

Protein-kinase Inhibitors and Transporters

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Protein tyrosine kinases are a popular target in oncology drug development. These proteins are upregulated in tumor cells, playing a role in the development and maintenance of the tumor, making them an ideal target for selective tumor therapy. In the recent years 8 protein kinase inhibitors (PKIs) have been approved for the treatment of various cancers, and many more are in the drug development pipeline.

In a recent review by van Erp et al (Cancer Treatment Reviews, 2009) the ADME properties of these marketed PKIs have been summarized [1]. These compounds reach their maximum plasma level fast and are extensively distributed. They are primarily metabolized by CYP3As and are secreted via the feces. All PKIs were shown to interact with ABC transporters, more specifically MDR1 (ABCB1, P-gp) and BCRP (ABCG2, MXR). Many PKIs are substrates of one or both of these transporter proteins, which may largely influence their ADME properties and also efficacy.

ABC transporter proteins are using the energy of ATP to transport molecules across the plasma membrane even against a concentration gradient. Transporters are localized at the main pharmacological barriers where they can modify the ADME properties of xenobiotics. MDR1 and BCRP are also commonly expressed in cancer cells, with their expression levels increasing during drug treatment. By lowering the effective intracellular concentration of drugs MDR1 and BCRP are able to decrease the efficacy of the

treatment. **Testing your PKIs for MDR1 and BCRP interactions is important for ensuring that the compound will not be the victim of drug resistance and it reaches the target tissue, or alternatively it will not cause unwanted side effects.**

In the last two years several publications focused on the interaction between PKIs and ABC transporters (a list of references can be found below). A commonly used assay system is determination of in vivo pharmacokinetics in knockout mice (*mdr1a* *-/-*, *mdr1b* *-/-* and *bcrp* *-/-*) mostly focusing on brain penetration of the given PKI; in vitro monolayer assays using MDR1 and BCRP transfected cells are also utilized to confirm the findings in rodents for the human protein [2-4]. In all investigated cases deletion of one or all ABC transporter at the site of the blood-brain barrier dramatically increased the brain penetration of the drug. In humans this would translate to DDI and may manifest in unwanted adverse effects and toxicity, or in other cases the presence and activity of ABC transporters may hinder brain penetration, thus decrease efficacy of the drug.

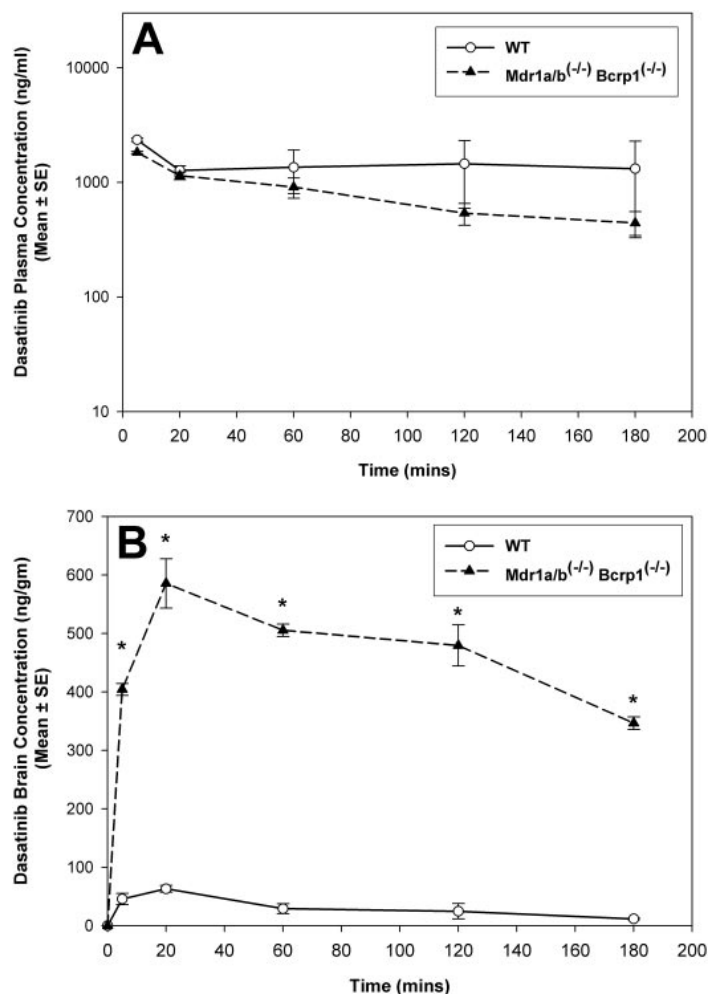


Figure 1: “P-glycoprotein and breast cancer resistance protein influence brain distribution of dasatinib.” Chen Y, Agarwal S, Shaik NM, Chen C, Yang Z, Elmquist WF. J Pharmacol Exp Ther. 2009 Sep;330(3):956-63.

At SOLVO Biotechnology, we utilized in vitro assays to evaluate the interaction between marketed PKIs and ABC transporters, MDR1 and BCRP. The dye extrusion assays, the Calcein and the Hoeschst assays can be used to pre-screen the compounds for transporter interaction. All studied PKIs inhibited the

dye efflux from the cell in a dose-dependent manner. The ATPase assay was then used successfully to identify substrates of the above transporters among the studied molecules (EC₅₀ determined = transporter activation). Inhibitors were also identified (IC₅₀ determined = transporter inhibition).

	MDR1 ATPase		MDR1 Calcein
	Activation <i>EC₅₀</i> (max%)	Inhibition <i>IC₅₀</i> (max%)	Inhibition <i>IC₅₀</i> (max%)
Erlotinib	0.83 μ M (100%)	-	4.0 μ M (120%)*
Gefitinib	0.75 μ M (100%)	-	6.4 μ M (100%)
Lapatinib	-	1.45 μ M (100%)	0.4 μ M (145%)*
Nilotinib	-	0.42 μ M (100%)	0.26 μ M (125%)*
Sorafenib	-	4.35 μ M (90%)	1.8 μ M (50%)
Sunitinib	11.5 μ M (50%)	30.8 μ M (80%)	- (65% at highest concentration)

	BCRP ATPase		BCRP Hoechst
	Activation <i>EC₅₀</i> (max%)	Inhibition <i>IC₅₀</i> (max%)	Inhibition <i>IC₅₀</i> (max%)
Erlotinib	0.015 μ M (100%)	-	0.29 μ M (120%)*
Gefitinib	0.22 μ M (50%)	-	0.36 μ M (95%)
Lapatinib	-	0.11 μ M (100%)	0.22 μ M (130%)*
Nilotinib	-	0.08 μ M (100%)	0.05 μ M (120%)*
Sorafenib	-	0.06 μ M (100%)	0.08 μ M (80%)
Sunitinib	-	2.15 μ M (100%)	0.82 μ M (110%)*

* - higher than 100% inhibition is achieved on a relative scale where 100% inhibition represents the effect of 60 μ M Verapamil (MDR1) or 1 μ M Ko134 (BCRP), respectively.

SOLVO Biotechnology offers you a range of assays for testing the interaction of Kinase Inhibitors with transporters. These Assays are designed for identifying substrates and inhibitors of MDR1 and BCRP, to help you to get the answers you need regarding you PKI.

The recommended assays include in vitro and in vivo systems focusing on human and rodent transporters:

- **Membrane assays**
 - ATPase assay (BCRP, MDR1)
 - Vesicular transport assay (BCRP, MDR1)
- **Cellular assays**
 - Dye extrusion assay (BCRP, MDR1)
 - MDCKII Monolayer assay (BCRP, MDR1)
 - Rat Brain Endothelial Cell monolayer assay (Mdr1a), Bcrp1 assay is under development)
- **Rat in vivo studies (for CNS indications/adverse effect evaluation)**
 - Brain microdialysis (Bcrp1, Mdr1a with specific inhibitors)

References

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