An Overview of Biotechnology Product Development: What to do When and Why

Dr. Zahra Shahrokh

Hosted by the NINDS CREATE Bio Program

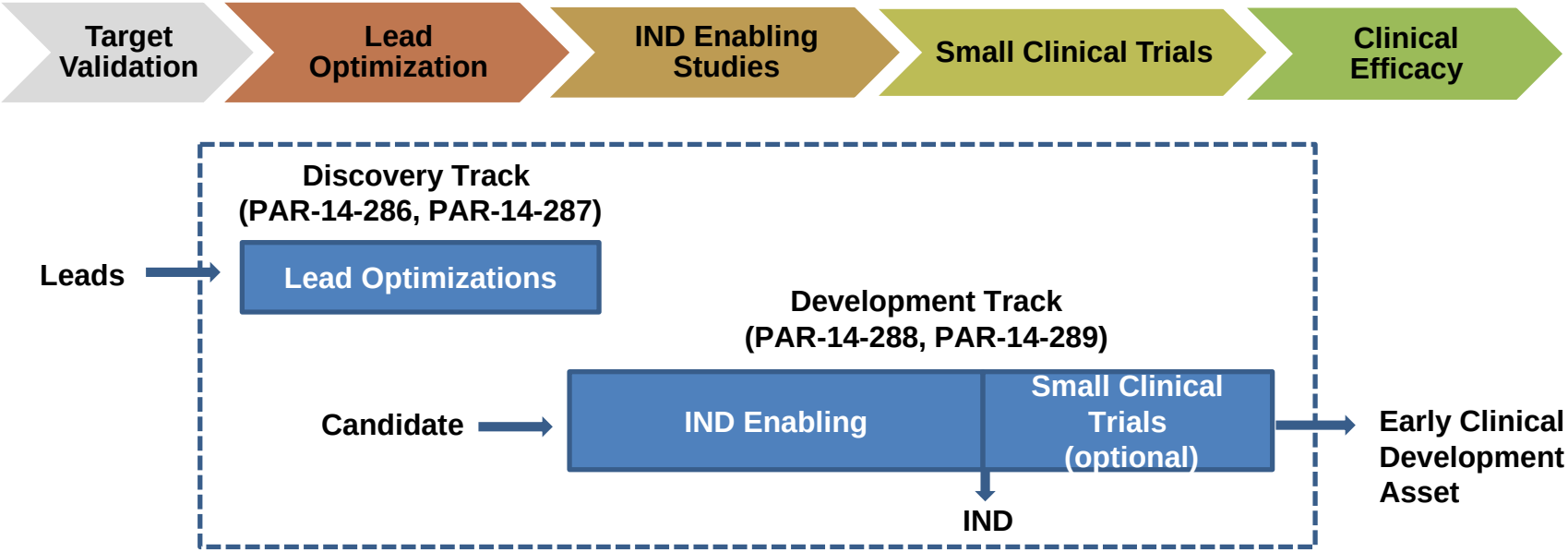
Contact: Hao Wang, PhD, Program Director
wangh16@ninds.nih.gov

NINDS Cooperative Research to Enable and Advance Translational Enterprises Biotechnology Products and Biologics (CREATE Bio)

Scope:

- Disorders that fall under NINDS mission
- Biotechnology products and biologics
  - Peptides, proteins, oligonucleotides, gene therapies, cell therapies, and emerging entities
- Lead optimization to early clinical trials

CREATE Bio Program



Purpose

- Discovery: Optimization of therapeutic leads to enable candidate selection
- Development: IND-enabling studies for IND submission and conduct early phase clinical trials

Cooperative agreement (U01, UH2/UH3, U44)

Next Application Date: February 11, 2016

National Institute of Neurological Disorders and Stroke

Logistics and Announcement

- The focus of this webinar is primarily on recombinant proteins
- This webinar will be archived on our website
- You will be muted during the webinar
- Please write your questions in the chat box to everyone
- Questions will be answered during Q&A session at the end of the webinar
- **2016 CREATE Bio Application Date:**
  - **February 11, 2016**
  - **Aug 11, 2016**

Dr. Zarah Shahrokh

- Zahra Shahrokh has 25 years experience in developing biotechnology products, with track record of global regulatory approvals of 5 recombinant proteins and clinical development of dozens of therapeutics. She is currently Chief Development Officer at STC Biologics and a CMC Consultant. Prior to that she was at Shire for ten years as Head of Pharmaceutical and Analytical Development and also as Head of CMC Program Management, where she built organizational capability from a start-up towards a multi-billion dollar commercial organization. Prior to that, she had technical and leadership roles at Genentech's Pharmaceutical R&D department.

- Zahra has a PhD in Biophysics from UC, Berkeley, dual BA in Chemistry and Physics from University of Pennsylvania, Philadelphia, and holds several patents and over 60 papers & invited presentations. She is an active Organizing Committee member for WCBP's "CMC Strategy Forum" that brings together regulators and biotech industry to present and debate current topics in product development.

Disclaimer

- The views expressed in this presentation are the views of the presenter and do not necessarily represent the views of the NIH.

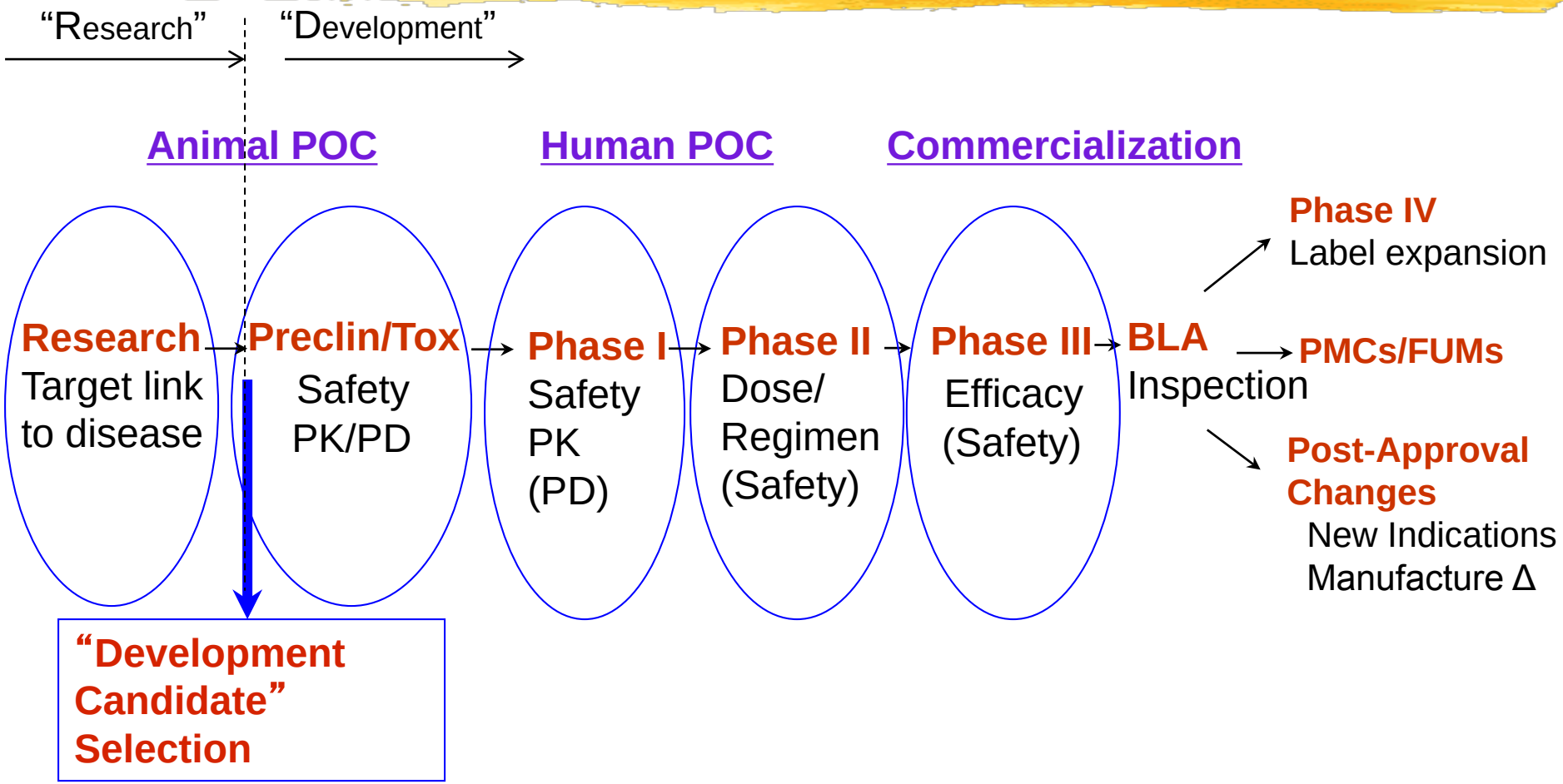
Presentation Outline

- Biologics Product Development Phases
  - Key questions and activities
- "Research" to "Development" Transition: *Development Candidate* Selection
- Pharmacokinetics-Pharmacodynamics 101
- Case Examples
  - Mis-steps in product development

Some Definitions

- TPP (Target Product Profile): intended indication and *in vivo* behavior of the drug, how made/stored, etc. (similar to "Package Insert" Information)
- PK (Pharmacokinetics): a drug's concentration over time, from administration until it disappears from blood compartment and tissue compartment of interest
- PD (Pharmacodynamics): a drug's impact on the disease state during the time period the drug is in the body
  - PD can be determined by either efficacy signal or a change in a biomarker
- "relevant species": an animal species whose tissues cross react with the drug candidate developed for humans
- Therapeutic window: The dose/regimen range which is efficacious but not toxic

Product Development Phases



IND = Investigational New Drug Application; BLA=Biologics License Application
PMC = Post-marketing commitments to the FDA; FUM = Follow-up-Measures for EMEA

Transition from Research to Development: "Development Candidate" Selection

Research

- *In vitro* POC
- Animal POC

Preclinical

Res "Development Candidate" Selection Dev

Development

- Manufacturing POC
- Human POC

- Link target to disease
- Screen different compounds for effect on Target (*in vitro*)
- Lab scale (µg to mg) prep of compound
- Test compound(s) in animal efficacy model(s)

- Define TPP
- Develop suitable cell line
- Identify compound with adequate stability, solubility, and PK
- Bench scale (mg to gram) prep of compound
- Check tissue cross reactivity to identify relevant animal species
- Define route, dose, regimen
- PK/PD/
- Pilot safety studies

- GLP Toxicology studies
- Scalable process development (gram to kg)
- Market demand, COG target
- GMP drug manufacture
- Link product characteristics to biology
- Clinical program detailed

TPP = Target Product Profile

Target Product Profile

- Intended to direct thinking about information needed to register a drug
- Living document throughout the development cycle
- Regulatory Agencies like to see TPPs (package insert items) in submissions

Preclinical Stage Level of Information

Efficacy related:

- Primary indication
- Target population (adult? what specific condition?)
- Treatment duration (acute, chronic?)
- Route of administration (IV, SC?)

  Regimen (daily? Monthly? bolus or infusion?)
- Efficacy (endpoints? biomarkers?)

CMC:

- Dosage form (liquid in vial? Prefilled syringe?)

Safety: Risk/Side Effect

Package Insert Level of Information

Efficacy related:

- Indications and Usage

  Dosage and Administration

Dosage Forms and Strengths

Contraindications

Warnings and Precautions

Adverse Reactions

Drug Interactions

Use in Specific Populations

Drug Abuse and Dependence Overdosage

Description

Clinical Pharmacology

Nonclinical Toxicology

Clinical Studies

How Supplied/Storage and Handling

Patient Counseling Information

index:

safety

Efficacy

CMC

*March 2007 Guidance for Industry and Review Staff, "Target Product Profile — A Strategic Development Process Tool"

6 Key Preclinical Steps to Development

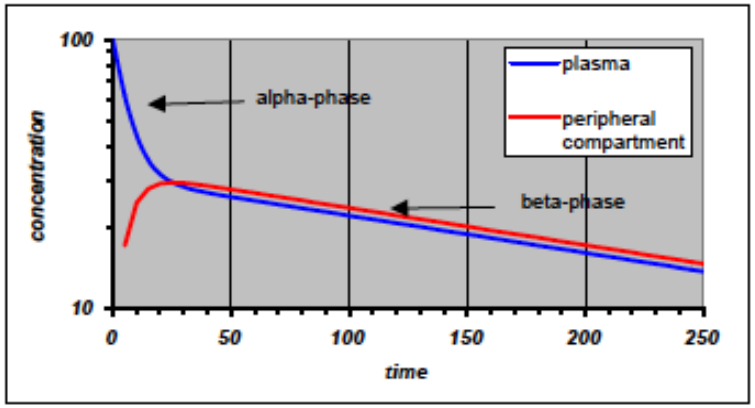
1. Identify a target that is related to a disease –literature, natural history, or own studies
2. Show that a ligand of the target (i.e. your compound) has a dose-dependent effect on this target
   - *in vitro* binds to it (is agonist or antagonist)
   - In *in vivo* animal models shows efficacy
3. Determine IC50 and IC90 of the ligand's binding to the target
   - IC90 should be the trough level when dosed *in vivo*
   - IC50 related to the efficacious dose at the site of action
4. Conduct tissue cross reactivity to identify "relevant species" for safety and PK-PD studies

Key Preclinical Steps to Development –continued...

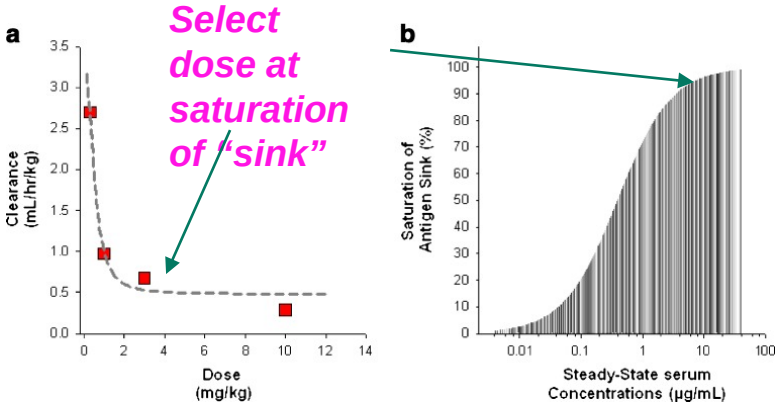
5. Conduct a single dose PK study (at different doses) in a small relevant species
   - determine MTD and select maximum dose below the MTD
   - plot AUC vs dose to identify where saturation of *in vivo* binding sites occurs (i.e. PK is linear); select minimum dose just at saturation
6. Conduct multiple dose study in diseased animal model
   - Establish PK-PD relationship to define regimen (the frequency of dosing where trough level reaches IC90, there is no drug accumulation, and efficacy is observed)
   - Determine “therapeutic window”, dose range/regimen where efficacy is observed but not toxicity

PK-PD Basics

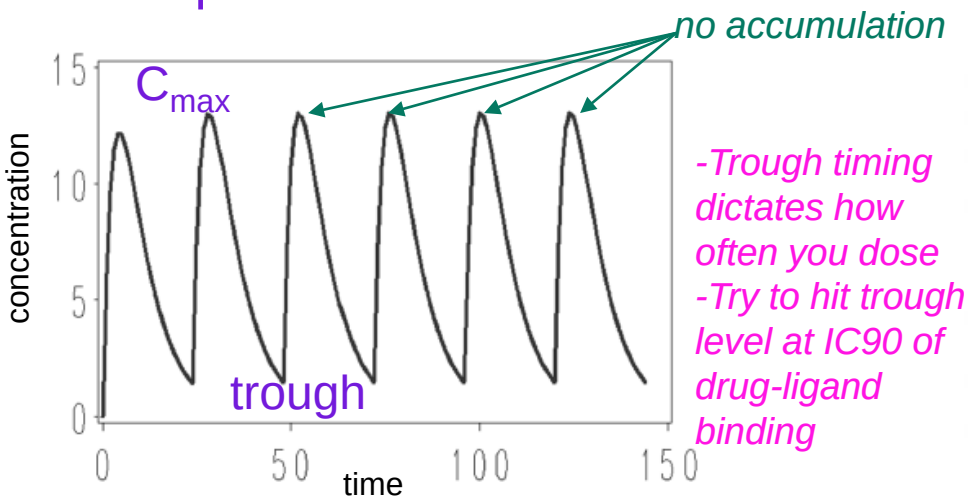
1. Single dose, two-compartment PK *typical of biologics*



2. Non-linear PK

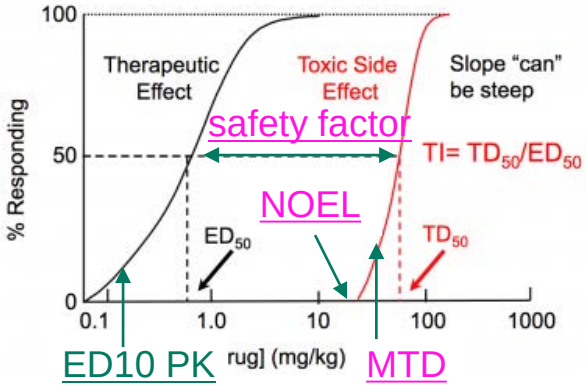


3. Multiple dose PK



-Trough timing dictates how often you dose
-Try to hit trough level at IC90 of drug-ligand binding

4. PK/PD/Toxicity



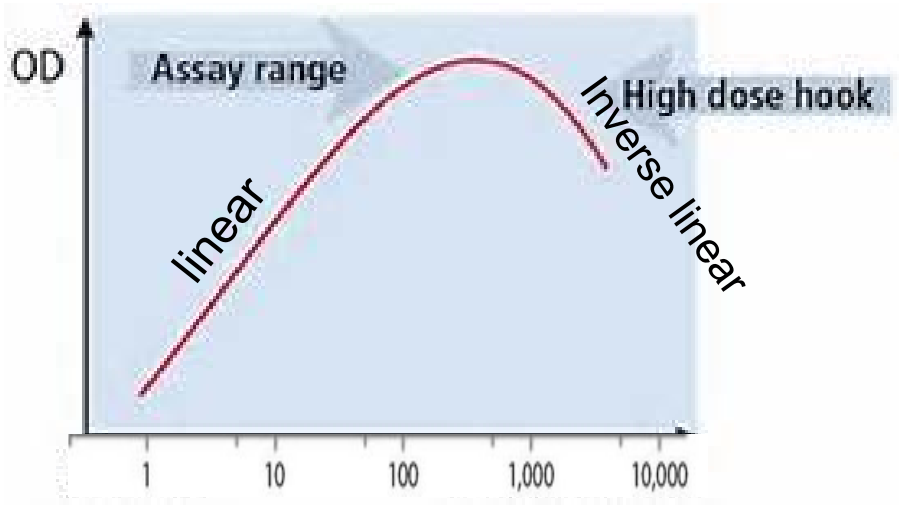
Case Example: When you can't detect tissue binding of drug

- Problem: Proteins may denature and lose native epitopes when added to formaldehyde-soaked tissue → loss of tissue binding
  - Hence you can't identify "relevant species"!

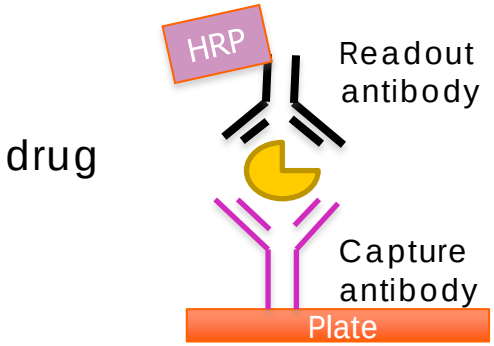
- Solution: Show biodistribution using autoradiography or PET imaging

A most common mistake in ELISA assay measurements: Hook Effect

- Hook effect problem: at too high concentration of analyte, the response is underestimated (inverse linear part of the curve)
- Solution: dilute the sample in the assay until you are in the linear concentration-response portion of the curve



Typical PK Assay Format

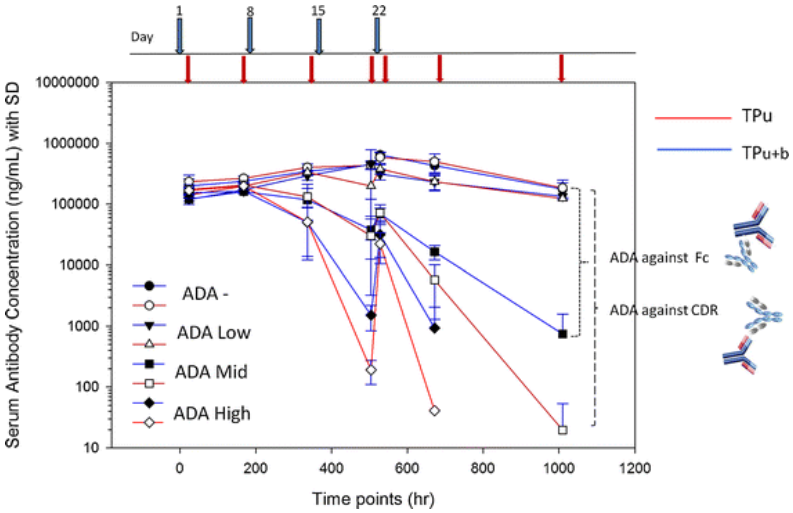


PK Assay Complications: Case of Circulating Blood Antigens/Ligands

- Many therapeutics have circulation ligands, which interfere with quantitation of the drug or its ligand
  - Need to measure “bound” and “free” drug
  - Need non-blocking antibodies
- When your drug is a human antibody, how do you do the readout?
- Example: Trastuzumab (anti-HER2); Patients have shed antigens (HER2)
  - To measure bound, capture with anti-HER2 that does not get blocked by trastuzumab-bound HER2 bound, then readout with anti-human IgG1
  - To measure free, capture with HER2 and readout with anti-human IgG1
- Example: anti-eotaxin IgG4 product; blood eotaxin interferes with PK assay
  - Use eotaxin to capture Bert, readout with monoclonal anti-Bert that does not detect human IgG4. Sample has to be diluted to reach low enough eotaxin for assay to work

What is ADA assay and why develop it?

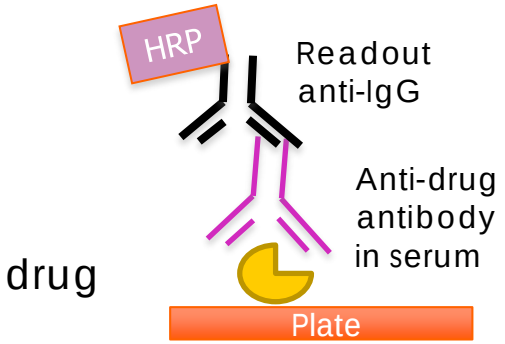
- ADA = anti-drug antibody
- Immunogenicity is a safety concern of biologics
- Upon repeat injection of biologics, anti-drug antibodies may be formed
- The ADAs may neutralize the activity of drug, leading to loss of efficacy
- The ADAs might result in allergic or anaphylactic reaction
- Leads to unusual PK profile



ADA Assay Complications

- Where do you get anti-drug antibodies from to develop the assay?
- Start early –immunize different species (including monkeys)
- Per FDA requirements, the sensitivity of the assay has to be 250-500 ng/ml, so need a good anti-drug antibody to develop the assay
- The readout antibody has to be specific (not recognize other IgGs in human serum)

Typical ADA Assay Format



Toxicology Studies for Recombinant Protein Therapeutics

- Identify "relevant species"
  - Cell/receptor binds with similar IC50 as human cells
  - Tissue cross reactivity (30 key organ types) –identify potential organ specific toxicity (do safety pharmacology)
- ICH S6: For recombinant proteins two relevant species should be used*.
  - May justify one relevant species if two is not found, or for long term tox studies when both species show similar tox profile
- Non-relevant species not required.
  - typically cynomulgus or rhesus monkey is used
  - if poor cross reactivity, may need to use Chimpanzee or transgenic animals
- Include a dosing period and a recovery period
- In contrast, small molecules require tox studies in one relevant species (e.g. dog for specific tox findings) and one non-relevant species (e.g. rodents for general tox findings)

ICH S6(R1) Preclinical Safety evaluation of Biotechnology-derived Pharmaceuticals

Case Example: Can't find a cross reacting species?

- **Problem**: Human recombinant protein did not react with tissues of any animal species
  - Cannot predict toxicity in animal species

- **Solution 1**: Make animal version of protein to do tox studies
- **Solution 2**: Humanize the mouse to do tox study (KO mouse receptors and knock-in human gene)

Early tox studies

- Find relevant species (shown tissue reactivity)
- Age and physiological state
- Can use non-naïve animals
- n=3 per dose male animals for starts
- Try three doses: low, mid (efficacious), high (near MTD)
  - go factors of 10 (or 3)
  - For most proteins MTD is not reached; find NOAEL (no observed adverse effect level)
  - Maximum feasible dose (based on solubility) can be justified if dosing is multiples of potential human dose
- Clinical observations, blood chemistry, histopathology
- Immunogenicity (to interpret the data)

Don't Ignore Data

- Early research studies showed a drug resulted in eye loss in drosophila
- Drug proceeded to preclinical and clinical stage
  - Clean tox findings
  - Good efficacy
- Some patients went blind!!

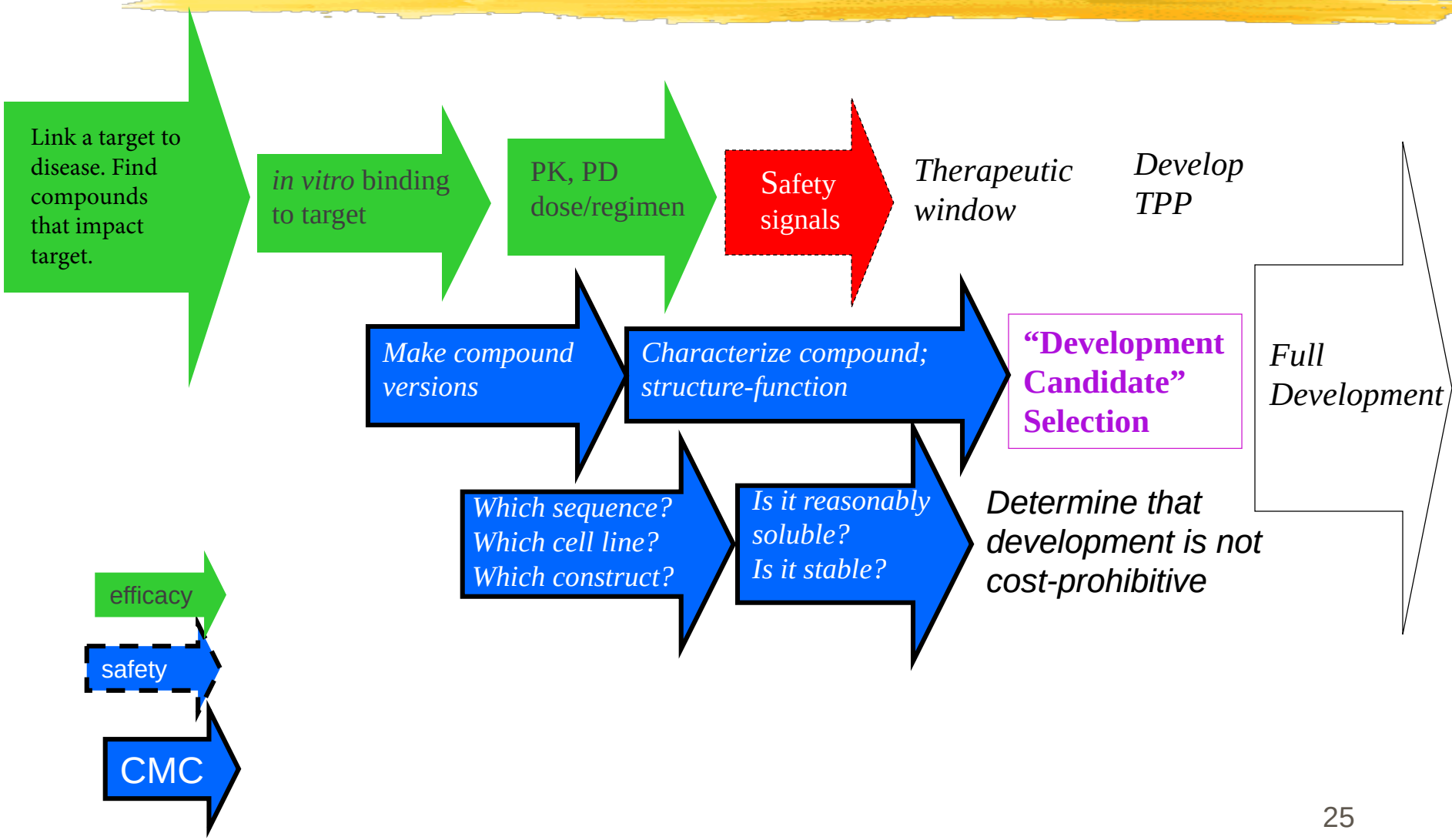
If something unusual is observed, investigate it –especially when safety is implicated

Solicit CMC experts early in your programs

- CMC =chemistry (*characteristics, physicochemical properties*), manufacturing (*how to make it*), controls (*how to keep it reproducible and traceable*)

- What do CMC folks bring to the table?
  - Know how to make cell lines that are stable
  - Know how to grow cells to yield high titer reproducibly
  - Know how to purify proteins reproducibly with high purity
  - Know what buffer/excipients to put in the protein and how to handle it so it is happily stable reproducibly
  - Know how to characterize the protein's structure and relate it to bioactivity (MS, HPLC, glycans, impurities, biophysical properties)

Linking Preclinical and CMC Studies



Case Example: IND-enabling Development "No-Go"

- Primate tox studies showed severe nephrotoxicity; program dropped
- Biophysical studies had shown human cytokine trimerizes human receptors (required for bioactivity), but only dimerizes monkey receptors
- Studies showed that dimerized receptor by monkey cytokine results in cellular toxicity (not seen with human cytokine)
  - Single amino acid difference between human and monkey cytokine impacted receptor oligomerization state and subsequent toxicity profile
- Monkey cytokine was made and used for repeating monkey tox studies
  - Clean tox studies observed --> program was revived and proceeded to clinical development

Formulation and analytical/biophysical characterization can rescue a program

Case Example: Uninformed "Go" Decision

- Enzyme showed great efficacy in animal models
- Program moved to IND-enabling process development stage
- Molecule made in PD did not show animal efficacy and had significant solubility issues
- Investigation indicated that previous efficacy studies were done with mouse enzyme, while PD was developing human enzyme
- Human enzyme had significantly different physicochemical properties (different charge variants and glycosylation) than mouse enzyme
- Human enzyme did not have the same binding affinity to mouse cells *in vitro*
- 1.5 years and $1.5 mil spent, program dropped

Poor basis of Development "go decision" with no analytical characterization studies

Case Example: Intrathecal Delivery of an Enzyme for ERT

- Clinical needs:
  - Dose no more than once per month
  - Delivery volume per injection < 1 mL
  - Need a device in children
  - Should filter solution prior to administration

- CMC implications:
  - Achieve 100 mg/ml solubility (and stability)
  - Limited formulation composition to deliver into the CNS
  - Endotoxin limits for CNS delivery is 20x lower than for IV: 0.2 EU/kg -→0.05 EU/mg is challenging to manufacture
  - Develop an implantable IT device that is small and is flexible to grow as the child grows

Feasibility Hurdles for IT delivery of an Enzyme

- Issues:
  - Half life the natural enzyme only 2-3 days in the CNS cells
    - Should one engineer the molecule for longer half life in the CNS?
  - Solubility of the natural enzyme only 15 mg/ml
    - Should one develop a molecule with higher solubility?
  - Only salt and water has been approved as CNS formulations
    - Conduct pilot tox studies with other formulations to make the program feasible

Case Example: Cytokine Development Requiring "CMC" Knowledge

- A promising anticancer cytokine was identified based on in vitro studies
- Issues:
- Several variants were being investigated; but variable/inconsistent efficacy results were observed in animal models
  - Purified proteins were kept refrigerated until dosing (stability not known)
  - Animals dosed via Alzet mini-pump delivery system (drug at 37° C in PBS for 20 days)
- Investigations/Solutions:
- pH-temperature stability studies showed aggregation at 37° C in PBS (pH 7.2); pH 5 was optimal
- New formulation provided consistent animal study results
- Additional structure and stability studies of variants also helped identify optimal "development candidate"
- Project moved into IND-enabling development stage

Summary: Preclinical Studies with "Development" line site

- Target ID: characterization tools
- Efficacy models and *in vitro* biology
- Molecule design: structure-activity
- Expression system: for research purpose vs "developability"
- Producing materials: scale, stability, purity, and reproducibility
- Identifying dose and regimen: PK/PD analysis for "developability"
- Safety assessment: *in vitro* and preliminary *in vivo* studies

Summary: Common Mis-Steps in Product Development

- "R" & "D" disconnect
- Wrong molecule as basis for development go decision
- **Little to no formulation development**
- **Inadequate analytics and product characterization**
- **Little structure-function understanding**

Effective Biotech Product Development integrates diverse disciplines of biological, CMC, and clinical sciences

Questions?

Hao Wang, PhD

wangh16@ninds.nih.gov

CREATE

Cooperative Research to Enable and Advance Translational Enterprises

http://www.ninds.nih.gov/funding/areas/translational_research/CREATE-Bio.htm