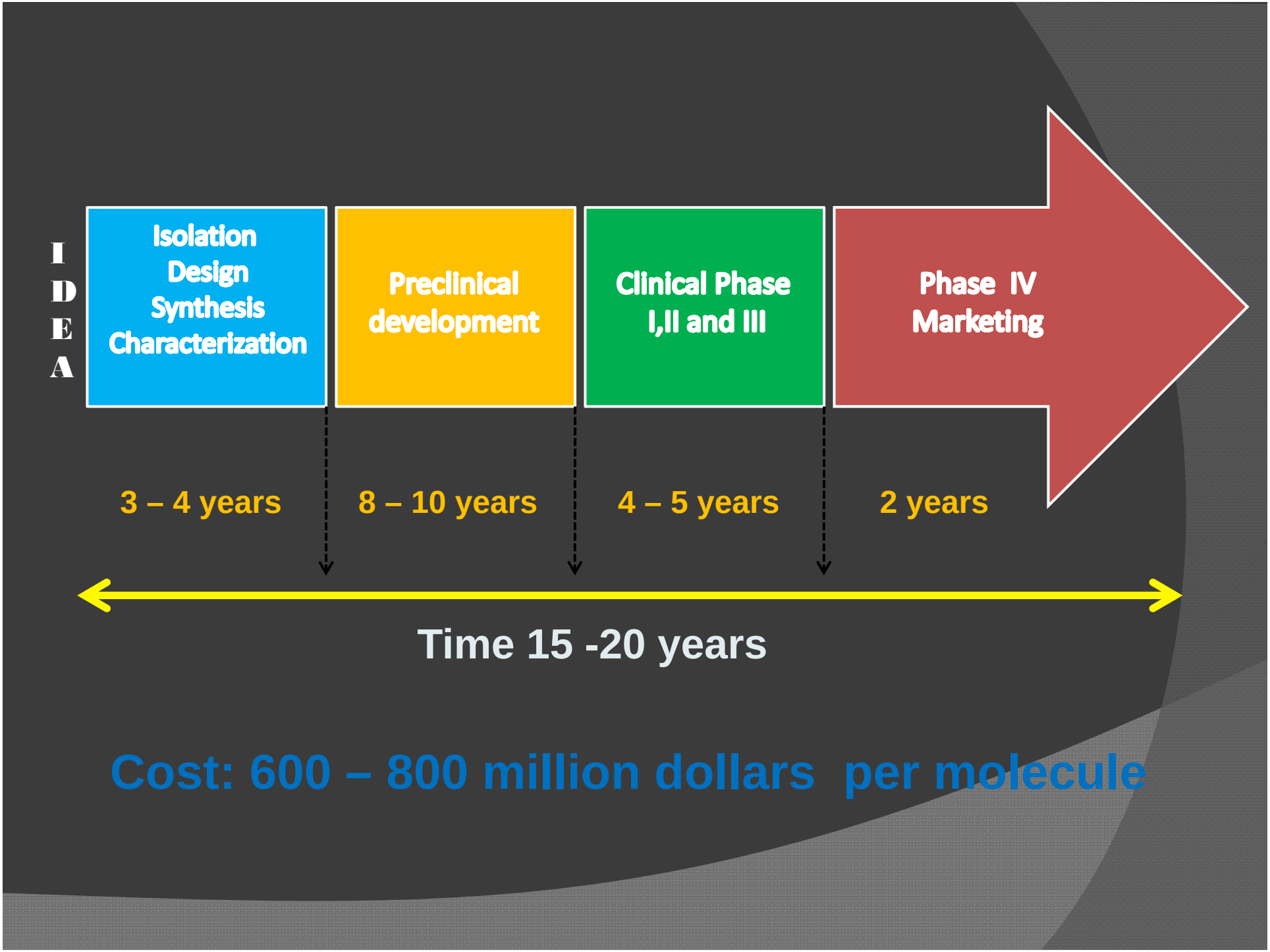
RED LATINOAMERICANA DE DESARROLLO DE METALOFÁRMACOS. (CYTED)

DESARROLLO DE FÁRMACOS DE BASE METÁLICA: TÉCNICAS BIOLÓGICAS DE EVALUACIÓN

OBJETIVO

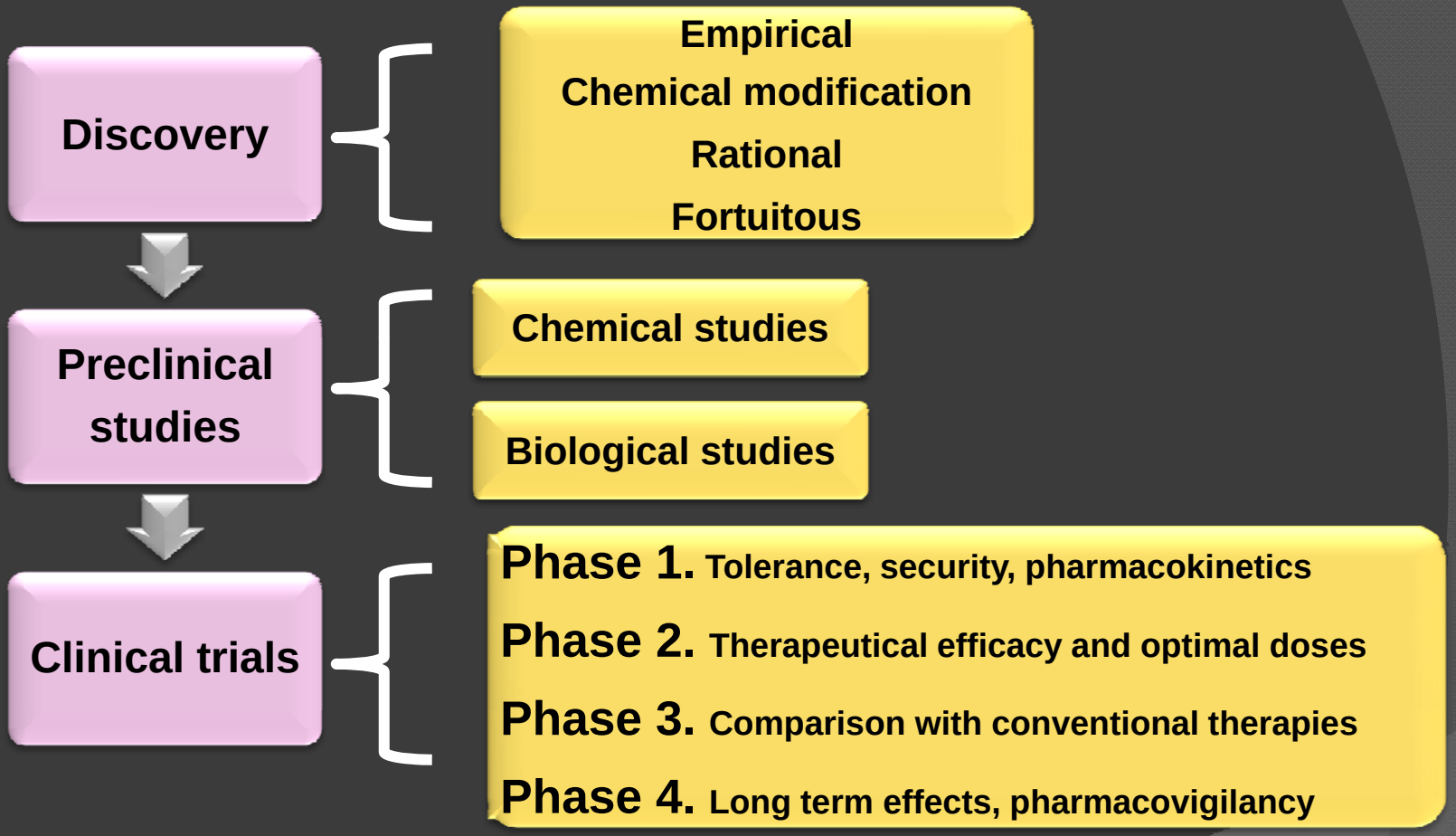
- **The aim of this course is to provide the students with a general overview of the basic techniques in biological screening (*in vitro* and *in vivo)* for possible therapeutic agents and the preclinical studies required to their development.**

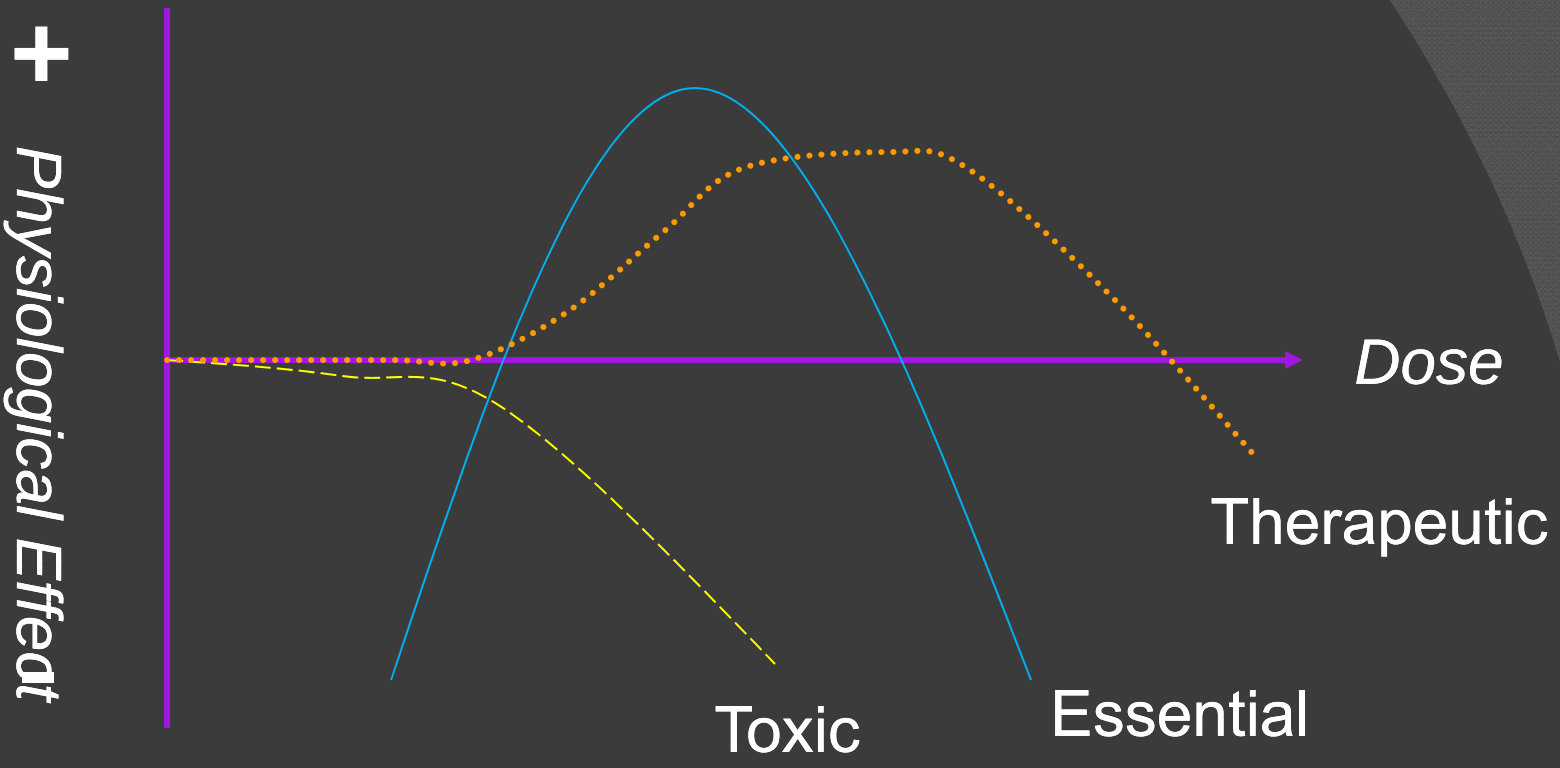
Módulo I. Screening test,
Módulo II. Toxicity,
Módulo III. Mode of action,
Módulo IV. Pharmacokinetics,
Módulo V. Structure Activity Relationships (SAR & QSAR).



Cost: 600 – 800 million dollars per molecule

PRECLINICAL AND CLINICAL STUDIES

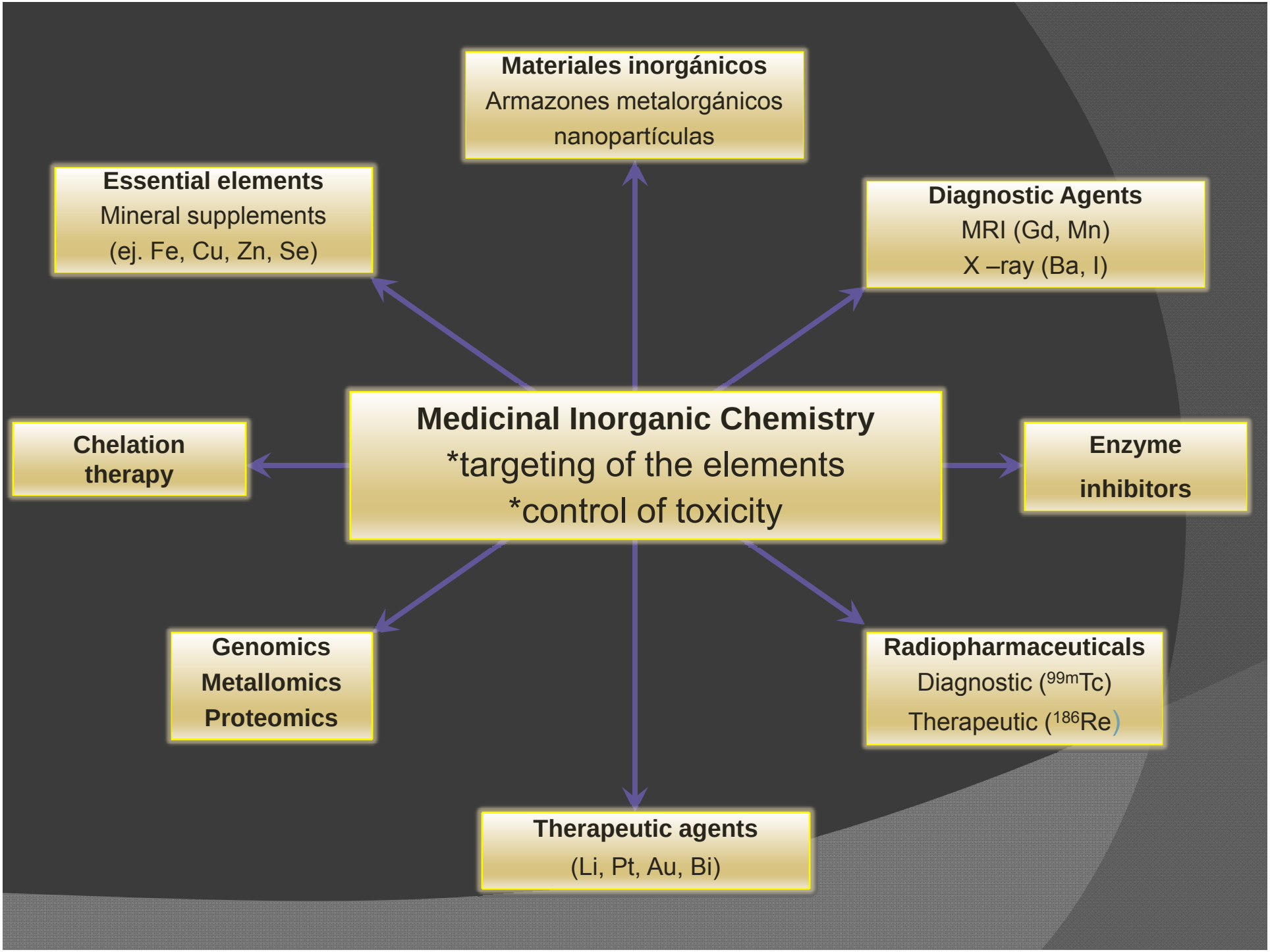




*Bertrand diagram adapted from **G. Bertrand**, 8th Int. Congr. Appl. Chem 28 (1912) 30*

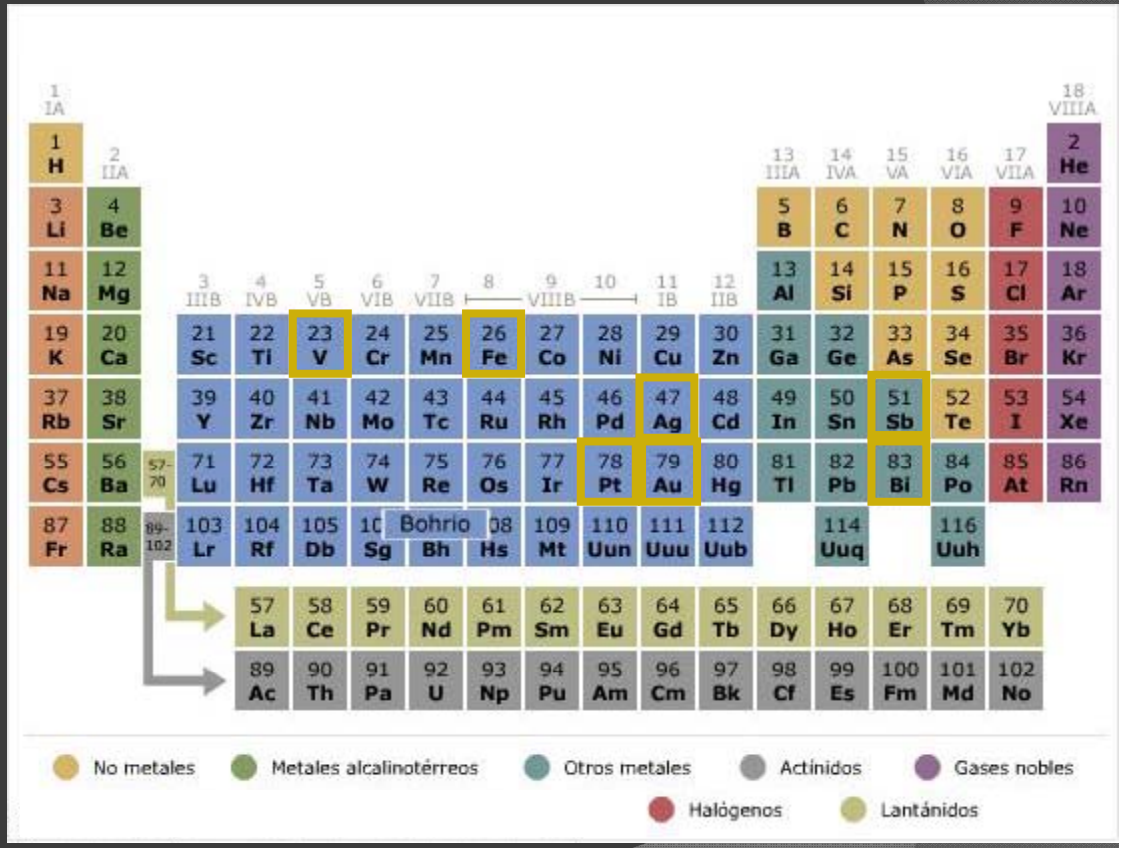
*"**The dose makes the poison**"*

Paracelsus (1493-1541)


Materiales inorgánicos
Armazones metalorgánicos
nanopartículas
Essential elements
Mineral supplements
(ej. Fe, Cu, Zn, Se)
Diagnostic Agents
MRI (Gd, Mn)
X –ray (Ba, I)
Medicinal Inorganic Chemistry
*targeting of the elements
*control of toxicity
Chelation therapy
Enzyme inhibitors
Genomics
Metallomics
Proteomics
Radiopharmaceuticals
Diagnostic (^{99m}Tc)
Therapeutic (^{186}Re)
Therapeutic agents
(Li, Pt, Au, Bi)

Importancia de los metales en medicina

- **Ag** (antimicrobiano)
- **Au** (Antirheumatoid arthritic)
- **Bi** (antibacterial, antiulcer)
- **Sb** (antiprotozoic)
- **V** (antidiabetic)
- **Fe** (antimalaric)
- **Pt** (anticancer)



Ruili Huang, Anders Wallqvist, David G. Covell. Anticancer metal compounds in NCI's tumor-screening database: putative mode of action. *Biochemical Pharmacology* 69 (**2005**) 1009-1039.

The basic questions that must be addressed when design and develop metal based drugs is:

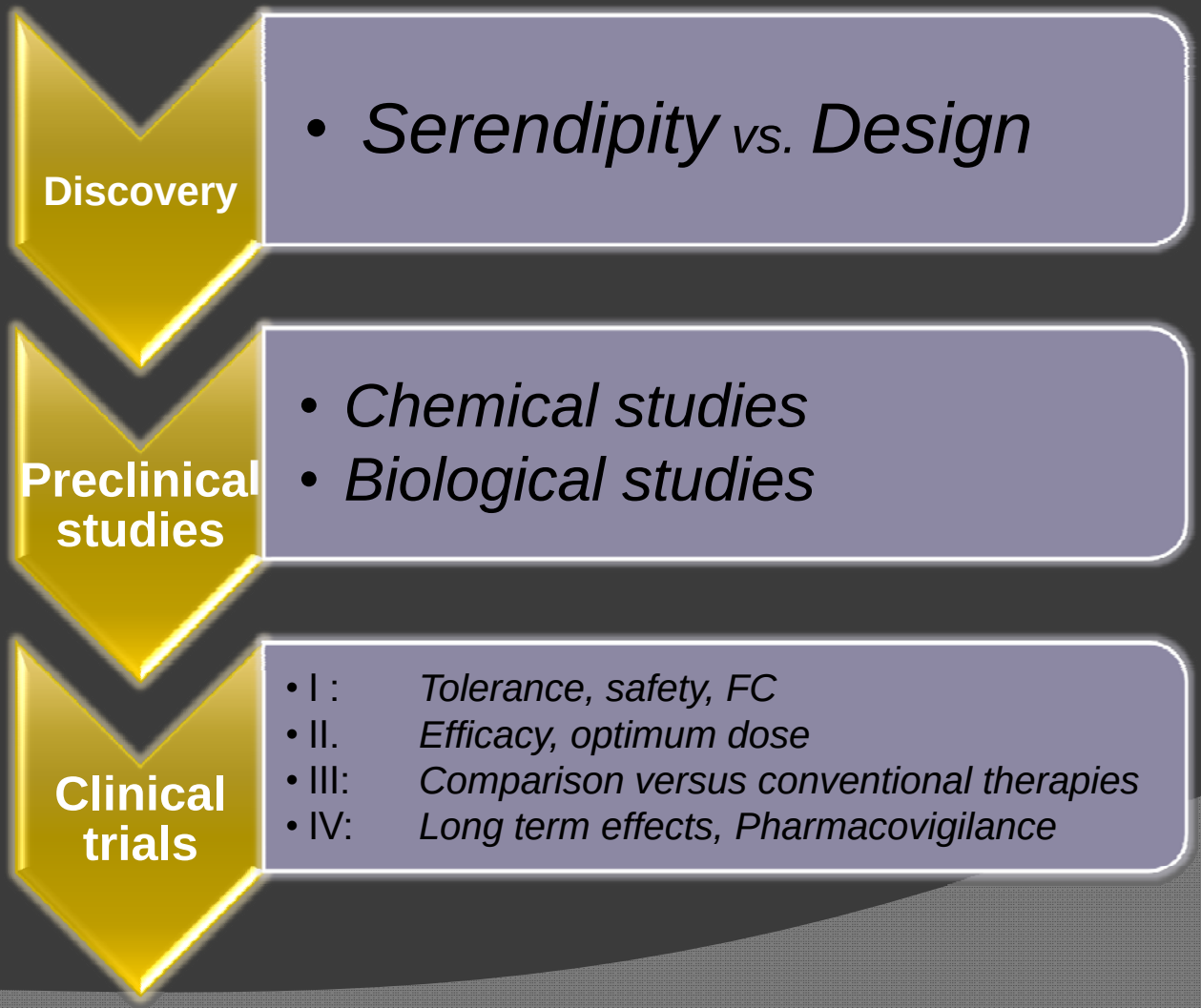
Which parts of the active compound are essential for activity (Pharmacophore):

- Is the metal essential for activity?
- Is the intact complex responsible for activity?
- Is the metal itself?
- Is the metal plus some of the released ligands?
- Is only the ligands?

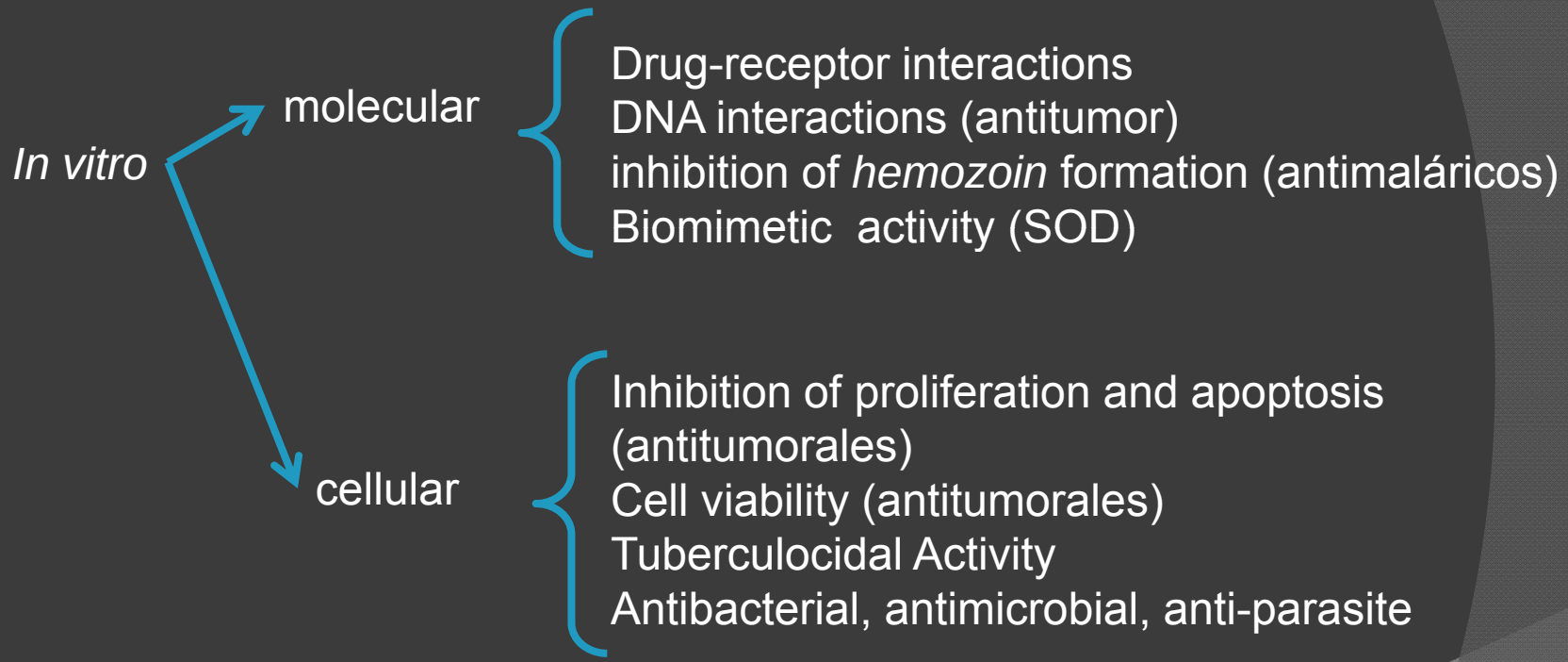
Clasification according to metal role

- The metal has a functional role
- The metal has a structural role
- The metal is a carrier for active ligands that are delivered *in vivo*
- The metal compound behaves as a catalyst *in vivo* (ROS) that cause cell damage
- The metal compound is photoactive and behaves as a photo-sensitizer.

Introducción



Screening Test



Cellular and Murine Models to Evaluate Novel and Conventional Therapeutic Strategies for Cancer

Screening Anticancer Agents

In Vitro *Human Tumor Cell Line Screen*

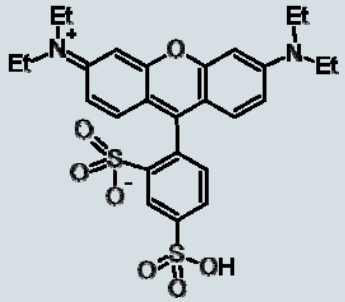
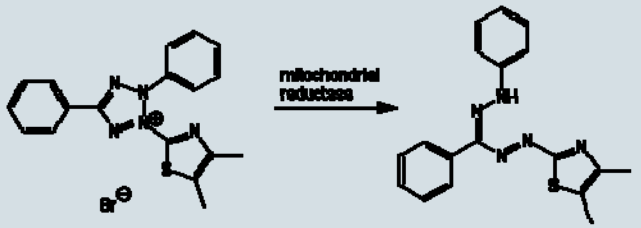
PRESCREENING PANEL
MCF-7 (breast carcinoma), NCI-H460 (lung carcinoma), and SF-268 (glioma).

remove inactive compounds from unnecessary and costly full-scale evaluation

Xenografts
60 different human tumor cell lines

❖the failure of drugs in the clinic is often associated with a poor PK profile or drug toxicity.
❖ generally cultured for years, losing much of their heterogeneity.

Sulforhodamine B (SRB) *vs.* MTT assays

SFB	MTT
Total protein content 	Ability of viable cells to reduce tetrazoliums to formazans 
Anionic protein stain, electrostatic interactions	Chemical reduction of tetrazolium by the test agents
100 times more sensitive than Lowry o Bradford	Chemical interferences with cellular reductants
Might overestimate the survival fraction	Metabolic conditions (pH, glucose, etc)

Rubinstein, L.V. et al. *J. Natl. Cancer Inst*. 82:1113-1118, 1990
Skehan, P. et al. *J. Natl. Cancer Inst.* 82:1107-1112, 1990

Ensayo de inhibición de la proliferación celular

10^6 cells/mL

20μL/well + 100μL Media/SFB

1. 37°C
5%CO_2/24Hr
2. Vaccum

90μL media D-MEM/SFB + 10μL test solution

1. 37°C
5%CO_2/24Hr
2. Vaccum

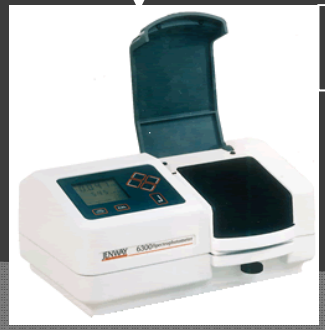
100 μL Tricloroacetic acid 10%

1. 4°C/1Hr
2. Rinse w/H_2O

100 μL Sulforhodamine-B 0.4%

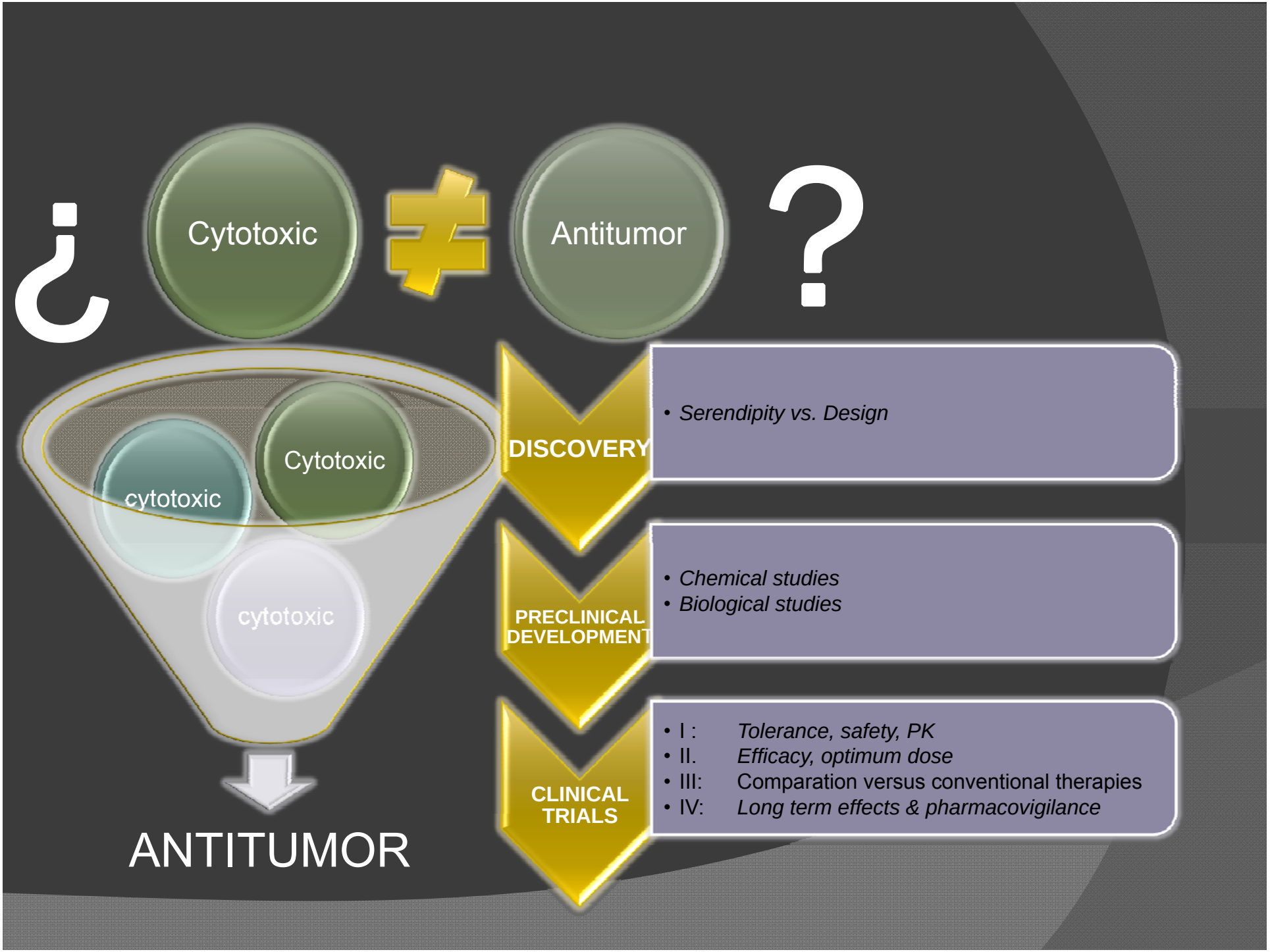
1. TA./30min
2. Rinse w/acetic acid 1%

100 μl Tris base 10μM pH 10.5



564 nm

Calculate Cl_{50} (mol/L), Probit


¿
Cytotoxic
≠
Antitumor
?
Cytotoxic
cytotoxic
cytotoxic
ANTITUMOR
DISCOVERY
• Serendipity vs. Design
PRECLINICAL DEVELOPMENT
• Chemical studies
• Biological studies
CLINICAL TRIALS
• I : Tolerance, safety, PK
• II. Efficacy, optimum dose
• III: Comparation versus conventional therapies
• IV: Long term effects & pharmacovigilance

Antitumor Efficacy Testing in Rodents

- ✓Identification of an Appropriate Species for Assessing Efficacy
- ✓Selection of a Proper Tumor Model
- ✓Experimental Design Considerations
- ✓Development of a Treatment Plan
- ✓Choice of Endpoints
- ✓Appropriate Statistical Evaluation of Tumor Growth Data

Factors involved in variation in activity

Biochemical heterogeneity among races

Species differences: Biochemical heterogeneity of animal and human

Individual differences in response: heterogeneity due to sex and age.

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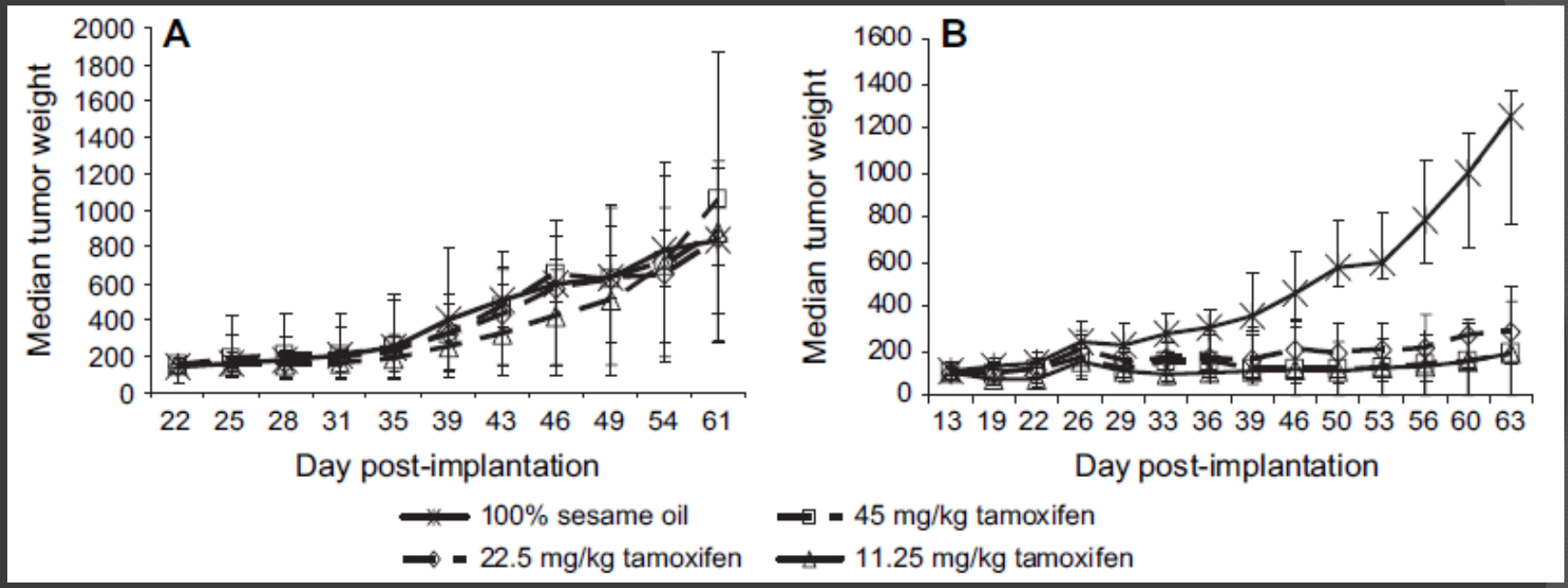
Activity of tamoxifen in hu man tumor xenografts in mice.



A) MDA-MB-435 estrogenreceptor – negative melanoma xenografts. **B) MDA-MB-361**estrogen receptor – positive breast cancer xenografts. Cells of both lines were implanted orthotopically into the mammary fat pad of athymic nu/nu NCr mice (Animal Production Program, NCI-Frederick), and treatment was initiated when the tumors reached 150 – 175 mg in size. The MDA-MB-361 tumor-bearing mice were treated weekly with estradiol cypionate (20 µ g per mouse) to support tumor growth. Exogenous estradiol is not required for progressive growth of MDA-MB-435 xenografts. For both studies, the vehicle control was 100% sesame oil given by oral gavage once daily for 20 days (n = 20 mice). Tamoxifen was administered by oral gavage once daily for 20 days at a dose of 45, 22.5, or 11.25 mg/kg (n = 10 mice per dose). Individual tumor weights were calculated as weight in mg = (length × width 2)/2. Data are plotted as median tumor weight ± the 95% confi dence interval of the median.

J Natl Cancer Inst 2008;100: 1500 – 1510

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Experimental design considerations

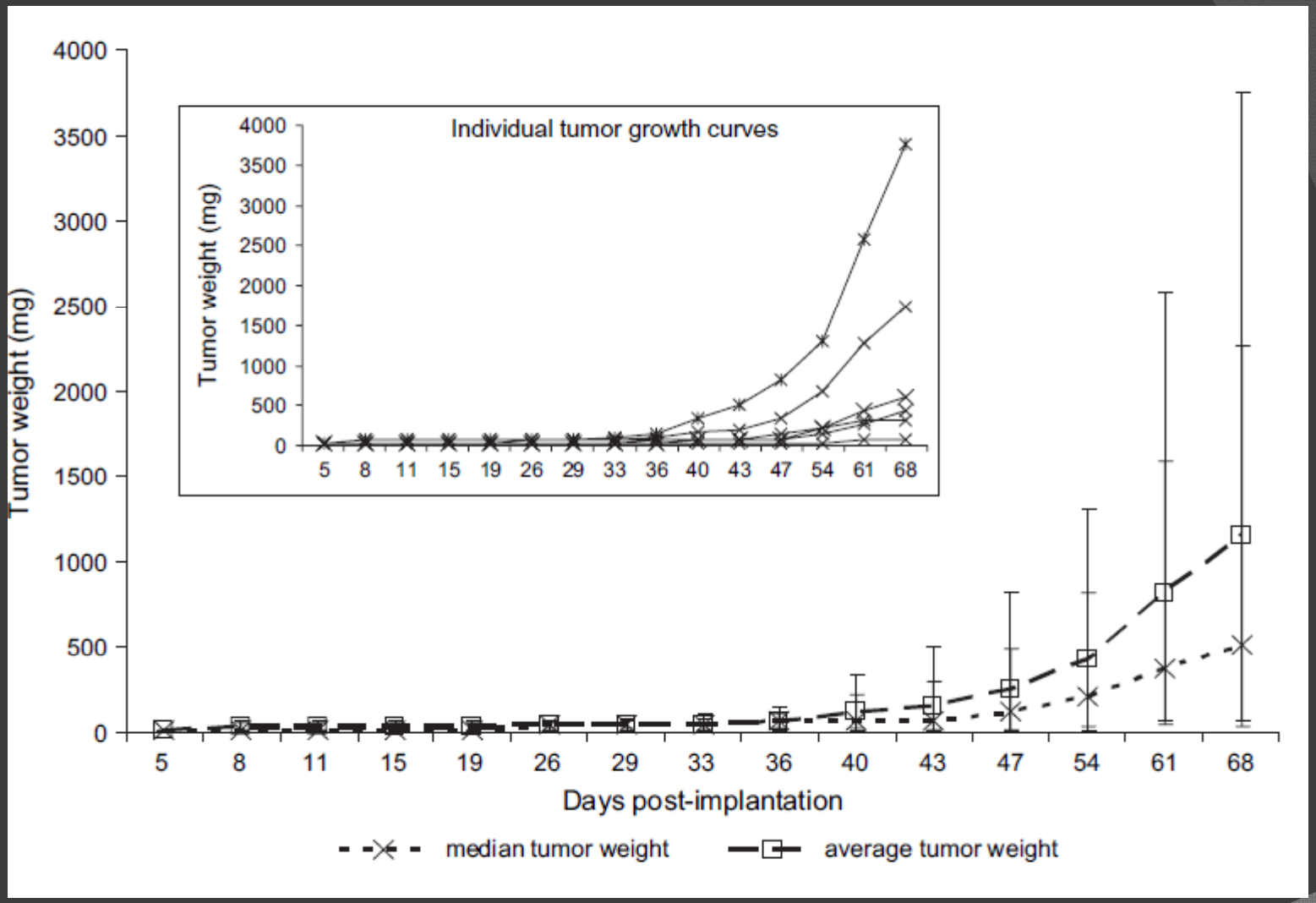
A. **Bioavailability (tumor growth site, the vehicle and treatment route, the solubility and stability of the test material, uptake, metabolic, and excretion pathways)**

B. **Required therapeutic exposure**

GOALS:

1) *To achieve a target plasma concentration,*
2) *To maintain a minimum exposure time, or*
3) *To administer the maximum amount of test agent that does not cause Inacceptable toxicity.*

C. **The number of test animals per group**



Tumor weight plots for MDA-MB-361 human breast tumors implanted subcutaneously in athymic nude mice. The main graph presents the median and average tumor weights for a group of six mice (nu/nu Ncr; Animal Production Program, NCI-Frederick), each implanted with 1 × 10 7 cells in 0.1 mL. The inset presents the individual growth curve for each of the six mice. Individual tumor weights were calculated as weight in mg = (length × width 2)/2. The **error bars indicate the** 95% confi dence intervals of the averages or the medians, as appropriate.

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Tumor models

- *Ascites Tumors*
- *Solid Human and Murine Tumors*
- *Sequential Tumor Model*
- *Human Tumor Stem Cell (HTSC) Assay/Clonogenic Assay*
- *Screening Using Human Tumor Xenografts in immunodeficient Mice*
- *Humanized Mice*
- *Orthotopic Tumor Models*
- *GEMs*
- *Autochthonous Tumor Models*

Outcome Criteria for Animal Tumor Models

Ascites Tumors mouse models

In 1955, it was suggested that a correlation existed between efficacy against transplanted tumors and clinical activity.

- L1210 leukemia cell line
- B16 melanoma and
- Lewis lung carcinoma

Solid Human and Murine Tumors

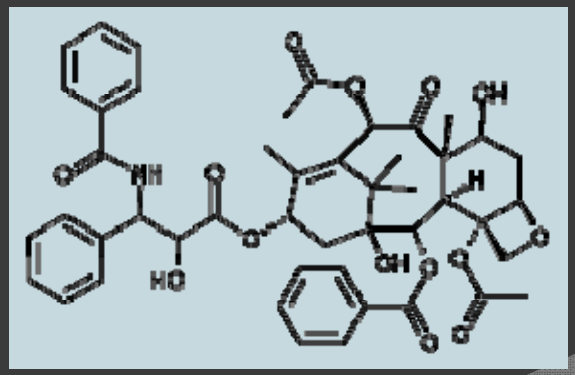
1976 NCI tumor panel

syngeneic models

murine L1210 leukemia and
B16 melanoma
inoculation
of tumor cells by i.p.,
subcutaneous (s.c.), or
intravenous (i.v.) routes

human tumor xenografts

breast, colon, and lung,
Inoculation under the renal
subcapsule.



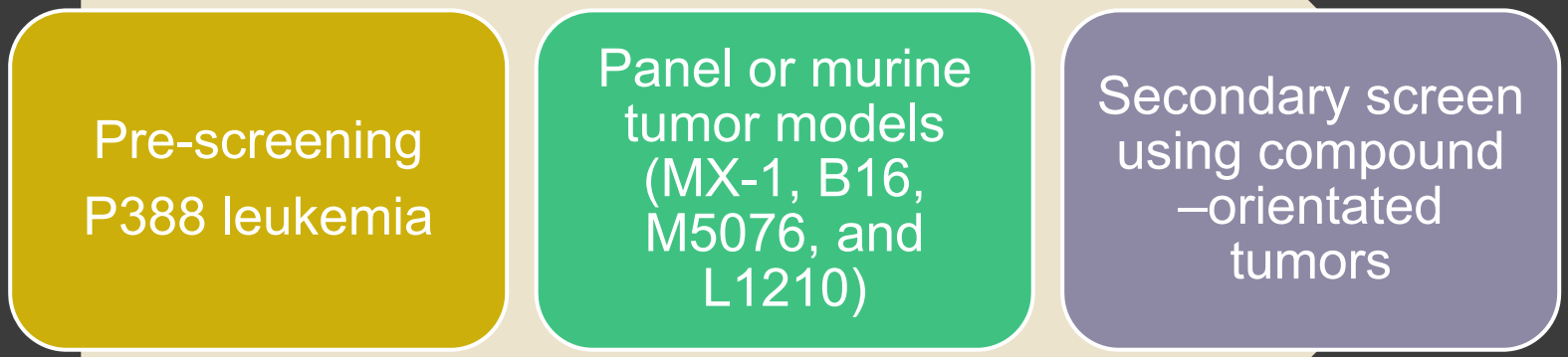
Low correlation between preclinical and clinical efficacy

Provide an evaluation within the context of an intact immune system and host stroma and extracellular matrix

Sequential Tumor Model

Screenign strategy in 1982.

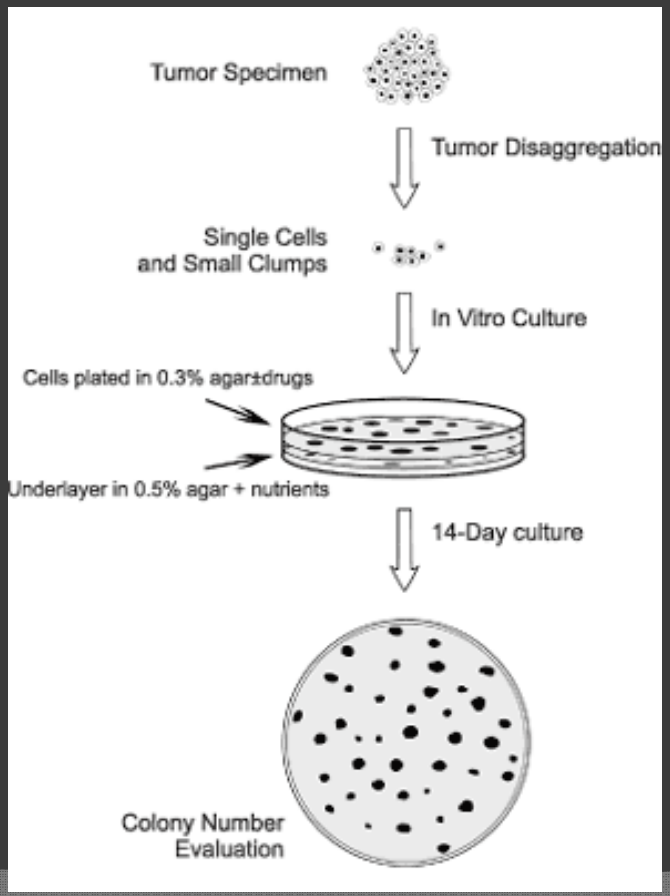
progressively more rigorous models

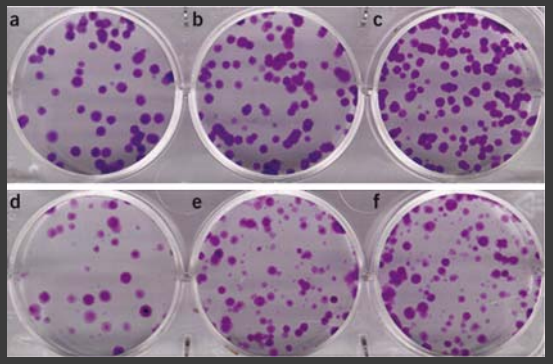


Did not demonstrate a correlation based on tumor histiotype

Human Tumor Stem Cell (HTSC) Assay/Clonogenic Assay

The HTSC assay was disease-orientated using soft agar colony growth of freshly explanted human tissue with outcomes based on growth inhibition.





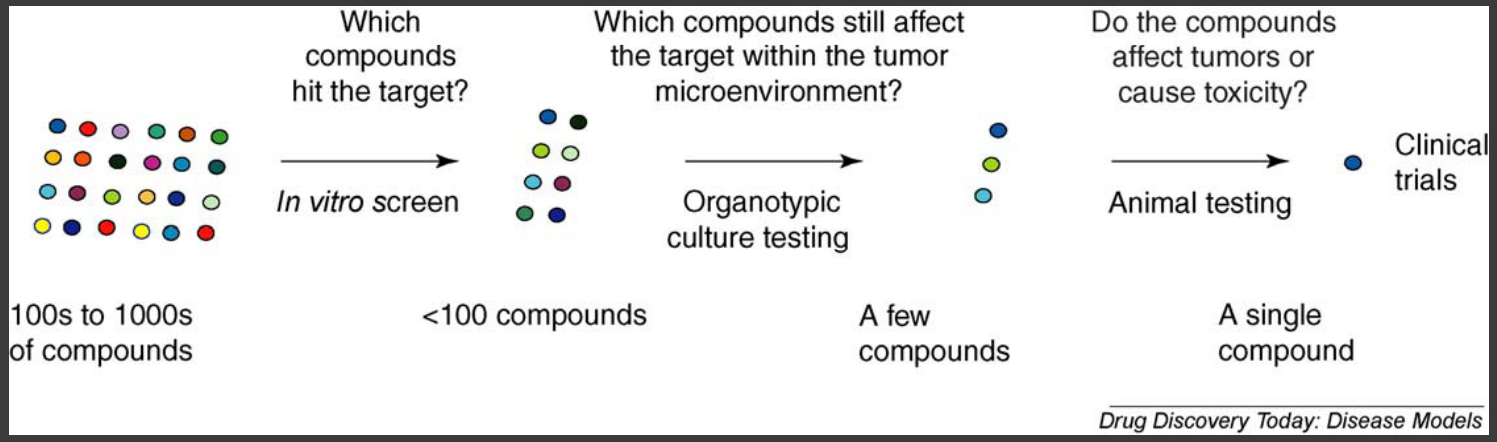
Drawbacks:

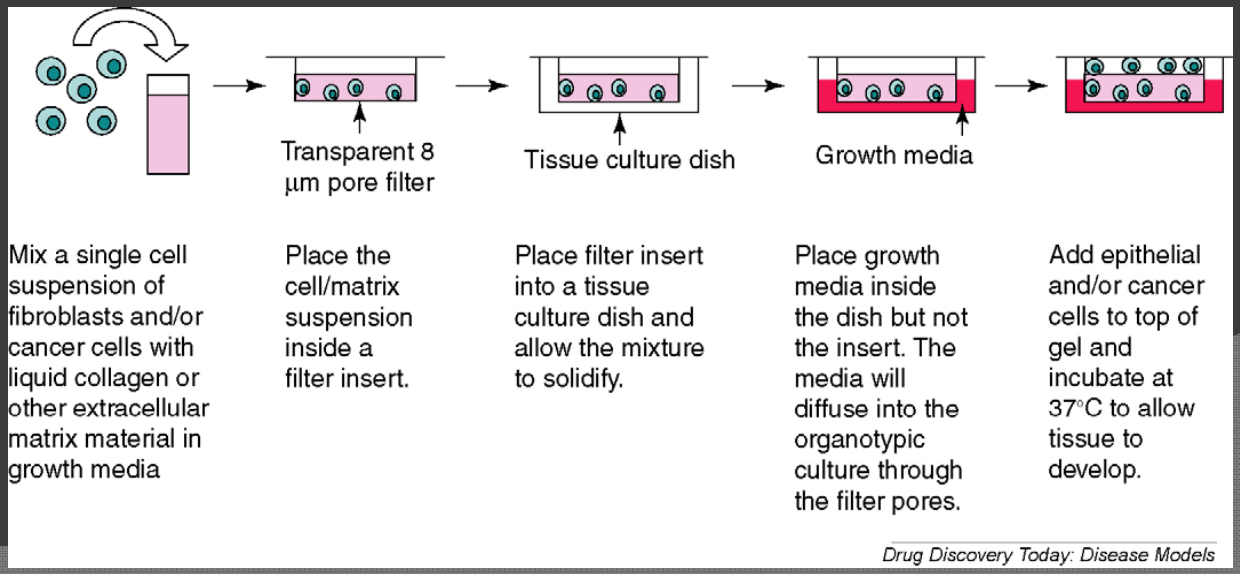
- ❖Low plating efficiency of most solid tumors and the poor availably of tumor tissue.
- ❖Lack of immune system and biotransformation

Although these models predict responsive histiotypes, no clinical analysis of individualized therapy has demonstrated a significant increase in survival compared with empirically determined standard treatment

Organotypic cultures

Drug Discovery Today: Disease Models Vol. 3, No. 2 2006

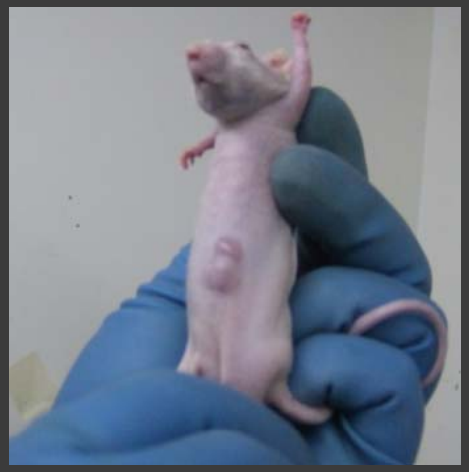




Screening Using Human Tumor Xenografts in Immunodeficient Mice

Characteristics:

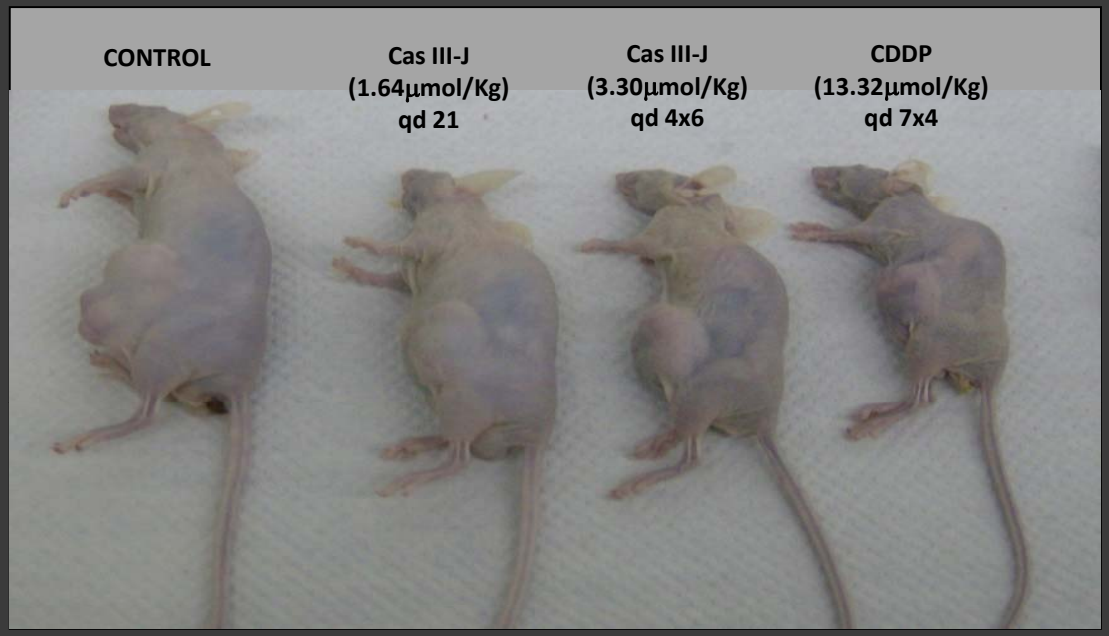
✓These studies require nude (athymic) or severe combined immunodeficient (SCID) mice that are T- and B-cell-deficient.
✓Clinical relevance is obtained only if careful attention is paid to the experimental conditions.
✓The neoplasm and mice must be keeped patogen-free.



Drawbacks:

➢*In vitro* culture for several years might select for clones that are no longer representative of the original tumor.
➢Compensatory increase in innate immunity, most notably increased NK activity and tumoricidal macrophages.
➢Lack human stroma and immune cells, which are important to the metastatic process.
➢Poorly predictive of a specific histological response
➢Murine xenograft models are not ideal for cancer drug development.

Antitumor activity



Treatment groups	ANTITUMOR FUNCTION		
	DIA 7	DIA 14	DIA 21
CONTROL NEGATIVO	100	100	100
CDDP (13.32 mmol/Kg) qd 7x4	20.5	28.3	33.7
Cas VIII-gly (1.64 mmol/Kg) qd 21	57.2	52.3	85.0
Cas VIII-gly (3.30 mmol/Kg) qd 4x6	106.0	71.6	72.9
Cas III - J (1.64 mmol/Kg) qd 21	42.9	35.7	37.6
Cas III - J (3.30 mmol/Kg) qd 4x6	37.8	30.7	26.8

[lenght (cm) x width^2 (cm^2) x π] / 6

Tumor relative volume (TRV) = (V day *x*) / (V day 0) x 100

AF = ($TRV_{tratado}$) / ($TRV_{control}$) x 100

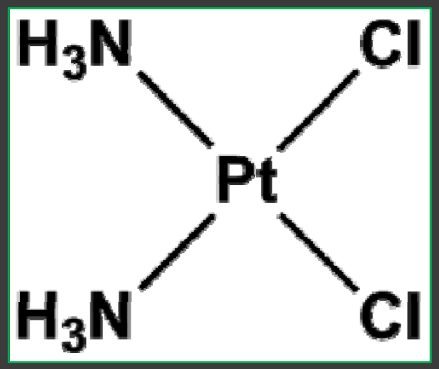
diimine	$L_{sec.}$	LD50 mM/Kg
3,4,7,8-tMe	Acac	18.89[1] 16.23 ± 2.63[2]
3,4,7,8 t-Me	Gly	16.45[1]

1. Hernández de la Paz, A.L., F. Química, UNAM, 2008
2. Bravo-Gómez, M.E. et al. **Journal of Inorganic Biochemistry**. DOI information: 10.1016/j.jinorgbio.2008.10.006

Humanized mice

- Immunodeficient mice reconstituted with human stem cells or lymphocytes transplantation of human thymi and/or BM before stem cell injection to provide a human stromal environment.

- Insertion of a human gene into the mouse genome (GEM's)



induced or steady-state CYP2E1 levels
and a comparison
to knockout and CYP2E1-humanized mice

Hepatotoxicity

Additional work and validation remain before they can be routinely and confidentially used in drug development.

Orthotopic Tumor Models

The organ environment can influence the response of tumors to chemotherapy.

Orthotopic implantation of human tumor cells from surgical specimens into nude mice

carcinomas (into the wall of the colon), renal cell cancers (into the kidney), melanomas (into the skin), mammary carcinomas (into the mammary fat pad), bladder carcinomas (into the bladder wall), prostate carcinoma (into the prostate), pancreatic carcinoma (into the pancreas), and lung cancer (into the bronchi)

Advantages:

✓Rapid growth of local tumors and in several tumor models, distant metastasis.

✓Representative of the primary tumor site.

Drawbacks:

•Their utilization is hindered by a need for a high level of technical skill, time, and cost.

•Therapeutic efficacy is also more difficult to assess with orthotopic models in contrast to the relative ease of s.c. tumor measurements

Autochthonous Tumor Models

Spontaneously occurring tumors and chemical, viral, or physical carcinogen-induced tumors

Advantages

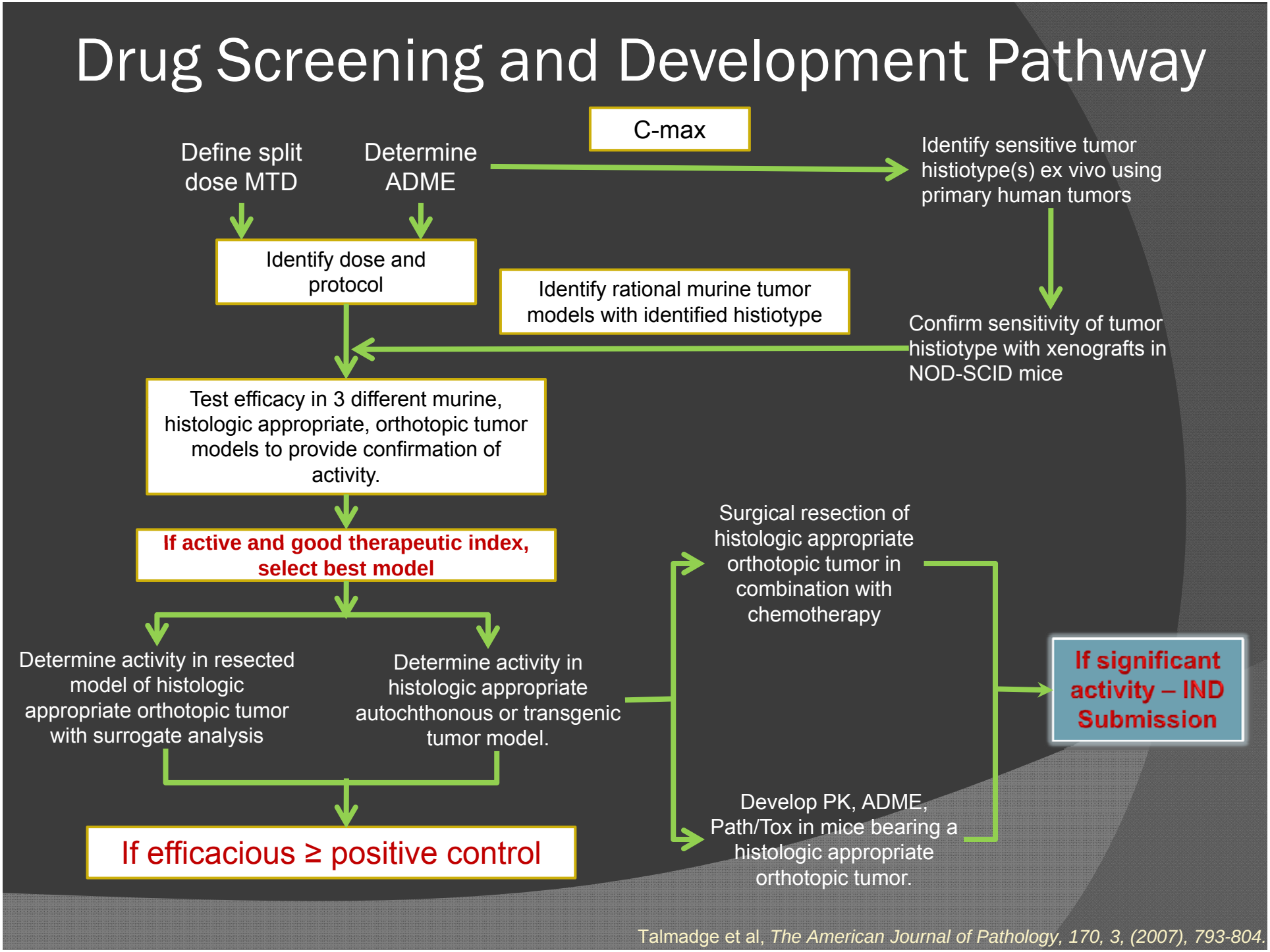
✓Believed to model human tumors more closely than transplanted tumors
✓Orthotopic growth
✓Metastasis via lymphatic and vascular vessels surrounding and within the primary tumor

Drawbacks

▪Inherent variability in the time to and frequency of tumor induction, number of tumor(s) induced, and thus the number of animals required for a study.
▪Time frames of several months to a year for a single experiment, as opposed to weeks with transplanted xenograft models.

RESERVED FOR CONFIRMATION STUDIES,

Drug Screening and Development Pathway



Talmadge et al, *The American Journal of Pathology, 170, 3, (2007), 793-804.*

Measurements of Outcomes in Animal Models

Endpoint	Comment
In vivo	
Tumor onset	Time to palpable tumor mass of predetermined size
Tumor growth rate	Assessment of tumor volume throughout time
Number of tumor-bearing animals	Frequency of cure
Tumor burden in vivo at set time	Weight of tumor or organ with metastases
Tumor growth delay	Volume estimated (mm3) two-dimensional measurement
	Delay of time for tumor to reach specific volume
Tumor cell kill	Log10 total tumor cell kill
	Net log10 tumor cell kill
Incidence of metastasis	Gross count (lungs)
	Cell count, resistance, florescence, 125IUdR uptake…
Survival-life span	Increase in median survival time
Survival-number alive	Percent cure at predefined time
Ex Vivo	
Gross pathology	Ulceration/central necrosis
	Invasion or tissue distribution and gross lesions
	Metastasis
	Angiogenesis
Histopathology	H&E staining
	Morphometrics
	Inflammatory cell infiltration
	Mitotic index, cellular apoptosis
Immunohistochemistry	T cell, macrophage, and DC infiltration
	Angiogenesis and lymphoangiogenesis
	Tumor cell apoptosis
	Enzyme and cytokine levels
Molecular pathology	Cytokines/chemokines or enzymes in serum or qRT-PCR of tmor, blood, spleen
Hematology	Complete blood count, platelets, spleen, marrow
	Blood/spleen/marrow/thymus differential
Immunology	Phenotype spleen, blood, tumor-infiltrating nonparenchymal cells and their function including qRT-PCR