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DRUG DISCOVERY UPDATES #11

DDup

AI-DRIVEN ANTIBODY DISCOVERY AT EVOTEC

From concept through to IND and beyond

09/2021

evotec — DDup

What's inside this issue?

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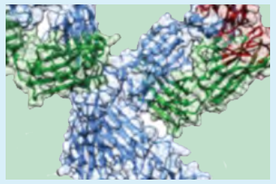
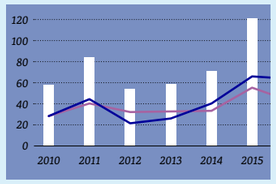
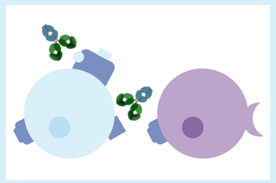






evotec

DEAR FRIENDS
OF EVOTEC

A message from Evotec CEO
Dr Werner Lanthaler

Welcome to this eleventh issue of DDup, an Evotec publication providing you with more insights into the company and its capabilities. Historically, Evotec has always been well-known as a leading company in small molecule drug discovery and development, but has in recent years, perfectly complemented by the acquisition of Just Biotherapeutics, built extensive expertise and capabilities in biologics, too.

In this DDup edition, we would like to introduce our exciting new developments in creating a biologics lane on the multi-modality Autobahn. With the combination of Evotec's long history in drug discovery coupled with state-of-the-art technologies such as Artificial Intelligence (AI) and Machine Learning (ML), we now have the opportunity to transform biotherapeutic discovery and development.

For the biologics industry, Evotec's multi-modality or "Digital Autobahn" provides a fast lane to drug discovery and development solutions. No matter where the project lies on the idea-to-IND continuum, Evotec's experience supports all activities from target identification through to IND submission, with high-end manufacturing completing this one-stop-shop. This comprehensive offering enables our partners to work with one experienced partner, which makes the processes less complicated, more efficient, more productive, less expensive, faster, and finally leading to more flexible ways of discovering, developing and manufacturing biotherapeutics. Our clients and collaboration partners can make use of our industry-leading, long standing experience in a broad range of target classes. The breadth and depth of the extensive disease biology, the availability of biology-relevant and mechanism-driven assays and models are applied in a rational and efficient way. But let me mention, that we also apply this unique platform to work on proprietary and co-owned assets, too, e.g. our FFmab platform where you can find more information about in this DDup.

Other highlights of this DDup edition feature Evotec's discovery technologies, such as our fully established hybridoma-based mAb generation and screening platform, as well as J.HAL℠, our novel AI-derived Just Humanoid Antibody Library, which is offered as part of our data-driven end-to-end biologic development platform, J.DESIGN.

Thank you for reading this latest edition of DDup – we hope you find it of interest. We welcome your thoughts and input, and hopefully we will get the opportunity to collaborate in this exciting area of transformational science in the future.

Yours sincerely,
for the management of Evotec
Werner Lanthaler,
CEO of Evotec SE

CHAPTER 01

INTRODUCTION
TO ANTIBODIES

WHAT ARE ANTIBODIES?

Antibodies, or immunoglobulin molecules, are glycoproteins produced by the plasma B cells of the adaptive immune system with the aim to recognise and neutralise foreign antigens, such as microorganisms and viruses (e.g. SARS-CoV-2). The specific binding of antibodies to their target molecules activates downstream immune responses leading to the elimination of the intruders. Thus, antibodies play a pivotal role in the immune system's defence against infection and disease.

Using a powerful and highly sophisticated combination of genetic recombination, somatic hypermutation and clonal selection, the adaptive immune response continuously generates novel antibodies against new antigens, thus making antibodies the most versatile among the currently known classes of binding molecules.

Full-length antibody (IgG)

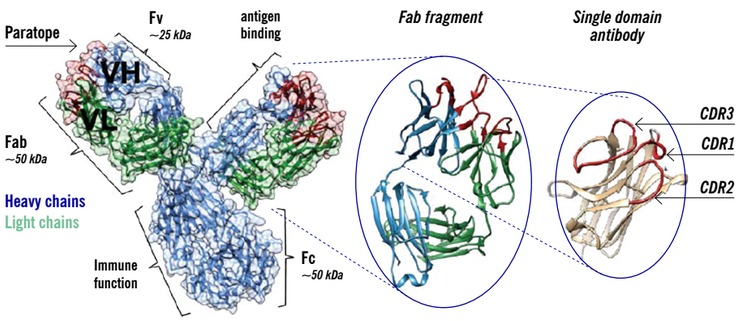


Figure 1, source: DOI: 10.1039/c8cs00523k– 2018-3rd generation antibody discovery methods: in silico rational design. On a structural view, antibodies are tetrameric proteins with a characteristic Y-shaped structure, consisting of two pairs of heavy and light chains. The tips of the Y are formed by the variable antigen-binding fragment (Fab) region, containing the paratopes, which are located within six binding loops, referred to as the complementary determining regions (CDRs). The paratopes mediate the interaction with the target regions of the antigens, also known as epitopes. The base of the antibody, also called the crystallisable fragment (Fc) region, defines the antibody subclass and regulates the communication with parts of the immune system that are important for effector function and serum half-life.

MONOCLONAL ANTIBODIES AS THERAPEUTIC AGENTS

The first use of the term "antibody" occurred by Paul Ehrlich in his article "Experimental Studies on Immunity", published in October 1891. In 1986, the first therapeutic monoclonal antibody was approved by the US Food and Drug Administration. Since then, more than 100 monoclonal antibodies have been designated as drugs, as they are very effective therapeutic agents. The high specificity of antibodies makes them ideal to reach their intended target and thus is useful to treat many different disease states. Due to their potential for high affinity and specific binding to a large variety of molecular targets, antibodies have been the focus of a wide range of technological developments aiming for the isolation, production and optimisation of these molecules for specific targets of interests in various indications. Antibodies are key tools in research and diagnostics, but also represent the fastest-growing class of biotherapeutics on the market due to the naturally favourable attributes such as specificity, potency and metabolic stability.

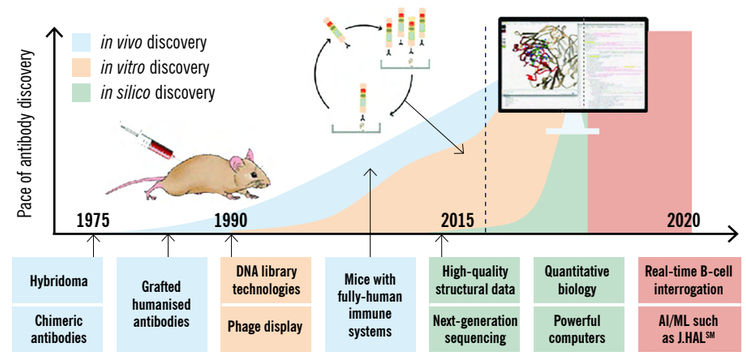


Figure 2, source: see figure 1. In vivo approaches harness the power of an immune system to generate antibodies, traditionally through animal immunisation and more recently also from human patients. In vitro approaches, on the other hand, rely on the construction of large libraries of antibody sequences mimicking the diversity achieved by the immune system, and thus likely to contain some binding molecules for each given antigen. The third generation covers the in silico attempts of designing and optimising biotherapeutics.

HISTORY OF ANTIBODY DRUG DISCOVERY PLATFORMS

Antibody drug discovery refers to the process of identifying new therapeutic antibodies to combat various diseases, such as cancer, autoimmune disease, viral infections, and many others. Over the last four decades, three different generations of antibody discovery technologies have been described. Importantly, the different approaches are not mutually exclusive but are highly complementary to each other.

The first major breakthrough in the development of antibodies for research applications was the production of monoclonal antibodies in 1975 by Köhler and Milstein. Their technique involved removing B-cells from the spleen of an animal that had been challenged with an antigen, and subsequently fusing them with an immortal myeloma tumour cell line. This resulted in a single-cell hybrid known as a hybridoma and allowed for the first time the purification of homogenous preparations (monoclonal antibodies; mAbs) of in which every antibody in the product is identical in its protein sequence, and thus every antibody is expected to have the same antigen recognition site, affinity, biological interactions, and downstream biologic effects.

More recently, the direct interrogation of single antibody-secreting B cells upon immunisation or virus challenge using technologies, such as fluorescence-activated cell sorting or the more modern ultra-high-throughput (UHT) microfluidic is rapidly evolving to become commonplace in the antibody discovery industry. The importance of this technology was recently highlighted by the real-time isolation of neutralising mAbs from convalescent COVID-19 patient's memory B cells that may serve as a promising intervention to SARS-CoV-2 infection.

In parallel to these *in vivo* (B-cell derived) discovery efforts, *in vitro* approaches relying on recombinant antibody technologies that can be used for the creation of large libraries of antibody sequences evolved. These antibodies are supposed to mimic the diversity achieved by the immune system, and in combination with the power of *in vitro* technologies, such as phage display, the selection of antibodies specific for any given target can be achieved.

As a third generation technology, the *in silico* design or engineering (molecular optimisation) of antibodies has emerged more recently. While being highly affine and selective for their targets, antibodies also need to exhibit different biophysical features, such as stability and solubility. Often these traits are conflicting as some of the mutations may cause advantages for some features but correlate at the same time with worsening others. Therefore, simultaneous optimisation of all of these features is required. For this, computational approaches offer a promising avenue, as they could drastically reduce time and costs of antibody discovery.

CHAPTER 02

AI-DRIVEN ANTIBODY DISCOVERY AT EVOTEC

As introduced above, antibody discovery may be driven by the interrogation of two primary sources: (1) *in vivo* (animal-derived B cells) or (2) *in vitro* (library-derived antibody display platforms). Evotec's strategy for the optimal path to lead candidates is to offer access to both, *in vivo* and *in vitro* sources of antibodies for discovery, coupled with the exploitation of state-of-the-art technologies to ensure success for a broad range of targets and disease states. In addition, if needed, selected lead candidates can be further optimised using powerful computational platforms such as our proprietary Abacus™ *in silico* tool suite to enhance productivity, manufacturability, and formulation stability.

AUTOMATED, HIGH-THROUGHPUT HYBRIDOMA PLATFORM

For the *in vivo* antibody discovery, we are harnessing the power of the immune system to generate antibodies, combining the traditional hybridoma technology with automated devices for high-throughput clone selection, screening as well as recombinant expression and purification.

As source for best-in-class human antibodies, we are using the ATX-Gx™ platform, a suite of immunocompetent transgenic mice from our collaboration partner, Alloy Therapeutics (www.alloytx.com). Antigens are first injected into the host to elicit the expansion of antigen-specific B cells. After the humoral response has been mounted, splenocytes are harvested and the antibody-producing B cells are fused with a highly proliferative, immortal myeloma cell line via strategies such as electroporation or polyethylene glycol (PEG) treatment. Subsequent clonal selection results in a single-cell hybrid known as hybridoma. The B cells confer antibody production capability, while the myeloma cells enable hybridomas to divide indefinitely and to grow well in cell culture. A hybridoma cell line secrets only one antibody type, effectively ensuring an infinite supply of antibodies selective for a single epitope, which are also known as monoclonals.

At Evotec, we have shortened and simplified this process of hybridoma establishment by coupling the traditional technology to an automated clonal selection of hybridoma colonies from methylcellulose-based, semi-solid medium using the cell separation robot CellCelector™. Thereby generated monoclonal hybridoma colonies are screened using high-throughput screening technologies, such as the iQue® Advanced Flow Cytometry Platform that enables rapid, high-content, multiplexed analysis of cells and beads in suspension. Once promising hits are identified, a next-generation sequencing protocol is used to obtain the VH/VL DNA sequence information of these potential lead antibodies. Availability of the immunoglobulin sequence information is a pre-requisite for the recombinant production of the mAbs. Additionally, it allows for the *in silico* analysis (using our proprietary Abacus™ software tool) of antibody sequence information to rank candidates in terms of diversity, clades, germline background and pairing, potential post-translational modifications (PTM), missing or inserted residue errors, potential immunogenicity, isotype, subtype, and can drive the engineering modifications necessary to repair or modify antibody sequences. After sequencing, selected VH/VL genes are synthesised and subjected to a transient, high-throughput expression and purification platform that allows the small-scale production of up to several hundred mAbs in parallel. The high quality material can then be used for further downstream characterisation, such as functional activities that allow the selection of potential lead candidates.

Hybridoma-based mAb generation and screening

Streamlined workflow from immunisation to recombinant mAb

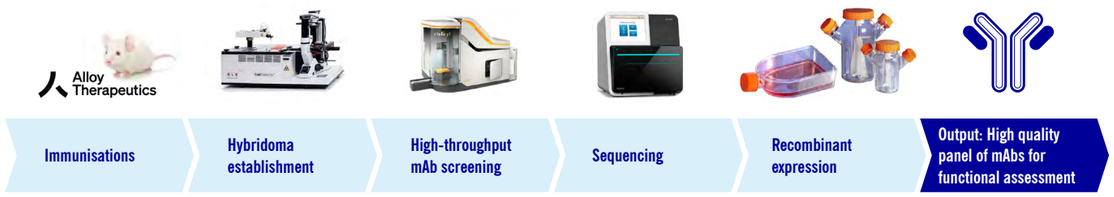


Figure 3

J.HAL℠ – A NOVEL HUMANOID ANTIBODY LIBRARY

Human-like antibodies designed and sequence-biased in silico for superior efficiency and developability features

For the *in vitro* antibody discovery, Just – Evotec Biologics exploits artificial intelligence (AI) and machine learning (ML) to generate novel, humanoid antibody sequences that both represent natural repertoires and are biased towards desirable features.

Antibody-based biotherapeutic discovery is optimised by enabling efficacy, epitope diversity, and suitable developability during the discovery process. High costs and long development times present key challenges in the global accessibility of monoclonal antibody therapeutics. *In vivo* discovery methods deliver therapeutically relevant antibodies, but often with limitations in epitope coverage and with no selective pressure toward developability. To enable broad target and epitope engagement, focused efficacy, and a bias toward developability, we have developed an Antibody-GAN (Generative Adversarial Network), a new synthetic approach to designing a novel class of antibody therapeutics which we term humanoid antibodies (Amimeur, T., et al. (2020). https://doi.org/10.1101/2020.04.12.024844).

The Antibody-GAN architecture utilises competing deep layer neural networks to learn and produce the features of the mature human antibody repertoire, including sequence characteristics and structure properties, allowing for the encoding of key properties of interest into diverse libraries for a feature-biased discovery platform. Our Antibody-GAN architecture (1) captures the complexity of the entire variable region of the standard human antibody sequence space, (2) provides a basis for generating novel antibodies that span a larger sequence diversity than is explored by standard *in silico* generative approaches, and (3) provides transfer learning (continued training of a model with a subset of data with specific desirable characteristics). This last method is of critical utility towards antibody discovery. It provides a method to bias the

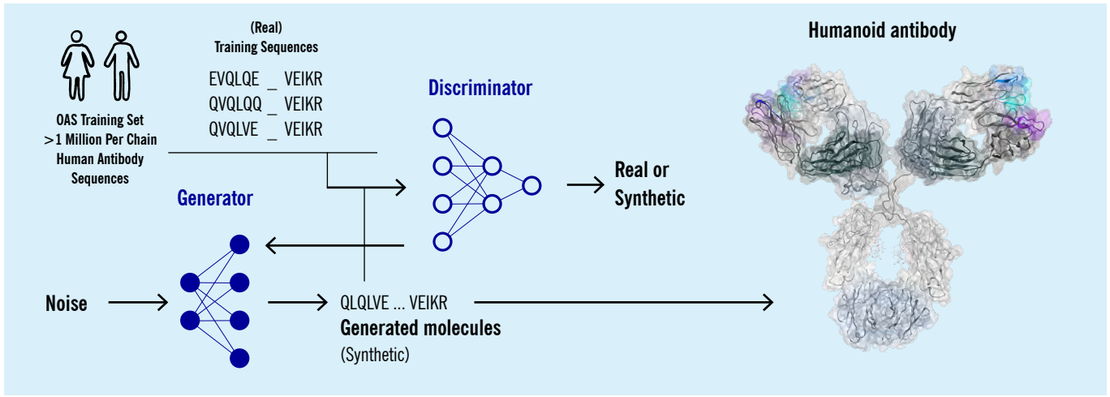


Figure 4

physical properties of the generated antibodies toward broader efficacy traits such as CDR lengths and surface properties, improved developability such as improved thermal and pH stability, and diverse chemical and biophysical properties.

A GAN operates by utilising competing, or adversarial, neural networks, as shown in Figure 4. The discriminator network is trained using human sequences to recognise real versus fake, while the generator network generates fake sequences in an attempt to fool the discriminator and is trained by feedback from the discriminator. We filtered the training sequences to remove recent immunisation and infection bias, as well as early recombination sequences, to obtain a set of training sequences which represent broad and somatically hypermatured antibodies. Over training, the two networks get progressively better at their tasks. After full training, the Antibody-GAN generator is eventually able to produce fully human, novel antibody sequences for the germline for which the GAN was trained. We have utilised the Antibody-GAN to generate a discovery library we call J.HAL℠, the Just Humanoid Antibody Library. This novel library contains an initial set of 25 sub-libraries across a variety of germline backgrounds, all containing both framework and CDR diversity representative of matured human antibodies. This initial library represents human repertoire response and is applicable to therapeutic antibody discovery. Additional library expansion is occurring through creation of sub-libraries focused on a broader range of efficacy and developability. Biasing toward longer and shorter HC-CDR3s, building out a range of surface properties to engage antigen via electrostatic or hydrophobic-driven binding, and expanding the representations of germline pairings, all while limiting the impact of potential immunogenicity provide enhanced library characteristics.

Biasing toward developability comes from GAN transfer learning using stable sequences obtained from ultra-high-throughput Fab library stressing. Improved conformational stability may impact many areas of process development such as increased titre and yield, decreased aggregation propensity, decreased particulation and sub-visible particle formation, improved yield from low pH viral inactivation, improved ability to withstand freeze-thaw cycles, improved room temperature stability, and has even been shown to improve serum stability, thereby extending half-life. The J.HAL℠ platform is also being extended to include single-domain VHH libraries to broaden functional epitope diversity as well as to provide a platform for multi-specific modalities.

These libraries are being physically realised as Fab-displayed phage to limit loss from conversion to full-length antibodies, to enable the measurement of biophysical properties at the library level, and to allow for library-level stressors

to enable transfer learning. This Fab-based phage library is applicable to standard library panning methods for target-binding antibody discovery, as shown in Figure 5.

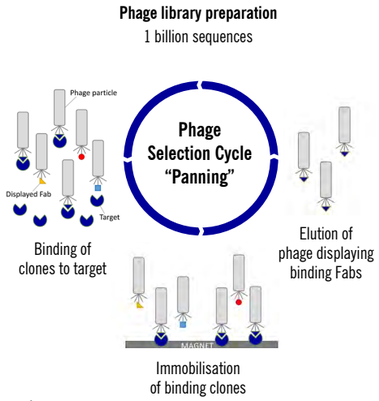


Figure 5

Multiple rounds of panning with increasing stringency results in enriched binders which are then translated to a recombinant system for affinity and activity verification. Target specificity and species cross-reactivity screening is also performed as this stage of antibody panel refinement. The library sequences are also being built as a scFv-displayed yeast library to enable direct conversion to scFv modalities such as CAR-Ts and multi-specific platforms, as well as full-length yeast display to enable direct antibody discovery and small-scale production directly from the library screen output. The full-length display and secretion library lends itself to microfluidic discovery strategies, thus enabling direct interrogation of binding and activity during the discovery process, as well as direct biophysical characterisation without the need for recombinant conversion. These workflow platforms are displayed in Figure 6.

To validate the utility of the initial J.HAL℠ suite of libraries, we chose a few targets of interest. The first target to be used in our antibody discovery platform was the SARS-CoV-2 wildtype variant Receptor Binding Domain (RBD) of the spike protein. Three rounds of phage panning resulted in the enrichment of 22 unique binders as determined by phage ELISA and DNA sequencing. Of these, eight were also found to bind to the B.1.1.7 variant spike protein. All enriched Fabs were transiently expressed in HEK293 cells as full IgG antibodies. Nine of these antibodies exhibited blocking activity of the spike protein to the ACE2 receptor in an *in vitro* functional assay.

Another panel of enriched binders isolated from a second phage panning enrichment and NGS selection process are being screened in a similar fashion and have approximately doubled the number of active leads. Most of these antibodies will be tested in pseudovirus assays to assess their potential as therapeutics.

The J.HAL℠ discovery platform is functionally diverse, developable, fully human, and under continual expansion.

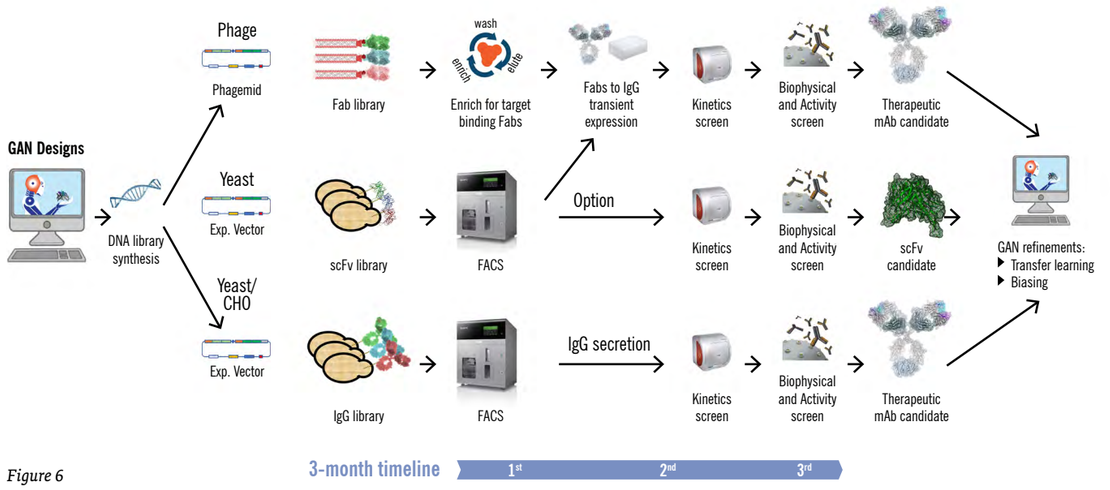


Figure 6

FROM CONCEPT TO IND

Less expensive, faster, and more flexible ways of discovering, developing and manufacturing biotherapeutics

A perfect therapeutic antibody is, most of all, a new molecule leading to a significant improvement for patients. This can result from either its ability to reach a novel target, the fact that its mode-of-action differs from already available drugs, or simply because it provides significant advantages to an existing antibody, such as an improvement of its efficiency, a reduction of its costs, or a lowering of its side effects.

In the previous sections of this document, we had laid out the antibody discovery platforms we offer at Evotec to support the selection of such novel biotherapeutics. These capabilities can be accessed as stand-alone services on demand. However, in addition, through our extensive drug discovery know-how and experience, we can also offer seamlessly integrated antibody drug discovery capabilities. No matter where the project lies on the idea-to-IND continuum and beyond, Evotec's experience supports all activities from target identification through to IND submission and beyond.

Our clients and collaboration partners can make use of our industry-leading, long standing experience in a broad range of target classes, the breadth and depth of the extensive disease biology, the availability of biology-relevant and mechanism-driven assays and models applied in a rational and efficient way. Evotec has in-depth disease expertise in anti-infectives, respiratory diseases, immunology, oncology, metabolic diseases and neurology. Depending on the therapeutic area and the target, Evotec employs relevant translational *in vitro* or *ex vivo* assays that assess desired functional effects of the candidate molecules. We develop suitable pharmacodynamic read-outs that either provide a target-proximal read-out, target engagement, or that quantify desired downstream effects. If available and desired, candidate molecules can also be tested in relevant disease models to assess efficacy in animal models. Evotec's pre-clinical department offers the full range of *in vitro* and *in vivo* GLP and non-GLP pre-clinical evaluation studies to thoroughly assess the safety profile of the drug candidate. Our clients and collaboration partners can benefit from our extensive understanding of PK/PD relationship for human dose prediction. Finally, Evotec can perform Biomarker discovery and Biomarker studies to enable diagnosis, assessment of target engagement and pharmacodynamic effects, patient stratification or prediction of therapeutic success. In this context, Evotec offers a comprehensive proteomics and ligand binding assay platform to support Biomarker sciences from discovery through to the clinic.

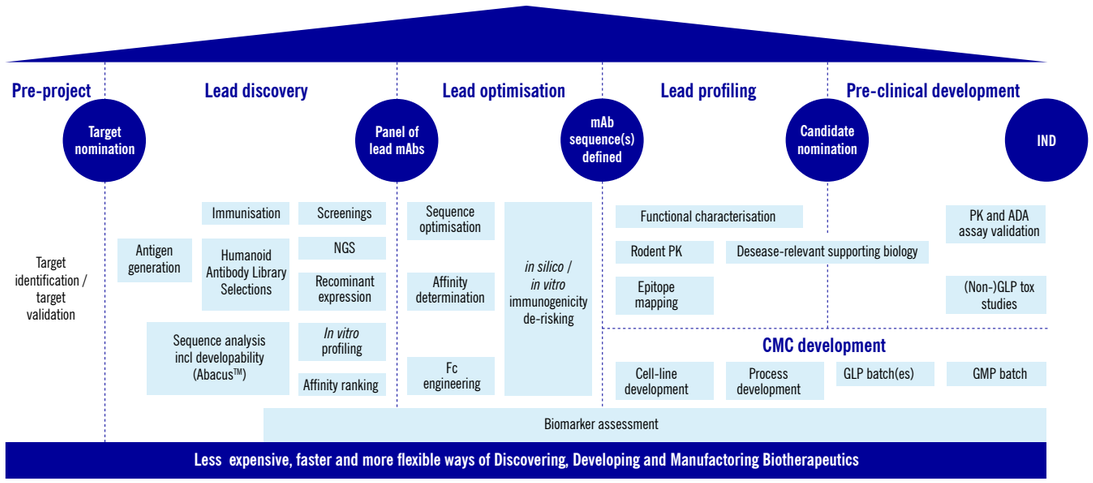


Figure 7

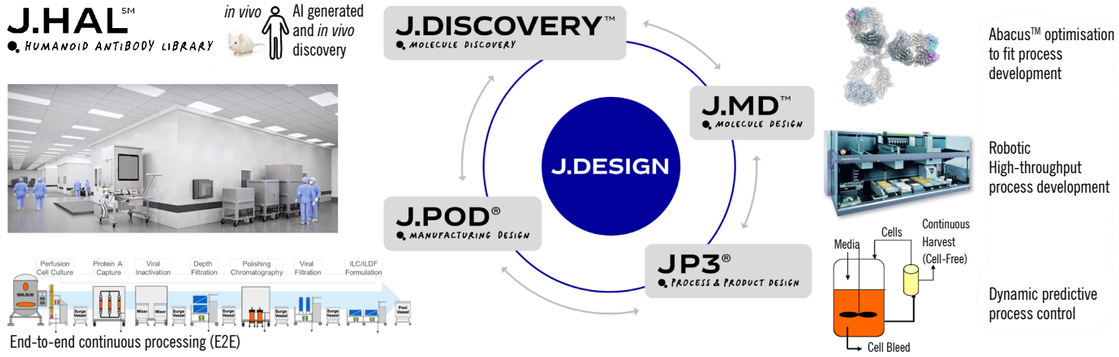


Figure 8

All of this is paralleled by data-driven solutions to biologics design and manufacturing to ensure smooth transition into development and reduced risk of downstream attrition and delay. With the acquisition of Just Biotherapeutics in 2019, Evotec now provides all capabilities for the discovery and development of optimal biotherapeutics. With decades of industry experience, the goal is to utilise our in-house, integrated technology platform to design and manufacture biologics (J.DESIGN). Utilising J.DESIGN, we can accelerate development and provide superior manufacturing process control for higher quality molecules.

The discovery, development, and manufacturing of biologics is complex and expensive. As the number of biologics modalities increase to target specific biology directly, such as multi-specifics, antibody fusions, and multi-domain antibody-based constructs, the complexity and costs increase. To drive the technologies required to increase speed and capacity, decrease costs, while maintaining high quality, an integrated approach to discovery and development is crucial. This breaks down the silos in technology development which allows for technology innovation across functions and is the basis for Just – Evotec Biologics' technology platform, J.DESIGN.

At the core of J.DESIGN is a common data management system that centralises and integrates the highly complex data sets generated from the distinct activities involved with the discovery, development and manufacture of biologics – at Just – Evotec Biologics we refer to these as Discovery, Molecular Design, Process and Product Design, and Manufacturing Design – into a singular biologics design space.

J.DISCOVERY™ contains the large, diverse, manufacturable, and developable AI-designed discovery libraries, including J.HAL℠. Molecular design, J.MD™, provides analysis, humanisation, and optimisation of parental antibody sequences to enhance manufacturability and stability, saving time and costs during process development. J.MD™ uses an in-house suite of computational tools called Abacus™ in conjunction with structural tools that can assist in designing the best molecules and predict the best conditions for development. JP3® involves the design of the process and product, spanning cell line development, upstream bioreactor, downstream purification development, analytical method development, drug product and formulation development, as well as processing formats, such as intensified fed-batch and continuous processing. Our JP3® scientists utilise high-throughput robotic solutions for process and product development and leverage data for learning and prediction. Process and long-term storage, which are traditionally determined through formulation activities, are defined in J.DESIGN during molecule optimisation activities, pushing the evaluation and lead selection of biotherapeutics earlier in the development cycle. Finally, the biotherapeutic is manufactured using the defined process in a J.POD®, a small footprint facility using disposable technologies and intensified processes resulting in flexible, deployable, and relatively low cost biomanufacturing. Our first J.POD® facility for biologics development and commercial manufacturing will be fully operational in Redmond, Washington this year.

FACTS & FIGURES

ANTIBODIES

CHAPTER 03

IMPORTANT HISTORICAL MILESTONES

- **1974:** Köhler and Milstein develop methods for the isolation of monoclonal antibodies (mAb) using hybridoma cells and were awarded with the Nobel Prize in 1984 for their work
- **1985:** George P. Smith laid the foundation for phage display, which is the first and still the most widely used technology for *in vitro* antibody selection
- **1986:** Approval of first therapeutic murine mAb, Orthoclone Okt3 for human use
- **1995:** Approval of first chimeric mAb Abciximab
- **1997:** First humanised mAb, Daclizumab, approved
- **2002:** Approval for Adalimumab, the first fully human mAb

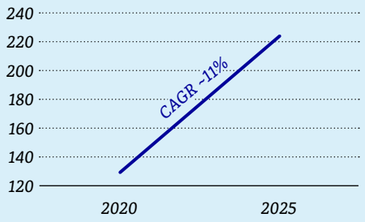
The FDA approved its 50th antibody in 2015, 29 years after the first one. It took just 6 more years to reach number 100 in April 2021.

In 2021, 3 antibodies have already been approved.

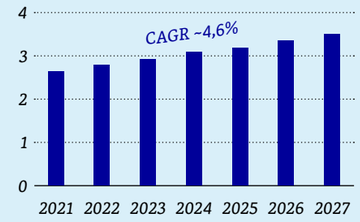
More than 15 antibodies are currently being reviewed by either the FDA or the EMA.

Constantly growing markets

Global antibody market development in $ bn



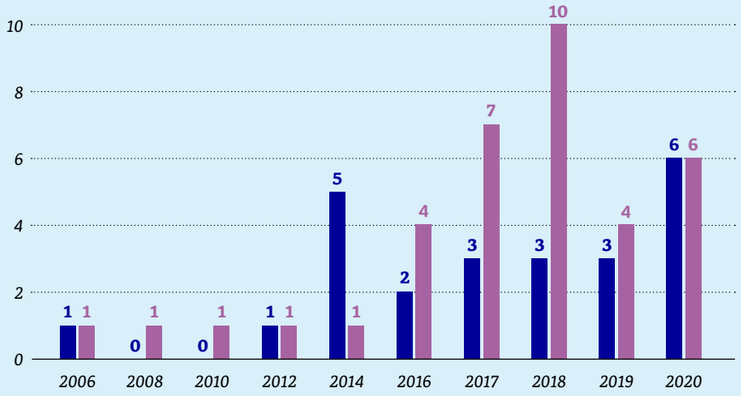
Global antibody discovery services market in $ bn



Monoclonal antibodies and their affiliated products represent one of the fastest growing segments of the pharmaceutical industry, but it is also worth highlighting that other antibody-based products, such as bispecific antibodies, ADCs and antibody fragments, are steadily gaining traction in the biopharmaceutical industry. The opportunity associated with such novel antibody formats is also significant; for instance, the bispecific antibodies market alone is expected to reach $ 7 bn by 2030, growing at a CAGR of 33%, between 2018 and 2030.

Number of antibody therapeutics granted a first approval in either the US or EU from 2006–2020

Cancer *Non-cancer*
Source: antibodysociety.org

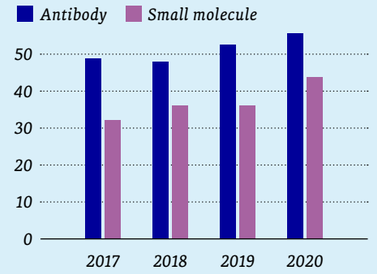


A STEADILY GROWING MODALITY WITH A BROAD APPLICABILITY

Commercial success

Monoclonal antibodies are on the way to dominate the pharmaceutical market, Humira (Adalimumab) became the world's top-selling drug in 2012 and has remained No. 1 ever since. If the drug claims the top spot in 2021, Humira will be on its way to overtaking Lipitor as the world's all-time best-selling drug.

Top 5 drug sales 2017–2020 in $ bn



The yearly Top 5 antibody therapeutics regularly outperformed the Top 5 small molecule drugs from 2017–2020.

The Top 10 best-selling drugs in this timeframe were dominated by antibodies.

The Top 10 best-selling antibodies achieved revenues of ~$ 75 bn in 2020.

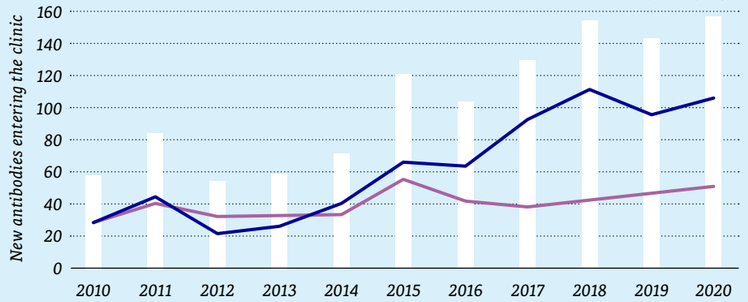
It is expected that antibodies will obtain their dominance in the top-selling drug lists in the upcoming future.

Clinical pipeline

Up until 2014, drug developers advanced roughly the same number of cancer and non-cancer antibodies into the clinic each year. In the past 5 years, however, cancer programmes have pulled ahead. In 2020, for instance, more than twice as many cancer programmes entered the clinic as non-cancer programmes (106 versus 51).

New antibodies entering the clinic, by year

Cancer *Non-cancer*
Source: antibodysociety.org



Overall, there are now nearly 870 antibodies in clinical development, but about 36% of these act on another short list of just ten validated and novel targets.

Top 5 investigational mAb targets:
1. PD1/PDL1
2. CD3
3. HER2
4. CTLA4
5. SARS-CoV-2

Despite having reached a landmark 100th approval, the target space these approved biologics cover is more limited. Just ten targets – counting ligands and their receptor pairs together – account for 42% of the approvals to date. So, there is a lot of room for new targets and novel approaches in this exciting and growing area of antibody drug development!

CHAPTER 04

FUNCTION-FOCUSED ANTIBODY (FFmab) SCREENING

AN ANTIBODY-BASED PHENOTYPIC SCREENING PLATFORM DRIVEN BY FUNCTION

FFmab SCREENING PLATFORM OVERVIEW

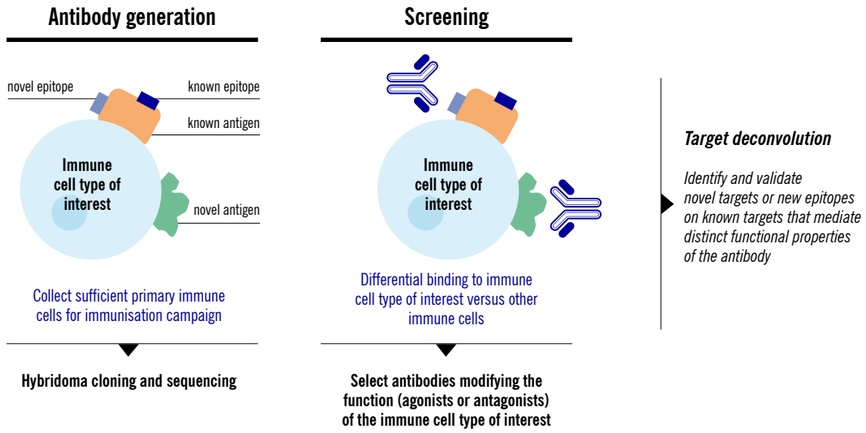


Figure 9: Overview of FFmab screening platform

WHAT IS FFmab SCREENING?

FFmab screening is a platform for identifying novel functional antibodies that change disease-modifying phenotypes of immune cells. The platform identifies innovative therapeutic antibodies that mediate differentiated mechanisms-of-action by binding either novel targets or new functional epitopes on known targets (Figure 9).

HOW DOES IT WORK?

The FFmab screening workflow consists of three main phases preluded by identifying an immune cell type as a key mediator of a disease phenotype. In Phase 1 adequate amounts of the primary human immune cell are generated, immunisations therewith are performed and antibodies that bind selectively to this immune cells type are identified. These cells

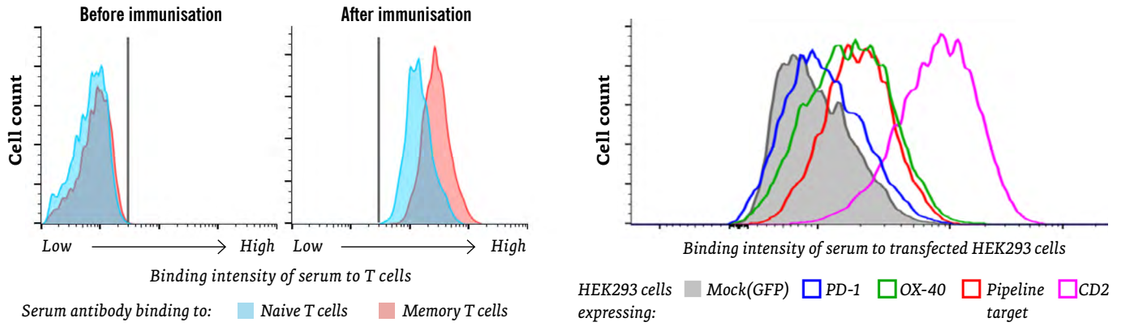


Figure 10: Using proprietary immunisation protocols, robust antibody response with selectivity for memory T cells was achieved. Antibodies against multiple immune modulatory targets were detectable in serum.

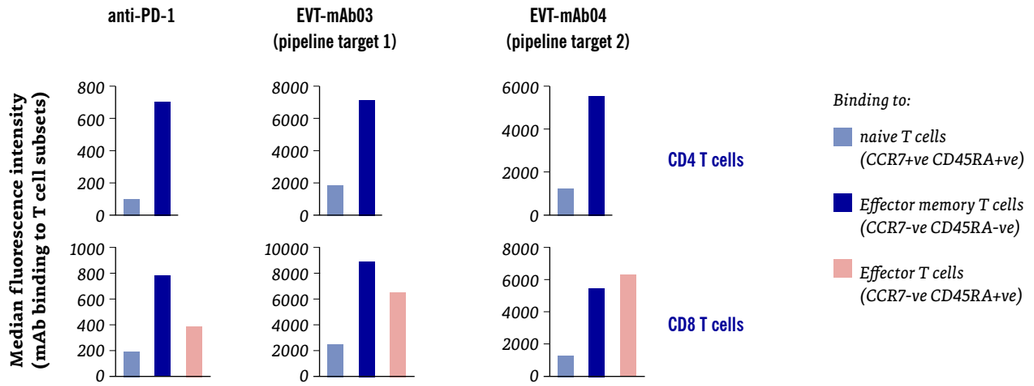


Figure 11: Screening for binding to memory T cells and simultaneous counterscreening against naïve T cells enrich for effector memory/effector T cell-selective antibodies.

may be purified from peripheral blood samples of healthy donors and patients or derived and expanded from a precursor cell type e.g. iPSCs. For a robust FFmab campaign, 2 x 10^9 cells of the immune cell type of interest are required. Immunising mice with these cells provokes a broad range of antibodies against cell surface antigens *in vivo* (Figure 10). The success of the immunisation is monitored. Here, in addition to classical titre tests that measure the quantity of antibody response, we perform assays to qualify the response of individual animals.

After verifying the success of the immunisation campaign, we generate antibodies from selected responder animals by state-of-the-art hybridoma technology. Using high-throughput flow cytometry-based screening of ~10,000 hybridoma clones, we identify those antibodies that bind selectively to the cell type of interest. This is accomplished by cell-multiplexing. Selective antibody clones are isolated and sequenced (Figure 11). After identification of a large panel of adequately diverse and developable antibodies, the project enters into the second phase.

Phase 2 involves functional assessment of the antibodies in relevant *in vitro* assays using primary immune cells (Figure 12).

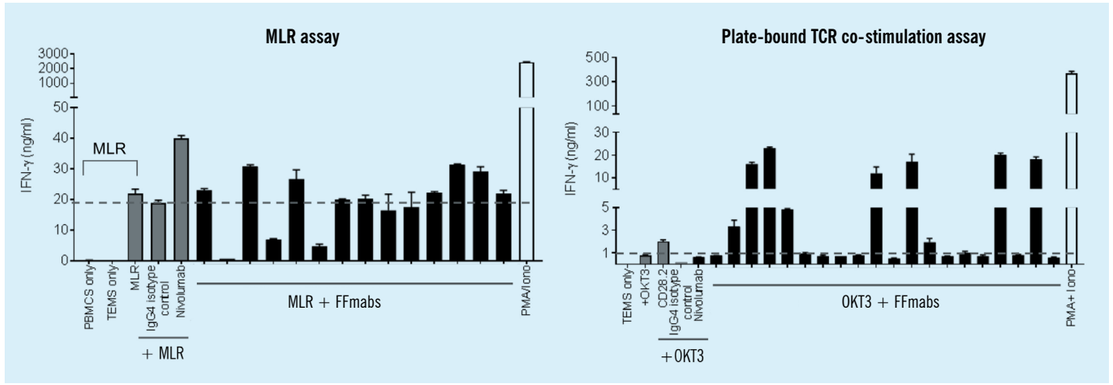


Figure 12: Bespoke functional assays are used to identify immune modulatory FFmabs

For this purpose, we convert the antibodies into a human isotype-matched format to exclude confounding effects mediated by different Fc types. Depending on the desired mode-of-action, we design the recombinant antibodies to exert normal, attenuated or enhanced Fc effects. Optimised processes for generating expression constructs and a high-throughput transient CHO antibody expression platform provide high quality material for the functional screens. Identified functional antibodies undergo further characterisation by epitope binning and assessment of their binding to a panel of known pre-clinical and clinical targets in the field of the disease indication in question.

The third phase of the project focuses on target deconvolution using the functional antibodies identified in the previous phase. To increase the likelihood of identifying novel targets, selected functional antibodies are first counter screened against "usual suspect" targets in the indication area. Then, using the functional antibodies as basis for identification of their respective targets, we employ the services of Retrogenix and DualSystems for target deconvolution. We perform in-house validation of any candidate target identified in this way by verifying that the respective antibody binds to the target expressed in cells recombinantly (Figure 13). At this stage, the project team may decide to generate an additional panel of antibodies with comparable functional properties using a target-specific discovery campaign. For selected clinical candidate antibodies, we perform further experiments demonstrating their therapeutic mechanism-of-action *in vitro* and *in vivo* in order to generate a strong pre-clinical data package.

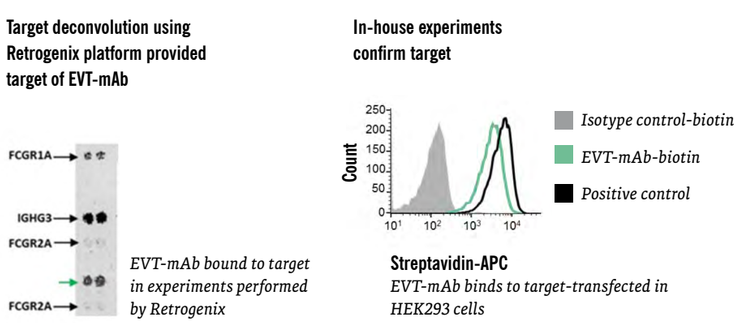


Figure 13: Target deconvolution

WHAT HAS BEEN DONE SO FAR?

The first project run on the FFmab screening platform focused on effector memory T cells. We selected effector memory T cells (TEM) as this T cell subset plays a key pathologic role in cancer and autoimmune diseases. For this pilot project, we immunised mice with TEM cells collected from PBMCs of 10 healthy donors. Immunisations resulted in serum responses against known immune modulatory targets, like PD-1, CD2, and OX-40 (Figure 10). We screened the generated hybridoma clones for TEM-selective antibodies versus antibodies that also bound to naïve T cells. We functionally tested recombinant antibodies in a TEM-based plate-bound TCR co-stimulation assay and a mixed lymphocyte reaction (MLR) assay. In this pilot project, we have identified an agonistic antibody against a novel target belonging to the immunoglobulin superfamily and an antibody against a new epitope on a known, clinically validated target. The unique epitope of the latter antibody enables its use in a different disease indication with a highly differentiated mechanism-of-action. Both antibodies are currently in pre-clinical development as first-in-class opportunities (Figure 14).

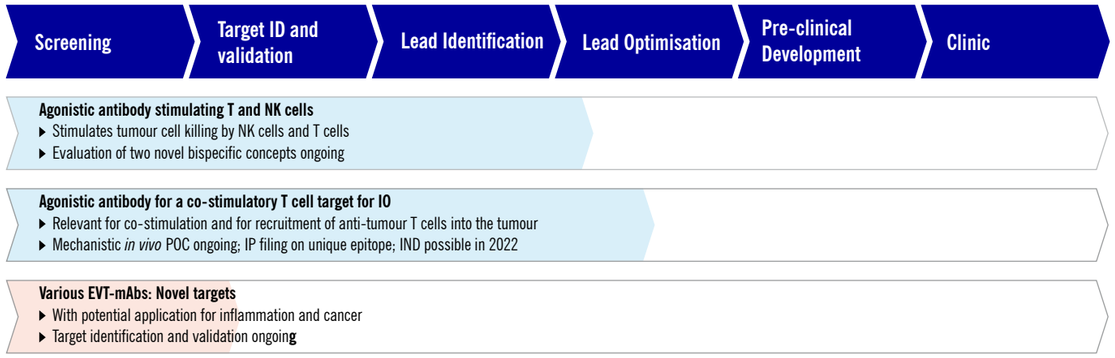


Figure 14: Pipeline arising from pilot project using effector memory T cells

The pilot study delivered a robust workflow optimised for speed and yield and provided proof-of-concept for the platform. This has encouraged initiating another screen focused on a different immune cell population that is currently underexploited for immune oncology.

WHAT IS IN PROGRESS?

Currently we are using the FFmab screening platform to identify novel functional antibodies directed against myeloid-derived suppressor cells (MDSCs). MDSCs are key suppressor cells in the tumour microenvironment. Patients with high MDSCs have poorer survival rates. Circulating MDSCs predict advanced cancer stage and increased risk of resistance to immune checkpoint inhibitor (ICI) therapy. MDSCs have hence emerged as a pivotal hurdle for more widespread clinical success of current ICI approaches. Nevertheless, MDSC-targeting therapeutic approaches are currently limited due to the dynamic and poorly defined

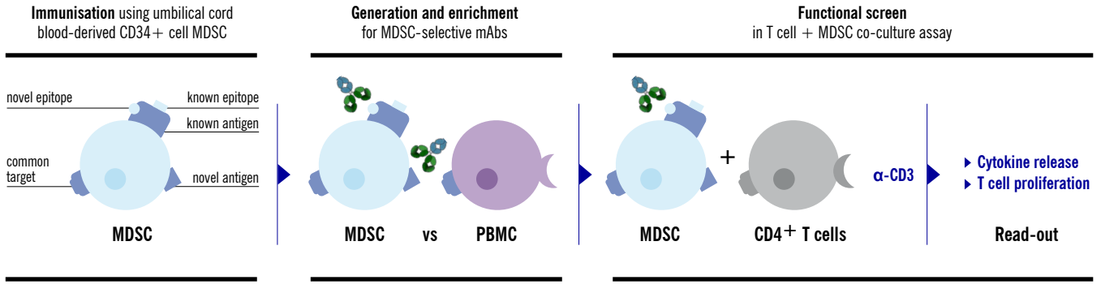


Figure 15

phenotype of these cells.
We have developed protocols to generate and expand MDSCs and characterise their suppressive phenotype in T cell co-culture assays. By immunising mice, we have generated hundreds of MDSC-selective antibodies with high sequence diversity.

The first recombinant antibodies are currently entering functional screening. We will focus on selecting antibodies that:
- block the suppressive activity of MDSCs and/or
- reprogram MDSCs from an immune-suppressive to an immune-stimulatory phenotype

Highly MDSC-selective antibodies that have neither of those two functional properties may still be functionalised by conjugating them to a payload for MDSC depletion.

Dr Welbeck Danquah

In 2012, Welbeck received his PhD in immunology selecting and characterising functional llama nanobodies against the inflammation-mediator P2RX7 ion channel in the Fritz Nolte lab at the University Medical Center in Hamburg, Germany. The dissertation was awarded the Heinrich Pette Dissertation Award in Neurology and Immunology. Following a short Post-Doc stint, also in the Fritz Nolte lab, Welbeck joined Evotec in 2014 nucleating and leading a pioneer team of scientists dedicated to antibody research in the company. Building on Evotec's track record in small molecule phenotypic screens, Welbeck and colleagues conceived the FFmab screening platform in Q4 2016 kicking off with a pilot project based on effector memory T cells.

As project lead, Welbeck was instrumental in establishing key components of the workflow using this pilot project. To further validate the platform, Welbeck proposed and leads the ongoing MDSC screen.

CHAPTER 05

5 MINUTES WITH

DR JIM THOMAS AND KAREN LACKEY

James N. Thomas, Ph.D.

Executive Vice President, Global Head of Biotherapeutics and President U.S. Operations

Jim is leveraging his extensive experience in biotechnology and scientific leadership to help create global patient access to vital medicines through technological innovation.
In his current role at Just-Evotec Biologics, Jim is helping to build biologics capability into the broader offering and capabilities of Evotec SE. Jim served as CEO and founding partner of Just Biotherapeutics. Prior to Just, Jim served as vice president of Process and Product Development within the Translational Sciences R&D organisation at Amgen. In this role, he led the development and application of all process, analytical and formulation technologies used to manufacture both clinical and commercial large molecule products. Jim was first exposed to biotechnology as a postdoctoral fellow at MIT almost 40 years ago, then worked as a scientist and leader at Genentech, Immunex and Amgen. Over the course of his career, he has contributed to the advancement of many important therapeutics including Activase®, Vectibix®, Enbrel®, Prolia®/Xgeva® and Repatha™.
Jim has built teams, departments and functions passionate about creating and using innovative technologies to deliver to the needs of patients, and he and his team at Just - Evotec Biologics are continuing this important mission.

Karen Lackey

Global Head of Integrated Drug Discovery, Evotec

Karen is responsible for >1,100 scientists covering chemistry, biology, computational drug discovery and pharmacology in >12 therapeutic areas of disease expertise. Prior to joining Evotec, she spent six years in Academic Drug Discovery most recently as the Director, Arizona Center for Drug Discovery (ACDD) at the University of Arizona that serves as an organisational hub for supporting academic-based drug discovery throughout the state. Karen was also an entrepreneur in early stage biotechnology, contributing to 5 start-ups in platform technologies (e.g. PyramidBio). Her pharmaceutical roles (>25 years) included Vice President of Discovery Chemistry at Hoffmann-La Roche (USA) and Global Vice President in the Molecular Discovery Research Division of GlaxoSmithKline.

What role do Biologics play in the context of novel therapeutics?

Jim: Biologics are a significant and growing portion of the arsenal of therapeutics used to fight serious disease today. There are literally thousands of biologics in the development pipelines of companies around the world, and the number in development is projected to approximately double by 2025. Commercial approval of these drugs should also double in this time frame.
Just - Evotec Biologics is using and continually improving a powerful platform to address the growing demand for biologics in the industry. We call this platform, J.DESIGN, and it will be applicable to greater than 70% of all biologics that will be discovered and developed over the next several years.

What are the challenges of developing well-behaving biotherapeutics and how does Just - Evotec Biologics overcome these challenges?

Jim: The molecular complexity of recombinant biologics has driven a very different approach to their discovery, development and manufacture when compared to small molecule drugs. Just - Evotec Biologics is capitalising on the broad functionality and molecular similarity of antibodies to build a powerful engine for novel protein therapeutics. The systematic approach we've taken with J.DESIGN spans from the AI-driven design of J.HAL℠, our Just Humanoid Antibody Library used in discovery, to the design and operation of commercial manufacturing facilities that can be deployed to different geographies. We call these facilities J.POD®s. Normally placed in multiple functional silos in large biopharma organisations, we've connected the components of J.DESIGN through a common and curated data reservoir. The core context and use of these data are for the design, selection, development and manufacture of antibodies or antibody-like molecules. The Key Performance Indicators that drive continuous improvement of J.DESIGN are product quality, speed to the clinic, lower development costs, lower manufacturing costs and deployable facility design to catalyse global access to biologics.

What is Evotec's unique selling point with regard to the discovery and development of biotherapeutics?

Jim: Applying machine learning algorithms across this broad design space promotes efficient learning from the least expensive and most abundant data encoded in the DNA of antibodies, to validation of this learning through less abundant, more expensive, but most relevant data from GMP manufacturing at full commercial scale. This is a systems approach to platform definition and continuous improvement and it is unique in the industry, made possible by a number of factors that will be difficult for others to replicate.
While enormously powerful for the reasons mentioned, the J.DESIGN platform will be most valuable when coupled to high quality therapeutic biology, whether that's internally at Evotec or from external sources through partners. Central to our future success in biologics will be the building of efficient bridges between both internal and external biology and J.DISCOVERY™, as we engage J.DESIGN through selection of the best therapeutic candidates from J.HAL℠. With the right partners, we will efficiently move successful clinical candidates to the commercial market and help supply patients worldwide through a global J.POD® network.

Going forward – what is your vision, how will Evotec and Just Evotec Biologics complement each other?

Jim: In the future, when we couple J.DESIGN to predictive tools like Evotec's EVO*panOmics* and EVO*panHunter*, and fully harness Evotec's pre-clinical development engine represented by INDiGO, we will have a powerful and very fast biologics lane on the Evotec digital autobahn to cures.

Historically, Evotec is well-known as small molecule player, what was the rationale behind building antibody discovery capacities?

Karen: We have created fully integrated, data-driven research and development capabilities from project ideation to fully enabled IND drug candidates (EVO*iR&D*). Our approach incorporates key elements to successful projects such as translational models in major disease areas, such as oncology, neurology, infectious diseases, women's health, aging, inflammation, fibrosis, metabolic diseases, and biomarkers. It was a natural next step to expand beyond small molecules to incorporate multiple therapeutic modalities to align with our extensive target validation capabilities. Our aim is to deliver high quality drug candidates with ideal drug intervention strategies to make the greatest impact in treatment options for patients.

There are a lot of well-established antibody players, how does Evotec's offering fit in this quite crowded space?

Karen: It is very important to apply the appropriate technology to solve the specific needs of the drug intervention strategy. The reason so many technologies exist is because there is no one technology that fits all the needs to discover and develop a biologic. Evotec invested in and supports the most promising approaches that will likely work in a majority of the biological targets. However, there are additional techniques being applied in the industry that are fit-for-purpose in subsets of targets. For those, we support the projects through our platforms such as pharmacokinetics, toxicity, immunogenicity, therapeutic translational models, and biomarkers. By being a comprehensive EVO*iR&D* organisation, we are able to bring as many high quality drug candidates to the clinic in a variety of partnership models.

Looking into the future – what is your vision, how will Just – Evotec Biologics/Evotec's capabilities and capacities look like in 5 years?

Karen: The vision for the future is both exciting and attainable: deliver high quality biologics with comprehensive data packages to support clinical trials in patients in a lean process to ensure that the industry lives up to the promise of the investments made in the area. As you can see from the information shared in this DDup, many years of creative research have gone into the field – and we are at the stage of translating that into medicines that are affordable. Just a few years ago, biologics were considered high-end expensive medicines, accessible only to certain people. With the Just technologies in discovery and manufacturing, it is very real to bring this to many more diseases and smaller patient populations.

What are your USPs (or unique selling points) with regard to antibody discovery services?

Karen: As mentioned above, the USP for Evotec in this space is the capital efficient access to an autobahn of technologies that allows for a broad range of partnership options: from end-to-end discovery of the biologic agent to manufacturing with all of the necessary characterisations, translational models, and toxicity evaluations to support the project, to opportunities to supplement with just the components needed to drive a partner's project to success.

CHAPTER 06

4 QUESTIONS

DR BARBARA BACHLER-KONETZKI

SHORT SUMMARY OF SCIENTIFIC CAREER

Barbara received her PhD in Biomedicine and Biotechnology from the University of Veterinary Medicine in Vienna, Austria in collaboration with the Harvard Medical School / Dana-Farber Cancer Institute in Boston, USA where she focused on the profiling of protective anti-HIV antibody responses upon vaccination. After moving back to Europe, she continued to work on antibodies at MorphoSys, a biotech company based in Munich, Germany, well-known for the discovery and development of therapeutic antibodies using human antibody libraries by applying the *in silico* phage display technology. During this time, Barbara successfully discovered a novel acting therapeutic antibody, and progressed it all the way from concept to pre-clinical candidate nomination. In 2021, this molecule entered clinical phase 1 testing in immune oncology. Joining Evotec in 2018, she played a key role in the establishment of the streamlined hybridoma platform and the implementation of the ATX-Gx™ mice from Alloy as source of fully human antibodies. She is now leading a group of scientists and technicians supporting internal and external antibody programmes from target identification to lead candidate nomination.

1 What were the limitations of previous hybridoma-based approaches?

Although the development of the hybridoma technology was a landmark event, the methodology still faced the limitation of generating mouse monoclonal antibodies, which are suboptimal as therapeutic agents due to rejection events caused by the human immune system. For example, while OKT3, the first therapeutic mAb against T-cell expressed CD3 has proven effective for preventing host-versus-graft disease, the antibody itself elicited an immune response that resulted in accelerated drug clearance. Later it turned out that this unwanted feature was induced by the non-human sequences of the murine OKT3 antibody. To overcome this problem, researchers developed techniques to transform rodent antibodies into structures that would more closely mimic human antibodies, without the loss of binding or functional properties. First, mouse monoclonal binding sites were combined with human antibody sequences to create so-called chimeric or humanised antibodies. Later, these strategies were even further expanded to create genetically engineered mice to produce antibodies with human sequences, allowing the immunisation of mice with fully-human immune systems (such as the ATX-Gx™ platform from Alloy Therapeutics) and therefore selection of therapeutically well-suited antibodies.

2 What is so special about the ATX-Gx™ mice and how will this collaboration improve the offering for Evotec's clients?

Alloy Therapeutics is a biotechnology company allowing other companies access to their tools, technologies, and services empowering the global scientific community to make better medicines together. The ATX-Gx™ platform comprises a suite of highly immunocompetent transgenic mouse strains that allow the selection of fully human antibodies upon immunisation with an antigen of choice. For me as a scientist, it was very important that their existing technologies are continuously being improved. As Alloy Therapeutics is re-investing all of their revenue in innovation, I am convinced that this will allow them to grow and expand their human therapeutic antibody discovery platforms going forward. As a matter of fact, they are already working on additional mouse strains, for example the ATX-HyperImmune strain to allow generation of human antibodies against high-homology targets or their ATX-CLC platform, a suite of common light chain mice that allow better and faster bispecific antibody discovery.
The technology partnership between Evotec and Alloy Therapeutics has expanded our existing antibody discovery capabilities, offering a comprehensive service with both *in vivo* and *in vitro* technologies. Our clients and collaboration partners will have access to this cutting-edge mouse platform and thereby will enable best-in-class *in vivo* discovery of fully human monoclonal antibodies across many different disease areas.

3 What is so special about Evotec's integrated antibody discovery and development approach?

In my experience, companies are usually focusing on the discovery OR the development of novel antibody drug molecules. In the past, companies have successfully developed great antibody libraries, they came up with better ideas of how to efficiently produce mAbs or they were very good in understanding the molecules' pharmacological behaviour in a certain indication of interest. However, there are not many success stories of where all of these capabilities can be combined under one roof. Three years after joining Evotec, I am still impressed with the breadth of possibilities and the expertise one company can offer to clients or collaboration partners as a contract research organisation. For example, we are currently seeing many virtual companies that approach us to support their highly valuable programme from target validation up to IND and even market access. They trust our knowledge in drug discovery and hand over their precious ideas to turn them into valuable drugs. For integrated programmes that may run over several years, Evotec assembles a project-dedicated team of scientists that supports all of the required activities needed to fully understand the target biology, the mode-of-action needed and the intrinsic features of the generated lead molecules. This allows us scientists and project leaders to build a trustworthy relationship with the client or collaboration partner, and feel closely connected to the programme. I am convinced that this one-stop-shop idea increases the flexibility of the programme and in the end the quality of the resulting product.

4 Tell us about your vision – what do you expect from therapeutic antibodies in the future?

For me the recent COVID-19 pandemic made it clear how advanced research and development has become in the context of therapeutic antibodies. Anti-SARS-CoV-2 antibodies have been selected from convalescent patient B cells, screened for their broadly neutralising behaviour, and manufactured for therapeutic application basically in real-time. As a community, we have been getting better, faster and more cost-effective in all of the antibody-relevant technologies, no matter if you look at the advances within the discovery or the manufacturing of biologics. I strongly believe, that with the combination of best-in-class antibody technologies, biomarker discovery, and the support by AI-methods, we will be able to design the next generation of antibody drugs, which will offer even better treatment options and hopefully make a huge difference for patients in the future. I am proud to be part of this journey and excited to see how our integrated offering will transform the discovery and development process of therapeutic antibodies in the near future.

CHAPTER 06

4 QUESTIONS

DR RANDAL R. KETCHEM

SHORT SUMMARY OF SCIENTIFIC CAREER

Randy received his PhD in Molecular Biophysics from The Florida State University, focusing on experimental protein structure determination, resulting in the first membrane-bound protein structure solved by Solid State NMR. As part of this effort he developed computational methods for the structure calculation and refinement of membrane-bound proteins. Randy continued his training in structural biology in a postdoctoral fellowship at The Scripps Research Institute where he expanded his experimental and computational efforts into solution-based protein structure. Randy then joined Immunex in 1997 to apply his expertise in protein structure modelling and analysis to efforts in the development of biologic-based therapeutics. During his many years of experience at Immunex, Amgen, and Just Biotherapeutics he has led efforts to engineer protein therapeutics, invented novel therapeutic modalities and computational methods for therapeutic design, and developed computational and experimental approaches to understanding and controlling biophysical properties of biological macromolecules. Randy has made significant contributions in antibody design, epitope mapping, molecular assessment, stability engineering, construct design, protein structure analysis, and protein engineering. At Just – Evotec Biologics, Randy leads the Molecular Design team, integrating biologics discovery and molecular design strategies into the entire therapeutic pipeline.

1 How does optimisation of antibodies improve development?

Just – Evotec Biologics has developed a molecular optimisation platform for monoclonal antibodies to increase productivity, eliminate potential degradative modifications, and decrease inherent instability that may manifest during processing or long-term storage as aggregation, precipitation, viscosity, and chemical instability. This optimisation platform has been proven to decrease process development time and significantly improve yields, while reducing the potential need to re-engineer a late-stage molecule due to sequence issues. Molecular optimisation begins with evaluation of the antibody sequence and molecular structure using Just's proprietary Abacus™ *in silico* design suite. Abacus™ evaluates stability, germline background and pairing, potential post-translational modifications (PTM), missing or inserted residue errors, potential immunogenicity, and can drive the engineering modifications necessary to repair or modify antibody sequences. Abacus™ also drives germline switching applicable to both improving germline pairings and to humanisation by understanding residue positions required for core fold stability as well as potential antigen interaction. Characterisation of recombinant material using high-throughput biochemical, biophysical analytical, and mass spec tools are also used to help guide an understanding of molecular behaviour and can be used to guide optimisation designs.

2 What is the limitation of *in vivo*-derived antibodies?

In vivo-derived antibodies are matured from germline through somatic hypermutation. While this process allows for the generation of a very large range of antibody sequence space, which in turn allows for tremendous levels of antibody specificity and activity, hypermutation can also introduce mutations that lead to poor stability. This stability decrease can manifest itself in multiple ways, such as poor folding stability, leading to poor expression, increased aggregation, increased particulation, decreased resistance to a large range of pH exposure or agitation, decreased thermal stability, and other issues. We have developed a machine-learned neural network method, RANDAb (Residual Artificial Network for the Design of Antibodies), to evaluate the sequences for fitness and potential engineering to optimise *in vivo*-derived antibodies. Another issue is epitope biasing in which the B-cell response is focused on a dominant epitope. This leads to a loss of therapeutic diversity that a library screen could overcome.
RANDAb uses a deep learning approach to capture higher-order interactions between every residue in an antibody sequence to model pairwise interactions. Using a model architecture and training procedure inspired by the latest research in the field of natural language processing, the core model of RANDAb encodes information about the properties of amino acids and the structure of antibodies, learned from millions of curated, matured human antibody sequences. RANDAb uses this model to assign probabilities to each amino acid in an antibody sequence and provides suggested mutations where these probabilities are low. These low probability residues represent places in an antibody sequence where the current amino acid is predicted to be a bad fit with the surrounding residues, offering attractive targets for molecular optimisation. The RANDAb method is fully automated for detection and residue replacement suggestion. Optimisation positions from RANDAb, structure evaluation, surface properties, and post-translational modifications then involve expert evaluation and structure-based protein engineering design to produce a set of potential combinatorial variants in an effort to improve antibody properties. Once optimisation positions for both stability and post-translational modifications are identified they are used within Abacus™ to build combinatorial variants to optimise the sequence for therapeutic application while simultaneously maintaining function. This often may be accomplished in a single round of engineering and subsequent testing.

3 What were the limitations of previous library approaches and what makes J.HAL℠ so special?

J.HAL℠ is by design representative of a human response, but with the added ability to bias toward specific efficacy and developability characteristics such as HC-CDR3 length, surface properties, stability, avoidance of determinantal post-translational modifications, and so on. Historically, libraries were built using random mutagenesis, resulting in most of the library being unusable due to clipping, stop codons, and un-manufacturable antibodies. More modern libraries generally utilise germline frameworks with CDR diversity. While this approach improves the library quality, it does not represent a human response with proper diversity in full somatic hypermutation. Some libraries further use positional frequency analysis to generate artificial CDR diversity which does not represent a multi-dimensional human sequence response. By using the J.HAL℠ GAN methodology we are able to generate clean sequences with true human representation while being able to bias toward desired properties.

4 Tell us about your vision – what do you expect from therapeutic antibodies in the future?

The use of J.HAL℠ not only results in a robust discovery platform, but also gives the ability to explore antibody biology in a hypothesis-driven manner. For example, Fv-directed mechanisms which impact pharmacokinetics, tissue interactions, clearance rates, degradation, titre, aggregation, particulation, intracellularisation, blood-brain barrier passage, and more. Coupling an understanding and purposeful engineering of these properties with multi-specific capabilities, such our J.HAL℠ expansion into VHH domains, will allow for both target and epitope-specific therapeutic modality designs with engineered biological functionality.

For any further questions on Evotec's antibody discovery platform, please contact:

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