Genetic Toxicology Mini-Adverse Outcome Pathways (mini-AOPs)

Azeddine Elhajouji

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ILSI Health and Environmental Sciences Institute

Outline

- **Introduction to AOP**
- **Mode of action and the need for mini-AOPs concept**
- **Mini-AOPs in genetic Toxicology**
- **Examples of mini-AOPs**

ADVERSE OUTCOME PATHWAYS (AOP)

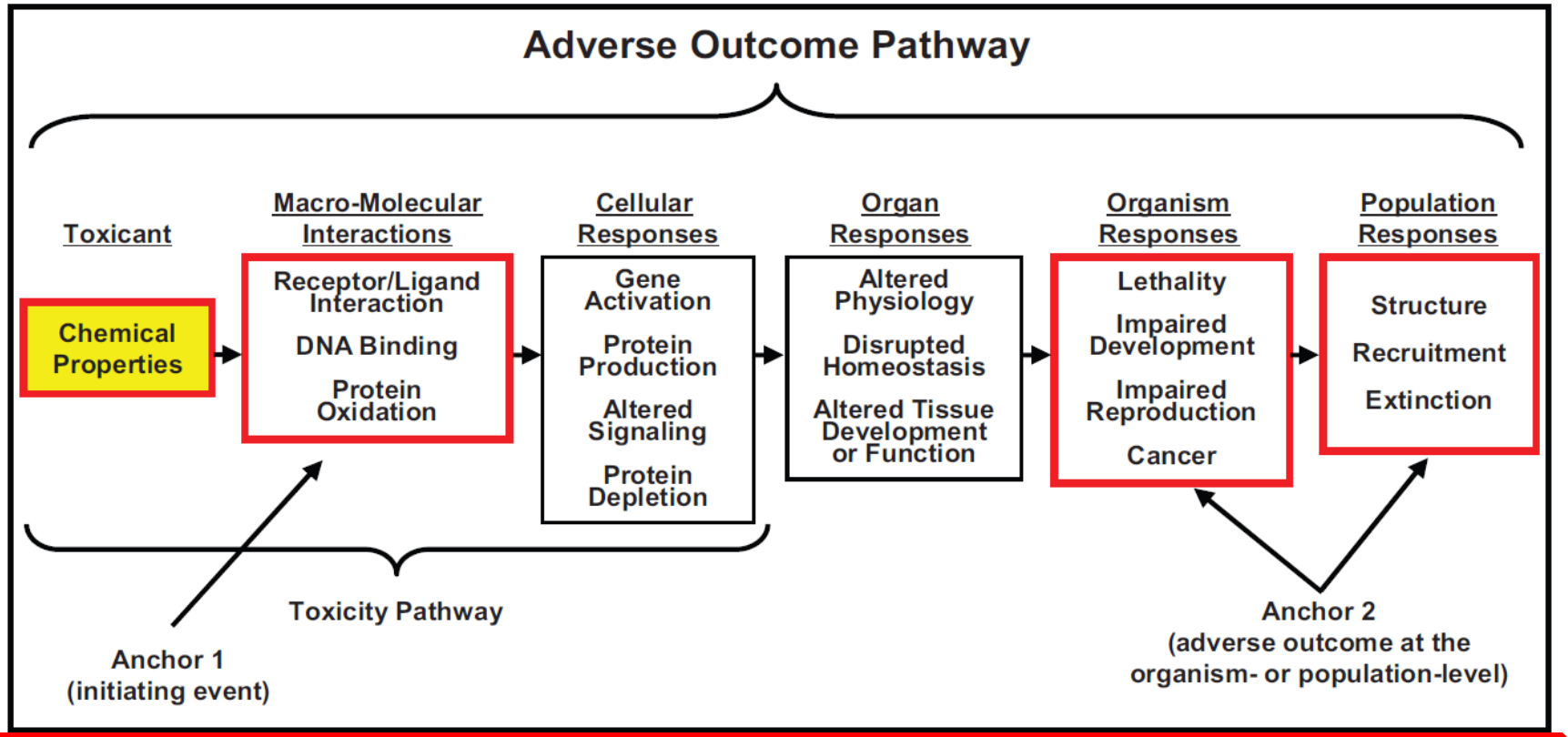
- An Adverse Outcome Pathway (AOP) is a structured representation of biological events leading to adverse effects that is considered relevant to risk assessment.

- The AOP links in a linear way existing knowledge along a pathway of causally connected **key events** (KE) between 2 points – a **molecular initiating event** (MIE) and an **adverse outcome** (AO).

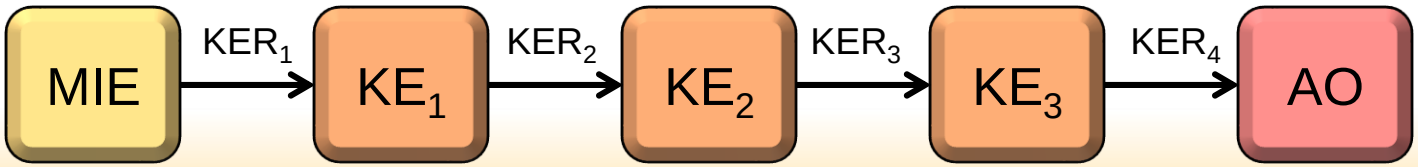
- The events occur at levels of biological organisation relevant to risk assessment e.g. molecular, cellular, tissue/organ, organism and/or population.

- The linkage between the events is described by **key event relationships** (KERs) that describe the scientifically established causal relationship between the chain of key events.

ADVERSE OUTCOME PATHWAYS

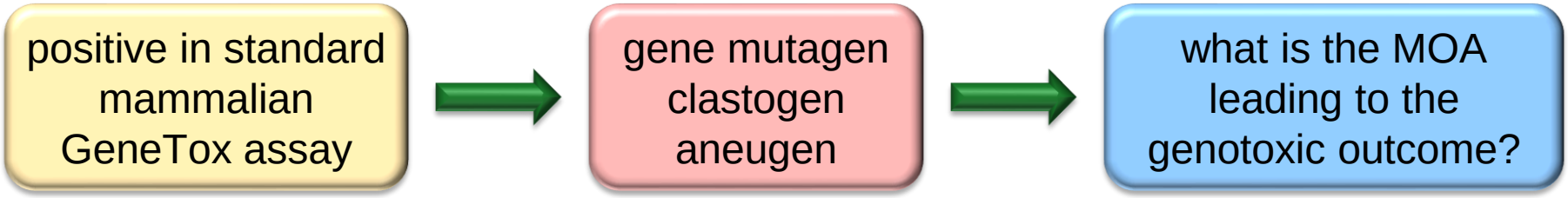


Ref: Ankley *et al*. (2010) Environ. Toxicol. Chem. 29: 730-741



“THE NEED FOR mini-AOPs CONCEPT”

BETTER UNDERSTANDING OF MOA

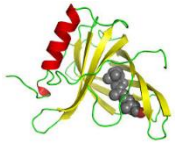


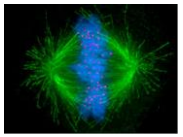
- genetox assays good for hazard identification
- not designed to provide detailed mechanistic information
- do not identify molecular target
- have often unclear structure activity relationship information
- are not designed to provide dose-response information

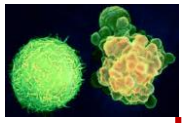
WHY MINI-AOP AND NOT AOP?


Exposure Source

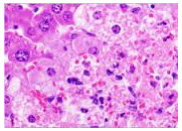

Exposure

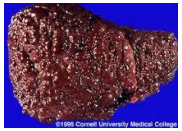

Molecular Initiating Event


Organelle/ Molecular Effects


Cellular Effects

Toxicity Pathway (mini-AOP)


Tissue Effects


Organ Effects


Individual Effects

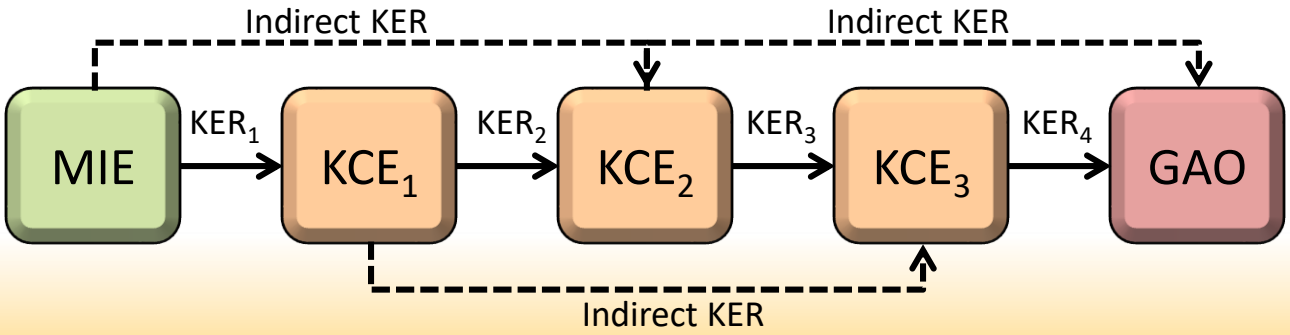

Population Effects

Adverse Outcome Pathway

- In vitro cellular effects can be more easily measured
  - More information available
  - Easier to hit dose-response relationships
  - Easier to address data gaps
- Tissue effects often a combination of different cellular effects
- Adverse health outcomes (i.e. cancer) do not always have a clear relationship
  - not clear that tubulin inhibitors initiate cancer
  - most genotoxins require significant toxicity before you see genotoxicity
  - threshold effects
  - sensitivity of cancer bioassay?

mini-AOPs DEFINITIONS

Molecular initiating event	MIE	A specialized type of key event that represents the initial point of chemical interaction on the molecular level within the cell that results in an event that starts the mini-AOP.
Key event	KCE	A change in biological state that is both measurable and essential to the progression of a defined biological perturbation leading to a specific genotoxic outcome.
Key event relationship	KER	A scientifically-based relationship that connects one key event to another, defines a directed relationship between the two (i.e., identifies one as upstream and the other as downstream), and facilitates inference or extrapolation of the state of the downstream key event from the known, measured, or predicted state of the upstream key event.
Genotoxic Outcome	GO	A specialized type of key event that is generally accepted as being of significance for the genomic integrity of the cell and is expected to result an apical endpoint in an accepted regulatory guideline toxicity test.



https://aopwiki.org/aops?utf8=%E2%9C%93&search=tubulin&commit=Search

WEIGHT OF EVIDENCE FOR KEY EVENT RELATIONSHIPS

Evolved Bradford Hill Considerations	Defining Questions
Biological Concordance	Does the hypothesized AOP conflict with broader biological knowledge? How well established is the AOP?
Essentiality of Key Events	Is the sequence of events reversible if dosing is stopped or a key event prevented?
Concordance of Empirical Observations	**Dose response** – Are the key events observed at doses below or similar to those associated with the apical effect? **Temporality** – Are the key events observed in hypothesized order? **Incidence** – Is the frequency of occurrence of the adverse effect less than that for the key events?
Consistency	Is the pattern of effects across species/strains/organs/test systems what would be expected based on the hypothesized AOP?
Analogy	Would the mode of action be anticipated based on broader chemical specific knowledge?

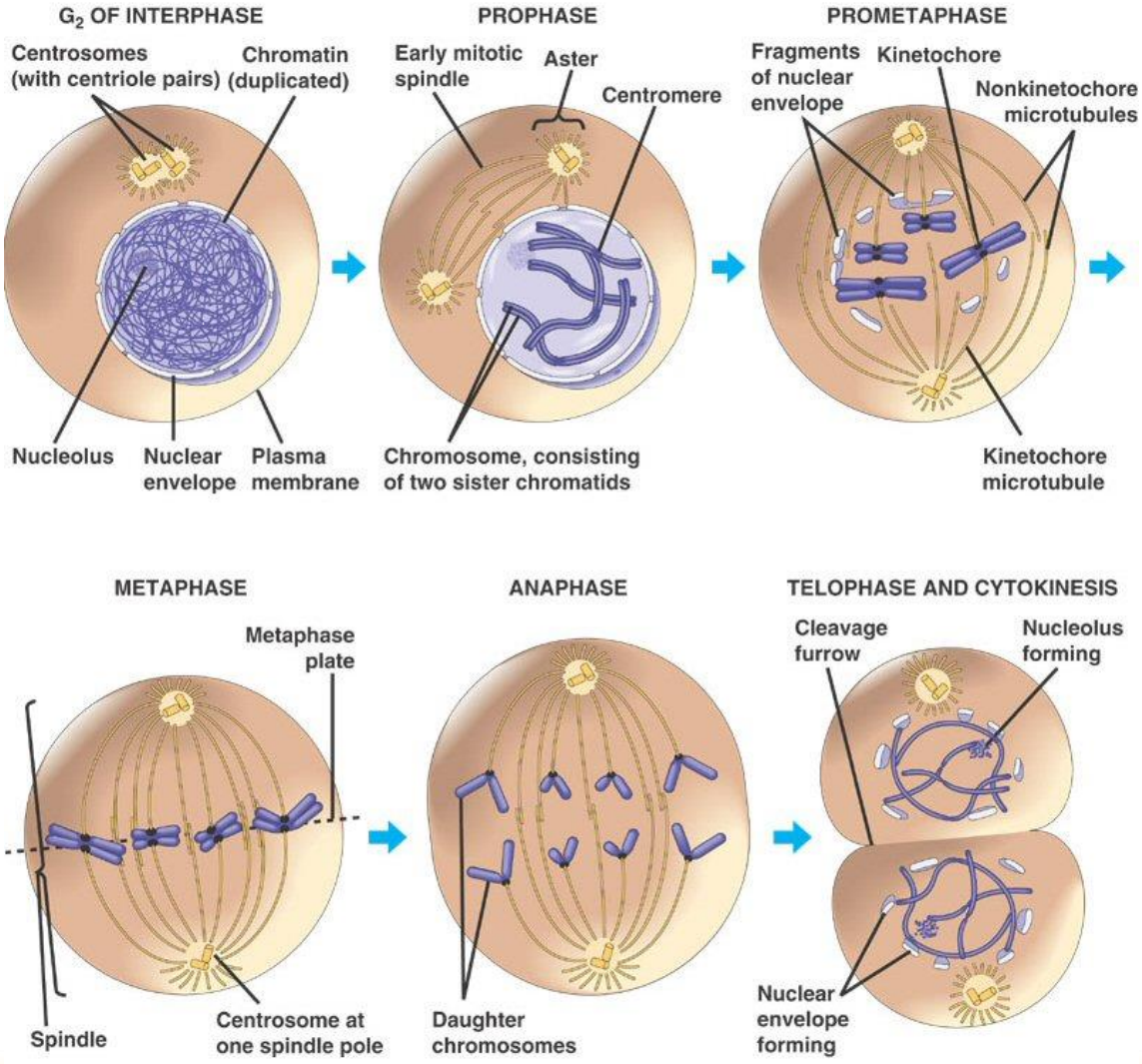
Meek et al., 2014

MOLECULAR INITIATING EVENTS IN GENOTOXICITY

Aneugenic MOA	Clastogenic MOA
• Tubulin binding • Aurora inhibition • Polo kinase inhibition • CDK 1 inhibition • NEK2 inhibition • Actin depolymerization • HSP90 inhibition	• DNA alkylation • Topoisomerase I & II inhibition • DNA intercalation • Nucleoside analogues • Reactive oxygen species • PARP Inhibition • HDAC inhibition • DNA Repair inhibition • Oxidative DNA damage

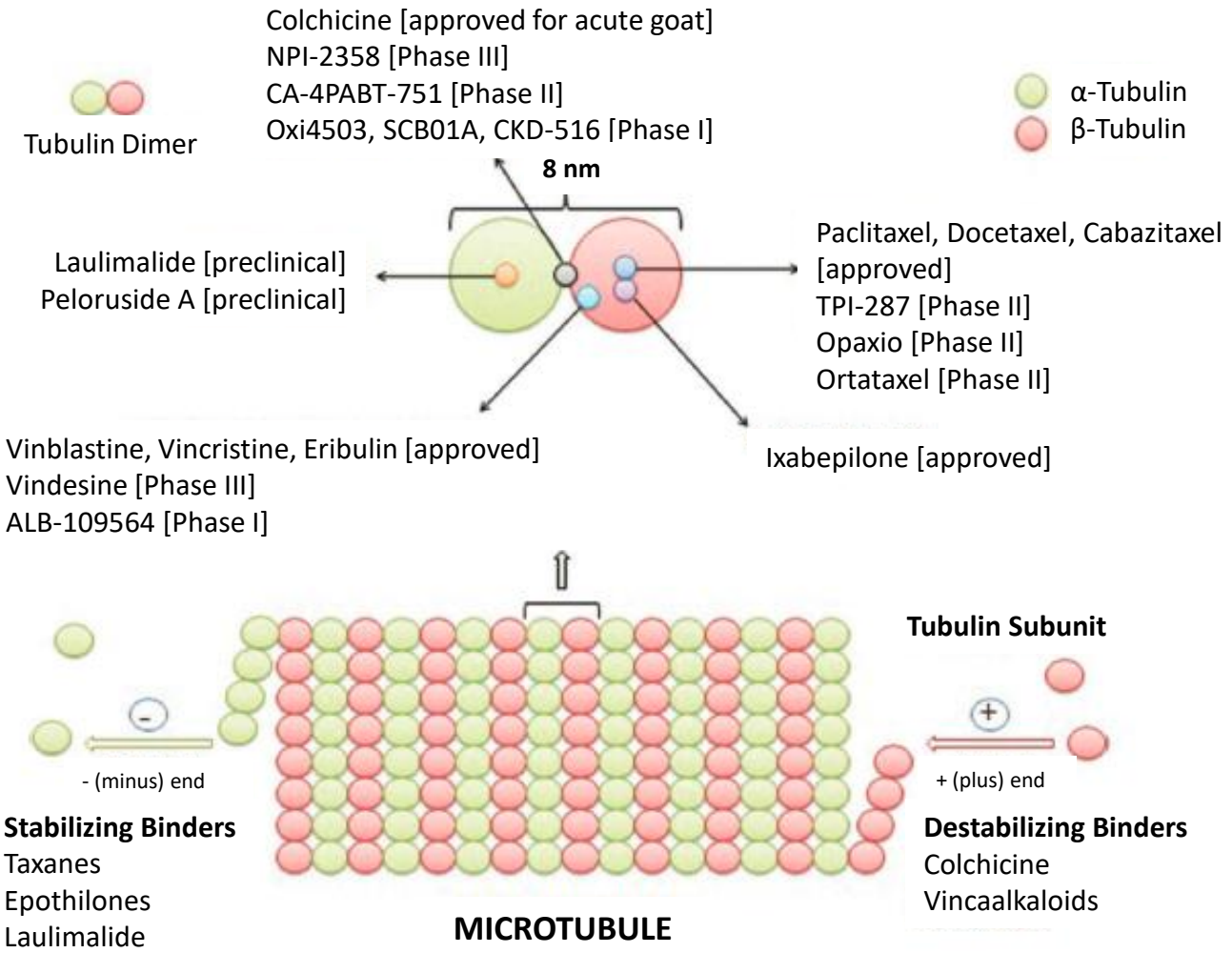
Example of mini-AOPs: Tubulin Binders & Topoisomerase II Inhibitors

MITOSIS OVERVIEW

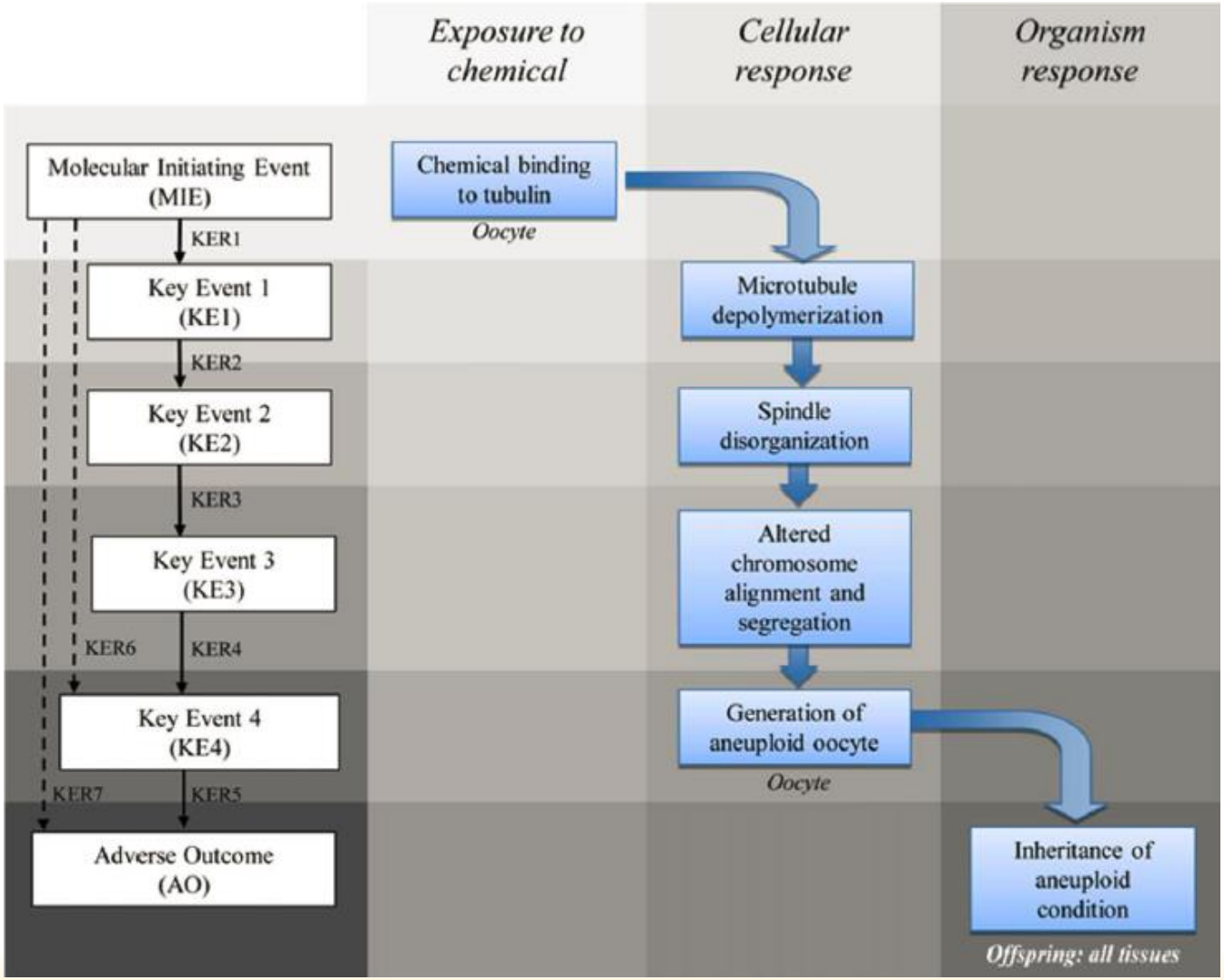


http://fhs-bio-wiki.pbworks.com/w/page/12145788/Mitosis

MICROTUBULI & TUBULIN BINDING SITES

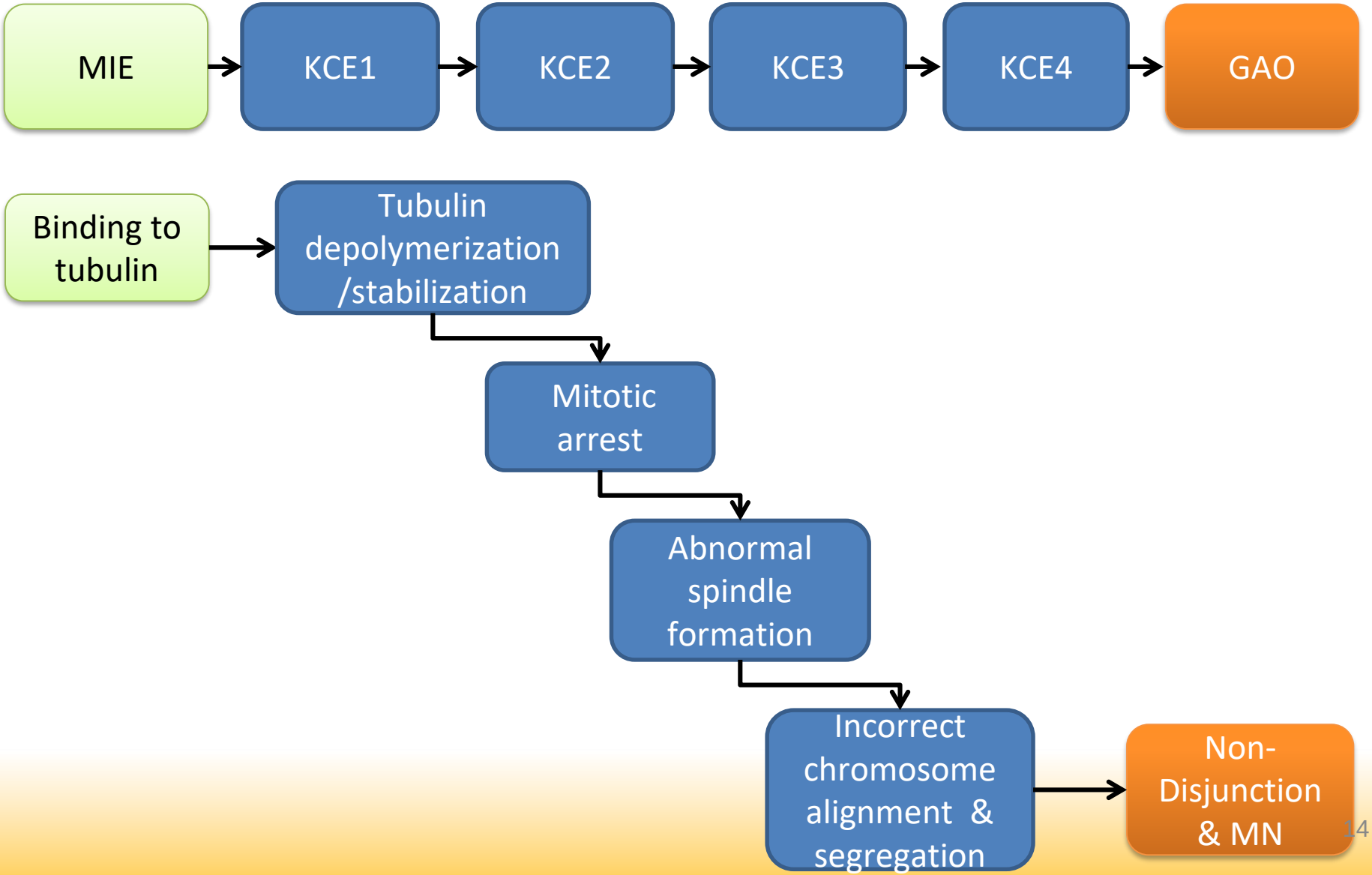


TUBULIN BINDER ADVERSE OUTCOME PATHWAY



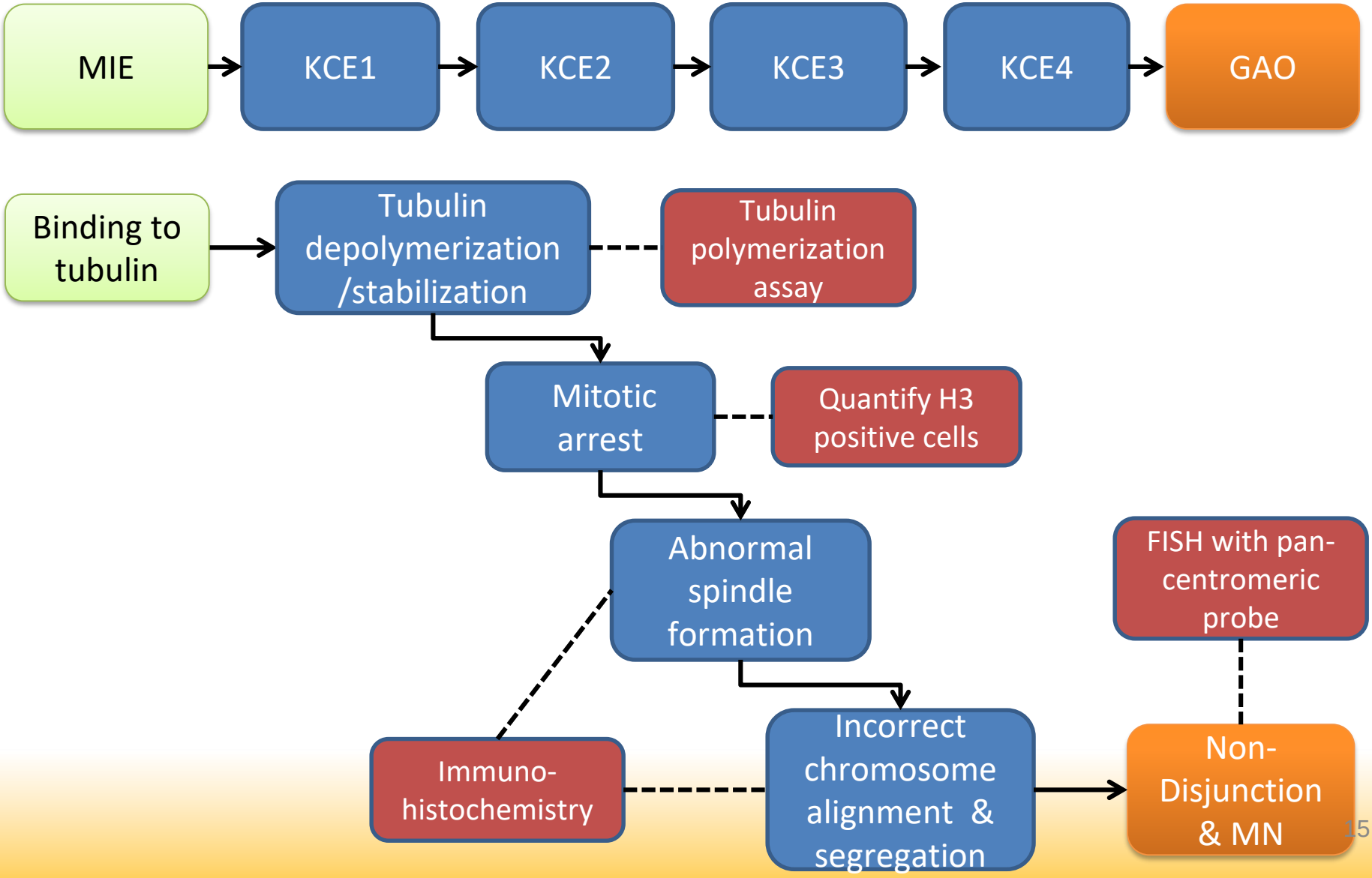
Marchetti et al., Environ Mol Mutagen 2016 ;57(2):87-113

mini-AOP: TUBULIN BINDERS & ANEUPLOIDY INDUCTION

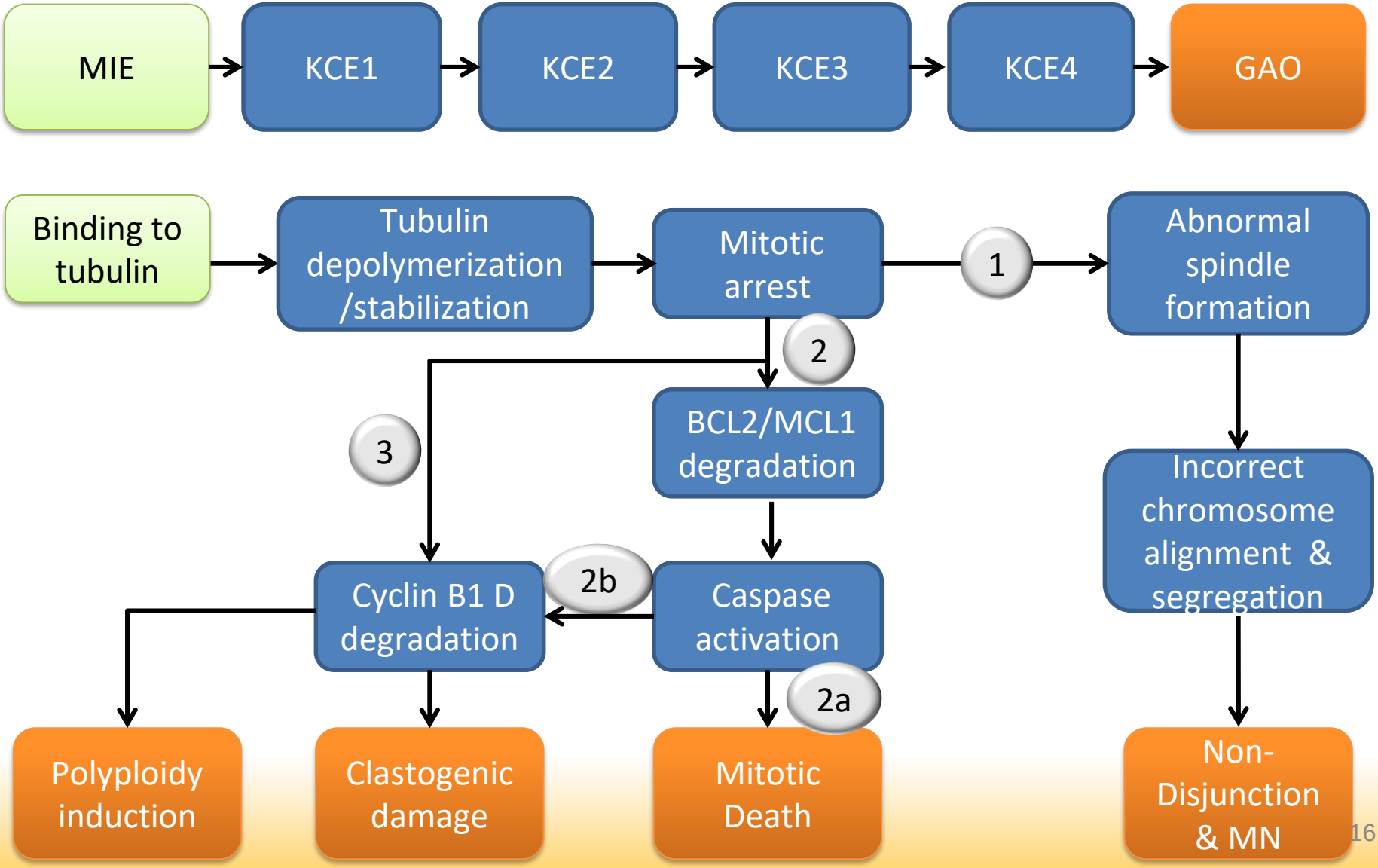


mini-AOP : TUBULIN BINDERS & ANEUPLOIDY INDUCTION

ASSAYS AND TOOLS (RED BOX)

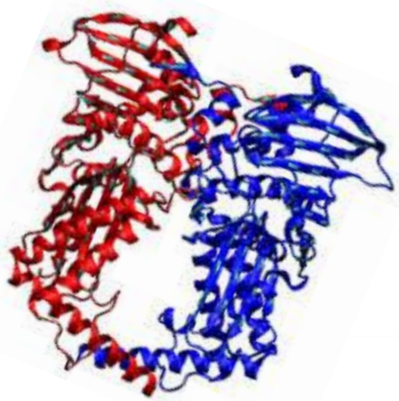


mini-AOP : TUBULIN BINDERS INDUCE MULTIPLE GAOs



TUBULIN BINDERS
KEY EVENT RELATIONSHIPS –KERS-

KCE (upstream)	Relationship type	KCE (downstream)	Level of Confidence in KER
Tubulin Binding	Directly leads to	Microtubuli de-polymerization/stabilization	strong
Microtubuli de-polymerization/stabilization	Directly leads to	Extended Mitotic arrest	strong
Extended mitotic arrest	Leads to	Mitotic abnormalities	medium
Mitotic abnormalities	Directly leads to	Chromosomal loss and non-disjunction	strong
Extended mitotic arrest	Leads to	Caspase activation	strong
Caspase activation	Leads to	Mitotic death and/or clastogenicity	medium
Extended mitotic arrest	Leads to	Cyclin B1 degradation	strong
Cyclin B1 degradation	Directly	Mitotic slippage	strong



Mini-AOP: Topoisomerase II Inhibition Leads to Clastogenicty & Tranlocations

Functions of topoisomerases II

- Resolve the problems associated with the topological constraints of the genetic material (*i.e.*, DNA under- or overwinding, knotting, and tangling)
- Transiently cleave both strands of the double helix
- Play essential roles in a number of genetic processes, including DNA replication, transcription, and recombination, as well as chromosome segregation.

Two main mechanisms of topoisomerases II inhibitors

- *Topoisomerase II poisons kill cells*
  - By increasing levels of covalent topoisomerase II-cleaved DNA complexes
  - By interfering with the ability of the enzyme to religate cleaved DNA molecules

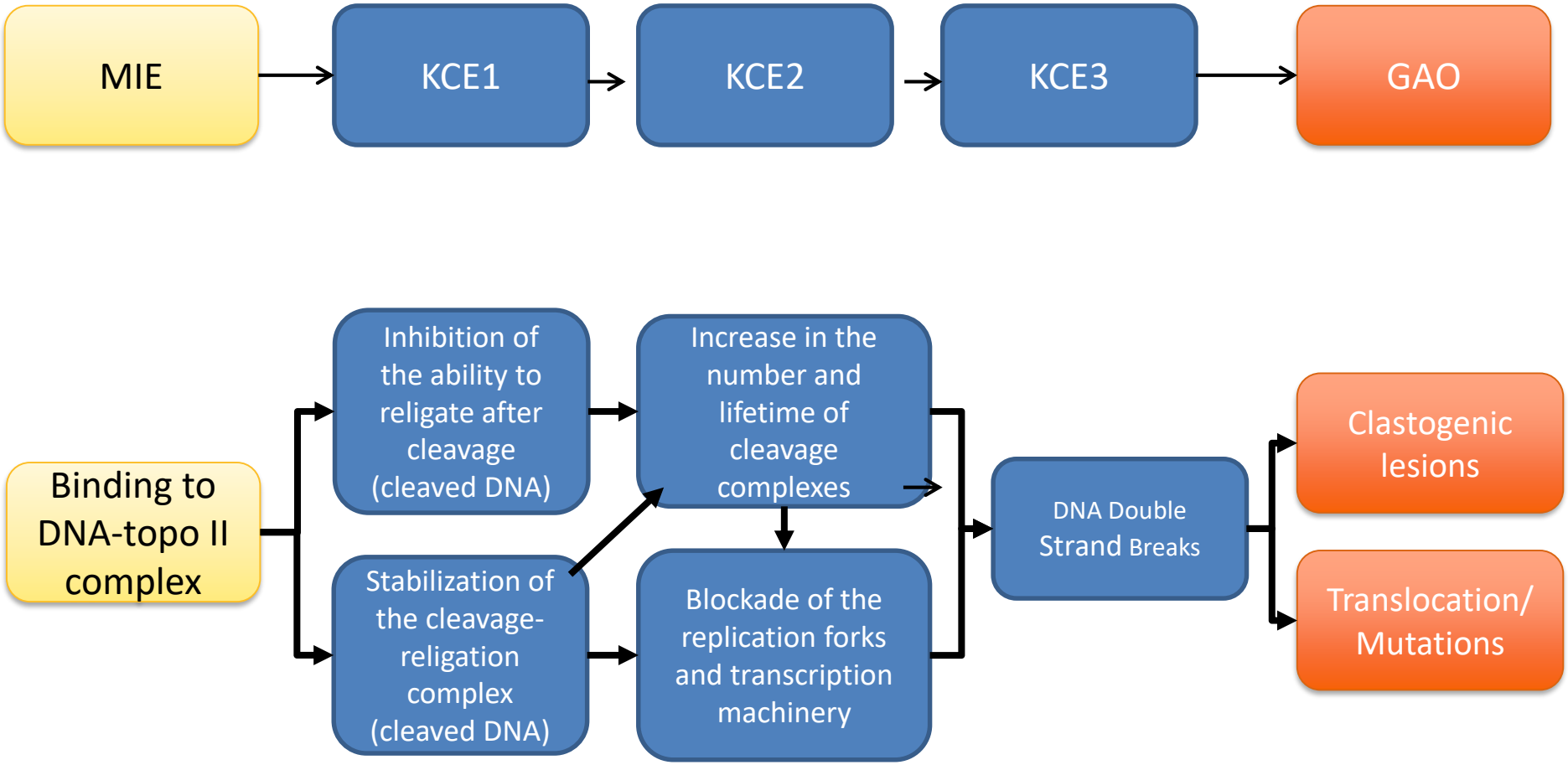
  → Resulting in [stabilization of] cleaved DNA

- *Topoisomerase II catalytic inhibitors*
  - By robbing the cell of the essential catalytic functions of the type II enzymes
  - By a variety of steps of the topoisomerase II catalytic cycle, including [inhibition of] DNA cleavage

  → Resulting in inhibition of Topoisomerase II functions and inhibition of DNA cleavage.

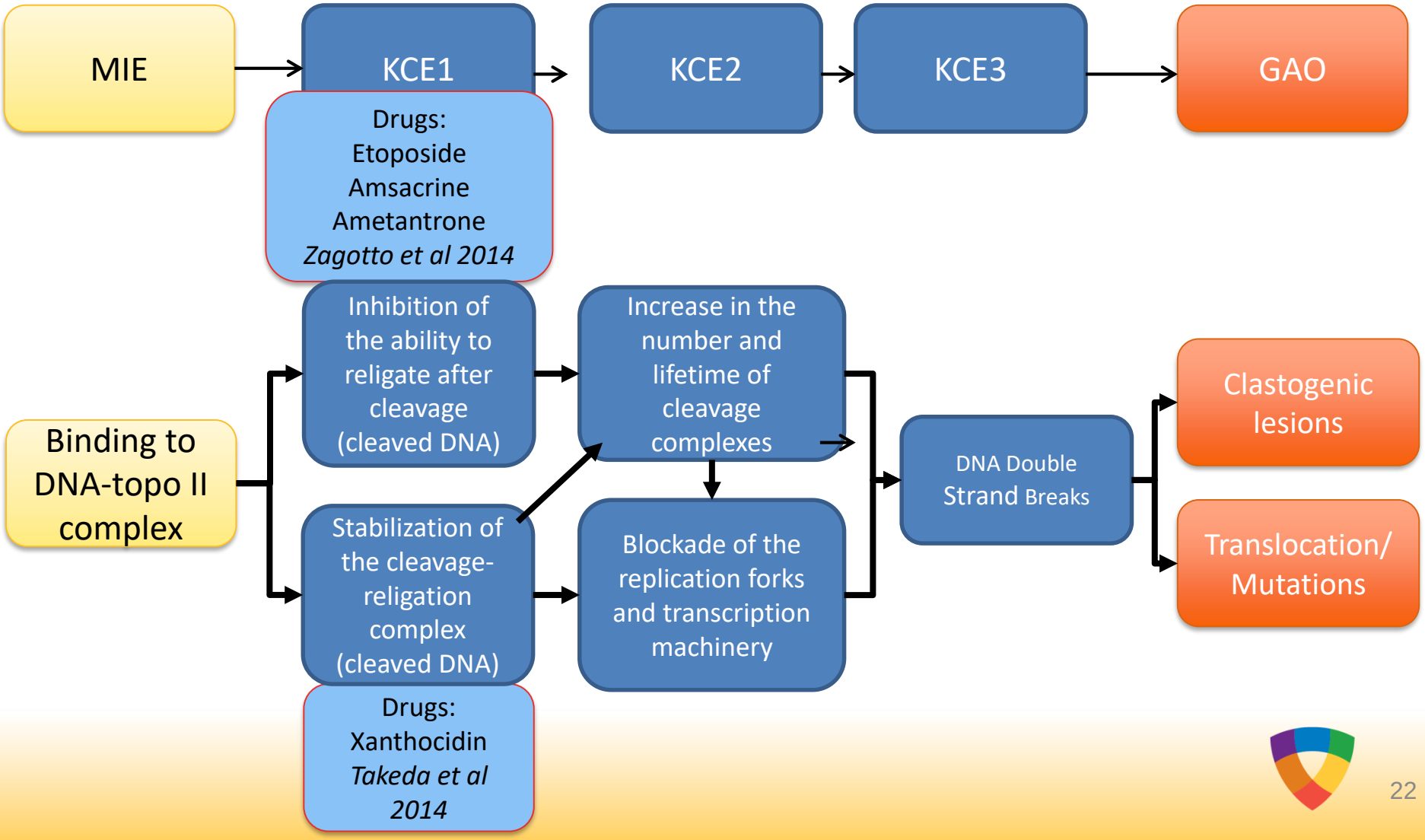
TOPOISOMERASE POISONS

Binding to DNA-topoisomerase II complex leading to genotoxicity (clastogenic lesions, gene mutations)

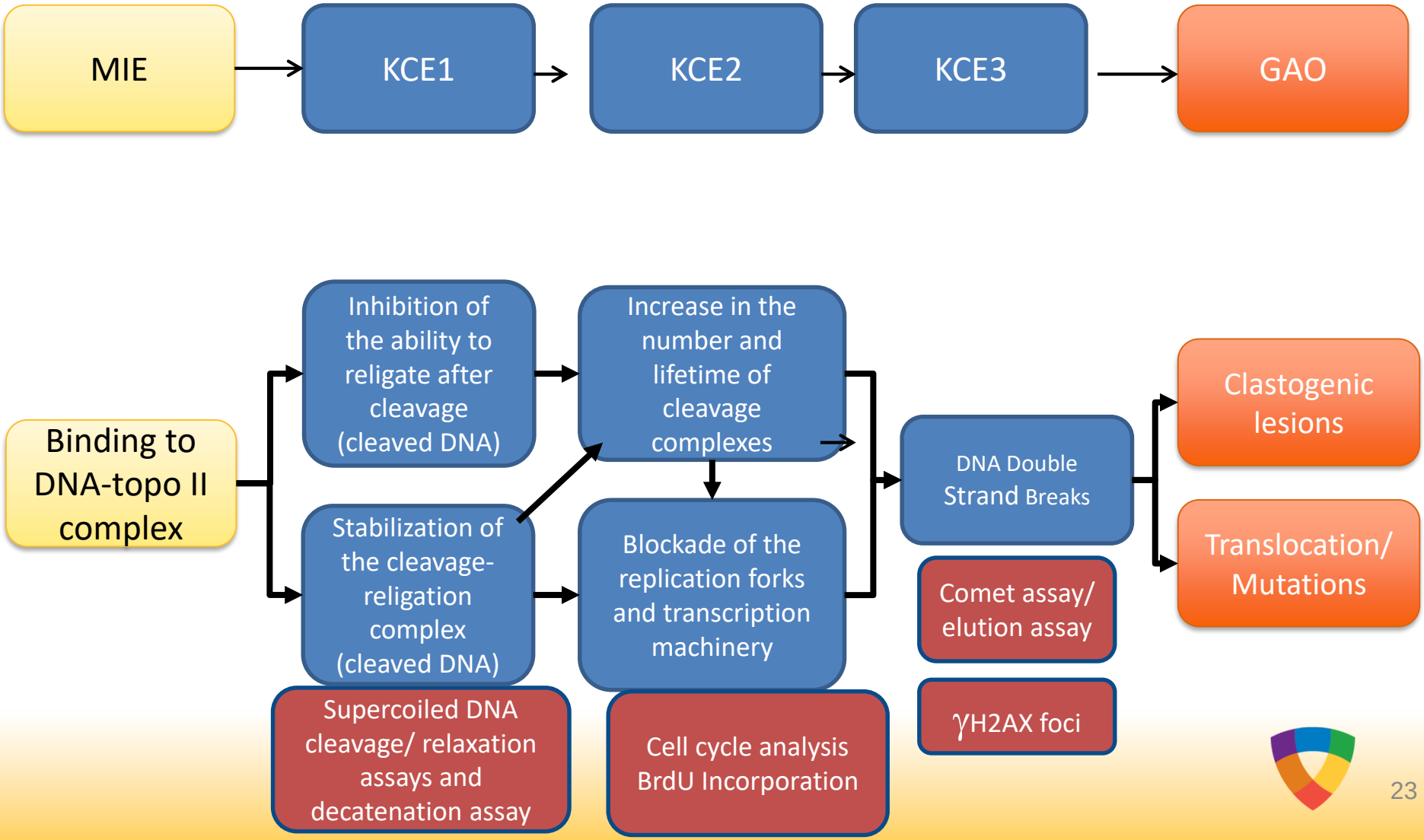


Topoisomerase Poisons

Stressors



TOPOISOMERASE POISONS
ASSAYS/TOOLS



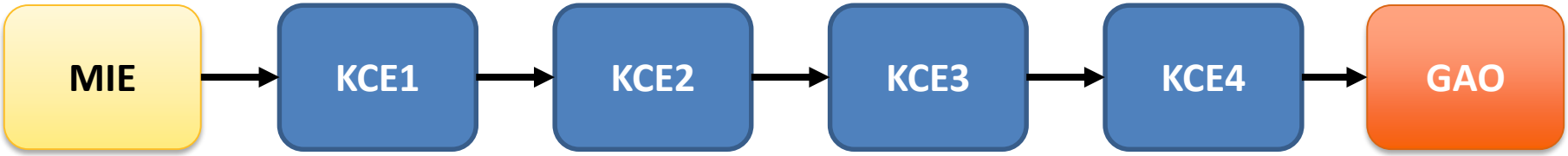
Topoisomerase Poisons

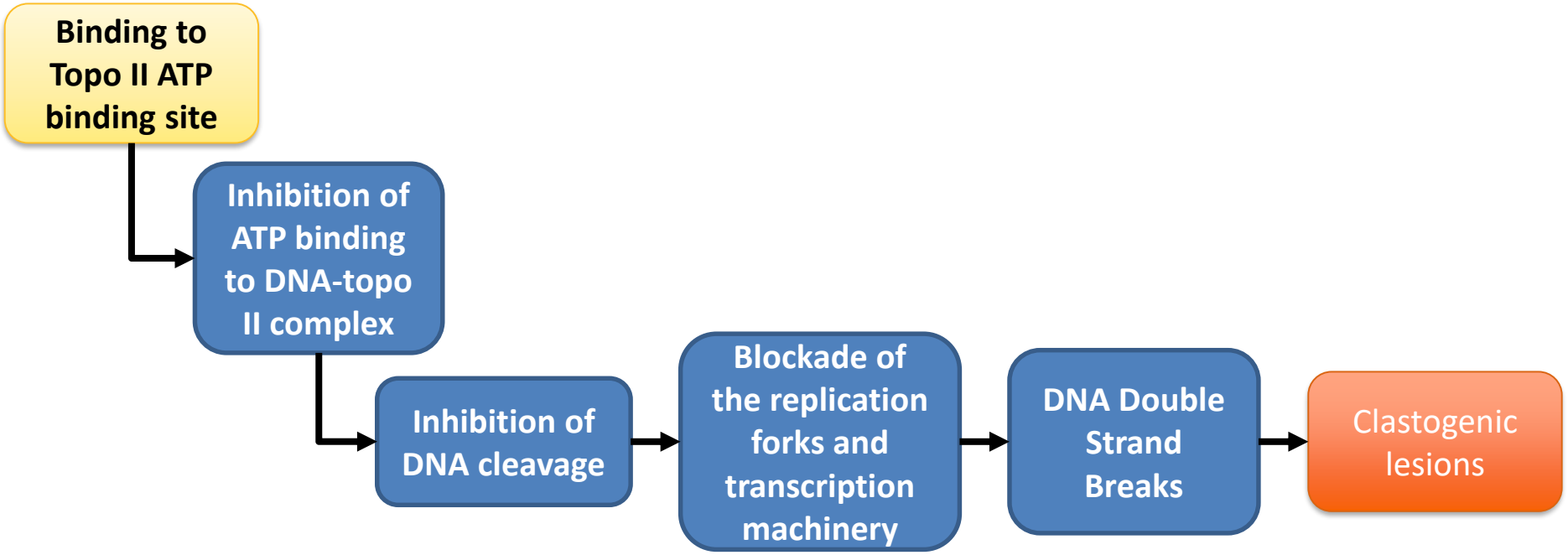
Key Event relationships –KERs-

KCE (upstream)	Relationship type	KCE (downstream)	Level of Confidence in KER
Binding to DNA-topo II complex	Directly leads to	Inhibition of the ability to religate after cleavage (cleaved DNA)	Strong
Binding to DNA-topo II complex	Directly leads to	Stabilization of the cleavage-religation complex (cleaved DNA)	Strong
Stabilization of the cleavage-religation complex (cleaved DNA)	Directly leads to	Blockade of replication and transcription	Strong
Blockade of replication and transcription	Directly leads to	DNA Double Strand Breaks	Medium
Inhibition of the ability to religate after cleavage	Directly leads to	DNA Double Strand Breaks	Strong
Stabilization of the cleavage-religation complex	Directly leads to	DNA Double Strand Breaks	Strong
DNA Double Strand Breaks	Directly leads to	Fixation of DSBs into mutations through Error prone repair (NHEJ)	Strong
Fixation of DSBs into mutations through Error prone repair (NHEJ)	Leads to	Clastogenic lesions/Gene mutations	Strong

TOPOISOMERASE CATALYTIC INHIBITORS

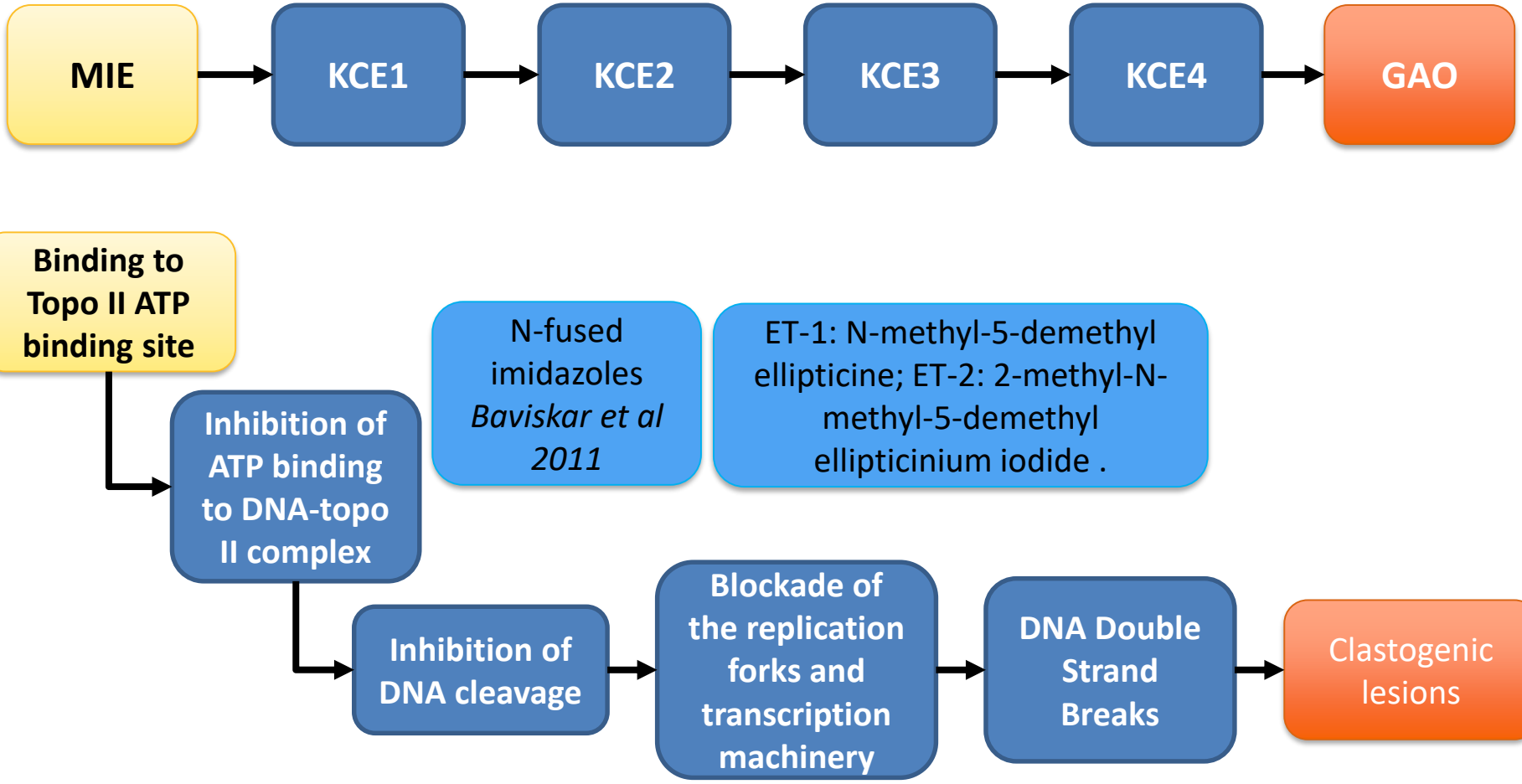
Catalytic inhibition of topoisomerase II leading to genotoxicity





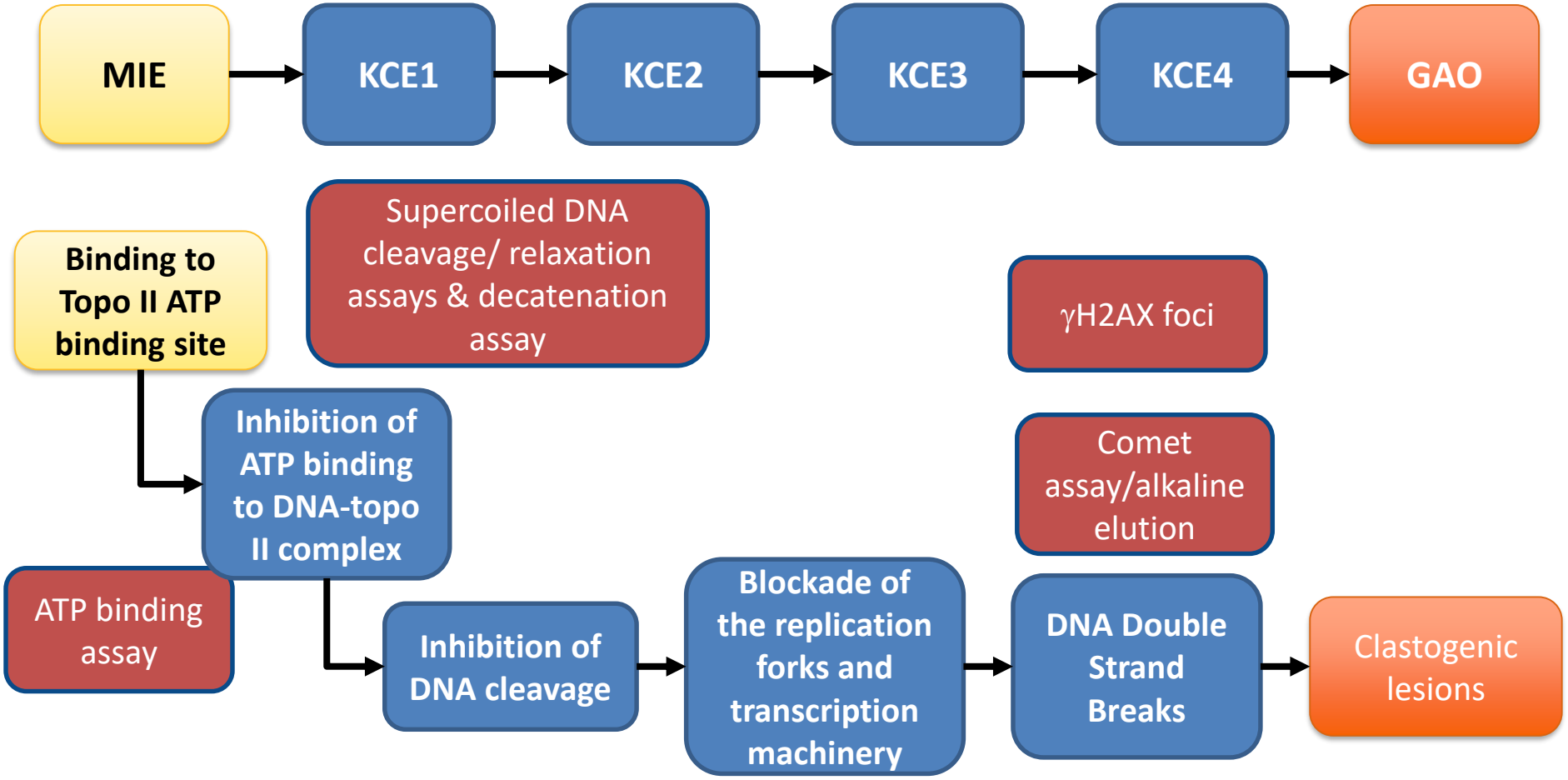
TOPOISOMERASE CATALYTIC INHIBITORS

Stressors



TOPOISOMERASE CATALYTIC INHIBITORS

Assays/Tools



Topoisomerase Catalytic Inhibitors

Key Event relationships –KERs- 1/2

KCE (upstream)	Relationship type	KCE (downstream)	Level of Confidence in KER
Binding to Topo II ATP binding site	Directly leads to	Inhibition of topoisomerase II binding to DNA	Strong
Inhibition of topoisomerase II binding to DNA	Directly leads to	Inhibition of DNA cleavage	Strong
Inhibition of DNA cleavage	Directly leads to	Blockade of the replication forks and transcription machinery	Strong
Blockade of the replication forks and transcription machinery	Directly leads to	DNA Double Strand Breaks	Strong
DNA Double Strand Breaks	Directly leads to	Fixation of DSBs into mutations through Error prone repair (NHEJ)	Strong
Fixation of DSBs into mutations through Error prone repair (NHEJ)	Leads to	Clastogenic lesions	Strong

HOW mini-AOPs COULD BE USEFUL IN GENETIC TOXICOLOGY?

- Identify MIE by characterizing cellular biomarkers (e.g. tubulin binders)
- Better understand structural basis of molecular interactions (e.g. structural features of kinase inhibitors)
- Provide alternative endpoints for in vivo follow-up testing (e.g. polyploidy assessment in histopathology samples for aurora B inhibition)
- Better understand dose-response relationships (nature of POD for topoisomerase II inhibition) and potential use of safety margins
- Use PD/PK modeling to predict in vivo and human relevance (e.g. high dose effects not relevant to human exposure)