**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 4 known proteins incorporated.

**Example: Antibody Discovery for CXCR5**

B6/C mice were immunized with CXCR5 antigen virions and mice with sufficient anti-CXCR5 titer were sacrificed and their v-genes extracted for an immunized phage library that was used to pan on CXCR5 antigen virus.

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 a modified Scatchard method.

**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 v-genes extracted for an immunized phage library.

Two antibodies were humanized and then tested for drug-conjugated cell killing using FabFc-ZAP as compared to controls. Both exhibited titratable cell killing of PreB-CXCR5 cells.

**Conclusions**

Poxvirus display of complex membrane antigens, including GPCRs, Ion Channels and ECDs in their native conformation is a versatile tool for antibody discovery. Immunization with recombinant poxviruses elicits specific anti-antigen antibodies in mice and can additionally be used for discovery methods such as immunized phage display libraries, single B cell analysis or plasma cell analysis. The use of two antigenically distinct poxviruses with limited membrane diversity facilitates successful antibody selection post-immunization by eliminating any background anti-viral antibodies from the mice. Selected antibodies show high specificity, affinity and functionality for difficult targets such as CXCR5 and P2X2.