P-glycoprotein targeting: a unique strategy to selectively eliminate immunoreactive T cells

Martin Guimond, Antonia Balassy, Mélanie Barrette, Sylvie Brochu, Claude Perreault, and Denis Claude Roy

T lymphocytes have been found to harbor P-glycoprotein (Pgp) and to demonstrate modulation of its ion channel transporter function according to the state of activation of T lymphocytes. We hypothesized that cytotoxic chemicals that are extruded by Pgp could be used to specifically eliminate immunoreactive T-cell populations. In this study, we evaluated the capacity of 4,5-dibromorhodamine methyl ester (TH9402), a photosensitizer structurally similar to rhodamine, a dye transported by Pgp, and which becomes highly cytotoxic on activation with visible light to selectively deplete alloreactive T lymphocytes. Stimulation of T cells with mitogens or allogeneic major histocompatibility complex–mismatched cells resulted in the preferential retention of the TH9402 rhodamine-derivative in activated T cells, both CD4+ and CD8+. Photodynamic cell therapy of TH9402-exposed T cells led to the selective elimination of immunoreactive T-cell populations. In addition, this treatment preserved resting T cells and their capacity to respond to third-party cells. Inhibition of Pgp enhanced cellular trapping of the dye in nonactivated T cells and resulted in their depletion after exposure to light. Targeting of Pgp-deficient cells may therefore represent an appealing strategy for the prevention and treatment of graft-versus-host disease and other alloimmune or autoimmune disorders. (Blood. 2002;100:375-382)

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Rhodamine enters all cells and is extruded from the intracellular milieu through P-glycoprotein (Pgp) active transport.\textsuperscript{26} Pgp, the product of the multidrug-resistance-1 (MDR1) gene, is a protein expressed not only in normal stem cells, but also in T lymphocytes.\textsuperscript{27–30} Investigators have proposed that T-cell activation may actually lead to the inactivation of Pgp.\textsuperscript{29} Thus, activated T cells should fail to extrude rhodamine. Although rhodamine is not cytotoxic, 4,5-dibromorhodamine methyl ester (TH9402), a rhodamine derivative, was found to harbor important photosensitizing potential.\textsuperscript{31–33} Its phototoxicity is mediated primarily by singlet oxygen production, with oxidative damage concentrated to mitochondria by the virtue of drug localization.\textsuperscript{31,34} The structural similarity between rhodamine and TH9402 prompted us to evaluate the capacity of the latter photosensitizing agent to be preferentially retained in Pgp-deficient activated T cells and, thus, lead to their selective elimination after exposure to visible light (514 nm).\textsuperscript{35}

In the present study, we found that photodynamic cell therapy (PDCT) with TH9402 was highly toxic against CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells activated in response to mitogens or MHC-mismatched antigens. This PDCT selectively preserved resting T lymphocytes and their ability to proliferate and to demonstrate cytotoxicity toward third-party antigens. PDCT may therefore have clinical utility for the selection of non–MHC-mismatched antigens. This PDCT selectively preserved resting T lymphocytes and their ability to proliferate and to demonstrate cytotoxicity toward third-party antigens. PDCT may therefore have clinical utility for the selection of non–MHC-reactive T cells to prevent GVHD and accelerate immune reconstitution after transplantation or for the treatment of immunoreactive disorders. Moreover, our findings identify targeting of MDR1 inhibition as a unique physiologic approach to specifically eliminate activated T cells.

**Materials and methods**

**Human cells**

Blood samples were obtained with the informed consent of healthy donors under clinical protocols approved by the Human Subjects Protection Committee of the Maisonneuve-Rosemont Hospital. Peripheral blood (PB) samples were collected in preservative-free heparin and mononuclear cells separated by Ficoll-Hypaque density gradient centrifugation (Ficoll-Paque; Pharmacia, Piscataway, NJ). The T-lymphoblastic cell line CEM and the T-lymphoblastic cell line Pgp-expressing KG1a cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD).

**Phytohemagglutinin stimulation**

Peripheral blood mononuclear cells (PBMCs) were cultured for 72 hours at a concentration of 3 \times 10^6 cells/mL in flasks (Nunclon, Nunc, Roskilde, Denmark) with 2 \mu g/mL phytohemagglutinin (PHA; Sigma, St Louis, MO) in X-Vivo 15 medium (Bio-Whittaker, Walkersville, MD) supplemented with 15% human AB serum (HAB; Sigma), 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/mL penicillin, and 100 \mu g/mL streptomycin (all from Gibco, Grand Island, NY).

**Allogeneic T-cell activation**

Activation of responder (A) T lymphocytes against stimulator (B) cells was conducted in a one-way mixed lymphocyte reaction (MLR).\textsuperscript{36} A and B individuals presented 3 major HLA mismatches. Briefly, responder cells from subject A were cultured for 4 days at 37°C with the same number of irradiated PBMCs from subject B (AB\textsuperscript{+}) (50 Gy; 157Cs; Gamma Cell, Atomic Energy of Canada, Ottawa, ON, Canada) in medium supplemented with 50 U/mL recombinant human interleukin 2 (rhIL-2; R & D Systems, Minneapolis, MN).

**Photodynamic cell therapy with TH9402**

After in vitro activation, cells were harvested, washed, resuspended at a final concentration of 1 \times 10^6 cells/mL, and incubated at 37°C with 10 \mu M TH9402 (Theratechnologies, Montreal, QC, Canada) in X-Vivo 15 medium with 2.5% HAB. After a 40-minute incubation, cells were centrifuged and dye efflux favored by resuspending cells in TH9402-free medium for 90 minutes. At the end of the latter dye efflux period, cells were exposed to a fluorescent light-scanning device (PDCT-Xerox Series 4, Theratechnologies) delivering 5 J/cm^2 at a wavelength of 514 nm.

**T-cell proliferation assay**

Proliferative activity of responder cells exposed to photodynamic therapy and nontreated controls was assessed on day 5 of an MLR in a standard 3H-thymidine labeling assay. The total number of cells (responder [A] and irradiated stimulator [B] cells) present before PDCT was not adjusted after PDCT. These cells were restimulated with a fixed number (1 \times 10^6) of irradiated stimulator (B\textsuperscript{*}) or third-party (C\textsuperscript{*}) cells at different responder/stimulator cell ratios (2:1, 1:1, 1:2, and 1:4) in 96-well U-bottomed microtiter plates (Nunc). Cultures in triplicate were labeled with 1 \mu Ci (0.037 MBq) 3H-thymidine (Perkin Elmer, Woodbridge, ON, Canada) per well for 18 hours and harvested onto glass fiber filter mats; 3H-thymidine incorporation was measured using a liquid scintillation counter (Wallac, Gaithersburg, MD).

**Cytotoxic T-lymphocyte precursor and limiting dilution assays**

Limiting dilution assays (LDAs) were used to calculate the frequencies of responding cytotoxic T-lymphocyte precursor (CTLp) cells and clonogenic CEM and KG1a cells in treated and untreated conditions using previously described methods.\textsuperscript{37,38} Briefly, to determine CTLp frequency, 24 replicates of graded numbers of treated or untreated responder (A) cells (3 \times 10^4 to 450 cells/well) were seeded in 96-well microtiter plates in the presence of 1 \times 10^3 irradiated (50 Gy) fresh PBMC stimulator cells (B or C). Control wells consisted of stimulator cells only. After 9 days of culture in medium supplemented with 50 U/mL rhIL-2, each well was tested for cytolytic activity against 5 \times 10^3 initial stimulator (B) and third-party (C) cells using a standard 51Cr-release assay. The supernatant (100 \mu L) was harvested from each well and counted in a \gamma counter. Spontaneous release was less than 15%. Results for individual wells were expressed as a percentage of specific lysis calculated as follows: percent specific lysis = 100 \times (experimental release – spontaneous release [medium only])/(maximum release [1% Triton X-100] – spontaneous release). To measure CEM and KG1a, clonogenic cell frequencies, cells were grown in a similar LDA (from 5 \times 10^3 to 0.5 cells/well) in RPMI 1640 medium (Life Technologies, Gaithersburg, MD) supplemented with 10% fetal bovine serum, fed every 4 days, and scored for growth under an inverted phase microscope.

**Immunophenotypic analysis**

Expression of cell surface T-cell antigens was evaluated by direct immunofluorescence using standard techniques.\textsuperscript{39} Monoclonal antibodies (mAbs) used in this study were anti-CD3–fluorescein isothiocyanate (UCHT1), anti-CD25–phycoerythrin (B1.49.9), anti-CD3–allophycocyanin (APC; UCHT1; Coulter Immunology, Hialeah, FL), anti-CD4–APC (RPA-T4), and anti-CD8–APC (RPA-T8; Pharmigen, San Diego, CA). Nonspecific binding was determined using appropriate isotypic controls. Immunofluorescence reactivity was determined by automated multiparameter flow cytometry analyzing at least 10^4 cells in each sample (FACSCalibur; Becton Dickinson, Mountain View, CA) and processed using Cell Quest software (Becton Dickinson).

**Hematopoietic progenitor cell assay**

Peripheral blood cells from healthy donors mobilized with granulocyte colony-stimulating factor (G-CSF; Amgen, Thousand Oaks, CA) underwent PDCT and were plated in methylcellulose medium (MethoCult H4434; Stem Cell Technologies, Vancouver, BC, Canada) on 35-mm plastic culture dishes, according to the manufacturer’s instructions. Colony-forming units–granulocyte-macrophage (CFU-GMs) and colony-forming units–mix (CFU-mix) were scored after 14 days of culture at 37°C in a fully humidified 5% CO\textsubscript{2} atmosphere.\textsuperscript{38}
Functional evaluation of Pgp-170

Pgp substrate efflux modulation by cyclosporin A (CSA) and verapamil was determined in an accumulation assay using TH9402. Cells were stained with 10 μM TH9402 for 40 minutes, washed, and resuspended in either medium alone, with 1 μg/ml CSA (Novartis Pharma, Dorval, QC, Canada) or 5 μg/ml verapamil (Sabex, Boucherville, QC, Canada). Cellular retention of the dye was assessed by flow cytometry (FACSCalibur, Becton Dickinson).40,41 Calibration beads were used in all experiments to ensure stable energy delivery (Calibrate3 and -APC, Becton Dickinson). Positive controls for functional Pgp expression consisted of KG1α cells.

Results

Photodynamic elimination of T cells

To assess the sensitivity of proliferating T cells to TH9402 PDCT, the lymphoblastic T-cell line CEM was used as target. Cells were incubated for 40 minutes with 10 μM TH9402, washed, and incubated with TH9402-free medium for 90 minutes and then exposed to light (5 J/cm²). These parameters were previously found to eliminate more than 99.9% of malignant K562 cells, while preserving more than 50% of hematopoietic progenitor cells.32 TH9402 PDCT when compared to untreated controls depleted more than 99.97% of CEM T cells measured by LDA (Figure 1A).

The potential of this TH9402-based PDCT method to eliminate activated T lymphocytes was evaluated by comparing proliferative responses of treated (PDCT) versus untreated PHA-activated normal PBMCs toward MHC-mismatched stimulator cells. In untreated controls, PHA-stimulated cells were able to proliferate when subsequently exposed to MHC-disparate stimulatory cells in an MLR (Figure 1B). In contrast, TH9402 PDCT completely abrogated the response of PHA-stimulated cells to MHC-mismatched cells. The specificity of PDCT for activated cells was evaluated by treating resting PBMCs, incubated in IL-2 containing medium only, and then measuring proliferative response in an alloimmune mismatch MLR (Figure 1C). Interestingly, PDCT did not affect the response of these resting cells, a finding that indicates a higher level of sensitivity to PDCT for primed versus resting T cells.

Depletion of alloreactive T-lymphocyte subsets

The clinical application of PDCT in the context of allogeneic transplantation must rely on both specific elimination of T cells that are reactive toward host cells and preservation of T cells capable of subsequent response to infectious or other foreign antigens. To clarify this issue, PBMCs (individual A) were first exposed for 4 days to allogeneic stimulator (individual B) cells mismatched at 3 MHC loci (A, B, and DR). After this activation process, cells were exposed to PDCT and then presented with either the same stimulator (B) cells for a second time or with third-party (C) cells in a conventional 3H-thymidine incorporation assay (Figure 2). Increasing concentrations of TH9402 and light intensity induced gradually decreasing proliferation toward stimulator B cells. In contrast, the capacity of residual cells to proliferate when exposed to third-party C cells was preserved except at the highest treatment intensity. Moreover, to discriminate between the effect of TH9402, light, and PDCT, A cells were treated with either TH9402 alone, light alone, or TH9402 PDCT. Proliferative responses were preserved after exposure to either TH9402 without light or to light alone (data not shown; P = NS). The highest TH9402 concentration (10 μM) and light intensity (5 J/cm²) were selected for all subsequent experiments because these achieved maximum elimination of specific alloreactivity and only slightly affected response to third-party C cells.

Immunophenotypic analysis

To evaluate the specificity of PDCT for activated T lymphocytes, the expression of the inducible chain of the IL-2 receptor (IL-2R; CD25) was measured on CD4+ and CD8+ T-cell populations from treated samples and untreated controls (Figure 3). At the end of 4-day MLR, cells were exposed to TH9402 PDCT or medium and immunophenotypic analysis performed after a 3-day culture in medium containing IL-2. At least 98% of CD8+CD25+ cells and 96% of CD4+CD25+ cells were eliminated by PDCT when measured by flow cytometry (Table 1). In contrast, most unactivated (CD25−) T cells were spared; their increased proportion after PDCT confirms the selectivity of PDCT for activated lymphocytes. In addition, CD25− T cells also stained negatively for propidium iodide, a finding that indicates preservation of T-cell integrity (data not shown).

CTLp frequency after PDCT

To confirm the specificity of PDCT for antihost T cells, CTLp cells were enumerated following treatment with TH9402 or medium using LDA. The number of CTLp cells active against B and C cells was determined after TH9402 PDCT of A cells MLR-primed against B cells (AB+) (Figure 4A). In untreated samples, more
stimulator cells from the same host or (B) third-party cells for 5 days and proliferation
TH9402 and light intensity. (A) After treatment, cells were cocultured with irradiated
cells in a one-way, 4-day MLR, and then treated with increasing concentrations

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The specificity of TH9402-mediated killing for activated T cells
Kinetics of dye retention

The specificity of TH9402-mediated killing for activated T cells could be due to a differential accumulation or retention of the dye in resting versus activated T cells. To test this hypothesis, TH9402 influx/efflux kinetics were evaluated in PHA-stimulated and resting lymphocytes. At the end of the incubation period, retention of the dye was higher in PHA-stimulated CD3+ cells than in resting lymphocytes (Figure 5A). In addition, activated lymphocytes continued to sequester more TH9402 over time than resting lymphocytes, even after reaching the plateau phase (P < .05).

Moreover, after an MLR, CD25+ expressing T lymphocytes, whether CD4+ or CD8+, retained more TH9402 compared to CD25− T cells (P < .05; Figure 5B-C). These data indicate that both TH9402 accumulation and TH9402 retention are increased in the proliferating and activated T cells.

Interestingly, resting T cells not only incorporated lower levels of TH9402 than PHA-activated cells, but also a large proportion of the former cells demonstrated a second peak of lower fluorescence intensity (Figure 6A). This bimodal distribution indicates the existence among resting cells of 2 populations with a different propensity to eliminate the dye. Similar results obtained using MLR-activated T cells (data not shown) indicate that both TH9402 accumulation and TH9402 retention are increased in the proliferating and activated T cells.

Pgp involvement in TH9402 efflux

To study mechanisms of retention of the TH9402 rhodamine derivative, we focused on Pgp, which has been previously described as the main channel involved in rhodamine efflux.43,44 In resting T lymphocytes, inactivation of Pgp by CSA led to a disappearance of the peak of lower fluorescence intensity (M2; mean fluorescence intensity (MFI) = 32) and gave rise to a single peak of TH9402 fluorescence (M1) demonstrating slightly higher retention of the dye (MFI = 600) than the M1 peak of the CSA-unexposed sample (MFI = 400; Figure 6A). In contrast, inactivation of the MDR1 channel had no major impact on retention of the dye in PHA-stimulated lymphocytes. Moreover, the effect of CSA on TH9402 efflux was durable and prevented the appearance of CD3+ T cells with low concentrations of dye for more than 2 hours (Figure 6B).

To determine if the higher retention of the dye caused by Pgp inhibition translated into higher cytotoxicity, we incubated resting T cells with verapamil and exposed them to PDCT. This MDR1 inhibitor significantly enhanced the PDCT elimination of resting T cells (P < .01; Figure 7A). To investigate the extent of MDR1 involvement in TH9402-mediated effects, we repeated the same experiment using the KG1a cell line, which demonstrates high levels of constitutive expression of Pgp.45 Phototherapy with a lower dose (5 μM) of TH9402 was not cytotoxic to KG1a cells, but the addition of verapamil led not only to increased retention of the dye (data not shown), but also to depletion of 99.99% of clonogenic cells (Figure 7B). When used without PDCT, verapamil did not deplete T or KG1a cells. These findings clearly identify Pgp as the principal modulator of TH9402 cellular concentration and photodynamic cytotoxicity.

Discussion

Selective elimination of donor T-cell subsets recognizing host histocompatibility antigens represents an appealing strategy to eradicate GVHD. However, to limit complications with viral and fungal infections, graft rejection, and relapse, which occur after
Although the scant number of CD25$^+$ cells detected could represent activated T cells escaping photodynamic eradication, it is also possible that they correspond to cells bound to die from lethal damage of PDCT-mediated oxidative damage. Alternatively, these lymphocytes could represent nonactivated T lymphocytes, such as regulatory T cells, which have been found to constitutively express CD25.$^{52,54}$ The preservation of a regulatory T-cell population would be particularly useful because it has been shown to play an important role in induction of tolerance to alloantigen via costimulatory blockade.$^{55}$ The latter scenarios would explain why the evaluation of the impact of PDCT on CTLp cells demonstrated greater elimination of antithost clonogenic cytotoxic precursors (Figure 4A) than of CD25$^+$ T-cell populations (Figure 3). Moreover, the 3 logs of depletion of CTLs observed with the LDA is of the same order as the threshold of 2 to 3 logs of T-cell depletion thought to be required for the prevention of GVHD.$^{56}$

Because T-cell–receptor diversity after transplantation is decreased according to the number of T cells present in the graft, it is crucial that as many T cells as possible be spared.$^{57}$ Our results demonstrate that although phototherapy using TH9402 is highly toxic for activated T cells, it remains selective and preserves a large proportion of the CD4$^+$ and CD8$^+$ cells that do not express the IL-2 high-affinity receptor and other cell lineages such as myeloid and erythroid progenitors. Interestingly, the administration of such T cells has the potential to restore T-cell–receptor diversity through expansion in response to homeostatic signals of the host and to reconstitute the peripheral T-cell pool.$^{58,59}$ Indeed, nonactivated T cells remain immunocompetent and able to proliferate in response to new antigenic stimuli, whether previously cultured in IL-2 only or mitogens, or stimulated with allogeneic cells. This is corroborated by the ability of TH9402-exposed cells to generate CTLp cells against third-party antigens, a finding that also confirms the selectivity of PDCT. Future studies will challenge us to determine if the small decrease in reactivity toward third-party cells observed after PDCT could reflect the elimination of T-cell clones with dual specificity, which demonstrate the capacity to react toward both host and third-party cells.$^{60,61}$

Because only T cells recognizing an antigen expressed by a stimulator cell will be eliminated, PDCT should spare T cells recognizing tumor antigens (developmentally regulated antigens or leukemia-specific antigens) provided care is taken to exclude neoplastic cells from the stimulator cell population.$^{62}$ Natural killer (NK) cells also express high levels of Pgp and should be protected from PDCT toxic effects.$^{28,64}$ Although we demonstrate here that PDCT is effective at eliminating T cells reactive to stimulator cell MHC antigens, the effect of PDCT on minor histocompatibility antigen (mHA)–stimulated T cells has yet to be addressed.$^{36,66}$ In addition, the observation that TH9402-treated T cells respond to third-party MHC antigens indicates preservation of T-cell signaling and effector pathways that should translate into elimination of viral and fungal invaders.$^{22}$ Moreover, the addition of donor T cells, although nonreactive toward host MHC antigens, could help lower the incidence of graft rejection associated with T-cell depletion.$^{3,5,8,20}$ Indeed, T cells present after PDCT have the potential to act as veto cells to block antidoor reactivity of host T cells without requiring recognition of host alloantigens.$^{66,67}$ In future studies, it will be important to delineate the nature of the various T and NK cell populations.

**Table 1. Impact of TH9402 PDCT on activated and nonactivated CD4$^+$ and CD8$^+$ cells evaluated using flow cytometry**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CD25$^+$ (10$^6$ cells)</th>
<th>CD8$^+$ cells (10$^6$ cells)</th>
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<tbody>
<tr>
<td>Medium</td>
<td>1.68 ± 0.61</td>
<td>0.96 ± 0.69</td>
</tr>
<tr>
<td>TH9402</td>
<td>0.060 ± 0.011</td>
<td>0.21 ± 0.09</td>
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</tbody>
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*After exposure to TH9402 PDCT or medium, AB$^+$ cells were cultured for 3 days in medium supplemented with IL-2. Absolute cell numbers are expressed in million cells (mean ± SEM of 3 experiments).*
that escape elimination by TH9402 PDCT and their contribution to the prevention of immunologic and infectious complications after transplantation.

Our findings indicate that TH9402 PDCT does not exhibit a broad antiproliferative effect, but rather acts specifically against activated T cells according to intrinsic physiologic properties of target cells. Modulation of Pgp activity, which results in differential retention and cytotoxicity from TH9402, could reflect biomechanical modifications of such channel transporters with the activation process. Interestingly, Pgp could also be inactivated by PDCT itself, an inhibitory mechanism that would augment retention of the photosensitizer in those activated T cells with partial inhibition of MDR1 function, without affecting resting T cells that have already extruded most of the dye at the time of light application. The increased retention of TH9402, and potentially of its photoproducts, in activated cells could enhance the efficacy and specificity of the treatment. Although we cannot exclude a contribution of metabolic changes induced by T-cell activation to altered mitochondrial targeting by this rhodamine derivative, our findings clearly indicate that Pgp plays an important role in the intracellular handling of TH9402 and identify a novel approach that takes advantage of the functional inhibition of this pathway of resistance to selectively eradicate activated T cells.

The current photodynamic approach could be applied directly for the ex vivo treatment of stem cell grafts or donor lymphocyte infusions to prevent GVHD in the context of MHC-mismatched allogeneic transplantation. Moreover, recent identification and sequencing of several MiHAs and improvements in immunization strategies using dendritic cells should facilitate the activation of effector cells directed at host MiHAs. This may broaden the applicability of this PDCT to allogeneic HLA-matched transplant strategies. Finally, the selectivity of TH9402 PDCT for activated T lymphocytes could be exploited for the targeted elimination of both alloreactive T-cell clones that develop after solid organ transplants and autoreactive clones responsible for diseases such as lupus erythematosus, rheumatoid arthritis, and systemic sclerosis.

Figure 5. Kinetics of incorporation of TH9402 in resting and activated lymphocytes. (A) TH9402 dye retention was analyzed in the CD3+ cells from samples incubated (diamonds) or not (circles) with PHA. (B) Dye retention was also measured in activated (CD25+) and nonactivated (CD25−) CD4+ and (C) CD8+ cells within the same MLR-activated sample. MFI = SEM of 3 to 6 experiments and P < .05 for all evaluations.

Figure 6. Impact of Pgp inhibition on TH9402 content in resting and activated lymphocytes. (A) PHA-stimulated and resting lymphocytes were stained with TH9402 for 40 minutes and resuspended in medium alone or with CSA. Flow cytometric assessment of TH9402 content in CD3+ cells was performed 90 minutes after the end of the incubation period. Numbers in parentheses indicate the MFI of corresponding cell populations. (B) The impact of CSA exposure on the proportion of PHA-stimulated lymphocytes capable of eliminating TH9402 (MFI < 100 U) was measured over time, starting after completion of the 40-minute incubation period. The results are representative of 3 experiments.

Figure 7. Down-modulation of MDR1 function enhances TH9402-mediated cytotoxicity. (A) Resting PBMCs were exposed to 10 μM TH9402 in medium supplemented or not with verapamil. Elimination of CD4+ and CD8+ cell populations was measured 3 days after PDCT using flow cytometry and compared with untreated controls. Inhibition of MDR1 function increased the photodynamic elimination of T cells. (B) Cytotoxicity of PDCT on KG1a cells, an MDR1-expressing cell line, was measured using an LDA. Verapamil alone or PDCT with 5 μM TH9402 had no effect on KG1a cells but combining the inhibition of MDR1 with verapamil to TH9402 PDCT resulted in the elimination of more than 3 logs of clonogenic cells. Results are expressed as mean ± SEM of at least 2 experiments.
Acknowledgments

The authors thank Drs B. Leonard, N. Beauger, and G. Krosi for insightful scientific advice; Dr M. A. Caligiuri for critical review of the manuscript; and C. LeHouiller and the members of the Cell Therapy Laboratory and Apheresis Unit for their excellent technical assistance. We thank all scientists at Thera-
technologies Inc. for their close collaboration and technical support.

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