Preclinical safety program Regular - ICH M3(R2)

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In particular, before proceeding, input from a specialist in drug metabolism and pharmacokinetics (DMPK) is advisable

Relevant guidelines

- **ICH M3(R2)** e.g. for
  - Guidance regarding **timing** of nonclinical studies relative to clinical development
  - **Phase 0 clinical studies** (exploratory clinical trials) with micro-dosing
- **Other general preclinical ICH safety guidelines**, in particular
  - ICH S5(R2) (reproductive and developmental testing)
  - ICH S2 (genotoxicity testing)
  - ICH S3 (pharmaco-/toxicokinetics PK/TK)
  - ICH S7A/B (testing for safety pharmacology)
  - Etc.
- **National/regional guidelines**

General remarks – ICH M3(R2)

- **Justified deviations** from standard development plans are possible
- For **clinical Phase 0 studies** (exploratory clinical trials) with micro-dosing see tables in ICH M3(R2)
- **Dose levels** for preclinical safety:

  HD generally maximal tolerated dose (MTD) or large exposure multiples (at least 50 x AUC*) or saturation or maximal feasible dose (MFD)
  Limit generally 1000 mg/kg (at least 10x clinical exposure, clinical dose < 1 g/d)

  For further details see backup

  * For carcinogenicity studies 25 x based on AUC values or 150 x based on mg/kg in animals vs. mg/person in humans might be sufficient

Compound for pivotal studies

- GMP grade or GLP analysis
- Compared to compound used for clinical trials
  - Not purer
  - No impurities which were not tested in animals
- Formulation
  - Oral: to achieve exposure similar to exposure intended in humans
  - Other routes of administration: generally similar or identical to that intended to be used in humans

Duration of repeat dose toxicity studies – ICH M3(R2)

Human application	*≤ 2 wks*	*> 2 wks – 1 mo*	*> 1 mo – 3 mo*	*> 3 mo – 6 mo*	*> 6 mo*
During clinical development	**2 wks**	**Same as intended treatment**			**6 mo** (rodents) **9 mo** (non-rodent)
For marketing authorization	**1 mo**	**3 mo**	**6 mo**	**6 mo** (rodents) **9 mo** (non-rodents)*	**6 mo** (rodents) **9 mo** (non-rodents)*

All studies have to be done in rodents (generally rats) and non-rodents (for topical development often minipigs
wks = weeks – mo = month(s)
* In many countries 6-month chronic studies are sufficient
Studies do not have to be repeated for marketing authorization

For clinical Phase 1 *(usually 2-4 weeks)*

Pharmacology including safety pharmacology

Pharmacology (MoA, dose-response, treatment schedule, biomarker)

- Primary PD
- Secondary PD

Safety pharmacology - ICH S7A&B

Core battery:

- *in vitro* hERG (may not be needed, if *in vivo* cardiovascular CV dog data are available)
- CV telemetry dog
- Respiratory rat
- Functional observation battery (FOB)/Irwin rat

Supplementary studies (e.g. *in vitro* off-target effects, Purkinje) as needed

Toxicology

***In vitro* mutagenicity** ICH-S2

- Ames for single dose
- *In vitro* or *in vivo* chromosomal aberration test, e.g. *in vitro/in vivo* MNT

Toxicity studies (2-4 weeks) in rodents and non-rodents

Including recovery and TK (ICH-S3A) with investigation of accumulation

NO(A)EL needed

Duration depending on intended human treatment duration (ICH M3)

Further **immunotoxicity testing**, if needed based e.g. on findings in regular toxicity studies

Abuse liability*, if indicated e.g. for CNS drugs - May be delayed

* E.g. testing for drug discrimination, self-administration or withdrawal symptoms

For clinical Phase 1 *(usually 2-4 weeks) – continued 2*

Toxicology (continued)

Local tolerance (if appropriate)
May be part of regular toxicity study
See also ICH M3(R2)

Phototoxicity* - ICH S10 - May be delayed
In silico or in vitro

No acute toxicity testing (replaced e.g. by dose-range-finding DRF studies)

Embryo-fetal development (Segment II) in rats and rabbits* including TK, with the following exceptions:

- No women of child-bearing potential (WoCBP) in clinical trial
- If WoCBP in only short duration clinical trials (e.g. 2 weeks): intensive control of pregnancy risk. Particularly, if targeted disease predominantly in women
- Knowledge of MoA
  Type of test drug
  Extent of fetal exposure very low
  Difficulty of conducting developmental toxicity studies in an appropriate animal model

* 1) Photochemical properties (e.g., photoabsorption and photostability) of the molecule
2) Information on the phototoxic potential of chemically related compounds
3) Tissue distribution (might become available only later)
4) Non-clinical (and later clinical) findings indicative of phototoxicity

For clinical Phase 1 *(usually 2-4 weeks) – continued 3*

Absorption/distribution/metabolism/excretion (ADME) – related activities

Develop and validate (according to GLP) **analytical method** in different species including humans

1. Pharmacokinetic (PK) behavior

A) Limited **PK** data in dedicated studies using the same 2 species as in toxicity studies (ICH S3B) Route of administration as intended in clinical studies and intravenously (i.v.) for investigation of bioavailability (BAV)

B) *In vitro* permeability (e.g. Caco 2) - May be delayed

C) Consider **further ADME-related studies**, such as e.g.
- Food effect
- Target organ exposure

May be delayed

D) See also TK in general and reproductive toxicity studies, especially regarding accumulation

2. Metabolism

A) *In vitro* **metabolic stability** e.g. in hepatocytes or microsomes (toxicity species and man)

B) Possibly determination of metabolites. May be delayed

C) Possibly cytochrome P450 (CYP) profiling (indicates the potential for being a “victim” of other drugs). May be delayed

D) See also parameters of 1 above

3. Drug-drug interaction *(DDI)*

A) *In vitro* **plasma protein binding** and blood-plasma distribution in toxicity species and man. May be delayed

B) Possibly **CYP inhibition/induction** (“perpetrator” for other drugs → DDI) - May be delayed

In silico **modeling** as needed

For clinical Phase 2 *(usually 4-12 weeks)*

Toxicology

Genotoxicity studies - Core battery - If not done for clinical Phase 1

- Tests as for clinical Phase 1 (repeated with new batch; not always necessary) *plus in vivo* micronucleus test (if not done earlier)

OR

- Ames (if repetition needed)
  plus in vivo cytogenetic test in 2 different tissues - One test may have been done earlier

Toxicity studies (4-12 weeks) in rodents and non-rodents, including recovery and TK
Duration ≥ duration of clinical trials up to maximum duration of the repeat-dose toxicity studies (6/9 months) – ICH M3(R2) – see also below

Embryo-fetal development (Segment II) in rats and rabbits including TK*
(if not done earlier)

Dedicated **immunotoxicity testing,** if needed, see also ICH M3(R2) and ICH S8
(if not done earlier). May also be delayed

* For WoCBP with effective birth control measures and negative DRF Segment II rat and rabbit studies (at the minimum investigated in 6 animals/group)
- USA: full Segment II study can be delayed until before clinical Phase 3
- EU/J: full Segment II study required, if clinical trials > 3 months and with > 150 WoCBP

For clinical Phase 2 (usually 4-12 weeks) – continued 2

ADME-related activities

1. PK behavior

A) Synthesis of radiolabeled compound

B) See also "1/2. PK behavior and metabolism" in slide for Phase 1

C) Consider **further ADME-related studies**, such as

- Placental transfer - May be delayed
- Milk transfer - May be delayed
- Food effect (if not done earlier)
- Effect of further formulations
- Multiple dose PK (if accumulation etc.)

2. Metabolism

A) **CYP isoenzyme profiling** (victim?) (if not done earlier)

B) **Enzyme induction** e.g. *in vitro* with isolated enzymes, including mRNA induction (self-induction? perpetrator → DDI?) (if not done earlier). Can be postponed for Phase 3

C) Determination of **metabolites** (if not done earlier)

D) See also above under 1./2. PK behavior and metabolism" in slide for Phase 1

1./2. PK behavior and metabolism – May be delayed

- *Rodent:* mass balance/excretion, metabolites, distribution
- Non-rodent: at least mass balance/excretion

Acute i.v. (if not done earlier) and per os (p.o.)

For clinical Phase 2 (usually 4-12 weeks) – continued 3

ADME-related activities (continued 2)

3. *Drug-drug interaction* (DDI)

A) **Protein binding** *in vitro*, in toxicity species and man (if not done earlier)

B) **Blood distribution** *in vitro*, in toxicity species and man (if not done earlier)

C) **Enzyme inhibition** (perpetrator → DDI?) (if not done earlier). Can also be postponed for Phase 3

- e.g. *in vitro* with isolated CYP enzymes
- Increasingly also: inhibition of transporters (see also later)

D) Enzyme induction see previous slide

For clinical Phase 3 *(often > 13 weeks)*

Toxicology

Chronic toxicity including recovery and TK
- Rodent (26 weeks)
- Non-rodents:
  - US & J: 39 weeks according to ICH S4
  - EU: 26 weeks – See also ICH M3(R2)

Depending on intented human use

Non-clinical characterization of **human metabolites**, if exposure ≥ 10% of total drug exposure AND human exposure significantly larger than maximum exposure in animals* – ICH Q3A/B(R2)
May be delayed until before registration

Local tolerance to cover unintended injection route

Dedicated **immunotoxicity testing,** if needed, see also ICH M3(R2) and ICH S8
(if not done earlier)

Dedicated **phototoxicity** study/ies only, if indicated, see also ICH M3(R2) and ICH S10
(if not done earlier)

Abuse liability, if indicated e.g. for CNS drugs (if not done earlier)

Rat **fertility and early embryonic** development (Segment I) including TK. Includes also male fertility

Embryo-fetal development in rats and rabbits (Segment II) (if not done earlier)

* For drugs with a daily administered dose <10 mg greater fractions might be more appropriate
Some metabolites are not of toxicological concern (e.g., most glutathione conjugates) and do not warrant testing

For clinical Phase 3 *(often > 13 weeks) – continued 2*

ADME-related activities

1. PK behavior

Placental / milk transfer (if not done earlier). May also be postponed to registration

1./2. PK behavior and metabolism (if not done earlier)

- *Rodent:* mass balance, excretion, metabolites, distribution
- *Non-rodent:* at least mass balance

In general: acute p.o. and i.v.

2./3. Metabolism and DDI

A) **Enzyme inhibition/induction** including e.g. *in vitro* studies with isolated enzymes (if not done earlier)

B) **Other ADME-related studies** – as needed – with substrates, inhibitors and partly inducers, e.g.

- *Phase-1 enzymes*: flavin-containing monooxygenases (FMO)*
- *Phase-2 enzymes*: especially UDP-glucuronosyltransferases (UGT)
- *Transporter proteins*, especially
  OAT1/3 (> 25% hepatic/biliary excretion)
  OATP1B1/3, OCT2 (> 25% renal excretion)
  P glycoprotein (gp), breast cancer-related protein (BCRP)

Human ADME with ‘hot’ compound - Possibly already done for clinical Phase 2

* CYP isoenzyme work done earlier

For registration

Toxicology

Rat and mouse carcinogenicity studies including TK (preceded by mouse DRF and pilot study)
ICH S1A-C - If concerns: to be conducted prior to clinical trials

If concerns: consider **photocarcinogenicity** study (but generally not considered being useful)

Juvenile toxicity testing (for many countries) - Should be available before starting clinical trials in children*

Toxicity studies with **human-specific metabolites** ICH M3(R2) - Preferably done already for clinical Phase 3
See also safety characterization of impurities: depending on amount and daily dose (ICH Q3B(R2)), generally if > 25 % of parent and/or > 10 % of total (EU)

Genotoxicity - If repetition needed

Pre- and postnatal in rat including TK (Segment III)

Embryo-fetal development (Segment II) (if not done earlier)

Environmental impact/occupational safety

ADME-related activities

Human ADME with 'hot' compound (if not done earlier)

* If drug is developed directly in children: see e.g.
- EMA Guideline on the Need for Non-clinical Testing in Juvenile Animals of Pharmaceuticals for Paediatric Indications of January 14, 2008
- FDA Draft Guidance for Industry - Pediatric Study Plans – July 2013

Backup

High dose selection

High dose HD for in vivo toxicity studies

- Maximum tolerated dose (MTD); not essential to reach in every study
- Other aspects:
  - Large exposure multiples
  - Saturation of exposure
  - Maximum feasible dose (MFD)
- Limit doses: 1000 mg/kg/day
  - If mean exposure margin < 10x clinical exposure and clinical dose > 1 g/day: limit dose 10x or 2000 mg/kg/day or the MFD, whichever is lower
  - If at 2000 mg/kg/day test exposure < clinical exposure, a higher dose up to the MFD can be considered
- In any case: HD with a 50x margin of exposure (usually based on group mean AUC values) of the parent drug or the pharmacologically active molecule of a pro-drug are generally considered acceptable

For further details see ICH M3(R2)

Evaluation of the risk of drug-drug-interactions (DDI) - Regulatory requirements

- **Metabolism**
  - ***Enzyme inhibition*** as **perpetrator** inhibiting the metabolism of other drugs, e.g.
    - Direct and time-dependent inhibition
    - CYP **1A2,** 2A6, **2B6, 2C8, 2C9, 2C19, 2D6,** 2E1, **3A**4
    - UGT1A1, UGT2B7
  - ***Enzyme induction*** as **perpetrator** increasing the metabolism of other drugs, e.g.
    - CYP **1A2,** 2B6, **3A**4
  - ***Metabolic pathways*** **profiling** to detect compounds being **victim** of inducing/inhibiting effects of other drugs
- **Transport**
  - ***Inhibition*** as **perpetrator** inhibiting the uptake/excretion of other drugs, especially
    - **P gp, OATP1B1, OATP1B3,** OCT1**, OCT2, OAT1, OAT3, BCRP,** BSEP, MATE1, MATE2
  - ***Induction*** as **perpetrator** increasing the uptake/excretion of other drugs, e.g.
    - P-gp (co-induction with CYP 3A)
  - Being the **victim** of inducing/inhibiting effects of other drugs

Systems, specific substrates/inhibitors/inducers for *in vitro* experiments and decision trees for clinical DDI studies are specified in corresponding guidelines

Co-medication in fixed formulation ICH M3(R2)

- *Compounds with clinical experience (≥ clinical Phase 3):*
  No further preclinical data needed
- *At least one compound without adequate clinical experience (≤ clinical Phase 2), but no significant toxicological concern:*
  Non-clinical combination study/ies generally only recommended before large-scale or long-term combination trials (generally clinical Phase 3), as well as for marketing
- *Only compounds without adequate clinical experience:*
  - Development of individual compounds: one nonclinical combination toxicity study in one species may be sufficient. Duration equivalent to that of the clinical trial or new drug application (NDA, FDA), but maximally 90 days
  - Development of combination: full program with combination