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# Transfer of the *MDR1* Gene into Haematopoietic Cells

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## INTRODUCTION

THE MULTIDRUG resistance gene *MDR1* encodes a cell-membrane protein, Pgp, which acts as an energy-dependent pump able to extrude many compounds out of cells [1]. Pgp expression in a wide variety of human cancers results in pleiotropic resistance to anticancer drugs including anthracyclines, mitoxantrone, vinca alkaloids, epipodophyllotoxins, actinomycin D, and paclitaxel [1, 2]. In normal tissues, Pgp is expressed on the lumenal surface of epithelial cells of the liver, kidney, small and large intestines, and pancreatic ductules, in the adrenal cortex and steroid secreting glands of the endometrium, and on endothelial cells in testis and brain [1]. Its presence in these locations suggests that this transporter contributes to the excretion of xenobiotics or toxic endogenous substrates, to steroid secretion, and as a component of the blood–brain barrier [3]. Pgp is also expressed at low to moderate levels in haematopoietic cells [4–6] and its potential role in the protection of stem cells from toxic insults and as a transporter of cytokines, cytotoxic effector molecules, or inflammatory mediators in leucocytes has been discussed [7]. Whether this low normal *MDR1* expression results in an intrinsic multidrug resistance of Pgp-expressing haematopoietic cells remains unclear [8]. However, clinical myelosuppressive side-effects of chemotherapeutic agents, including Pgp-expelled cytotoxic drugs, clearly limits dose-intensification in the treatment of solid tumours.

Transfer of the *MDR1* gene into haematopoietic cells has been achieved *in vitro* and *in vivo* in animal models, resulting in protection from cytotoxic Pgp substrates in *MDR1*-expressing cells [9]. This suggests the potential use of *MDR1* to protect the bone marrow of patients undergoing high-dose chemotherapy for solid tumours [3]. Moreover, efficient gene therapy of genetic diseases of the haematopoietic system is limited by the low proportion of successfully transduced or transfected cells and the usual impossibility of selecting the targeted cells based on transgene expression. *MDR1* may allow selection of haematopoietic cells co-expressing an otherwise non-selectable transgene [3, 9].

In this review, the pattern of Pgp expression in normal bone marrow (BM) and peripheral blood (PB) haematopoietic cells, and *in vitro* and *in vivo* transfer experiments of the human *MDR1* gene into haematopoietic cells will be reviewed. Finally, the potential use and limitations of *MDR1* in gene therapy will be discussed.

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### *Physiological expression of the* MDR1 *gene in haematopoietic cells*

A lineage specific pattern of Pgp expression and function is observed for haematopoietic cells as assessed by immunostaining and/or RT-PCR for detection of *MDR1*-message RNA and/or the use of functional assays. These latter assays, based on measuring the efflux of a fluorescent Pgp substrate (e.g. rhodamine 123, Rho) are sensitive and useful assays for the detection of low levels of functional Pgp such as that seen in haematopoietic cells.

In normal human BM, Pgp expression correlates with CD34 antigen expression and cell clonogenic potential, both characteristic of progenitor cells [10]. Approximately 20% of isolated stem cells (CD34+) display low uptake of Rho corresponding to expression of functional Pgp. This subpopulation is thought to contain the most primitive cells, which are the origin of both myeloid and lymphoid cells, while precursor cells characterised by high Rho uptake might be more mature and limited to precursors of lymphoid cells [11, 12].

*MDR1* expression is down-regulated during normal myelopoiesis [13–15]. There is no difference between pluripotent progenitor cells (CD34+/HLADR−) and CD34+/HLADR+ subpopulations, but committed early and late myeloid precursors (CD33+/CD34+ and CD33+/CD34− cells) express lower levels of Pgp than earlier CD34+ cell populations [13, 15]. BM cells at a later myeloid differentiation stage and PB granulocytes are negative for cell-surface Pgp, fail to show functional Pgp [13, 14, 16–18], and contain no [15] or low levels of *MDR1* mRNA [16, 19]. The lack of measurable Rho transport and cell surface staining in granulocytes, in spite of a moderate *MDR1* mRNA level, suggests that there may be little or no Pgp in these cells on the plasma membrane [16]. BM monocytic cells are negative for cell-surface Pgp [9] and do not contain detectable amounts of *MDR1* mRNA [15] whereas PB monocytes show low levels of Pgp immunostaining [14, 17], low levels of *MDR1* mRNA and slight Rho efflux [15]. In erythroid progenitors (glycophorin A+), no clear evidence for *MDR1* expression has been obtained in PCR or Rho efflux studies [15] and no Pgp expression in the megakaryocytic lineage has been reported so far.

In contrast to myelopoiesis, maturation of normal B lymphocytes might be paralleled by upregulated *MDR1*

expression with low levels of *MDR1* mRNA in CD10+/CD19+ precursor B cells whereas CD10−/CD19+ B cells are highly positive for *MDR1* mRNA [15]. Mature PB T lymphocytes express Pgp [10, 13, 15, 17], with a higher expression level for NK cells (CD56+ natural killer cells) and T suppressor cells (CD8+), than for T helper cells (CD4+) and B lymphocytes (CD19+) [14, 15].

## TRANSFER AND EXPRESSION OF THE *MDR1* cDNA IN HAEMATOPOIETIC CELLS

Transgenic mice have been engineered by microinjecting fertilised eggs with expression vectors containing the *MDR1* cDNA [20, 21]. In some transgenic animals, constitutive expression of human Pgp was limited to BM with activation of the *MDR1* transgene probably occurring at a very early stage of BM differentiation, since cells of different lineages expressed the transporter. Expression levels were comparable with those seen in human cancers or normal epithelia which express Pgp, and resulted in protection from the myelotoxicity of Pgp-expelled drugs; several-fold higher doses of paclitaxel and daunorubicin could be administered safely to these animals compared with control mice [20–22]. No alteration of normal BM function was observed over a 2-year period [23].

Murine transplantation models provide additional evidence that *MDR1* can be transferred into normal haematopoietic progenitor cells and that the resulting expression of a functional human Pgp protects BM cells from the myelotoxicity of *MDR1* substrates and allows selection of targeted cells. First, human Pgp-expressing BM from *MDR1* transgenic mice was reinfused into lethally irradiated sensitive mice, which demonstrated a multidrug-resistance phenotype after engraftment [23]. Also, BM cells transduced with *MDR1* cDNA containing retroviral vectors *in vitro* were transplanted into irradiated sensitive mice. Subsequent administration of a cytotoxic Pgp substrate (paclitaxel) to recipient mice resulted in *in vivo* selection of chemoresistant BM, reflected in a substantial increase in the proportion of leucocytes containing the *MDR1* provirus [24, 25]. Similar experiments by other groups confirmed these results [26, 27]. Several lines of evidence suggest that stem cell transduction occurred [3]. First, cells of different haematopoietic lineages expressed Pgp as assessed by immunostaining [28] and analysis of proviral insertion in cells from thymus, PB granulocytes and BM of recipient mice suggested the same insert site, consistent with initial integration into a pluripotent stem cell precursor [24]. Further, long-term expression (4–8 months) [25, 28] of *MDR1* in transplanted animals and persistence of Pgp expression after serial transplantation from resistant to sensitive mice [26] suggest that very early precursor cells, the only ones with self-renewal capacity, were transduced. Targeting limited to more mature lineage committed progenitor cells would have resulted in transient gene expression, restricted to specific lineages. Cells mobilised from BM of splenectomised mice by the administration of haematopoietic growth factors [29] or cells from fetal liver (where normal haematopoiesis takes place at early stages of development) [30] have also been used as sources of haematopoietic cells for *MDR1* gene transfer experiments. Simian models have also been developed. CD34+ BM cells from Rhesus monkeys have been transduced *in vitro* with an amphotropic retrovirus and transplanted into irradiated monkeys [31]. In a preliminary report, one of the two recipient monkeys demonstrated relative protection against treatment-induced leucopenia and a 10-fold increase of transduced cells following repeated injections of docetaxel.

Human haematopoietic cells have been transduced *in vitro* using safety-modified recombinant *MDR1* retroviruses. In a recent study, transduction of selected BM CD34+ cells resulted in a 10% increase of Pgp expressing Rho dull cells [27]. Resistance to high doses of paclitaxel of transduced CD34+ cells and the presence of *MDR1* cDNA in erythroid and myeloid clones derived from transduced cells has been demonstrated [32]. An alternative to BM as a source of haematopoietic cells in humans is umbilical cord blood that is rich in progenitors and easily harvested without any deleterious consequences for the newborn [33]. Cord blood derived CD34+ cells might be more efficiently transduced than BM derived cells [34]. Haematopoietic progenitors mobilised by myeloablative chemotherapy and/or haematopoietic growth factor administration and harvested by cytapheresis during haematopoietic recovery are also suitable targets for gene transfer [35].

Retrovirus-mediated transduction of haematopoietic stem cells is difficult, mainly because most of these cells are not dividing *in vivo* which precludes provirus integration into the host cell genome [3]. The use of cells producing a high viral titre and the activation of stem cells by chemotherapeutic pretreatment of BM donors and by culture in the presence of growth factors and cytokines prior to transduction may improve the transduction efficiency in murine experiments [36, 37]. Coculture seems more efficient than the use of producer cell line supernatants for transduction procedures. In a recent study [28], gene transfer by coculture of murine sorted stem cells with producer cells resulted in expression of functional Pgp in more than 60% of stem cells. The benefit of stem cell isolation prior to transduction is unclear. For example, *MDR1* transfer efficiency after cocultivation in the presence of producer cells was not improved by CD34+ cell purification, whereas such isolation was found to be critical in order to obtain high level *MDR1* transfer and expression when supernatants of the same producer cells were used for transduction [34]. This may be due to the lower transduction efficiency using retroviral supernatants compared with cocultivation. In any case, the translation of this transduction technology to humans faces several logistic problems among which is the need to cocultivate a large volume of BM with the virus-producing cells prior to transplantation [34].

Other viral and non-viral gene delivery systems have been used to transfer the *MDR1* gene into sensitive haematopoietic cells. Murine haematopoietic progenitors can be transduced both *in vitro* and *in vivo* using recombinant adeno-associated virus 2 (AAV) particles [38, 39] and AAV-mediated transfer of *MDR1* into human CD34+ enriched BM and cord blood cells has been recently reported [40]. Transduction of haematopoietic cell lines and fresh normal BM specimens with SV40 *MDR1* pseudovirions has been successful as well [41]. Finally, cationic liposome-mediated transfection of murine haematopoietic cells has been achieved both *in vitro* and *in vivo* following intravenous injection of expression plasmid/liposomes complexes in recipient mice [42, 43].

## ADVANTAGES AND LIMITS OF *MDR1* GENE TRANSFER IN HAEMATOPOIETIC CELLS

Numerous experiments have demonstrated that *MDR1* expression confers drug resistance to targeted cells which can be selected by subsequent exposure to Pgp-expelled cytotoxic

agents both *in vitro* and *in vivo*. Compared with other resistance genes, *MDR1* presents attractive features such as a wide spectrum of Pgp substrates and the ability to achieve various degrees of resistance. Current clinical trials are evaluating whether transplantation of autologous haematopoietic cells transduced with *MDR1* safety-modified retroviruses into patients with advanced breast or ovarian cancers will allow treatment dose-intensification [44–46]. In one protocol, PB stem cells and BM cells of patients with metastatic breast cancer without evidence of BM involvement and metastatic bone disease, and who responded at least partially to 4–5 cycles of chemotherapy are harvested [45]. Some of these cells are used for isolation of CD34+ progenitors, transduced *in vitro* with a retroviral vector expressing the human *MDR1* cDNA and reinfused into patients along with remaining non-transduced cells after high-dose chemotherapy. Patients with residual or progressive disease after transplantation are treated with paclitaxel or vinblastine, two Pgp substrates with clinical activity in the treatment of breast cancer. Studies are being performed to analyse whether *MDR1* expression confers myeloprotection and whether chemotherapy amplifies the proportion of haematopoietic cells containing the *MDR1* provirus. Some very preliminary results have been reported for one ongoing trial [47]. Nine ovarian or breast cancer patients have been transplanted with *MDR1* transduced CD34+ cells following intensive combination chemotherapy. Exposure of CD34+ cells to *MDR1*-retroviral vectors had no deleterious effects on haematopoietic recovery, which occurred after normal delays following transplantation (on average, 13 and 22 days, respectively, for recovery to $0.5 \times 10^9$ neutrophils/l and $20 \times 10^9$ platelets/l).

One major concern is that malignant cells may contaminate the transplant and acquire drug resistance post-transduction. Also, the *MDR1* gene may be expressed either intrinsically or subsequent to chemotherapy administration in patients who are being treated using gene therapy with *MDR1*-based vectors. Therefore, a means to distinguish the Pgp expressed by the gene therapy vector from endogenous Pgp would be extremely desirable. Several point mutations in *MDR1* cDNA are known to change substrate specificity and therefore alter the pattern of drug resistance and sensitivity to MDR modulators (non-cytotoxic Pgp substrates competing with chemotherapeutic agents for binding and/or transport) [45]. In cases where a resistant cancer develops or persists following *MDR1* transduction, differences in reversion or resistance pattern between endogenous and transduced Pgps might be useful to render inadvertently transduced contaminating cells sensitive to chemotherapy without deleterious effects on normal tissues expressing Pgp, or to treat residual resistant cancer cells without toxicity for the *MDR1* transplant [3]. In addition, generation of *MDR1* vectors which provide a specific advantage to transduced BM cells compared to multidrug resistant cancers (i.e. higher levels of resistance to drugs subsequently used for cancer treatment) would be very valuable. Other approaches to specific reversal of drug resistance are also feasible, including the use of monoclonal antibodies specific to altered residues on the surface of mutant transporters, and the introduction of 'suicide' genes or sequences which can be used to eliminate unwanted transduced cells [3, 48].

Gene therapy for haematopoietic disorders is hampered by the low efficiency of gene transfer, low and/or unstable expression of transgenes and the frequent impossibility of selecting the targeted cells based on the transgene expression [49]. Bicistronic vectors, in which *MDR1* cDNA is linked to a second transgene of interest via a picornavirus internal ribosome entry site sequence (IRES), have been designed [43, 50, 51]. IRES sequences are able to promote ribosome binding and initiate translation at internal sites within a mRNA [52]: two genes linked by an IRES sequence are transcribed as a single mRNA driven by the same promoter, but are translated independently. This system presents some advantages compared with splicing vectors, vectors containing multiple transcription units, or vectors leading to the production of chimeric proteins especially when a selection marker is involved and when the final cellular locations of the transgene products differ [50]. NIH3T3 cells, transfected or transduced with *MDR1*-containing bicistronic vectors and selected with *MDR1*-substrates, expressed the otherwise non-selectable transgenes transferred together with *MDR1* cDNA (the human α-galactosidase or human glucocerebrosidase cDNAs) [43, 50, 51]. The use of *MDR1* to select cells which cannot be selected on the basis of their expression of a second transgene of interest may be applied to haematopoietic cells. Gene therapy targets may be whole BM or sorted subpopulations in which the deficient gene product is normally expressed or in which the product of the transgene may enhance favourable cell properties. Modified haematopoietic effectors may be efficient tools for therapy of cancer and other diseases; for example, co-introduction of a selectable drug-resistance gene would allow *in vivo* selection of tumour-infiltrating lymphocytes (TIL) or other cytotoxic T lymphocytes (CTL) transduced with cytokine expression vectors, enhancing cell killing capability.

## CONCLUSION

Transfer and expression of the human multidrug transporter encoded by the *MDR1* gene into haematopoietic cells can be achieved and results in a resistant phenotype of the targeted cells which can be selected *in vitro* and *in vivo* based on *MDR1* expression. The use of *MDR1* in gene therapy protocols involving haematopoietic cells in humans is the subject of ongoing preclinical and clinical investigations.


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