

Biologics Drug Discovery: Steps to producing an antibody drug candidate



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About GenScript





Antibody and Protein Engineering

- Single domain antibody generation
- Antibody sequencing
- Affinity maturation and humanization



In-vitro Pharmacology

- CellPower™ custom stable cell line for assays
- GenCRISPR™ custom knock-out or knock-in cell lines
- Cell-based assays and Ion channel and GPCR assays



In-vivo Pharmacology

- Tumor models including SC xenograft, orthotopic and syngeneic
- Bioluminescence imaging of tumors
- Fibrosis models



Frank Fan, M.D. Ph.D.

- ◆ Director, Antibody and Protein Engineering at GenScript
 - Highly customized antibody libraries
- ◆ Human B cell immunologist specialized in antibody tolerance research
 - Published over 40 papers, in journals such as *Nature Medicine*
- ◆ Development of new immunology assays:
 - Live-cell fluorospot
 - Single B-cell RT-PCR of BCR VH and VL





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- 3 Antibody discovery process
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Growing Trend for Biologics



- ◆ **Biologics accounted for one-third of new medicine approvals in the past decade**
 - **Antibody-based** – anti-VEGF, anti-IL-12 and IL-23
 - **Vaccines** – Human papilloma virus (HPV)
 - **RNAi** – Duchenne Muscular Dystrophy (DMD)
 - **Cell-based** – Autologous cellular immunotherapy for castrate-resistant prostate cancer
 - **Gene therapy** – adeno-associated virus (AAV) delivery of neurturin, to restore damaged cells in Parkinson's patients

- ◆ **Growing trend for more biologics based therapies, especially monoclonal antibodies**
 - 338 monoclonal entities currently in clinical trials
 - 170 monoclonal antibodies in development for cancer

Data from Medicines in development: Biologics 2013 Report. Presented by America's Biopharmaceutical Research Companies (PhRMA).

Challenges in Antibody Discovery



- ◆ Human anti-mouse antibody (HAMA) response
- ◆ Non-specific targeting of antigens on healthy cells
- ◆ Toxicity caused by binding to surface antigens shed into circulation
- ◆ Limitations in biological activity due to location of binding and stimulation of immune response
- ◆ More costly to develop than small molecule therapeutics

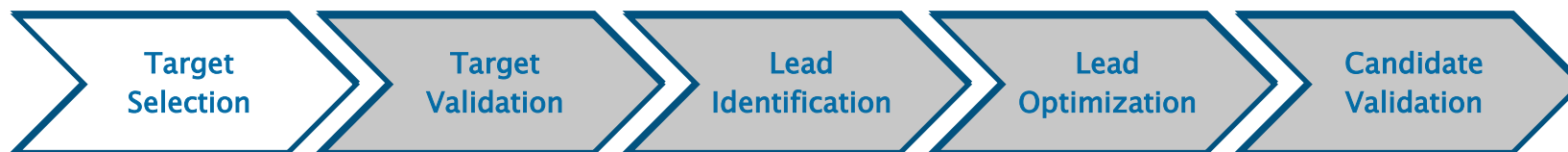
Antibody Discovery Process



◆ Factors to consider:

- Where is the process starting from? Is the target already validated?
- Which antibody platform to use?
- How many leads to move forward into optimization?
- What are the best optimization approaches?
- How do we maximize translation of efficacy into the clinic?

Target Selection



Target identification: Identifying druggable targets, determining whether targets are good antibody candidates.

◆ Factors to consider:

- Efficacy
- Safety
- Meet clinical and commercial needs
- Is the target viable for antibody-based therapeutic?

Is there enough evidence linking the target to the disease?



◆ Data mining of available biomedical data using bioinformatics approach

- Data sources: publications ; patent information; gene expression data, proteomics data, transgenic phenotyping and compound profiling data.

◆ Perform phenotypic screening to identify disease relevant targets

- ◆ Use phage-display antibody library to isolate human monoclonal antibodies (mAbs) that bind to the surface of tumour cells.

◆ Hot targets linking human genetics data to diseases in 2014

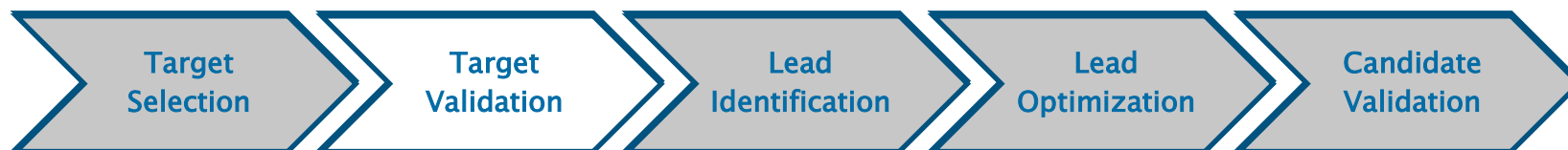
- Mutations on ZnT8 can protect against type 2 diabetes
- Loss-of-function of NaV1.7 channel voltage sensor suppress pain and itch
- Loss-of-function mutations in APOC3 can lower triglycerides and prevent coronary disease

Is the target viable for antibody-based therapeutic?



◆ Antibody targets should be easily accessible:

- On the cell surface – G-protein coupled receptors (GPCRs), ion channels
- Extra-cellular – Vascular endothelial growth factor (VEGF) family of proteins
- Freely circulating – Cytokines such as interleukin-12 (IL-12) and interleukin-23 (IL-23)
- Need the antibody to cross blood brain barrier ?
 - Single domain Ab (sdAb) / nanobody.
 - Bispecific sdAb to enhance penetrating blood brain barrier



Multi-validation approach is very important

◆ **Factors to consider:**

- Collect enough evidence to support target rationale
- Experimentally validate target (e.g. animal model)
- Obtain key stakeholder's approval
- Establish project plan and form project team



- ◆ Knock-out cell lines and animal studies support target validation
 - CRISPR/Cas9 allows for genome scale knock-out screening in human cells¹
 - Using CRISPR/Cas9 to develop animal models allows for multiple genes to be knocked-out simultaneously. Also shortens development timeline²

¹Shalem O et al. **Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells**. *Science*. Jan 2014. 343(6166):84-87

²Wang H et al. **One-step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR/Cas-mediated Genome Engineering**. *Cell*. May 2013. 153(4):910-918

Lead Identification



◆ Factors to consider:

- **Which is the best antibody platform to move forward with?**
 - Monoclonal, single domain, rabbit (phage display), full human naïve Fab library or synthetic antibody/monobody library?
- **Are the appropriate *in vitro* screening assays established?**
 - ELISAs to screen for binding to target antigen
 - Proof-of-principle efficacy assays
- **Have the appropriate *in vivo* PK/PD models been established?**
 - Tissue distribution studies to evaluate PK in several species
 - Efficacy endpoints to evaluate PD



Screening preparation:

- ◆ Generate reagents for assay development and screening
- ◆ Develop Proof-of-principle efficacy assays such as binding (ELISA) and functional assays, ADCC & CDC assays
- ◆ Develop animal efficacy models
- ◆ Define and execute strategies for evaluating non cross-reactive antibody e.g.
 - Generate surrogate antibody,
 - Establish transgenic mouse models expressing human target



Screening criteria

- ◆ **Specificity:** Clearance of cross-reactivity with human tissue/organ sections unrelated to the target by radiolabeled antibody (or non-radiolabeled with high sensitivity)
- ◆ **Species cross-reactivity:** Binding (and relative affinity) of the antibody to antigen counterparts in mouse, rat, and monkey.
- ◆ **Selectivity:** 100 fold or better over closest class/family members
- ◆ **Affinity (KD):** 1 nM or better
- ◆ **Potency:** 10 nM or better



Immunization strategy:

Best approach for antigen generation/immunization, by target type:

- Single pass membrane bound –
 - ECD protein, Stable expressing cell line, DNA immunization
- Ion channel & GPCR receptor
 - DNA immunization, VLP, Stable expressing cell line /membrane prep
- Soluble protein
 - IM injection with/without adjuvants

Which is the best antibody platform to move forward with?



Monoclonal antibodies (hybridoma)

Pros: Lots of clinical evidence to support. Cost effective compared to other platforms. Easy to produce.

Cons: Requires humanization to avoid HAMA

Best for: All types of target, diseases (cancer, immunological, neurological)

Monoclonal Antibody Services

- Positive Western Blot result guaranteed
- Fast turnaround time: as soon as 45 days
- Competitive price: starting from \$3,800



Which is the best antibody platform to move forward with?

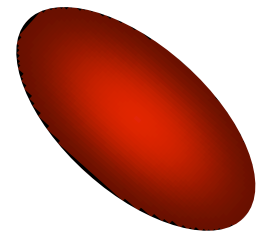


Camelid single domain antibodies (phage)

- **Pros:** Small ~13kD size, high stability, easy to engineer, Economy in production, excellent safety (low risk), Better tissue penetration.
- **Cons:** Pharmacokinetics require extensive engineering
- **Best for:** Diseases (cancer, immunological, neurological)



V_HH



Which is the best antibody platform to move forward with?

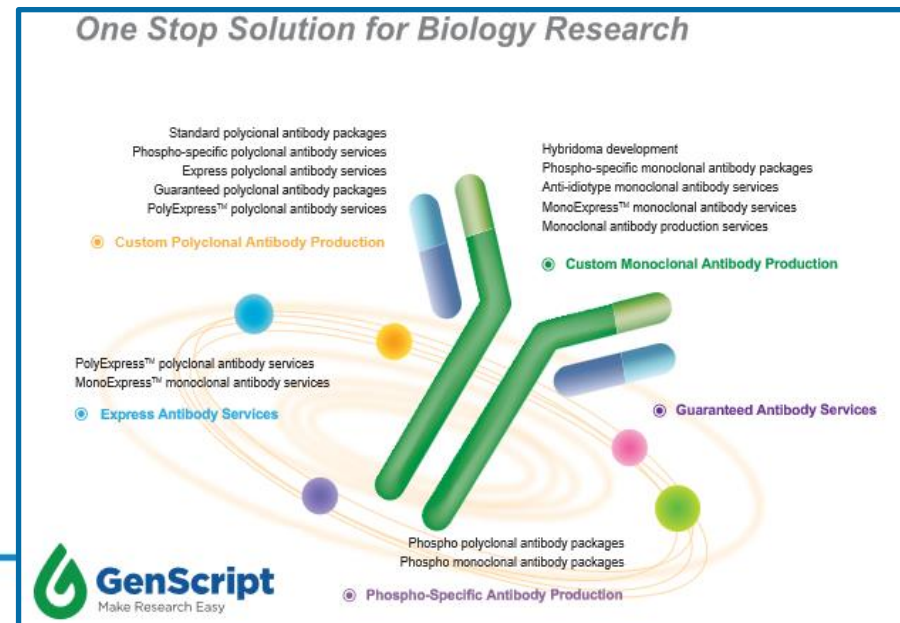


Rabbit mAb (phage display library)

Pros: Very high affinity, superior for unique epitope

Cons: Need antibody reconstruction and humanization which is technically challenging,

Best for: All types of target, especially good for phosphorylated protein target



Which is the best antibody platform to move forward with?

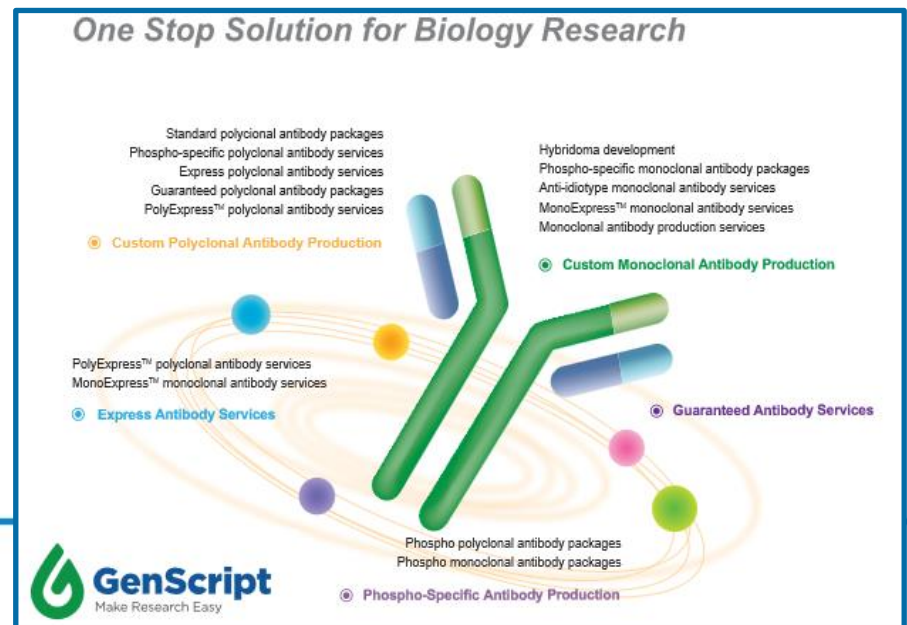


Human naïve phage library

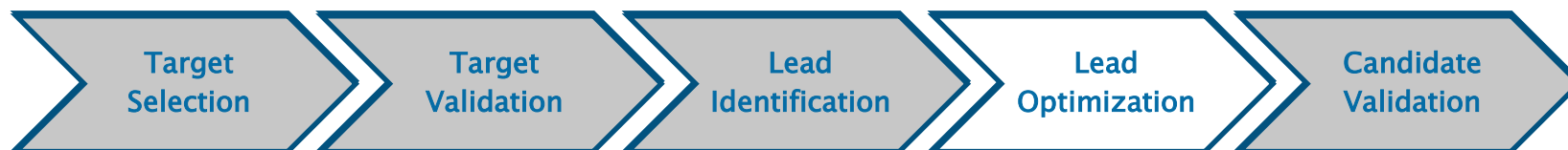
Pros: Fully human antibody, fast turnaround time, easy to engineer

Cons: Low affinity and specificity, high background

Best for: Vaccine for infectious disease, reagent antibody development



Lead Optimization



Generate mutant libraries via antibody engineering

- Humanization
- Affinity maturation
- Fc engineering
- Improve biochemical and biophysical characteristics
 - expression, solubility, stability, aggregation, glycosylation
- Exploratory PK/PD
- Construction of bi-specific or multivalent antibody

Biochemical and biophysical characterization of hits



◆ GenScript FASEBA platform

FA **FAst**

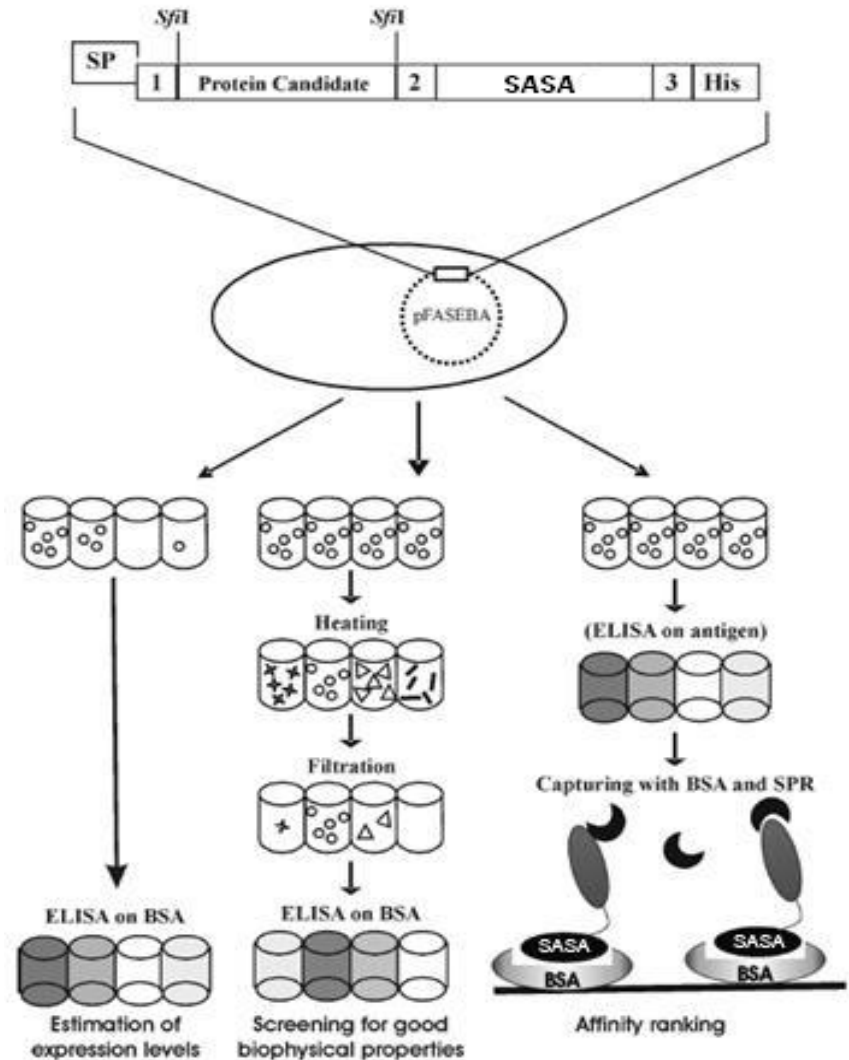
S **Screening of**

E **Expression**

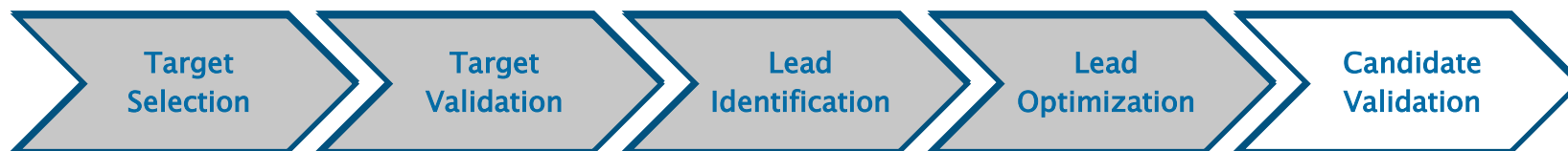
B **Biophysical-properties and**

A **Affinity**

FASEBA is a patented technology for the selection of the best protein binders, based on their expression level, biophysical properties and binding affinity, from a large number of protein candidates in a high throughput fashion without actually purifying these proteins.



Candidate Validation



◆ Scale up antibody production:

- Stable cell line generation
- Antibody protein production

◆ *In vivo* efficacy assays:

- Tumor models – Syngeneic models – to test the efficacy of immuno-oncology antibody leads
- SC xenograft and orthotopic (bioluminescence imaging)

Candidate Validation before clinical trial



- ◆ How do we maximize translation of efficacy into the clinic?
 - Examination of scientific data package
 - Risk assessment
 - Safety assessment
 - Commercial assessment
 - Legal evaluation
 - Clinical plans
 - Clinical biomarker plan
 - Patient stratification plans
 - Regulatory plans
 - Manufacturing plans

One-stop Antibody Drug Development



Antibody Drug Development

Therapeutic Hybridoma

- Antigen production
- Immunization
- *In vivo* transfection
- Cell fusion
- Hybridoma screening
- Hybridoma stabilization

Antibody and Protein Engineering

- mAb sequencing
- Epitope mapping
- Ab characterization
- Antibody humanization
- Affinity maturation
- sdAb development
- Phage display
- Customized synthetic library

In vitro pharmacology

- Cell proliferation
- Growth inhibition
- ADCC
- CDC
- Screening & profiling

In vivo pharmacology

- Xenograft model
- Syngeneic model
- Tumor model dev
- *In vivo* efficacy evaluation

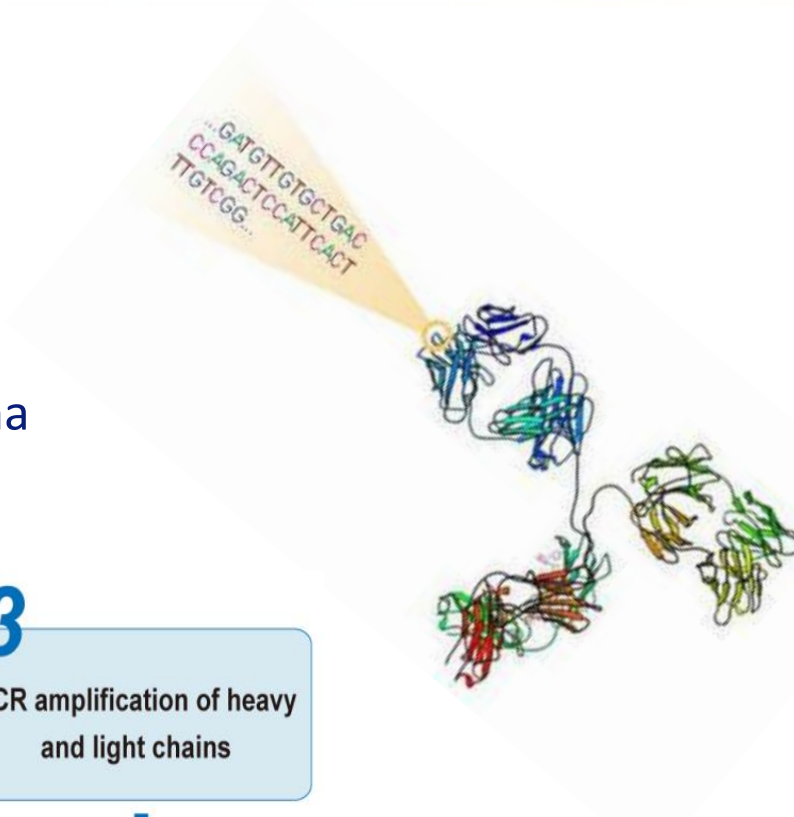
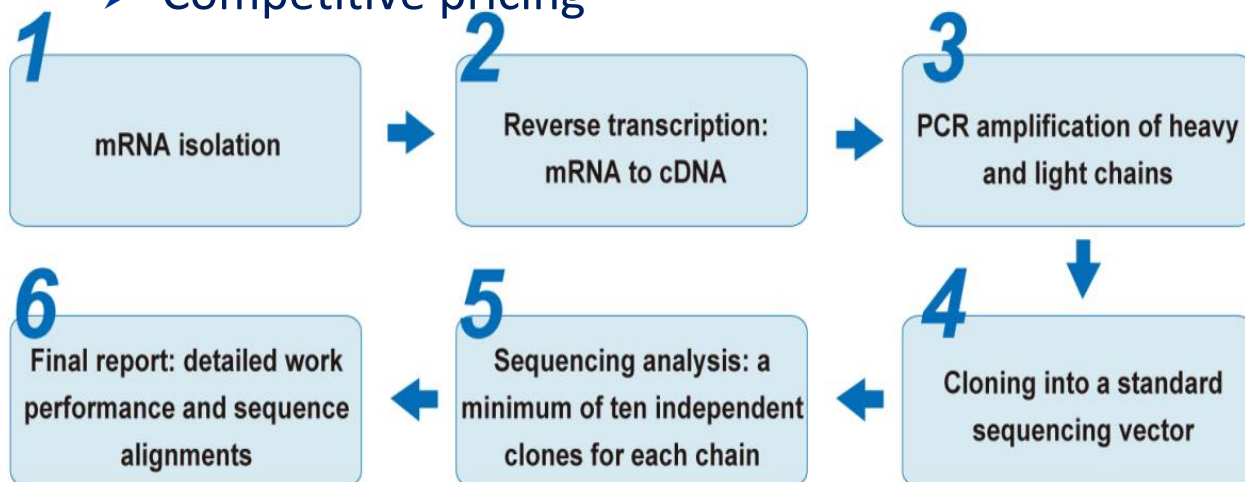
Bio-Production

- Transient expression
- Stable expression
- Cell line dev
- Process dev
- Scale up

mAb Sequencing Service



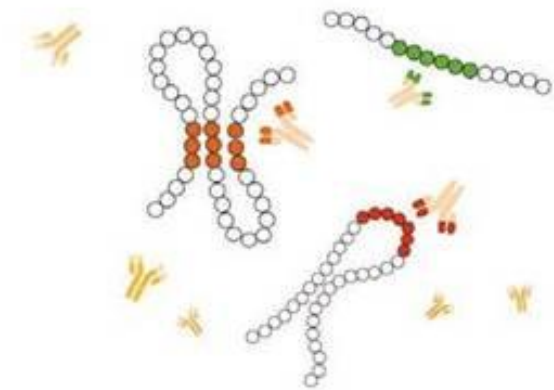
- **Quality**
 - A record of 100% success rate
- **Fast turnaround**
 - Express services available
- **Versatility**
 - Human, mouse, rat, rabbit, and llama
- **Economy**
 - Competitive pricing



Epitope Mapping Service



- **Epitope information is critical for**
 - **Identifying the binding site of therapeutic or surrogate mAbs**
 - **Securing patent position**
 - **Facilitating vaccine development**
- **Unparalleled accuracy**
 - **Proprietary FlexPeptide™ technology ensures quality of peptide library synthesis and assay development**
- **Fast delivery**
 - **4 weeks by average**



Antibody Optimization



- **Services**

- Humanization
- Affinity maturation
- Effector-function enhancement
- Half-life extension
- Stability enhancement



- **Expertise**

- Leadership: Chuan-Chu Chou, PhD, inventor of humanized anti-IL-5 mAb, soluble IL-10 receptor, co-inventor of MCP-1-Ig fusion proteins (Schering)
- Advisor: Leonard Presta, PhD, inventor of Herceptin and Avastin, key contributor to the success of Xolair (Genentech); inventor of mAbs to IL-23, IL-23R, IL-17A, TSLP, GITR, IL-10, CD40, IFN- α , TGF- β , MDL-1, and CD200R, and co-inventor of MCP-1-Ig fusion proteins (Schering); key contributor to the success of Lambrolizumab (Merck)

Antibody Humanization Service

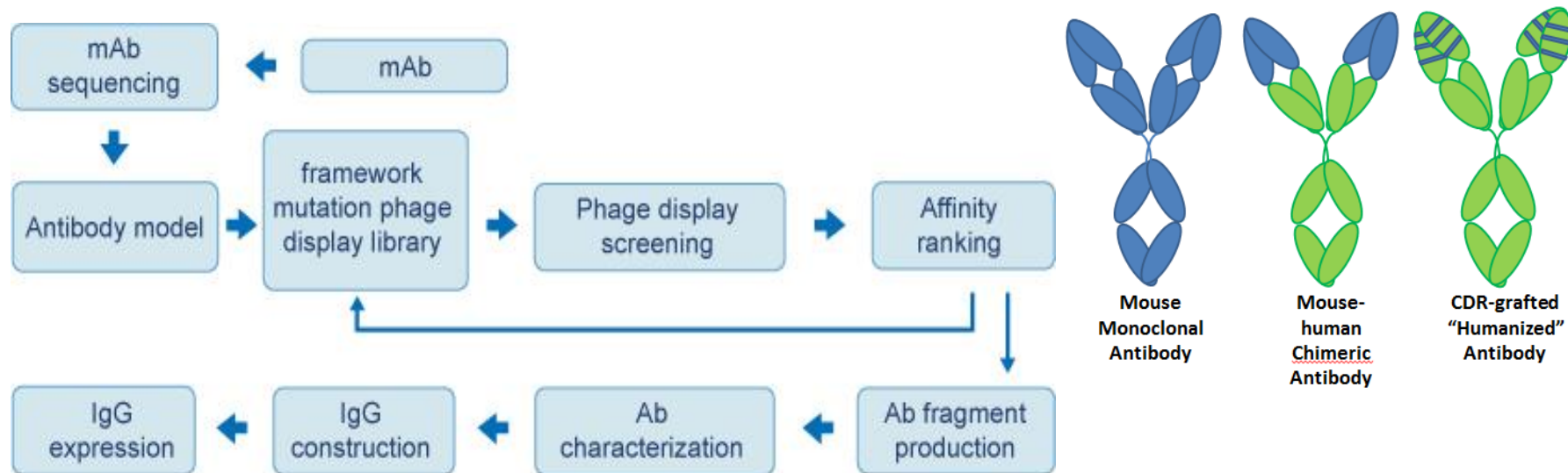


❖ Antibody Humanization – (Almost) the ultimate platform

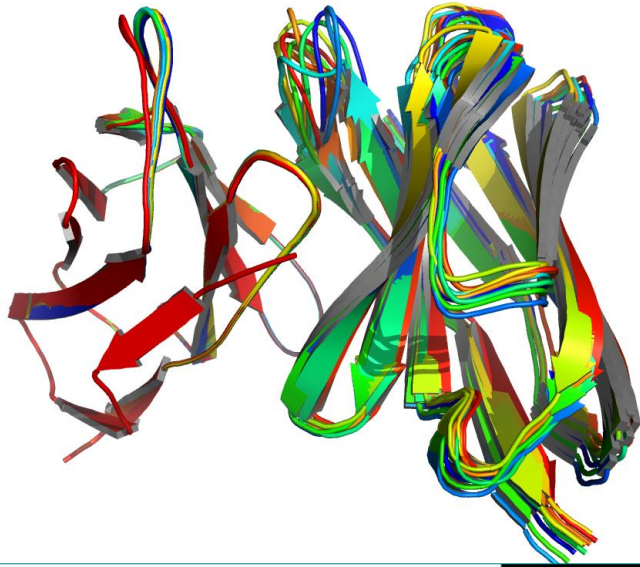
- Proprietary FASEBA technology for ensured protein stability
- Extensive bioinformatics analysis for selection of the best human recipient frameworks
- Retaining affinity of parental antibody

❖ Our real advantage

- Our understanding that an antibody has to meet all criteria (expression levels, stability and affinity) to be considered as a drug candidate (Of course in vivo performance is also important, but this will mainly be affected by Fc region).
- Our understanding that stability is one of the keys, if not the key, in antibody engineering.
- We licensed a FASEBA (FAst Screening for Expression, Biophysical-properties and Affinity) from NRC, which allows us to screen expression and stability besides affinity.



Structure-based Antibody Humanization - Model Building

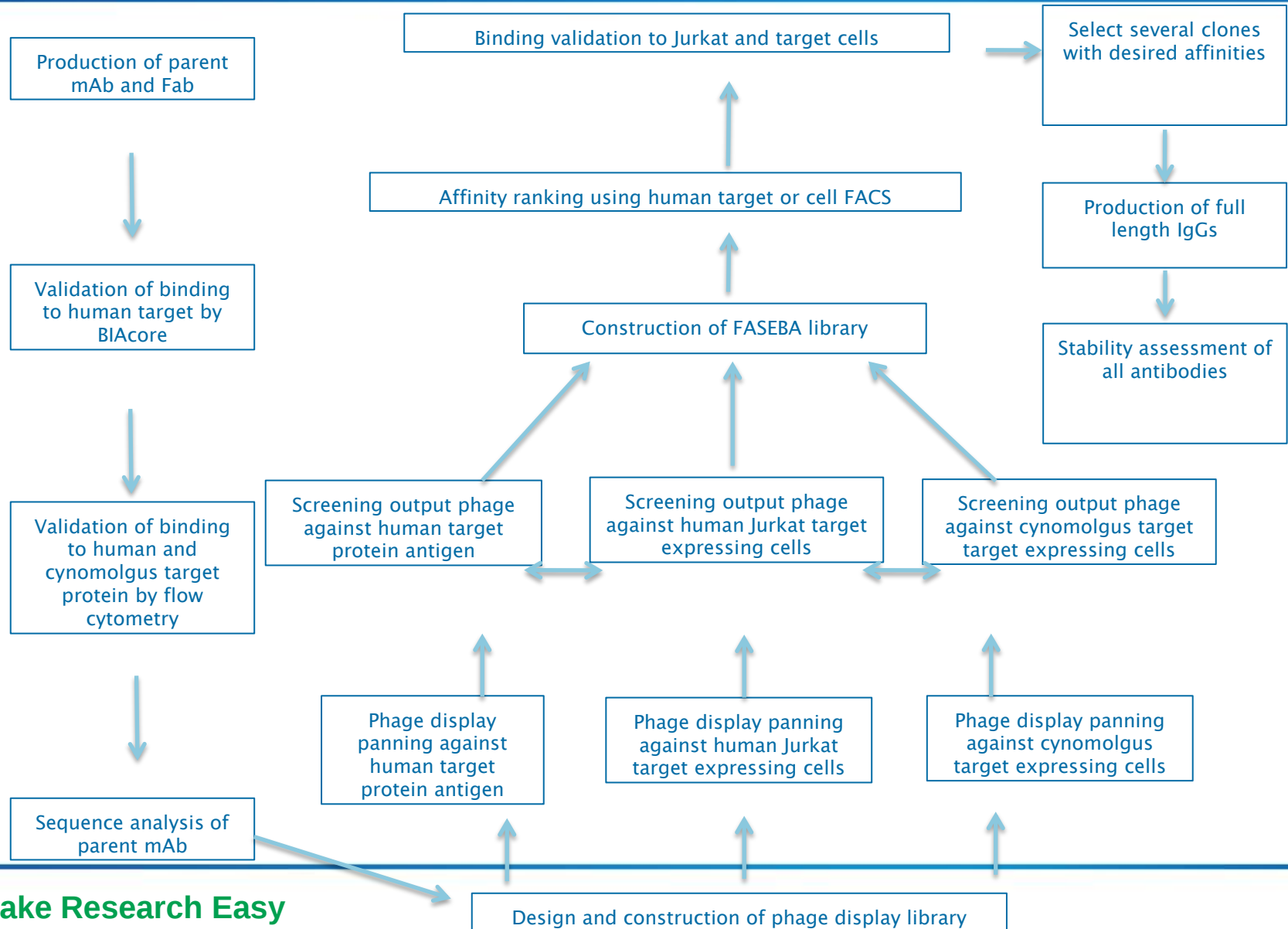


Successful engineering of an antibody often relies on the ability of structure modeling. Structural biologist at GenScript can generate models which help antibody humanization and affinity maturation.

Models of an humanized anti-TNF α antibody.



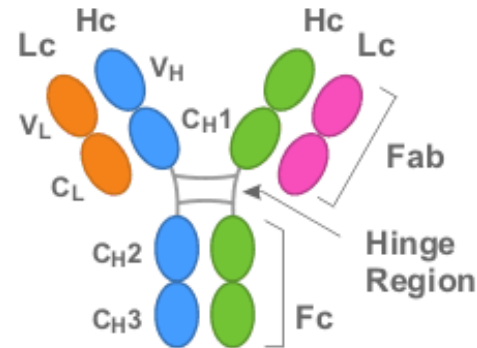
Highly customized service - R &D with our customers



Huge Demand for a New Generation of Antibody Drugs



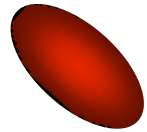
- ◆ Limitations of traditional antibodies call for new generation of antibody drugs.
 - **Long cycle** of lead optimization and modification, due to the complexity of molecular structure
 - **Inefficient penetration** into solid tumors as well as into the brain, due to large molecular size
 - **High cost** of manufacturing



Single-domain Antibody (sdAb): A Promising Solution

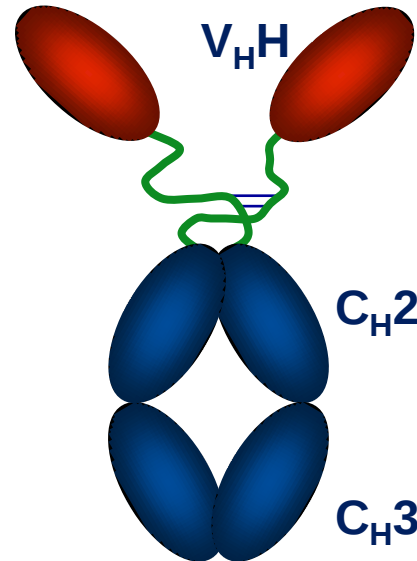


V_HH



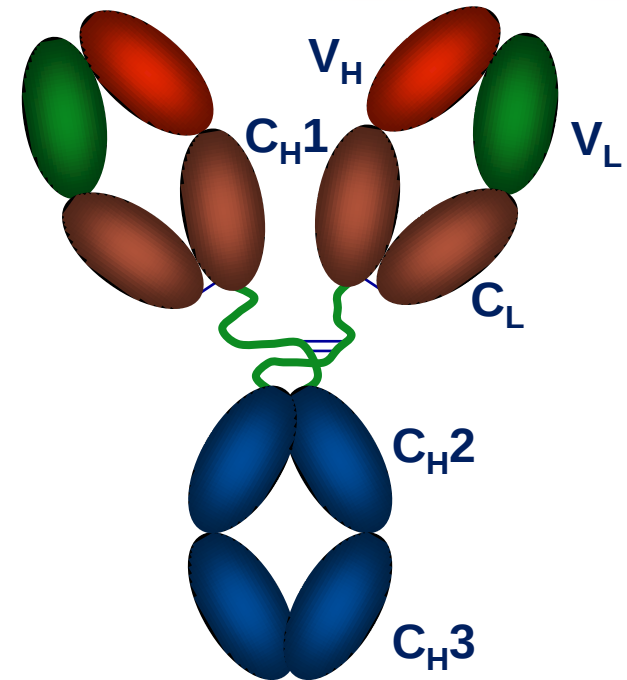
sdAb

One protein domain
No Fc



Camelid heavy chain antibody

Two heavy chains
No light chain
Six protein domains
Full Fc



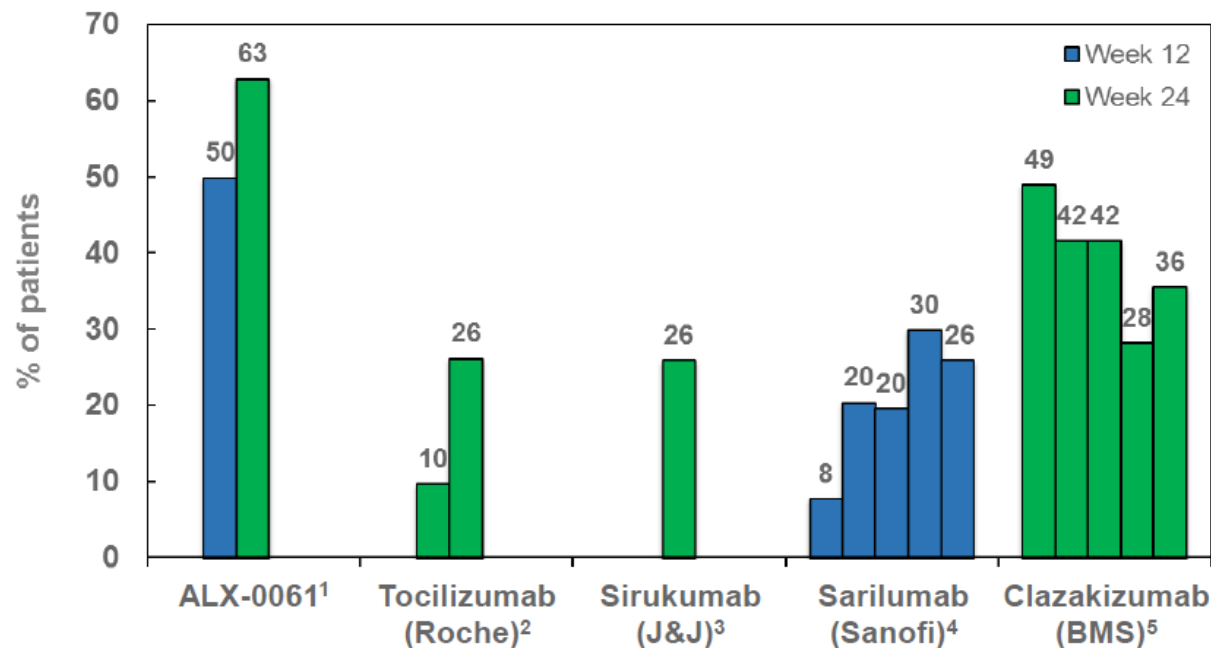
Conventional IgG

Two heavy chains
Two light chains
Twelve protein domains
Full Fc

Proof of Concept: sdAb Can Potentially Be Superior to 1st-Generation Biologics



Potential differentiating DAS28 remission profile



1. All unmodified ALX-0061 treated patients at week 24 (N=24) and pooled ALX-0061 data at week 12 (N=28) as reported in Oct 2012
2. Data estimated from ACT-RAY, OPTION, RADIATE, ROSE, SAMURAI, SATORI and TOWARD trials, for 4 and 8 mg/kg tocilizumab + MTX, Q4W. The data was described by a weighted non-linear regression model. [Quantify RA clinical database, Feb 2013]
3. Combined data, Phase II trial, EULAR 2012
4. Phase IIb MOBILITY trial; 100 mg Q2W, 100 mg Q1W, 150 mg Q2W, 150 mg Q1W, 200 mg Q2W (+MTX)
5. Phase II trial, sc Q4W: 25mg, 100 mg and 200 mg + MTX; 100mg and 200 mg without MTX

Public information of Ablynx

ALX-0061 is an anti-IL6R sdAb

GenScript: The World's Leading CRO of sdAb Drug Discovery Service



- Over 5 years of experience of developing sdAb
 - ✓ Immune and naïve libraries, sdAb engineering and production, and functional characterization
- Successfully delivered 2 therapeutic sdAbs and 8 reagent sdAbs
 - ✓ High affinity: K_D of 11 pM achieved
- Ongoing projects
 - ✓ 5 therapeutic sdAb projects and 6 reagent sdAb projects



Success is Assured by Capability of One-stop Service



- Antigen optimization
 - ✓ Antigen design
 - ✓ Gene synthesis and codon optimization
 - ✓ Peptide synthesis
 - ✓ Protein expression and purification
- Ab generation and optimization
 - ✓ Phage and yeast display library screening
 - ✓ Affinity maturation and humanization
 - ✓ Epitope mapping
 - ✓ Half life extension
- Functional and safety validation
 - ✓ Cell lines and assays
 - ✓ Animal models and PK/PD
 - ✓ Stability
 - ✓ Tissue cross-reactivity



Summary



- ◆ Antibody-based therapeutics has entered the center stage of drug discovery
- ◆ Major efforts in the biopharmaceutical industry are devoted to establishing sophisticated industrial processes for discovery and development of viable candidates, from target validation to lead candidate identification.
- ◆ GenScript, as one of the world's leading biology CROs, has established a comprehensive antibody drug discover platform to provide reliable one-stop service.
- ◆ GenScript's discover biology team provides superior services such as single domain antibody generation, antibody humanization, affinity maturation and antibody sequencing.

Thank you for your participation
We wish you all success in your research
Email me: Maxine.Chen@GenScript.com

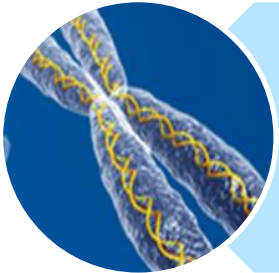


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October 29, 2014/ 8:00 am and 2:00 pm EST

Codon optimization: Why & how to design DNA sequences for optimal soluble protein expression – *Rachel Speer, Ph.D.*



November 5, 2014/ 8:00 am and 2:00 pm EST

Design high specificity CRISPR/Cas9 gRNAs: principles and tools – *Heidi Huang, Ph.D.*