WHITE PAPER

Antibody-based drug discovery at the speed of light

The combination of the PentaMice® platform and single B cell screening with the Berkeley Lights Beacon® Optofluidic system increases speed to market for monoclonal antibody therapeutics

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Capabilities and technology combine to provide First-to-Human antibody discovery, development and clinical manufacturing. Speed, scientific expertise and efficiency can surmount the high attrition rates of early antibody discovery and achieve first-to-market delivery of new therapeutics.

Slow processes that generate a limited number of recombinant monoclonal antibodies hinder success. For a blockbuster $1B per annum biologic in an increasingly competitive market, every month of delay in getting to market can result in the loss of up to $83 million in revenue per month. Worse, any delay increases the chance of a competitor filing a patent claim for the sequence and utility of the antibody you have discovered before you do.

Technologies and tactics that provide for First-to-Patent with comprehensive sequence claims, and First-to-Human opportunities, are of increasing interest to the biopharmaceutical industry.

The speed with which a company can move from an idea to proof-of-concept for a therapeutic provides significant competitive advantages and allows resources to be focused on those approaches most likely to deliver beneficial clinical outcomes.

First-to-Human antibody therapeutics

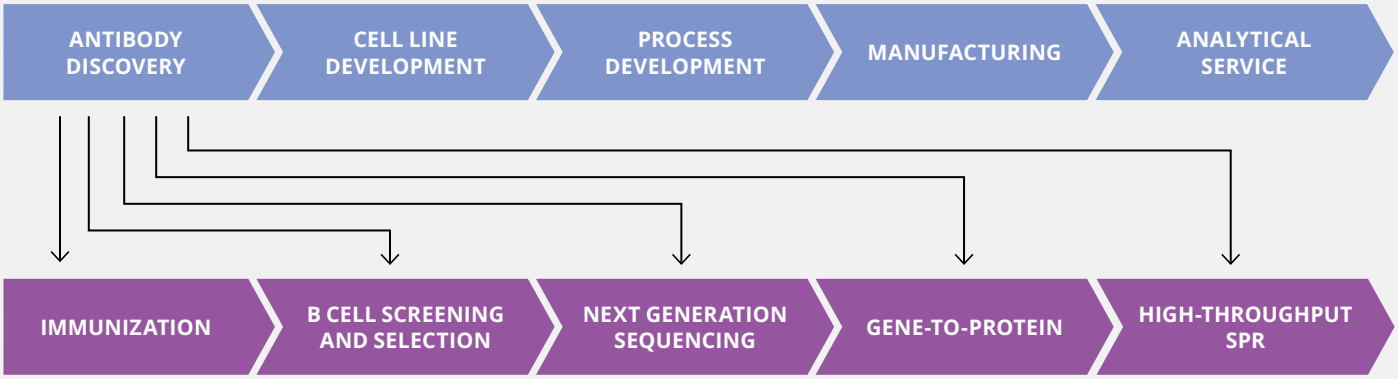
The development of enhanced mouse systems for antibody generation and high-throughput single B cell screening, combined with next generation sequencing (NGS) and rapid recombinant production of milligram to gram quantities of purified monoclonal antibody (mAb), significantly accelerates identification of development candidate leads.

With the antibody discovery workflow detailed here, which leverages the PentaMice® platform, the Berkeley Lights Beacon® Optofluidic system, NGS and rapid gene-to-protein (GTP) production, it is possible to conservatively shave six months off the development timeline of a mAb candidate when compared to traditional approaches, which may take a year or longer. Critical to the expedited timeline is Curia's ability to deliver on right-first-time immunizations: single PentaMice cohorts of just 10 animals and smart immunization protocols can deliver maximized antibody plasma titers from multiple animal strains in as little as 17 days, resulting in a diverse immune response in efficient time frames. Combining the power of the PentaMice platform with high-throughput B cell screening by Beacon, NGS and rapid GTP production, a synergistic acceleration of antibody discovery transpires, providing First-to-Patent and First-to-Human opportunities to those using these technologies.

The workflow detailed in this white paper greatly reduces timelines for antibody discovery through these steps:

- PentaMice mice and/or human antibody–producing transgenic mice are immunized
- Single B cells are isolated by AI-driven algorithms and up to 80,000 cells can then be directly screened using the Beacon system
- High-throughput processing is enabled by automated screening coupled with unique barcoding to multiplex hits
- NGS derives the variable heavy- and light-chain sequences of the monoclonal antibody produced by each B cell hit
- Custom bioinformatic analysis is used to map the diversity of sequences and assess antibody developability, providing information for candidate selection for downstream processing
- An optional rapid mAb re-expression step in 293T cells may be used to validate hits by ELISA, flow cytometry or a functional assay
- Reformatting and rapid recombinant expression of 48–96 high-priority mAbs using our TunaCHO℠ transient antibody production process yields milligram quantities for lead selection assays. With a "start in CHO, stay in CHO" approach, early access to CHO-derived mAbs allows a seamless transition to later-stage stable, high-yield production under cGMP
- Kinetic analysis and epitope binning by surface plasmon resonance is performed using Carterra® LSA® instruments

Figure 1. Optimized workflow with the PentaMice platform and the Beacon system



Antibody discovery is the first step in the process of developing a successful mAb therapeutic.

Once promising mAbs have been ranked, their sequences can be considered for incorporation into patent claims and lead development candidates nominated for progression into stable cell line development, process development and manufacturing for advanced preclinical and clinical studies. CDMOs such as Curia, who are able to provide the full complement of antibody discovery, development and manufacturing services, provide their partners with an accelerated path to market. Execution of proactive tactics, such as formulation development, stability, *in vitro* safety and downstream process studies using transiently CHO-expressed development candidates in parallel with stable cell line development provides additional opportunities to accelerate selection of a clinical candidate and manufacturing of drug substance and drug product under cGMP. This combination of proprietary, state-of-the-art technology and modern tactics provides true First-to-Patent and First-to-Human opportunities.

PentaMice

Immunization — PentaMice platform for antibody generation

Enhanced mouse systems for antibody generation generally fall into two categories:

- Transgenic, human antibody-producing mice
- Immune response-optimized wild type mice

At Curia, both systems are available for immunization strategies. All animals are maintained in our onsite AAALAC-accredited vivarium.

HUMAN ANTIBODY-PRODUCING TRANSGENIC MICE

This system eliminates the need to humanize antibody candidates. Curia works with major human transgenic mouse platforms for antibody discovery. Curia can also immunize a client's proprietary mouse system, if desired.

THE PENTAMICE PLATFORM — IMMUNE RESPONSE-OPTIMIZED MICE

Curia's unique PentaMice system, combined with our optimized rapid immunization strategy, provides a large and diverse starting pool of antibodies. The PentaMice platform provides a collection of five strains of immunologically diverse wild-type mice covering nine different MHC class II haplotypes. Compared with conventional C57BL/6 mice that express just a single MHC II heterodimer, PentaMice wild-type mice can present antigen on 42 different class II heterodimers, providing effective CD4 T cell help for a wide range of targets to drive high-affinity antibody maturation *in vivo.*

The diversity of MHC haplotypes represented in the PentaMice platform combined with optimized immunization protocols increases the chances of successfully discovering antibodies with the desired specificity, affinity and function from a single cohort of just 10 animals. For more information on the PentaMice platform, see Hybridoma.com.

Single B cell screening with the Beacon system

The antibody discovery process is further accelerated by screening antibody-secreting B cells directly to find immunoglobulins with the desired binding characteristics. No additional library construction is required.

The Beacon system is an innovative package using the technology of light, nanofluidics and refined AI algorithms to identify and orchestrate the movement of single B cells into a grid of NanoPen™ chambers for screening. It uses multi-channel time-lapse fluorescence imaging and computer-assisted software to automate hit identification.

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Multiplexed barcoding of hits and next-generation sequencing facilitates high-throughput analysis of antibody sequences.

Individual cells are arrayed through automation on a nanofluidic chip. Each chip can store up to 20,000 B cells, with each cell residing in its own NanoPen. The cells are bathed in growth medium, which allows them to express and secrete antibodies detectable within minutes. Assay reagents are then added using microfluidics to identify individual cells producing an antibody with the desired activity. A built-in migrating imaging system captures the presence or absence of these antibodies by localized fluorescent blooms above the NanoPens.

Combining the use of the PentaMice platform with the Beacon system can greatly increase the number of high-quality antibodies, improving the chances of finding effective antibodies against difficult targets.

Following the identification of B cells expressing target-binding antibodies — of which there may be many — cDNA from the candidate B cells is exported from the chip with antibody sequence recovery in as little as one week.

ALLOWS SEQUENTIAL ASSAYS

Following the initial antigen-binding screen, which can be multiplexed, additional assays can be run sequentially by flushing the channels above the NanoPens and importing new reagents.

Secondary assays include:

1. Epitope competition
2. Neutralization
3. Cell binding

IMPROVED EXPLORATION OF AVAILABLE EPITOPE REPERTOIRE

The Beacon system and workflow allows as many as 40,000 B cells to be screened per run or 80,000 B cells to be screened per sample to identify and then sequence the antibody gene variable heavy- and light-chain regions of those that produce antibodies with the desired binding characteristics. Multiple runs expand the number of B cells to be screened in relatively short order, providing opportunity for an enhanced exploration of the available epitope and epitope-binding repertoire. This can increase the chance of identifying antibodies with rarer characteristics in a reasonable time frame, such as those with high affinity against a particularly challenging antigen or with a unique binding interaction/epitope. Sequence and cross-binding reactivity analysis provide additional insights into relationships between the antibody hits discovered, allowing for more effective prioritizations for further evaluation.

Sequence identification

HIGH-THROUGHPUT HIT SELECTION AND NEXT GENERATION SEQUENCING OF ANTIBODY GENES

Once a B cell candidate has been identified, early generation Beacon technology would use light to move the single cell out of its NanoPen into a well of a PCR plate, and subsequent Sanger sequencing reveals the heavy- and light-chain sequences. However, Curia has embraced the latest Antibody Discovery 4.0 workflow to provide an NGS-based solution that is a more cost-effective and higher-throughput sequencing approach. The heavy and light chains for up to 1,152 clones are barcoded and prepared for multiplexed sample analysis. Pooled IgG cDNA libraries are sequenced using Illumina® instrumentation. Specialized software deconvolutes the sequences and associates the heavy- and light-chain sequences to their cognate NanoPen. In as little as four days post-cDNA export, high-quality sequences can be generated for hundreds of IgGs.

Custom bioinformatics

Bioinformatic analysis pairs the identified heavy- and light-chain sequences and determines paired sequence diversity and developability among the antibody hits. A lineage tree of the discovered antibodies shows how closely related they are based on sequence identity. Sequences with high levels of identity are placed into sibling groups. In our hands, sibling groups comprising five or more clones can have 100% confirmation rates of on-chip screening activity. *In silico* analysis of developability is based on Curia's Z^2 score, which is a scoring system to contextualize predicted sequence-dependent liabilities, such as oxidation sites in the complementarity-determining regions, that can impact antibody production and manufacturability. Other analyses, such as homology modeling and hydrophobicity analysis, can be used to remove sequences with predicted risk for

The production can be tailored to a project's specific need, starting with as small as a 0.01 L scale culture, seven-day process

CASE STUDY

DISCOVERY OF DIVERSE MONOCLONAL ANTIBODIES THAT BIND SARS-COV-2 DELTA AND OMICRON SPIKE PROTEINS

Leveraging Curia's PentaMice platform and rapid immunization protocols, maximum plasma titers against SARS-CoV-2 Delta spike protein were achieved within 17 days. CD138+ plasma B cells were enriched and loaded into 60,000 NanoPens for on-chip screening against Delta spike protein. More than 1,000 antigen-specific B cell hits were identified. The hits were multiplexed via the Antibody Discovery 4.0 workflow, and high-throughput single-cell RNA NGS led to high-quality paired heavy- and light-chain sequences in a week. A diverse set of more than 240 unique mAbs was identified spanning 170 clonal families utilizing 67 distinct VH genes, with most mAbs possessing robust developability scores using Curia's Z^2 system. Upon rapid re-expression, 23% of the antibodies demonstrated cross-reactivity to Omicron (B.1.1.529 and/or BA.2) spike proteins (Figure 2).

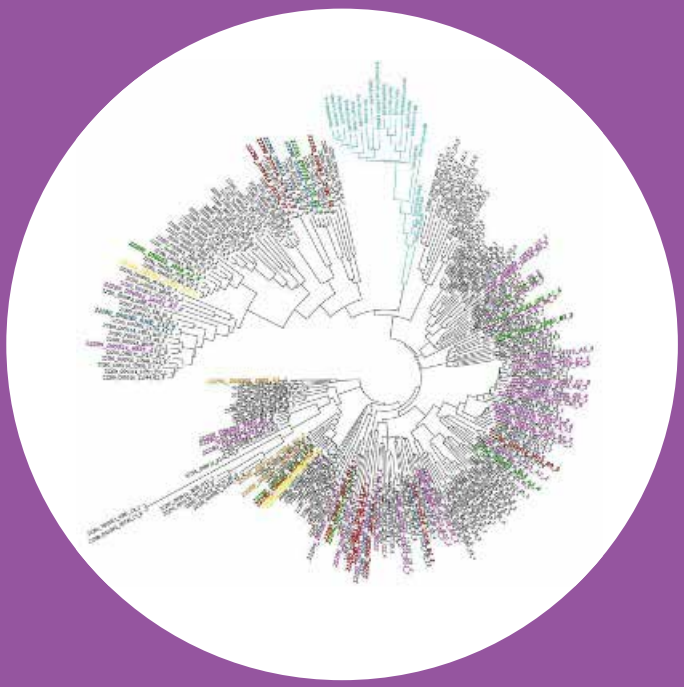


Figure 2. Single B cell antibody discovery for SARS-CoV-2 Delta spike. A polar dendrogram analysis of 248 unique Delta spike-binding antibody sequences identified at Curia by the Beacon system is shown. The dendrogram is rooted at the top with 20 unrelated FDA-approved mAbs (colored in teal). Other colors represent different cross-reactivity binding profiles against spike proteins from the originating SARS-CoV-2 virus and Omicron (B.1.1.529 and BA.2) variants. A diverse set of 170 clonal families of Delta-binders was identified.

development. Furthermore, Curia also determines the immunogenicity score of potential leads based on the likelihood of peptide sequences that could be presented by MHC molecules. Thus, comprehensive sequence analysis encompassing diversity, developability and immunogenicity all contribute to selecting optimal antibody candidates for rapid re-expression and validation.

Gene-to-protein strategies

RAPID RE-EXPRESSION IN HEK293T

Secondary assays following primary screens and NGS sequencing can confirm hit selection and facilitate a narrowing of a hit list. Curia's process includes a rapid re-expression step, where heavy- and light-chain variable regions are PCR amplified and expressed from transiently transfected HEK293T cells. Within five days, conditioned media with secreted IgG is subjected to informative validation assays (e.g., ELISA, cell binding by flow cytometry,

biofunctional activity). Validation assay results are integrated with the computational sequence analyses to provide a data package for each mAb to enhance prioritization of mAb candidates for larger scale recombinant monoclonal production and more advanced testing.

HIGH-THROUGHPUT RECOMBINANT EXPRESSION IN TunaCHO PROCESS

While rapid re-expression in HEK293 cells may be useful for intermediate confirmation, recombinant expression of top clones in CHO cells at an increased scale facilitates biophysical characterization, evaluating productivity and testing of purified antibodies. From clone cDNA, variable heavy and light chains are subcloned into a proprietary expression plasmid and reformatted. DNA transfection and recombinant antibody production using our proprietary TunaCHO process, which has been optimized for rapid high-concentration transient expression, affords purified mAbs for characterization.

The production can be tailored to a project's specific need, starting with as small as a 0.01 L scale culture, seven-day process. Larger volumes and longer production periods, such as a 0.25 L 14-day process, can produce up to 50–75 mg of mAb, while maintaining similar rapid timelines of five weeks from receipt of the DNA sequence for dozens of mAbs in parallel. In expedited cases, this means the entire process from immunization to delivery of pure recombinant mAb with the desired characteristics can be completed in as little as three months (Table 1).

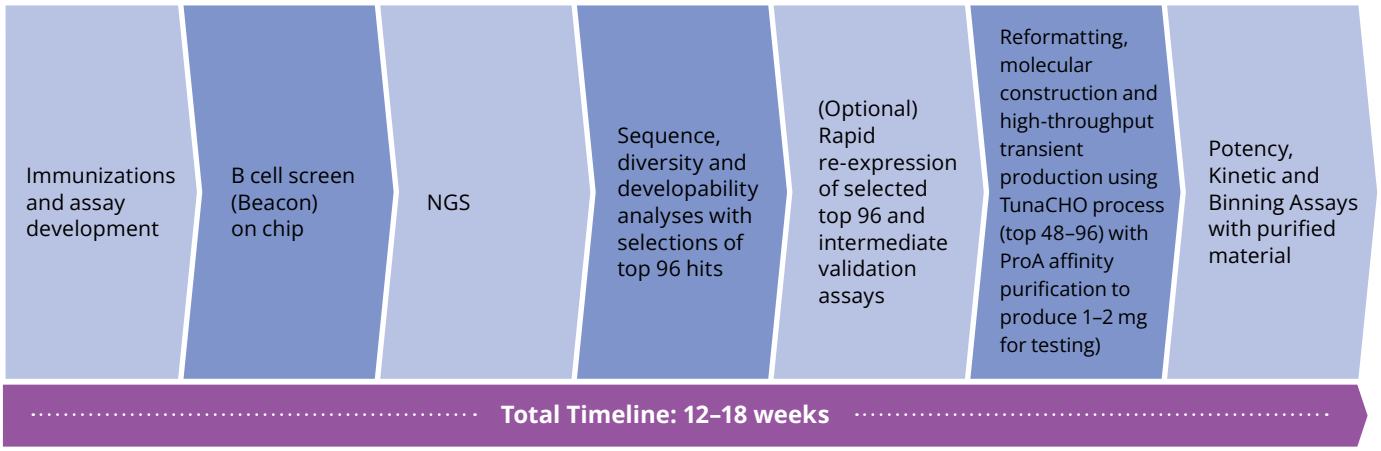
HIGH-THROUGHPUT SURFACE PLASMON RESONANCE (SPR) TESTING WITH CARTERRA LSA PLATFORM

Kinetic (k_{on} and k_{off}) and affinity data can drive lead selection, honing in on equilibrium constants (KD). Microgram quantities of mAb are used to measure binding kinetics and determine affinity by SPR; with Carterra LSA technologies, parallel and high-throughput analysis is performed against multiple antigens of interest. With purified mAbs and in the same experiment, subsequent epitope binning analysis of the mAbs provides head-to-head competition analysis of antigen binding. Coupled with EC_{50} potency and IC_{50} inhibition assay data, characterization of purified mAbs allows us to narrow down candidates from more than one hundred to the best dozen.

HUMANIZATION AND BEYOND

The mAbs generated using the PentaMice platform can be humanized at this point. Diagnostic antibodies and tool surrogate mAbs for *in vivo* studies are

Table 1. Sample timelines from immunization to purified, characterized mAbs



Timelines may vary depending on the project.

often sufficiently mature at this point, while mouse antibodies intended for therapeutic use often require humanization. Commonly, grafting of mouse complementarity-determining regions (CDRs) onto human framework regions generates engineered variable regions, where a humanized IgG would require reformatting with a human antibody Fc (fragment crystallizable) region. Because the original CDRs are retained and careful algorithms are used to select humanized designs, such as homology modeling with Curia's T20 scoring[1], antigen specificity and affinity can be retained even while transforming a mouse mAb into a humanized mAb.

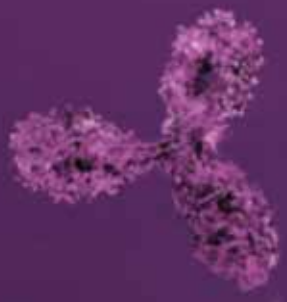
Beyond engineering sequences, Curia offers stable cell line development, upstream and downstream process development, manufacturing and analytics, all of which can be performed following antibody customizations and *in vivo* studies.

Lighting the path to discovery when speed counts

The PentaMice platform and Beacon technology, combined with next generation sequencing and gene-to-protein services, provide an efficient, rapid development option for those endeavoring to be First-to-Patent and First-to-Human. At Curia, the addition of these new technologies complements established methodologies of hybridoma and phage- and yeast-display, and further deepens our capabilities and expertise for antibody discovery.

Combined with exceptional cell line development, process R&D and GMP drug substance and drug product manufacturing, Curia provides a seamless First-to-Human mAb therapeutic development and manufacturing solution.

Learn how our experts can help shorten timelines to advance your First-to-Human drug discovery at www.curiaglobal.com/beacon/



ABOUT CURIA

Curia is a Contract Development and Manufacturing Organization with over 30 years of experience, an integrated network of 29 global sites and over 3,500 employees partnering with customers to make treatments broadly accessible to patients. Our biologics and small molecule offering spans discovery through commercialization, with integrated regulatory and analytical capabilities. Our scientific and process experts and state-of-the-art facilities deliver best-in-class experience across drug substance and drug product manufacturing. From curiosity to cure, we deliver every step to accelerate and sustain life-changing therapeutics. ***Learn more at curiaglobal.com***

Reference

1 Gao SH, Huang K, Tu H, et al. Monoclonal antibody humanness score and its applications. *BMC Biotechnol* 2013;13:55. https://bmcbiotechnol.biomedcentral.com/articles/10.1186/1472-6750-13-55#citeas

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