FDA Expectations for Toxicology Support of Clinical Trials and Marketing

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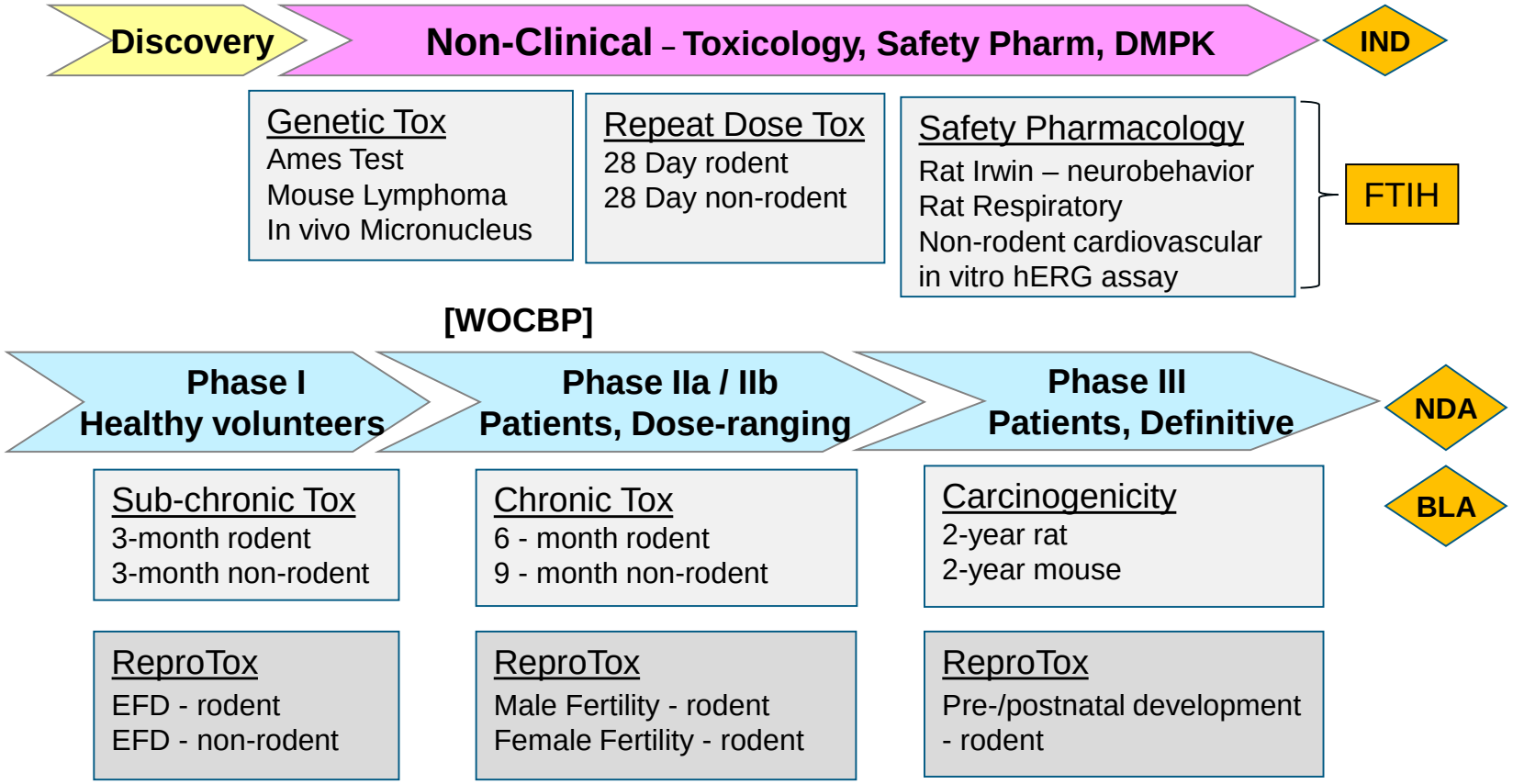
Outline

- 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



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

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Standard Duration of Nonclinical Toxicity Studies to Support Clinical Trials (ICH M3(R2))

Max Clinical Trial Duration	Pivotal (Definitive) Toxicology Study Duration	
	Rodents	**Non-rodents**
≤2 Weeks	2 weeks	2 weeks
2 Weeks to 6 Months	Same as clinical trial	Same as clinical trial
Greater than 6 Months	6 months	9 months (6 in EU)

Toxicity Study Durations Required for Marketing
(ICH M3(R2))

Duration of Indicated Treatment	Toxicology Study Duration	
	Rodents	**Non-rodents**
up to 2 Weeks	1 month	1 month
>2 Weeks to 1 Month	3 months	3 months
> 1 Month to 3 months	6 months	6 months
> 3 months	6 months	9 months (6 in EU)

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 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

ICH Quality Guidelines	
Q1A - Q1F - Stability	Q7 - GMPs
Q2 – Analytical Validation	Q8 – Pharmaceutical Development
Q3A - Q3D - Impurities	Q9 – Quality Risk Management
Q4 – Q4B - Pharmacopoeias	Q10 – Pharmaceutical Quality System
Q5A – Q5E – Biotech Products	Q11 – Dev/manufacture Drug Substance
Q6A – Q6B - Specifications	Q12 – Life Cycle Management
M7(R2) – Mutagenic Impurities	

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M7(R2) – Mutagenic Impurities	

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

Thresholds from ICH Q3A

Maximum Daily Dose	**Reporting Threshold**	**Identification Threshold**	**Qualification Threshold**
≤ 2g/day	0.05%	0.10% or 1.0 mg per day intake (whichever is lower)	0.15% or 1.0 mg per day intake (whichever is lower)
> 2g/day	0.03%	0.05%	0.05%

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

Standard	Oncology (including Biologics) – S9
• 1-month Ph1 Studies up to 6/9 months for NDA – NOAEL required • Safety Pharm and Genetox needed • Starting dose based on NOAEL • Full DART package • Carcinogenicity studies	• 1-month studies Ph1, Ph2 3-month studies for Ph3 and NDA – No NOAEL required • Safety pharm "bolted on" to general tox; No gene tox needed • Starting dose based on 10% of severely toxic dose in animals (STD 10) – Dose-escalate above animal NOAEL to MTD in humans • DART - only need EFD study – one species only if positive

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

From: Cavagnaro (2002) Nature Reviews Drug Discovery, 1:469-475

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CDER
ICH S6 + M3

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CBER
Not ICH S6

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Differences Between Biologics and Small Molecules

Small Molecules

- Small: < 700 daltons
- Generally lipophilic - Can cross biological membranes, including the placenta and/or VYS
- Well defined structures and relatively stable
- Rapidly metabolized; require daily dosing
- Toxic response related to chemical structure and exaggerated pharmacology
- Less likely to illicit an immune response (be immunogenic)
- More likely to have activity in multiple species

Biologics

- Large Macromolecules:
  - Peptides – ~ 1,000 to 10,000 dal
  - Proteins – ~ 20,000 to 60,000 dal
  - mAbs - ~150,000 dal
- Less lipophilic - Generally either can't cross membranes or use receptor-mediated mechanisms
- Complex physiochemical characteristics and heat sensitive
- Degraded over time, can be very long acting; may need intermittent dosing
- Toxic response related to exaggerated pharmacology
- More likely to be immunogenic
- More often show species selectivity

See: Cavagnaro (2002) Nature Reviews Drug Discovery, 1:469-475

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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?

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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

Special Considerations for Biologics, cont

- 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

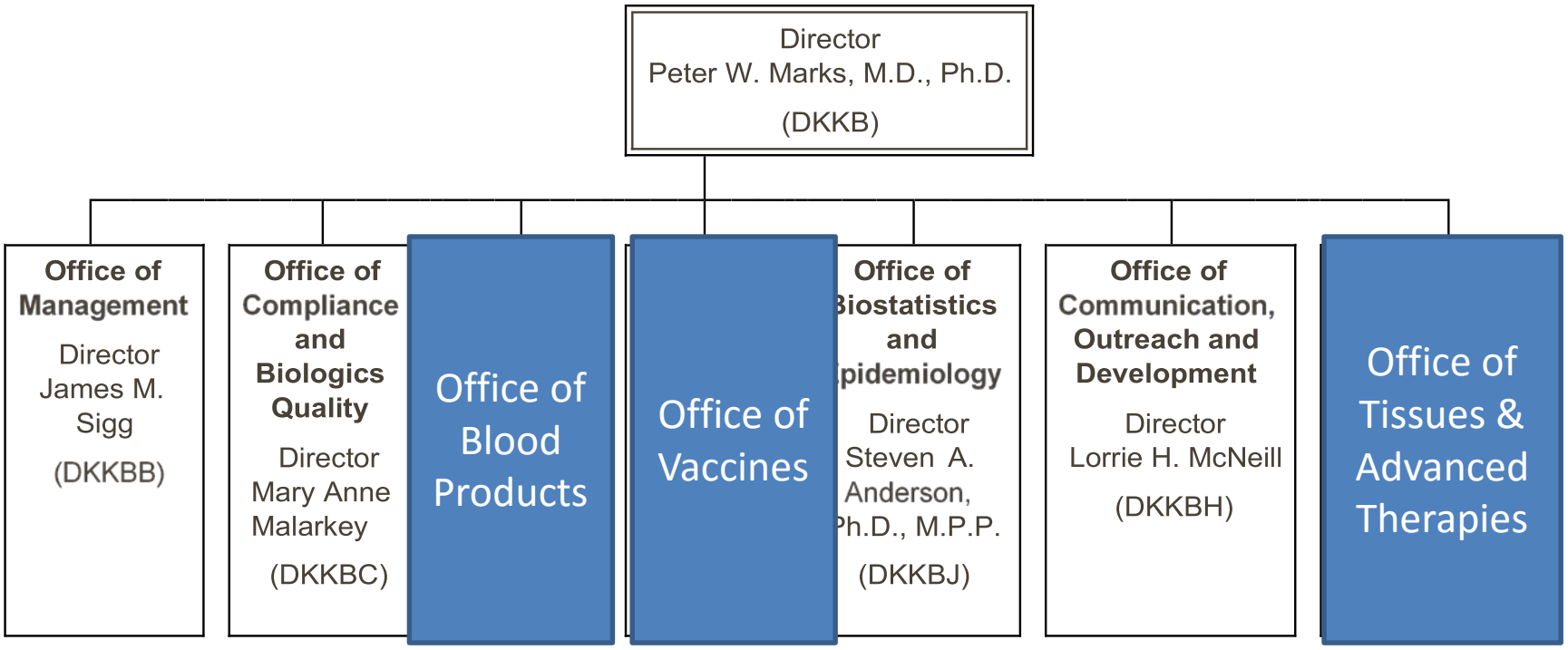
- Safety Pharmacology – some assessment expected, but **could be bolted on to general toxicity studies** – long half life for mAbs
- Genotoxicity Assessment – **not applicable to biologics**
- Carcinogenicity
  - Weight-of-evidence assessment for level of concern should be conducted – pharmacology class, 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 insufficent 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

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

Biologics - CBER

CBER Office of New Drugs Organizational Chart



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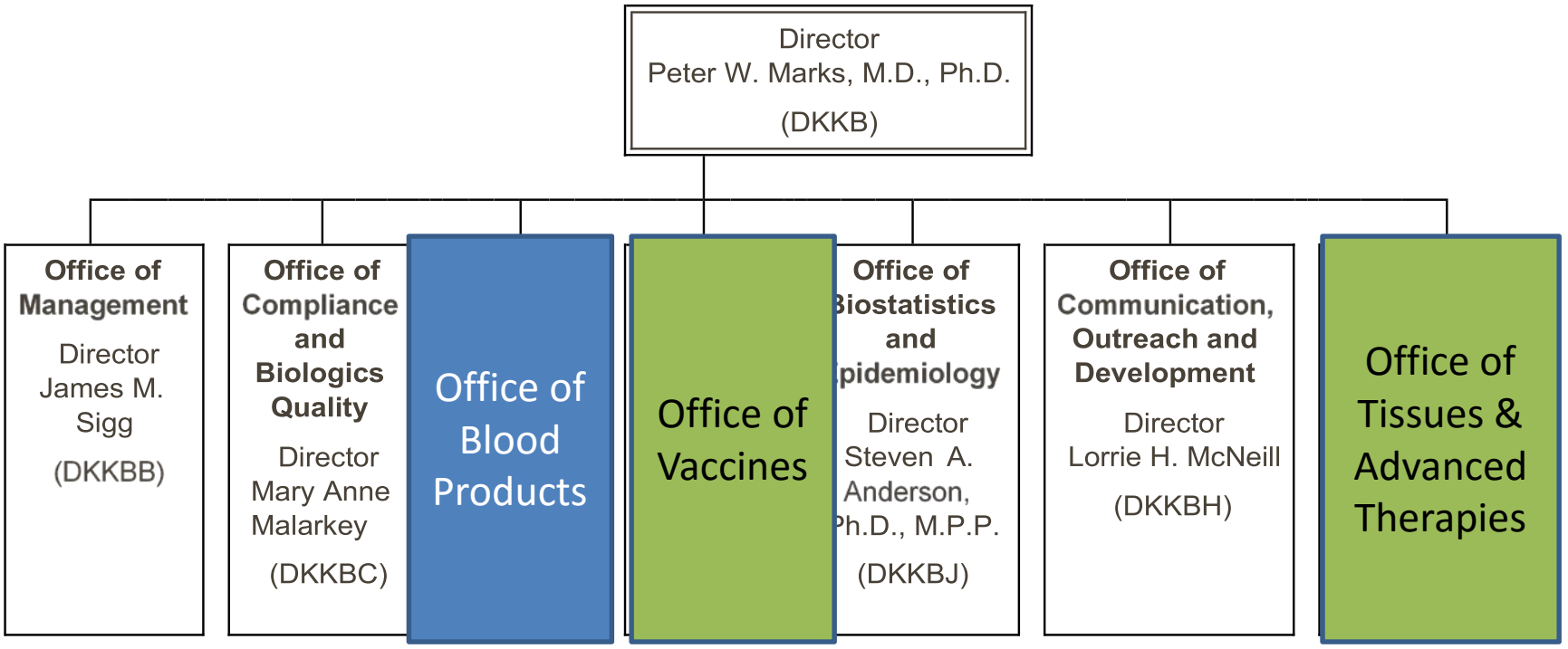

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CBER - Office of Tissues & Advanced Therapies

- 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**

CGT Product Preclinical Study Recommendations

- 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

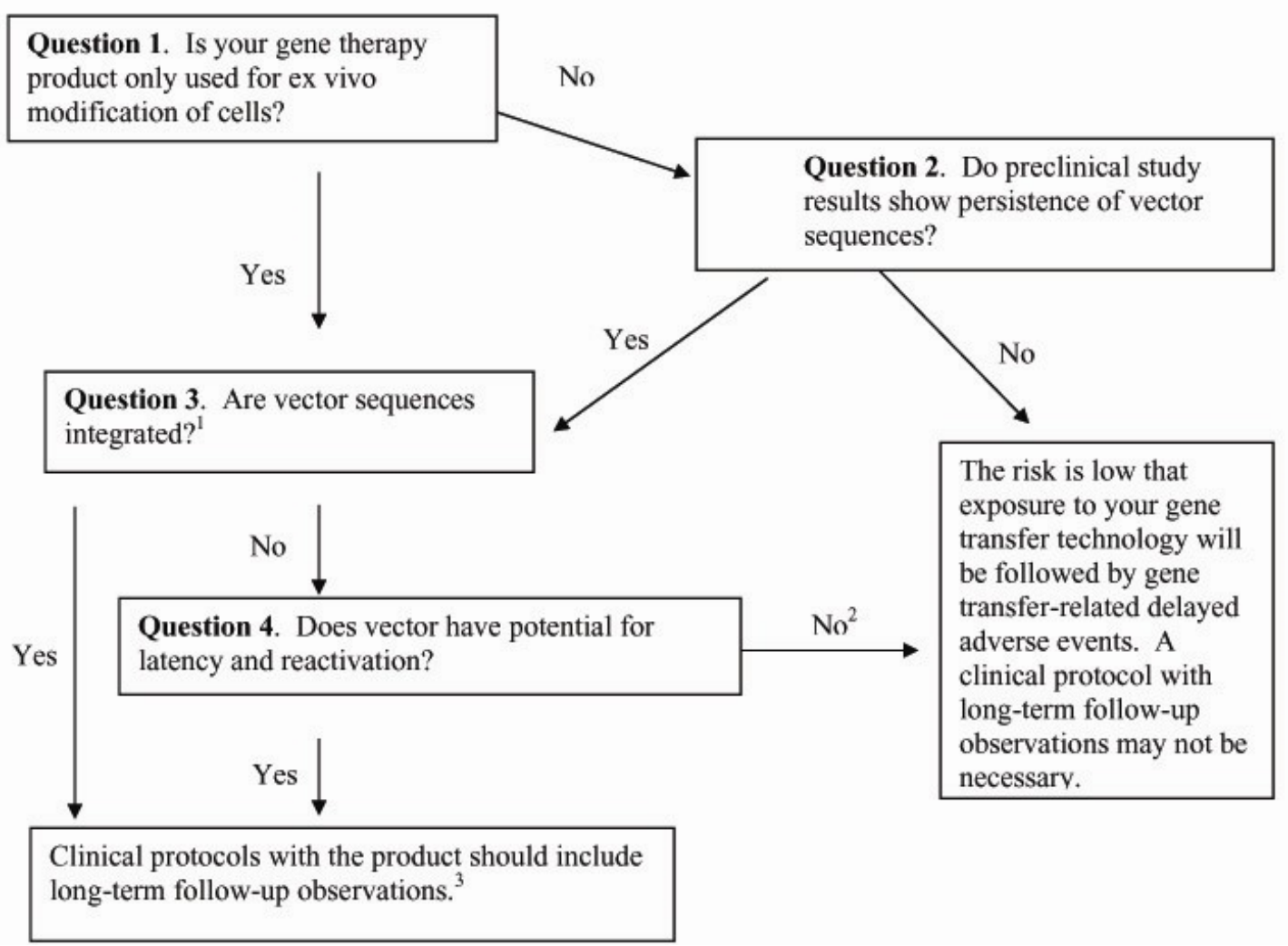
Gene Therapy – Biodistribution

- 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.
  - Guidance for Industry: *Gene Therapy Clinical Trials – Observing Subjects for Delayed Adverse Events, Nov 2006*
  - Multiple necropsy groups to test persistence and distribution across time

GT - Decision Tree for Length of Clinical Follow-up



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Guidance for Industry: *Gene Therapy Clinical Trials – Observing Subjects for Delayed Adverse Events, Nov 2006*

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
  - *WHO Guidelines on nonclinical evaluation of vaccines, WHO Technical Report Series, No. 927, 2005*
- 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 ?