Regulatory genotoxicity: From the bench to the dossier

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June 2016

Who and Why

Who I am?

- Working in pharmaceutical drug development for 16 years
- The last 7 years working at Innoqua Toxicology Consultants providing support in the preclinical development to small and medium size companies
- I belong to the reduced and endangered species: Spanish Regulatory Toxicologist

Why I am here?

- Give you the perspective from the industry
- Innoqua has been involved in more than 100 projects: from impurities qualification to MAA.
- 95% of the programs are from the pharmaceutical industry.
- 60-70% of these projects have required genotoxic assessment based on guideline requirements

Main objective of the talk: to share with you our experience

Background

Genotoxicity is an inherent property of a compound which has to be assessed

There are currently international regulations which establish how and when this assessment has to be done during the development to register any new substance to be marketed.

Positive results in genotoxicity assays mean a “red flag”.

Overall, understanding the mechanisms behind the genotoxicity potential does not necessarily mean to lower the red flag from a regulatory point of view

However key guidelines allows evaluating the biological relevance of a positive result in a genotoxicity study

Background

Genotoxicity assessment should be performed with any new compound intended to be used in pharmaceutical (human and veterinary), cosmetic, chemical or agrochemical industry.

The tests used for the genotoxicity evaluation are the same and very well standardized and regulatorirly accepted following OECD protocols.

A regulatory study means meeting:

Regulatory requirements: in agreement with guidelines
Normative/quality requirements: in agreement with GLP
Scientific requirements: very well trained personnel and background data

The main objective of a regulatory study is to support the safety of a new compound being submitted to the Regulatory agencies.

The talk will be focussed on pharmaceutical drugs genotoxicity assessment

Toxicology assessment of new compounds

- Repeated dose toxicity
- Reproductive and developmental toxicity
- Genotoxicity
- Carcinogenicity.
- Local tolerance
- Phototoxicity
- Immunotoxicity
- Abuse liability
- Other endpoints applicable to the particular development

The main toxicology issues in preclinic/clinic can be managed in some cases

- Dose repeated toxicity
  - Hepatotoxicity of paracetamol
  - Rhabdomyolysis, statins
  - GI and Liver effects, NSAID
- Reproductive and developmental toxicity
  - Thalidomide (now used in Multiple myeloma treatment)
  - Imidazoles (i.e: ketoconazole, voriconazole)
  - Antimalarian agents (i.e: mefloquine)
- Carcinogenicity
  - PPAR gamma agonist, urinary bladder hyperplasia
  - Proton pump inhibitors (i.e lansoprazole), gastric mucosa and testicular Leydig cell hyperplasia
- Abuse liability/withdrawal syndrome
  - Benzodiazepines (i.e diazepam)
  - Opioids
- Immunotoxicity/hematotoxicity
  - Antitumoral drugs, Hydralazine (blood pressure), Isoniazid (antimicrobial)

- Genotoxicity: let's see …

Genotoxicity meaning from a regulatory point of view

Genotoxicity tests can be defined as **in vitro and in vivo tests** designed to detect compounds that induce genetic damage by various mechanisms. These tests **enable hazard identification** with respect to damage to DNA.

Compounds that are positive in tests that detect such kinds of damage have the **potential to be human carcinogens and/or mutagens**

- Development is a continuous risk benefit-balance: *"walking on the edge"*
- However positive results in a regulatory battery of genotoxic studies, normally means a red flag and the "climber" falls down

Genotoxicity assessment within the development of a new drug

- Active ingredient

  How — ICH S2(R1) *Guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use*

  When — ICH M3 *guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals*

- Impurities

  ICH guideline *M7 on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk*

  ICH Q3A-B *Impurities in new Drug substance /New Products*

Genotoxicity assessment within the development of a new drug

- Active ingredient: Overall picture

Standard approach (option 1) two in vitro test before Phase I and an in vivo test before Phase II

Option 1

i. A test for gene mutation in bacteria.
ii. A cytogenetic test for chromosomal damage (the in vitro metaphase chromosome aberration test or in vitro micronucleus test), or an in vitro mouse lymphoma Tk gene mutation assay.
iii. An in vivo test for genotoxicity, generally a test for chromosomal damage using rodent hematopoietic cells, either for micronuclei or for chromosomal aberrations in metaphase cells.

Option 2

i. A test for gene mutation in bacteria.
ii. An in vivo assessment of genotoxicity with two different tissues, usually an assay for micronuclei using rodent hematopoietic cells and a second in vivo assay. Typically this would be a DNA strand breakage assay in liver, unless otherwise justified

Genotoxicity assessment within the development of a new drug

- Many compounds that are mutagenic in the bacterial reverse mutation (Ames) test are rodent carcinogens. This test has been shown to detect relevant genetic changes and the majority of genotoxic rodent and human carcinogens

- A battery approach is needed because no single test is capable of detecting all genotoxic mechanisms relevant in tumorigenesis.

- Several in vitro mammalian cell systems are widely used and can be considered sufficiently validated:
  metaphase chromosome aberration assay,
  micronucleus assay
  mouse lymphoma assay (MLA)

- These three assays are currently considered equally appropriate and therefore interchangeable for measurement of chromosomal damage when used together with other genotoxicity tests in a standard battery for testing of pharmaceuticals

Genotoxicity assessment within the development of a new drug

- In vivo test: mainly micronucleus , UDS, comet Assay in rodents (first option)
- A positive result in any assay for genotoxicity does not always mean that the test compound poses a genotoxic/carcinogenic hazard to humans
- Biologics according to ICH S6 should not be tested for genotoxicity
- Antitumoral drug (for late stage patients) according to ICH S9 should not be tested for genotoxicty to enable clinical testing

Positive results

- Overall picture

Positive in one in vitro test, negative in the two in vivo tests 

Positive in two in vitro tests  depend on in vivo data

Positive in one in vitro test and positive in one in vivo test 

Positive in the two in vivo tests  

Positive results, however….

Always case by case

Regulations have evolved to avoid in vitro false positive results and unrealistic data

Not all positive results are so bad. You can deal with some in vitro positive results, because:

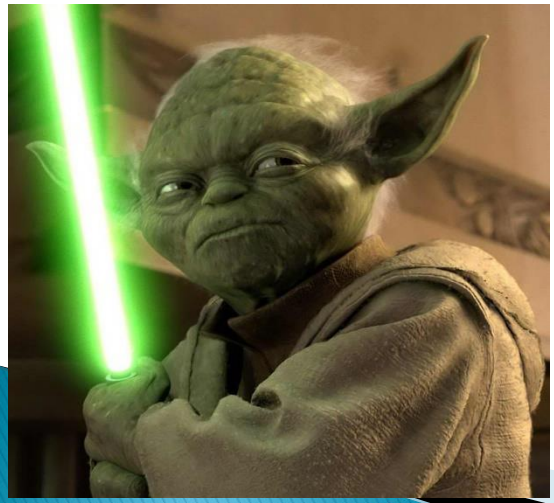
- In vivo assays are more relevant than in vitro ones. Always try to get information from in vivo in case in vitro positive results.
- Small increases that are statistically significant compared with control values, but are within the confidence intervals of historical control values of the testing facility
- Too high concentrations tested, far away from pharmacology/toxicology
- Solubility and cytotoxicity issues
- Impurities
- Metabolism not really relevant in humans, S9 activation system
- Others….

Positive results not well understood

Guidelines allow further assessment, always depend on the sponsor but turning  to  is not a easy task:

try to get weight of evidence
try to show the finding is irrelevant to humans
try to convince your investors “it is not really a problem”
try to convince the regulators “ it is not as bad as its seems”

You need: time, money and keep your fingers crossed



May the force be with you

Some notes

- Perform the genotoxicity assessment as early as possible in the regulatory development.

- In our experience, very few compounds give positive results in genotox assays once entered into regulatory toxicology. The preliminary screening tox has discarded candidates with “red flag”.

- It is thrue, genotoxicity assays are imperfect models, but regulators trust the results from standard assays applied to a very high number of compounds.

- If a red flag is raised and confirmed, do not be so blind and “let the candidate peacefully die”, your competitors will probably not have a genotox issue.

- You should give a lot of justifications in a Due Diligence or to the regulators when submitting a CTA

Some notes

- Project managers believe that everything should be as planned because is written in a development plan approved by management.

- However, in case you have positive result in a in vitro study: stop the engine, perform the *in vivo* studies and do not take unnecessary risks

- Do not forget: behind a regulator there is a person with a family and friends, and he/she may unconsciously think: Would I give this drug to one of me beloved?

- Neither forget: behind a Due Diligence inspector evaluating your program there is a person with a family and friends, and he/she may unconsciously think: Would I give this drug to one of me beloved?

- Case by case approach in case of appropriate risk benefit:
  antitumoral drugs

Genotoxic assessment of impurities (overall picture)

- Impurities above a threshold (defined by ICH Q3A-B) should be assessed for:

  - Repeated dose toxicity in one single species covering the posology up to a maximum of 3 months

  -Genotoxicity in two in vitro assays (Ames + mammalian cells)

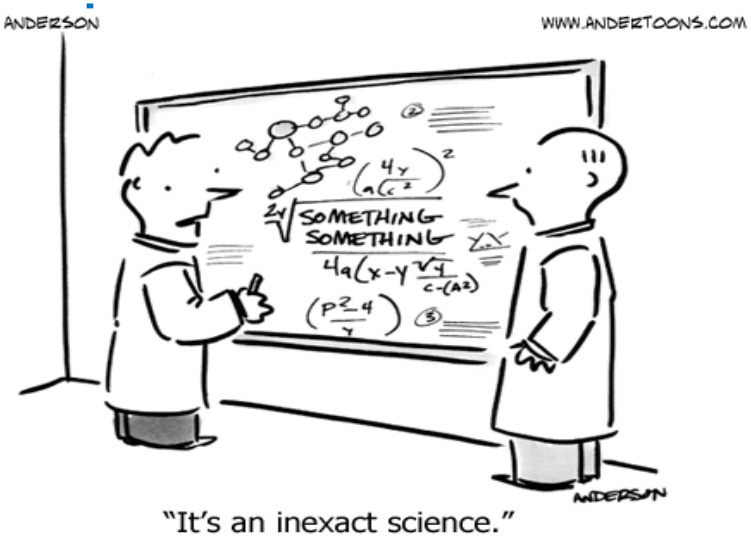
  If positive genotox: modif nd keep the impurity below the TTC (1.5µg/

- There was previous EMA a guidelines on geno rites. We lived under a beautiful blue s *one ring was giv*

Three Rings for th
Seven for the Dwarf-lo e,
Nine for Mortal Men doomed t
One for the Dark Lord on his dark throne
In the Land of Mordor where the Shadows lie.
One Ring to rule them all…

J.R.RTolkien

Introducing ICH M7 also known as Th



- The one ring entered in to force quite
- All known and putative impurities sho
- That's mean you should lock in a roor putative impurities based on the starti the route of synthesis.
- They will be allowed to leave the room once having a reasonable (science-based) list of impurities
- It can be a never ending task: scientific rationale should be applied.
- The MRDD (Maximum Recommended Daily Dose) should be considered
- The LOD/LOQ (Low limit of detection/quantification) should be considered
    - Quality and genotox criteria are combined

M7, very rough picture: supporting clinical trials

- For clinical trial of short duration (phase I clinical trial <14 days): using intermediated or starting materials not genotoxic or keeping the known genotox impurities under the acceptable limit is enough.

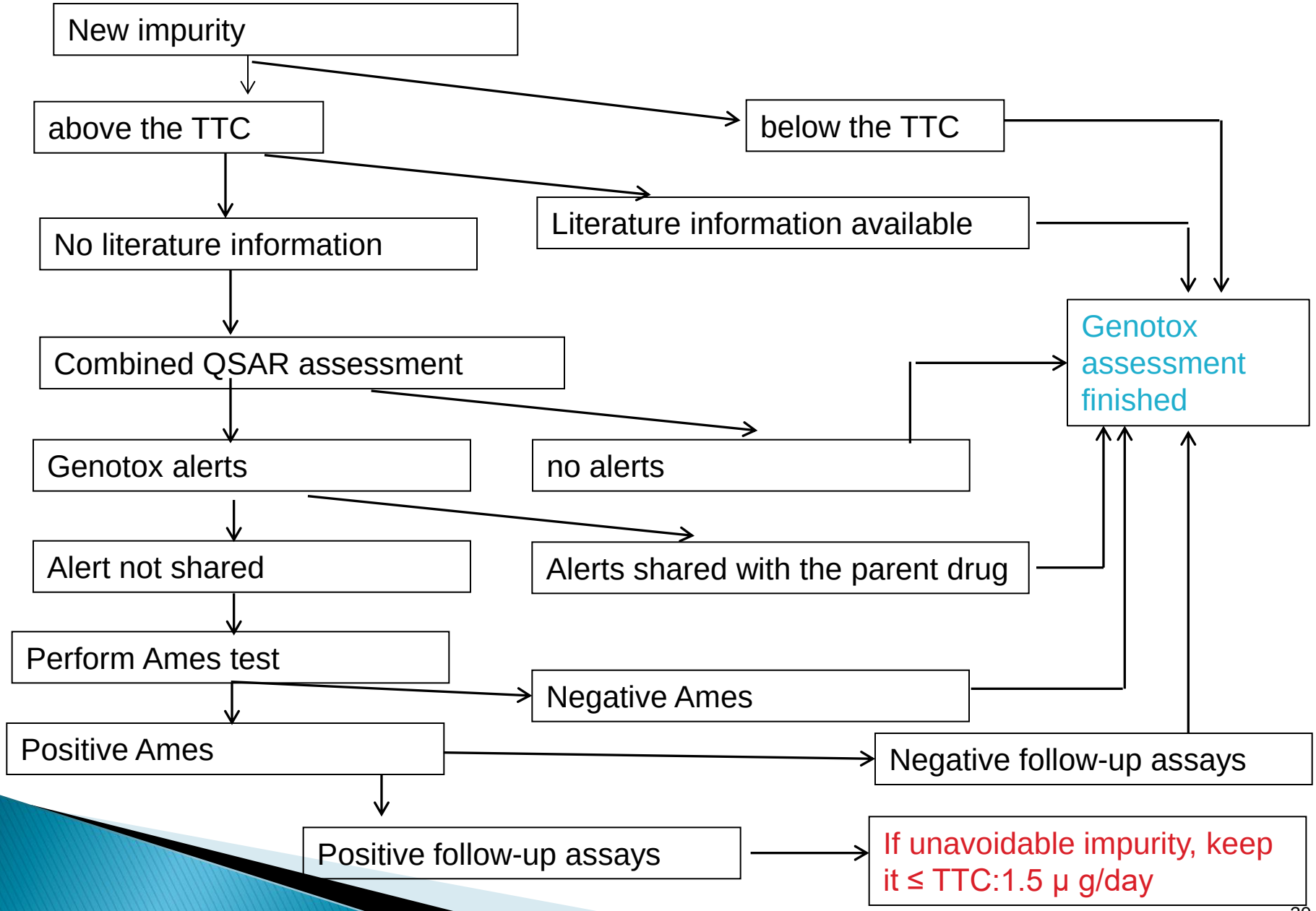
  Other impurities are considered as non genotoxic

- LTL (less than lifetime) exposure

Table 2: Acceptable Intakes for an Individual Impurity

Duration of treatment	≤ 1 month	>1 - 12 months	>1 - 10 years	>10 years to lifetime
Daily intake [µg/day]	120	20	10	1.5

M7, very rough picture: supporting MAA (Marketing Authorization Application)



Summary

- Genotox is an inherent property of a compound
- Pharmaceutical drugs should be checked for genotoxicity according to well standards and regulatory accepted protocols
- Positive results in genotox assays means a red flag. Case by case approach.
- This is a competitive world, competitors may not have your genotox issues
- Known and putative impurities should be also checked for genotoxicity.
- Regulator always prefer clean results based on standard studies than a long list of mechanistic studies justifying the lack of relevance. Case by case approach.

Thank you / Gràcies / Gracias

Thanks to Eduardo Cunchillos for his suggestions and comments

Questions?

Learn from yesterday, live for today, hope for tomorrow. The important thing is not to stop questioning

Albert Einstein