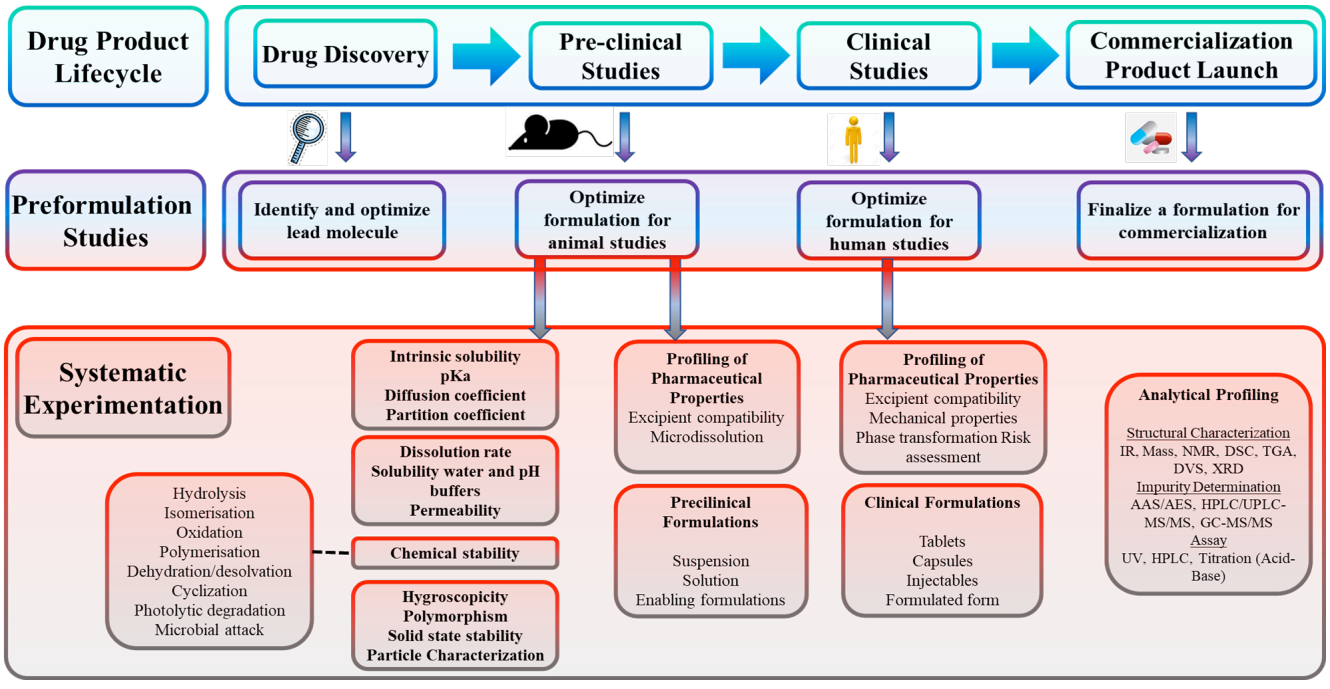
Preformulation Strategy – Inception to Completion

Harsh S. Shah
harsh.shah@jstar-research.com
Shanming Kuang
shanming.kuang@jstar-research.com

Overview

Drug discovery is an exceedingly complex process. Discovering a new chemical entity is in itself a huge accomplishment, which is often possible only after almost two decades of hard experimental work as well as advanced simulation efforts. Therefore, the next stage of the process, determination of toxicity becomes even more crucial as most new chemical entities (NCE) are disqualified if the NCE shows a potential toxic effect, despite any possible therapeutic effect. Once the NCE is determined to be safe, the next goal is to ensure availability of the NCE at the site of action *in vivo* upon administration through an appropriate route. Once bioavailability is confirmed, therapeutic effect must be demonstrated. Practically speaking, these most important challenges require a NCE to undergo animal studies in before its administration into humans, where efficacy is measured. To summarize, a NCE can be deemed effective, safe and stable, if it possesses desirable physico-chemical, pharmacokinetic, and pharmacodynamic properties thoroughly characterized by robust design of experiments.

A NCE can then be claimed to be an active pharmaceutical ingredient (API). However, it is not yet a drug or therapy. Proper administration of API usually requires suitable pharmaceutical formulation: API bioavailability, stability, and pharmacokinetics all depend upon formulation. Preformulation studies occur concurrently with and inform this activity and involve a multidimensional approach at various stages during the drug product lifecycle. Knowledge of the pharmacology, toxicology, biochemistry, chemistry (solid state, medicinal, and analytical), clinical pharmacy, and pharmaceutics are closely intertwined. The figure below shows activities usually performed step-wise during diverse stages of the drug product life cycle. The overall goal of preformulation studies can be divided into two parts: 1) Promoting a NCE to an API supported by knowledge of proper administration and dosing to the desired target *in vivo* by formulating practical drug products for animal, as well as, human studies; 2) Improving stability of the drug product by appropriate design and stabilization of the API both itself and in drug product toward environmental de-gradation while also completely characterizing the developed drug product.



Introduction

Once a new molecule is discovered, different physico-chemical properties of the drug substance must be investigated and characterized. This scientific approach is referred to as preformulation studies. The majority of newly discovered drug candidates have limited physiological solubility and bulk permeability; therefore, candidates that are not ionizable, or weak bases or acids require a robust solid form as either anhydrates, hydrates, solvates, salts and/or cocrystals. These pharmaceutical solid forms require a method of preparation and characterization before advancing to oral solid drug product development. In addition to this, drug substance compatibility assessment with inactive ingredients (excipients) in tablets or capsules is often required. Intravenous formulations can be even more challenging as limited API solubility may require non-aqueous solvents or emulsifying agents under sterile conditions.

Step-By-Step Preformulation Studies

- Suitable assay method development to determine concentration and purity of the drug substance (*e.g.*, suitable spectroscopic or chromatographic methods)
- Solid form screening and selection (anhydrates, hydrates, solvates, salts and/or cocrystals) and computational materials science
- Solubility, including pH dependent solubility and dissolution rate investigation of drug substance and corresponding solid forms (anhydrates, hydrates, solvate, salts and/or cocrystals) in aqueous and organic systems, along with particle size effects thereupon
- Solid-state stability (crystallinity, melting point, polymorphic changes and/or hygroscopicity) of drug substance and corresponding solid forms.
- Chemical stability of drug substance and corresponding solid forms (anhydrates, hydrates, solvate, salts and/or cocrystals) both in solution and the solid state
- Photostability of drug substance
- pK_a determination
- Obtention of drug substance lipophilicity (*i.e.*, oil:water partition coefficient, expressed as K_d)
- Optimizing particle morphology and determining milling capabilities
- Bulk characterizations, *e.g.*, bulk density, powder flow, angle of repose, etc.

Assay Method Development for Determination of Concentration and Purity of the Drug Substance

One of the first and an important step in preformulation studies is to develop analytical methods that can specifically and quantitatively analyze drug during formulation development and related studies. Validation may not be necessary at this point; however, a validated method may be of more use going forward due to its demonstrated rigor and integrity. The most commonly used assay methods include chromatographic and spectroscopic techniques, as well as, thermal methods. Chromatographic methods may include, but are not limited to, reversed-phase high performance

liquid chromatography (RPHPLC), normal phase chiral chromatography (NPLC), Liquid chromatography coupled with mass spectroscopy (LC-MS) and others. Similarly, spectroscopic techniques may include Raman spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy and many others.

For oral solid formulations, the method should be unaffected by excipient interference. Note, it might be advantageous if the same assay technique can be used for bioanalytical assay to analyze blood concentrations from clinical studies after gastrointestinal tract absorption. Conversely, percutaneous absorption is a key parameter for topical drug products. A successful topical therapy is dependent on absorption through the skin. Therefore, the specificity, sensitivity and robustness of any analytical method becomes even more crucial. A robust analytical method is required for investigation of molecular properties that includes solubility, dissolution rate, physico-chemical stability studies, partition co-efficient, pK_a studies, and similar investigations for a new drug substance.

Solid Form Screening and Selection

While discovery of a new active molecule is a notable achievement, additional optimization at a molecular level is often required to produce a successful drug. These modifications of a lead candidate can improve physicochemical traits of the molecule, including those associated with good bio-availability, stability and manufacturability. Modifications may be achieved by preparing anhydrates, hydrates, solvates, salts, cocrystals, amorphous materials or a combination thereof. Polymorphism is defined as a chemically identical molecular entity with two or more distinct crystalline forms. In general, only one polymorph is thermodynamically stable at a particular temperature. In an event of the existence of different polymorphs, they can be

enantiotropic or monotropic. Physical characteristics and stability are a function of a polymorphic form. Therefore, the decision to choose a particular solid form may be really important and bioavailability improvement may not be the only consideration. J-Star's research capabilities make such determination facile.

Salt formation is a most preferred approach to improve physicochemical properties like solubility, dissolution rate, hygroscopicity, chemical stability, and/or thermo-mechanical properties. Similarly, cocrystal formation can also give improvement in properties such as compressibility, hygroscopicity, as well as solubility. Hydrates or solvates are typically considered only when anhydrate does not lead to acceptable physicochemical properties. If anhydrate solid form has a tendency to convert to hydrate due to environmental exposure such as wet granulation or in solution state *in vivo*, hydrate may be selected as a lead solid form. With J-Star's expertise in solid form screening, a suitable solid form can be identified rapidly and economically to meet client needs. Our state-of-the-art characterization tools include: X-Ray Powder Diffraction (XRPD), Differential Scanning Calorimetry (DSC),

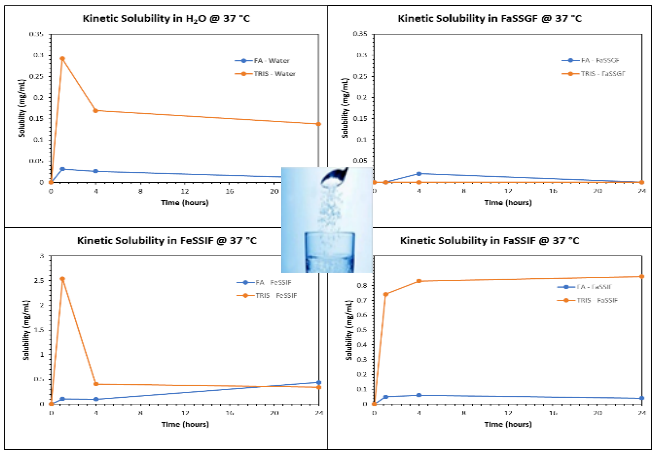
DSC

TGA

Thermogravimetric Analysis (TGA), Dynamic Vapor Sorption (DVS), Polarized Light Microscopy (PLM), Particle Size Distribution (PSD), NMR, Raman spectroscopy, FT-IR, and HPLC to support complete polymorph screening studies.

SOLUBILITY AND DISSOLUTION RATES INVESTIGATION

For a drug substance to be sufficiently bioavailable, it has to solubilize in body fluids. Various body fluids have different pH, therefore, it becomes important to study solubility under expected physiological pH conditions. This can be achieved by using standard aqueous buffers and biorelevant media. The drug substance is agitated at constant temperature, *e.g.*, 25 °C or 37 °C. Once the equilibrium is achieved, the thermodynamic solubility is determined. For compounds with ionizable groups, this equilibrium solubility of the un-ionized form is known as the intrinsic solubility. Usually, solubility studies begin with intrinsic solubility measurement in neutral, acid and alkaline environments. These conditions may include 0.1 M HCl, water, and 0.1 M NaOH at different temperatures (*i.e.*, 4 °C, 25 °C, 37 °C and an elevated temperature, *e.g.* 50 °C). Solubility numbers obtained from these experiments will provide insight into expected solubility under *in vivo* conditions, *e.g.* as it passes through the gastrointestinal tract, and circulates through various cellular and organ components, arteries and veins, and excretory fluids such as bile and urine. The solubility profile at different pH's may also drive the aqueous solvent selection for parenteral formulations (*e.g.*, parenteral injection, nasal or ophthalmic drops, oral solutions). This information can be used to further exploit the possible effect of aqueous dissolution media, *e.g.*, tablet processing, enteric and film coating. Newly discovered compounds possessing inherently low intrinsic solubility in aqueous media may have increased bioavailability upon addition of a water-miscible solubilizing agent, such as polysorbates, ethanol or polyethylene glycol (PEG) to aqueous systems. Similarly, during preformulation studies, investigation of the efficacy of various solubilizing agents such as methylcellulose or cyclodextrin becomes important given their relatively low



risk effects in humans.

Dissolution

Although knowledge of intrinsic solubility is very important, characterization of dissolution *rate* at which a drug is expected to dissolve in a particular medium is also needed. Consideration of the intrinsic dissolution rate along with surface area may be fruitful in this regard. If the dissolution rate is inherently slow, the API can be formulated into a sustained release drug product with once-a-day drug administration. Dissolution rate is dependent on many factors, which include particle size distribution and particle porosity. The dynamic surface area during dissolution drives wettability of

the particle surfaces, along with the nature of the dissolution fluid, its polarity, rheological properties and the degree of stirring or agitation during dissolution. When considering different variables present in dissolution experimentation, the initial focus should be on designing an experiment that uses a pharmacopoeial paddle as part of the dissolution apparatus at constant temperature and pH starting with API of similar particle size run-to-run. A discriminatory medium can be further optimized to study the effect of surface area, pH or particle size as drug product development progresses. Dissolution experiments can further probe for possible complications that may arise with oral formulations, especially when solubility is highly pH dependent. The pH difference between the stomach and small intestine is significant. Gastric pH can range from pH 1-2 in fasting state, whereas it ranges from 3-5.5 in fed state. Similarly, intestinal pH in fed state ranges between 5-6 and around 6-7.5 in fasting state. These pH differences lead to rapid solubilization of basic substances in the stomach, which then typically precipitate in the intestine. This pH swing behavior may negatively impact drug absorption. Often, the potential for this behavior needs to be studied prior to animal toxicity studies, because incomplete absorption in the animal may not truly characterize toxicity of the NCE. Generally, a drug substance exhibiting intrinsic dissolution rates greater than 1 mg/min/cm^2 has minimal likelihood of absorption problems during formulation development (Kaplan, 1972). Therefore, low volume two-stage dissolution studies will provide deeper insight toward understanding the dissolution behavior of a drug substance in small animals like the rat, mice and guinea pigs.

SOLID-STATE STABILITY

Stability Chambers

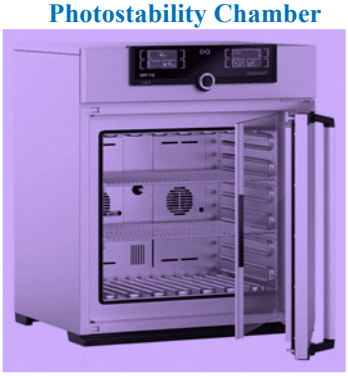
Once desired solubility and dissolution rates are achieved, it is important to ensure that the selected solid form is still chemically and physically stable. Solid-state stability includes assay potency, appearance, flowability and consistent crystallinity and crystal form. Once a drug product is manufactured, it is usually anticipated to have a shelf-life of 1-5 years. Initial stability study duration may range from 1-4 weeks within a temperature range from 4 to 75 °C with static moisture sorption at relative humidity levels between 20-90%; conditions are typically chosen to reveal long-term stability to some degree. During shelf life, a typical stability criterion is less than 5% chemical decomposition with no significant physical change under normal storage conditions. In addition, hygroscopicity of a drug substance determines the stability of a lead solid form towards water, as the water activity of a crystal form may vary with water content. Furthermore, hygroscopic material may deliquesce and form hydrate(s) or mesophases which may have completely different physicochemical properties. Dissolution rates and solubility may thus be impacted in some cases. Finally, hydrolysis of a molecule may turn an active compound into a therapeutically inactive molecule. Therefore, it is essential to determine the hygroscopicity of the lead solid form during preformulation studies.

CHEMICAL STABILITY AND PHOTO-STABILITY

All NCEs are expected to meet both accelerated and long-term ICH chemical stability conditions. If a different solid form is subsequently identified and expected to be developed further, the lead solid form (*i.e.*, salts, cocrystals, anhydrate, hydrates or solvates) as well as, the free form are studied, wherein the free form is considered as the control. Solution stability at pH values ranging from 1 to 11 at ambient temperature and 37 °C are also required. In a few cases, chemical degradation in presence of solubilizing agents is also investigated. Sensitivity to UV and visible light, and to oxygen exposure is also measured. The degradation rate under these short-term accelerated conditions will help the scientist to predict the degradation rate under routine storage conditions.

DVS

Photostability Chamber



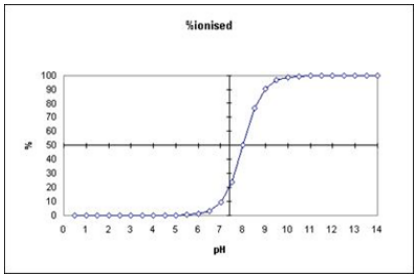
Drug substances likely to undergo degradation at low pH should be prevented from release in the stomach by using a modified release technique, such as enteric coating. This would allow release of the drug into the intestine.

The overall goal of chemical stability testing during the preformulation stage is to determine which possible challenges a lead solid form may face during the future drug product cycle. As the drug product progresses to the next stage, more definitive and rigorous long-term (1-5 year) stability studies will be required for regulatory purposes.

pK_A DETERMINATION

The aqueous solubility of a drug compound is a nearly always a function of its ionizability. The extent of ionization can be calculated by the Henderson–Hasselbach equation, which for weakly acidic compounds (HA) is

$pKa = pH + \log[HA]/[A^-]$
and for weakly basic compounds (HB) is
$pKa = pH + \log[BH^+]/[B]$

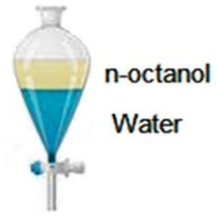


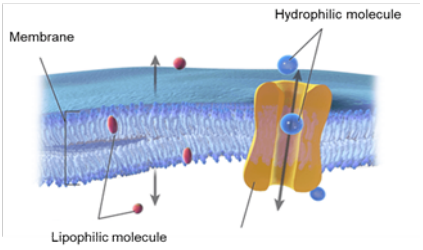
Volumetric Titrator

Intrinsic solubility data can be used to calculate the pK_a of a drug substance. Other techniques such as conductivity, potentiometry and spectroscopy can also be used to deduce pK_a. The pK_a of a molecule can serve as an indicator of possibility of ionization of drug substance in the gastro-intestinal tract as noted above and thus its impact on absorption characteristics and bioavailability of the drug substance. Lastly, if the lead solid form is a salt or cocrystal, the chemical nature and concentration of the counter ion *e.g.*, chloride or sulfate, or cocrystal former may significantly influence solubility. These studies made during preformulation work support further development.

Partition Coefficient (Log P)

A drug substance may be freely soluble in aqueous systems at physiological pH, but absorption and distribution of a drug in bodily fluids is directly proportional to its ability to cross cell membranes, which all contain lipophilic domains surrounding and within the cell. During this process, the drug substance may need to pass through many different tissues and lipid barriers. Therefore, choice of lipidic phase is critical for obtaining relevant Log P values. For many decades, n-octanol has been a preferred lipidic phase choice for preformation studies. The reasoning behind choosing n-octanol is its close similarity to many biological short chain hydrocarbon lipids present *in vivo*. The





lipophilicity of a drug substance can be gauged using the following equation to calculate its partition coefficient as a function of the equilibrium distribution of un-ionized drug between an organic and an aqueous phase.

$$P_{O/W} = \left(\frac{C_{Octanol}}{C_{water}}\right) at\ equilibrium$$

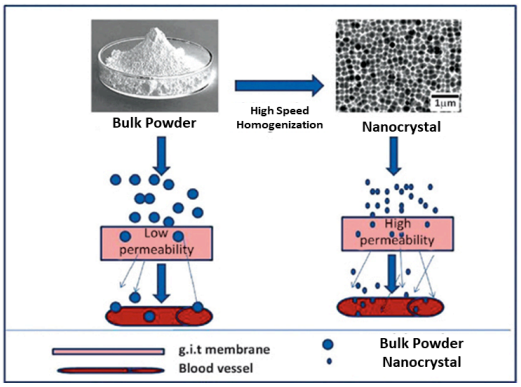
The shaken flask method is commonly used for this, wherein an aqueous phase saturated with a quantitated amount of drug substance and an equal n-octanol volume is agitated until equilibrium is attained. The concentration in each phase is determined and the amount of drug partitioned from the aqueous into the lipid layer quantified. While only a qualitative gauge of lipophilicity *in vivo*, Log P determination can even so be a useful tool for optimization of a lead compound during preformulation development.

Material Conservation and Strategic Drug Product Design

Optimizing particle morphology and determining milling capabilities.

Jet Mill

During the preclinical stage, the goal is to quickly find a formulation which can be administered into animals as well as humans by keeping in mind Good Laboratory/Manufacturing Practices (GLP/GMP) compliance. Drug product stability may not be crucial as it is administered immediately upon preparation. Formulations for animal and first in human (FIH) studies comprise solution, injection, suspension, enabling forms, powder in bottle, powder in capsule, and formulated dosage unit. Small and uniformly sized particles of the drug substance obtained through milling are ideal for these. The resultant particles would form a free-flowing powder that can avoid blend uniformity issues upon mixing with excipients. A majority of newly discovered drug substances tends to be hygroscopic crystals



with relatively low melting point, which makes their physical or chemical integrity vulnerable to mechanical milling and/or environmental conditions. Challenging, difficult to handle solid forms during preformulation include those which are amorphous or glassy, waxy due to the presence of hydrophilic polymer, or micronized or nanoparticles susceptible to electrostatic charges. These solids definitely require additional work as the API advances through the drug product cycle, since tablets or capsules are the most acceptable dosage forms.

Drug product approaches and selection

It should be noted that the typical preclinical formulation meant for animal toxicity studies is relatively different from first-in-human or clinical formulation. This is the case because during preclinical studies, the main goal is to be able to study toxicity, which requires higher dosing compared to that anticipated for humans. Toxicity studies will allow determination of the "no observed adverse effect level" (NOAEL) and the maximum tolerated dose (MTD).

Powder-in-Bottle (PIB) is a quick, material conserving, and often cost-effective approach. However, GMP compliance requirements for PIB and the associated Analytical methods developed during early phase may or may not remain usable and effective without further work if the final formulation changes. The reconstitutable powder comprised of drug substance and minimal inactive ingredients can be swiftly mixed and shipped to the clinical facility. Professionally trained healthcare staff can disperse the pre-weighed powder (single dose) into a commercially available vehicle and administer it to the patient in the form of a suspension or solution. The dispersing liquid may also include water or certain palatable juices. Although a pre-weighed single dose PIB is less susceptible to dosing errors, a multi-dose PIB may be more convenient if drug substance stability has been shown post suspension or dissolution in the desired administration vehicle. PIB approach works best for BCS Class I and III drugs, and may not be equally acceptable for BCS Class II and IV drugs due to limited availability of solubility improvement options. Note, the taste of a drug substance may be challenging, also. Overall, dosing flexibility and time constraints in the early stage, may make PIB the preferred choice of formulation at that time.

Powder-in-Capsule (PIC). PIC shows similar advantages to PIB. Importantly, organoleptic issues can be avoided with PIC, enhancing patient compliance. In PIC, human error in weighing or re-constitution can be avoided due to its single dose nature. As PIC needs the additional step of capsule filling, characterization by content uniformity testing becomes crucial, particularly at low strength dosing. Capsule filling can be done by hand, semi-automated, or fully automated. Capsule sizes typically range from size 4 (smallest) to 00 (large). Capsule shells can be made of gelatin (BSE/TSE free) or hydroxypropylmethyl cellulose. As capsule shell is part of the drug substance in PIC, it is important to characterize its disintegration in biorelevant media, as capsule shell may not be innocent (*e.g.*, gel formation) with regard to drug substance, excipient, or biorelevant component. This may result in incomplete drug release. If all the aforementioned factors are addressed appropriately, PIC can be very workable option.

Formulated Dosage Units. In specific cases, PIC or PIB may not be used due to inherent properties of a drug substance. Such properties include poor solubility, bioavailability, flowability, or anticipated modified drug release. In these situations, the drug substance requires pre-processing or co-processing with another functional excipient or polymer. Such use necessitates additional formulation development, typically one to three months, before being able to make final drug product as a tablet or soft gel capsule. Excipient compatibility studies and process development are essential and may lengthen lead time significantly. Although shelf life may not be of immediate concern for, *e.g.*, FIH testing, since tablets exhibit longer shelf life in general, this type of formulation may actually facilitate rapid progress in phase II and III clinical studies. Prototype stability data *can* be used for IND filing and additional complicated analytical testing may or may not be required; if so, data generated can be qualified later and used for both release and stability studies. To summarize, long lead times and high cost in early stage development may be considered a built-in long-term investment meeting corporate goals right from Phase 1.

Conclusions

A newly discovered drug substance can only become a potentially successful pharmaceutical if it is determined to first be safe and effective. Systematic "step-by-step" pre-formulation safety and efficacy studies are thus very critical to the long-term clinical success of potential drug.

Carefully designed solid form selection via salt and polymorph screening supported by scientific data is very important in early phase work. Thorough elucidation of drug substance bulk properties can inform an effective choice of delivery technologies and formulations in a time- and cost-effective manner. Degree of preformulation characterization is directly proportional to transition rate from preclinical to clinical studies; *i.e.*, extensive, in-depth preformulation work speeds transition into later stage activities. Choice of drug product may require weighing corporate factors such as time and resource requirements.

Succinctly, J-Star can offer the best strategy and best scientific resources for developing your compound while always keeping in mind client needs and costs. J-Star Research can be a great partner in advancing your drug candidate from concept to reality, making new therapies possible.

Reference

Kaplan, Stanley A. "Biopharmaceutical considerations in drug formulation design and evaluation." Drug Metabolism Reviews 1, no. 1 (1972): 15-33.