Genotoxicity risk assessment during lead optimization phase in pharmaceutical drug development

Alessandro Casartelli, Illaria Masotto, Dario Salerno and Michela Pecoraro

Aptuit Srl, an Evotec company, Campus Levi-Montalcini. Via Alessandro Fleming 4, 37135 Verona, Italy

Rationale

Genotoxicity risk assessment plays a critical role in the lead optimization phase of pharmaceutical drug development. As potential therapeutic candidates progress through preclinical evaluation, it becomes essential to evaluate their potential to induce genetic damage, thereby minimizing the risk to human health. This work provides an overview of the genotoxicity risk assessment strategies employed by Evotec during the lead optimization and candidate selection phases, highlighting the importance of integrating these assessments into the drug development process. Any issue rising during the late lead optimization or candidate selection phase should be managed by a risk-based approach: the toxicologist can develop a tailored approach and eventually identify the most appropriate strategy to close the gap between the lead optimization and the following development processes. In such cases, the decision making is mainly driven by the previous data available, the mechanism of action, the pharmacological class and any data on structurally related known compounds. A decision tree model is adopted, to drive the decision with a risk-based approach. The genotoxicity risk assessment process must comply with regulatory guidelines, including those provided by ICH and FDA: these guidelines specify the minimum battery of tests required to evaluate the genotoxic potential of pharmaceutical compounds that enter in the full development. The integration of genotoxicity risk assessment tools in the lead optimization and the compliance to the regulatory guidelines would ensure a more robust genotoxicity assessment in an earlier phase, improving drug safety and success rates in clinical trials.

Regulatory framework

ICH M3(R2): provides the overall framework of the toxicology assessment

ICH S2(R1): provides the relevant information for the genetic toxicology strategy in preclinical development of pharmaceutical products

ICH S9: toxicology testing in anticancer pharmaceuticals: the genetic toxicology assessment is generally not required unless healthy volunteers instead of patients are expected during clinical trials

Genetic toxicology assessment overall strategy

	Drug Discovery	Lead Optimization	Candidate Selection	Clinical Enabling
Number of molecules	Many (>50)	<10	2-3	1
Suggested technology	*In silico*	*In vitro* Screening	*In vitro* “GLP like”	Full GLP test
Endpoint	*In silico* (mainly on genic mutation)	Genic mutation assessment	Genic mutation & chromosomal aberration	Genic mutation & chromosomal aberration
Tests	*In silico*	Ames test screenings	Ames + *in vitro* micronucleus tests	Ames + *in vitro* micronucleus tests

Rationale for the follow up of potential issues raising during the Lead optimization or Candidate Selection phases

Genic mutation:
positive Ames test follow up

- To discharge any possible artefactual issue, an accurate evaluation of the test procedure and study conduction is suggested (i.e. number of positive doses, test conditions and study design, cell viability, colony morphology, chemical stability of the test molecule, contaminants or impurities…)
- A true positive Ames may be acceptable only if considered appropriate on the basis of the risk-benefit analysis. This may be established on the basis of
  - the importance of the drug (i.e. life-saving drugs)
  - the drug indication
  - the target population
  - the duration of treatment.
- For sub chronic or chronic therapies, a true positive Ames test is generally a showstopper, as very difficult to follow up.
  - *In vivo* tests able to detect genic mutations are very specialistic, and would not ensure an effective follow up
  - Carcinogenicity studies may help, but requiring very high costs
- A strong rationale is anyway required.

Chromosome aberrations:
positive *in vitro* micronucleus test follow up

- To discharge any possible artefactual issue, an accurate evaluation of the test procedure and study conduction is suggested (i.e. cell toxicity, pH, number of positive doses …)
- Micronuclei can be formed by means of:
  - a DNA damage (clastogenicity)
  - an aneugenic effect on the cell cycle (i.e. mitotic spindle interferences)
- Clastogenic compounds are genotoxic
- Aneugenic compounds are NOT genotoxic (as DNA is not the target).
- A scientific rationale based on the mechanism of action and aimed to identify any potential interference with the cell cycle (and suggesting a potential aneugenic mechanism) would pose the basis for the next evaluations.
- New data should be produced to characterize the finding, and demonstrate the aneugenic vs genotoxic potential. This can be done with a tailored testing strategy.
- Aneugenic compounds are considered not mutagenic, and therefore in the same way as done for other toxicological findings (can be accepted if an exposure threshold is set in the IND enabling *in vivo* tests).

Conclusion

- The genotoxic potential is one of the major risks associated to potential pitfalls in drug development
- True positive results in the genotoxicity screening observed during the Lead Optimization phase not always represent a showstopper, but need to be carefully characterized
- In this scenario, the toxicologist could provide a strategy enabling the access to the IND enabling phase, ensuring the lowest attrition and the ethical use of animals
- In this work, a flowchart describing the overall strategy adopted in Evotec is presented

Follow up of positive tests

Bridging from Lead Optimization / Candidate Selection **to** IND enabling full development

Positive Ames test

The scientific rationale supporting the further development should be based on:

- Importance of the drug Life-saving drugs
- Drug indication
- Target population
- Duration of treatment

Weight **O**f **E**vidence

Different strategies are available depending on the information supporting the rationale – may vary from no action to tailored *in vivo* studies

OUTCOME: ?

Positive *In vitro* Micronucleus test

Is the observed positivity due to:
an ANEUGENIC (= non genotoxic) effect
OR
a CLASTOGENIC (= genotoxic) effect?

Different tailored strategies:

In vitro mechanistic test to demonstrate the aneugenic vs clastogenic effect

In both cases no additional animals are required!

Two *in vivo* tests: if negative they can counterbalance the positivity seen *in vitro* (ICH S2)

Centromere staining *in vitro* study

In vitro chromosome aberration study

A combined Comet/ Micronucleus assessment is included in the preliminary MTD/Dose Range Finding *in vivo* rodent study

Produces evidences enabling the demonstration of an aneugenic vs clastogenic effect

Detects the clastogenic potential only. If negative, the clastogenic potential can be excluded (likely aneugenic)

Achievements:
- Risk discharge vs the following phases
- No need to add new studies - reductions in the animals’ use

If considered CLASTOGENIC

If considered ANEUGENIC

If *in vivo* study outcome is NEGATIVE

If *in vivo* study outcome is POSITIVE

- Anticipate the GLP *in vivo* micronucleus study before the IND
- Assess the safe exposure threshold
- The selection of the clinical trials highest dose will be based on the safe exposure threshold and the NOAEL

OUTCOME:

Anticipate the GLP *in vivo* genetic toxicology study before the IND: run a combined Comet / Micronucleus *in vivo* GLP standalone study