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# Recommendations for Successful IND Approval of RNA-LNP Drugs

Pharmaceutical companies must successfully file regulatory documentation before conducting clinical trials on cutting-edge novel medicines. Therefore, submitting an Investigational New Drug (IND) application for RNA therapeutics to the FDA is a significant milestone.

## IND APPLICATIONS OVERVIEW

INDs are required before conducting clinical studies for

- Unapproved new molecular entities
- Studying approved drugs different than the approved labeling, such as
  - New dosage
  - New Indication
  - New age group

The IND application process must be meticulous and adhere to the rules; failing to gather and report the necessary chemical, manufacturing and control (CMC) data and process specifics may result in a Clinical Hold. For successful IND submissions, it is significant to conduct general studies to evaluate the toxic effects of the new drug at various doses over specific periods. The data from the studies will determine the safe starting dose and the range of doses that can be used in the clinical trials and establish parameters once in human trials.

Once the IND application is submitted, the FDA review team members use their expertise to assess if all the following requirements for taking the nanomedicine to the clinic have been fulfilled.

- 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 an 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, they emphasize 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 10-12 years. However, with RNA therapeutics, the nanomedicine 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 BioNTech/Pfizer and Moderna could 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. Another contributing factor was the companies leveraging good practices and strong collaboration with the regulatory agencies. The existing regulatory system is ambiguous for this new and rapidly evolving class of medicines. Therefore, it is crucial to prepare early for regulatory filing that matches the rapid development of RNA therapeutics.

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# PRECLINICAL DRUG DEVELOPMENT WORKFLOW AND IND GUIDELINES

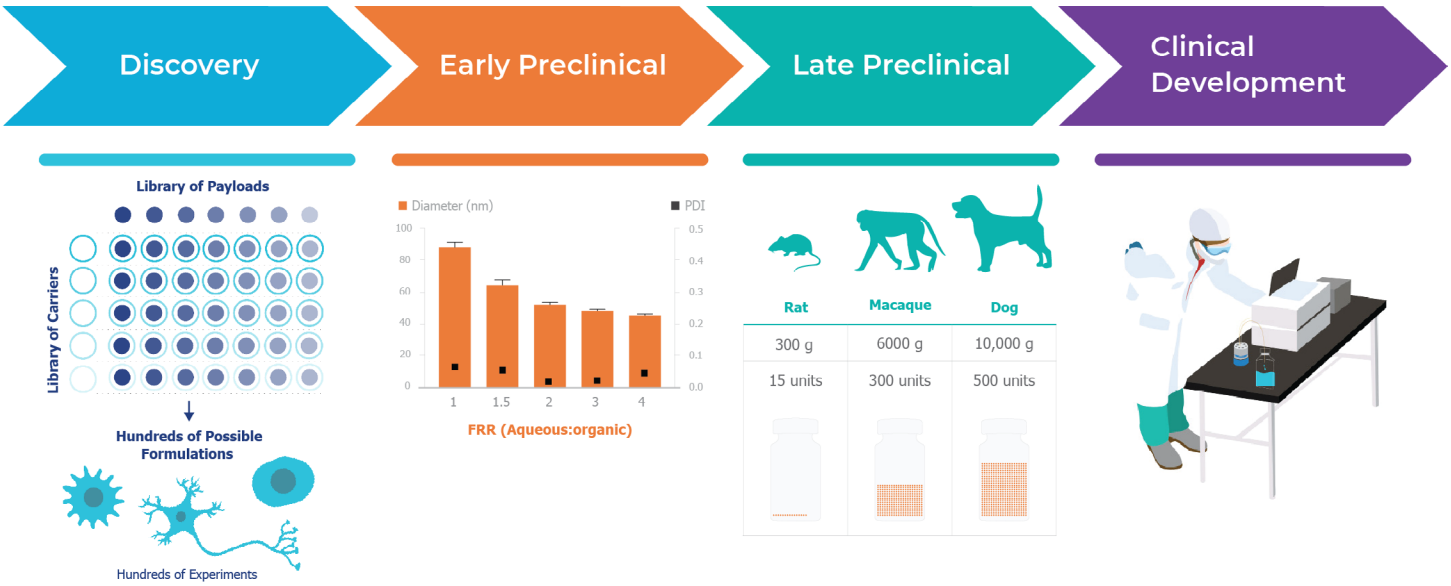


***Figure 1.*** *Drug development stages*

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 is vital to understand each stage and its purpose in regulatory approvals.

For a successful IND filing, make wise decisions to scale and validate production processes during the development phase. The FDA expects to see a step-wise approach to IND review. Therefore, tightening the acceptability of testing for the following processes while moving from the discovery phase to clinical is necessary.

- Product characterization and stability
- Analytical and process validation
- Good Manufacturing Practices

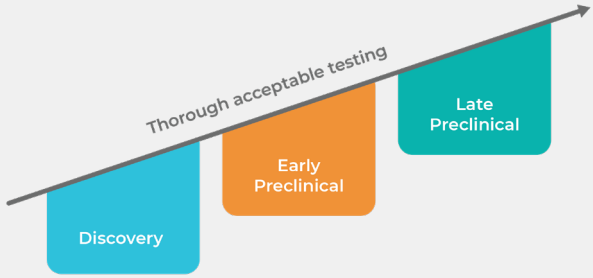


***Figure 2.*** *Step-wise approach for IND filing*

The quality of tests and data is essential and should be according to the development stage at which FDA regulations require the documentation. Below is an overview of how the information must be presented in the IND filing application at various drug development stages for RNA therapeutics.

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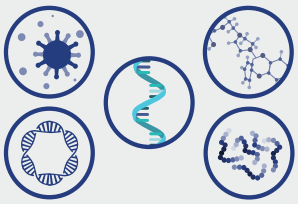
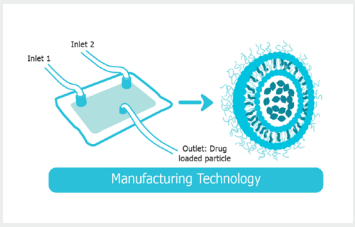
| Information for an IND Application | Drug Development Stage | | |
|---|---|---|---|
| | Discovery Phase | Early Preclinical | Late Preclinical |
| **Physical, Chemical & biologic description**  | Description of active ingredient. | a. Details on nucleic acid sequences and side-chain modifications.<br>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 of process changes, scale changes and if subsequent unit operations remained consistent at all scales. |
| **Acceptable limits & analytical methods to verify quality**  | Brief description of the tests and specifications that inform analytical methods and their validation and acceptable limits for release testing and stability monitoring. | a. Data representing analytical comparability assessment by release, comprehensive characterization and stability testing.<br>b. Process performance comparability assessment by in-process controls (IPCs) and critical process parameters (CPPs) evaluated against expected ranges or proven acceptance ranges (PARs).<br>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.<br>b. All extended analytical characterization results conformed to the expected comparability range.<br>c. Data supporting that the manufacturing process parameters and quality attributes were comparable across the manufacturing scales. |

| Information for an IND Application | Drug Development Stage | | |
|---|---|---|---|
| | Discovery Phase | Early Preclinical | Late Preclinical |
| **Stability data**  | a. A series of tests designed and performed to obtain information on the stability that support the analytical procedures, the shelf-life and storage conditions.<br>b. Data representing raw material quality. | a. Developmental Assessment and Reproductive Toxicology.<br>b. Non-GLP Repeat Dose Toxicity and Immunogenicity Study.<br>c. Other Supportive Toxicology Studies.<br>d. Biodistribution Study.<br>e. Clinical Diagnostic Assays Used to Support Primary Clinical Efficacy Endpoints. | a. GLP toxicology studies in animal models that includes clinical chemistry, pathology, hematology, ophthalmology measures of cardiac and pulmonary safety, urinalysis, bioanalysis, toxicokinetic analysis, and statistical analysis.<br>b. Non-clinical pharmacology study reports typically done in mice, rats, hamsters and non-human primates.<br>c. Updated stability and efficacy analyses data.<br>d. Stability data from multiple batches at accelerated scale stresses.<br>e. Immunologic mechanism that confers benefits of the drug.<br>f. Immunogenicity assays validating reports. |

***Table 1**. Overview of data requirements for IND application*

The present FDA regulation allows flexibility in the volume and depth of data; however, it is strongly recommended to have all research data and processes documented. With a strong understanding of the chemistry, manufacturing, and control information, it is 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 the strategies. The below strategies provide 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*


***Figure 3.*** *IND Filing Strategies Across Drug Development Stages*

## Drug Discovery

The drug discovery stage is to kick-start the validation of novel treatment modalities or formulations. Active pharmaceutical ingredients (APIs), drug delivery systems and mechanistic understanding of target compounds are key questions to be answered in this stage. Rational and practical considerations in the drug formulation development process include target product profile (TPP). TPP must be designed based on attributes such as indications, targeted population, clinical efficacy, safety and tolerability, stability, route of administration, dosing frequency and cost, and development timelines. They 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] Efficient screening while preserving precious materials can be a delicate balance as scientists define an appropriate TPP.

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### Considerations to accelerate this phase:

1. Access to 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. Speedy, consistent LNP preparation technology.
5. LNP production with near complete sample recovery, avoiding wastage of raw materials.

### Preparing for successful manufacturing and scale up:

1. Leverage 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 the analytical procedures, the 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. Easy-to-use automated instrument operations that reduce human error and minimize batch-to-batch variability.

### *Strategies for a successful drug discovery to move towards Preclinical Studies*

Access a well characterized lipid nanoparticle portfolio, like the one offered by Precision NanoSystems, to rationally design LNP formulations for validation of active pharmaceutical ingredients (APIs), generating proof-of-concept and efficacy data to progress lead candidates quickly through screening and *in vitro* preclinical studies.

Start operating mRNA-LNPs formulations on instruments that can integrate into the full workflow. The NanoAssemblr® Spark™ 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 use NxGen™ mixing technology, which maintains the same architecture as the equipment scales to produce larger batches. NxGen 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.

### Planning for IND filing

Many key elements for an IND filing can be identified and documented in this stage:

- Intended clinical target of the therapeutic, including the pathogen, targeted and disease prevented, the anticipated clinical use and the target populations.
- Description of the mRNA-assess mRNA sequences, length, capping and nucleotide modifications, the rationale for its selection, any proteins that are encoded and their contributions to the proposed mode of action for the therapeutic.
- Mechanistic description of the mRNA mode of action (i.e., vaccine applications would include immune responses).
- Manufacturing processes for the mRNA.

- Scheduling a pre-IND meeting with FDA. The pre-IND meeting is optional but 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.
- More importantly, some common mistakes can be avoided. Successful strategies to consider during the discovery phase include:
- Build a comprehensive preclinical program with IND filing in mind, 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.
        - Literature searches to find similar models or mechanisms already published or in clinical trials.
        - 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 Pharmacokinetics/Pharmacodynamics (PK/PD) models.
- Be realistic when planning timelines and milestones.It is 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 is 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, formulation development, and some refinement of the API. This 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. It requires thorough characterization of the drug substance and drug product properties of the 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).

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**Considerations to accelerate this phase:**

1. Consistent and reproducible LNP production with quick formulation times at scales appropriate for small animal studies.
2. Access to 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 to LNP expertise for design of experiment (DoE) studies to optimize manufacturing parameters.
4. Access to LNP formulation expertise for process development.
5. Invest in expertise in upstream and downstream processes.

**Preparing for successful RNA-LNP manufacturing and scale-up:**

1. Evaluation and qualification 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. Utilize 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 Attribute (CQA) 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***

Model unit operations and maintain the same process parameters when scaling to simplify clinical development and manufacturing transition. The NanoAssemblr® Ignite™ and Ignite+™ enable lipid nanoparticle-controlled and precise 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 and the physicochemical properties and *in vivo* biological outcomes. This process identifies optimized lead formulations while defining acceptance criteria for CQAs and CPPs.

Establish partnerships to leverage technical knowledge and experience in payload design and lipid-based delivery systems. Modifications in the chemical structure and ratio of the LNP components affect its 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. 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.

Precision NanoSystems 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, offering expertise in lead candidate optimization, process development, and scale-up.

**Planning for IND filing**

Many key elements for an IND filing can be identified and documented in this stage:

- 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.
- Successful strategies to consider during the early preclinical phase include:
- Invest 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.
- Do not underestimate the challenges 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 is 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. Thus, 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 this phase:**

1. Scaling up lipid and RNA manufacturing, scaling up 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 and characteristics at the time of manufacture and the effect of environmental factors on a formulation's purity, efficacy, and structure. Precision NanoSystems offers stability testing services that complies with the regulatory requirements for clinical trials.
3. Developing analytical methods that enable qualification of input and output materials.
4. Procure GMP-grade drug substance/reagents to manufacture a non-GMP batch as Engineering run or GLP batch.

5. Collaborate with partners that have GMP scale LNP formulation instruments to reduce process and formulation redevelopment when changing scales, compared to conventional batch-based methodologies.
6. Team up with trusted partners 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, pre-clinical evaluation for critical quality attributes and continuing throughout the clinical development phase, including post-approval. Although animal toxicological studies need to be carefully performed to generate relevant data to minimize safety risks in human subjects, testing is important at each stage, including CMC. Carry out the necessary and relevant tests and keep records (manually or by recording instruments) that verify all required sampling, inspecting, and testing procedures. Also, maintain records and investigate deviations while moving to GLP, GMP, and LNP manufacturing. Take advantage of Precision NanoSystems' analytical capabilities to perform *In vitro* Potency Assays, including lipid and nucleic acid-specific analyses, to generate data insights for accelerated clinical trials. Proven instruments like Ignite+™, Blaze™ and Blaze+™ are suitable for process development of both the upstream and downstream processes in late-preclinical testing and allows formulation protocol and technology transfer to GMP manufacturing minimizing optimization step and reducing risk and time requirements. Furthermore, the parameters can be directly transferred across instruments to fit different production scales. Access the lipid portfolio to save time scaling novel lipid formulations and collaborate with Precision NanoSystems' biopharma services to use their expertise in scaling lead candidate formulations for GLP studies and developing qualification assays.

Before IND submission, carry out non-GMP batch as engineering run or GLP batch scale, which may reveal CPPs that need to be modified and mitigate any risk in Process Performance Qualification (PPQ) batches as part of process validation.

**Planning for IND filing**

Key elements for an IND filing can be identified and documented in this stage:

- Clinical protocol
  - Describe intended dosing and the route of administration, justifications for dose selection based on dose-response, PK/PD data.
    - Provide summary of pharmacokinetics preclinical studies.
    - Include any safety / efficacy data from similar clinical studies.

  - 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.
    - Recording and reporting of 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 is suggested
  - Include clinical batch analysis
  - Labeling of proposed drug product
- 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 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 ADME (absorption, distribution, metabolism and excretion) differences between animal models and humans.
  - Create a dedicated prefabricated facility space or nested into existing facility space and find a partner 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 successful filing.
  - Build a collaborative relationship with the FDA.

**Successful strategies to consider during the late preclinical phase include:**

While IND submissions can be long and resource-intensive, planning and preparation during each preclinical phase can streamline the overall 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 IND continues as the data from IND is used to support Biologics License Application (BLA) or New Drug Applications (NDA) and for post-marketing commitments and studies to support new uses of supplemental NDAs.

Partner with Precision NanoSystems' Biopharma Services and get started with a well-defined project plan, milestones, deliverables, timeline, and budget for successful IND submissions customized to your needs.

Contact a specialist to discuss more specific projects.

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