

Early Development of Antibody Therapeutics: key aspects of advancing an antibody drug from research to clinic

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Introduction

Depending on the companies and their decision processes, Early Development can be defined as the timeframe of a candidate drug from Research to Proof-of-Concept in human (typically end of Phase II). This article intends to focus on activities in early development that translates a research molecule into clinical development candidate. By limiting the final deliverable as a successful submission of Investigational New Drug (IND) application, I hope to highlight key aspects of advancing an antibody drug from research to clinic, including:

- Generate data in research to support the molecule moving forward
- Support indication selection and dose regimen selection for clinical studies
- Identify and generate data needed for regulatory filing (IND with the FDA in the United States or EMEA in Europe)
- Understand the cost and maximize the value of a therapeutic antibody

Early Stage Research

The decision to pursue an antibody (versus a small molecule) as therapeutics lies in research, because the target for intervention will, to a large extent, influence the modality of the therapeutics. Antibody therapeutics typically target cell surface molecules, such as receptors or antigens expressed in tumor cells, host immune cells, or other host cells (for viral infection). Research owns the decision to intervene a disease pathway with certain pharmaceutical modalities.

At the research stage, the need for antibody capable of addressing two or more targets may also be assessed, leading to engineering requirement for bi-specific or dual-acting antibodies. Typical activities in early stage research are as following:

Target Assessment and Validation

Like small molecule drug discovery and development, the first step is to define the molecular target and validate the target. This can be achieved through mouse genetics, through expression patterns in human primary tumor or primary cells, as well as by other means (more details below). Typically a monoclonal antibody recognizing the mouse ortholog of the intended human protein is obtained as a surrogate antibody to facilitate target validation. Overall, research biologists define the role of the target in disease

processes, factoring into consideration if the target is already validated in the clinic; generate research reagents: surrogate antibodies, transgenic animals, and cell lines; and understand the biology using knockout, transgenic animal or RNAi techniques. Intellectual property evaluation should be considered at this early stage, too. The criteria for a sufficiently validated target vary by company.

Lead Discovery

Lead discovery in antibody therapeutics is quite different from traditional small molecule lead discovery. Granted, one needs to set up screening assays. The screening library here, however, consists of cDNA or proteins instead of small molecular chemicals. Biology researchers generate proof-of-principle efficacy data in relevant *in vitro*/*in vivo* model with surrogate agent, select appropriate molecules or isotype (e.g. IgG1, IgG2, IgG4, receptor-IgG, etc.), conduct initial pharmacokinetics (PK)/tissue distribution studies with the research antibody to confirm suitability for *in vivo* use and to estimate preliminary dose regimen(s), and consider plan for PK/PD (pharmacodynamics) assay for analysis of preclinical studies.

Indication Searching

Indication searching at this early stage takes into consideration of the target biology and its expression in human normal or cancer tissues, or human peripheral blood lymphocytes (PBLs).

Late Stage Research

Once the target is sufficiently validated, a decision is made to generate human or humanized antibody. Such decision triggers the transition of a molecule from Early Stage Research to Late Stage Research. Typical activities in late stage research are as following:

Lead Optimization

Lead optimization with antibody therapeutics includes antibody production/humanization, characterization of biochemical properties, characterization of cell biological properties *in vitro*, and *in vivo* pharmacology.

Humanization or fully human antibodies are thought to reduce the immunogenicity of antibody therapeutics that could lead to anti-therapeutic antibody (ATA). Antibody engineering experts can achieve humanization of the mouse monoclonal antibody, screen phage libraries for

fully human antibodies, or generate fully human antibodies using mice genetically engineered to carry the germ line loci for human IgG.

During lead optimization, antibody characterization goes hand-in-hand with antibody production, either via transient expression or via stable cell lines. This is because characterization studies require initial material. In return, once a lead molecule emerges from a panel of well characterized humanized or human antibodies, it calls for a larger supply for *in vivo* studies.

Characterization of the antibody candidates is a critical step in prioritizing the candidate and eventually producing a lead. Biochemical properties to characterize include affinity, specificity, selectivity and the epitope. Cell biological and other *in vitro* characterizations include potency, antigen density of target, internalization, ADCC (Antibody-Dependent Cell-Mediated Cytotoxicity) and CDC (Complement-Dependent Cytotoxicity). Many of these assays may be adopted later as initial analytical characterization of lead molecule with Process Development. This is an area where intensive collaboration takes place between Research and Process Development, or between the future Research subteam and CMC subteam (CMC stands for Chemistry, Manufacturing, and Controls).

In vivo pharmacology at the late stage research phase includes proof-of-concept efficacy, mechanism of action, pharmacokinetics (PK)/pharmacodynamics (PD), and preliminary toxicological studies. The following activities involve close collaboration between Research and Pharmacology, the latter being typically a division in the Development organization.

- Develop PK assays for antibody
- Conduct PK studies in rodent/cynomolgus monkey
- Identify potential predictive and PD marker
- Supply a list of safety considerations to assist in toxicological study design
- Characterize species cross-reactivity profile to enable *in vivo* efficacy and toxicology models
- Conduct preliminary toxicological studies (single dose, short term)
- Study normal human tissue expression of target (pilot study)
- Establish reproducible, dose dependent efficacy at pharmaceutically relevant doses
- Establish efficacy in more than 1 relevant disease model (if available)

Indication Prioritization

Indication prioritization serves the purpose of defining target relevance and clinical relevance in patient populations, and identifying potential predictive markers to support clinical indications. Typically, indication prioritization utilizes data from in vivo efficacy models, including disease or tumor xenograft models (with or without transgenic or knockout mice), expression in diseased tissues, blood/serum/PBLs, pathway activity in diseased tissues, genetic/mutational analysis in diseased tissues, and initial diagnostic strategy to identify which known biomarkers might be informative and/or devise a preliminary research plan to discover new biomarkers.

Target Candidate Profile (TCP)

Specific requirements for certain properties of antibodies may be captured by a Target Candidate Profile (TCP). The parameters or specs for the lead candidate of antibody therapeutics are defined by a team of experts in areas including Research, Pharmacology, Clinical Sciences, Regulatory, and Commercial. Included in the TCP are parameters governing the affinity of antibody to its target, requirement for the effector function (or lack of), half-life compatible with once a month or once a quarter injection, and the following:

- Target
- Scientific Rationale
- Indication
- Route of administration
- Efficacy
- Dose and schedule
- Combination treatment
- Safety/toxicology
- Clinical pharmacology
- Freedom to operate
- Competition

Development

To limit the scope of this article, I will discuss development-related activities leading to IND-filing only. These are conducted mainly in the functional areas of Preclinical Development, Process Development and Manufacturing, and Clinical Development, under the organization of the Early Development Team and the leadership of EDTL (Early Development Team Leader).

Preclinical Development

Non-IND Enabling Studies

Non-IND enabling studies for lead characterization and optimization may be carried out in late stage research phase or in early part of the development (considering a development candidate is declared by now). Typically pharmacokinetics (PK), pharmacodynamics (PD) and assay groups in the Development organization (instead of Research organization) are responsible to:

- Complete predictive marker and PD assay development
- Complete a pilot PK/PD study in one primate species (primates are used because a therapeutic antibody cross-reacts more readily with the same antigen in primates than in other mammals; here as well as in toxicology studies, cynomolgus monkey is preferred over other monkey species because of its smaller size requiring less drug for study)
- Finalize PD plans for Phase I
- Develop relevant PK/Ab/potency assays by leveraging the know-how from Research
- Optimize and run relevant PK/PD assays for pre-clinical and clinical studies
- Determine relevant tissue biodistribution as a function of dose; correlate with efficacy
- Conduct preclinical combination therapy with standard of care drugs as needed

IND Enabling Studies and Activities

As part of the IND planning, Early Development team will consider studies to be conducted by the standard of Good Laboratory Practice (GLP) and included in the non-clinical section of IND, such as:

- GLP normal tissue cross-reactivity
- Monkey PK/PD study for guiding dose and regimen selection
- GLP Toxicology studies - dictated by clinical program design
- Hemolytic potential
- Plasma stability, etc.

Other activities to support IND filing and Phase I clinical trial include:

- Documentation of key research data to be used for IND filing

- Transfer potency assays to Process Development
- Confirm manufacturing feasibility
- Complete PK, PD and Immunogenicity assay development & validation for monkeys to support IND-enabling primate studies
- Complete PK, PD and Immunogenicity assay development for humans to support Phase I studies
- Establish viable therapeutic window including projected human dosing using the IND-enabling tox study and PK/PD study results

The IND-enabling toxicology study is usually on the critical path to IND filing. Meanwhile, initiation of the IND-enabling tox study is dependent on availability of material for such a study from the CMC (Chemistry, Manufacture and Controls) team in the Process Development and Manufacturing functions.

Process Development and Manufacturing

Process Development is the bridge between Research and Manufacturing, working in parallel with Development. Research discovers genes and provides DNA sequences (such as those for the selected monoclonal antibody therapeutics). Process Development starts with plasmid construction for high yield expression in appropriate host cells (*E. coli* or mammalian cells). They take care of transfection and cell cloning. They develop stable cell lines and appropriate media for cell culture. Recovery group in Process Development handles Harvest (including homogenization, centrifugation and filtration), Purification (including chromatography, characterization, validation and viral removal), and Analyses (including developing assays, determining structure and testing for activity). Formulation experts are called for to study stability, excipients, degradation and delivery. Finally, Quality and Scale-Up groups in Process Development are to ensure consistency, reproducibility, manufacturability and cost saving in manufacturing.

The main activities for the CMC subteam that oversees Process Development and Manufacturing are as following:

- Set initial process development, formulation, stability, QC specifications
- Bank master cell line
- Manufacture and characterize tox lots, Phase I material, and surrogate molecule (Placebo manufacturing may also be required for Phase I in non-oncology indications)
- Develop potency test (via collaboration with Research and Assay group in Preclinical) and validate it in time to manufacture Phase I material
- Provide stability data for Drug Substance and Drug Product
- Generate Certificate of Analysis (CofA) for Phase I materials (Certificate of Test or CofT is usually adequate for material used in GLP tox studies)
- Develop relevant sections in IND for IND filing
- Release product to clinic to start trial

Clinical Development

Prior to getting to human, the main responsibility for Clinical Development is to collaborate with Research and Pharmacology in planning for the trial. The typical deliverables from clinical scientists (medical training required) and clinical operations are:

- Indication selection
- Clinical Development Plan, with more details on the Phase I study design, including intended clinical route of administration (for antibodies, typically intravenous or subcutaneous), dosing frequency and anticipated clinical doses or exposure. The final clinical dose regimen will be determined or influenced by data from IND-enabling toxicology studies with a “dose multiple” safety factor
- Phase I trial site selection and contract negotiation
- Phase I trial budget and timelines
- Phase I protocol synopsis
- Final protocol and Investigator’s Brochure (IB)

Strategic Planning

Strategic planning is a key cross-functional activity at Early Development, particularly of therapeutic antibodies. It may be done in a streamlined fashion, perhaps involving 1-2 consultants if the company is small and does not have necessary expertise, for example, in the Commercial arena. However, it is best done in a rigorous way to determine the feasibility of developing a selected molecule for stated indication(s) based on scientific rationale, commercial viability, internal and external competitive landscape, and feasibility of developing, manufacturing and gaining approval in a manner that adds value to the pipeline and benefits patients by addressing unmet medical needs. It also outlines key uncertainties/risks that need to be addressed and recommends risk mitigation plan, including specifying decision-making gates and criteria to ensure high quality decision-making in the future.

The reason that strategic planning is critical for antibody therapeutics is because developing antibody therapeutics demands huge upfront cost, mostly concentrated in process development and manufacturing. It is estimated that fully burdened development cost, including process development and manufacturing, from a molecule exiting Research (i.e. declared as the lead antibody) to reaching the IND filing, is in the range of \$20-30 M. Thoughtful planning and good understanding of resource commitment and return on investment are critical.

A key deliverable of strategic planning (sometimes called Development Assessment) is the Target Product Profile (TPP). TPP provides a clear description of the attributes necessary to produce a clinically meaningful and successful product. It serves as a decision-making tool to allow the team to assess the likelihood that continued investment in the molecule will result in an impactful product. It aligns teams, management and the collaboration partners around desired product features. It serves as a contract for what the team plans to deliver to the organization and the market. Last but not least, it guides the content of the preliminary package insert that then serves as the bases of negotiations with the FDA on the content of the package insert at license application.

The TPP describes as objectively as possible the critical attributes and expected performance of a target product which best balances: 1) the current scientific knowledge of the molecule, 2) properties required for the molecule to be a commercially attractive product, and 3) demonstration of safety and efficacy that will support successful license applications to regulatory agencies. The TPP is not a description of current reality—it should describe the safety and efficacy, pharmacokinetics and other bars that the intended drug is supposed to meet in forthcoming studies (especially Phase III pivotal trials) in order to be considered a worthy product at the anticipated time of entry into the market.

Additional activities as listed below may be called for to facilitate strategic planning, such as:

- Overall timeline with clearly identified milestones (typically it takes 12-18 months to move the antibody molecule from Research to Clinic)
- Overall cost from exiting Research to filing IND, possibly even to product launch, with cost breakdown by phase of trials and by functions/departments
- Market research using draft TPP if necessary
- Commercial opportunity evaluation, including possibly revenue projection from launch to peak year
- Complete clinical development plan
- PTS (probability of technical success) estimate

Value proposition for molecule (including risk/return ratio, productivity metrics, etc.)

IND filing

An Investigational New Drug Application (IND) is a request for authorization from the Food and Drug Administration (FDA) in the US or its equivalent in other countries to administer an investigational drug or biological product to humans. Such authorization must be secured prior to interstate shipment and administration of any new drug or biological product that is not the subject of an approved New Drug Application (NDA) or Biologics/Product License Application (BLA/PLA).

From a broader sense, the IND content includes the following:

- Animal pharmacology and toxicology studies--Pre-clinical data to permit an assessment as to whether the product is reasonably safe for initial testing in humans. Also included are any previous experiences with the drug in humans (often applications for use in foreign countries or for additional indications).
- Manufacturing Information--information pertaining to the composition, manufacturer, stability, and controls used for manufacturing the drug substance and the drug product. This information is assessed to ensure that the company can adequately produce and supply consistent batches of the drug.
- Clinical Protocols and Investigator Information -- detailed protocols for proposed clinical studies to assess whether initial-phase trials will expose subjects to unnecessary risks. Also, information on the qualifications of clinical investigators--professionals (generally physicians) who oversee the administration of the experimental drug—to assess whether they are qualified to fulfill their clinical trial duties. Finally, commitments to obtain informed consent from the research subjects, to obtain a review of the study by an institutional review board (IRB), and to adhere to the investigational new drug regulations.

For more Information on Submitting an Investigational New Drug Application, please visit the official Web site of the FDA:

<http://www.fda.gov/BiologicsBloodVaccines/DevelopmentApprovalProcess/InvestigationalNewDrugINDorDeviceExemptionIDEPProcess/ucm094309.htm>

Summary

Advancing a therapeutic antibody from research to clinic requires close collaborations among Research, Development (including Non-clinical, Clinical and Regulatory), Manufacturing and Commercial, as well as many other functions. Compared with small molecules in the transition from research to clinic, attributes affecting manufacturability, dose and administration are uniquely at the center of early development of antibody therapeutics. Therefore efforts in early development need to characterize these attributes and have plans to address these issues before moving to clinic. In summary, success in early development of antibody therapeutics is measured by the following:

- Analytically characterized lead molecule with proposed backup if needed
- Feasible manufacturing process
- Lead indication(s)
- Viable therapeutic window including projected human dosing
- Target Product Profile, phase I study design, and diagnostic plan (if needed)
- High level understanding of the overall development path and cost
- IND filing and initiation of first-in-human trial



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