Characterizing antibody libraries by high throughput Surface Plasmon Resonance – from haystack to lead

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Symphogen - a Servier Company

Antibody Center of Excellence

- Close to Copenhagen and part of a leading Northern European life science hub
- Since 2020 part of the **Servier Group** with 21800 employees in over 150 countries, > 20% revenue invested in research
- A research and early development site with a focus on innovative therapeutic substances
- Optimized for antibody discovery and development from antibody discovery to IND and PhI
- Efficient processes for identification & selection of monoclonal and bi-specific Abs with unique MOAs
- Proven track record of providing an average of 1 IND per year since 2015
- Currently ~135 employees and ~ 20 consultants in Copenhagen

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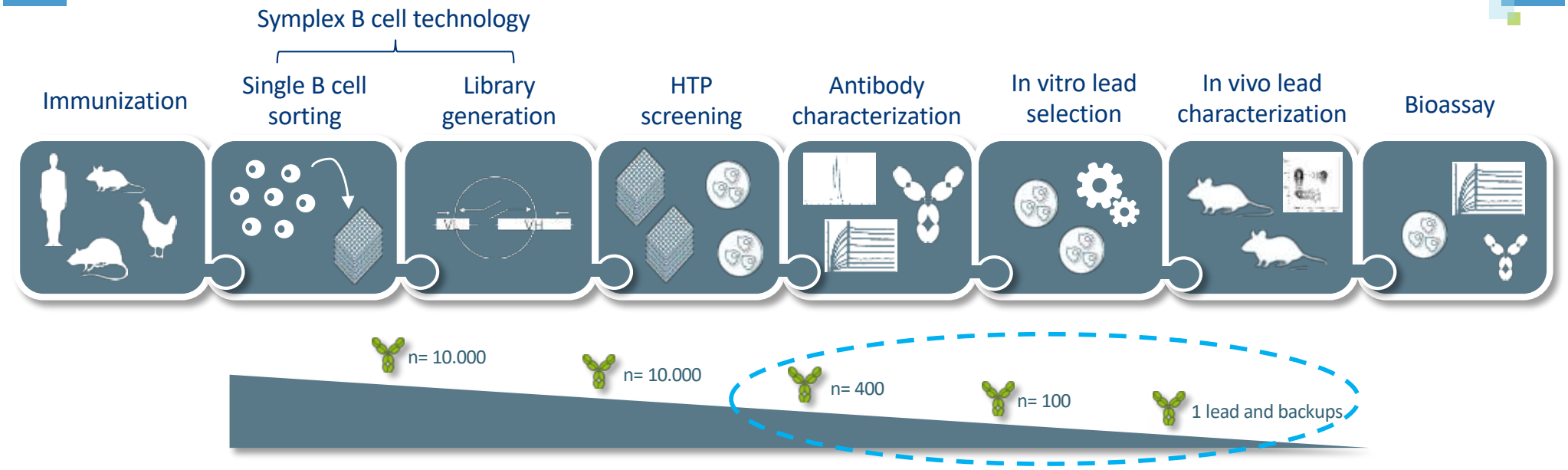
Integrated high throughput workflows for antibody generation



- Antibody characterization by HTP SPR
- Worked with Carterra LSA since late 2019

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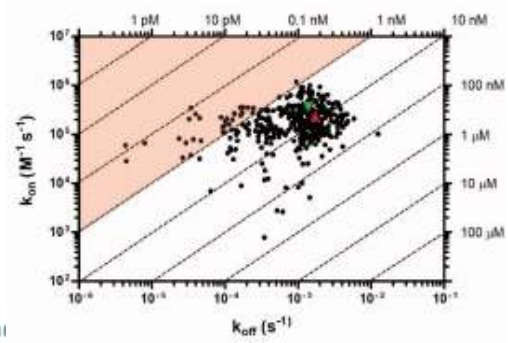
Antibody / antigen biosensor characterization

Antibody characterization

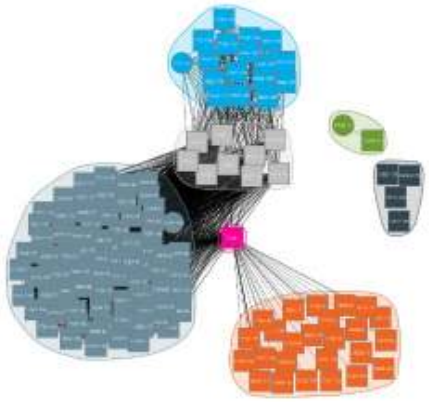
- Affinity ranking
- Binding kinetics (on-rate/off-rate)
- Cross-reactivity
- Epitope binning
- Epitope mapping

Carterra LSA SPR

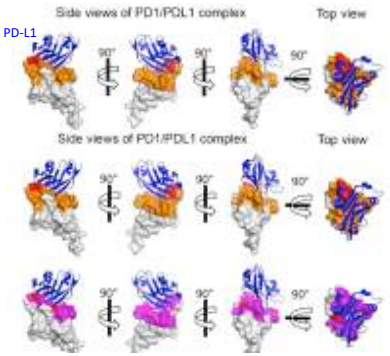
Binding kinetics & affinity (SPR, N=384 mAbs)



Epitope binning (N=384 mAbs)



Epitope mapping (N=~7-10 mAbs)



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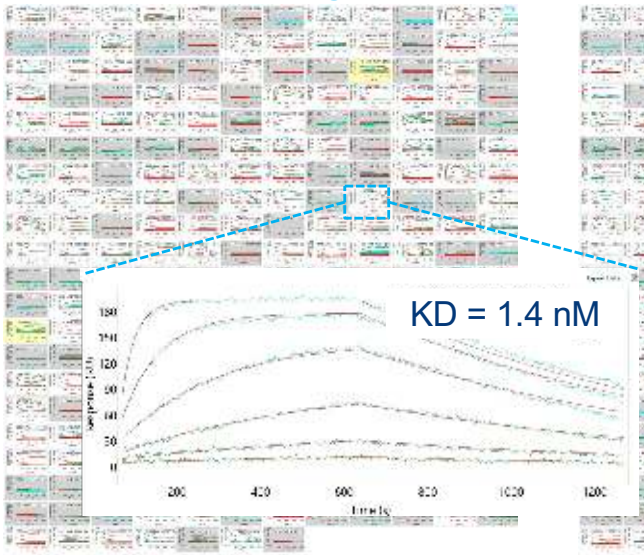
Affinity and cross-reactivity screening

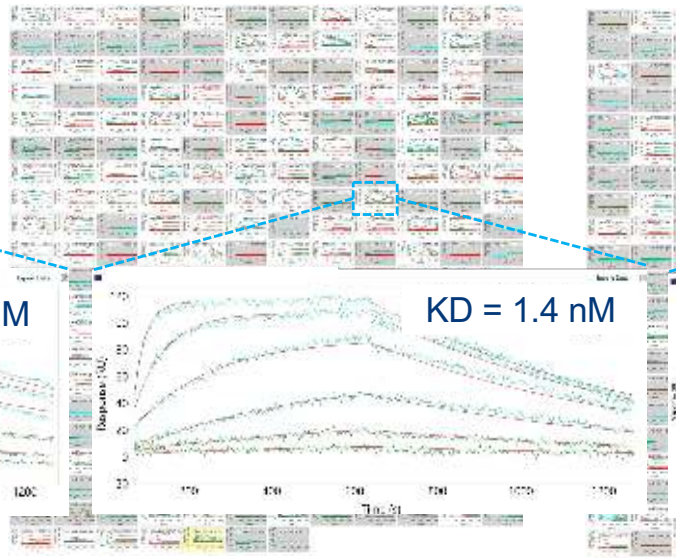
- Affinity ranking and binding kinetics to target antigen of interest
- Cross-reactivity to relevant animal target for non-human safety studies

Human antigen



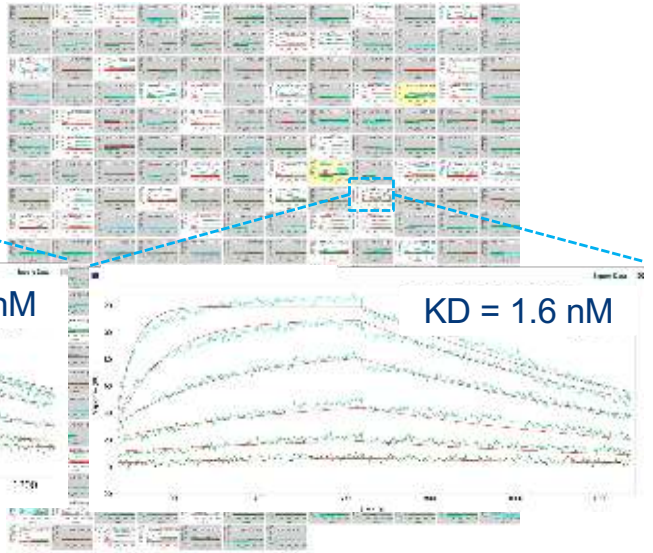
73% binders,
30% pM, 40% 1-10 nM

Cynomolgus antigen



69% binders,
13% pM, 52% 1-10 nM

Mouse antigen



29% binders,
2% pM, 40% 1-10 nM

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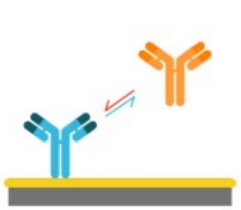
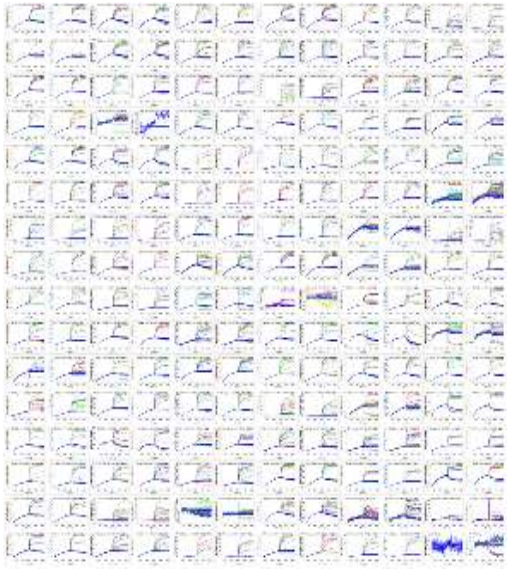
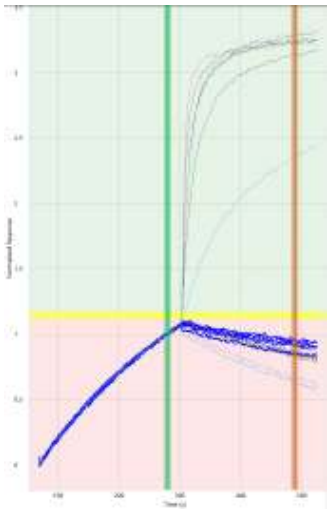
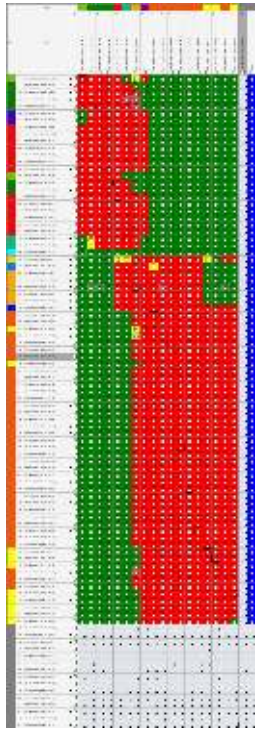
High-throughput epitope binning

- HTP epitope binning – few analytes selected to have sequence diversity
- Classical sandwich setup with amine coupling of mAbs
  - Loose app. 10-20% of mAbs upon immobilization









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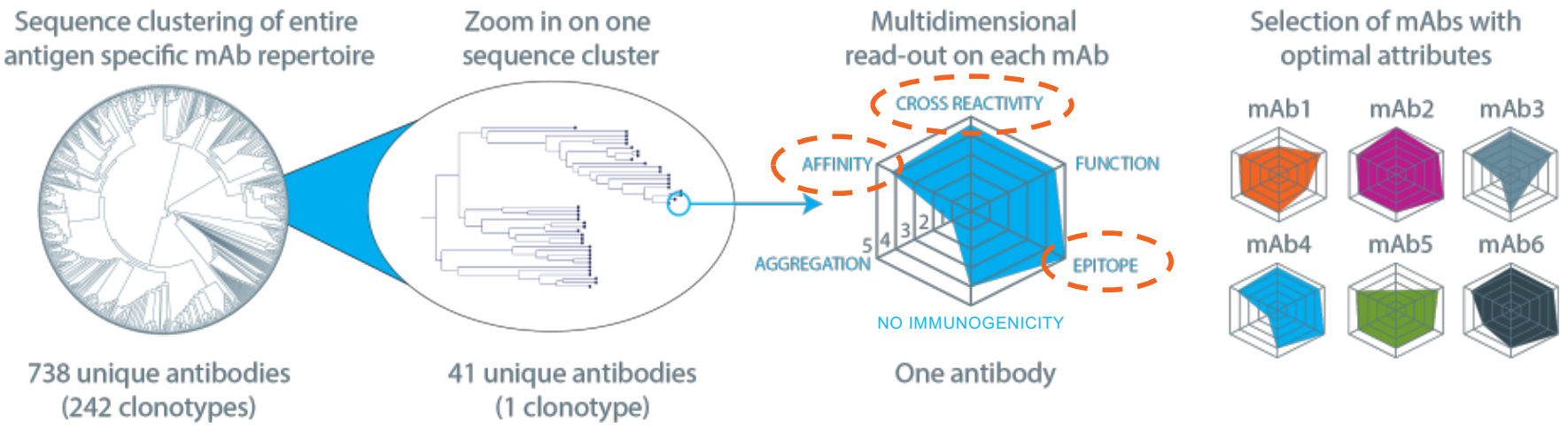
Symphogen monoclonal antibody platform delivers multidimensional assessment of all unique antigen specific antibodies



- HTP SPR combined with multidimensional read-out enables rapid and efficient selection of top lead candidate antibodies for further evaluation

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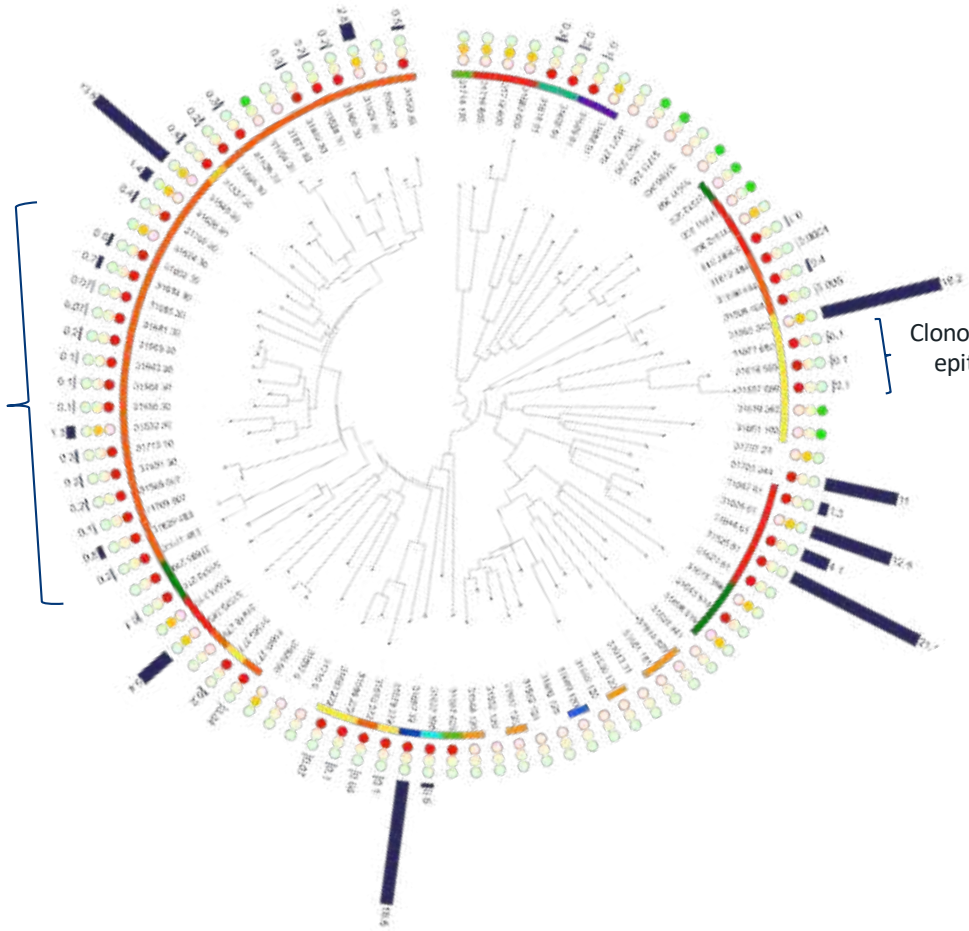
Correlation of sequence diversity, affinity, epitope and functional data

Affinity
KD<10nM, 10nM>KD<50nM,
KD>50nM

Epitope bin 1-13

Functional data, IC50



Clonotypes binding functional epitope with high affinity

Clonotypes binding functional epitope with high affinity

Clonotypes binding less favorable epitope

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Case study: Fc silent anti-CD40 antibodies with strong synergistic activity (SYM P161)

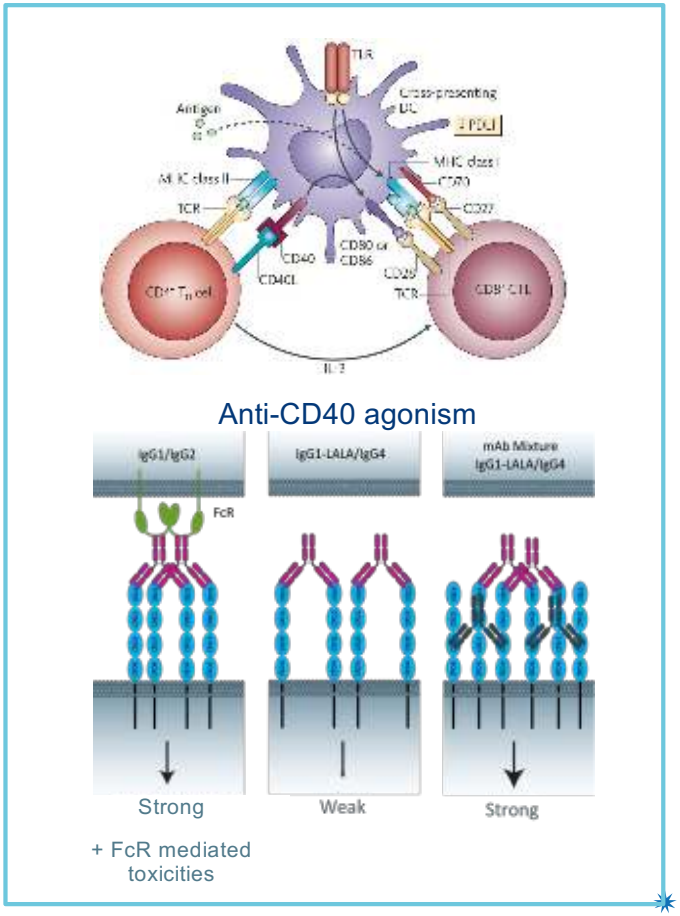
CD40 Target rationale

CD40 is a key regulator of antigen presenting cells

- CD40 is expressed on a variety of antigen presenting cells (APC) such as B cells, DCs, monocytes and macrophages
- CD40L is trimeric and believed to initiate CD40 activation by clustering of the receptor in the cell membrane
- Upon activation, CD40 promotes T cell priming and anti-tumor activity
- Targeting CD40 with agonistic antibodies have shown to induce clinical activity but is also associated with serious adverse events
- **Toxicities are likely driven by FcγR interactions** and have proven an issue for the development of anti-CD40 mAbs

Mixture strategy with Fc attenuated isotype

- An agonistic mAb mixture that stimulates CD40 activity without Fc receptor interactions expected to ***improve toxicity index***



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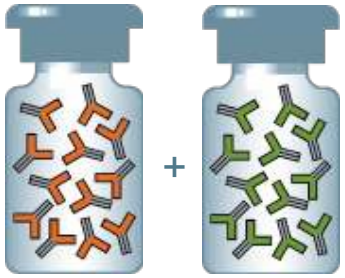
mAb combinations against same target requires non-overlapping epitopes

mAb combination



Two drug products
Two epitopes

mAb mixture

One drug product
Two epitopes

Bi-specific Ab

One drug product
Two epitopes

mAb combinations against same target requires non-overlapping epitopes

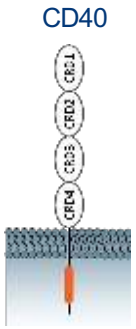
- First FDA approval of two co-formulated mAbs in 2020

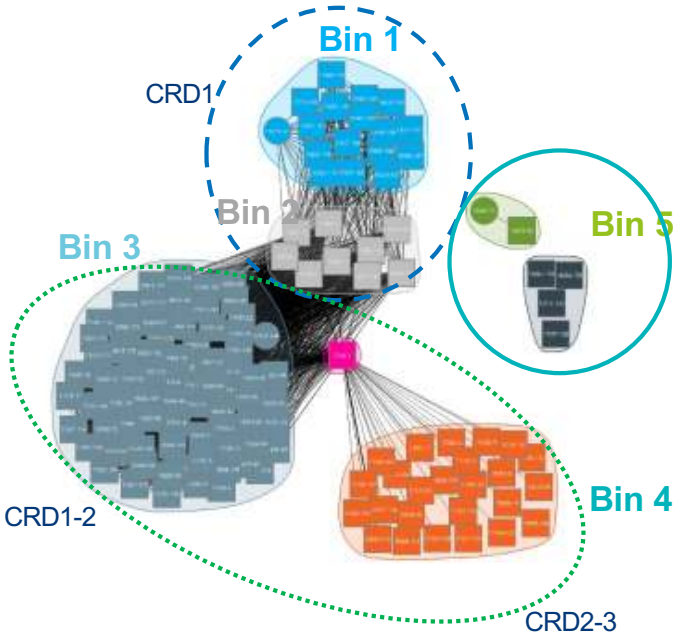
FDA approved fixed-dosed combinations of co-formulated antibodies

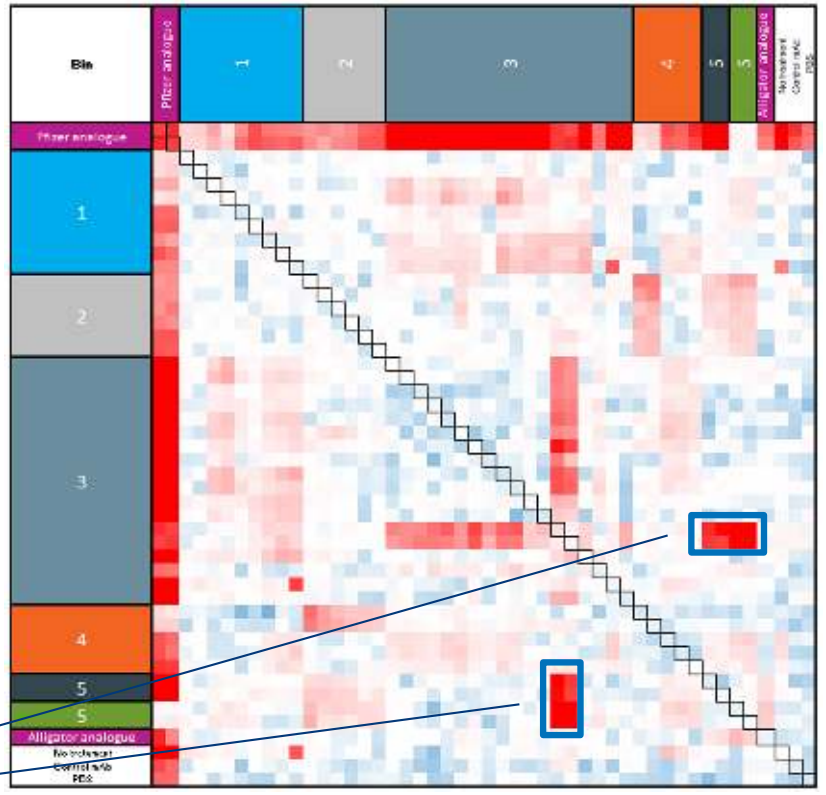
Name	Company	Combined mAbs	Indication
Phesgo®	Roche/Genentech	trastuzumab, pertuzumab	HER2-positive breast cancer
Inmazeb ®	Regeneron	atoltivimab, maftivimab, odesivimab	Zaire ebolavirus infections
Opdualag™	Bristol Myers Squibb	relatlimab, nivolumab	Advanced melanoma
REGN-COV, Ronapreve™	Regeneron	casirivimab, imdevimab	Prevention and treatment of COVID-19

Table from D. Krieg *et al, It is never too late for a cocktail - Development and analytical characterization of fixed-dose antibody combinations, J Pharm Sci, May 2022*

Synergistic Activity Between Antibodies From Non-Overlapping Bins







Strong synergistic activity between antibodies from Bin 3 and Bin 5

Symselect was performed in NFkB reporter assay and primary B cell proliferation assay

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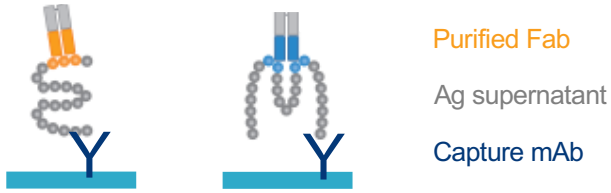
High throughput epitope mapping by SPR

- Epitope mapping based on HTP expression of 100 - 200 Ag mutants
- Mutants analyzed by SPR (KD)
  - Linear 10AA epitopes defined by chimeric sequence scanning in human Ag
  - Contact residues defined by alanine scanning
- Up to 7-10 mAbs mapped in parallel
- 100% success rate and differentiation of lead epitope to competitors
- 7 patents filed with epitope claims
- Epitope mapping project usually takes 2-3 months

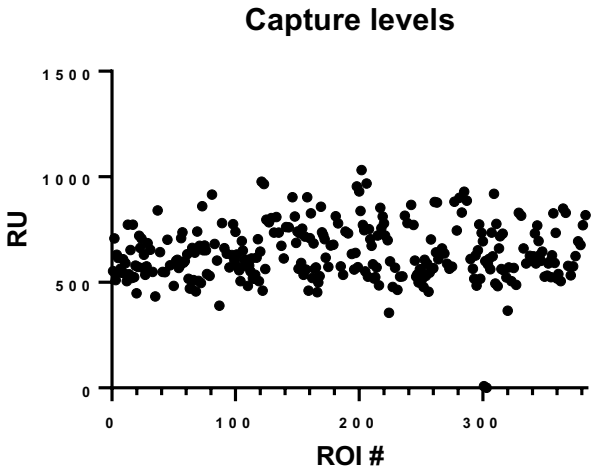
SPR based epitope mapping

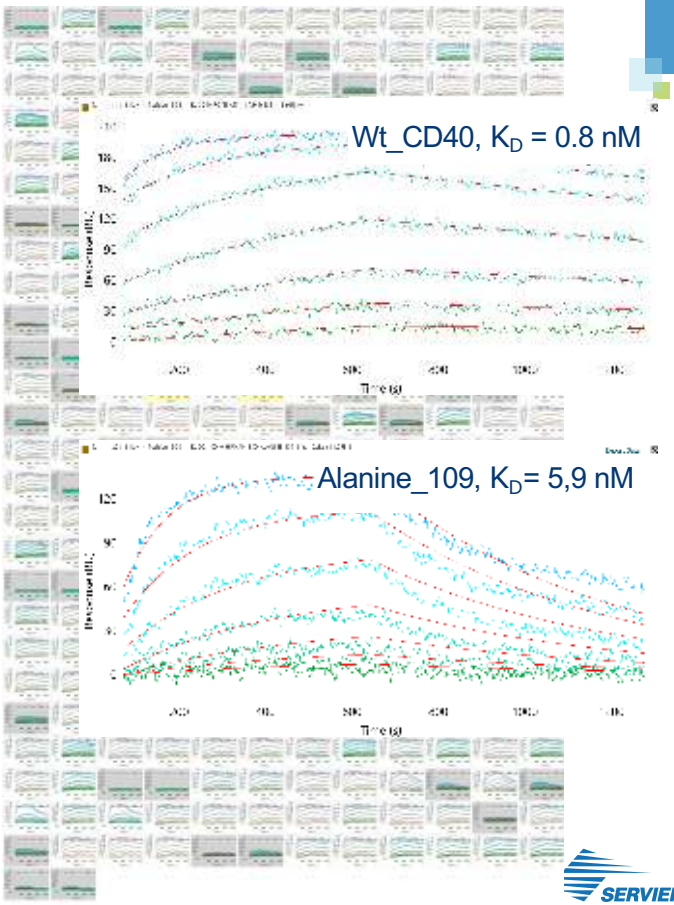
Carterra LSA SPR



High throughput epitope mapping by SPR

- 141 mutants in duplicates captured from supernatant by anti-huIgG coupled on a HC200M sensor
- Binding kinetics fitted to 1:1 binding
- Sorted for common inactive
- Epitope defined as : No binding or ΔK_D (wt/mut) > 5





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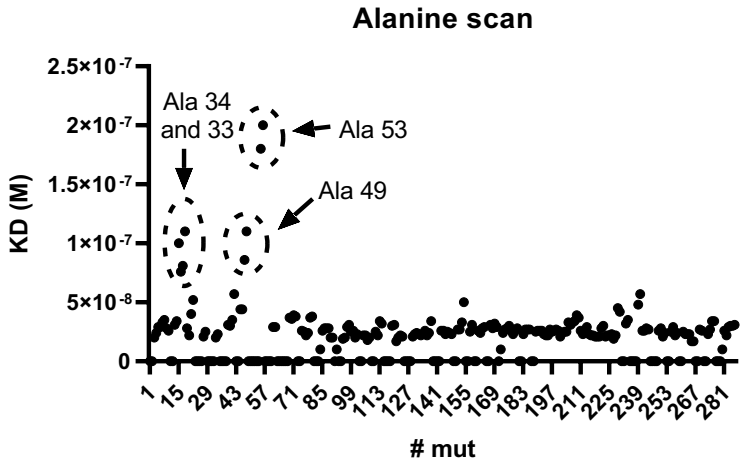
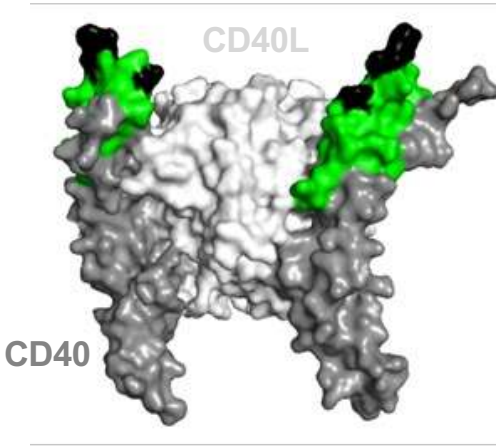
High throughput epitope mapping by SPR

- Key ammino acids identified and mapped on CD40:CD40L ligand complex
- Linear and conformational epitopes overlaps





Conformational Epitope

Linear Epitope

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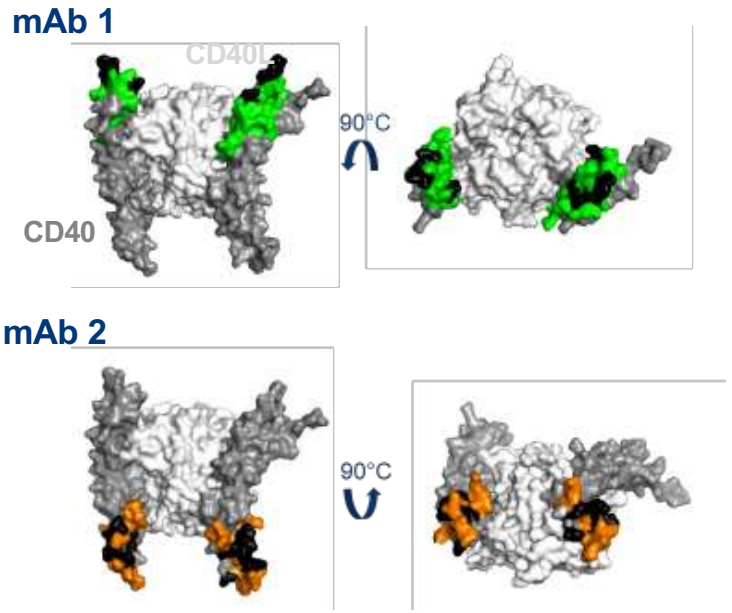
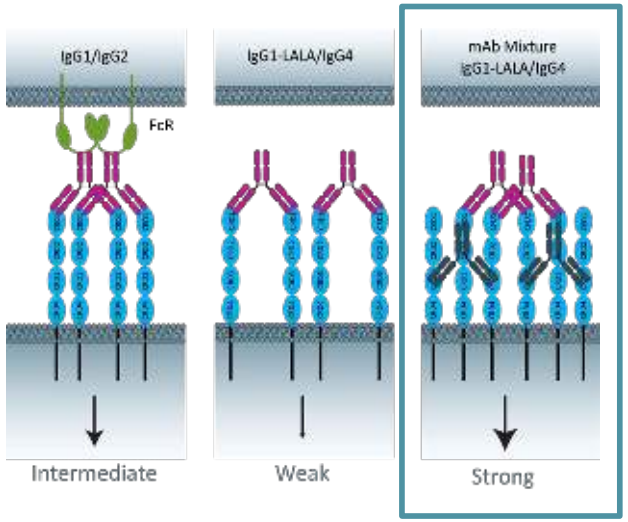
High throughput epitope mapping by SPR

- Non-overlapping mAbs from bin 3 and bin 5 binds CRD1 and CRD3 of CD40
- Agonistic mAb mixture acts synergistic and stimulates CD40 activity without Fc receptor interactions

Conformational Epitope

Linear Epitope





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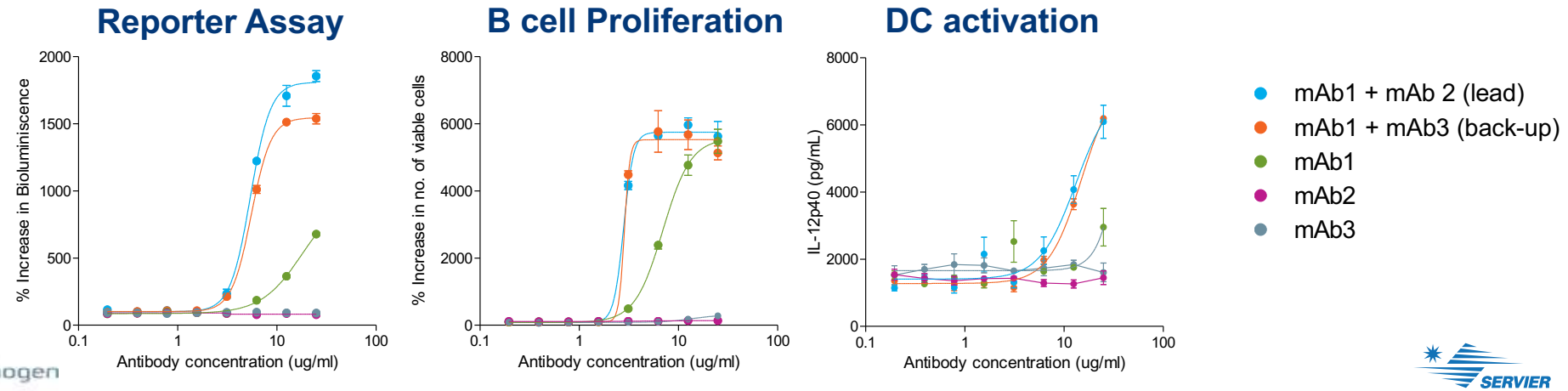
Strong Synergistic Activity of Lead and Back-up mAb Mixtures in Several Assays

- Lead mixture can stimulate antigen presenting cells (B cells and DCs) and does not block activity of the natural ligand
- Symphogen has identified Fc silent anti-CD40 antibodies with strong synergistic activity → Novel mode of action



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Highlights - SYM P161 agonistic mAb mixture

- HTP SPR key method to characterize mAbs affinity, cross-reactivity, the epitope landscape, and to map epitopes of lead anti-CD40 mAb mixture
- Strong agonistic synergy between mAbs from non-overlapping epitope bins binding CRD1 and CRD3 of CD40
- Superior or similar anti-tumor activity compared to competitors at clinically relevant dose (data not shown)
- Fc silent CD40 agonistic mAb mixture can improve therapeutic window due to lower toxicity

Acknowledgements

Thanks to colleagues at Symphogen and Servier,

Thanks for listening!

Future Servier building, Paris-Saclay

Symphogen building, Copenhagen-Ballerup

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