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Optimizing DART Assessments for Regulatory Success & Emerging Modalities

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INTRODUCTION

The regulatory expectations for Developmental and Reproductive Toxicology (DART) testing are evolving rapidly, driven by guidance from the International Council on Harmonisation (ICH). Specifically, ICH S5(R3) introduces a more flexible approach to reproductive risk assessment, emphasizing scientifically robust methodologies that balance comprehensive safety evaluations with ethical considerations regarding animal use.

At the heart of this evolution is the weight-of-evidence (WoE) approach. It allows sponsors to integrate multiple data sources—i.e., alternative assays, existing pharmacological data, and toxicological findings—into their risk assessments. By shifting away from a reliance on traditional animal testing, this framework supports more predictive and human-relevant evaluations of reproductive hazards.

European and U.S. regulators have embraced these advancements through initiatives like the Drug Development Tools (DDT) qualification program. This program provides pathways for validating innovative methodologies that align with the principles of reducing, refining, and replacing animal testing. Key regulatory priorities include:

- **Human relevance:** Prioritizing data that directly translates to human risk assessment, particularly for biopharmaceuticals where species-specific mechanisms may limit traditional models.
- **Exposure-based dose selection:** Using pharmacokinetic/pharmacodynamic relationships rather than arbitrary dose multiples to refine study designs.
- **Patient population-specific risk assessment:** Tailoring reproductive safety evaluations to specific clinical populations based on disease severity and therapeutic need.

For biopharmaceuticals targeting well-characterized pathways, regulators increasingly recognize that reproductive risks can often be predicted based on a drug's mechanism of action (MOA). This knowledge-based approach reduces unnecessary animal studies while accelerating development timelines and optimizing resources. Novel modalities such as antibody-drug conjugates (ADCs) and antisense oligonucleotides (ASOs) particularly benefit from this flexibility, as traditional testing paradigms may not adequately capture their unique risks.

1. LESSONS FROM IND & NDA SUBMISSIONS

Regulatory submissions for Investigational New Drugs (INDs) anvd New Drug Applications (NDAs) provide critical insights into the challenges sponsors face when conducting Developmental and Reproductive Toxicology (DART) assessments. These submissions highlight the complexities of evaluating reproductive risks, particularly for large molecules like biologics, peptides, and gene therapies. They also highlight the importance of strategic planning, robust analytical methods, and proactive engagement with regulatory agencies to navigate these challenges effectively.

Managing Delayed Findings or Unexpected Reproductive Effects

One of the most significant hurdles in DART testing is addressing delayed findings or unexpected reproductive effects that emerge during preclinical studies. Small molecules manage these challenges more easily due to their simpler structures and predictable pharmacokinetics (PK). However, large molecules bring additional complexities, such as species specificity, immunogenicity, and intricate pharmacodynamic (PD) profiles. These factors necessitate customized study designs tailored to each molecule's unique attributes.

Pre-IND meetings are invaluable for mitigating these risks. These discussions provide a platform for sponsors to align with regulators on study design elements such as species selection, dosing regimens, and endpoints specific to the molecule's pharmacological profile. Pre-IND consultations often extend to analytical and bioanalytical method validation for large molecules. These methods are essential for confirming exposure levels and assessing critical parameters like placental transfer, milk excretion, and immunogenicity.

Chemistry, Manufacturing, and Controls (CMC) Considerations

CMC considerations also play a pivotal role in early development. Ensuring product consistency across batches used in preclinical studies is crucial for both small- and large-molecule therapies. Addressing CMC challenges early helps prevent delays or inconsistencies that could compromise study outcomes or regulatory timelines. For example, manufacturing process variability can impact toxicology data's reproducibility, making early resolution critical to maintaining development schedules.

Tailoring Nonclinical Programs

Regulators emphasize tailoring nonclinical programs to align with the intended clinical route and dosing schedule. This requires a thorough understanding of the molecule's mechanism of action (MOA) and its potential impact on reproductive health. Gene therapies targeting specific pathways may require bespoke study designs that account for unique biological interactions. Sponsors must also consider patient population-specific factors when designing DART programs, particularly for therapies targeting life-threatening conditions where risk-benefit considerations differ significantly.

Insights from Accelerated Regulatory Approvals

While accelerated approval pathways are relatively rare, they offer valuable opportunities for therapies addressing serious conditions with unmet medical needs. These pathways—including fast-track designation, breakthrough therapy designation, accelerated approval based on surrogate endpoints, and priority review—enable expedited access to life-saving treatments while maintaining rigorous safety standards.

For sponsors pursuing accelerated approvals, DART strategies should align with compressed timelines. This often involves conducting reproductive toxicity studies in parallel with early clinical trials rather than sequentially. Such approaches are particularly relevant for orphan drugs or therapies targeting life-threatening conditions where traditional timelines could delay patient access. The rapid development of COVID-19 vaccines is a recent example of how accelerated pathways can facilitate drug development without compromising safety or efficacy.

To support accelerated approvals effectively, sponsors should adopt a WoE approach. Based on the molecule's target biology, mechanistic studies can provide insights into potential reproductive risks. Surrogate models may be employed when traditional species are irrelevant, while advanced analytics can enhance understanding of PK and placental transfer dynamics. Strategic communication with regulators throughout this process ensures alignment in study designs and data requirements.

2. INNOVATIVE APPROACHES TO DART TESTING

DART testing is a critical component of drug development, and while animal models are required for regulatory approval, technological advancements show immense promise. Improved predictive accuracy, reduced reliance on animal testing, and streamlined development are only a few of the advantages to exploring novel approaches. Methodologies that rely heavier on in vitro models, microphysiological systems (MPSs), and artificial intelligence (AI) are beginning to reshape DART testing—although integrating them into regulatory approvals is very challenging.

In Vitro Models for Early Screening

In vitro models, especially those derived from human stem cells, have become valuable tools for reproductive toxicity screening. These models allow researchers to study cellular responses to drug exposure in a controlled environment, providing insights into developmental risks without relying on animal studies. An example of their potential are trophoblasts—i.e., human stem cells that mimic aspects of placental function—allowing scientists to evaluate drug transfer and toxicity at the maternal-fetal interface. These models cannot replace in vivo studies but can provide preliminary data to refine study designs and reduce the scope of subsequent animal testing.

Despite their promise, regulators are hesitant to accept in vitro data alone. Concerns about in vitro's ability to replicate complex biological systems accurately have proven daunting. However, the DDT qualification program provides a pathway for validating these methodologies for specific uses. As in vitro models evolve and gain validation, their ability to reduce reliance on animal models while maintaining scientific rigor is a cause for optimism.

Organ-on-a-Chip Technology

MPSs, including organ-on-a-chip (OOAC) technology, represent another innovative frontier in reproductive toxicology research. These systems aim to replicate human organ functions on a microscale using engineered tissues and fluidic devices. Researchers are working toward developing chips that simulate reproductive organs and placental function for DART testing. However, these systems face significant technical hurdles in replicating the complexity of human reproduction and development. Achieving regulatory approval will require extensive validation and consensus among global regulatory bodies.

OOAC systems are used primarily in exploratory research rather than regulatory submissions. However, their value lies in complementing traditional methods by providing mechanistic insights into drug effects on specific tissues or processes. Reliability across drug classes remains OOAC's biggest challenge—the technology cannot deliver valid regulatory data until this problem is solved.

AI & Computational Toxicology: Unlocking Predictive Modeling

AI and computational toxicology offer transformative potential for DART studies. These tools can identify patterns and predict reproductive risks from vast historical datasets, enabling more informed decision-making during drug development. Specifically, machine learning algorithms can analyze endpoint data from previous studies to uncover correlations between molecular structures and reproductive outcomes.

Despite their promise, AI-driven tools face data accessibility and standardization challenges. Much of the existing DART data is siloed within individual organizations or inconsistently formatted, limiting its utility for large-scale analysis. Efforts to centralize and harmonize data repositories could significantly enhance the effectiveness of AI-based predictive modeling.

Current Usage & Regulatory Perspectives

AI, in vitro, and MPS approaches are gaining traction in research settings, but their adoption in regulatory submissions remains limited. Regulators see traditional animal models—and their established predictive capabilities—as the gold standard for DART assessments. However, initiatives like the DDT qualification program are a starting point for integrating novel methodologies into drug development. Each approach offers unique advantages despite distinct limitations:

- In vitro models excel at early-stage screening but require complementary in vivo studies for comprehensive risk assessment.
- MPSs provide valuable mechanistic insights but face technical challenges that limit their immediate applicability.
- AI tools streamline data analysis and improve predictive accuracy but depend heavily on robust datasets and standardized methodologies.

Combining these innovative approaches with traditional methods is the only way sponsors can optimize DART strategies and maintain compliance with regulatory expectations. But these technologies are not far off. As they mature and gain broader acceptance, they could transform DART assessments.

3. DART CONSIDERATIONS FOR ADVANCED MODALITIES

Advanced modalities, including antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), biologics, and antibody-drug conjugates (ADCs), present unique challenges in DART assessments. These challenges stem from their complex pharmacology, species specificity, and limited historical data. To address these complexities, the ICH's WoE framework allows sponsors to tailor their study designs to the specific attributes of these innovative therapies.

Challenges with ASOs and siRNAs

Oligonucleotide therapeutics ("oligos"), including ASOs and siRNAs, are designed to target specific genetic sequences, which can lead to unintended effects on gene expression pathways critical for reproduction and development. The potential for adverse effects due to on-target activity or exaggerated pharmacology underscores the need to characterize these molecules carefully.

DART evaluations for oligos typically include studies on fertility and early embryonic development (FEED), embryofetal development (EFD), and pre- and post-natal development (PPND). While rodents are commonly used for FEED and PPND studies, EFD assessments are conducted in rodents and rabbits. In some cases, existing data, including mechanistic insights into the molecule's mode of action (MOA), phenotypic data from genetically modified animals, or human genetic information, may provide sufficient evidence to predict reproductive risks without requiring additional animal testing. However, when oligos lack pharmacological activity in standard species, surrogate molecules or transgenic animal models may be necessary to generate valuable data.

Challenges with Biologics

Biologics introduce additional complexities due to their large molecular size and species-specific MOA. Placental transfer of biologics is generally limited during early pregnancy but increases in later stages through active transport mechanisms such as Fc receptor-mediated endocytosis. This is particularly relevant for immunoglobulin G (IgG)-based therapeutic proteins. However, data on drug exposure during pregnancy and lactation remain scarce, especially for newer monoclonal antibodies (mAbs). This lack of pharmacokinetic (PK) data complicates risk assessments for women of childbearing potential enrolled in early clinical trials.

To address these gaps, sponsors should consider studies that evaluate placental transfer using humanized models or in vitro systems where feasible. Published data on similar biologics can also help contextualize potential risks. Regulatory agencies recommend tailoring DART study designs to align with the molecule's specific attributes while adhering to ICH S6(R1) guidance on biotechnology-derived pharmaceuticals.

Challenges with ADC Payloads and Immune-Modulating Therapies

ADCs combine the specificity of mAbs with the cytotoxicity of small-molecule payloads. This dual nature introduces unique challenges because both components must be evaluated independently and in combination. The payload's cytotoxicity can affect rapidly dividing cells involved in reproduction and fetal development, while the antibody component may influence placental transfer dynamics.

For immune-modulating therapies targeting young cancer patients—both male and female—data on reproductive risks are particularly limited. In these cases, sponsors should prioritize studies that assess fertility impacts alongside EFD and PPND endpoints. Mechanistic studies can provide valuable insights into how the therapy's intended pharmacological activity might influence reproductive outcomes.

Regulatory Recommendations

Given the complexity of advanced modalities, early engagement with regulatory authorities is essential to ensure that study designs are scientifically justified and aligned with current guidelines. Pre-IND meetings allow sponsors to discuss critical elements such as species selection, dose regimens, and study endpoints tailored to the product's unique attributes. For example:

- When oligos or biologics lack cross-reactivity in traditional species, surrogate molecules or transgenic models may be required.
- Additional safety considerations may be necessary for ADCs with highly potent payloads to address potential off-target effects.

By adopting a flexible approach informed by WoE principles and product-specific considerations, sponsors can effectively navigate the challenges associated with advanced modalities while meeting regulatory expectations for reproductive safety assessments

4. OPTIMIZING STUDY DESIGN FOR SMALL MOLECULES VS. BIOLOGICS

Developmental and Reproductive Toxicology (DART) study designs differ significantly between small molecules and biologics due to their distinct pharmacological properties, MOAs, and species specificity. These differences require tailored approaches to ensure that reproductive risks are accurately assessed while meeting ICH regulatory expectations.

Study Requirements for Small Molecules

Small molecules, characterized by their low molecular weight and ability to diffuse across biological membranes, follow well-established DART testing paradigms. These compounds are typically assessed in standard species, such as rodents and rabbits, as outlined in ICH S5(R3). Standard studies include evaluations of fertility and early embryonic development (FEED), embryofetal development (EFD), and pre- and post-natal development (PPND). These studies collectively assess potential impacts on reproduction and development across all reproductive cycle stages.

Small molecules' relatively simple pharmacokinetic (PK) profiles allow straightforward dose selection based on systemic exposure levels. However, their ability to cross the placenta necessitates comprehensive evaluations during organogenesis to identify teratogenic risks. Regulatory agencies expect these studies to provide robust data on potential reproductive hazards, making DART programs for small molecules relatively standard compared to biologics.

Unique Challenges for Biologics

Biologics, including mAbs, peptides, and other large-molecule therapeutics, present unique challenges in DART testing due to their complex structures and species-specific activity. Unlike small molecules, biologics often do not readily cross biological membranes or the placenta, limiting fetal exposure during early pregnancy. However, certain biologics, such as IgG-based therapeutic proteins, can cross the placenta via Fc receptor-mediated transport during later stages of gestation. This variability complicates risk assessments for biologics and requires careful study design.

Because many biologics exhibit pharmacological activity only in humans or closely related species, traditional rodent models may not be relevant for assessing reproductive risks. In such cases, alternative approaches are required. For example, surrogate molecules that mimic the biologic's activity in standard species may be developed. Genetically modified animals expressing human target receptors can also provide a means to assess pharmacological activity.

Non-human primates (NHPs) may be used selectively for EFD or PPND studies when no other species is pharmacologically relevant. However, regulatory agencies discourage routine use of NHPs due to ethical considerations and limited availability.

Regulatory Flexibility for Biopharmaceuticals

ICH S6(R1) emphasizes a case-by-case approach for biopharmaceuticals based on the molecule's attributes. For example, if a biologic is active only in NHPs but sufficient precautions can prevent pregnancy during clinical trials, DART studies may be deferred until Phase III. Labeling may reflect these risks without requiring extensive nonclinical studies for biologics with MOAs that raise significant concerns for EFD. This flexibility ensures that study designs are scientifically justified while minimizing unnecessary animal use.

Key Considerations in Study Design

Sponsors must consider several critical factors when designing DART programs for small molecules or biologics. For example, rats and rabbits are typically sufficient when selecting species for small molecules. For biologics, however, pharmacological relevance often dictates the use of surrogate molecules or transgenic models. Small molecule doses are based on systemic exposure levels relative to human therapeutic doses in dose selection. For biologics, dosing strategies must account for immunogenicity and PK behavior. Finally, the timing of DART studies related to clinical development varies depending on the molecule's attributes and intended patient population. For small molecules, reproductive toxicity studies are generally conducted early in development to identify potential risks before large-scale clinical trials begin. For biologics, however, study timing may differ significantly. For example, EFD studies may be deferred until Phase III if adequate precautions are taken during earlier clinical trials to prevent pregnancy among participants.

CONCLUSION

DART studies are advancing rapidly, with regulatory updates, new technologies, and complex drug modalities impacting reproductive risk assessment. Optimizing DART strategies requires a thorough understanding of regulatory frameworks like ICH S5(R3) and S6(R1) and the ability to tailor study designs to the unique attributes of small- and large-molecule drugs.

Integrating new technologies like AI-driven modeling and in vitro systems opens a complex corridor of considerations. These advancements improve the predictive accuracy of DART assessments and help reduce unnecessary animal testing. However, the complexity of designing and executing DART programs—particularly for emerging modalities like ADCs, ASOs, and cell-based therapies—can be overwhelming.

Species selection, dose optimization, and regulatory expertise require dedicated technical expertise that drug sponsors may not have in-house. This is where partnerships can make all the difference. Trusted lab partners can provide end-to-end support for DART studies, ensuring that regulatory expectations are met, timelines are secured, and resources are safeguarded.

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