AI-driven development of pH-selective antibodies targeting human FOLR2 for tumor-specific therapy

MindWalk™

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Targeting tumors at their weak spot: pH

The acidic microenvironment of tumors offers a unique opportunity for selective therapy. Using MindWalk's LensAI AI-driven design and *in silico* modeling, we developed pH-selective antibodies against human FOLR2, achieving precise tumor targeting without requiring target crystal structures. Our lead candidate, Talem Ab B 3.1, shows selective binding at acidic pH, no binding at neutral pH with no cross-reactivity to human FOLR3—paving the way for next-generation precision immunotherapies.

Project goal:

Generate *in silico* engineered variants of hFOLR2 antibodies that show:

- Preferred binding to hFOLR2 at pH 6.0 with reduced or no binding at pH 7
- No cross reactivity with hFOLR3.

Input sequences

- Publicly disclosed hFOLR2 binding (pH=7) mAb sequences: n = 3
- Panel of hFOLR2 binding mAb sequences internally sourced: n= 12

Starting position

No crystal structure available for the following:

- hFOLR2 in acidic conditions
- hFOLR3 in acidic and neutral conditions
- Ab-Ag complex for human FOLR2

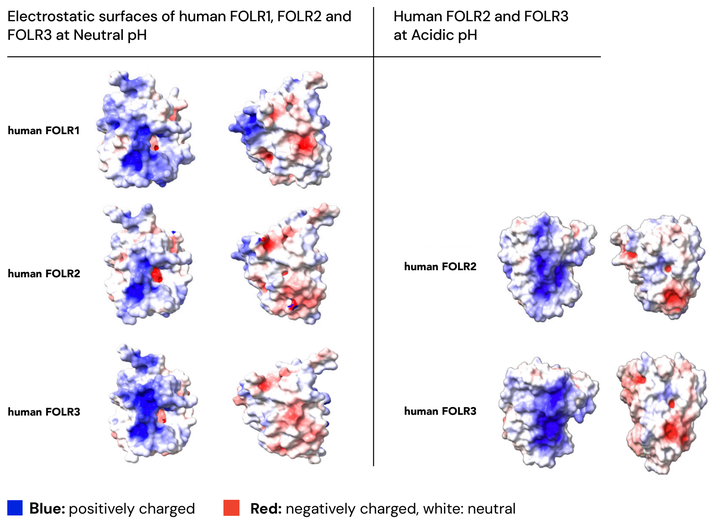
Rational *in silico* design delivered pH-selective variants with enhanced specificity

Phase I: Epitope—paratope evaluation

- Target modeling: FOLR2 and FOLR3 at pH=6 and pH=7, assess stability
- Predict epitope for all parental Abs
- Determine the residues critical for stable Ag-Ab binding at atomic level. Account for glycosylation in the Ag-Ab interaction
- Determine residues that are suitable for mutation to create selective binding at pH=6

Outcome: 6 parental antibodies suitable to move to next phase

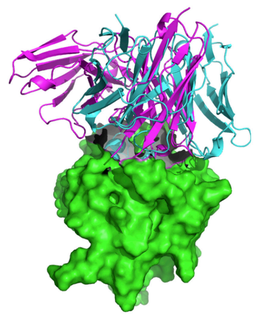
Advanced modeling accurately predicted human FOLR2 and FOLR3 structures at two pH levels, despite limited crystal references



Molecular dynamics and docking offered insightful analysis of antigen flexibility and epitope binding

Structure of hFOLR2 at pH6.0: results of 1200ns MD simulation

Two flexible regions, 60-75 in orange and 111-121 in blue



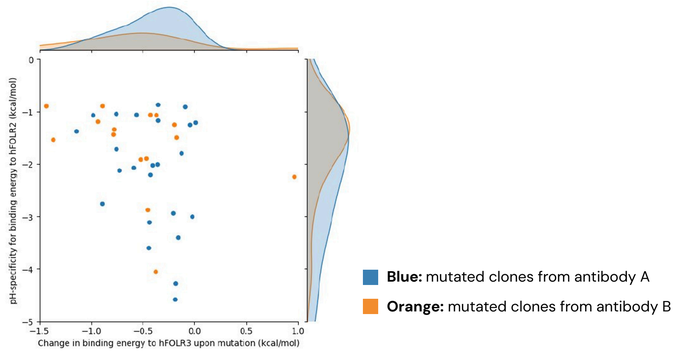
Talem B 3.1 (in cyan) and a public hFOLR2 antibody (in magenta) bound to hFOLR2 (green). Both antibodies bind to similar epitope.

Phase II: *In silico* mutagenesis design and evaluation

- 62 single-point mutations of key residues at different pH conditions evaluated
- 181 multi-point mutations of combined key residues at different pH condition evaluated
- Check cross reactivity with FOLR3
- Check immunogenicity of the mutated clones

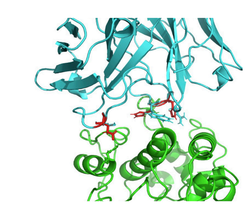
Outcome: 40 mutants were moved forward for wet lab testing

For the mutants of the two parental antibodies, which showed some prior cross-reactivity with hFOLR3, top clones for wet lab validation were selected based on change in affinity for hFOL3 and pH-specificity towards hFOLR2

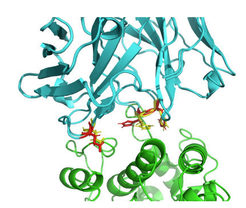


Gain in pH-specificity for "FOLR2" vs "Decrease in binding to FOLR3" as criteria of selection of the clones. Each point corresponds to a particular mutated Ab.
x-axis: change in binding energy to hFOLR3 upon mutation at normal pH conditions (similar as acidic conditions).
Zero means no changes, negative values correspond to stronger binding.
y-axis: difference in binding energy to hFOLR2 between pH 6.0 and pH 7.0.
Negative values correspond to increased pH-specificity.
The histograms on the top and right show the distribution of changes

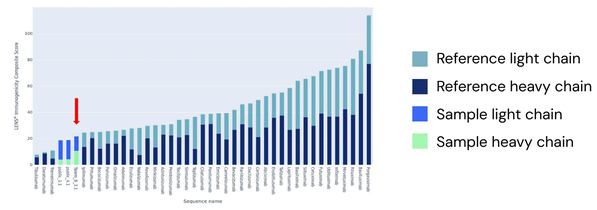
Paratope-driven modeling accurately predicted binding energy differences between mutant and parental antibodies across pH conditions



Mutated residues (red side chains) of Talem B 3.1 vs parental residues of Talem B (cyan side chains) at pH 6.0 in complex with hFOLR2 (green)



Mutated residues of Talem B 3.1 at pH6.0 (red side chains) vs Talem B 3.1 at pH 7.4 (yellow side chains) in complex with hFOLR2 (green)



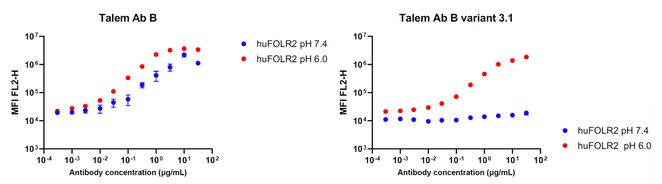
Stacked ranking bar-chart of antibodies: Immunogenicity score of Talem B 3.1 in comparison to a set of therapeutic antibodies

Phase III: Wet lab validation

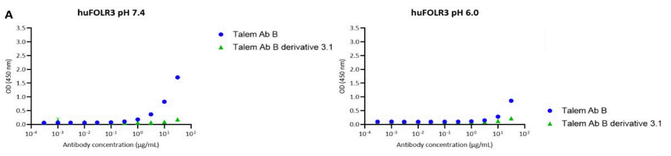
- Flow cytometry for target binding at different pH condition
- Indirect ELISA for cross reactivity with hFOLR3
- Affinity evaluation
- HP-SEC evaluation

Outcome: Successful identification of 3 in silico engineered mAbs with pronounced pH-selective binding to hFOLR2 and no cross-reactivity to hFOLR3. One best clone, Talem B3.1 is shown here.

Talem B 3.1 maintains binding strength with hFOLR2 at pH 6.0, but not at pH 7.4, demonstrating its pH-selective feature

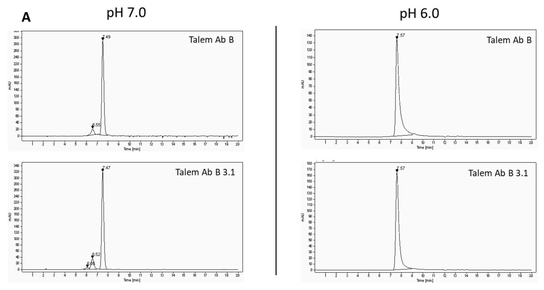


Talem B 3.1 showed differentiated binding to hFOLR2 at different pH conditions



Binding of Talem B3.1 with hFOLR3 was significantly reduced in both pH conditions compared to parental Talem B.

Talem Ab B3.1 shows good purity and stability with no aggregation in both pH conditions, similar as its parental clone



HP-SEC profiles of the Talem Ab B 3.1 and its parental antibody in different pH buffer

Antibody	KD(M) pH7.0	KD(M) pH6.0	Binding to Cyno FOLR2 by Flow	Cross-reactivity with hFOLR3 by ELISA
Talem Ab B Parental	E-08	E-06	Yes	Yes
Talem Ab B 3.1	ND	E-06	Yes	Near background levels

Talem Ab B3.1 maintains pH 6.0 affinity with hFOLR2 with reduced binding at neutral pH. Binding with hFOLR3 was also significantly reduced.

MindWalk delivers integrated, end-to-end solutions that unite AI, data, and advanced lab research to accelerate antibody discovery and development. LensAI *in silico* platform powered by patented HYFT technology achieved successful molecular engineering of pH-selective mAbs. Despite the absence of crystal structures, LensAI's advanced algorithms and proprietary docking technology successfully estimated the epitope of the antigen-antibody interaction. Molecular dynamics simulations at different pH levels guided the design of targeted mutations, resulting in antibody variants that met the project goal: reduced binding at neutral pH and preserved FOLR2 specific binding at pH 6.0. This innovative approach demonstrates the potential for rationally engineering pH-selective antibodies while maintaining specificity and favorable developability.