Editorial

Clinical Trials Involving Multidrug Resistance Transcription Units in Retroviral Vectors

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Cowan et al. (1) have reported in this issue of Clinical Cancer Research the results of a clinical trial that was designed to evaluate the feasibility of using retroviral vectors to transfer the MDR-1 gene to hematopoietic cells to protect them from the effects of chemotherapy. The MDR-1 gene codes for p-glycoprotein, an ATP-dependent membrane protein known to transport many of the complex alkaloid chemotherapy agents, such as the anthracyclins, the pertussis toxins, and the epipodophyllotoxins from inside to the outside of cells. By reducing the achievable intracellular concentrations of each of these drugs, the p-glycoprotein protects the cells from the toxicity of these drugs. The presence of high levels of the p-glycoprotein is associated with high levels of resistance to many epithelial tumors. In addition, Chaudhary and Roninson (2) had shown that the level of expression of the MDR-1 gene decreases as hematopoietic cells matured in the marrow. This data provided one possible mechanism through which tumors can acquire resistance to multiple chemotherapy agents through the overexpression of a single gene. In addition, it provided one of many possible mechanisms through which immature hematopoietic cells can survive doses of chemotherapy that are lethal to hematopoietic cells belonging to more mature stages of hematopoietic differentiation. Finally, this data suggested to many that the transfer of the gene for the MDR-1, in a transcription unit controlled by a promoter that is active hematopoietic progenitor cells. In this concept into the clinic (1, 8–10). The conditions of transduction involved either cocultivation of the producer cells lines with the hematopoietic cells or exposure of the hematopoietic cells in suspension or on stromal monolayers to cell-free supernatants of the viral particles. After transplantation of these vector modified cells into lethally irradiated recipient mice, it was shown that: (a) the transplanted MDR-1-transduced hematopoietic cells were resistant to much higher concentrations of the drug in clonogenic progenitor assays than in the cells of unmodified donors (4–7); (b) the administration of posttransplant chemotherapy was associated with increased levels of the vector-modified cells in the animals transplanted with MDR-1 vector-transduced cells (4); and (c) the MDR-1 vector-transduced cells were capable of serially transplanting up to six successive cohorts of mice, whereas unmodified cells would only successfully serially engraft three successive cohorts of irradiated mice (6–7). The hematopoietic maturation appeared normal in all of these transplant experiments, and no evidence of myelodysplastic states were associated with this vector modification. A cryptic splice acceptor side was also discovered in the MDR-1 gene, which resulted in a variable percentage of the transductants containing functionless truncation mutants of p-glycoprotein (4, 6–7). The most encouraging aspects of these preclinical trials were the long-term engraftment and the increases in the levels of vector-modified cells during the administration of posttransplantation chemotherapy, which compensated, in part, for the low percentage of transplantable hematopoietic cells that were MDR-1 vector positive immediately after transplant (4).

These data led to an interest all over the world in testing whether such vector delivery systems could be used to deliver MDR-1 transcription units to patients with epithelial malignancies. The interest in developing such programs was further heightened by the advent of the taxanes, which were associated with dose-limiting hematopoietic toxicity, along with neurotoxicity.

In the initial trials, institutions in the United States (NIH Bethesda, MD), Columbia University (New York, NY), and the University of Texas M.D. Anderson Cancer Center (Houston, TX) secured approval from the NIH Recombinant DNA Advisory Committee and the Federal Drug Administration to carry this concept into the clinic (1, 8–10). The conditions of transduction in each case involved incubation for several days in medium supplemented with serum and late-acting hematopoietic growth factors. All three groups used the autologous transplantation model for testing of the engraftment of MDR-1 vector-modified cells (1, 7–9), and two of the trials studied, in addition, the effect of posttransplant chemotherapy in the context of the transplantation of MDR-1 vector-modified cells (1, 8, 10).

The first trial to be published, which involved 20 patients, showed only short-term engraftment of vector-modified cells.
and that *in vitro* transduction of the clonogenic progenitor cells was not predictive of the presence of posttransplant MDR-1 vector-positive hematopoietic cells (7). This data suggested that at least the subset of the clonogenic progenitors, which were transduced, did not have significant engrafting capability (8, 10). This result (8) confirmed earlier mouse studies by Uchida *et al.* (11), which had suggested that the clonogenic progenitors had limited self renewal capability.

The next group to publish the results of their MDR-1 vector modification trial also showed only very short-term engraftment of the MDR-1 vector-mediated cells after transplant (9). The next publication showed that the delivery of posttransplant chemotherapy immediately after engraftment, in the context of the MDR-1 chemoprotection trial, converted some breast cancer patients from a partial response after transplant to a complete clinical response (10). Two of the patients achieving partial response after the pretransplant intensive chemotherapy and autograft, and then a complete response from the posttransplant chemotherapy, are still disease-free in unmaintained remissions over 3 years after the initial transplant (10). Thus, although the vectors used for the MDR-1 delivery were not as successful as anticipated, the results of this trial suggested that genetic chemoprotection could be of potential utility in the use of high-dose therapy and autographs.

Cowan *et al.* (1) now report the results of their trial, which involved seven patients transplanted with MDR-1 vector-modified cells. Their results show that only short-term engraftment with MDR-1 vector-modified cells was seen after transplant. However, one of their patients exhibited stable, but low-level, engraftment with the MDR-1 vector-positive cells for several months after transplant (1). A normal pattern of hematopoietic differentiation was maintained in this patient. Cowen *et al.* (1) discussed the reasons for the short-term, but not long-term, engraftment of MDR-1 vector-positive cells in the marrow of patients participating in the human MDR-1 vector transplant trials. After the initiation of these clinical trials, multiple groups have shown that the conditions used in the early three MDR-1 chemoprotection trials (interleukin-3, stem cell factor, and serum) led to induction of maturation of the early hematopoietic stem cells and decreased levels of engraftment of the *in vitro*-manipulated cells (12). Orlic *et al.* (13) have shown that the receptors needed by the vectors to infect the target cells are not represented on early human stem cells to the degree that they are present on mouse stem cells, or more mature human hematopoietic cells. The transcriptional regulatory elements of the Maloney and Harvey vectors undergo methylation in the early hematopoietic cells, which could have suppressed the level of expression of the MDR-1 genes in the stem cells. The doses of posttransplant chemotherapy used to attempt to create a selective advantage for the MDR-1 vector-modified cells were set for safety reasons at too low a level to confer a selective advantage on cells that were positive for the MDR-1 vector transgene.

Several developments have occurred in vector design and in systems available for the *in vitro* incubation of human hematopoietic cells, which have potentially addressed many of the problems that may have contributed to the failure to achieve long-term engraftment and chemoprotection in these early trials:

(a) *Ex vivo* serum-free culture conditions that support the survival and self renewal of stem cells have been developed (14, 15), which will enable future trials to avoid the conditions used for transduction in the early MDR-1 trials (incubation in serum containing medium supplemented with late-acting growth factors, such as interleukin-3, in the presence of SCF), which have been shown to induce maturation of stem cells and to, therefore, reduce their self renewal potential in transplantation settings;

(b) The development of pseudotyping of the retroviral vectors have allowed newer vectors to be created, which display ligands on their surface for which receptors are present on stem cells. These vectors show much higher transduction frequencies of the stem cells than obtainable in earlier studies;

(c) Baum *et al.* (16) have developed vectors in which the introduction of transcriptional regulatory elements of the mouse embryonic stem cell virus, which is not methylated in stem cells, contribute to much higher levels of expression of the vector transgenes in hematopoietic stem cells than was the case with the Maloney and Harvey vectors used in the early MDR-1 trials in which the transcriptional elements of the virus are inactivated by methylation and, therefore, the expression of the retroviral transgenes are only of short duration;

(d) The lentiviral vectors (17) have been developed, which retain the ability to transport the vector cDNA through the intact nuclear membrane of the nondividing cell, whereas the Maloney and Harvey vectors could not deliver their cDNA through the intact nuclear membrane of the nondividing cell (this limited the transduction of reconstituting early hematopoietic stem cells, most of which are nondividing, in the early trials). These lentiviral vectors have been shown to result in modification of at least 80% of the CD34+CD38− cells, and the retroviral vectors developed in serum-free medium have been shown to result in the modification of 50% of these cells;

(e) An entire series of additional chemoprotection transcription units, in addition to MDR-1, have been introduced into vectors and shown to protect hematopoietic cells;

(f) Newer and more powerful growth factors, which are specific for the early cell, have been cloned (such as FLT 3 ligand), which are now available for creating serum-free *in vitro* transduction conditions that preserve the integrity of the hematopoietic stem cells;

(g) Cornetta and his coworkers (18) have used the fibronectin fragment of David Williams to increase the transduction frequency of hematopoietic cells and the percentage of MDR-1 vector-positive cells posttransplant;

(h) Several new vector and animal models are now available for the study of the engraftment capabilities of genetically modified stem cells: the NOD X SCID transplant model (17) and the fetal sheep model of Zanjani (19, 20). Experiments such as serial transplantation need to be carried out so that the true self renewal potential of these modified cells can be assessed.

The early MDR-1 vector modification studies (1, 8–10), such as that of Cowan *et al.*, published in this issue of *Clinical Cancer Research* (1), have clarified the logistics and the hurdles that need to be overcome for genetic modification of hematopoietic stem cells to become relevant to clinically important end points. Although old questions remain about the ability to ultimately succeed in stem cell gene therapy and new questions have arisen about the impact of overexpression of MDR-1 transcription units in hematopoietic cells (21), the developments
in the field of virology and the biology of the hematopoietic stem cell, which have occurred since the inception of these trials, may make this dream a reality in the very near future and lead to a new round of preclinical trials to test the feasibility of using vector-mediated gene transfer as a means of reducing the risk and cost of administering chemotherapy for the treatment of epithelial neoplasms, and for correcting constitutional and acquired abnormalities of the hematopoietic cells.

REFERENCES


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