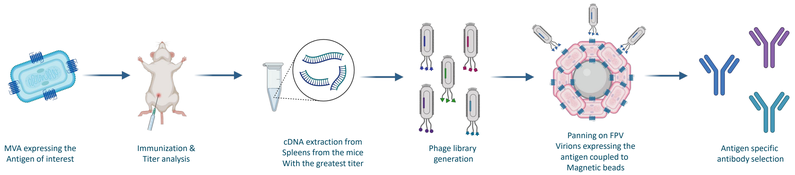



MVA antigen virions contain fewer non-antigen membrane proteins than conventional VLPs.

Western blot of VLPs and MVA virions surface biotinylated and loaded at equal protein concentrations. Detection is with streptavidin-HRP.

## Antibody Discovery Methods

Cohorts of 12 female Balb/c mice were immunized with Modified Vaccinia Ankara (MVA) expressing the human antigens CXCR5, Claudin18.2 or P2X7. Sera titer was analyzed after two or three doses by flow cytometry. Custom phage libraries were created from six mice immunized with antigen virions that displayed sufficient titer and were used to pan on the alternate strain of poxvirus, Fowlpox virus (FPV) also expressing the human antigen of interest. The v-genes from the bound phage were cloned into a mammalian secreted IgG expression vector and tested for antigen specificity by flow cytometry. Select antibodies were purified and tested further for affinity and functionality against the target of interest.

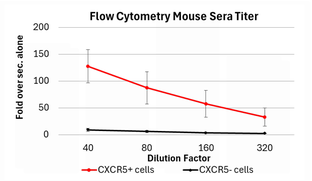


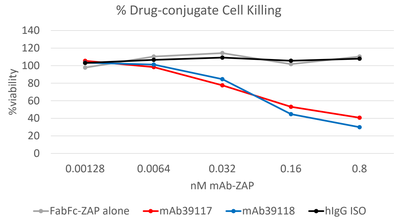

Images created with Biorender.com

## Discovery of functional human antibodies for CXCR5

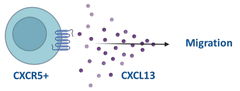
CXCR5 is a GPCR chemokine receptor expressed on mature B cells and involved in cell migration. CXCR5 is a potential oncology target as it is expressed in Burkitt's Lymphoma.

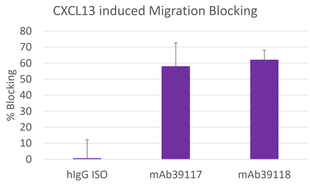
Unique antibodies discovered by immunized library phage panning that bound specifically to human CXCR5 were humanized and the top two mAbs were purified. Functionality as a potential malignant B cell depletion therapeutic was investigated using a pseudo-ADC reagent FabFc-ZAP (Advanced Targeting Systems) and Human PreB-697 cells over-expressing CXCR5. Specific cell killing was evident over Isotype Control




The ligand for CXCR5 is CXCL13. Cells expressing CXCR5 will migrate towards CXCL13 and can be evaluated in a standard transwell migration assay. The humanized antibodies were able to block migration to CXCL13.



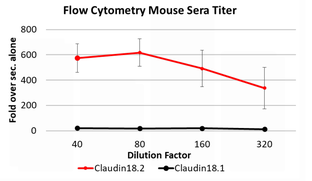

**CXCR5 Antibody Discovery Summary**

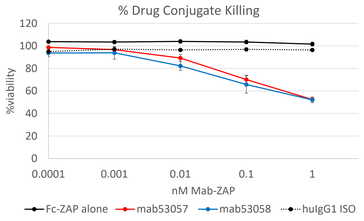
| | |
|---|---|
| **Antigen type** | GPCR |
| **Unique Antibodies Found** | 266 |
| **Unique HCDR3s** | 29 |
| **Affinity Range** | 0.04-1.7 nM |
| **Functionality** | Internalization, Migration Blocking |

## Discovery of functional human antibodies for Claudin18.2

Claudin18.2 is a membrane tetra-spanner involved in tight-junction formation and a target for gastric and esophageal cancer and metastasis. Multiple clinical trials using anti-Claudin18.2 antibody therapeutics are ongoing, with methodologies including ADCs, bispecifics and CAR-T.

As for CXCR5, the ability of the two best Claudin18.2 specific antibodies were tested for their ADC potential using FabFc-ZAP. Both antibodies showed the ability to internalize and kill HEK293-Claudin18.2 cells.



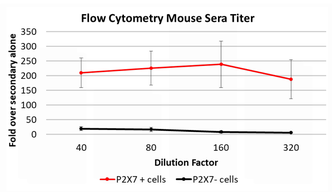

**Claudin18.2 Antibody Discovery Summary**

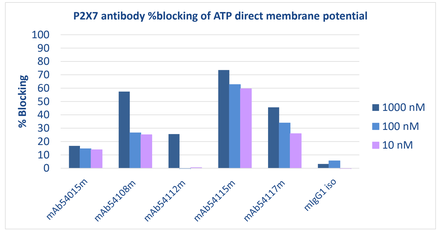
| | |
|---|---|
| **Antigen type** | 4-pass |
| **Unique Antibodies Found** | 27 |
| **Unique HCDR3s** | 11 |
| **Affinity Range** | 0.4-1.1 nM |
| **Functionality** | Internalization |

## Discovery of functional antibodies for P2X7

P2X7 (P2X purinoreceptor 7) is an Ion Channel with two transmembrane domains and a large extracellular domain. P2X7 is an ATP binding molecule being researched for therapies in immunology, oncology, neurology and cardiology. In the ATP rich tumor micro-environment, activation of P2X7 may influence tumor cell metabolism, survival and metastasis.

Several unique P2X7 antibodies were discovered, and the top five were purified and tested for the ability to block ATP induced membrane potential in HEK293 overexpressing P2X7. Variability was seen among the antibodies with mAb54115m showing significant blocking. Humanization and further characterization of this antibody is in progress.




**P2X7 Antibody Discovery Summary**

| | |
|---|---|
| **Antigen type** | Ion Channel |
| **Unique Antibodies Found** | 124 |
| **Unique HCDR3s** | 16 |
| **Affinity Range** | 0.05-0.43nM |
| **Functionality** | ATP Blocking |

## Conclusions

Antibody selection for difficult targets such as GPCRs and Ion Channels remains challenging due to limitations in antigen availability for traditional methods of discovery such as hybridoma or in vitro panning. Immunization can be performed with whole cells, VLPs, or molecular methods like DNA or RNA, but suitable antigen reagents are still required for assessing titer and antibody selection from the animal immune repertoire. In addition, whole cells and VLPs have known problems with specificity due to the variety of antigens they present in addition to the target of interest.

Antigen virion membrane presentation in two antigenically distinct strains overcomes challenges to specificity often encountered with other membrane protein reagents including VLPs and whole cells. This technology has applications for both *in vivo* an *in vitro* antibody discovery for increasingly challenging antibody therapeutic targets.

Such functional, high affinity, specific humanized antibodies have therapeutic applications including passive immunotherapy, ADC conjugates, bispecific formats, and CAR-T. Antigen virions are a potent tool to develop novel therapeutics against previously unattainable oncology and immunotherapy targets.