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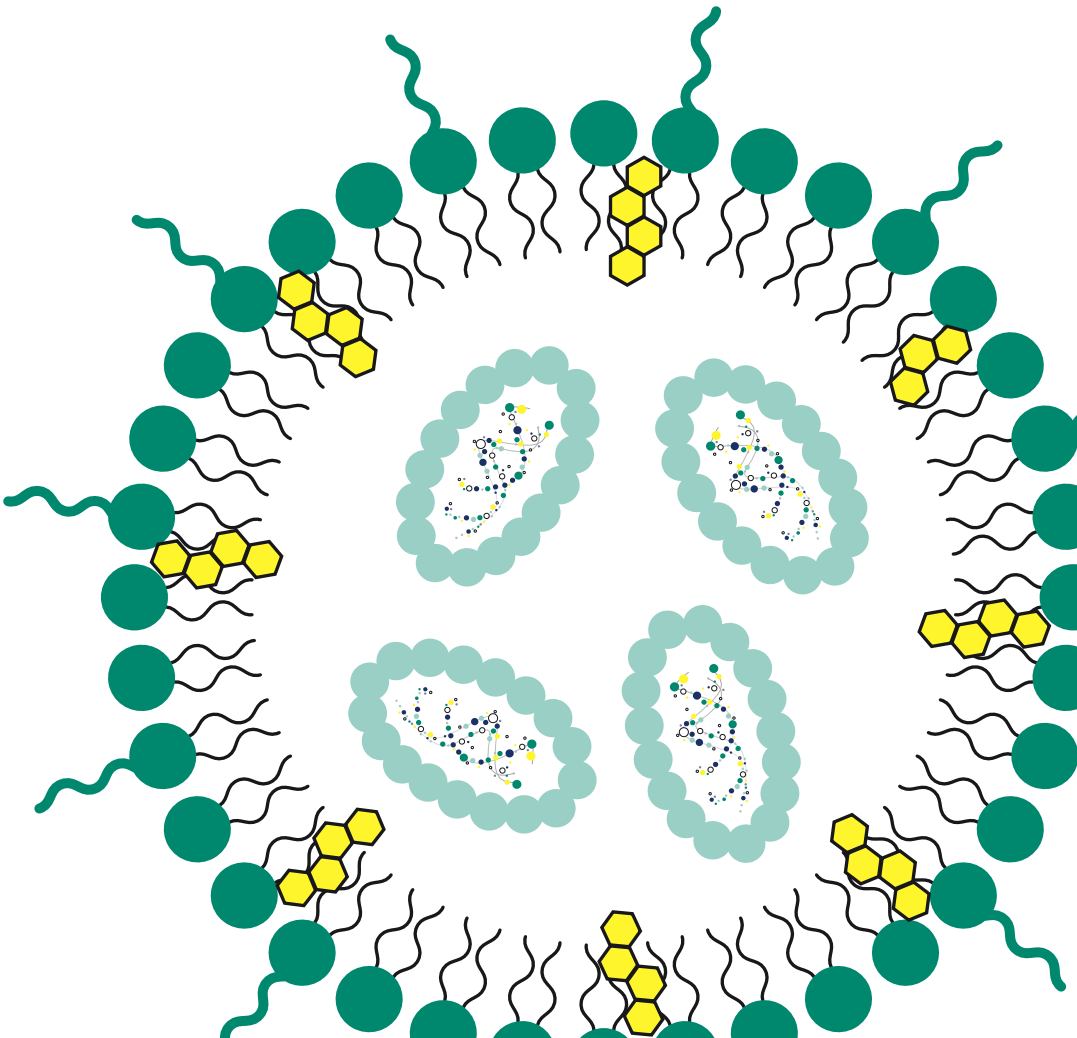
Recommendations for successful IND approval of RNA-LNP drugs

Before conducting clinical trials on cutting-edge novel medicines, pharmaceutical companies must file extensive regulatory documentation. This process takes rigorous work and planning, so submitting an Investigational New Drug (IND) application to the FDA represents a significant milestone in an RNA therapeutic's journey to patients.



IND applications overview

INDs are required before conducting clinical studies for:

- Unapproved new molecular entities
- Approved drug uses different than the approved labeling, such as:
  - New dosage
  - New indication
  - New age group

The IND application process must be meticulous. If you don't adhere to a regulatory body's stringent rules or fail to gather and report the necessary chemical, manufacturing and control (CMC) data and process specifics, it may result in a clinical hold. For successful IND submissions, studies evaluating the toxic effects of the new drug at various doses over specific periods are essential. The data from these studies will determine a safe starting dose and the range of doses that can be used in clinical trials, while also helping to establish key study endpoints and metrics to be measured and analyzed for both safety and efficacy.

Once an IND application has been submitted, an expert from the FDA will review it to assess if all the following key questions have been addressed prior to bringing a nanomedicine to the clinic:

- Has the drug product been characterized?
- Are there adequate specifications and test methods?
- Has the impurity profile been defined?
- Is there data that supports product stability through the clinical study?
- Is it reasonably safe to study this investigational drug in humans?
- Do the benefits of the investigational drug outweigh the risks?

The IND review focuses on establishing quality standards and checking critical pharmaceutical quality attributes including chemistry, formulation, stability, bioavailability, manufacturing process and product performance. In addition, there's an emphasis on quality by design and safety and efficacy. Thus, adhering to regulatory guidelines and workflows that produce successful, predictable outcomes are elements of good practice for drug development.

Generally, the average time to develop and approve a typical drug product is 10 to 12 years. However, nanomedicine-based RNA therapeutics can be designed and synthesized rapidly for clinical tests as soon as the chemical structure of the RNA and the delivery methods are established. This is why early developers of COVID-19 vaccines were able to design mRNA sequences, apply them in animal experiments, and conduct clinical trials quickly before releasing them to the market for emergency use in less than a year. The companies developing and producing these vaccines also leveraged good practices and strong collaboration with regulatory agencies to move through the process efficiently.

The existing regulatory system, however, is ambiguous for this new and rapidly evolving class of medicines. It's therefore crucial that nanomedicine developers stay ahead of RNA therapeutics' rapid pace and prepare for regulatory filing early.

Preclinical drug development workflow and IND guidelines

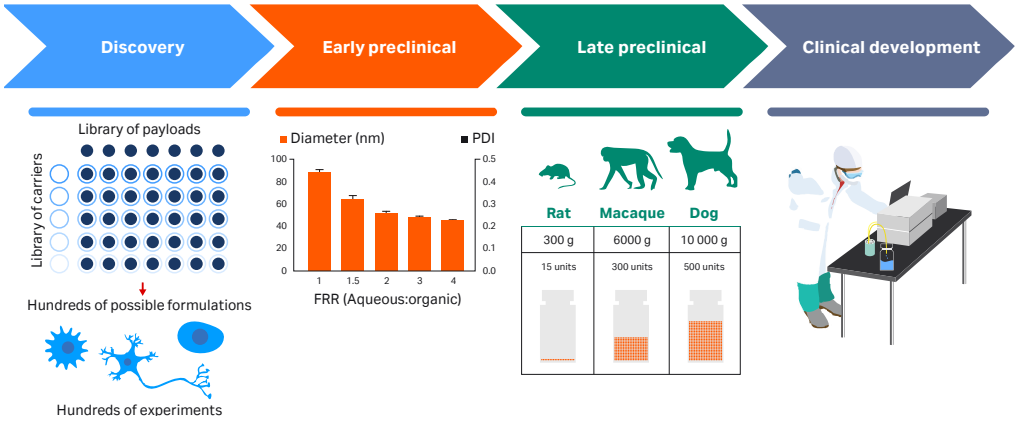


Fig 1. Drug development stages (FRR = flow rate ratio; PDI = polydispersity index).

Prior to submitting an Investigational New Drug (IND) Application to the FDA, there are three key stages in the drug development process: drug discovery, early preclinical, and late preclinical. To build a comprehensive submission package, it's vital to understand each stage and its purpose in regulatory approvals.

For a successful IND filing, it can be wise to scale and validate production processes during the development phase. The FDA expects to see a stepwise approach to IND review, and tightening the acceptability of testing for the following processes while moving from the discovery phase to clinical is key:

- Product characterization and stability
- Analytical and process validation
- Good manufacturing practices

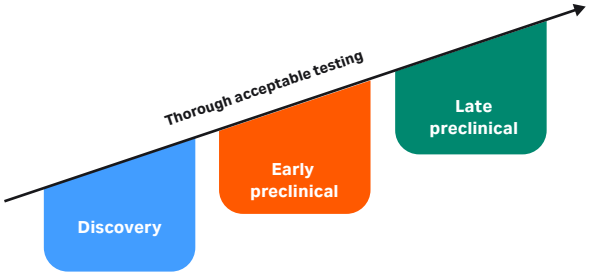
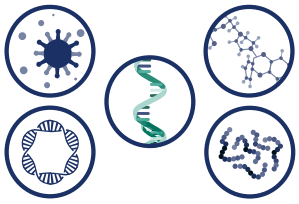
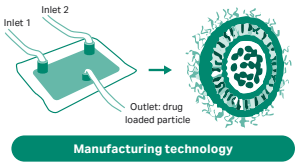


Fig 2. Stepwise approach for IND filing.

Test and data quality are essential and should align to the development stage at which FDA regulations require documentation. On the following pages is an overview of how the information must be presented in the IND filing application at various drug development stages for RNA therapeutics.

Overview of data requirements for IND application

Information for an IND application	Drug development stage		
	Discovery phase	**Early preclinical**	**Late preclinical**
Physical, chemical, and biologic description 	Description of active ingredient	**a.** Details on nucleic acid sequences and side-chain modifications **b.** Mechanistic description of the lipid nanoparticle modality	Specific information, including particle size, encapsulation efficiency, and biologic properties
Method of preparation 	Brief description of the manufacturing process, reagents, and a flow diagram to present the information	Data to support increases in manufacturing capacity and how the process underwent scale-related changes	Details related to process changes, scale changes, and if subsequent unit operations remained consistent at all scales
Acceptable limits and analytical methods to verify quality 	Brief description of the tests and specifications that inform analytical methods, along with their validation and acceptable limits for release testing and stability monitoring	**a.** Data representing analytical comparability assessment by release, comprehensive characterization, and stability testing **b.** Process performance comparability assessment by in-process controls (IPCs) and critical process parameters (CPPs) evaluated against expected ranges or proven acceptance ranges (PARs) **c.** Data to confirm that the analytical methods were developed concurrently with process development	**a.** All release results obtained for process performance qualification (PPQ) batches manufactured using commercial-scale process **b.** All extended analytical characterization results conformed to the expected comparability range **c.** Data supporting that the manufacturing process parameters and quality attributes were comparable across the manufacturing scales
Stability data 	**a.** A series of tests designed and performed to obtain stability information to guide analytical procedures, shelf-life, and storage conditions **b.** Data representing raw material quality	**a.** Developmental assessment and reproductive toxicology **b.** Non-GLP repeat dose toxicity and immunogenicity study **c.** Other supportive toxicology studies **d.** Biodistribution study **e.** Clinical diagnostic assays used to support primary clinical efficacy endpoints	**a.** GLP toxicology studies in animal models that include clinical chemistry, pathology, hematology, ophthalmology measures of cardiac and pulmonary safety, urinalysis, bioanalysis, toxicokinetic analysis, and statistical analysis **b.** Non-clinical pharmacology study reports typically done in mice, rats, hamsters and non-human primates **c.** Updated stability and efficacy analyses data **d.** Stability data from multiple batches at accelerated scale stresses **e.** Immunologic mechanism that confers benefits of the drug **f.** Immunogenicity assays validating reports

FDA regulation allows flexibility in the volume and depth of data, but you should still document all research data and processes. With a strong understanding of chemistry, manufacturing, and controls along with early action and thorough preparation, it's possible to accelerate genomic medicine development and its approval, which requires early action and thorough preparation.

In addition, establishing partnerships with a leader in lipid nanoparticle (LNP) technologies to build and qualify manufacturing processes early allows scientists to easily scale from discovery to the clinic, as the end goal of commercialization is built into strategies. In Figure 3, we've provided guidance for gathering required data for IND filings of an investigational RNA-LNP drug at different stages of drug development.

Strategies for successful IND filing of RNA-LNP drugs

Start with the end in mind

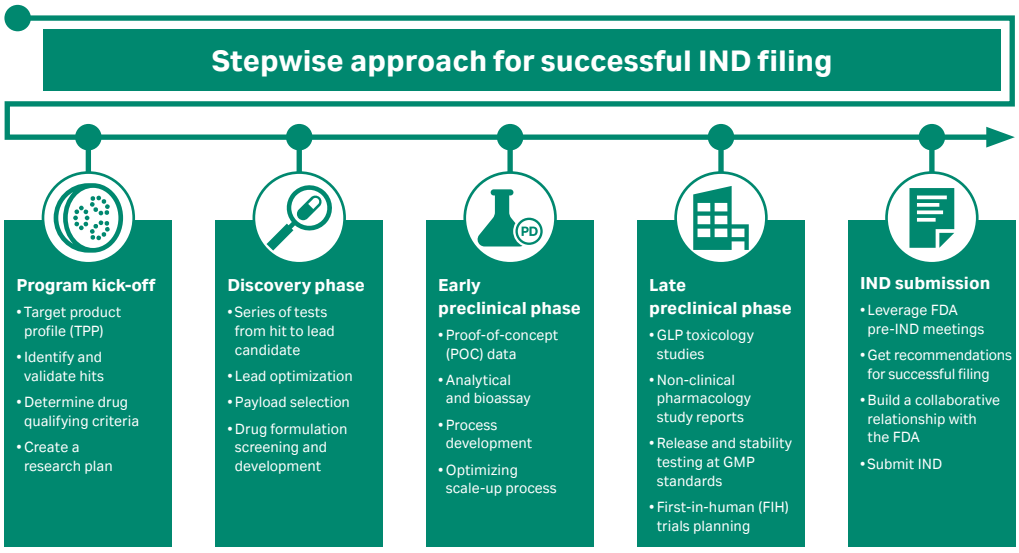


Fig 3. IND filing strategies across drug development stages.

Drug discovery

The drug discovery stage kick starts the validation of novel treatment modalities or formulations. Active pharmaceutical ingredients (APIs), drug delivery systems, and mechanistic understanding of target compounds are key elements to be addressed in this stage.

Rational and practical considerations in the drug formulation development process include a target product profile (TPP), with framework aligning with FDA and ICH Q8 (R2) guidelines for early-stage drug development. The TPP document is based on a drug's indications, targeted population, clinical efficacy, safety and tolerability, stability, route of administration, dosing frequency and cost, and development timelines. These key elements help identify a product's critical attributes prior to development and serve as a planning tool to facilitate discussions with regulatory agencies. The TPP also helps develop a business case for the characterized product. It can be adapted to incorporate new information or reflect significant product development changes. (3) Conducting efficient screening while preserving precious materials can be a delicate balance as scientists define an appropriate TPP.

Considerations to accelerate the drug discovery phase:

1. Access a well-characterized lipid nanoparticle portfolio to screen drug delivery formulations and assess the potency of RNA sequence, capping, and side chain optimizations.
2. Establish a method that is fit-for-purpose to quantify the potency of mRNA delivery and the subsequent translation and protein expression.
3. Procure optimized LNP reagent mixes or expertise-driven custom compositions to achieve tunable biological outcomes.
4. Find speedy, consistent LNP preparation technology.
5. Strive for LNP production with near complete sample recovery, avoiding raw material waste.

Preparing for successful manufacturing and scale-up:

1. Leverage a lipid library to license the same successful formulations found in discovery for clinical stages.
2. Develop reliable and robust *in vitro* bioassays for accelerating early screening of LNP-mRNA formulations.
3. Perform tests to obtain stability data that support analytical procedures, shelf-life, and storage conditions.
4. Invest in scalable LNP preparation technologies, utilizing the same architecture from µL batches in discovery to L batches during manufacturing.
5. Incorporate easy-to-use automated instrument operations that reduce human error and minimize batch-to-batch variability.

Strategies for successful drug discovery

Access a well-characterized lipid nanoparticle portfolio, like the one offered by Cytiva, to rationally design LNP formulations for validation of APIs. Once validated, you can move toward generating proof-of-concept and efficacy data to progress lead candidates quickly through screening and *in vitro* preclinical studies.

It's also helpful to start operating mRNA-LNPs formulations on instruments that can eventually integrate into the full workflow. The NanoAssemblr™ Spark system is ideal for screening novel genetic medicine candidates as formulations are ready in seconds and can be made on-demand in a sterile hood for immediate cell culture application. LNPs formulated on the Spark system use NxGen™ mixing technology, which maintains the same architecture as the equipment scales to produce larger batches. NxGen technology achieves this unique scalability by preserving the critical conditions at the point of formulation regardless of whether the throughput is µL/min or L/hour.

Many key elements for IND filing can be identified and documented in the discovery phase:

- Intended clinical targets of the therapeutic, including disease, pathogen, and population.
- Description of the mRNA, including sequences, length, capping, and nucleotides, as well as the rationale for its selection, any proteins that are encoded, and their contributions to the proposed therapeutic mechanism of action.
- Mechanistic description of the mRNA mechanism of action (i.e., vaccine applications would include immune responses).
- Manufacturing processes for the mRNA.

Avoid common mistakes by implementing strategies that will set the stage for success, including:

- Schedule a pre-IND meeting with the FDA: The pre-IND meeting is optional but provides an opportunity to educate the FDA on the drug and address issues that might lead to a clinical hold or program delay. Carefully crafted questions and meeting packages can facilitate meaningful responses. In addition, the regulators can share helpful advice concerning safety parameters to evaluate and submit in the IND that can guide the rest of the regulatory strategy. This type of information exchange can help in building a collaborative relationship.
- Build a comprehensive preclinical program with IND filing in mind, and design preclinical studies that will support clinical trial enablement.
  - Identify clinical indications, type/duration of treatment, and study populations, and then plan translational preclinical studies.
  - Start researching to select an appropriate animal model for the disease.
    - Conduct literature searches to find similar models or mechanisms already published or in clinical trials
    - Review previously approved nanomedicine therapeutics
    - Consider species selection.
  - Prepare to fully characterize the formulation with robust analytics:
    - Stability
    - Purity
    - Consistency
  - Plan to establish safety/toxicology metrics.
    - Prepare a strategy to develop dose-response relationships with broad ranges.
    - Begin research to identify relevant pharmacokinetic/pharmacodynamic (PK/PD) models.
- Set realistic timelines and milestones. It's easy to underestimate the time and resources required to get to an IND.
  - Science comes first and cannot be rushed (i.e., plan an appropriate timeframe for biological responses and assays).
- Review similar development programs and prepare mitigation strategies.
- Understand that it's a multi-disciplinary effort to commercialize a new drug product. Consult and collaborate with experts to leverage expertise, and streamline the process both scientific and regulatory).

Early preclinical studies

This stage focuses on *in vivo* proof-of-concept studies, formulation development, and some refinement of the API. It involves systematically varying lipid compositions and process parameters and determining the impact on the physical characteristics of the drug and the biological response in a small (usually rodent) model. You'll also be asked to thoroughly characterize the drug substance, drug product properties for formulation, and development of a functional biological readout to establish a relationship between composition, process parameters (i.e., inputs), and biological outcomes (i.e., outputs).

Considerations to accelerate the early preclinical phase:

1. Achieve consistent and reproducible LNP production with quick formulation times at scales appropriate for small animal studies.
2. Access a well-characterized lipid nanoparticle portfolio to have a clear path to the clinic. It offers an efficient, systematic approach to optimize the lead candidate's physicochemical properties and biological activity.
3. Access LNP expertise for design of experiment (DoE) studies to optimize manufacturing parameters.
4. Access LNP formulation expertise for process development.
5. Invest in expertise in upstream and downstream processes.

Preparing for successful manufacturing and scale-up:

1. Evaluate and qualify of raw materials and process consumables.
2. Invest in scalable mixing technologies that minimize batch-to-batch variability and future process re-development at larger scales.
3. Integrate easy-to-use instruments for automated synthesis of genomic medicines with minimal setup and training.
4. Define clinically relevant parameters and processes at small volumes (critical quality attributes [CQAs] and critical process parameters) to develop scale-optimized formulations that provide consistent data.
5. Establish analytical methods to evaluate LNP formulation.

Strategies for successful early preclinical studies

To simplify clinical development and manufacturing transition, model unit operations and maintain process parameters when scaling. The NanoAssemblr Ignite and Ignite+ systems enable controlled and precise lipid nanoparticle assembly using the highly scalable NxGen™ microfluidic technology. As a result, LNP formulations can be systematically explored and fine-tuned to establish relationships between CPPs, physicochemical properties, and *in vivo* biological outcomes. This process identifies optimized lead formulations while defining acceptance criteria for CQAs and CPPs.

Collaboration can also be a powerful tool for leveraging technical knowledge and experience in payload design and lipid-based delivery systems. Modifications in the chemical structure and ratio of the LNP components affect transport efficiency, potency, and biodistribution, making it critical to optimize lipid nanoparticles to be fit-for-purpose. Access to a diverse portfolio of lipid nanoparticle compositions that includes novel ionizable lipids and systematic categorization maximizes the chance of finding a composition well suited for the therapeutic application of interest. Furthermore, access to this lipid library eliminates the resource-intensive activity of developing and manufacturing novel ionizable lipids and compositions, thus accelerating formulation optimization and development.

Cytiva instruments and biopharmaceutical services support developing proof-of-concept studies in early preclinical stages and assist in developing custom analytical methods for nanoparticle formulations and raw materials. Our team offers expertise in lead candidate optimization, process development, and scale-up.

Many key elements for IND filing can be identified and documented in the early preclinical phase:

- Description of the formulation and all LNP excipients, including the rationale for including any novel excipients, supported by preclinical study data.
- Characterization of excipients, including stability, structure, and analytical assessment.
- Mechanistic description of the LNP modality (i.e., biodistribution, cellular uptake, drug delivery).
- Method of production, including a description of analytics and quality control processes for formulation.

Implement strategies that will set the stage for success, including:

- Investing time in formulation development
  - Optimize formulations and excipients, so they are fit-for-purpose, with the best efficacy and safety profiles.
  - Empirically assess formulations through all manufacturing processes, both upstream and downstream.
  - Document all analytical considerations at each stage to define metrics and maintain quality.
- Planning for the challenges associated with scale-up
  - Reagents: Lipid synthesis, mRNA production, stability, storage
  - Manufacturing: Analytical and production methods to maintain consistency and quality, characterized in preclinical studies

Late preclinical development

This stage focuses on process development, optimization, validation studies, and narrowing down a lead candidate while considering the practical considerations of formulations, analytics, and sourcing for later stages and manufacturing. It's critical to know what data is required for IND filing and the timelines to generate that data to plan and execute validation studies that will address the reviewer's questions the first time.

In summary, this phase generates all the data required for a successful IND application submission to the FDA, commonly referred to as IND-enabling studies. The following key areas need to be addressed in an IND application: animal model pharmacology, pharmacokinetics, and toxicity investigation, as well as clinical trial protocols and manufacturing processes.

Considerations to accelerate the late preclinical phase:

1. Scale up lipid and RNA manufacturing, as well as LNP production and downstream processing.
2. Perform stability testing, as this information is vital for the regulatory approval of new medicine. Stability testing will generate data that provides information on how long a product will maintain its properties, the characteristics at the time of manufacture, and the effect of environmental factors on a formulation's purity, efficacy, and structure. Cytiva offers stability testing services that comply with the regulatory requirements for clinical trials.
3. Develop analytical methods that enable qualification of input and output materials.
4. Procure GMP-grade drug substance/reagents to manufacture a non-GMP batch as an engineering or GLP batch.
5. Work with a collaborator that has GMP-scale LNP formulation instruments to reduce process and formulation redevelopment when changing scales, compared to conventional batch-based methodologies.
6. Team up with organizations and teams that have expertise in clinical protocols and manufacturing.

Preparing for successful manufacturing and scale-up:

1. Accelerate timelines by performing large-scale formulations.
2. Optimize and document manufacturing processes for technology transfer to GMP manufacturing.
3. Complete engineering batches with validated analytical method.

Strategies for successful late preclinical studies

Testing is exhaustive and continuous, beginning with screening, optimizing formulations, and pre-clinical evaluation for critical quality attributes, continuing throughout the clinical development phase and including post-approval. Although animal toxicology studies need to be carefully performed to generate relevant data and minimize safety risks in human subjects, testing is important at each stage, including CMC. It's important to carry out the necessary and relevant tests and keep records (manually or through instrument recording) that verify all required sampling, inspecting, and testing procedures. You should also be sure to, maintain records and investigate deviations while moving to GLP, GMP, and LNP manufacturing.

Take advantage of analytical capabilities at Cytiva to perform *in vitro* potency assays, including lipid and nucleic acid-specific analyses, to generate data insights for accelerated clinical trials. Instruments like our Ignite+, Blaze and Blaze+ systems are suitable for both upstream and downstream process development in late-preclinical testing, allowing for formulation protocol and technology transfer to GMP manufacturing, minimizing optimization steps, and reducing risk and time requirements. Furthermore, parameters can be directly transferred across instruments to fit different production scales. Access to the lipid portfolio also saves time scaling novel lipid formulations. Consider collaborating with the biopharma services team at Cytiva to leverage their expertise in scaling lead candidate formulations for GLP studies and developing qualification assays.

Before IND submission, you should carry out non-GMP batches as engineering or GLP runs at smaller scales, which may reveal CPPs that need to be modified to mitigate any risk in process performance qualification (PPQ) batches during validation.

Many key elements for IND filing can be identified and documented in the late preclinical phase:

- Clinical protocol
  - Describe intended dosing and the route of administration, justifications for dose selection based on dose-response, and PK/PD data.
    - Provide summary of pharmacokinetic preclinical studies.
    - Include any safety/efficacy data from similar clinical studies.
  - Address key toxicity considerations.
    - Provide summary of pharmacological and toxicological effects in preclinical studies (including excipients and formulations).
    - Include any toxicological data from similar clinical studies.
  - Provide description of possible risks and side effects.
    - Include data that proves drug benefits outweighs the risks.
    - List precautions and mitigation plans to prevent or monitor adverse events.
    - Record and report adverse events.
    - Include any relevant data from similar clinical studies.
- Method of manufacturing and packaging, including description of analytics and quality control processes for scaling up production, procuring GMP grade materials, and final specifications
  - Flow diagram suggested
  - Clinical batch analysis batch analysis
  - Labeling of proposed drug product

Implement strategies that will set the stage for success, including:

- Review similar safety/toxicology preclinical programs and prepare mitigation strategies.
- Review LNP excipients to determine if any additional toxicology studies are required beyond the drug substance requirements.

- Collect enough data across broad ranges to characterize a dose-response relationship and model first-in-human (FIH) dosing strategies.
- Relate preclinical data to clinical implications.
  - Generate translational data in relevant animal models and show how it relates to efficacy and toxicology.
  - Account for absorption, distribution, metabolism, and excretion (ADME) differences between animal models and humans.
  - Create a dedicated prefabricated facility space or a nested space in an existing facility, and find a collaborator in packaging and distribution of the finished product.
- Leverage FDA pre-IND meetings.
  - Address specific questions (i.e., Is there enough data? Is this the right data to include?).
  - Gain an understanding of the FDA's perspective on the preclinical program.
  - Get recommendations for a successful filing.
  - Build a collaborative relationship with the FDA.

While IND submissions can be long and resource-intensive, planning and preparation during each preclinical phase can streamline the process. Identifying clinical goals at the outset allows researchers to establish preclinical programs that generate supporting data and manufacturing strategies at each stage, strengthening the IND filing and accelerating the path to clinical development. Remember, the life of an IND continues as the data from the document is used to support a biologics license application (BLA) or new drug application (NDA), and for post-marketing commitments and studies to support new uses of supplemental NDAs.

Partner with BioPharma Services at Cytiva and get started with a well-defined project plan, strategies to meet milestones, deliverables, a timeline, and budget for successful IND submissions customized to your needs.

Contact a specialist to discuss more specific projects.

References

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