

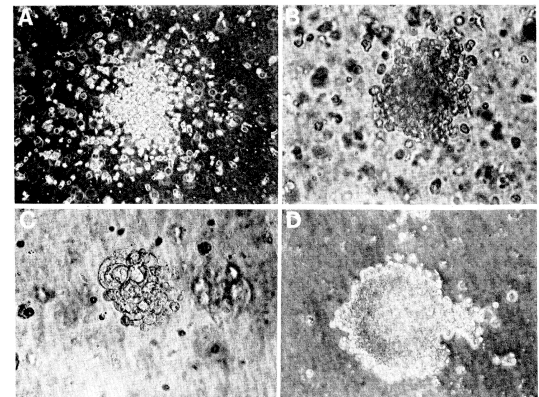


Advantages and Limitations of Cell Culture Models in Pediatric Drug Development

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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*





Tritiated Thymidine Incorporation

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



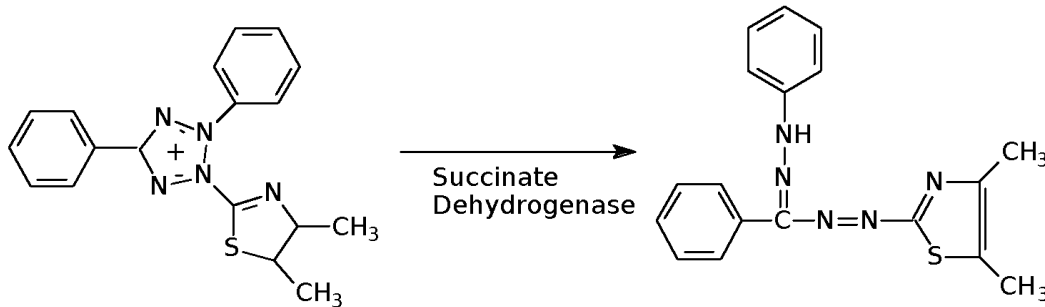
Historical *in vitro* Assays

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

Non-clonogenic Assays

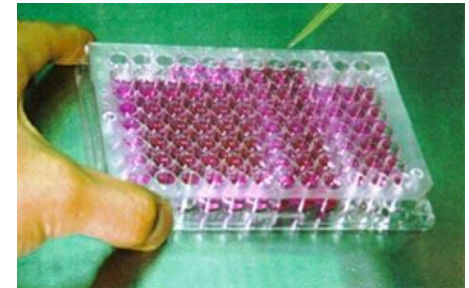
■ MTT Assay

- *Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays**

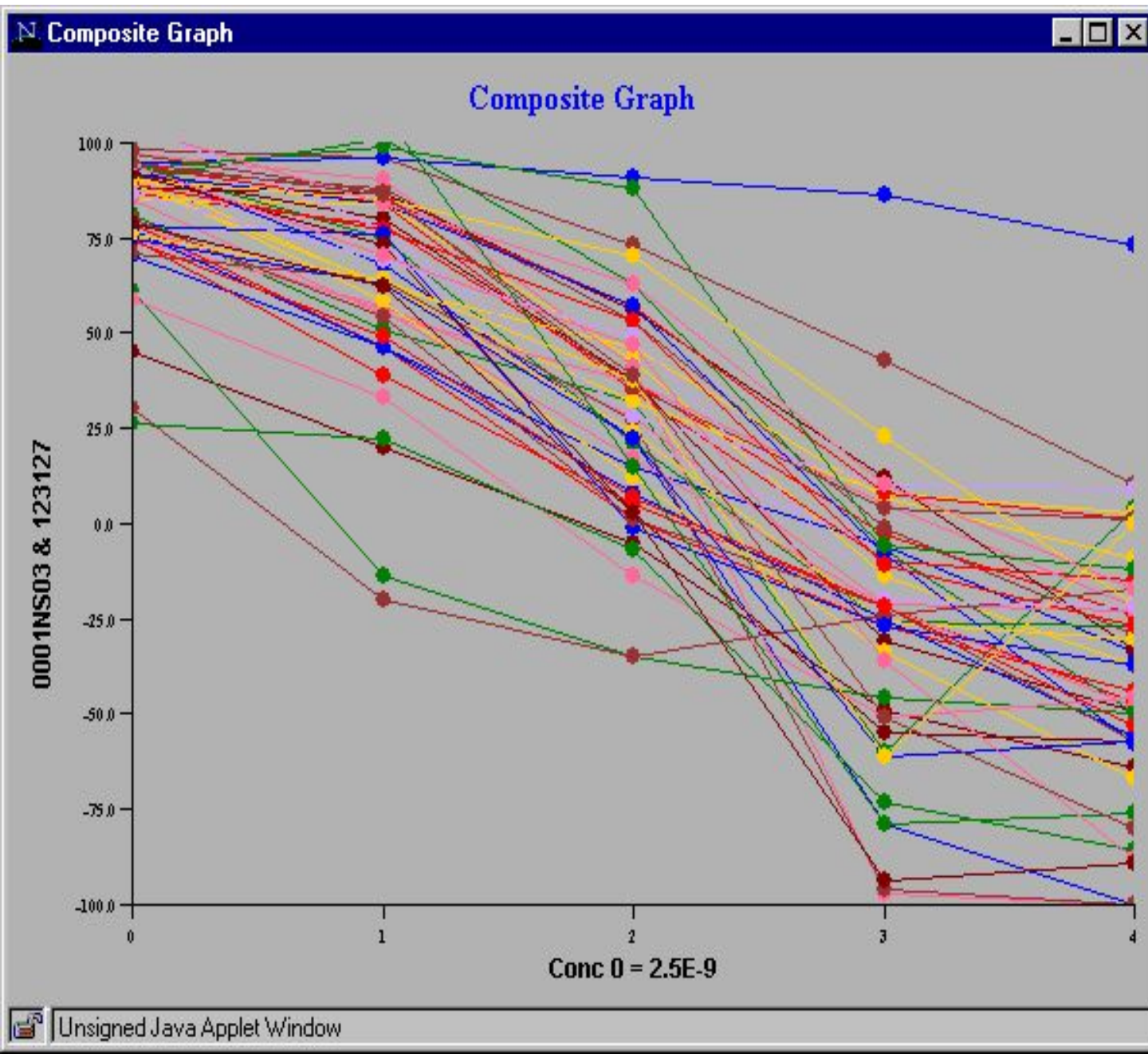


MTT

Formazan



NCI 60 Cell Line Screen





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 \approx

Viable cell number \approx

Clonogenic assay \approx

In vivo cell growth \approx

Tumor growth in patient



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

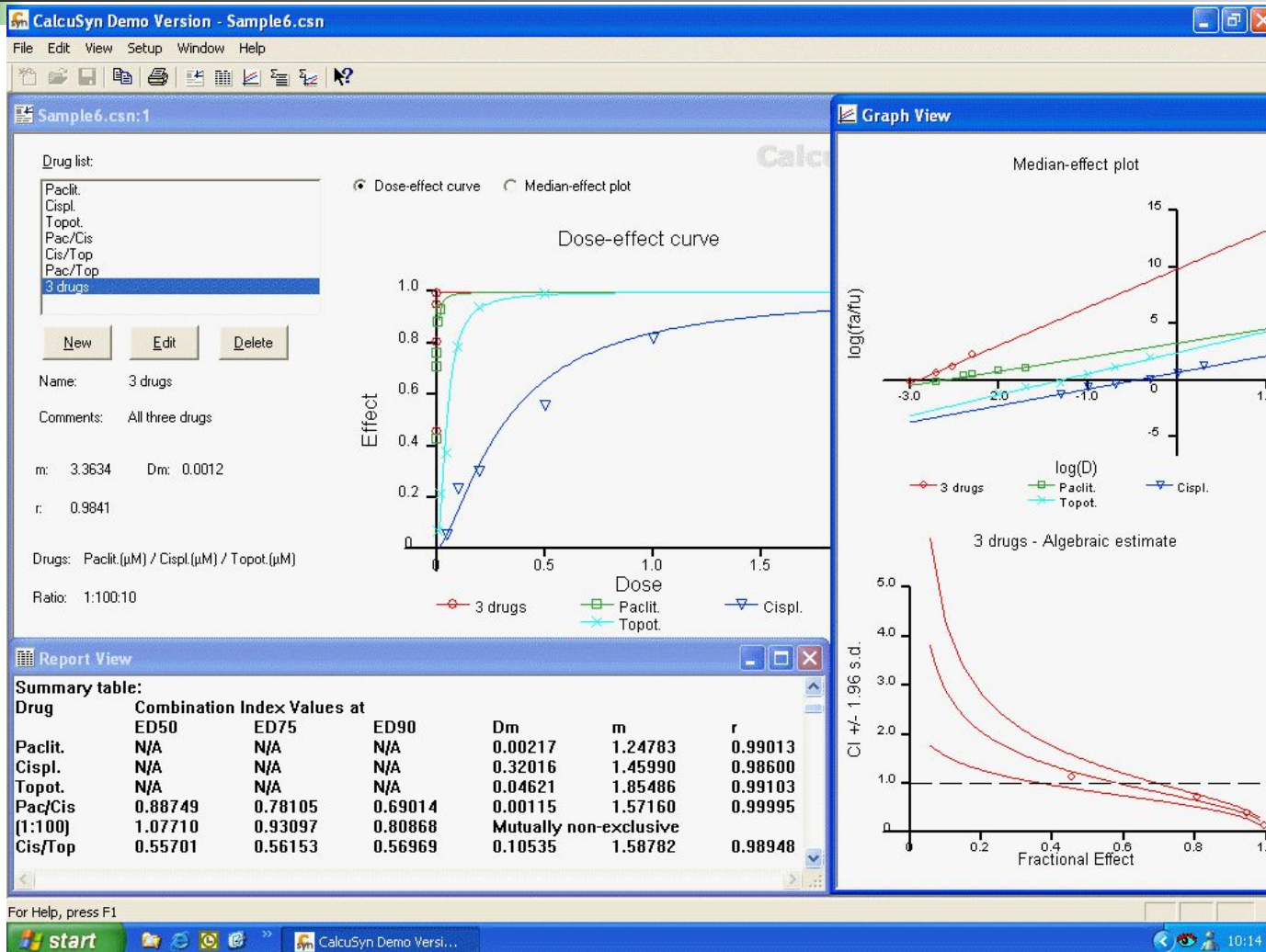


Example: Determination of Synergy

- 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



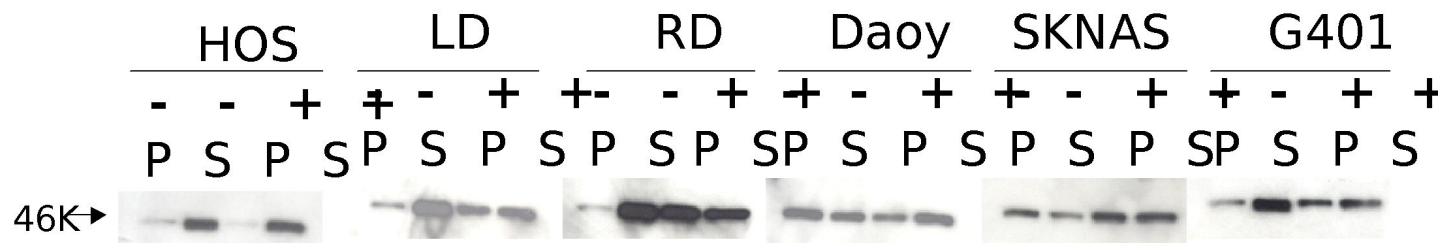


Example: Activity in Pediatric Tumors

- BMS 247550 is an analog of epothilone B that binds tubulin, stabilizes microtubules 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).

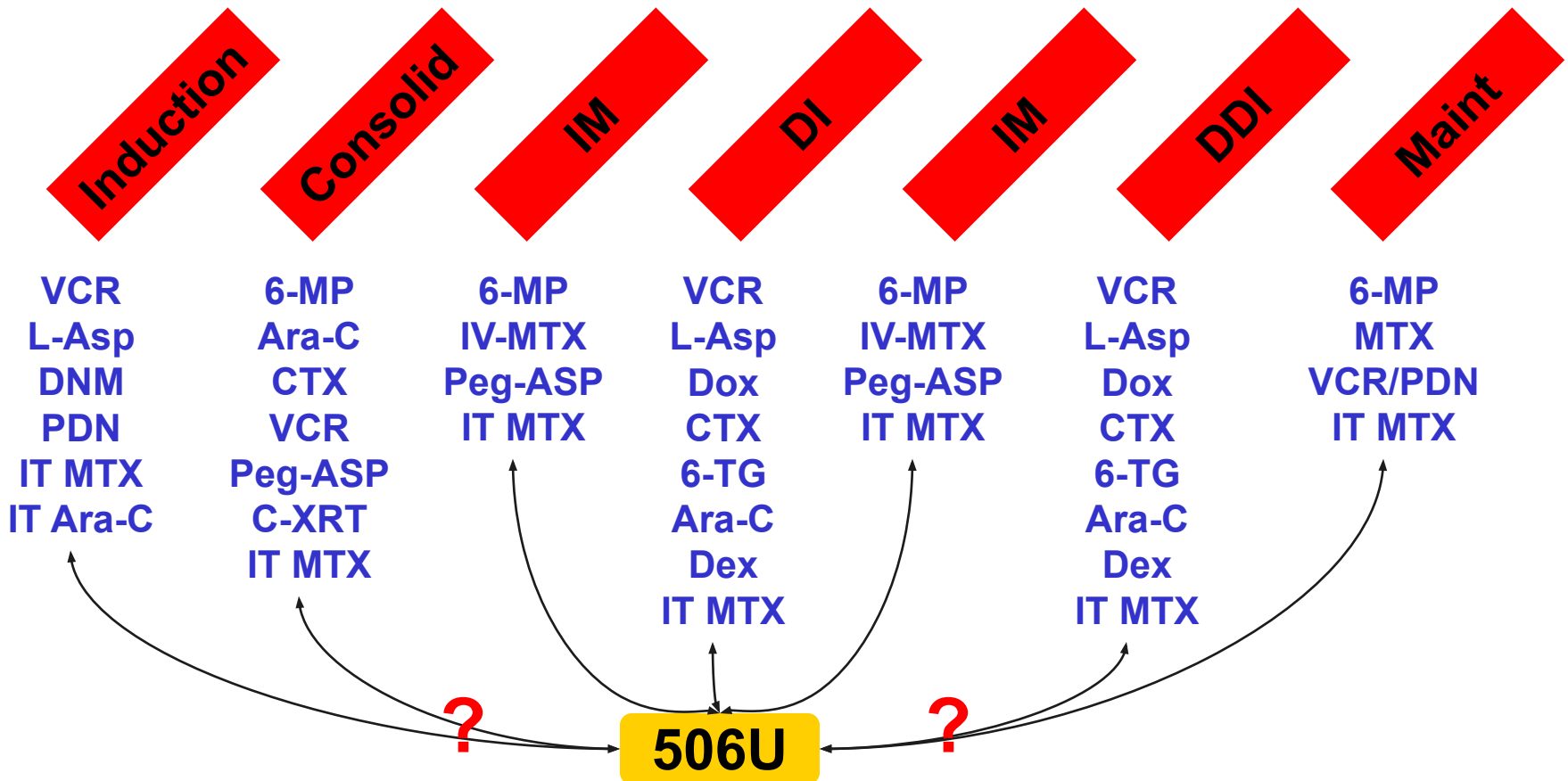
BMS 247550: Pre-clinical Activity

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

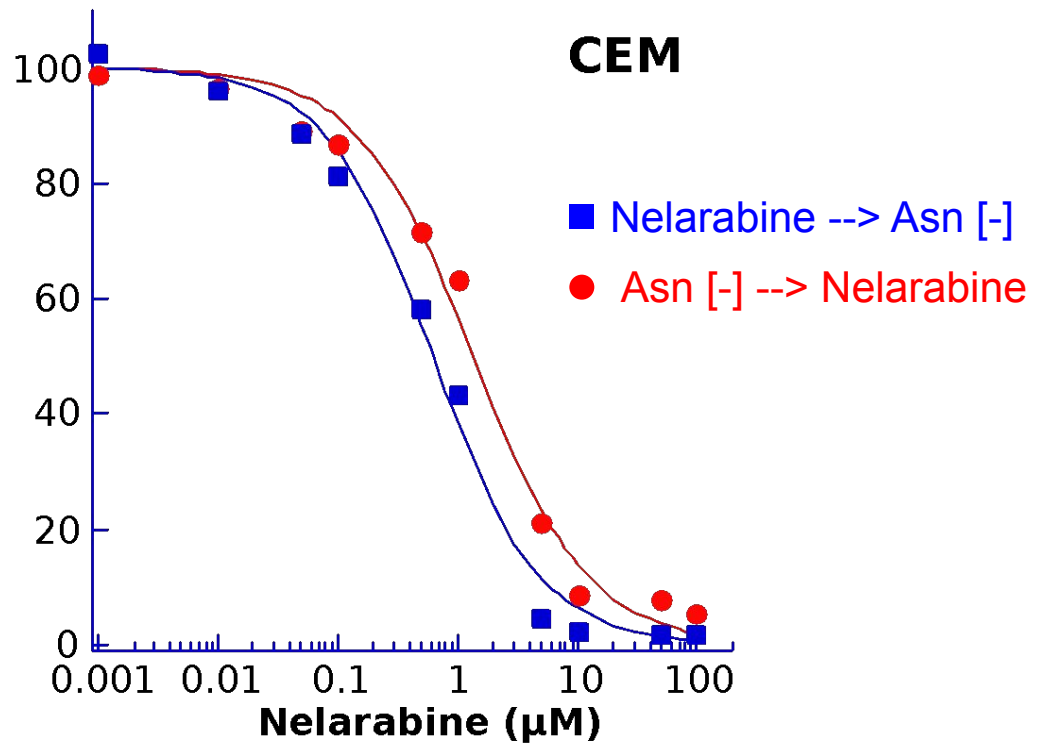
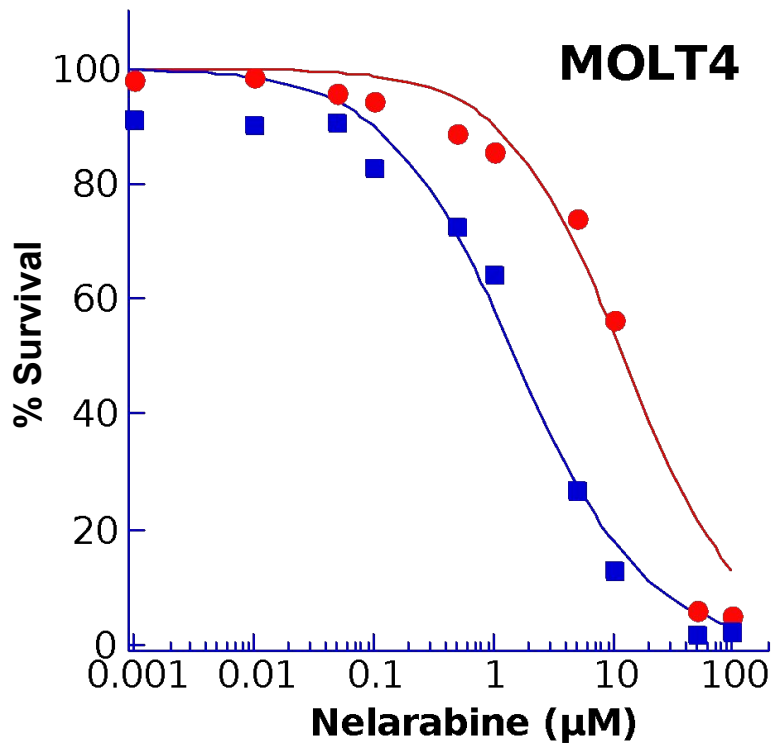


Example: Integration of New Agents

High-Risk ALL Therapy



Asparaginase + 506U





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*