Mrp1 Targeted Mutation Mice

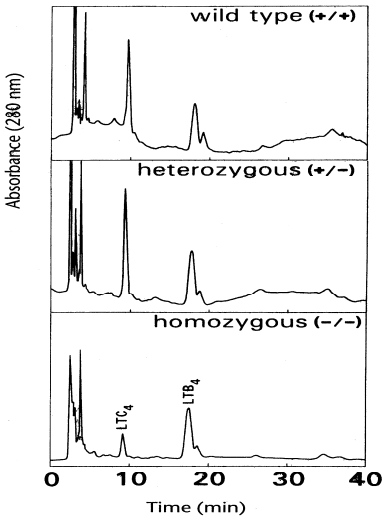
Disruption of the Abcc1a gene provides a mouse model with impaired cellular secretion of anticancer agents and of leukotriene C_4, with a corresponding sensitivity to cytotoxic drugs and a muted inflammatory response

Applications for the Mrp1 Targeted Mutation Mouse Model

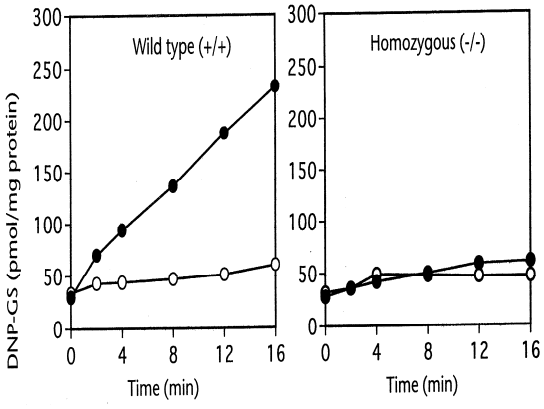
The Mrp1 Targeted Mutation Mouse model lacks a normal mechanism by which cells excrete a variety of substrates after their conjugation to glutathione, glucuronide, or sulfate. Specifically, *Abcc1a* encodes the protein, multi-drug resistance-associated protein (MRP1), which transports a selection of endogenous substrates (e.g., steroids, leukotrienes) as well as xenobiotic drugs (e.g., anticancer agents) out of cells. Without this transport pathway, Mrp1 Targeted Mutation Mice have a compromised cellular excretion of certain substrates. Potential applications of the model include:

- Exploring cellular transport mechanisms for anticancer drugs and other agents known to be excreted by MRP1
- Discovery of additional transported substrates for MRP1
- Defining excretion pathways (e.g., MRP1 versus P-glycoprotein) for xenobiotics and endogenous metabolites in normal and in cancer cells
- Defining co-transport requirements in the ATP-dependent excretion of molecules through MRP1 mechanisms
- Identification of tissue-specific patterns of MRP1-dependent transport
- Discovery and design of anticancer drugs that circumvent MRP1 transport
- Exploration of possible non-transport roles of MRP1 in anticancer drug sensitivity
- Characterization of leukotriene transport and metabolism in cells implicated in the inflammatory response
- Refinement of tissue-specific roles of leukotrienes and other endogenous MRP1-transported products in non-cancerous cells
- Elucidation of structure-function relationships of MRP1 proteins by gene replacement on the MRP1 null background

HPLC Analysis of Leukotriene Products Excreted from Bone Marrow-Derived Mast Cells of Wild-type and Mrp1 Targeted Mutation Mice (homozygous -/-)



Transport of 2,4-dinitrophenyl S-glutathione (DNP-GS) Across Erythrocyte Membrane Vesicles in Wild-type and Mrp1 Targeted Mutation Mice (homozygous -/-)



Above figures reproduced with permission from Wijnholds J., *et al.* (1997) **Increased Sensitivity to Anticancer Drugs and Decreased Inflammatory Response in Mice Lacking the Multidrug Resistance-Associated Protein.** *Nature Medicine* . 3:1275-9

Features of the Mrp1 Targeted Mutation Mouse (model 001486) [1,2]

- Homozygous for disruption of the *Abcc1a gene*
- Lack of functional MRP1 protein in a variety of tissues
- Normal fertility and viability of mice up to one year, with no gross physical or physiological manifestations
- Dramatic reduction (95%) in ATP-dependent cellular transport of representative glutathione-conjugated substrates (assessed in erythrocyte-derived membrane vesicles)
- Reduced excretion and increased intracellular accumulation of leukotriene C_4, an MRP1 substrate, by cultured mast cells derived from bone marrow
- Reduced localized inflammation in homozygous mice after topical application of leukotriene precursor (arachidonic acid)
- Persistence of wild-type inflammatory responses with injection of leukotriene C_4 or phorbol ester
- Sensitivity of bone marrow-derived mast cells to the anticancer drugs etoposide and vincristine, but not cisplatin
- Hypersensitivity of mice specifically to etoposide, resulting in tissue damage, body weight drop, and death on exposure to that drug at doses not fatal to wild type mice

Scientific Profile of the Mrp1 Targeted Mutation Mouse Model

The genetic mutation of Mrp1 Targeted Mutation Mice has rendered them unable to produce multi-drug resistance-associated protein (MRP1), an important transmembrane transporter for a variety of endogenous and exogenous compounds. The ability of cells to secrete certain lipophillic cellular products or xenobiotic compounds has been found to involve association with membrane proteins known as multispecific organic anion transporters (MOATs) or glutathione-conjugate (GS-X) pumps, such as MRP1s and P-glycoproteins (Pgps). MRP1s are a family of related, ATP-dependent transport proteins with affinities for several anticancer drugs and for compounds that are conjugated in the cell to glutathione, glucuronide, or sulfate. This mode of secretion has been demonstrated in healthy murine and human cells for certain leukotrienes, steroids, and bile salt derivatives; in cancer cells this excretion activity renders a diversity of drugs less potent.

In Mrp1 Targeted Mutation Mice, a targeted disruption of the *Abcc1a* gene eliminates the production of MRP1, but does not influence Pgp synthesis.[1] As a result of the loss of the MRP1 mechanism, alterations in transport of known substrates are seen, and additional MRP1 substrates can be sought. For example, glutathione-conjugated leukotriene C_4 is a pro-inflammatory cytokine released by mast cells that is known to be an important substrate of MRP1; the secretion of this product is diminished to about a fourth of normal levels in cultured bone marrow mast cells of Mrp1 Targeted Mutation Mice.[1] Correspondingly, intracellular concentrations of leukotriene are elevated. In erythrocyte membranes, transport of glutathione-conjugated ethacrynic acid and of 2,4-dinitrophenyl S-glutathione is dramatically reduced.[1]

The reduced cellular secretion of leukotriene C_4 observed *in vitro* may underlie a reduced *in vivo* inflammatory response of Mrp1 Targeted Mutation Mice. Mice that were experimentally exposed to topical arachidonic acid, a precursor of leukotrienes and prostaglandins, had a diminished inflammatory response (edema and vascular permeability) compared to wild-type mice.[1] Prostaglandin synthesis was artificially blocked in these experiments, leaving leukotriene synthesis and secretion as the primary mode of mast cell-mediated inflammation. This dampened inflammatory reaction in Mrp1 Targeted Mutation Mice awaits further characterization. The mice may be a suitable model for defining relative contributions to the inflammatory response of leukotrienes and other chemical mediators. Of particular note are mediators released by platelet activating factor (PAF) since Mrp1 Targeted Mutation Mice still were susceptible to lethal anaphylactic shock on exposure to PAF.[1]

Elimination of MRP1-mediated transport alters the ability of cells to excrete xenobiotic drugs, such as anticancer drugs. Indeed, MRP1 is believed to be responsible in large part (along with other transporters) for multi-drug resistance of cancer cells. MRP1 appears able to transport a diversity of drugs and eliminate them from the cytoplasm of intended cellular targets. Bone marrow-derived mast cells of Mrp1 Targeted Mutation Mice responded to the anticancer drugs, etoposide and vincristine (but not cisplatin), by an inhibition of growth in excess of that induced in wild-type cells. Ratios of surviving cells after drug treatment *in vitro*, in wild-type *versus* Mrp1 Targeted Mutation-derived mast cells, respectively, were 4.2:1 with etoposide and 2.4:1 with vincristine.[1]

A marked hypersensitivity to etoposide has been documented for Mrp1 Targeted Mutation mice. Exposure of 11-week to 13-week-old mice to etoposide (but not to vincristine) resulted in death, although the precise mechanism is unknown.[1] A separate study evaluating the anatomical and physiological changes correlated with etoposide toxicity in Mrp1 Targeted Mutation Mice revealed several specific alterations: hypotonic polyuria, damage to the oropharyngeal mucosa, localized alopecia, reduced leukocyte count by 7 days post-exposure, and lower testicular weight with abnormal spermatogenesis.[2] Each of these manifestations was absent or minimal in wild-type mice

and in drug-naïve mutant mice. This may be caused by excessive drug accumulation in mutant cells which, in wild-type mice, would normally depend on MRP1 transport to eliminate the drug. Tissues not damaged by etoposide presumably rely on non-MRP1 transporter mechanisms, or are not targets for the drug. The exact cause of death in etoposide-treated mutant mice remains undetermined.[2]

Origin of the Model

The *Abcc1a gene* was disrupted in the Mrp1 Targeted Mutation Mice by homologous recombination in the laboratory of Jan Wijnholds, Piet Borst, and colleagues at the Netherlands Cancer Institute. [1] A targeting construct containing a *hygromycin* cassette was introduced into E14 embryonic stem cells derived from 129/Ola strain mice. Homologous recombination resulted in a mutant allele lacking the coding region for amino acids in the first ATP-binding domain of the MRP1 protein.

Two stem-cell clones containing the disrupted allele were injected into 129/Ola blastocysts and gave rise to chimeric mice that transmitted the disrupted *Abcc1a* allele to F_1 offspring. Although the mutant allele was transcribed in some tissues, no MRP1 protein was detected.

The mice came to Taconic in October 1999 from the Borst Lab. The mice were backcrossed to FVB/N mice for six generations before arriving at Taconic. The mice were backcrossed to FVB/NTac for six additional generations (N12) and embryo transfer derived using heterozygous males and FVB/NTac females. The derived heterozygous were intercrossed to produce a homozygous colony. The foundation colony is maintained through homozygous brother x sister matings in a plastic film isolator. The production colony is maintained in a 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 Mrp1 Targeted Mutation Mice.

Related Mouse Models from Taconic

Taconic provides a number of mouse models relevant to cancer research and inflammatory responses, including mutational, targeted mutation, and microinjected models on a variety of backgrounds. Call or fax to inquire about the following additional models:

- **Bcrp Targeted Mutation Mouse (model 002767)** 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
- **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; useful in neurotoxicology and in studies of drug design, cellular transport and testing
- **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; useful in neurotoxicology and in studies of drug design, cellular transport and testing
- **Mdr1a/b-Bcrp Targeted Mutation Mouse (model 003998)** carries disruptions of three genes; *Abcb1a*,*Abcb1b*, and *Abcg2*, that incode for three drug-extruding transporters.
- **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.

References Cited:

1. Wijnholds J., Evers R., van Leusden M.R., Mol C.A.A.M., Zaman G.J.R., Mayer U., Beijnen J.H., van der Valk M., Krimpenfort P., Borst P. (1997) **Increased Sensitivity to Anticancer Drugs and Decreased Inflammatory Response in Mice Lacking the Multidrug Resistance-associated Protein.** *Nature Medicine*,. 3:1275-1279.
2. Wijnholds J., Scheffer G.L., van der Valk M., van der Valk P., Beijnen J.H., Scheper R.J., Borst P. (1998) **Multidrug Resistance Protein 1 Protects the Oropharyngeal Mucosal Layer and the Testicular Tubules Against Drug-induced Damage.** *Journal of Experimental Medicine*. 188:797-808.
3. Wijnholds J., de Lange, E.C.M., Scheffer, G.L., van den Berg, D-J., Mol. C.A.A.M., van der Valk, M., Schinkel, A.H., Scheper R.J., Breimer, D.D., Borst, P. (2000) **Multidrug Resistance Protein 1 Protects the Choroid Plexus Epithelium and Contributes to the Blood-Cerebrospinal Fluid Barrier.** *Journal of Clinical Investigation*. 105(3): 279-285.

Every Taconic Transgenic Model™ carries a label license granting you a license under Taconic's in-licensed patent right(s) to use the model in your research. TTM™s are produced and distributed under rights to patents that Taconic has licensed from various institutions, including exclusive distribution rights to Positive Negative Selection and Isogenic DNA gene targeting technologies. Taconic is the only commercial breeder that can supply transgenic models with these licenses for use in your research.

Conditions of Use for Taconic Transgenic Models™

TACONIC TRANSGENIC MODELS™ ("MODELS") are produced and distributed under rights to patents and intellectual property licensed from various institutions. Taconic grants to each purchaser a right under Taconic's rights in such licensed patents and intellectual property to use the purchased MODEL in consideration of purchasers' acknowledgement of and agreement to the Terms and Conditions of Sale and the following terms of use:

- Title to these MODELS and biological materials derived from them remains WITH TACONIC FARMS, INC.
- The MODELS will be used for research purposes only.
- The MODELS will not be bred except to obtain embryos or fetuses required for research purposes.
- The MODELS and biological materials derived from them will not be distributed to third parties or used for commercial purposes.

For more information or to place an order contact:

TACONIC
1 Discovery Drive, Suite 304
Rensselaer, NY 12144
Toll Free: 1-888-TACONIC
Phone: 518-537-6208
Fax: 518-537-7287
e-mail: custserv@taconic.com
Internet: http://www.taconic.com

in Europe: Taconic Europe
Bomholtvej 10 P.O. Box 39
DK 8680 Ry DENMARK
Phone: +45 70 23 04 05
Fax: +45 86 84 16 99
e-mail: TaconicEurope@taconic.com
Internet: http://www.taconic.com

in Japan: CLEA Japan, Inc.
Phone: 03-5704-7063
Fax: 03-3792-5298
e-mail: ad-import@clea-japan.com
Internet: http://clea-japan.com

Rev. 3/08

Taconic Transgenic Models
Publication Reference List
Mrp1 Targeted Mutation Mice

Allen, J.D., Brinkhuis, R.F., van Deemter, L., Wijnholds, J., Schinkel, A.H. (2000) **Extensive Contribution of the Multidrug Transporters P-Glycoprotein and Mrp11 to Basal Drug Resistance,** *Cancer Res.* 60:5761-5766.

Honig SM, Fu S, Mao X, Yopp A, Gunn MD, Randolph GJ, Bromberg JS. (2003) **FTY720 stimulates multidrug transporter? and cysteinyl leukotriene?dependent T cell chemotaxis to lymph nodes;** *J Clin Invest*, 111(5):627-37.

Lorico, A., Nesland, J., Emilsen, E., Fodstad, O., Rappa, G. (2002) **Role of the Multidrug Resistance Protein 1 Gene in the Carcinogenicity of Aflatoxin B1: Investigations Using Mrp11-Null Mice,** *Toxicology.* 171:201-205.

Robbiani DF, Finch RA, Jager D, Muller WA, Sartorelli AC, Randolph GJ. (2000) **The Leukotriene C4 Transporter MRP1 Regulates CCL19 (MIP-3â, ELC)-Dependent Mobilization of Dendritic Cells to Lymph Nodes.** *Cell*, 103:757-68.

Schultz, M.J., Wijnholds, J., Peppelenbosch, M.P., Vervoordeldonk, M.J.B.M., Speelman, P., van Deventer, S.J.H., Borst, P., van der Poll, T., (2001) **Mice Lacking the Multidrug Resistance Protein 1 Are Resistant to *Streptococcus pneumoniae*-Induced Pneumonia[1],** *J Immunol*. 166:4059-4064.

Ten Hove, T., Drillenburg, P., Wijnholds, J., Te Velde, A.A., Van Deventer, S.J.H. (2002) **Differential Susceptibility of Multidrug Resistance Protein-1 Deficient Mice to DDS and TNBS-Induced Colitis,** *Dig Dis Sci.* 47(9):2056-2063.

Verbon, A., Leemans, J.C., Weijer, S., Florquin, S., Van Der Poll, T. (2002) **Mice Lacking the Multidrug Resistance Protein 1 Have a Transiently Impaired Immune Response During Tuberculosis,** *Clin Exp Immunol.* 130:32-36.

Wijnholds J., Evers R., van Leusden M.R., Mol C.A.A.M., Zaman G.J.R., Mayer U., Beijnen J.H., van der Valk M., Krimpenfort P., Borst P. (1997) **Increased Sensitivity to Anticancer Drugs and Decreased Inflammatory Response in Mice Lacking the Multidrug Resistance-associated Protein.** *Nat Med.* 3:1275-1279.

Wijnholds J., Scheffer G.L., van der Valk M., van der Valk P., Beijnen J.H., Scheper R.J., Borst P. (1998) **Multidrug Resistance Protein 1 Protects the Oropharyngeal Mucosal Layer and the Testicular Tubules Against Drug-induced Damage.** *J Exp Med.* 188:797-808.

Wijnholds J., de Lange, E.C.M., Scheffer, G.L., van den Berg, D-J., Mol. C.A.A.M., van der Valk, M., Schinkel, A.H., Scheper R.J., Breimer, D.D., Borst, P. (2000) **Multidrug Resistance Protein 1 Protects the Choroid Plexus Epithelium and Contributes to the Blood-Cerebrospinal Fluid Barrier.** *J Clin Invest.* 105:279-285.