Case Study

LENS^{ai}™ Antibody Optimization

BioStrand
a subsidiary of IPA

Antibody engineering for tumor microenvironment targeting

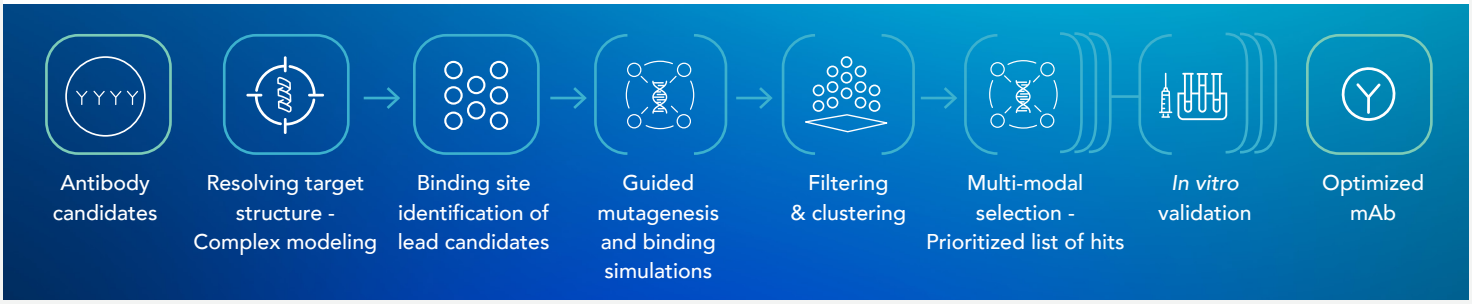
The Challenge:

Antibody (mAb) optimization comes with challenges, which are particularly true for paratope engineering. Determining amino acid replacements needs careful consideration to avoid loss of target binding and/or introducing poly-reactivity. Availability of experimentally determined antigen/antibody interaction sites are very helpful to guide residue substitution strategies, but these types of characterizations are time consuming, relatively expensive and not always successful.

The Approach:

In silico mAb engineering targeting a specific tumor microenvironment (TME) to yield a superior performing antibody with highly desirable properties

In this case study, the challenge was to introduce TME specificity while maintaining target selectivity in the absence of environment-specific crystal structures of the (complexed) target. Our LENS^{ai}™ methodology, incorporating structural model predictions and molecular dynamics, effortlessly considers environmental factors such as those found in the TME. This enables effective interrogation of the complexities of paratope optimization and expedites successful antibody engineering.



Engineering to introduce TME specificity

Objective: achieve engineered variants with the following characteristics

- Preferential binding to tumor microenvironment target 1 (TMET-1) in Tumor Condition A (TCA)
- Reduced or absent binding to TMET-1 in Tumor Condition B (TCB)
- Non cross-reactive with tumor microenvironment target 2 (TMET-2)

Input sequences

- Publicly disclosed TMET-1 binding mAb sequences: n=3
- Panel of TMET-1 binding mAb sequences sourced internally: n=12

Starting position

No crystal structure for the following:

- TMET-1 in TCA
- TMET-2 in TCA nor TCB
- Target-mAb complex

Program Summary

Method:

In silico structural modeling, epitope prediction, identification of interacting residues, mAb/target interaction engineering

Scale:

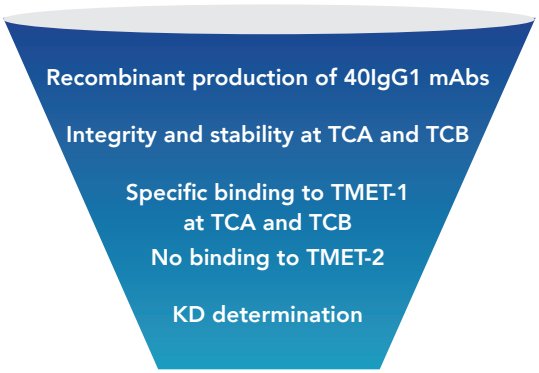
- 15 input parental mAbs
- Based on *in silico* modeling and epitope-paratope prediction: 6 out of 15 mAbs were suitable for *in silico* engineering to obtain TME specificity
- 181 variants designed *in silico*
- 40 mAbs derived from 5 parental mAbs prioritized for wet lab testing

Outcome

Three mutant mAbs preferentially binding target in the desired TME condition

Experimental validation

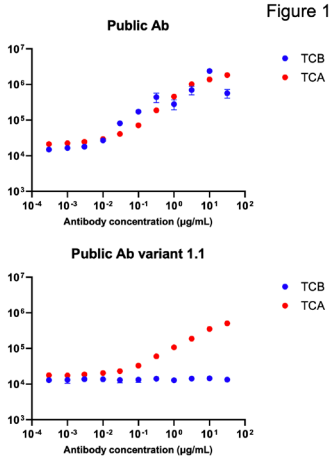
Overview: Forty mAbs prioritized for wet lab testing



Forty mutants with highest predicted TCA specificity and low off-target interaction were selected for *in vitro* validation. Each mAb variant had up to 5 amino acid substitutions, mostly across CDRs and predominantly in the heavy chain.

- All forty mutants were successfully produced recombinantly.
- HP-SEC analysis indicated that integrity and stability was not impacted by either tumor condition.
- Reactivity profiling using using both plate- and flow-based assays indicated that three mAbs had the desired characteristics.
- Affinity determination using high-throughput SPR was performed to determine KD under both conditions.

Figure 1



Result:

Successful identification of 3 in silico engineered mAbs with the desired properties

- Preferentially binding TMET-1 in TCA in a clear dose-dependent manner
- Absent/weakened binding in TCB

Figure 1 shows a representative mAb with newly engineered preferential binding to cell surface expressed TMET-1 under TCA over TCB compared to the parental mAb

- Demonstrated by flow cytometry

None of the 3 TCA-specific molecules showed:

- Cross-reactivity to TMET-2
- Non-specific binding to controls

Conclusion

BioStrand's LENS^{ai} *in silico* platform powered by patented HYFT® technology achieves successful molecular engineering of mAbs based on complex modeling in the absence of environment-specific crystal structures of the the target and mAb-target complex. In this case study, *in silico* redesigning of TMET-1 mAbs yielded 3 molecules with preferential binding under the desired TME condition, while maintaining TMET-1 specificity.

BioStrand
a subsidiary of IPA

For more information, contact: info@biostrand.ai