How Best to Communicate Toxicology Results & Needs

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Outline

- Purpose of Toxicology Studies
  - Hazard identification
  - Regulatory drivers
  - Support clinical development
  - Evaluate mechanism of toxicology/exaggerated pharmacology
- Key Communication from Toxicology Case Studies
  - Internal audience (clinical development): support for clinical pharmacology study
  - Line management (nonclinical safety): support for CMC manufacturing
  - External investigators: support for on-target pharmacology
- Conclusion

Hazard Identification

- Characterize the safety of a new molecule entity (NME) and identify the potential risks to humans
  - Determine on- and off-target toxicity

- Guide clinicians: translation of nonclinical toxicity to potential clinical adverse events (AEs)
  - Informs clinical monitoring/management plan
  - Provides exposure margins for identified toxicity signal(s)
  - Determines safe starting dose for first-in-human (FIH) clinical trial
  - If toxicity is **not** observed in animals, ➔ no guidance to clinicians/regulators

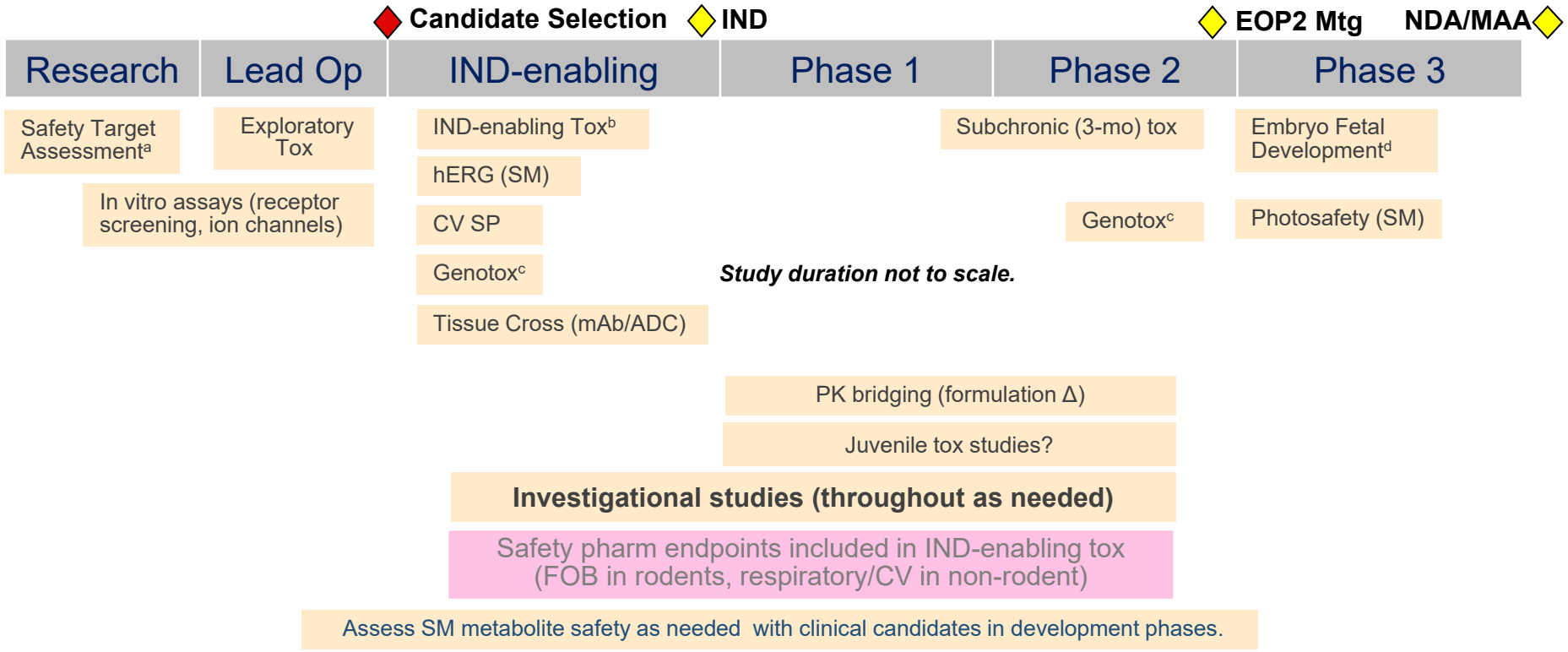
- Support clinical regimen and populations
  - Dosing regimen in tox studies should be commensurate with that in the clinic
    - Oncology: IND-enabling tox program generally supports Phase 1/2; subchronic 13-week duration supports Phase 3 (registrational or pivotal clinical trial)
    - Non-oncology: nonclinical duration precedes clinical; 6-month rodent/9-month non-rodent required for chronic duration
  - Special populations (not discussed)
    - Women/men of child-bearing potential (WOCBP/MOCBP)
    - Pediatrics

- Occupational Toxicology: setting safe exposure levels for workers, establishing health hazard categorization for CMC

Regulatory Drivers

- International Council on Harmonization (ICH) guidelines outlines approaches for drug/pharmaceutical development for US/EU/Japan
  - M3(R2): Non-oncology indications, may include adjuvant/neoadjuvant therapies in oncology (~5 yrs)
  - S9: Oncology (advanced cancer, life-threatening) where life expectancy is months to years (~2 yrs)
  - S6/S6 Addendum: Biotechnological-derived Products (e.g., mAbs, vaccines)
  - S7A/B: Safety pharmacology, potential for delayed ventricular repolarization (QT interval prolongation)
  - S5(R3): Reproductive and Developmental Toxicology
  - Other: Carcinogenicity (S1A-C), Genetic Toxicology (S2[R1]), Toxicity Testing (S4: duration of chronic testing), Immunotoxicology (S8), Photosafety (S10), Impurities (Q3A-D)
- Safety studies adhere to Good Laboratory Practice (GLP) and Organization for Economic Co-operation and Development (OECD in the EU) regulations
  - Test facility complies with GLP and/or OECD regulations
  - Non-GLP studies used for hazard identification; GLP studies make a claim of “safety” and basis for clinical dose setting/duration
- Environmental Risk Assessment (SMs)

Drug Development: The Right Study at the Right Time (Oncology Base Case)



[a] Safety Target Assessment (STA) typically includes context of target, indication, standard of care, and competitive landscape.
[b] Two species (small molecule [SM])/relevant species (biologics) to support clinical plan (generally ~28 days to support Ph 1/2)
[c] Genotoxicity (SM): in vitro for adjuvant setting or to support inclusion of healthy volunteers in clinical pharmacology studies.
[d] SM: not warranted for cytotoxic molecules or agents known to cause embryolethality or teratogenicity; staged approach with rodent first, followed by rabbit if rodent is negative.
SP = safety pharmacology; CNS=central nervous system; CV=cardiovascular.
IND=investigational new drug; EOP2=end of phase 2; NDA=new drug application; MAA= marketing authorization application.

Case Study #1: Communicating to an Internal Audience (Clinical Development)

Support for Clinical Pharmacology Study

Background for Toxicology Support in Clinical Pharmacology Studies

- H3B-6527, a covalent inhibitor of fibroblast growth factor receptor (FGFR) 4, in Phase 1 for hepatocellular carcinoma and intrahepatic cholangiocarcinoma (https://clinicaltrials.gov/ct2/show/NCT02834780)

- H3B-6527 in nonclinical species showed greater exposure in the fed versus fasted state in non-rodents

- In the IND-enabling repeat-dose toxicology studies, a no-observed-adverse effect level (NOAEL) was not determined

- Clinical development team wanted to assess food effect of H3B-6527 in healthy volunteer (HV) subjects vs. cancer patients

Strategy of the Nonclinical Safety Team: Conduct GLP 7-day Toxicology Studies

- In oncology, the severely toxic dose level in 10% (STD_{10}) in rodent and/or highest non-severely toxic dose (HNSTD) in non-rodent, rather than a NOAEL, used to determine the clinical starting dose
  - STD_{10} was the highest dose tested in rodents (>>HNSTD)
  - HNSTD used as basis of clinical starting dose of 300 mg QD

- Evaluating food effects on clinical PK during the dose expansion phase was estimated to be delayed and complicated by lack of standard high-fat meal in Asia vs. US/EU

- Requirements to assess food effects of H3B-6527 in HV subjects
  - Enable testing clinical PK at the highest pill burden: 200 mg strength
  - Determine NOAELs in both tox species and negative in vitro genotoxicity
  - Selected 7-day duration to ensure H3B-6527 reached steady-state concentrations and elicit comparable toxicities seen in previous studies

Key H3B-6527 Related Findings in the GLP 7-Day Toxicology Study in Rodents

Dose (mg/kg/d)	Low Dose	Mid Dose (NOAEL, F)	High Dose (NOAEL, M)
Day 7 AUC_{0-24} /C_{max} M,F (ng·h/mL / ng/mL)	M: 4,030 / 1,760 F: 12,400 / 2,670	M: 15,100 / 6,380 F: 20,400 / 3,740	M: 21,200 / 4,040 F: 30,300 / 4,310
Mortality	-- (all groups)		
Body Weight	--	↑ BW gain (F)	↑ BW (8.5% M rel to controls); ↑ BW gain (M/F)
Clinical signs	--	--	↑ FC (+107-135% on D 1-2, 2-3 for M rel to controls)
Ophthalmic findings (slit lamp; indirect ophthalmoscopy)	--	--	--
Clinical pathology	--	Mild ↑Phosphorus (F)	Mild ↑Phosphorus (F, M); Min ↓Glucose (M)
Histopathology	--	Corneal epithelial atrophy (min-mild in 5/12 F)	Corneal epithelial atrophy (min-mod in 9/12 F)

12/sex/group; no recovery animals.
"--" indicates no test article-related effect. BW = body weight; FC = food consumption.
Corneal atrophy was characterized by a reduction in the number of layers of corneal epithelium; no ophthalmic change correlated with this microscopic finding. Corneal atrophy completely reversed in the IND-enabling tox study.

Conclusion: Clinical Starting Dose of 200 mg QD Proposed for HV FE Study

- Proposed clinical dose of 200 mg QD
  - 1/10th NOAEL of MD mg/kg/day in female (F) rodents
  - 1/20th NOAEL of HD mg/kg/day in male (M)/F non-rodents
- Corneal atrophy noted in F, but not M, considered non-adverse due to incidence and severity at MD (NOAEL)
  - Completely reversible finding at MD in F in current 7-day study and at HD in M/F in the IND-enabling 28-day tox study
  - Corneal atrophy not observed in non-rodents ≥7 days
- AUC exposures in F rodents with corneal atrophy were ~160- or 270-fold higher than C_{max} or $AUC_{0\text{-}24h}$, respectively, in HCC patients given 300 mg QD
- 200 mg QD dose planned for male HV subjects (fasted or fed state) in food effects study considered safe based on the large AUC exposure margins
- FDA approved the clinical pharmacology protocol; study in HV proceeded

Case Study #2:
Line Management (Nonclinical Safety) & CMC

Toxicology Support for CMC Manufacturing

Background for CMC Issue

- Compound X is a potent and selective small molecule (SM) inhibitor that was in Phase I dose escalation clinical trial with promising activity
- Prior to clinical trials, SMs are assigned a default health hazard categorization (HHC) based on the minimal toxicity data and the HHC is refined based on the emergence of new data
- Due to the potency of Compound X, the HHC to manufacture large scale active pharmaceutical ingredient (API) was projected to become highly hazardous, requiring extraordinary personal protective equipment
- The estimation of occupational exposure limits (OELs) used by CMC are derived from NOAELs from GLP, IND-enabling tox studies with additional safety factors
  - 10-fold safety factor for: species extrapolation, inter-individual variability, study duration, NOAEL to NOEL, severity of toxicity

Health Hazard Categories

- A Health Hazard Category (HHC) is assigned during early stages of development

HHC 1:	No or slight hazard	≥ 100 µg/m^3
HHC 2:	Moderate hazard	< 100 – 10 µg/m^3
HHC 3A:	High hazard	< 10 – 1 µg/m^3
HHC 3B:	High hazard	< 1 µg/m^3 – 50 ng/m^3
HHC 4:	Very high hazard	< 50 ng/m^3

Category D (< 10 µg/m^3): Default category used if insufficient data are available to place a compound into one of the above categories.

- HHCs have associated chemical handling and exposure limit guidelines which are increasingly more rigorous as the hazard increases
- An inhalation OEL, or, at a minimum, a HHC is required prior to API scale up
- Compound X was estimated to be HHC 3B based on IND-enabling tox studies and considered highly hazardous from a CMC worker perspective

Strategy to Line Management: Early Conduct of EFD Study Would Refine HHC

- Nonclinical safety conducted an EFD study during Phase 1, rather than Phase 2/3
- Design: QD dosing on Gestational Day (GD) 7-17

Dose Group	Dose (mg/kg/day)	Number of Rats	Dose Volume (mL/kg)
I	0 (Control[a])	8 + 6[b]	5
II	Low Dose	8 + 6[b]	5
III	Mid Dose	8 + 6[b]	5
IV	High Dose 1	8 + 6[b]	5
V	High Dose 2	8 + 6[b]	5

[a] Control article = Methylcellulose/Tween (0.5%/0.2%) in reverse osmosis water.
[b] 3 rats/subgroup for TK sampling on GDs 7 and 17: predose (0) and 1, 2, 4, 8, & 24 hr postdose.

- Toxicity Assessment (routine): clinical observations, body weights (dams), food consumption with C-section on GD 21 (gross necropsy)
- Evaluation on dams and fetuses (see ICH S9/S5)

Conclusions of EFD Study and Impact on HHC

- Compound X resulted in maternal and fetal toxicity at MD and HD1
  - Maternal tox characterized by reduced BW and litter/# live fetuses per litter
  - Developmental toxicity occurred only at maternally toxic dose levels
  - Fetal toxicity comprised of embryofetal death, reduced body weights, and malformations at MD and HD1
- Maternal and fetal NOEL = LD (AUC exposure ~8 µM-hr)
- Impact on pregnancy label: Category C/D (pre-2015; pregnancy categories no longer used)
  - Exposures at NOEL > clinical MTD in Phase 1a
  - Exposures for maternal/fetal NOEL ~2.5X MTD based on AUC
  - For clinical trials, allows enrollment of up to 150 WOCBP with contraceptive measures
- Impact for CMC manufacturing: using NOEL in GLP EFD tox study rather than 10-safety factors applied to NOAEL from the 28-day tox study, Compound X designated HHC 3A and $M cost savings

Case Study #3:
External Investigators

Toxicology Support for On-Target Pharmacology

Background

- H3B-6545 is a selective, novel estrogen receptor (ER) covalent antagonist being developed for metastatic ER-positive, HER2-negative breast cancer

- The impact of ER inhibition on organ systems such as bone, dependent on the ER pathway, has become increasingly important to principal investigators

- As part of the target candidate profile, H3B-6545 should not have deleterious effects on bone

- The potential effects of H3B-6545 on bone turnover was evaluated in an ovariectomized (OVX) rat model to represent the post-menopausal setting

Communication Strategy to Line Management: Request an On-target Pharmacology Study on Bone

- 6-month-old female Sprague-Dawley rats underwent sham or OVX surgery followed by once daily (QD) oral gavage doses of H3B-6545, positive controls of tamoxifen (TAM) or estradiol (E2), or vehicle controls for 6 weeks based on the study design below:

Group	Surgery +Test article (mg/kg QD)	Dose Vol (mL/kg)	Dose Conc (mg/mL)	Number of Females:
1	Sham + Control[a]	5	0	20
2	OVX + Control[a]	5	0	20
3	OVX + 0.01 mg/kg E2[b]	5	0.002	20
4	OVX + 1 mg/kg TAM[b]	5	0.2	20
5	OVX + 3 mg/kg H3B-6545	5	0.6	20
6	OVX + 10 mg/kg H3B-6545	5	2	20
7	OVX + 30 mg/kg H3B-6545	5	6	20

OVX = ovariectomized rat; E2 = estradiol; TAM = tamoxifen
[a]Control vehicle = 0.5% (w/v) methylcellulose/0.2% (w/v) Tween 80 in water (MCT); H3B-6545 was formulated in MCT; [b]vehicle for E2 and TAM was corn oil.

- Routine toxicity/TK assessments with additional bone biomarkers (osteocalcin, C-telopeptide, deoxypyridinoline, and serum chemistry) and comprehensive bone loss evaluation (predose and Weeks 5/6)

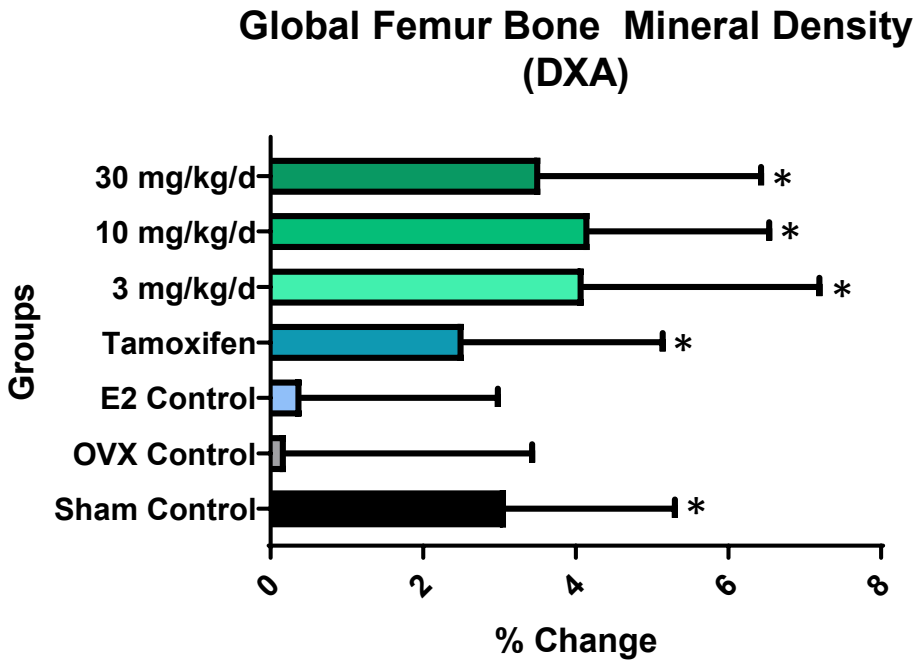
H3B-6545 Effects on Bone Turnover Biomarkers Were Comparable to TAM in OVX Rats

- Bone turnover biomarkers evaluated pretreatment, Weeks 4/ 6
  - Osteocalcin (OC, serum): bone formation
  - C-telopeptides of Type 1 Collagen (CTx, serum): bone resorption
  - Deoxypyridinoline (free DPD, urine): bone resorption (DPD normalized to urinary creatinine, measured in same sample)

Group (Surgery + TA)	Osteocalcin	CTx	DPD
Sham + Control	NA	NA	NA
OVX + Control	Mild ↑ (+105/+83%)*	slight ↑ (+64/+63%)*	mod ↑ (+302/+334%)*
OVX + E2	--^	--^	--^
OVX + TAM	slight ↓ (-31/-35%)^	slight ↓ (-14/-24%)^	slight ↓ (-27/-36%)^
OVX + 3 mg/kg H3B-6545	slight-mild↓ (-31/-35% in Wk 4 and -37/-44% in Wk 6)^	slight ↓ (-12/-26% in Wk 4 and -24/-26% in Wk 6)^	slight ↓ (-12/-26% in Wk 4 and -24/-26% in Wk 6)^
OVX + 10 mg/kg H3B-6545			
OVX + 30 mg/kg H3B-6545			

*relative to Sham control; ^relative to OVX control

H3B-6545 Prevented all Changes Related to Bone Loss Following 10 Weeks of E2 Deprivation



** Statistically different than OVX Control (p≤0.05). Exogenous E2 at 0.01 mg/kg/d did not prevent OVX bone-related effects. Data are presented as % change from pre-treatment*

OVX = ovariectomized, 6-month-old sham or OVX female rats (19-20/group) treated QD for 6 weeks.

- H3B-6545 partially reversed and reduced bone loss/strength at all examined sites with effects comparable or better than TAM
- H3B-6545 and TAM increased L4 trabecular/total BMC and BMD and had less effects on bone via biomechanical testing

Furman, D., et al. Covalent Era Antagonist H3B-6545 Demonstrates Encouraging Preclincial Activity in Therapy-Resistant Breast Cancer. DOI: 10.1158/1535-7163.MCT-21-0378.

Key Messages of Bone Loss Study with H3B-6545 to Clinical Investigators

- H3B-6545 administration to OVX females rats for 6 weeks resulted in non-dose-dependent prevention of bone changes related to 10 weeks of estrogen deprivation
  - Reduced bone formation and resorption markers
  - Prevented the increases in cancellous bone formation and resorption parameters
  - Partially reversed and reduced trabecular bone loss at all examined sites
  - Partially reversed decreases in L4 strength

- H3B-6545 in OVX female rats had similar effects on body weight/food consumption and uterine histopathologic findings as those in the IND-enabling toxicology study

- Bone turnover biomarkers and endometrial thickness/uterine volume via ultrasound will be evaluated in the ongoing Phase 1/2 clinical study (https://www.clinicaltrials.gov/ct2/show/NCT03250676)

Concluding Thoughts: Talking “Tox” When Others Don’t Speak the Language

- Align tox strategies with project needs and openly communicate
  - Understand the “why” and “what” of the other group/individual: internal and external
- Stay focused on the scientific rationale in a regulated environment
- Seek opportunities to partner with all disciplines/parties to solve a problem and advance projects
- Be committed to the ethical use of animals and to the 3Rs (Reduction, Refinement, and Replacement) in industry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