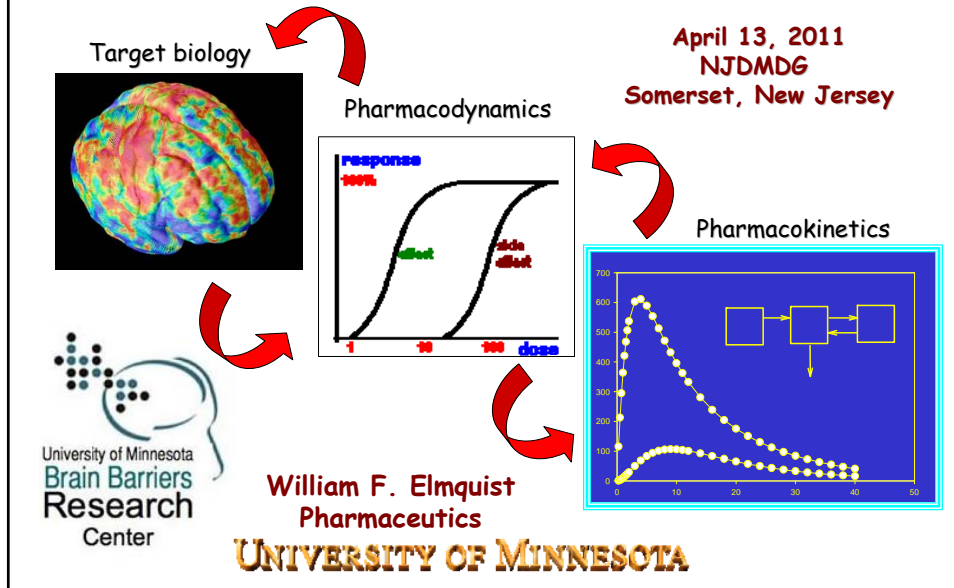


Factors Influencing Drug Delivery to the Brain: Multiple Mechanisms at Multiple Barriers



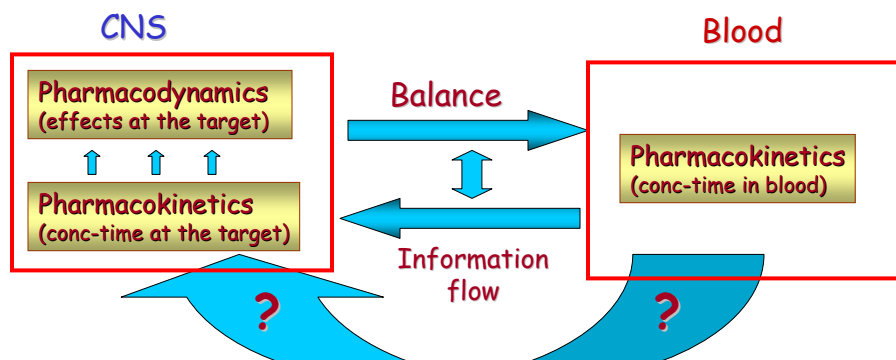
CNS drug development : Must keep in mind the big questions !

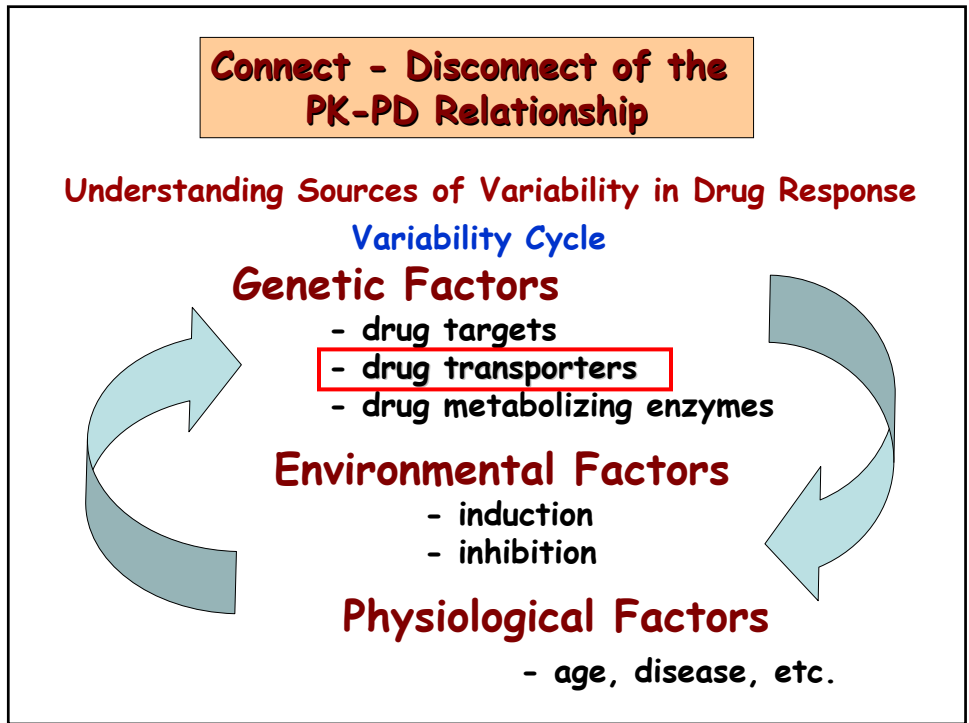
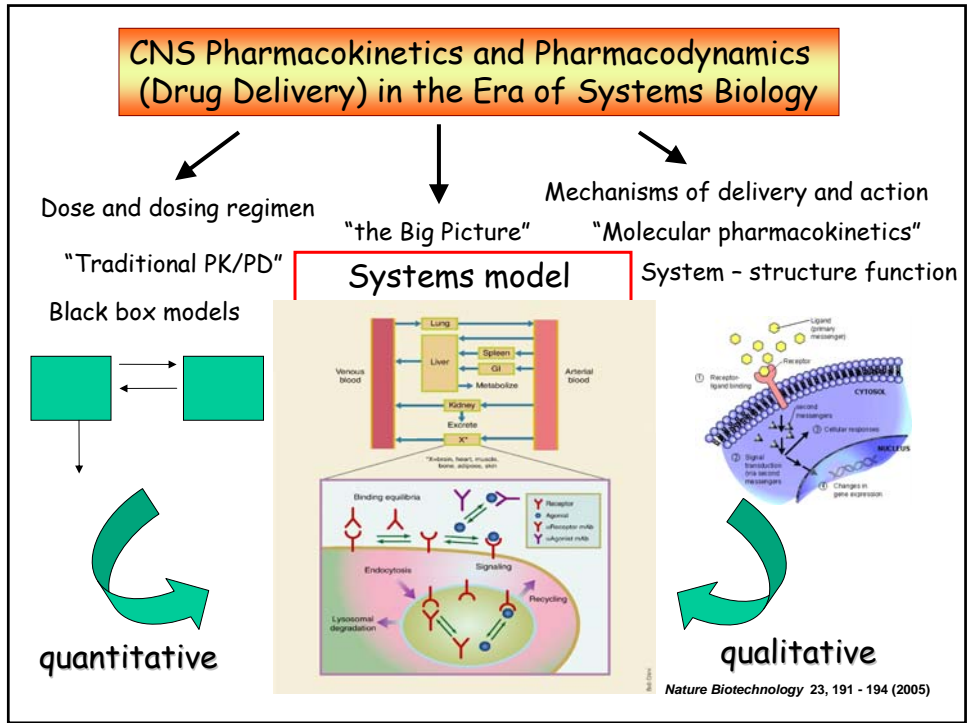
Why Does a Drug Work ??

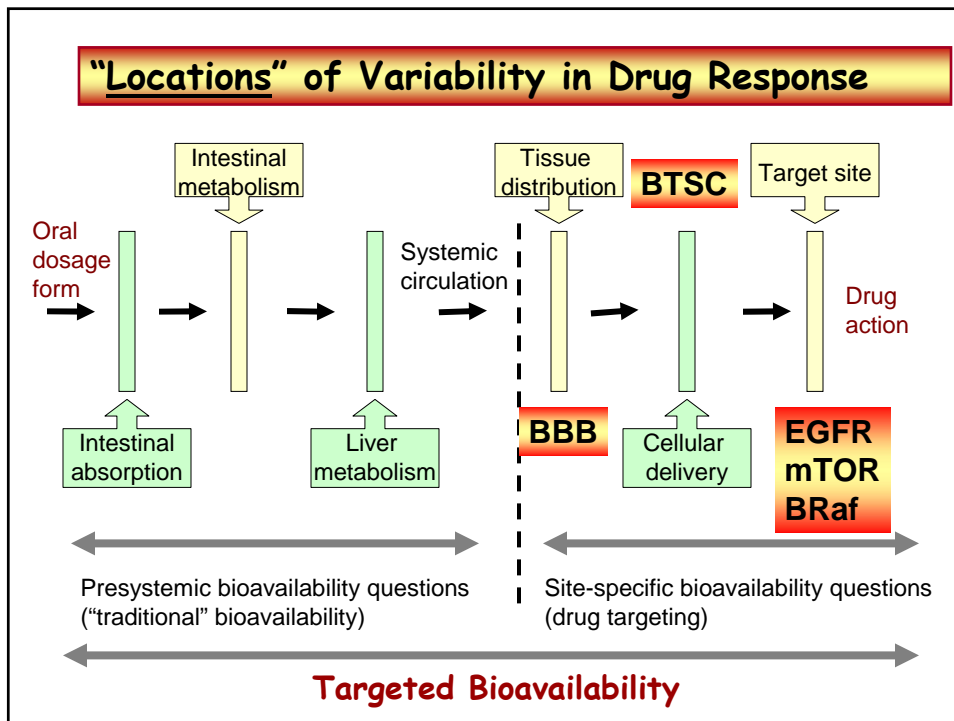
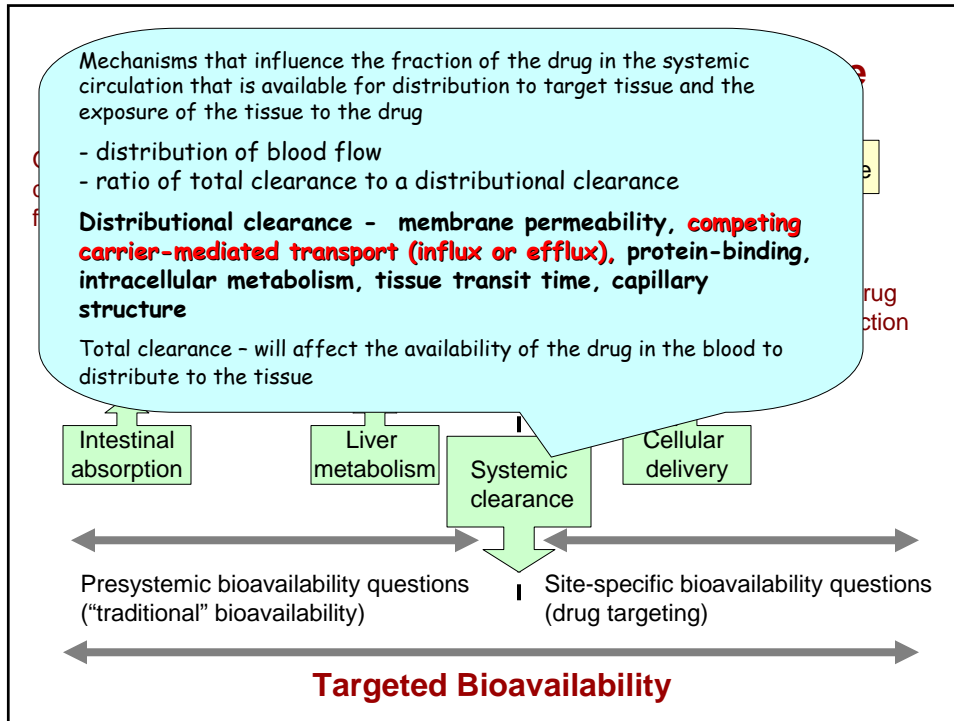
Why Doesn't a Drug Work ??

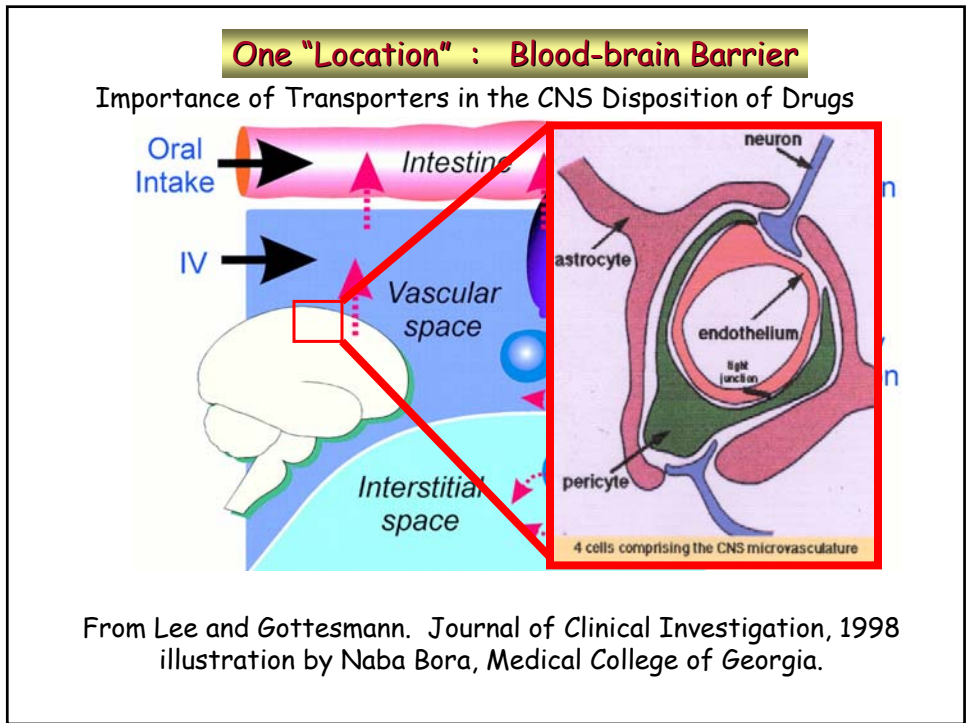
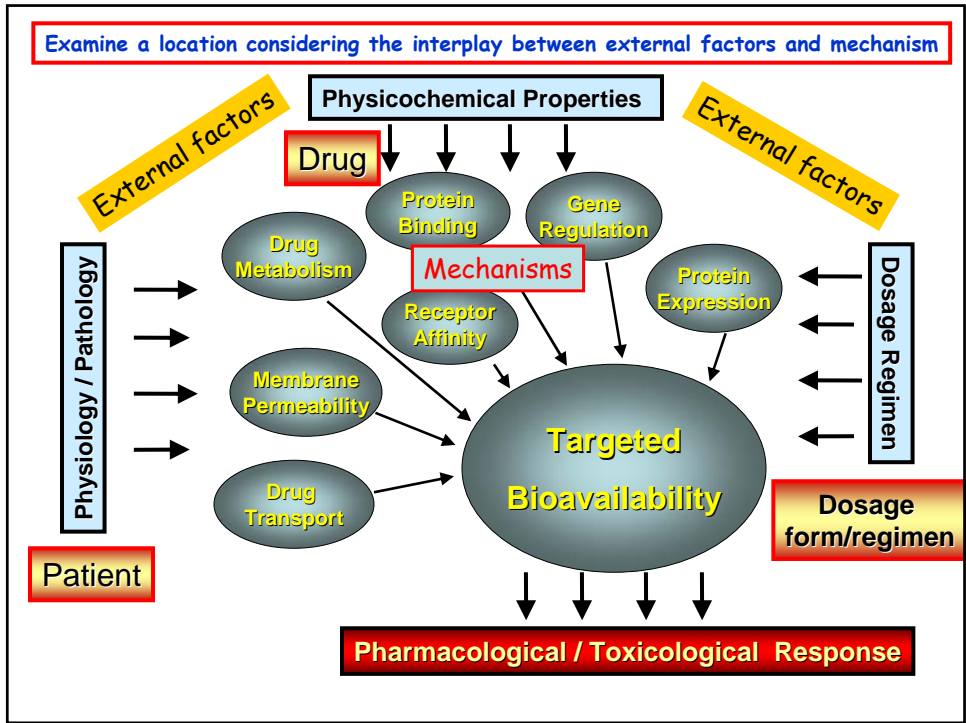
Why does this one work, and that one doesn't ??

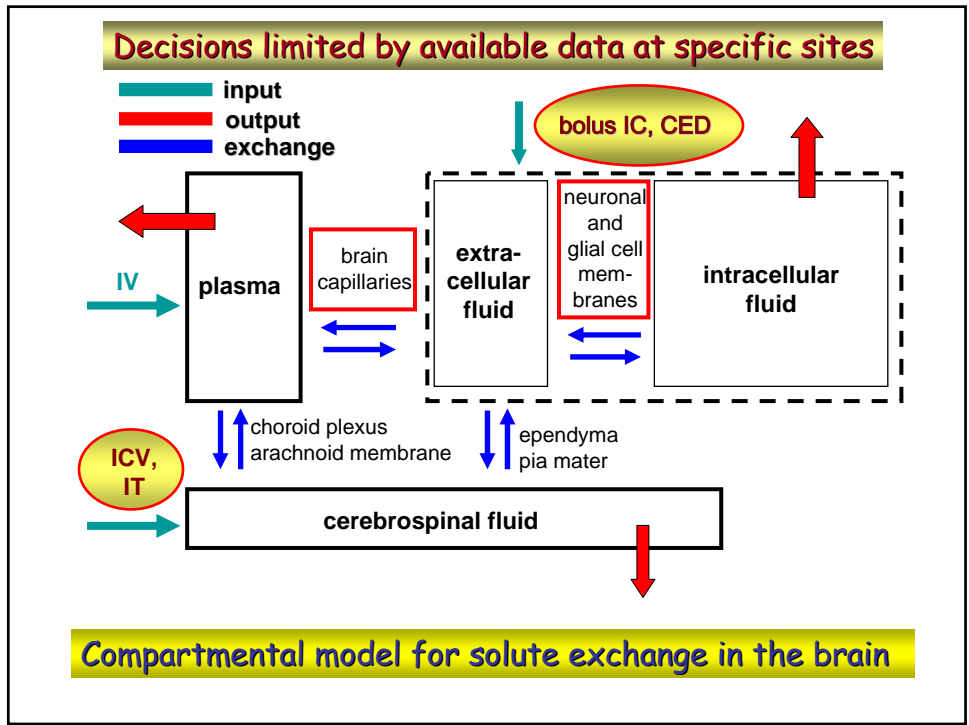
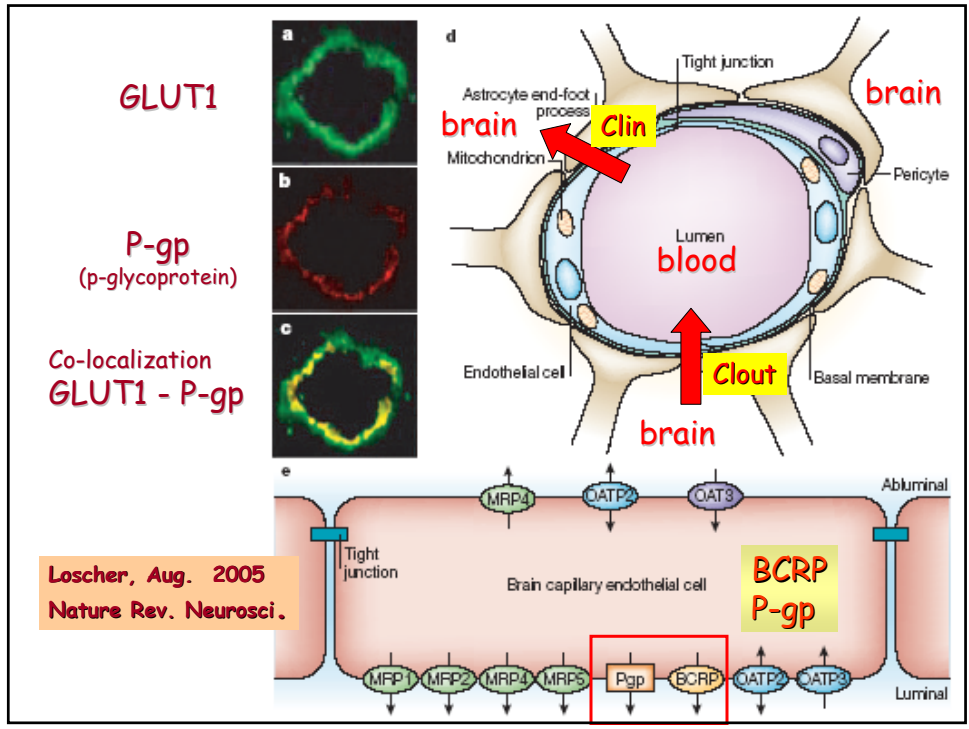
**Connect - Disconnect of the
PK-PD Relationship**

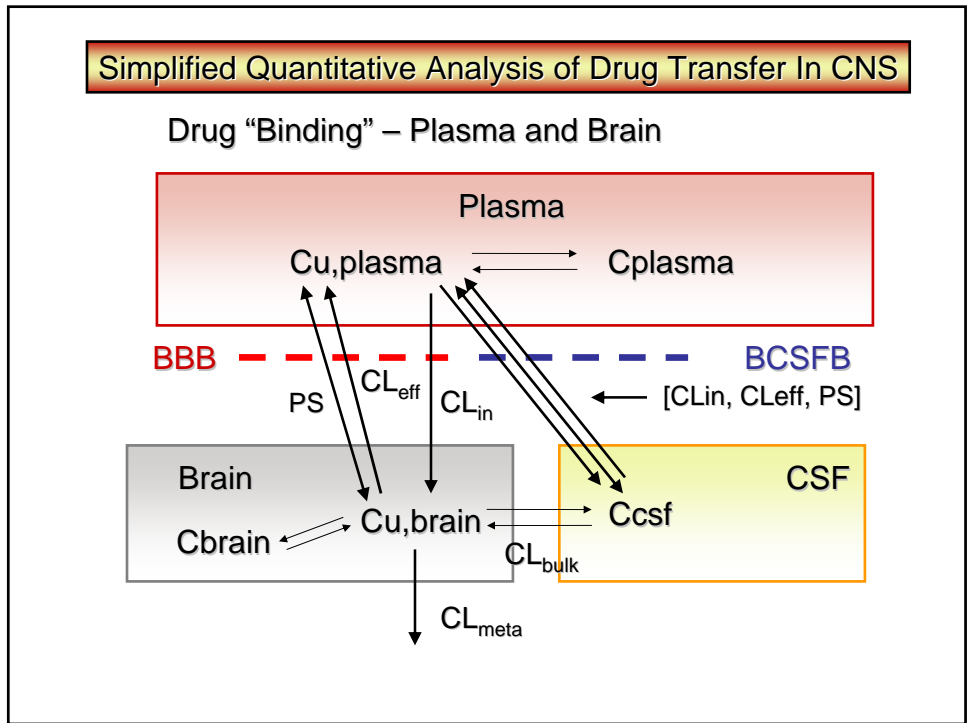
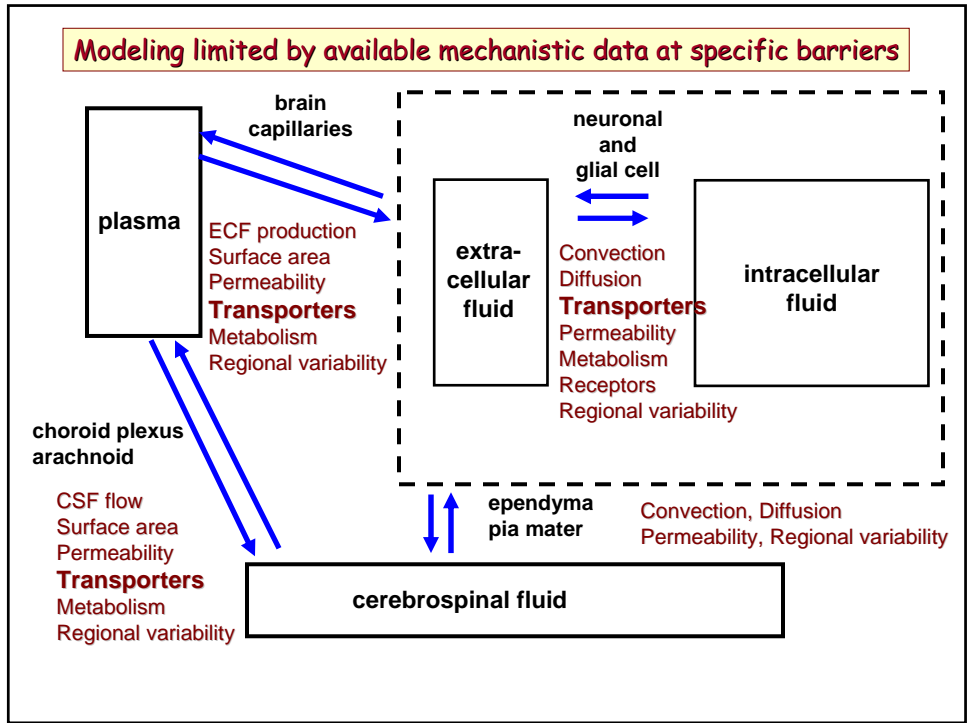












Simplified Quantitative Analysis of Drug Transfer In CNS

Extent - partitioning into brain parenchyma

$$K_{p,free} = \frac{PS + CL_{uptake}}{PS + CL_{efflux} + CL_{metabolism} + CL_{bulk}}$$

Diagram illustrating the components of the equation:

- PS** (Permeability Surface Area Product) is influenced by **Tight-junction opening** (top left) and **Tight-junction opening** (bottom left).
- CL_{uptake}** (Clearance due to uptake) is influenced by **Substrate for Influx Transporter** (top right).
- CL_{efflux}** (Clearance due to efflux) is influenced by **Inhibition of Efflux transporter** (bottom center).

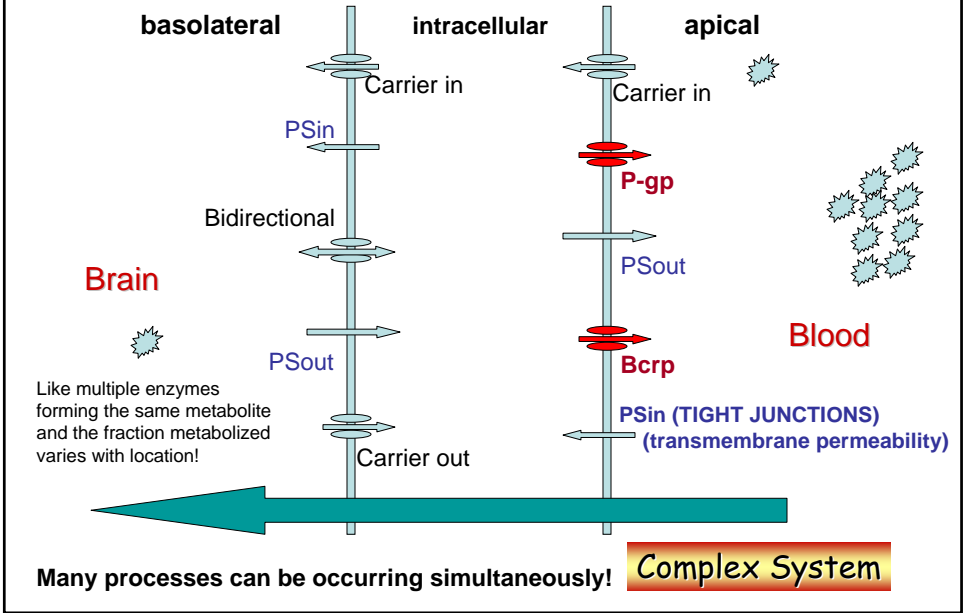
Drug Targeting Index (a measure of delivery)

$$DTI = \frac{AUC_{target,intervention} / AUC_{blood,intervention}}{AUC_{target,control} / AUC_{blood,control}}$$

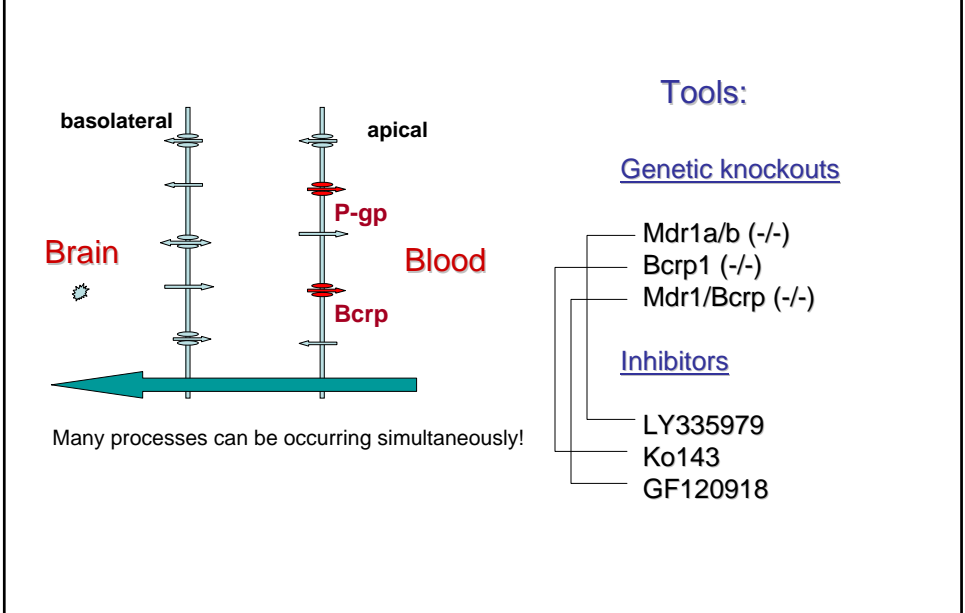
A neutral intervention would lead to a targeting index of unity, where positive effect would lead to a DTI greater than one, and a negative effect would result in a DTI less than one.

The critical issue in the quantitative assessment of drug targeting is the need to measure drug concentrations at the target site

Complexity of the Transporter Problem at Various Barriers



Complexity of the Transporter Problem at Various Barriers



Case Study :

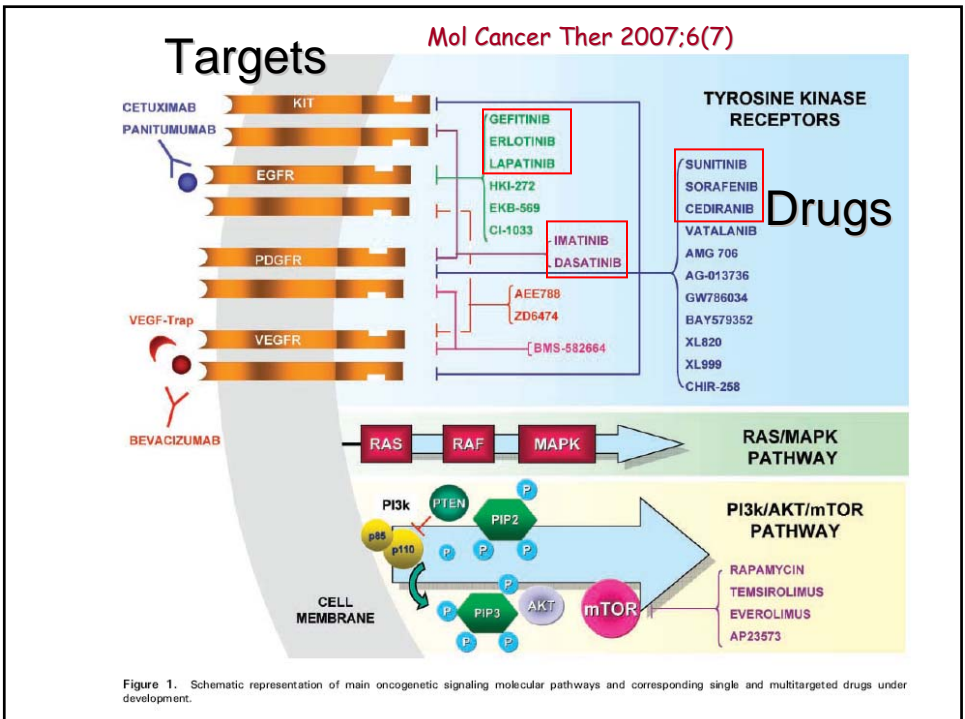
**Tyrosine Kinase Inhibitors for
Glioblastoma Multiforme (Glioma)**

"Molecularly-Targeted" Agents.
Can they find the target?

Numerous clinical trials with targeted
tyrosine kinase inhibitors for glioma have failed.

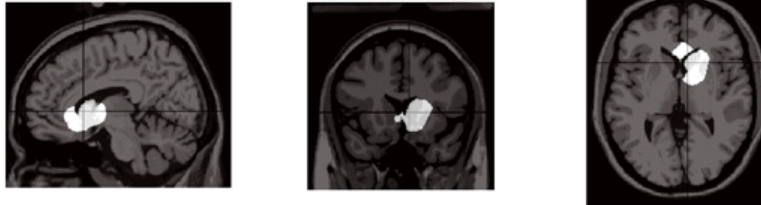
Is there a PK-PD disconnect for these drugs in glioma?

consilience : to give a purpose to understanding the details

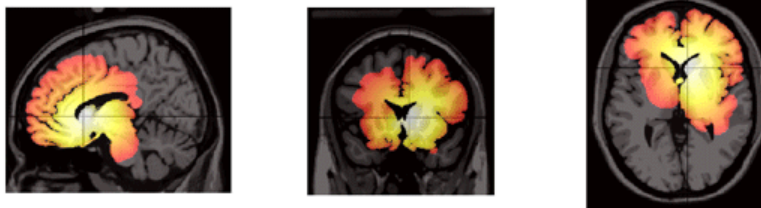


Target Locations

MRI-Detectable Portion of Tumor



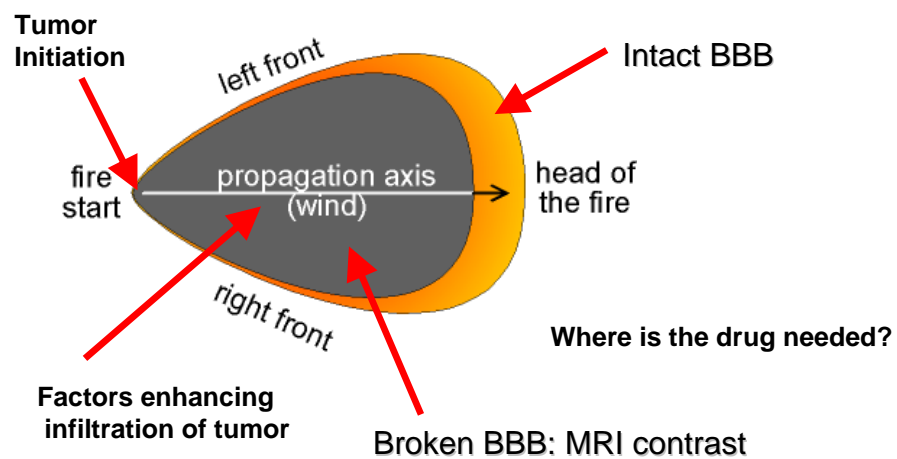
Actual Tumor

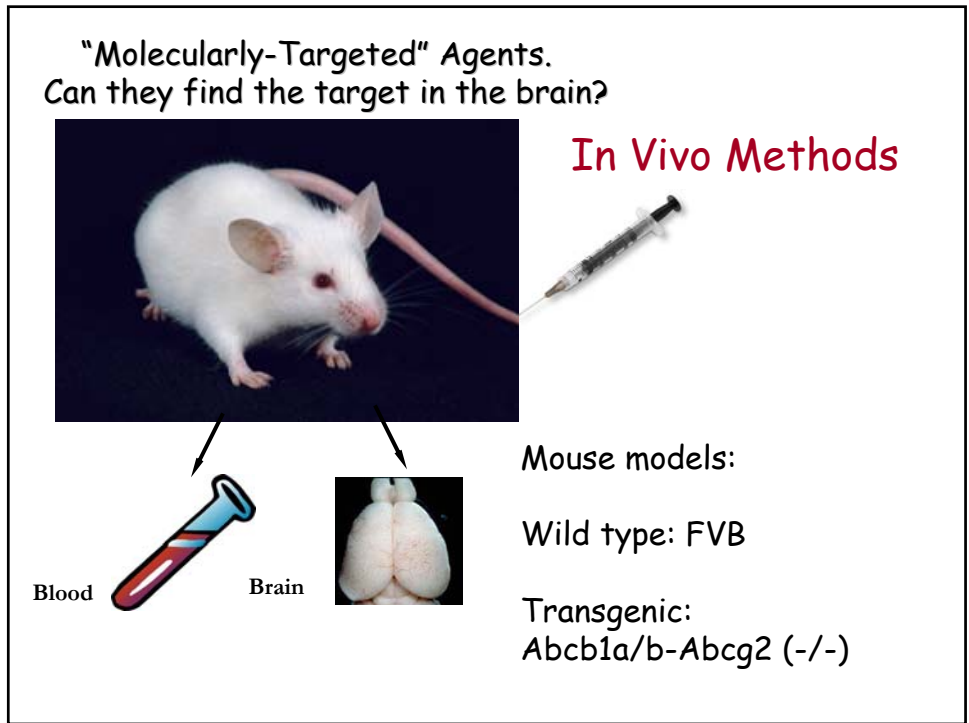
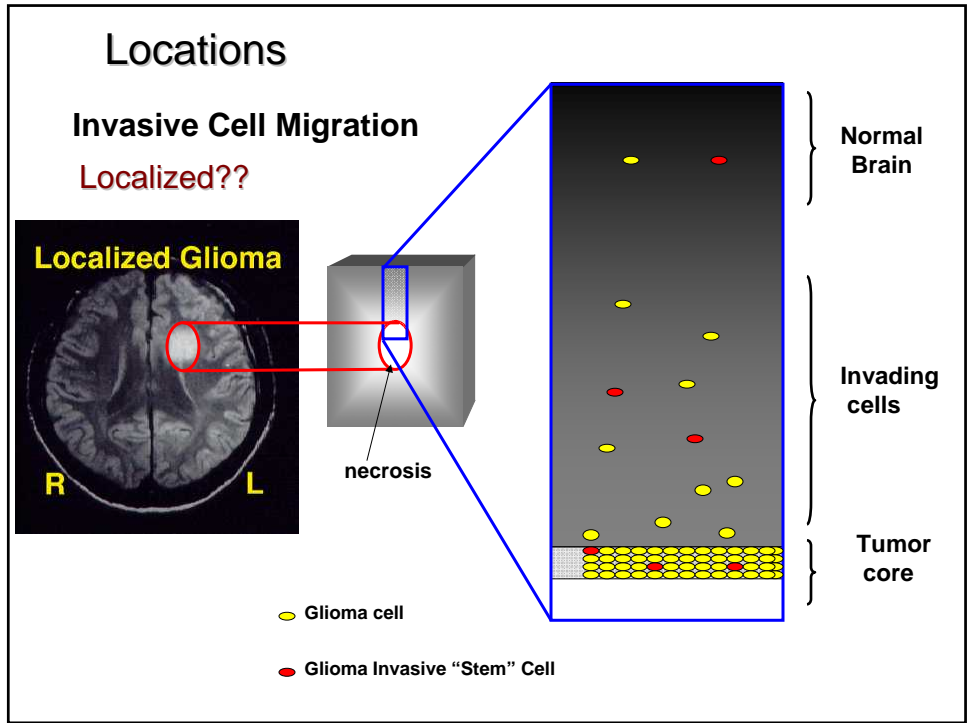


1033 days

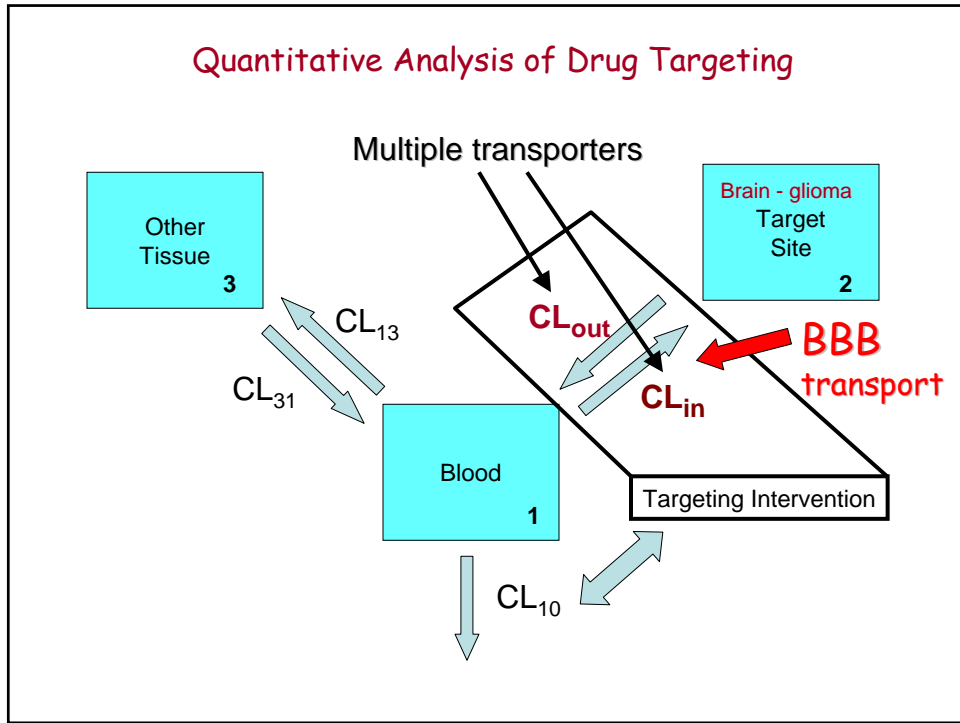
Kristin R Swanson, Ph.D., University of Washington, 2008

Like Fighting a Forest Fire

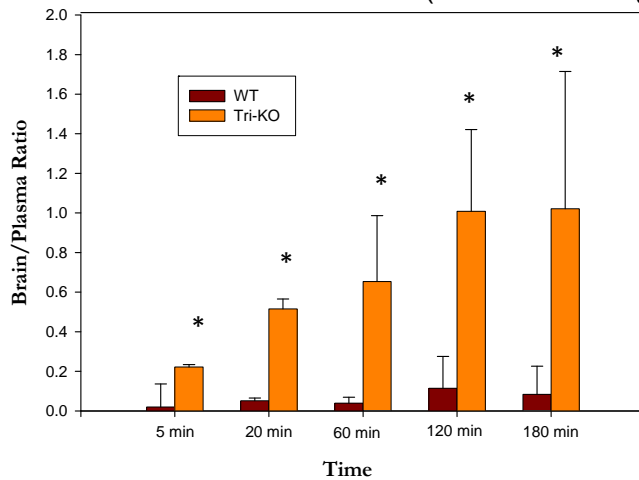




Quantitative Analysis of Drug Targeting



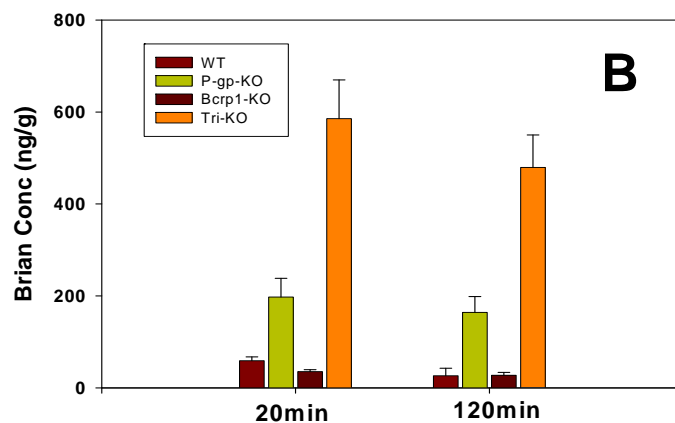
Dasatinib – Brain/Plasma ratios in triple knockouts (Abcb1a/b-Abcg2 (-/-))



The brain-to-plasma ratio of dasatinib in wild-type and triple-knockout FVB Mice
(* $p < 0.05$, $n=4$)

Chen Y. et al., JPET September 2009 vol. 330 no. 3 pgs. 956-963

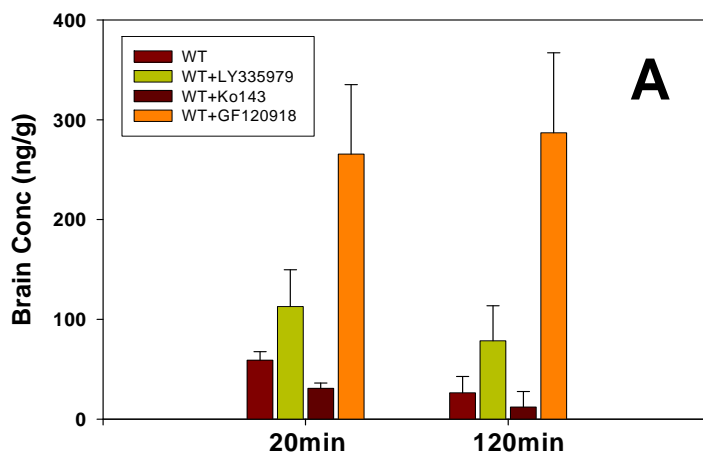
Dasatinib – genetic deletion of transporter genes



Brain concentration of dasatinib in WT mice with and without genetic deletion of efflux transport.

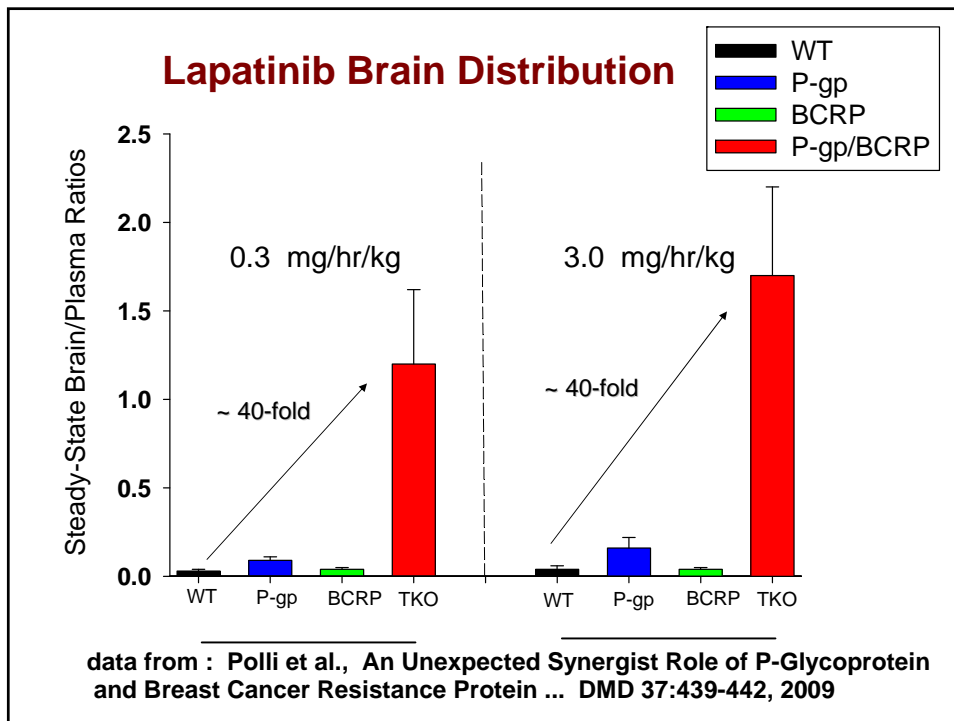
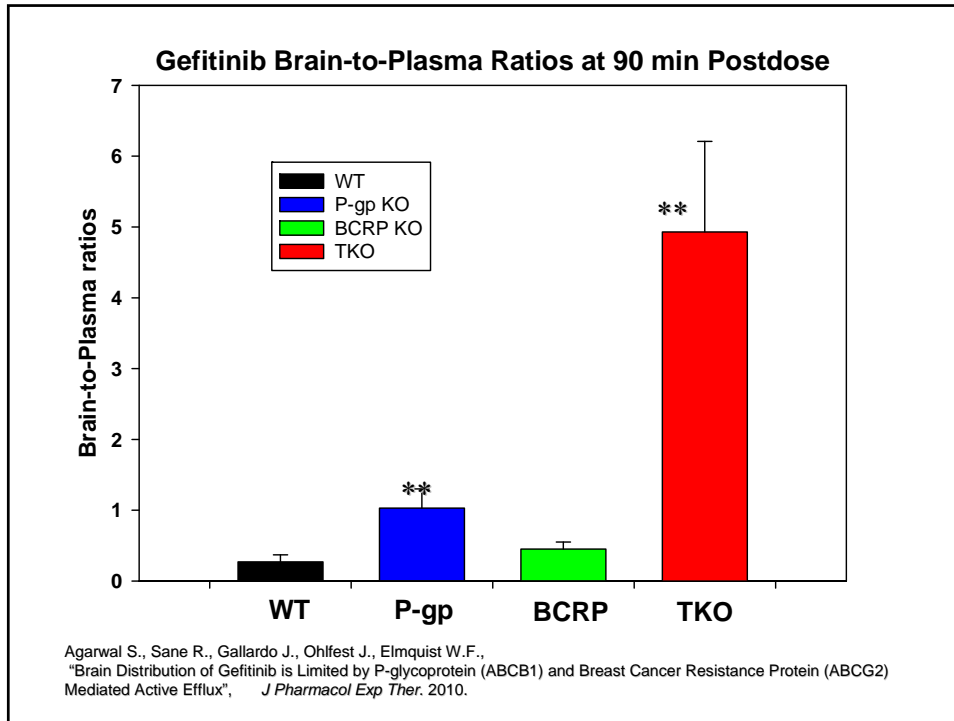
Chen Y. et al., JPET September 2009 vol. 330 no. 3 pgs. 956-963

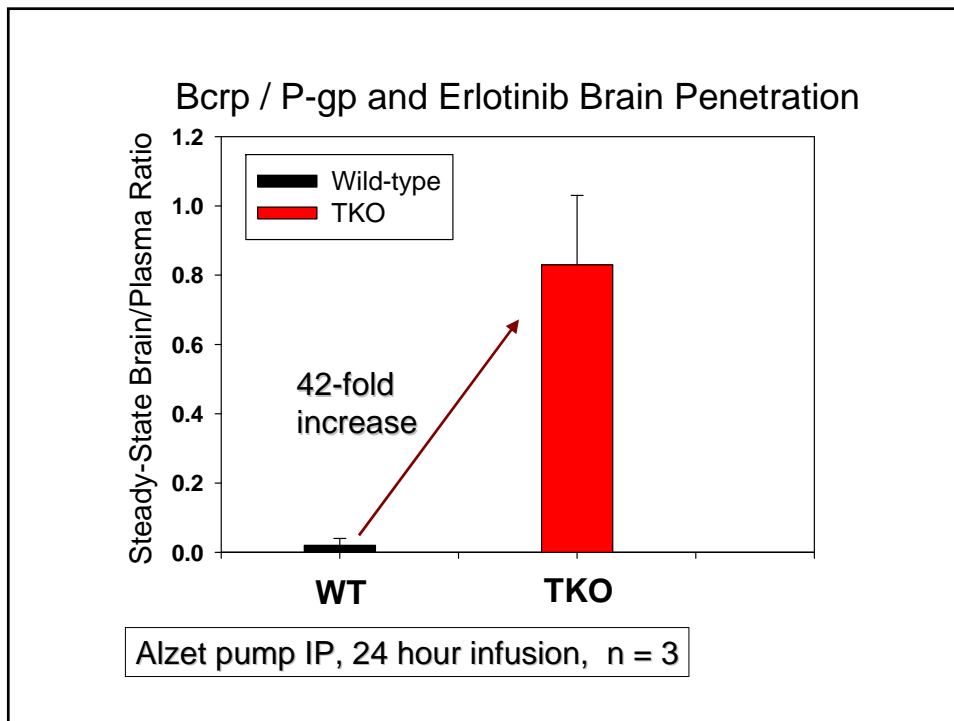
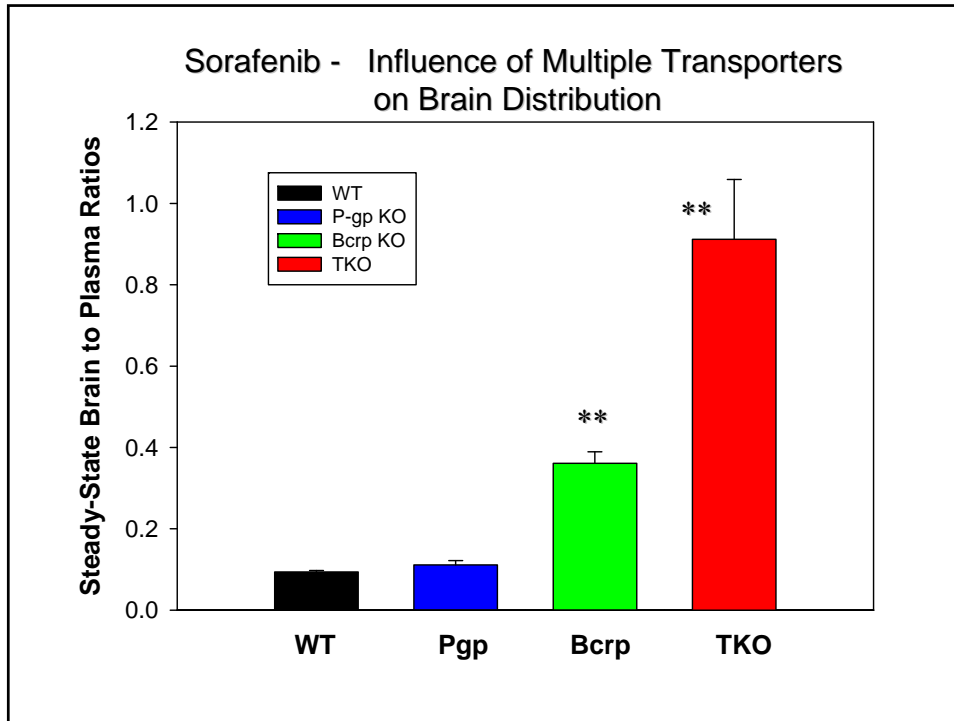
Dasatinib – pharmacologic inhibition of transport



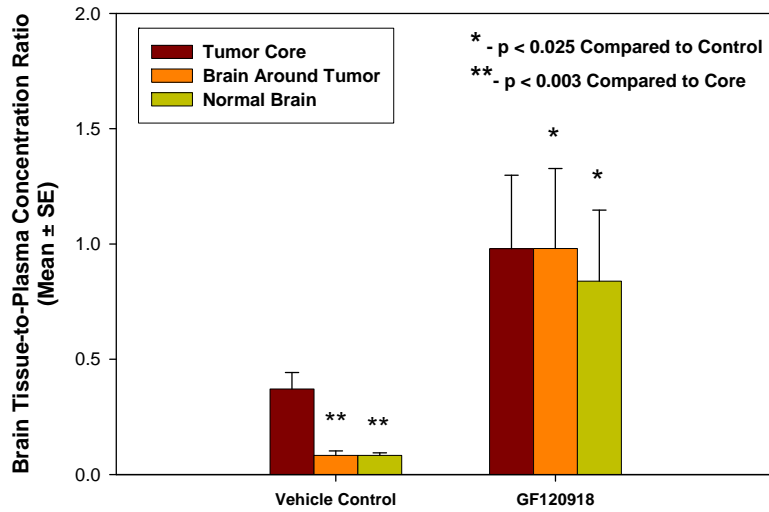
Brain concentration of dasatinib in WT mice with and without pharmacologic inhibition of efflux transport.

Chen Y. et al., JPET September 2009 vol. 330 no. 3 pgs. 956-963

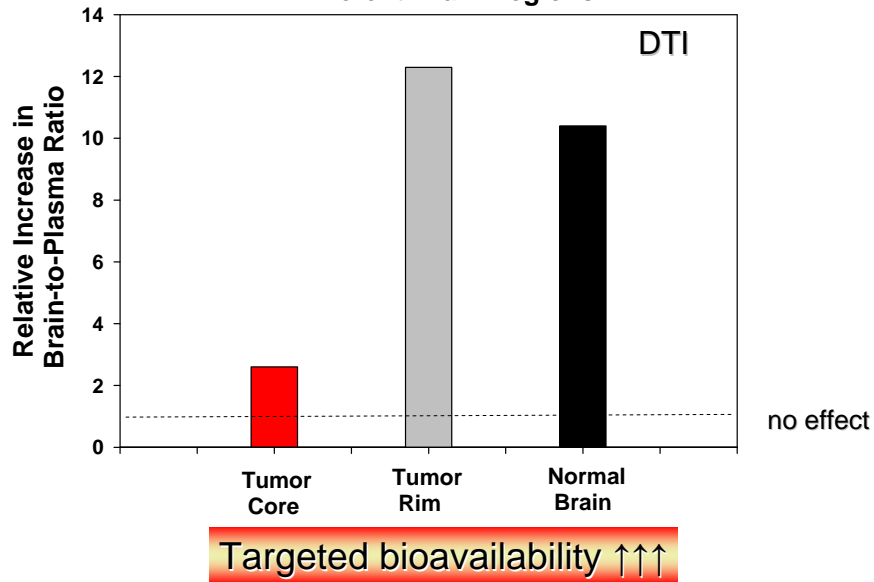




Erlotinib Brain-to-Plasma Concentration Ratio at Different Locations in Rat Brain Tumor



Influence of Elacridar on Erlotinib Brain Penetration in Different Brain Regions

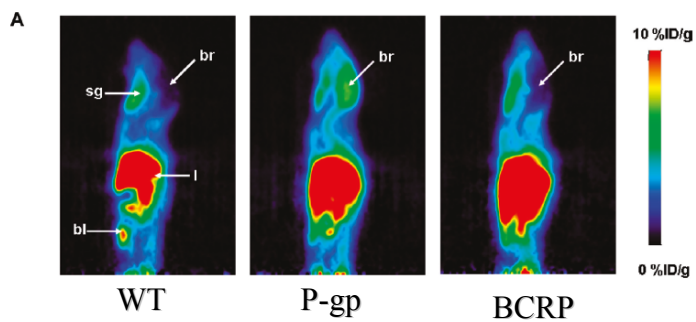


Differences in Brain Distribution Enhancement between Genetic Knockouts and Pharmacological Inhibition

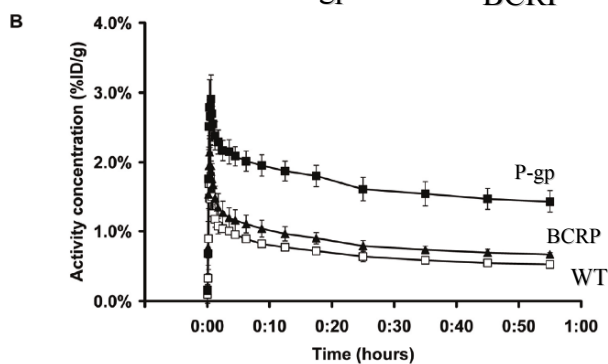
Generally knockouts show greater enhancement

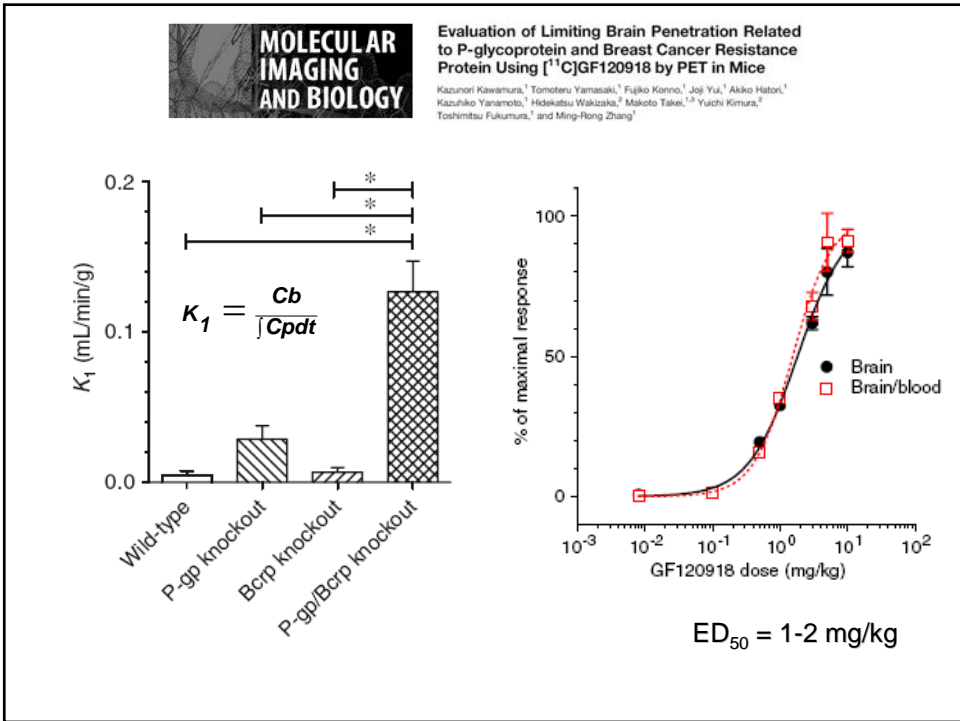
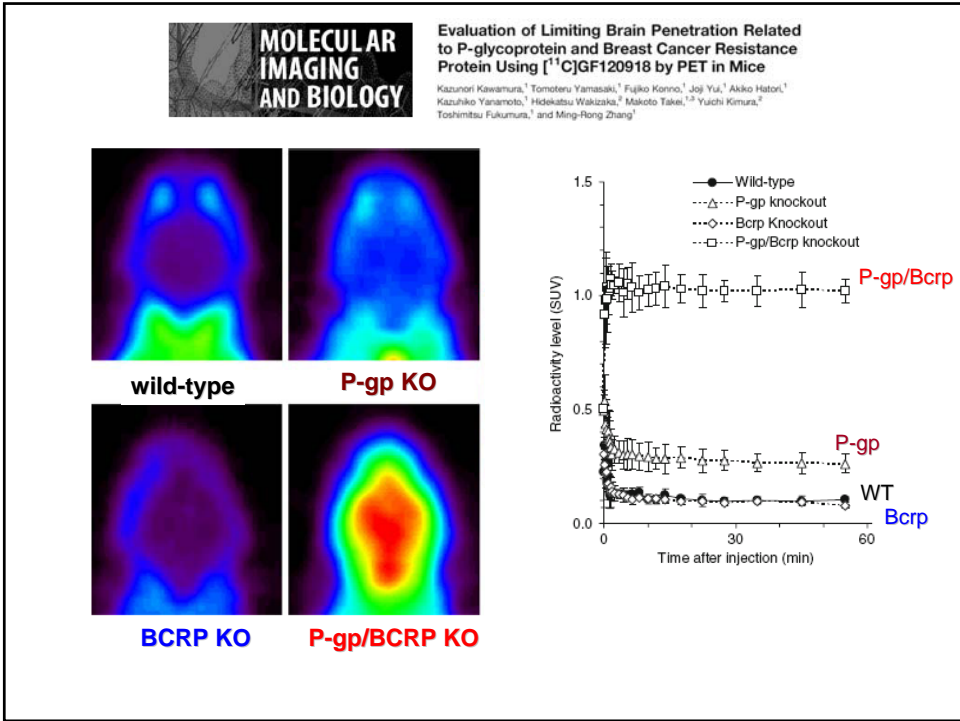
- 1) accessibility of inhibitors ?
(dose, potency, parenchymal concentrations)
- 2) locations of transporters ?
(BBB vs parenchyma)

^{11}C -elacridar
(GF120918)

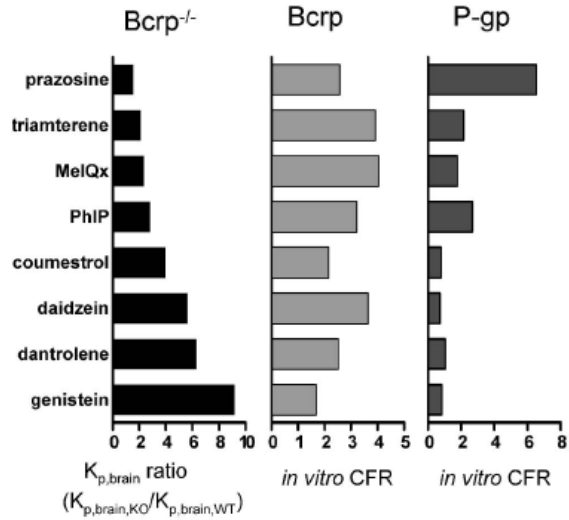


Dorner, 2009





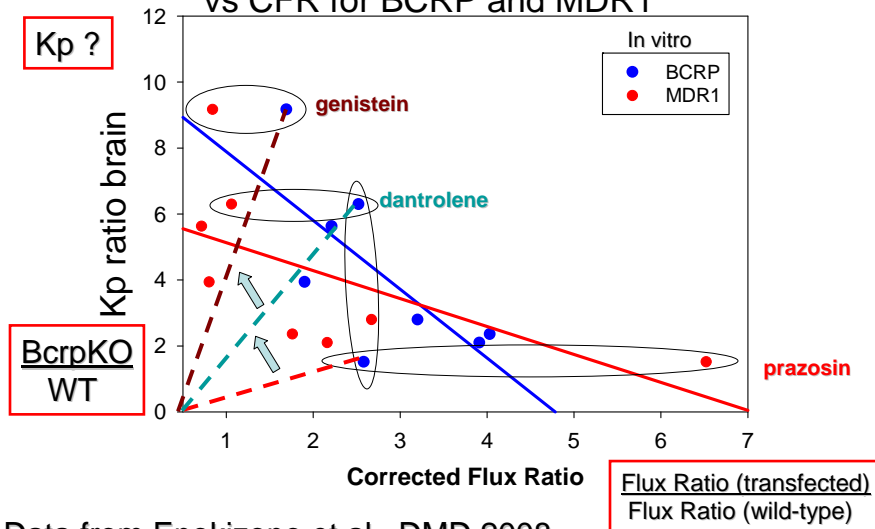
In Vitro- In Vivo Correlations, What about BCRP vs P-gp?



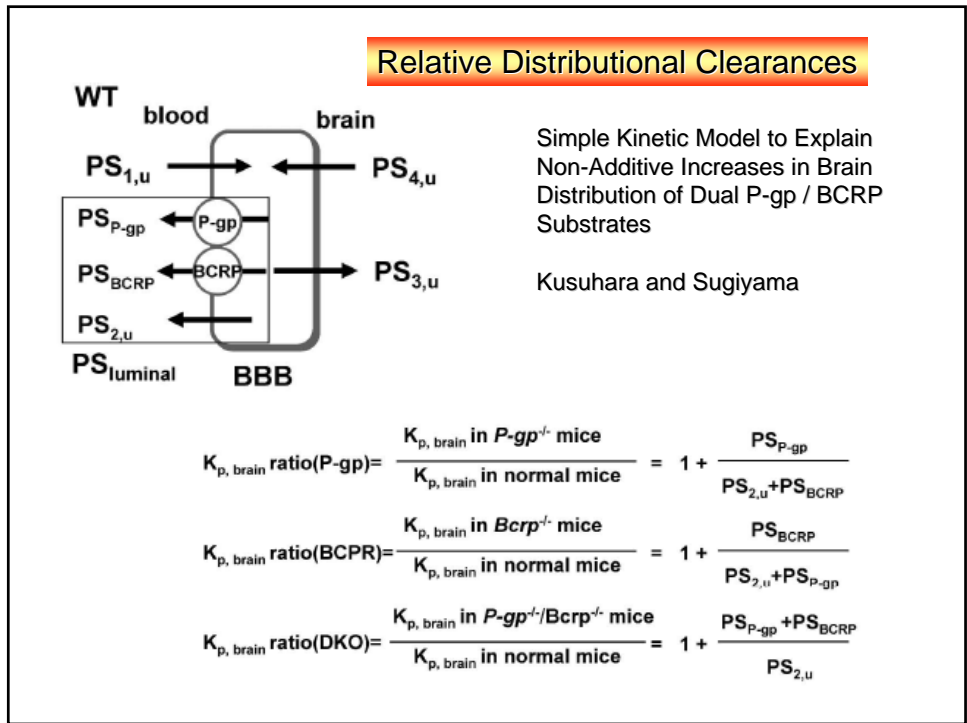
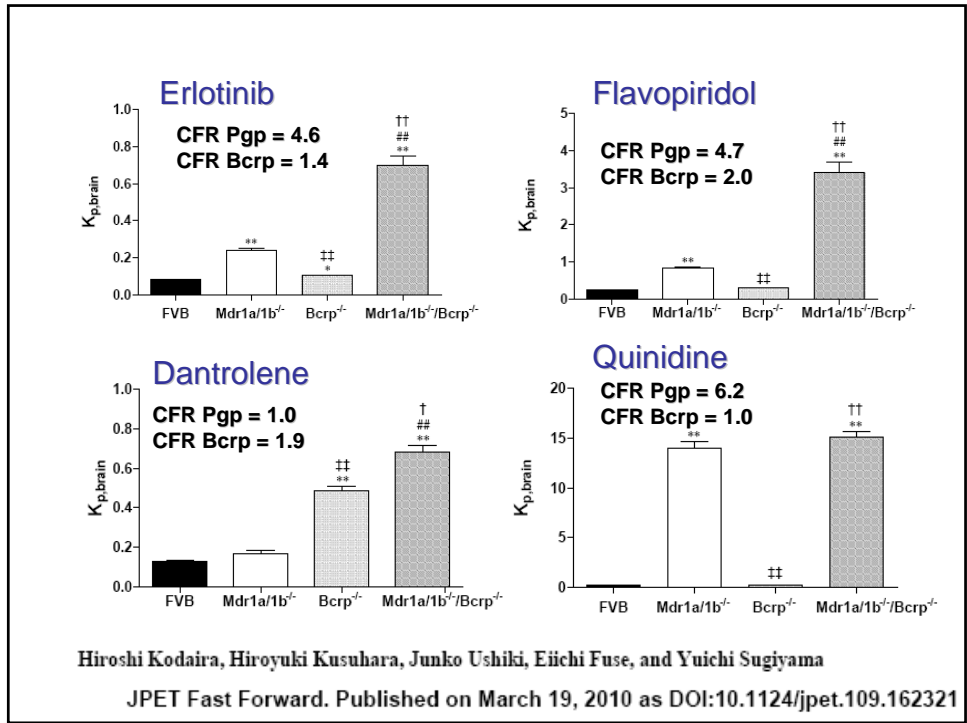
Kusuhara and Sugiyama

Is BCRP Important in the BBB? - Does BCRP KO tell the story?

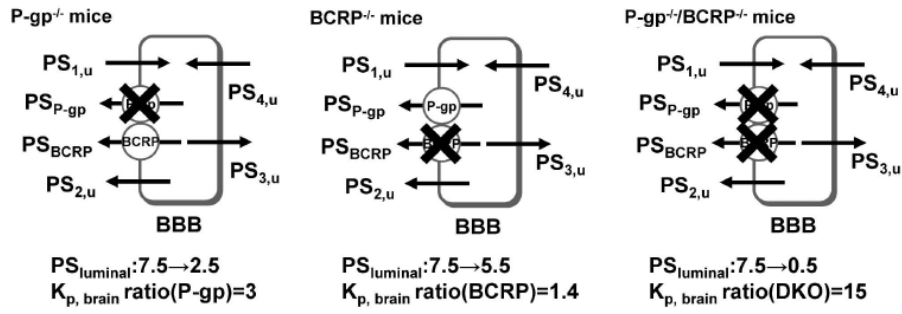
K_p (brain) Ratio (*Bcrp* KO/WT)ss vs CFR for BCRP and MDR1



Data from Enokizono et al., DMD 2008

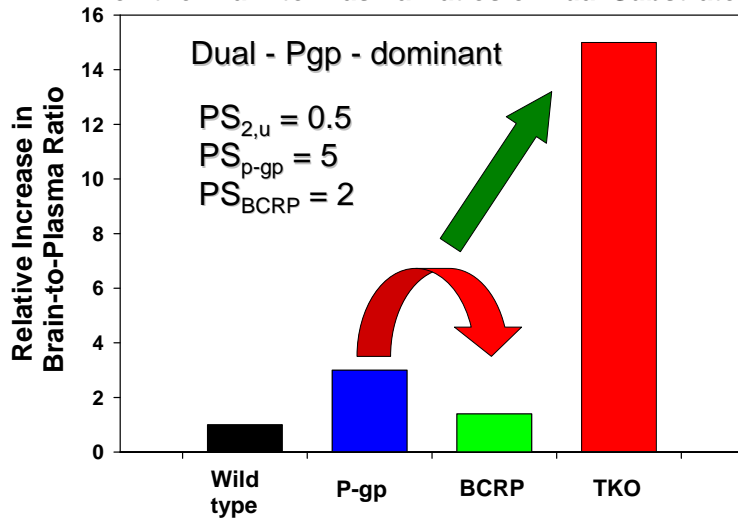


A hypothetical common substrate drug with following kinetic parameters;
 $PS_{2,u}=0.5$, $PS_{BCRP}=2$, $PS_{P-gp}=5$

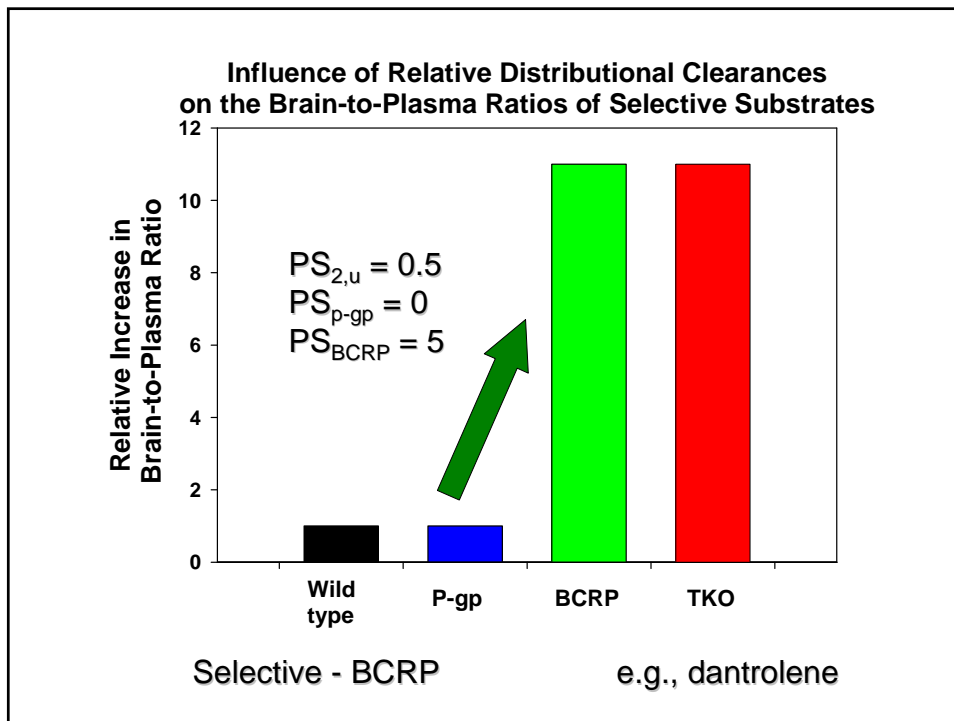
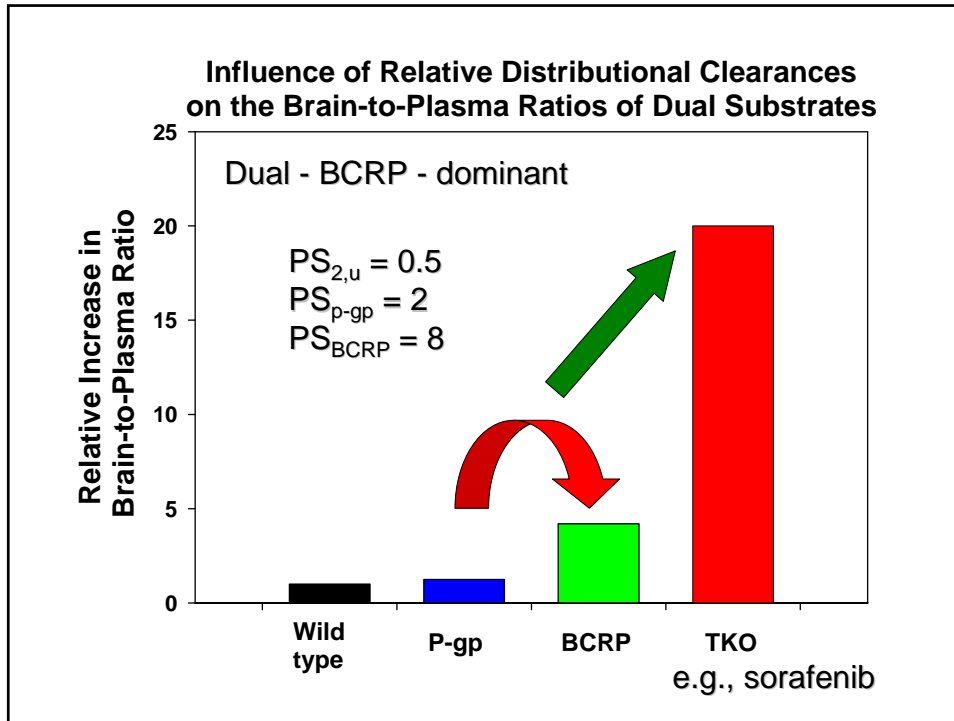


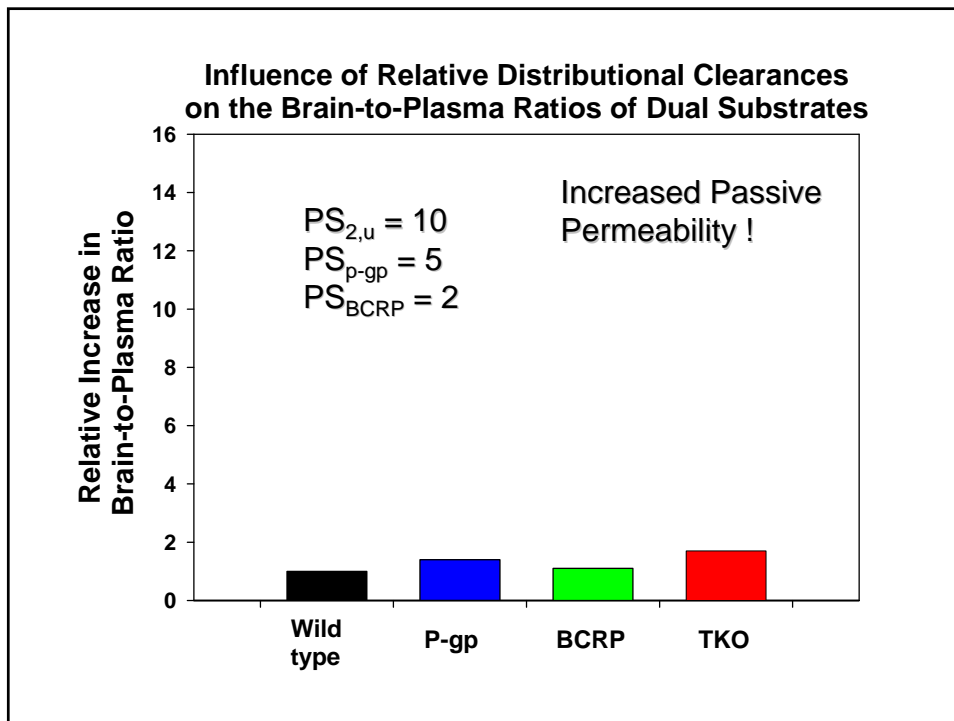
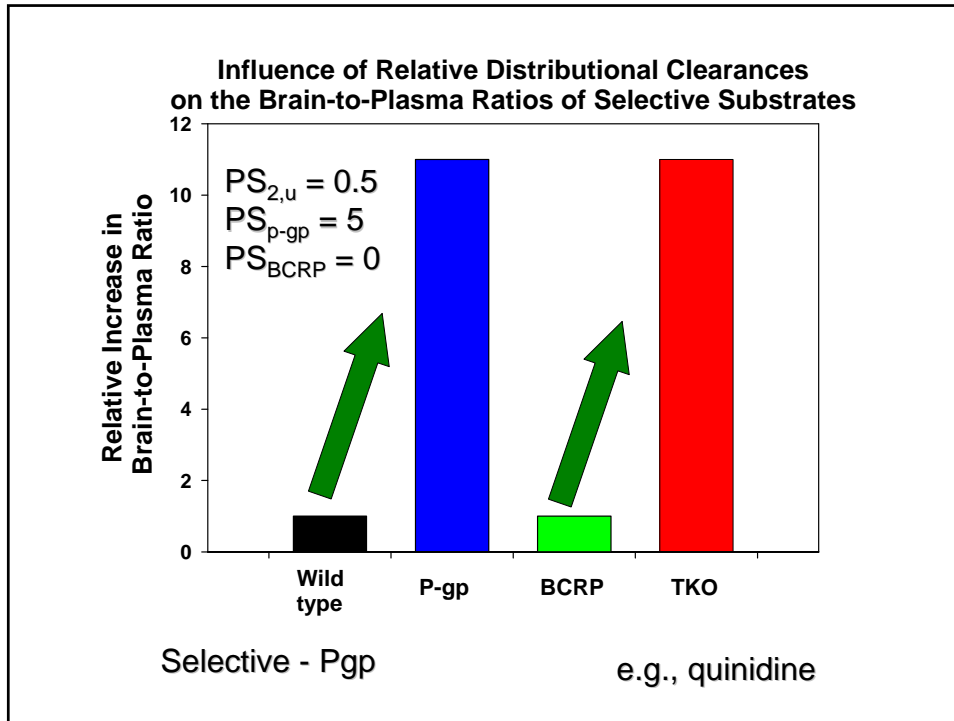
Kushuhara and Sugiyama, Drug Metab. Pharmacokint. 24 (1):37-52, 2009

Influence of Relative Distributional Clearances on the Brain-to-Plasma Ratios of Dual Substrates

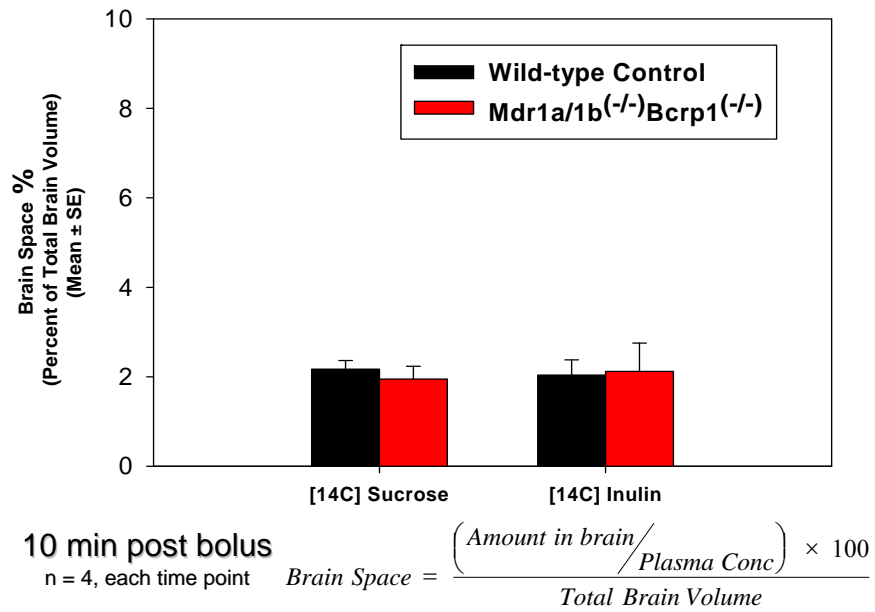


e.g., dasatinib, erlotinib, gefitinib, lapatinib (affinity and capacity)





Integrity of Tight Junctions in WT vs Triple Knockout Mice



De Novo Induction of Genetically Engineered Brain Tumors in Mice Using Plasmid DNA

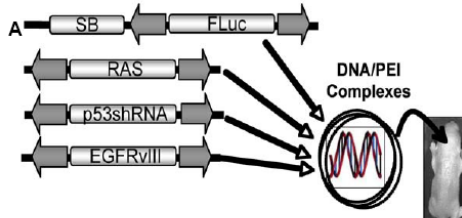
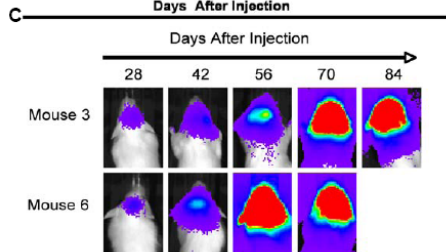
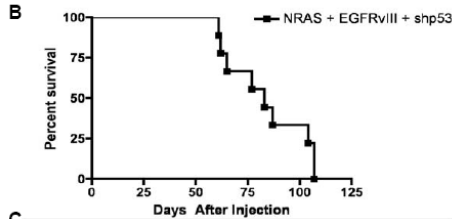
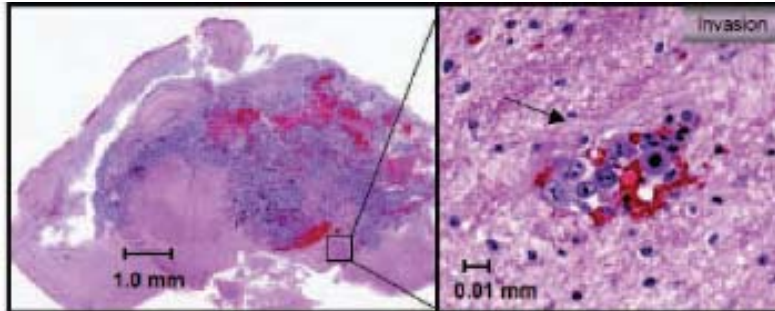


Fig. C.20 **A.** Tumors were induced by injection of oncogene PEI/DNA complexes into the lateral ventricle of neonatal mice. This is a co-transfection of four plasmids (250 ng each, 2 μ l volume). Grey arrows mark the transposon termini, similar to a retroviral LTR. SB transposase gene is encoded on the Luc vector to facilitate integration. **B.** Survival of mice from **A.** **C.** Mice were imaged to detect luciferase expression as a measure of tumor burden. **D.** Luciferin was injected to mark tumor growth via bioimaging.



John Ohlfest,
Brain Tumor Program, U of MN

Characterization of the NRAS/shP53/EGFRvIII model

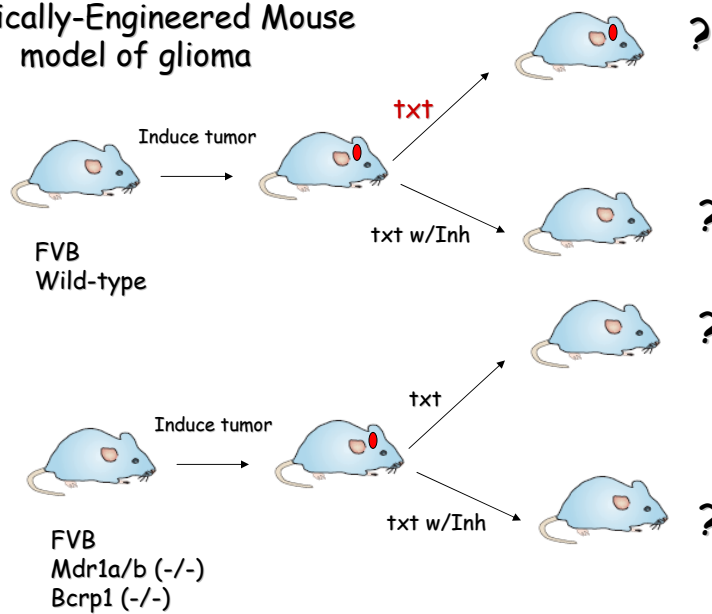


Large tumor in the right hemisphere invading the left hemisphere

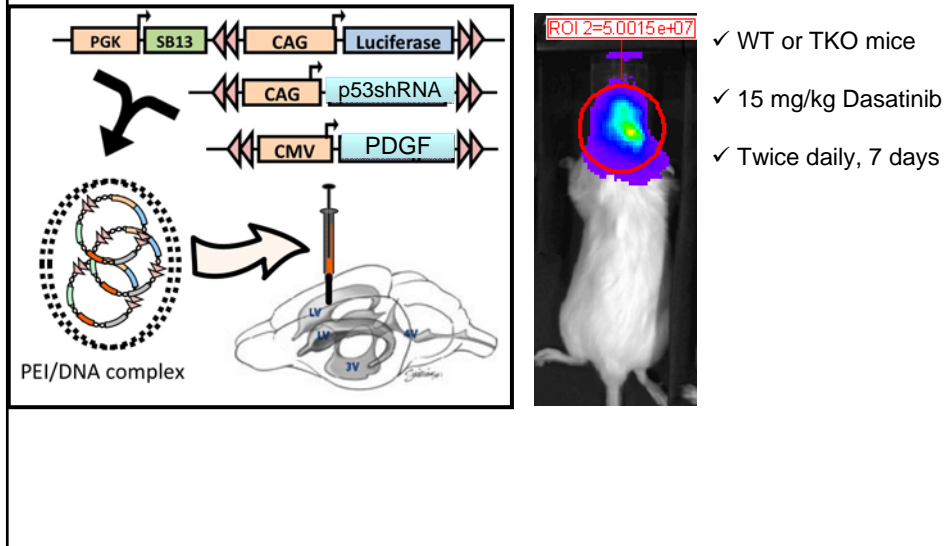
20x increase magnification showing infiltrating tumor in normal brain

John Ohlfest, Brain Tumor Program, U of MN

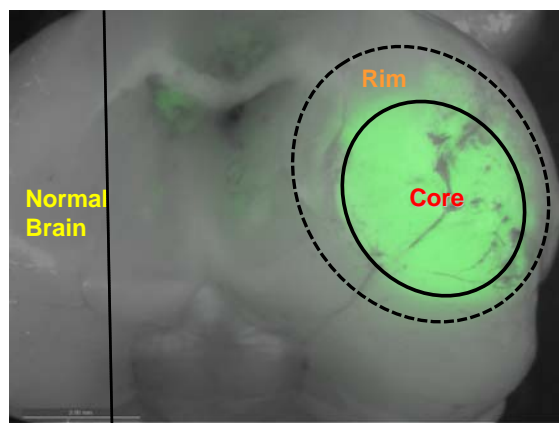
Genetically-Engineered Mouse model of glioma



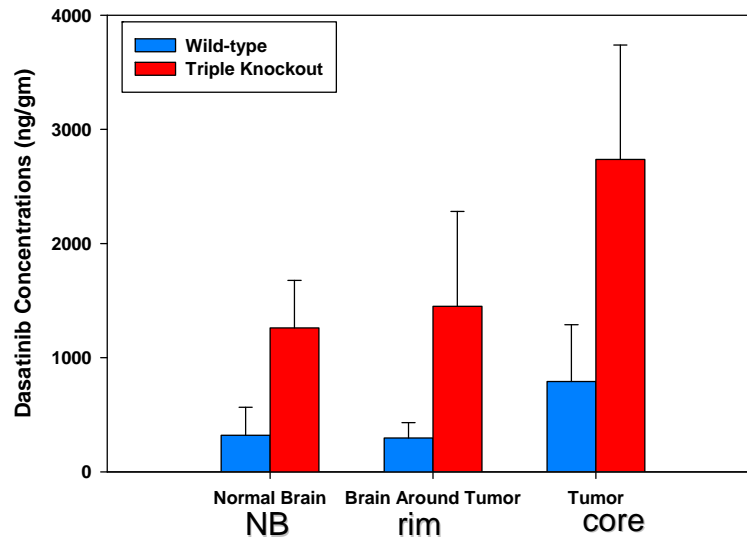
Experimental Design



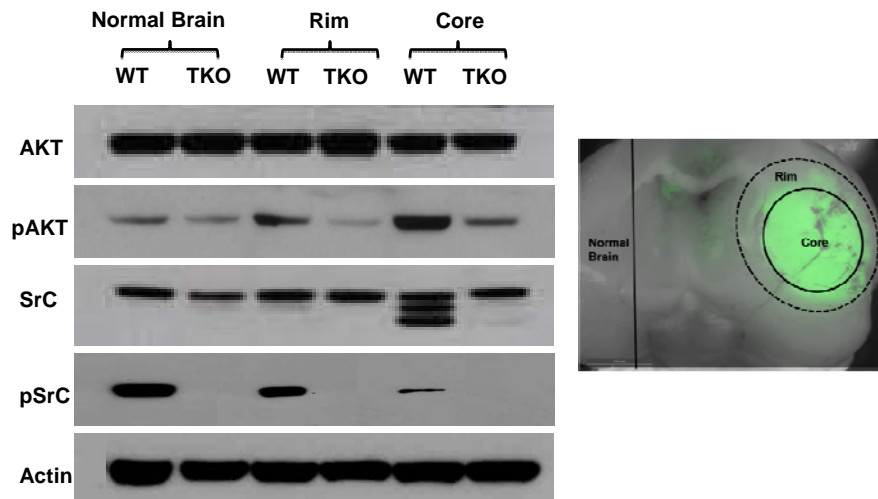
GFP-Guided Dissection Strategy



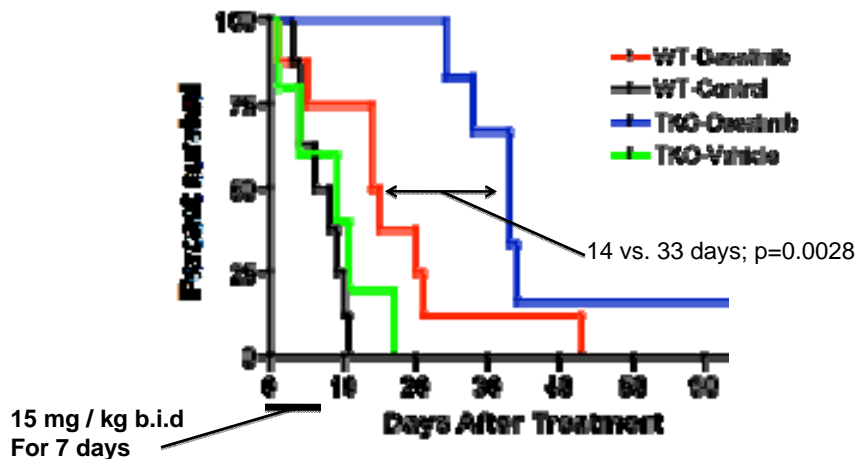
Dasatinib Regional Brain Distribution



Regional Effect on Signaling



Less Efflux = Superior Efficacy



Conclusions:

- 1) multiple mechanisms at multiple barriers may limit glioma treatment to invasive brain tumor stem cells (barrier 1 (BBB); barrier 2 (BTSC))
- 2) several "molecularly-targeted" drugs are substrates of critical transport systems that are in both barriers
- 3) "molecularly-targeted" drugs may be effective in glioma if delivery issues are overcome and allow personalized therapy depending on individual tumor
- 4) need dirty drug (s), sharp needle

Overview

Major Challenges (Opportunities) in Describing the Kinetics of Drug Distribution in the CNS (**systems biology to do list**)

1) limited knowledge of biochemical, anatomical, and physiological variables that influence drug transport and delivery in the CNS

(**to do: integrate locations and mechanisms**)

2) develop methods to measure time and space dependent changes in drug concentration in and around the target site

(**to do: use complementary existing technologies and strive to develop new measurement techniques**)

Overview

Major Challenges (Opportunities) in Describing the Kinetics of Drug Distribution in the CNS (**to do list**) *continued*

3) design appropriate experimental and mathematical models that incorporate critical transport or transformation mechanisms, allowing predictions of concentration-time and space profiles leading to the site of action

(**to do: make correlations between animal models and human application**)

4) incorporate pharmacokinetic and pharmacodynamic information to help design and implement more effect treatments for CNS diseases

(**to do: educate the many disciplines involved in the discovery, development and eventual use of new treatments**)

Acknowledgements

Graduate Students:

Sagar Agarwal, Ying Chen, Haiqing Dai,
Tianli Wang, Li Li, Ramola Sane

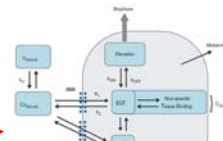
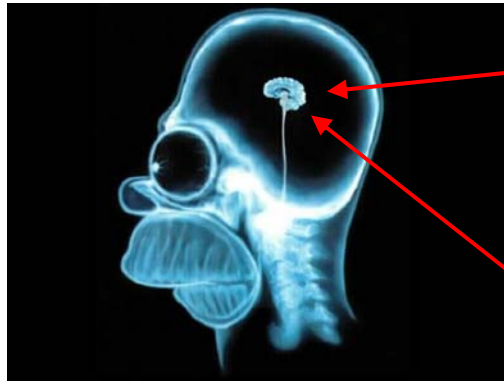
Collaborators:

John Ohlfest and group (Jose, Belle; Pediatrics and
Neurosurgery, UMN)
Mike Vogelbaum, Cleveland Clinic

Funding:

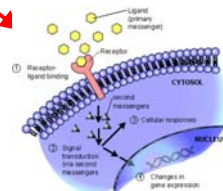
NIH-NCI, Leukemia Research Fund, BMS, Novartis,
Childrens Cancer Research Fund-Minneapolis, MN,
Brain Tumor Program-UMn, Masonic Cancer Center-
UMn, Academic Health Center-UMn

Consilience: Beware of the simplified boxes.



Empiric "Black-Box"
Pharmacokinetics

Systems Biology



Mechanistic "Molecular"
Pharmacokinetics

Make things as simple as possible,
but not simpler.

Albert Einstein