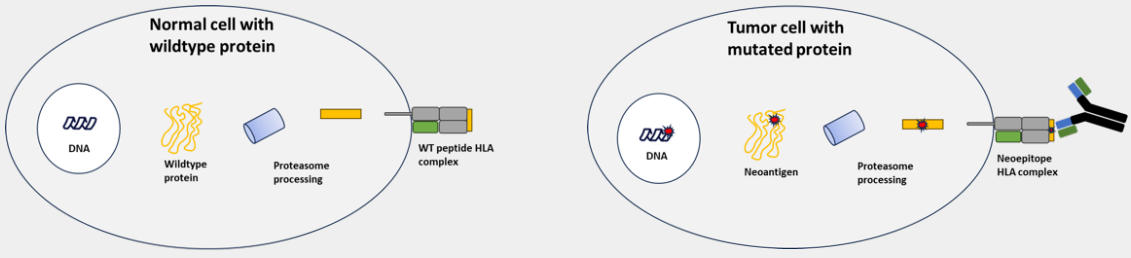
Case Study

Highly diverse human scFv libraries

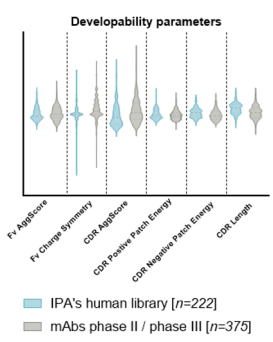
The Challenge

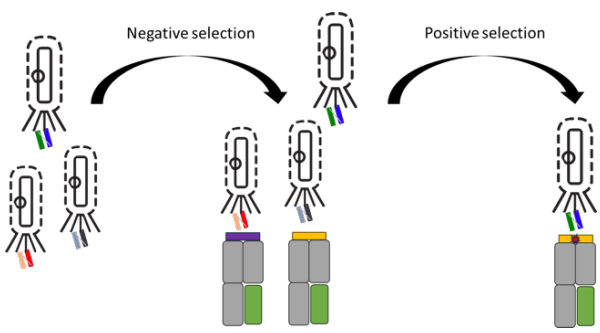
Neoantigens: promising, but challenging targets for developing cancer therapeutics. Due to their tumor cell-restricted expression, neoantigens are attractive targets for cancer therapy. Most neoantigens derive from intracellular proteins, making the intact neoantigen a difficult target for antibody-mediated therapy. However, neoantigens, like most endogenous proteins, are processed by the proteasome, and resulting neoantigen peptides may form complexes with HLA type 1 and 2 proteins, called neoepitopes, that locate to the plasma membrane for presentation to T-cells (figure on right) and are therefore potentially accessible to antibodies. However, due to the minor differences between neoepitopes and their non-mutated counterpart it is challenging to obtain neoepitope-specific antibodies through immunizations with recombinant neoepitopes. Rather, it requires antibody discovery techniques that allow for stringent selection procedures, such as phage display. IPA's highly diverse human scFv phage libraries have proven to be successful in obtaining antibodies against difficult targets and have the advantage of containing antibodies with favorable developability profiles. IPA's human phage libraries could therefore be an excellent source for clinically-developable neoepitope-specific antibodies. Here, we present a case study identifying antibody fragments (scFvs) against a common neoepitope by utilizing IPA's in-house human scFv libraries.



The Solution

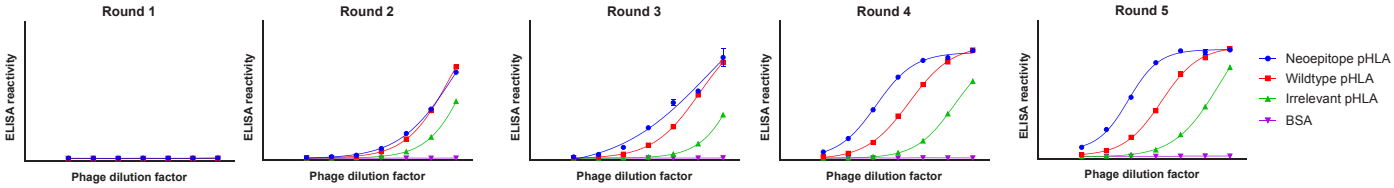
Tackling neoantigen antibody discovery challenges with fully human scFv libraries



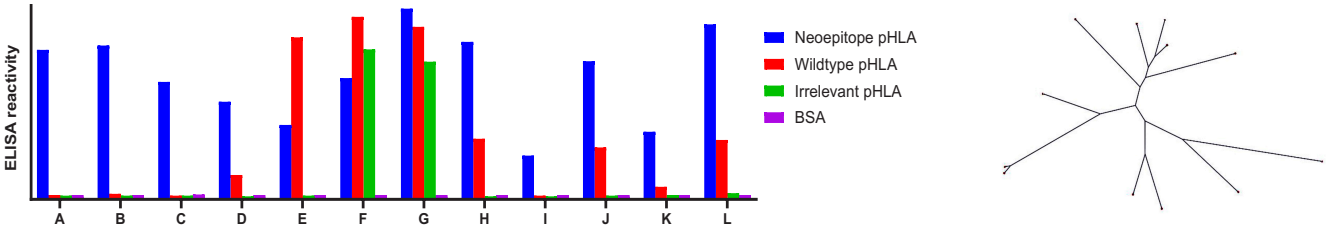


IPA-EU's human scFv phage libraries with favorable developability characteristics (figure on left) were subjected to five consecutive panning rounds consisting of negative selections against wildtype and irrelevant peptide HLA (pHLA) complexes and a positive selection against the neoepitope pHLA complex (figure on right). Selection and washing stringencies were increased after each round.

Output summary: accelerated neoepitope antibody discovery

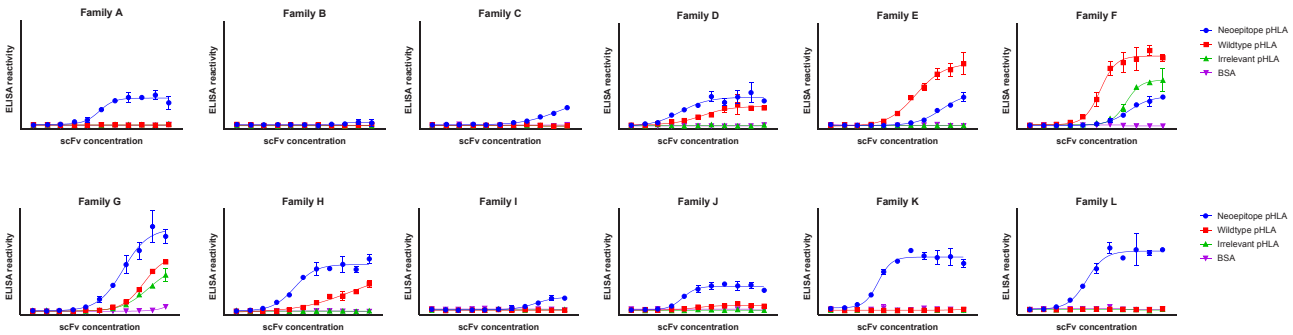


Polyclonal panning outputs from each selection round were screened for reactivity towards the neoepitope, wildtype and irrelevant pHLA complex and BSA as negative control in an ELISA. Neoepitope differentiating reactivity is apparent from selection round 3 on and increases until selection round 5. Round 4 and 5 panning outputs were selected for monoclonal scFv analysis.



ELISA-based reactivity analysis (figure above) and subsequent sequencing of monoclonal scFvs yielded clones with a range of specificities (from neoepitope specific to cross-reactive with all three pHLA complexes) that belonged to 12 unique sequence families, labeled A-L. Four of these families showed highly specific neoepitope pHLA complex reactivity. Interestingly, these four families (A, B, C and I) cluster closely as shown in a phylogenetic tree analyzing full VH and VL sequences (figure on right).

Streamlined workflow for rapid confirmation of neoepitope specificity



Each unique scFv was recombinantly expressed and purified, followed by screening for reactivity towards neoepitope, wildtype and irrelevant pHLA complex and BSA as negative control in an ELISA. In general, reactivity profiles were confirmed with some deviations (Family 2 and Family 11).

Conclusion

- IPA's highly diverse human scFv libraries were successfully used to identify neoepitope targeting antibodies, yielding 11 VH-CDR3 families, of which four showed highly neoepitope specific reactivity.
- Recombinant production and screening of scFvs confirmed the identification of 4 neoepitope specific antibodies.
- Phage display-mediated antibody discovery using fully human repertoires allows for rapid and accurate selection of specific, clinically-suitable neoepitope antibodies.

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