Native complex membrane antigen expression on poxvirus for antibody discovery

Vaccinex has developed a fusion protein technology to enable the direct incorporation of multi-pass membrane proteins such as GPCRs and ion channels into the membrane of two antigenically distinct poxviruses. The protein of interest can be 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 any anti-viral antibodies from being selected.

Antigen virions can also be used in vivo antibody discovery methods. Immunization with the viral strains produce potent antibody responses. The resulting immune cells can then be used for Single B Cell sorting, Plasma Cell interrogation, or to create an immunized phage library for in vitro panning. Here we describe the discovery of functional antibodies against CXCR5 and P2X2 utilizing our antigen virions post-immunization in mice by all three methods.

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 4 known proteins incorporated.

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.

### Examples of Successful Targets

**In vitro Antibody Discovery Methods**

**Example: Antibody Discovery for CXCR5**

Balb/c mice were immunized with CXCR5 antigen virions and mice with sufficient CXCR5 titer were sacrificed and their spleens and bone marrow taken for B cell sorting, immunized phage library generation and plasma cell ELISA screening.

Plasma cells were isolated from the spleens and bone marrow and seeded into 96 well plates at 100-1000 cells per well. Supernatants were then tested by ELISA on CXCR5 virion versus Negative virion coated ELISA plates.

Individual phage libraries were created from whole spleens, sorted B cells and CXCR5+ plasma cell pools and panned on CXCR5 antigen virions. DNA from the CXCR5 enriched phage was cloned into a CHO expression vector for transfection. Individual antibody clones were tested for binding to CXCR5+ cells (red histogram) vs Negative cells (black histogram) by flow cytometry.

A total of 30 antibodies were discovered. Antibodies exhibited functionality in the ability to prevent migration of CXCR5 expressing cells toward hCXCL13 and blockade of CXCL13 binding. Antibody affinity was determined by flow cytometry using the Scatchard method. Additional antibodies are pending purification and testing.

**In vivo Antibody Discovery Methods**

**Example: Antibody Discovery for P2X2**

Balb/c and C57Bl/6 mice were immunized with P2X2 antigen virions. Mice with sufficient anti-P2X2 titer were sacrificed and their spleens and bone marrow taken for immunized phage library generation and plasma cell ELISA screening.

Plasma cells were isolated from the spleens and bone marrow and seeded into 96 well plates at 100-1000 cells per well. Supernatants were then tested by ELISA on P2X2 virion versus Negative virion coated ELISA plates.

A total of 128 unique antibodies were discovered. Select antibodies were purified and tested for affinity and their ability to block ATP induced membrane potential changes.

### Conclusions

Poxvirus display of antigens is a versatile tool to express a variety of complex membrane proteins for antibody discovery, including GPCRs, Ion Channels and ECDs in their native conformation. The use of two antigenically distinct poxviruses with limited membrane diversity facilitates antibody selection by in vitro display technologies such as phage and yeast. Antigen poxvirus can additionally be used for in vivo discovery methods such as single B cell analysis, plasma cell analysis and hybridoma screening.