FDA Expectations for Toxicology Support of Clinical Trials and Marketing

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Nonclinical Toxicology
Aclairo Pharmaceutical Development Group, Inc.
Outline

- Relevant ICH Guidelines
- Standard Development – Small Molecules
- Cancer Indications
- Biologics - CDER
- Biologics and Novel Therapeutics - CBER
- Pediatric Indications – time permitting
FDA Follows ICH Guidelines

- **ICH M3(R2)** - Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals - Step 4
  - Describes the timing of all nonclinical studies needed to support each phase of clinical development and marketing
- **ICH S9** – Nonclinical Evaluation for Anticancer Pharmaceuticals
  - Describes specific considerations for oncology products
- **ICH S6 (R1)** - Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals - Addendum (R1): Step 4
  - Describes additional considerations for Biologics - CDER
Drug Development Phases

**Discovery**

- **Non-Clinical – Toxicology, Safety Pharm, DMPK**
  - **IND**
    - Genetic Tox
      - Ames Test
      - Mouse Lymphoma
      - In vivo Micronucleus
    - Repeat Dose Tox
      - 28 Day rodent
      - 28 Day non-rodent
    - Safety Pharmacology
      - Rat Irwin – neurobehavior
      - Rat Respiratory
      - Non-rodent cardiovascular
        - in vitro hERG assay

**Phase I**

- **Healthy volunteers**
  - Sub-chronic Tox
    - 3-month rodent
    - 3-month non-rodent
  - ReproTox
    - EFD - rodent
    - EFD - non-rodent

**Phase IIa / IIb**

- **Patients, Dose-ranging**
  - Chronic Tox
    - 6 - month rodent
    - 9 - month non-rodent
  - ReproTox
    - Male Fertility - rodent
    - Female Fertility - rodent

**Phase III**

- **Patients, Definitive**
  - Carcinogenicity
    - 2-year rat
    - 2-year mouse
  - ReproTox
    - Pre-/postnatal development
      - - rodent

**[WOCBP]**

**IND = Investigational New Drug application – permission to dose people**

**NDA = New Drug Application – permission to market drug**

**BLA = New Biologics Application – permission to market biologic**
# Standard Duration of Nonclinical Toxicity Studies to Support Clinical Trials (ICH M3(R2))

<table>
<thead>
<tr>
<th>Max Clinical Trial Duration</th>
<th>Pivotal (Definitive) Toxicology Study Duration</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Rodents</td>
</tr>
<tr>
<td>≤2 Weeks</td>
<td>2 weeks</td>
</tr>
<tr>
<td>2 Weeks to 6 Months</td>
<td>Same as clinical trial</td>
</tr>
<tr>
<td>Greater than 6 Months</td>
<td>6 months</td>
</tr>
<tr>
<td>Duration of Indicated Treatment</td>
<td>Toxicology Study Duration</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td></td>
<td>Rodents</td>
</tr>
<tr>
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</tr>
<tr>
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</tr>
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Nonclinical Toxicology Package Overview

- Evaluate 1 rodent (usually rat) and 1 non-rodent (usually dog) species
  - Should be pharmacologically active (at least one species)
  - Should have some ADME information for each
  - Monkey usually only used after de-selection of dog
- Range Finding Studies:
  - Goal – Select doses for definitive studies; usually non-GLP
  - Observe general toxicity, survivability, target organs, and TK (toxicokinetics)
  - Define non-toxic & toxic dosages
    - Ideally define the maximum tolerated dose (MTD)
    - Make sure to push the dose
Definitive/Pivotal General Toxicity Studies

• Goals:
  • **Identify toxicities** to guide clinical monitoring
  • Identify no-observed-adverse-effect-level (NOAEL)
  • Calculate safety margins relative to intended clinical exposures
  • Set safe starting doses in the clinic

• Study Design:
  • 3 Dose groups and vehicle control
  • Generally half-log spacing of doses (based on TK exposures – AUC)
  • N = 10/sex/group for rodents (could be larger for longer studies)
  • N = ~4/sex/group for non-rodents

• Endpoints:
  – Clinical pathology, ophthalmology, cardiovascular evaluations (non-rodent)
  – Terminal necropsy – full histopathology
  – Recovery groups (Control and HD) – on 1 study ≥ 4 weeks duration
    • N=5/sex/group rodents; 2/sex/group non-rodents
Selection of High Dose (ICH M3(R2))

- High dose should show toxicity (adversity) in each study – should be considered the maximum tolerated dose (MTD)
  - Justify based on results in earlier studies
  - Toxicities may occur at lower doses in longer studies - death
  - **ex)** liver toxicity – generally tolerated, doesn’t progress – use same dose
  - **ex)** cardiac toxicity – could get worse – consider lowering the dose

- Other options for low toxicity molecules (e.g., mAbs):
  - Maximum feasible dose – e.g., an i.v. formulation at the maximum solubility and dosing volume
  - Large exposure margins over intended clinical (~50-fold AUC)
  - Limit dose of 1000 mg/kg/day
    - Provided at least 10-fold clinical margin and clinical dose of < 1g; other wise limit dose of 2000 mg/kg/day
  - PD Target saturation and fold-multiples – biologics (mAbs)
Genotoxicity Studies – ICH S2

• To test for mutagenicity and clastogenicity (strand break) potential
• Generally conduct the following 3 tests:
  – In vitro Ames – mutation test in multiple strains of bacteria (+/- metabolic activation)
  – In vitro mouse lymphoma or human lymphocyte (+/- metabolic activation) – genetic damage
  – In vivo mouse micronucleus – genetic damage
• Some flexibility in how to conduct – can bolt in vivo test onto general toxicity study
Safety Pharmacology – ICH S7A / 7B

- Evaluates physiologic changes related to pharmacology (PD) that could cause acute effects in Ph1 subjects
  - Not conducted at MTD, but mild toxicity at high dose; doses can be in clinical range
  - 3 doses and control; generally single dose administered
- Acute Neurotoxicity (Irwin test) – rats
  - Functional observational battery – autonomic, sensory/motor, behavior
- Cardiovascular
  - In vivo in non-rodents – ecg, QTc prolongation, HR, blood pressure, etc.
    - Small Molecules - Latin Squares design – all animals get all doses
  - In vitro – hERG (human potassium channel), patch-clamp test
- Respiratory
  - Stand-alone in rodent, or bolted on to non-rodent CV study
Developmental and Reproductive Toxicity (DART) (ICH S5)

- Embryo-fetal development (EFD, Seg 2)
  - Rodent and non-rodent (usually rabbit)

- Fertility and early embryonic development (FEE, Seg 1)
  - Rodent
  - Can run as separate studies in males and females or combined

- Pre-/postnatal development (PPN, Seg 3)
  - Rodent
Carcinogenicity Studies – ICH S1

• For chronic indications
• Evaluates potential of drug to cause cancer
• Traditionally – 2 separate studies (mouse and rat)
  – Generally need 2-week and 3-month mouse tox studies to support dose selection
  – 2 years duration (life-time dosing)
• Can sometimes replace mouse study with shorter mouse transgenic (hRAS) study
• Start with 20-40/sex/group
• Special statistics needed to evaluate tumor production
Estimating Safe Starting Dose – Phase 1

- “FDA Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers, July 2005”

- Submit with IND

- Calculate Human Equivalent Dose (HED) of NOAEL in animals
  - Use $\text{mg/m}^2$ conversion factor ($k_m$) to account for body surface area differences
  - For certain drugs (e.g., mAbs) – use mg/kg without conversion
  - ex) rat NOAEL = 200 mg/kg/day; HED = 200 / 6.2 rat $k_m$ = 32 mg/kg (~1900 mg)

- First Ph 1 clinical dose should be ~10-fold lower than NOAEL HED
  - Apply a greater safety margin in certain cases (e.g., steep dose-response)
  - ex) start at 1900/10 = 190 mg
  - Dose-escalate to HED of animal NOAEL (ex, 1900 mg)
  - Not generally allowed to go above the HED of the animal NOAEL

- Note: Important to define minimum pharmacologically active dose (mPAD) and exposures in animals and predict human PAD/exposure (AUC)
  - ex) If predicted PAD in humans is << NOAEL, dose can be lowered in Ph1
CMC Considerations: Quality of Drug Substance
CMC / Pharmaceutical Quality (new name)

- CMC = Chemistry, Manufacturing, and Controls
- FDA takes quality very seriously! Drug should not contain contaminants that could be toxic – impurities, degradants, etc.
- Chemists and CMC Regulatory Affairs specialists should be consulted to comply with quality expectations

<table>
<thead>
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ACLAIRO
pharmaceutical development group, inc.
CMC for the Toxicologist

- Impurities must be tracked and controlled at specific levels by the time of the NDA – to set manufacturing specifications
  - Impurities = Starting materials, intermediates, degradants, solvents, etc.
- During drug development chemists and toxicologists must work together to ensure that the levels of all contaminants are “qualified” for safety
- ICH M7 – potential impurities should be tested with “in silico” methods to predict mutagenic potential (e.g., DEREK, Leadscope)
  - If “in silico” positive – must run Ames in vitro genotox test
  - If positive in Ames – must control at low levels in clinical trials and in the marketed batch
CMC for the Toxicologist, continued

- ICH Q3A – Q3D: Impurities in drug substance, impurities in drug product, residual solvents, and inorganic impurities
- At the time of the NDA – all non-mutagenic impurities must be reported, identified, or qualified if they reach certain levels
- Qualified = were present at that level in a toxicology study
- If not qualified – level (specification) must be dropped, or a toxicology study done with the impurity

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<tr>
<td>≤ 2g/day</td>
<td>0.05%</td>
<td>0.10% or 1.0 mg per day intake (whichever is lower)</td>
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<td>0.03%</td>
<td>0.05%</td>
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Example of Impurity Assessments

- Drug X has 3 impurities in the final batch: A, B & C
  - Impurity A is at **0.07%** of the drug substance
  - Impurity B is at **0.11%** of the drug substance
  - Impurity C is at **0.3%** of the drug substance
- Impurities B and C must be identified and reported (>0.1%)
- Impurity A must also be reported (>0.5%) – use HPLC RT
- Impurity B & C – must be evaluated for mutagenicity using “in silico tests”
  - If positive – do Ames test
  - If Ames test positive – control as a genotoxic impurity (ICH M7)
- Impurity C – must be qualified or controlled at 0.15%
  - 1-month toxicity study had 0.5% of Imp C
  - Therefore, Imp C is qualified - manufacturing specification can be set at 0.3%

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Oncology Indications – ICH S9
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• *ICH S9: Nonclinical Evaluation for Anticancer Pharmaceuticals, March 2010*

• “…for pharmaceuticals that are intended to treat cancer in patients with serious and life threatening malignancies… referred to as patients with advanced cancer.”

• Supports trials in patients for whom other treatments have failed

• Phase 1 in patients not healthy volunteers

• Minimal nonclinical work to initiate Ph 1

• Ph 2 can proceed without additional nonclinical studies

• **Not** for long-term treatments to reduce cancer recurrence (i.e., patient in remission)
  – Full nonclinical program would apply
## Standard vs. Oncology Package

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<th>Oncology (including Biologics) – S9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-month Ph1 Studies up to 6/9 months for NDA</td>
<td>1-month studies Ph1, Ph2 3-month studies for Ph3 and NDA</td>
</tr>
<tr>
<td>– NOAEL required</td>
<td>– No NOAEL required</td>
</tr>
<tr>
<td>Safety Pharm and Genetox needed</td>
<td>Safety pharm “bolted on” to general tox; No gene tox needed</td>
</tr>
<tr>
<td>Starting dose based on NOAEL</td>
<td>Starting dose based on 10% of severely toxic dose in animals (STD 10)</td>
</tr>
<tr>
<td>Full DART package</td>
<td>– Dose-escalate above animal NOAEL to MTD in humans</td>
</tr>
<tr>
<td>Carcinogenicity studies</td>
<td>DART - only need EFD study – one species only if positive</td>
</tr>
</tbody>
</table>
Biologic Therapies
Types of Biologics – Molecules found in/made by biological systems

• Monoclonal Antibodies (can inhibit or activate a target)
  – Mouse, chimeric, humanized, whole or fragments, etc.
• Cytokines and Growth Factors
  – Interferons, interleukins, colony stimulating factor
• Hormones
  – Growth hormone, insulin, erythropoietin
• Vaccines
  – Proteins or peptides, DNA plasmids
• Gene and Cell Therapy products
  – Viral and non-viral delivery systems, genetically engineered cells, stem cells
• Blood products
  – Albumin, thrombolytics, fibrinolytics, clotting factors

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### Differences Between Biologics and Small Molecules

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<th><strong>Small Molecules</strong></th>
<th><strong>Biologics</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Small: &lt; 700 daltons</td>
<td>Large Macromolecules:</td>
</tr>
<tr>
<td>Generally lipophilic - Can cross biological membranes, including the placenta and/or VYS</td>
<td>– Peptides – ~ 1,000 to 10,000 dal</td>
</tr>
<tr>
<td>Well defined structures and relatively stable</td>
<td>– Proteins – ~ 20,000 to 60,000 dal</td>
</tr>
<tr>
<td>Rapidly metabolized; require daily dosing</td>
<td>– mAbs - ~150,000 dal</td>
</tr>
<tr>
<td>Toxic response related to chemical structure and exaggerated pharmacology</td>
<td>Less lipophilic - Generally either can’t cross membranes or use receptor-mediated mechanisms</td>
</tr>
<tr>
<td>Less likely to illicit an immune response (be immunogenic)</td>
<td>Complex physiochemical characteristics and heat sensitive</td>
</tr>
<tr>
<td>More likely to have activity in multiple species</td>
<td>Degraded over time, can be very long acting; may need intermittent dosing</td>
</tr>
<tr>
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<td>Toxic response related to exaggerated pharmacology</td>
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<td>More likely to be immunogenic</td>
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<tr>
<td></td>
<td>More often show species selectivity</td>
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Considerations for Selecting an Appropriate Animal Model and Study Design for Biologics

• **Pharmacologic Activity**
  – Toxicity based on chemical structure is not expected, so a **must use pharmacologically relevant species**
  – mAbs, cytokines and growth factors, etc. – should cross-react with the appropriate target in the animal species
  – The pharmacologic target should have a similar function in the animal
  – Vaccines should elicit an appropriate immune response

• **Immunogenicity**
  – Are neutralizing antibodies (NAs) formed?
  – Would an immune response be elicited that would significantly impact the health or survival of the animal?

• **Toxicokinetics**
  – If NAs formed, can we still maintain adequate exposure?
  – How does TK determine my dosing regimen?
Special Considerations for Biologics – ICH S6(R1)

- Remember: ICH S6 – only applies to CDER-regulated biologics
- **Toxicity Studies** - must use pharmacologically relevant species!
  - ICH S6(R1) – prefer use of clinical candidate therapeutic
  - Use 2 species if both relevant (rodent and non-rodent)
  - Single species acceptable if only 1 species is relevant (e.g., NHP)
  - Animal homologues acceptable, but must be well characterized (considered a separate molecule) - best used if no other choice
  - Disease models can also be used to evaluate safety – low expressing targets (ex - Alzheimers – only expressed in disease)
- For FTIH studies – use 2 relevant species if possible
  - If species responses are the same, a single species can be used for longer studies (preferably rodent, if possible)
- Dosing frequency should be based on PK
mAbs against non-mammalian targets (bacteria, viruses)
- One short-term safety study in single species (no reprotox)
- Alternatively – safety endpoints collected in disease model

Immunogenicity – measure anti-drug antibodies (ADA)
- Used to explain changes in PK or PD or animal toxicity
- **Not** good indicator of human responses – Predict based on pharmacology

Tissue Cross-Reactivity Studies
- Were typically done in animals to predict toxicity or select species
- **Revised ICH S6** – *not of value in animals, but should be done on a panel of human tissues before Ph 1*
- To find a relevant species – pharmacology binding assay with species-specific target more useful than tissue cross-reactivity
Special Considerations for Biologics, cont

- Safety Pharmacology – some assessment expected, but **could be bolted on to general toxicity studies** – long half life for mAbs
- Genotoxicity Assessment – **not applicable to biologics**
- Carcinogenicity
  - Weight-of-evidence assessment for level of concern should be conducted – pharmacology class, target biology, transgenics, etc.
    - **Several immunosuppressive mAbs have cancer risk in humans!**
    - If mechanism raises concern (e.g., immunosuppressant, growth factor) – address with labeling and risk management practices
    - If this is insufficient information, some additional short-term studies may be warranted
    - **2-year animal carc study/transgenic mouse studies not considered warranted/practical**
- Reprotox – differences described later in talk
# Small Molecules vs. CDER Biologics

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<th>Small Molecules</th>
<th>Biologics (CDER)</th>
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<tr>
<td>• 2 species (rodent/non-rodent)</td>
<td>• Only pharmacologically relevant species for tox - could use 1 species; could use homologues</td>
</tr>
<tr>
<td>• In vivo and in vitro safety pharmacology</td>
<td>• No in vitro safety pharm; can bolt on safety pharm to tox study</td>
</tr>
<tr>
<td>• Genetox evaluations</td>
<td>• No genetox evaluations</td>
</tr>
<tr>
<td>• Carcinogenicity studies (chronic indications)</td>
<td>• No carcinogenicity studies, but weight of evidence evaluation expected – appropriate labeling</td>
</tr>
<tr>
<td>• Safe starting dose based on NOAEL of animal studies; HED based on mg/m² conversion</td>
<td>• Safe starting dose based on NOAEL and PAD or MABEL;</td>
</tr>
<tr>
<td></td>
<td>• HED based on mg/kg</td>
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Biologics - CBER
CBER Office of New Drugs Organizational Chart

Director
Peter W. Marks, M.D., Ph.D.
(DKKB)

Office of Management
Director James M. Sigg
(DKKBB)

Office of Compliance and Biologics Quality
Director Mary Anne Malarkey
(DKKBC)

Office of Blood Products

Office of Vaccines

Office of Biostatistics and Epidemiology
Director Steven A. Anderson, Ph.D., M.P.P.
(DKKBJ)

Office of Communication, Outreach and Development
Director Lorrie H. McNeill
(DKKBH)

Office of Tissues & Advanced Therapies
CBER - Office of Tissues & Advanced Therapies

• Formerly – Office of Cell, Tissue and Gene Therapies
  – Recently – blood cell products moved into this division

• Products covered:
  – Allergenics
  – Blood cells
  – Gene Therapy
  – Human tissues
  – Human Cellular Products
  – Therapeutic vaccines – against mammalian targets (ex – oncology)
  – Xenotransplantation Products (from animals)
  – Medical devices and tests used to keep blood and cells safe from viruses and other infectious agents
Guidance for Industry on Cells, Tissues and Genes

• Prior to 2013 – No FDA Guidance on development of these products

• **BioSafe** - preclinical section of **BIO** (Biotechnology Industry Organization)
  – **Organized annual F2F meetings with CBER starting in 2008**
  – Need for clear guidance on development of CBER products
  – Meetings designed to discuss current issues facing researchers/developers and get CBER input

• The following BioSafe working groups were formed and proposed topics for each meeting:
  – Blood products
  – Gene therapy
  – Cell therapy
  – Vaccines

• **Many of the topics discussed have been included in 2013 guidance!**
Cell, Gene, Tissue (CGT) Products – Safety Assessment Principles

• Guidance for Industry: *Preclinical Assessment of Investigational Cellular and Gene Therapy Products*, Nov 2013
  – Does not apply to – autologous human tissues or cells (put back into the same donor) [see 21 CFR Part 1271]
  – Does not apply to CDER-regulated biologics

• General Principles:
  – Intrinsic properties (materials and mechanisms of action) different from drugs
  – Typical ADME principles – may not apply
  – Traditional standardized safety testing for drugs not always applicable
  – CBER uses flexible, science-driven review process
    • Some aspects of ICH S6(R2) can be applied as appropriate
  – Recommendation – early and frequent communication with CBER staff

• Pre- Pre-IND meetings welcomed and expected!!
CGT Product Preclinical Study Considerations

• Preclinical objectives – Appropriate animal model:
  – Biologic plausibility
  – ID of biologically active doses in animals; and safe doses and dosing regimen for clinical trials
  – Reasonable safety and feasibility of the proposed route of administration
  – Patient eligibility
  – Physiologic parameters guiding clinical monitoring
  – Patient and public safety

• Combining of animal efficacy and safety studies encouraged
Use **final clinical CGT product and delivery system** in pivotal animal studies where possible

**Animal Model Selection Key** - Animal species must exhibit the following:
- Comparable physiology and anatomy to humans
- Similar infectivity/replication of viral vectors for gene therapy
- Immune tolerance to CT product or human transgene of GT product
- Feasibility of clinical delivery procedures
- **Note**: non-standard species (e.g., transgenics; unusual species) may be acceptable; could use a combination of species, but not mandatory
- All these attributes must be **demonstrated in pilot studies** to provide the rationale for species selection
- Animal surrogate product could be acceptable if no acceptable species

Disease models may be used for both efficacy and safety assessment in the same study
- Consider limitations of this approach (limited HC data, variability of model, etc.)

In vitro studies encouraged where possible to reduce animal use
CGT Product Preclinical Study Recommendations, cont

• **Proof-of-Concept (POC) Studies** – confirm: effective dose range; route of administration and dose schedule; putative MOA and biological outcome
  – Combination of in vitro and in vivo studies (disease model) recommended

• **Toxicology Studies**
  – Must use biologically active species
  – Use of disease models encouraged vs. traditional healthy animals
    • In addition to or instead of
  – **Mimic proposed clinical trial as closely as possible** – same dose route, dosing schedule, delivery system
  – **Multiple dose levels bracket the clinical dose** – rely on POC studies
  – Multiple sacrifice timings – capture acute, chronic, delayed-onset toxicity – could be done all in the same study
  – Traditional toxicity endpoints – clinical exams, BW, FC, clin path, histopath
  – Additional parameters specific to CGT product
CGT Product Delivery Systems

• CGT Products often have novel delivery systems – **devices**
• Should be identical to the clinical delivery device
• Safety must be established for the delivery device
  – IND submission – should state if a Device Master File (MAF) has been submitted to CDRH for the delivery device
    • **Note**: Sponsor must get permission to reference MAF
  – CBER consults with CDRH to ensure safe use in humans
  – If MAF doesn’t exist, CDRH recommends needed information
  – Large animals may be best to evaluate safety of delivery device
  – Published studies may also be referenced
CGT Product – Later Clinical Development

• Additional toxicity studies are not necessarily needed to support longer clinical trials

• Would need to conduct bridging study for the following reasons:
  – Change in manufacturing/formulation of product
  – Change in dosing regimen or patient population

• Reproductive Toxicity – not always needed; will depend on product type and/or patient population

• Carcinogenicity/tumorigenicity – no 2-year bioassays required
  – Specific recommendations for each type of product – see references in Guidance document
Cell Therapy – Specific Recommendations

- Types of CT Products:
  - Stem cell-derived
  - Mature/functionally differentiated
  - Induced pluripotent stem cells – have characteristics of both
  - Cell-device combinations, e.g., cells on scaffolding
    - Don’t forget biocompatibility assessment of device elements
Cell Therapy – Specific Recommendations

• Safety Concerns:
  – Theoretically more concerns with less differentiated products
  – Do they reach their target? Where else do they go?
  – Do they stay intact, or do they change, differentiate or transform?
  – Integration? Tumorigenicity?
  – Effect of scaffolding on nature of cells

• Study Design Elements:
  – Animal models to overcome immunogenicity with long-term testing
    • May need immunodeficient animals or animal homologue to test – requires thorough characterization
  – Need way to identify cells after implantation
    • PCR
    • Imaging – helps to follow cells over time in the same animal
Hot Topics in Cell Therapy

1. **Immune Responses to Cell Products** – Animal Model Selection
   - What happens when animal rejects human cells? - Cannot test long-term effects – may under-predict effects in humans
   - Immuno-suppressed or Immuno-compromised animal models may be needed to allow survival of cells for study
   - Immuno-compromised models could include:
     - Long-term drug-induced immunosuppressed large animal
       - **Pro** – tolerates human doses of cells and human delivery systems
       - **Con** – difficult to immunosuppress large animals – animals susceptible to lymphoma or infection
     - Drug-induced immunosuppressed or immunocompromised rodent – healthy or disease models
       - Immune-mediated pathology difficult to assess

2. **Techniques to distinguish transplanted cells from native cells**
   - Quantitative (Q-PCR) vs. qualitative (in situ hybridization)
   - Imaging techniques; gender-specific tissues; GFP genes within viral vectors
Gene Therapy – Specific Recommendations

• Types of GT Products:
  – Non-viral vectors (e.g., plasmids)
  – Replication-deficient vectors (e.g., adenovirus, AAV, retrovirus, lentivirus, etc.)
  – Replication-competent oncolytic vectors (e.g., measles, reovirus, adenovirus, etc.)
  – Microbial vectors (e.g., *Listeria*, *Salmonella*, *E. coli*, bacteriophage)
  – Ex vivo genetically modified cells
Gene Therapy – Specific Recommendations

• Animal Models should:
  – Be permissive to the viral vector similarly in animal and human
  – Show the same pharmacologic response to transgene or genetically modified cells

• Safety Concerns:
  – Toxicity to the formulation (e.g., liposomes, excipients)
    • Should be tested separately if a MAF does not exist
  – Aberrant localization to or viral vector replication in non-target cells/tissues
  – Persistence of vector and expressed transgene
  – Immune response to vector; or overall immune suppression or activation
  – Insertional mutagenesis or oncogenicity
  – Germline transmission
  – Transmission to family members or health professionals (shedding)
  – Vector-specific concerns – see guidance
  – Transgene-specific safety concerns
Gene Therapy – Biodistribution

• Biodistribution characterization considered very important!
  – Does it reach target organs? Where else does it go?
  – Does transgene expression persist? Is it intended to persist?
• Biodistribution study (BDS) is needed before dosing humans for:
  – New vector classes
  – Established vectors (EVs) with significant changes to:
    • Backbone
    • Formulation or route of administration changes
    • Dosing schedule
    • Vector dose levels
• Significant discussions have occurred between sponsors and the FDA about having to repeat BDS with well-characterized vectors (e.g., AAV)
  – Can justify not repeating based on past experience
Gene Therapy – Biodistribution

• Conduct BDS on the molecular level using quantitative PCR (qPCR) in all applicable organs, tissues, biological fluids
  – More limited for local injection

• **Important**: Make sure to use very clean techniques for necropsies (change scalpel between organs) to avoid false positives

• Ensure tissues are collected according to the following guideline:
  • BD used information to determine the length of follow-up needed in clinical trials.
    – Multiple necropsy groups to test persistence and distribution across time
Question 1. Is your gene therapy product only used for ex vivo modification of cells?

No

Question 2. Do preclinical study results show persistence of vector sequences?

Yes

No

Question 3. Are vector sequences integrated?\(^1\)

Yes

No

Question 4. Does vector have potential for latency and reactivation?

No\(^2\)

Yes

Clinical protocols with the product should include long-term follow-up observations.\(^3\)

The risk is low that exposure to your gene transfer technology will be followed by gene transfer-related delayed adverse events. A clinical protocol with long-term follow-up observations may not be necessary.
Hot Topics Gene Therapy

• **Immune Responses** against viral gene therapy vectors
  – Clinical trials have shown cellular immune responses with possible adverse responses
  – **Example** – AAV delivery of Factor IX for hemophilia B*
    • Long-term hemophilia correction in mouse and dog; but F.IX antibodies
    • Human: IM – no safety issues, but transgene expression low
    • IV (hepatic artery) – good transgene expression at 2 weeks
      – But - Subsequent rise of liver transaminases (toxicity) and reduction of transgene
    • Likely – T-cell mediated event likely targeting transduced cells
    • Need for immunosuppression in clinical trials
  – Similar immune-mediated toxicity with AAV trials for lipoprotein lipase deficiency (Kidney), alzheimers (brain)

• **Need better understanding of ways to predict these responses**

*Hasbrouck and High, Gene Therapy (2008) 15, 870–875
CBER - Vaccines
Vaccines – General Preclinical Principles

• FDA follows World Health Organization (WHO) vaccine guidelines – harmonized globally

• FDA has their own guidances as well:
  – e.g., Guidance for Industry: General Principles for the Development of Vaccines to Protect Against Global Infectious Diseases, 2011

• General Principles:
  – Clinical candidate (GMP), formulation, route of administration and frequency of administration should be used for animal studies
  – Animal models – must mount a similar immune response to humans
  – No TK needed, but PD response should be fully characterized
  – Adjuvants and excipients tested as for drugs if no MAF
Vaccines – General Preclinical Principles

- Toxicity studies:
  - Usually a single species studied – matched to efficacy species
  - Usually 1 dose level sufficient – clinical dose (mg basis) or higher
  - Human dosing regimen followed where possible
  - Standard toxicity assessments conducted – after each dose and after an off-dose period
    - Timing of assessments to correspond with peak Ab production
    - Evaluate injection-site reactions
- Reproductive Toxicity – not needed for childhood vaccines
  - Needed if patient population includes women of childbearing potential
  - Only embryo-fetal and postnatal development study (no fertility)
    - Generally a single study with separate arms
    - Postnatal development only followed through weaning
- Generally no need for carcinogenicity, genotoxicity
- Safety Pharmacology only based on cause for concern
Conclusions

• FDA follows ICH Guidelines (or WHO Guidelines) when available
• Standard toxicity study packages are expected for small molecules and for biologics that fall under CDER
• Abbreviated packages are acceptable for cancer indications – terminally ill patients
• CBER-regulated products – decided on case-by-case basis depending on nature of the product and pharmacology
• Most important challenges for all biologics is identifying a pharmacologically-responsive species
Thank you for your attention.

• Questions?