TACONIC
Models For Life.

# Bcrp Targeted Mutation Mice

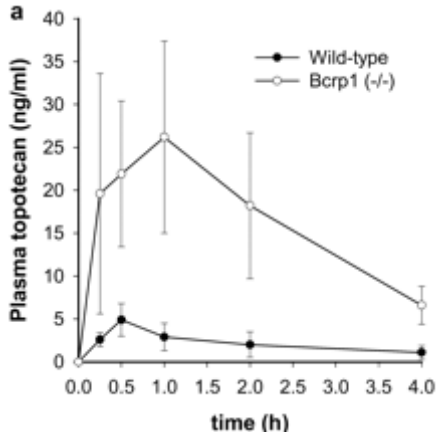
***Disruption of the Abcg2 gene provides a mouse model with impaired cellular secretion of anticancer agents and PhIP with a corresponding sensitivity to these compounds.***

## Applications for the Bcrp Mouse Targeted Mutation Model

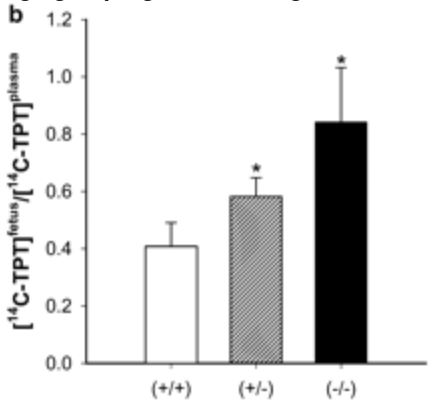
The Bcrp Targeted Mutation Mouse model lacks a normal mechanism by which cells export drugs and toxins. This model carries a disruption in the endogenous gene *Abcg2*, a member of the ATP-binding cassette (ABC) family of drug transporters, formerly referred to as *Bcrp*, known to transport anticancer drugs such as doxorubicin, mitoxantrone, topotecan, and daunorubicin, as well as known toxins including pheophorbide-a and 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP). Without this transport pathway, Bcrp Targeted Mutation Mice have compromised cellular excretion of certain substrates, and lactating females do not excrete normally concentrated substances in the breast milk. Potential applications of this model include:

- **Drug transport** Studies of *in vivo* drug disposition and definition of excretion pathways of compounds
- **Oral Bioavailability** Study the pharmacological activity associated with the use of transporter inhibitors
- **Teratology** Evaluate the barrier function of BCRP in placental transport and susceptibility to chemically-induced birth defects
- **Pharmacokinetics** Establish the principal contributions of BCRP transport to the pharmacokinetics of drugs of interest
- Investigation of the effect of BCRP action on the concentration in breast milk of drugs and pesticides applied during lactation for cows and humans
- Discovery of additional transported substrates for BCRP
- Exploration of the cellular transport mechanisms for anticancer drugs and other agents known to be excreted by BCRP
- Identification of tissue-specific patterns of BCRP-dependent transport
- Characterization of pheophorbide-a transportation and resulting light sensitivity
- Exploration of the effects of the inhibition of drug transport upon uptake and distribution of drugs and dietary and environmental toxins
- Elucidation of structure-function relationships of BCRP proteins by gene replacement on the BCRP null background

**Plasma Concentration vs. Time after oral administration of 1 mg/kg topotecan**




**Ratio of [$^{14}$C] topotecan concentration in fetus over maternal plasma at 15 min after i.v. administration of 0.2 mg/kg to pregnant dams, gestation day 15.5**




**Reprinted with permission.** Jonker *et al*. (**2002**). *The Breast Cancer Resistance Protein Protects Against a Major Chlorophyll-derived Dietary Phototoxin and Protoporphyria.* **Proc. Natl. Acad. Sci. USA** 99(24):15649-654. Copyright (2002) National Academy of Sciences, U.S.A.

## Characteristics of the Bcrp Targeted Mutation Mouse (Model 002767)[1,2]

- Homozygous for disruption of the *Abcg2* gene
- Lacks functional murine Bcrp1 protein and mRNA
- Normal fertility, life spans, and body weights, with no obvious phenotypic aberrations under standard housing conditions

- Upon exposure to pheophorbide-a, a chlorophyll-breakdown product, animals develop phototoxicity
- Animals develop a unique protoporphyria, with 10-fold higher erythrocyte levels of Protoporphyrin IX
- Pharmacokinetics affected for compounds known to be transported by Bcrp1, such as PhIP and topotecan. In general, bioavailability is increased, plasma clearance is decreased and hence tissue levels of transported compounds are markedly altered compared to wild type

## Scientific Profile of the Bcrp Targeted Mutation Mouse

**The genetic mutation of the Bcrp Targeted Mutation Mouse renders them unable to produce murine Breast Cancer Resistance Protein (Bcrp1), an important transmembrane transporter for a variety of compounds.** Bcrp1, and its human homolog BCRP, is expressed in strategic sites key to the regulation of distribution of compounds, including the small intestines, liver, kidney, blood vessels, and placenta.[1,3] BCRP is a member of the ATP-biding cassette family of drug transporters with affinities for several anti-cancer drugs. Both the human and mouse homologues transport a number of compounds including the anti-cancer drugs doxorubicin, mitoxantrone, topotecan, and daunorubicin[3] as well as environmental toxins such as pheophorbide-a[1] and PhIP[2]. In healthy mice and humans this mode of secretion offers protection from environmental and dietary toxins; in cancer patients this excretion activity renders a diversity of drugs less potent and less bioavailable.

**Without functional Bcrp1, mice show marked changes in oral availability and fetal accumulation of known transported substances such as Topotecan and PhIP.** In Bcrp Targeted Mutation Mice, oral availability of topotecan was increased ~6 fold higher and fetal accumulation of topotecan was ~2 fold higher than wild type, indicating a role for BCRP limiting intestinal uptake and placental penetration of certain substances.[1] BCRP has also been shown to efficiently transport PhIP, a potent mutagen found in protein-containing foods. In Bcrp Targeted Mutation Mice, accumulation of orally administered PhIP is 2.9 fold higher in the plasma and kidneys, with excretion shifted from fecal as in wild type to mainly urinary in Bcrp Targeted Mutation Mice.[2]

**Bcrp Targeted Mutation Mice become dramatically hypersensitive to light exposure with oral administration of the dietary chlorophyll breakdown product, pheophorbide-a.**[1] BCRP is efficient in limiting the uptake of pheophorbide-a from ingested food by preventing its absorption in the intestines and possibly directing its excretion via the liver and kidneys. Upon oral administration of pheophorbide-a at doses as low as 2 mg/kg/day and fluorescent light exposure, Bcrp Targeted Mutation Mice developed ear lesions within 2 days, severe edema of the head within 3 days, and became moribund, compared to wild type mice that never developed skin lesions with oral administration of pheophorbide-a doses up to 200 mg/kg/day and light exposure.

**Bcrp Targeted Mutation Mice develop a unique protoporphyria.**[1] Erythrocyte levels of Protoporphyrin IX (PPIX) were increased 10-fold in Bcrp Targeted Mutation Mice compared to wild type mice, regardless of diet. This is a previously unknown type of protoporphyria, as it is not caused by a genetic defect in the heme biosynthetic pathway like all other characterized protoporphyrias. This protoporphyria can be cured by a bone marrow transplant from wild type mice, indicating that the protoporphyria does not depend on Bcrp1 activity elsewhere in the body. Transplant of bone marrow from wild type mice decreases the photosensitivity of these animals, indicating that PPIX may contribute to the photosensitivity of the Bcrp Targeted Mutation Mice.

**Bcrp is expressed in the mammary glands of lactating mice, cows, and humans, and is implicated in concentrating of toxicologically important substrates in breast milk.**[4] A comparison of Bcrp Targeted Mutation Mice and Wild Type mice showed that while the i.v. administered substrates PhIP and topotecan were highly concentrated in the milk of wild type mice, the active milk secretion was abolished in Bcrp Targeted Mutation Animals. Elucidation of this function is important in understanding the role Bcrp plays in the excretion of drugs and pesticides that are applied during lactation in humans and cows.

## Origin of the Model

The *Abcg2* gene was disrupted in Bcrp Targeted Mutation Mice by homologous recombination in the laboratory of Alfred H. Schinkel and colleagues at the Netherlands Cancer Institute.[1] A 1.8-kb targeting construct containing *pgk-hygromycin* replaced a 5.1-kb fragment containing exons 3-6 encoding most of the ATP-binding domain in reverse-transcription orientation in E14 embryonic stem cells derived from the 129P2/OlaHsd strain mice.

Chimeric mice were generated by microinjection of two independently targeted embryonic stem cell clones into blastocysts. This gave rise to chimeric mice that transmitted the disrupted *Abcg2* allele to F1 offspring.

No Bcrp1 protein or mRNA was detected through Western and Northern blotting in homozygous mice.

The mice came to Taconic in July 2003 from the Schinkel lab. The mice were backcrossed to FVB/N mice for 7 generations prior to arrival at Taconic. At Taconic the line was embryo transfer derived using homozygous males and females. The foundation colony is maintained through homozygous brother x sister matings in a plastic film isolator. The production colony is maintained in the MPF™ (Murine Pathogen Free) Isolator Barrier Unit.

## Ready for Your Experiments

Taconic's Mouse Models are produced in Isolator Barrier Unit (IBU™) facilities. Mice are shipped in Taconic Transport Cages (TTC™) and come with an up-to-date health report documenting their Murine Pathogen Free (MPF™) health status. Barrier housing conditions are recommended for maintenance of Bcrp Targeted Mutation Mice.

## Related Mouse Models from Taconic

Taconic provides a number of mouse models relevant to drug transport. Call or fax to inquire about the following additional models:

- **HRN Mouse (models 007293 and 007353)** – carries a liver-specific deletion of the *Por* gene, resulting in a mouse that lacks hepatic cytochrome P450 activity.
- **Mdr1a Targeted Mutation Mouse (model MDR1A)** – carrying a disrupted *Abcb1a* gene, a multi-drug resistance-associated transport protein, conferring a deficiency in the blood-brain barrier.
- **Mdr1a/b Targeted Mutation Mouse (model 001487)** – carrying disruptions of two genes, *Abcb1a* and *Abcb1b* and lacking cellular transport mechanisms by their two multi-drug resistance-associated protein products, conferring a deficiency in the blood-brain barrier.
- **Mdr1a/b-Bcrp Targeted Mutation Mouse (model 003998)** – carries disruptions of three genes; *Abcb1a,Abcb1b,* and *Abcg2*, that encode for three drug-extruding transporters.
- **Mrp1 Targeted Mutation Mouse (model 001486)** – carrying a disruption of the *Abcc1a* (multi-drug resistant associated protein gene), an ATP dependent drug-extruding transporter.
- **Mrp2 Targeted Mutation Mouse (model 006621)** – carries a disruption of the *Abcc2* gene, which encodes the multidrug resistance protein 2.
- **Oct1/2 Targeted Mutation Mouse (model 006622)** – carrying a disruption of the *Slc22a1* and *Slc22a2* genes, which encode the organic cation transporters 1 and 2.

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3. Allen *et al*. (**2002**) *Multidrug Resistance and Pharmacological Protection Mediated by the Breast Cancer Resistance Protein (BCRP/ABCG2).* **Mol. Cancer Ther.** 1: 427-434.

4. Jonker *et al*. (**2005**) *The Breast Cancer Resistance Protein BCRP (ABCG2) Concentrates Drugs and Carcinogenic Xenotoxins into Milk.* **Nature Med.** 11(2): 127-129.

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**Rev. 3/08**

# Taconic Transgenic Models
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