Advantages and Limitations of Cell Culture Models in Pediatric Drug Development

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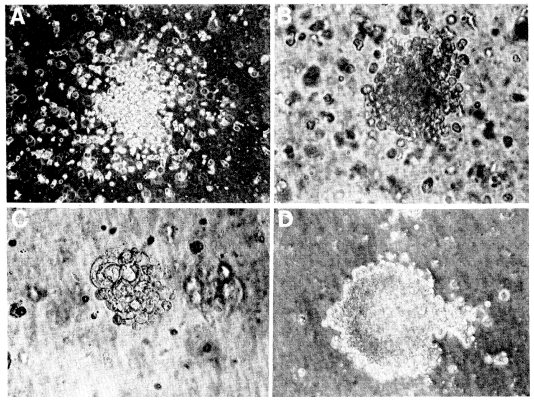
Clonogenic Assay

- *Primary Bioassay of Human Tumor Stem Cells**
  - Tumor stem cells are cell renewal source and serve as seed of metastatic spread
  - Cytotoxicity in clonogenic assay proportional to cytotoxicity *in vivo*



*Hamburger AW, Salmon SE. *Science*, 197 (4302) 461-463; 1977.

Tritiated Thymidine Incorporation

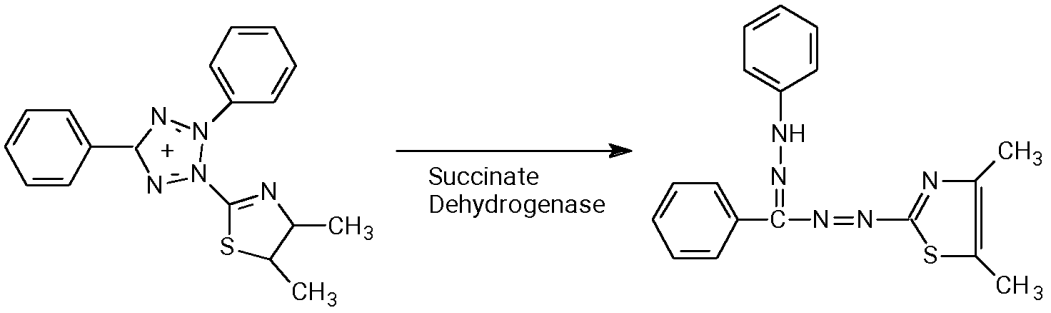
- ^{3}H-TdR measures cells in S-phase
- Quantifies cell number as cpm

Historical *in vitro* Assays

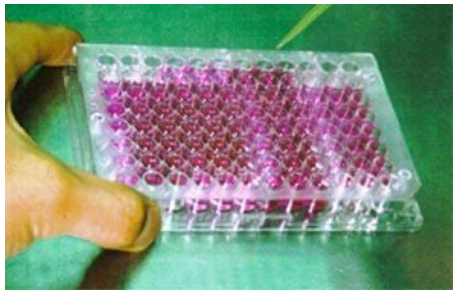
- Clonogenic Assay
  - Labor intensive
  - Not readily amenable to high throughput
- ^{3}H-TdR
  - Limitations of using radioactivity
  - Non-clonogenic method

Non-clonogenic Assays

- MTT Assay
  - *Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxcity assays**

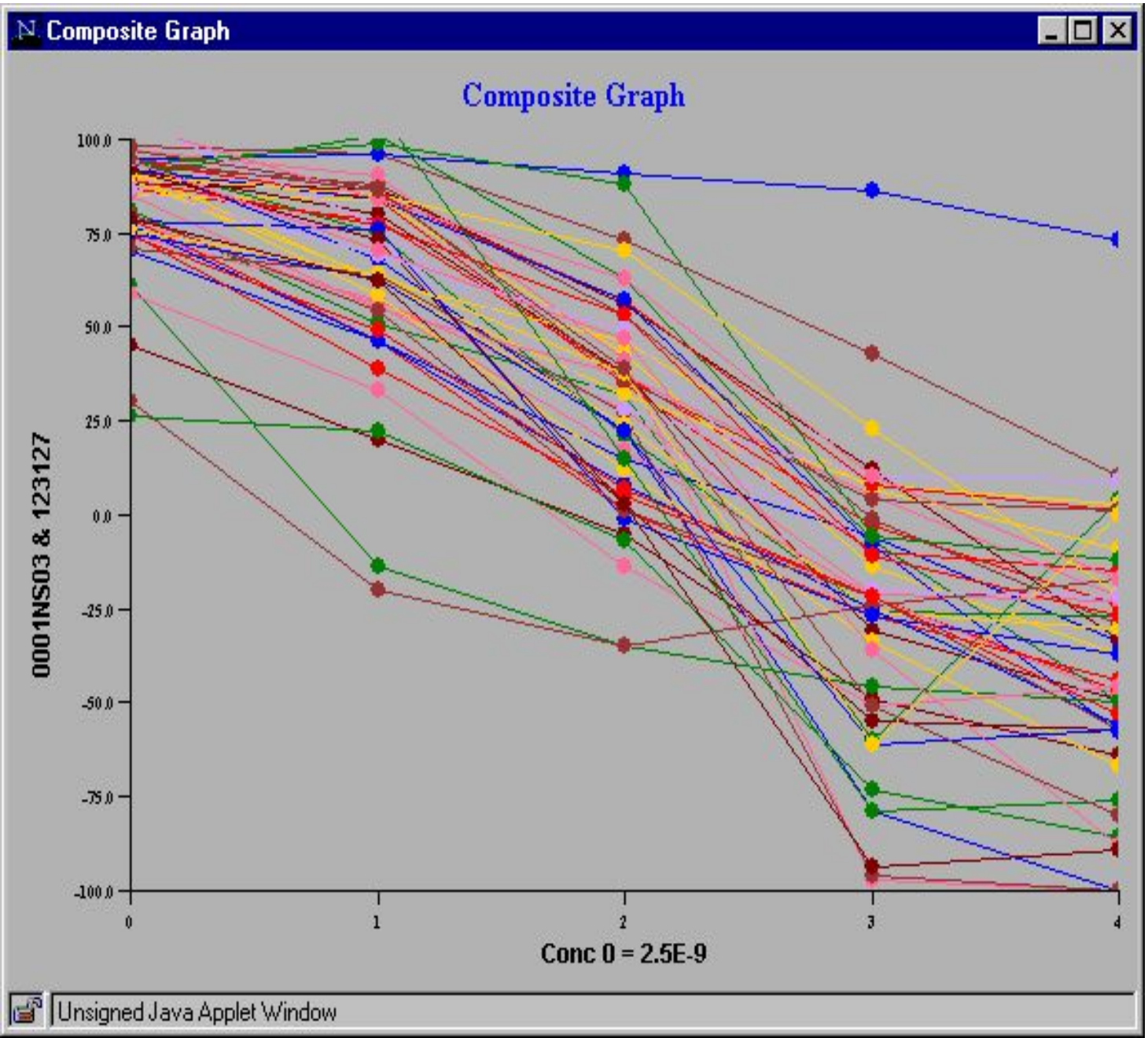


MTT **Formazan**



*Mossman T. J Immunol Meth 1983;65:55-63.

NCI 60 Cell Line Screen



- Leukemia
- NSCLC
- Small Cell
- Colon
- CNS
- Melanoma
- Ovarian
- Renal

Non-Clonogenic Assays

- MTT
- XTT
- SRB
- Trypan Blue
- DiscAssay
- FDA
- TACs Hoechst
- WST-1
- Acid Phosphatase
- DIMScan
- MTS
- Brd-U
- Luminescent-ATP

Non-Clonogenic Assays

Non-clonogenic assay ≈

Viable cell number ≈

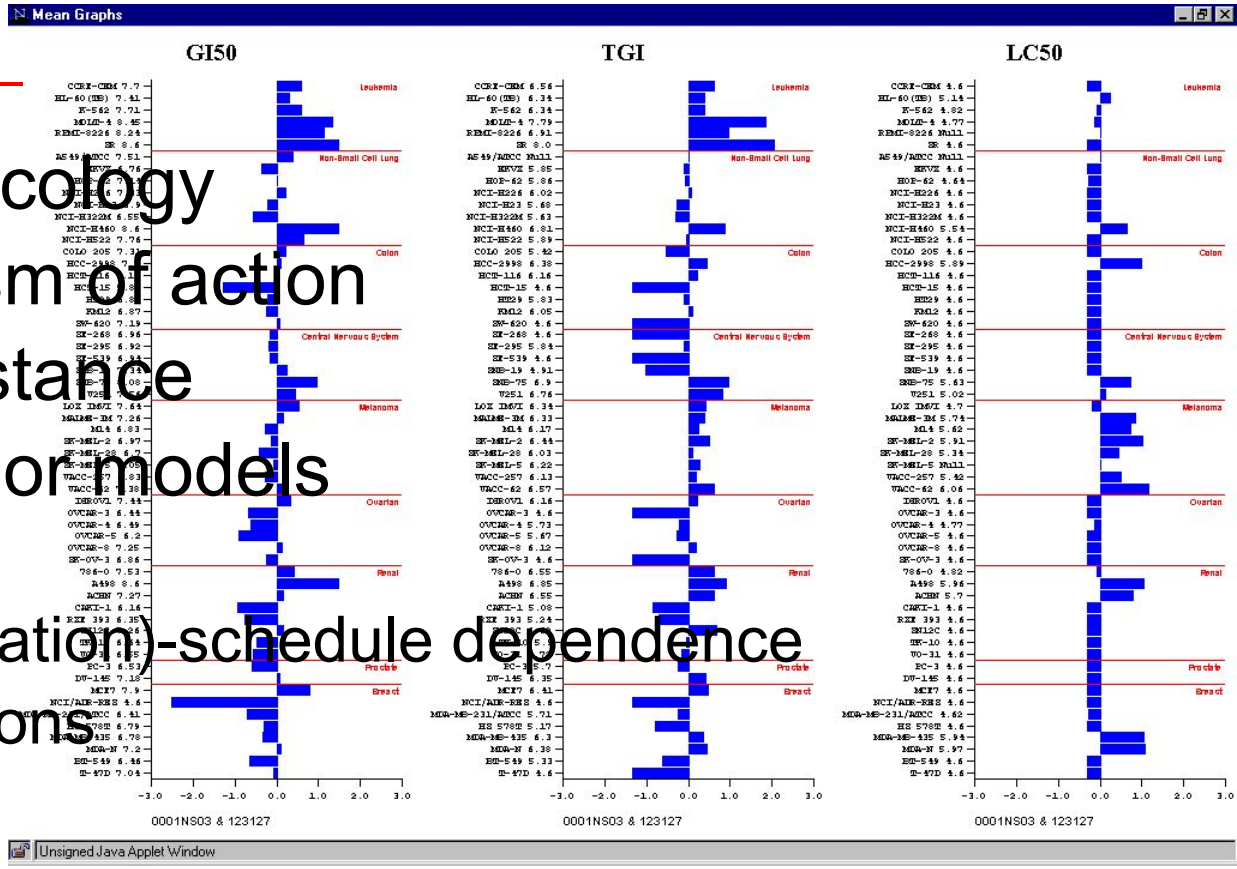
Clonogenic assay ≈

In vivo cell growth ≈

Tumor growth in patient

Use of Cell Culture Models

- ~~Drug discovery~~
- Cellular pharmacology
- Study mechanism of action
- Study drug resistance
- As pediatric tumor models
  - Drug activity
  - Dose (concentration)-schedule dependence
  - Drug combinations



Limitations of Cell Culture Models

- Cell lines undergo transformation to allow for *in vitro* growth
- Drugs may require metabolic activation or have active metabolites
- Potential differences in drug exposure
  - Protein binding
  - Drug disposition not modeled
- Differences in tumor micro-environment
  - Lack of vascularization
  - Hypoxia
- Other limitations…

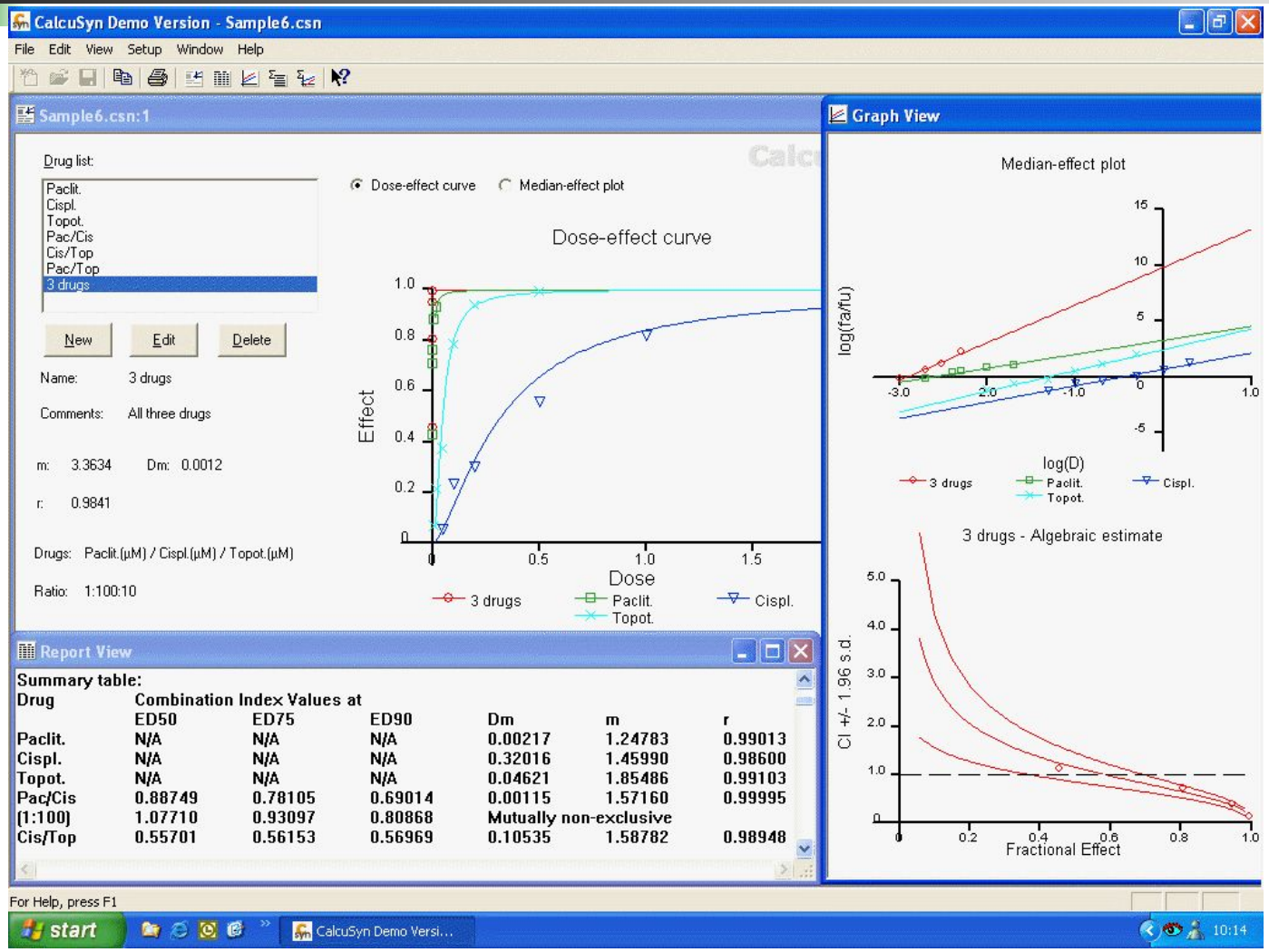
Advantages of Cell Culture Models

- Not labor intensive
- Relatively low cost
- Moderate throughput capabilities
- Ability to study multiple cell lines
- Ability to study multiple combinations of drugs
  - Only system that mathematically determines synergy, additivity, and antagonism

Example: Determination of Synergy

- Problems with the “addition” method
  - Drug A 25% cell kill
  - Drug B 25% cell kill
  - Drug A + Drug B > 50% cell kill - synergy?
- It’s not that simple
  - Drug A 70% cell kill
  - Drug B 70% cell kill
  - Drug A + Drug B = 140% cell kill?

Median Effect Model



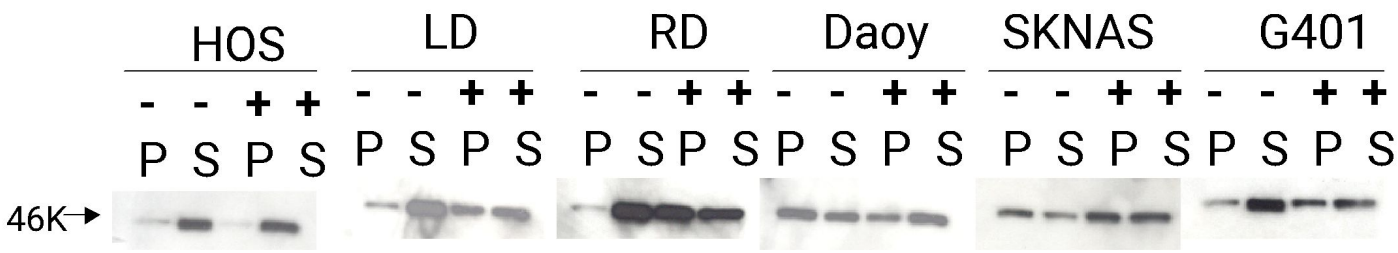
Example: Activity in Pediatric Tumors

- BMS 247550 is an analog of epothilone B that binds tubulin, stabilizes mictrotubules by inhibiting tubulin depolymerization, blocks mitosis and causes apoptosis.
- BMS 247550 is cytotoxic in taxane resistant tumors and tumor cell lines expressing the multidrug resistance phenotype (MDR).

Fox, Stover, Widemann, Fojo, Balis (AACR 2003)

BMS 247550: Pre-clinical Activity

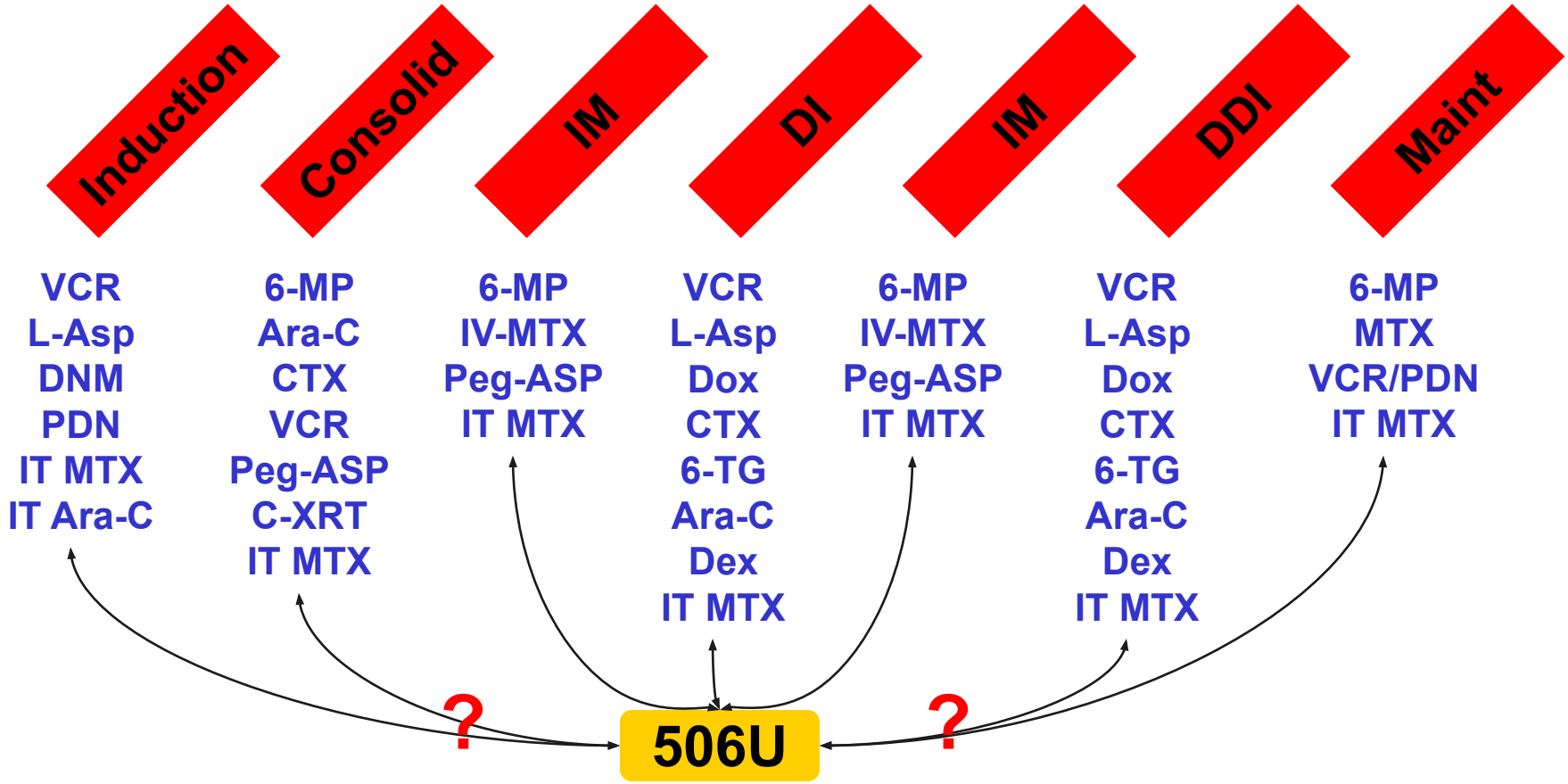
Cell Line	IC_{50} (nM)			
	BMS247550	Paclitaxel	Vincristine	Vinorelbine
HOS	8.6 ± 0.4	0.4 ± 0.03	44.7 ± 1.0	10.6 ± 0.4
LD	8.2 ± 0.4	2.0 ± 0.2	5.0 ± 0.5	4.9 ± 3.1
RD	16.8 ± 6.9	0.6 ± 0.03	38.4 ± 2.0	18.0 ± 0.6
Daoy	9.2 ± 0.2	14.4 ± 0.5	14.9 ± 0.4	20.1 ± 1.1
SK-N-AS	11.7 ± 1.3	8.6 ± 2.3	4.7 ± 0.4	0.8 ± 0.1
G401	7.9 ± 0.1	6.8 ± 0.5	5.2 ± 0.1	1.9 ± 0.2



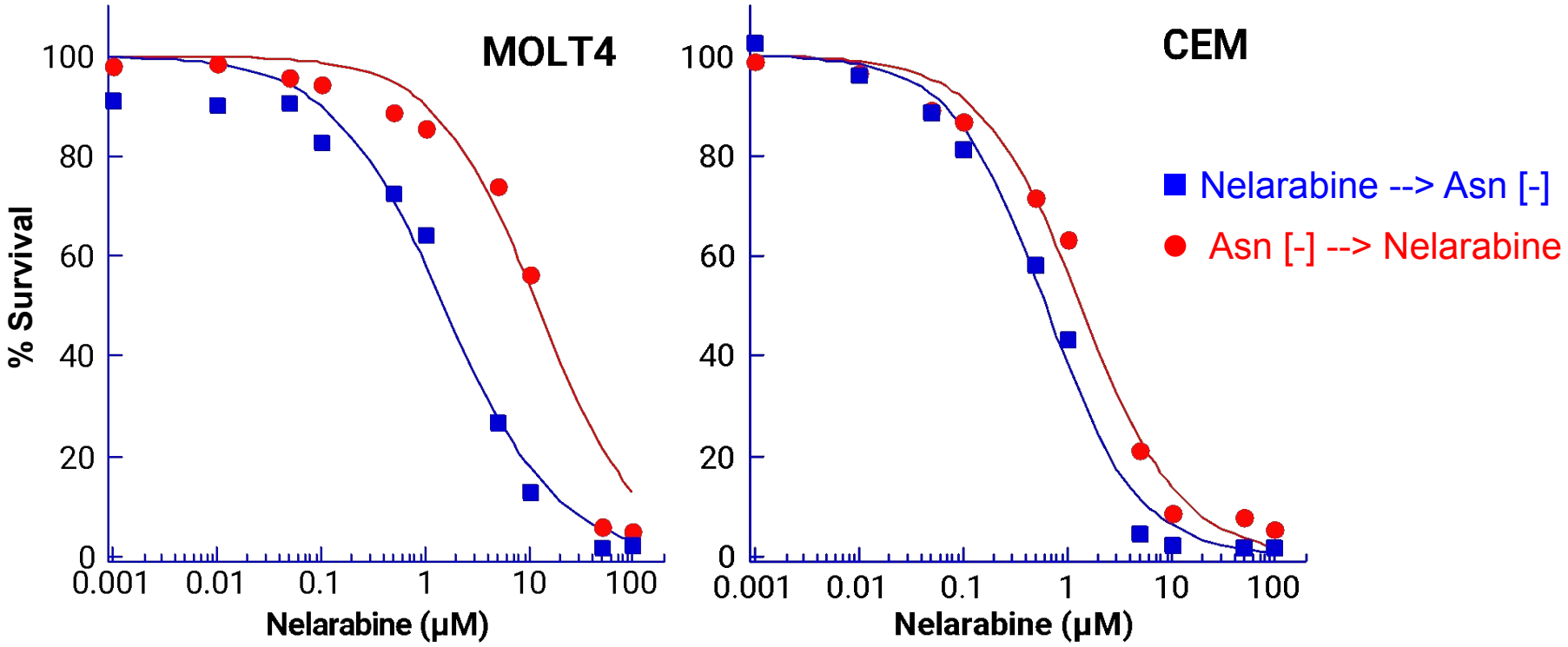
Fox, Stover, Widemann, Fojo, Balis (AACR 2003)

Example: Integration of New Agents

High-Risk ALL Therapy



Asparaginase + 506U



Jayaprakash, Adamson, Lampkin, Berg, Balis, Fox (AACR 2004)

Perspectives on Cell Culture Models

- *In vitro* models are a cost efficient method to search for activity, but mechanistic based approaches likely will have higher yield
- *In vitro* models can further our understanding of drug action in pediatric tumors
- Moderate throughput is advantageous, especially when studying drug combinations

Perspectives on Cell Culture Models

- For most cytotoxic agents, if it does not work *in vitro*, it will not work *in vivo*
- If it takes supra-pharmacologic concentrations *in vitro* to have an effect, it will likely not fare well *in vivo*
- If it works well *in vitro*, there is a reasonable likelihood that it will do absolutely nothing *in vivo*