Elimination of Clonogenic Tumor Cells from HL-60, Daudi, and U-937 Cell Lines by Laser Photoradiation Therapy: Implications for Autologous Bone Marrow Purging

By K.S. Gulliya and Shazib Pervaiz

Lymphomas are a heterogeneous group of tumors involving the lymphoreticular and hematopoietic systems. There were 42,000 cases reported in 1986. The incidence of non-Hodgkin’s disease is three times higher than Hodgkin’s disease in the United States. Like most cancers, lymphomas respond to high doses of chemotherapy at an early stage. However, the relapse rate is very high with subsequent involvement of bone marrow and the central nervous system. The utility of high dose chemotherapy is limited because of its cytotoxicity to the normal marrow. Autologous bone marrow transplantation is done to protect the marrow from cytotoxic effects of chemotherapeutic agents and because of the scarcity of HLA-matched donors in allogeneic transplantation. A recent study demonstrated that patients transplanted with their own cryopreserved marrow suffered more relapses than patients transplanted with marrow from identical twins. This indicates that the cause of relapse may be the tumor cells present in the marrow graft. Therefore, there is a need for methods that can eliminate the residual tumor cells with minimal toxicity toward normal cells. One possible mode of bone marrow purging is merocyanine 540 (MC540)-mediated, laser light-induced photoradiation therapy (LPRT). We recently reported that MC540-mediated LPRT is very effective in killing 99.999% of leukemic cells while 80% of the normal marrow cells and 40% of the granulocyte-macrophage colony-forming cells survived the treatment. Therapeutic efficacy of interferons has been established in various hematologic malignancies. The antiproliferative properties of recombinant interferon alpha (rIFN) on megakaryocytic progenitor cells have been reported recently. Interferons have been shown to alter the plasma membrane density and concentration of some plasma membrane glycoproteins. They also are known to alter the cell membrane fluidity. These interferon-induced alterations could be mediated by changes in membrane-associated functions of the treated cell. In this study we have used LPRT for the elimination of clonogenic lymphomas in an experimental model of simulated marrow graft. The effect of rIFN on LPRT of leukemic and lymphoma cells was investigated because (1) leukemia and lymphoma patients are potential candidates for rIFN therapy, and (2) LPRT has proven effective for the elimination of leukemia and lymphoma cells in an in vitro model.

Materials and Methods

Reagents. rIFN was obtained from Hoffman La Roche (Nutley, NJ). MC540 was obtained from Sigma Chemical Co. (St. Louis). Dilutions of rIFN were made in RPMI-1640 medium just prior to use. A stock solution of MC540 was prepared in 50% ethanol-water at 1 mg/mL and stored in small aliquots in the dark at −20°C. Final dilutions were made in RPMI-1640 medium to obtain desired concentrations.

Light source. A Spectra Physics model 171 ion-laser with model 270 power supply was used.

Cells. The Fab M2 type leukemia cell line (HL-60),3,21 histiocytic lymphoma cell line (U-937), and Burkitt’s lymphoma cell line were used.

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(Daudi) were used in these experiments. The cells were maintained in RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 25 mmol/L Hepes, 0.25 mmol/L L-glutamine, and 100 units penicillin/100 µg streptomycin (Gibco, Grand Island, NY). They were stored at 37°C in a humidified atmosphere of 5% CO2 in air. Cells were maintained in log phase with >95% viability. Normal bone marrow cells were obtained from patients participating in marrow transplantation research protocols.

Laser photoradiation therapy. All experiments were carried out in the presence of human albumin (AB). Human AB was used because in preliminary experiments we determined that in the presence of human AB serum complete elimination of leukemic cells was not attainable at all concentrations (1% to 20%) of AB serum tested. At low concentrations of human AB serum (1% to 5%) the survival of normal marrow cells was significantly reduced. Similar results were obtained with five different sources of human AB serum tested. In one set of experiments, tumor cells and normal bone marrow cells (3 × 106 cells/mL) in RPMI-1640 medium supplemented with 0.25% human AB were mixed with 20 µg/mL or 25 µg/mL of MC540. This cell dye mixture was then placed in 35 x 10 mm falcon petri dishes and exposed to different doses of 514 nm laser light. In an experimental model to test the efficacy of LPRT in eliminating tumor cells from contaminated marrow grafts, gamma ray-irradiated normal bone marrow cells (4,000 rad) were mixed with tumor cells in a ratio of 1:1 or 10:1. This mixture of cells was treated with LPRT as described above. During irradiation the temperature of the cell suspension remained constant because cells were kept on a ½" thick aluminum plate that acted as an effective heat sink. Following irradiation, the cells were washed with RPMI-1640 medium, resuspended in fresh feeding medium, and incubated overnight at 37°C. The viability of cells was determined by trypsin blue dye exclusion method. To assess the effect of IFN, the tumor and marrow cells were mixed with different doses of IFN (30 to 12,000 U/mL) before the addition of MC540 and laser light irradiation. For fluorescence polarization experiments, tumor cells and normal marrow cells were incubated overnight with different doses of IFN.

Colony formation assay. The formation of the bone marrow progenitor cell colony was performed as described in detail elsewhere. Briefly, 1 x 103 cells were added to each culture plate containing 1 mL of 0.8% methylcellulose (Methocel A4M, Dow Chemical Co, Midland, MI), 20% FBS, 0.6 mmol/L 2-mercaptoethanol, 100 units penicillin/100 µg streptomycin, and 100 µL of conditioned growth medium giant cell tumor (GCT) mix (Gibco). For stimulation of GEMM colony formation, 100 µL of phytohemagglutinin-stimulated leukocyte-conditioned medium (Collaborative Research, Bedford, MA) was added on day zero and erythropoietin (Canaught Laboratories, Willowdale, Ontario, Canada) 2.5 IU/mL was added on day 5. For BFU-E and CFU-C, erythropoietin 2.5 IU/mL was added on day zero. CFU-Cs were identified by their red color and discrete tightly packed colonies. Assays for liquid culture were done as described elsewhere. Briefly, treated and untreated bone marrow cells were transfected to gamma ray-irradiated flasks containing a monolayer of stromal cells. At 0-, 7-, and 21-day intervals the cultured cells were drawn and analyzed for CFU-GM as described above. Triplicate cultures were incubated in a humidified atmosphere of 5% CO2 in air. Colonies consisting of 30 or more cells were counted on day 10.

To assay clonogenic tumor stem cells, untreated and LPRT treated cells were plated as described above, except GCT mix was not added and colonies consisting of 50 or more cells were counted on day 7.

Fluorescence polarization. Tumor cells and normal marrow cells (2 x 10⁶ cells/mL) were treated with different doses of IFN (30, 300, 3,000, 6,000, 12,000 U/mL). After overnight incubation, cells were treated with 5 µmol/L (final concentration) of 1,6-diphenyl-1,3,5-hexatriene (DPH) for 30 minutes at 37°C. Fluorocence polarization measurements were taken at room temperature using a T-format polarization fluorometer. This polarizer was equipped with an xenon lamp excitor through a monochrometer set at 350 nm (3-mm bandwidth) and emission selected with Schott KV 389 cut-off filters. The output from the photomultiplier tube was amplified and interfaced to a microcomputer for computation of polarization values. The background signal obtained from cells only (no DPH) was subtracted to get corrected (net) polarization value.

Data analysis. All experiments were performed at least three times, and mean results with standard deviations are reported. The lower limit of accurate cell detection of hemocytometer count was found to be 3,750 cells as described elsewhere. To increase the accuracy of cell detection further, all cultures were followed for a period of 4 weeks, when a tenfold concentrate of the cells was mixed with an equal volume of trypan blue and examined for cell viability. The absence of a living cell was considered a 99.99% cell kill. In a cell-limiting dilution experiment, as few as 10 HL-60 and Daudi, and 15 U-937 cells regrew to an easily detectable number at the end of 4 weeks. In a separate experiment these tumor cells (2 x 10⁵) were photosensitized and viability was monitored for 2 weeks. No living cells were detected at the end of 2 weeks.

RESULTS

Figure 1 shows that in the presence of 20 µg/mL of MC540 and 93.6 J/cm² laser light, 99.999% of HL-60 cells are killed. However, to obtain the same percentage of tumor cell reduction for U-937 cells, an increased dose of MC540 (25 µg/mL) was required. For Daudi cells 31.2 J/cm² of laser light in the presence of 20 µg/mL of MC540 was sufficient for 99.999% cell kill. Under identical conditions the survival of normal marrow cells was 76% and 87%.
Lasers photoirradiation in bone marrow purging

Table 1. Effect of LPRT on Survival of CFU-GM Colony Forming Cells

<table>
<thead>
<tr>
<th>Treatment of Marrow Cells</th>
<th>Colony Formation</th>
<th>No. of Colonies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp. #1</td>
<td>Exp. #2</td>
</tr>
<tr>
<td>Untreated</td>
<td>111.9</td>
<td>110.9</td>
</tr>
<tr>
<td>20 µg/mL MC540</td>
<td>110.4</td>
<td>108.4</td>
</tr>
<tr>
<td>25 µg/mL MC540</td>
<td>105.15</td>
<td>100.5</td>
</tr>
<tr>
<td>31.2 J/cm² laser light</td>
<td>107.15</td>
<td>107.65</td>
</tr>
<tr>
<td>93.6 J/cm² laser light</td>
<td>102.6</td>
<td>99.65</td>
</tr>
<tr>
<td>20 µg/mL MC540</td>
<td>57.62</td>
<td>49.27</td>
</tr>
<tr>
<td>31.2 J/cm² laser light</td>
<td>52.1</td>
<td>64.9</td>
</tr>
<tr>
<td>25 µg/mL MC540</td>
<td>18.95</td>
<td>19.92</td>
</tr>
</tbody>
</table>

For CFU-GM assay 1 x 10⁶ cells were treated with MC540, laser light, or both and washed with RPMI 1640. After overnight incubation, cells were plated in 0.8% methyl cellulose, followed by the addition of GTC mix, as described in Materials and Methods. Colonies with 30 or more cells were counted on day 10. Three replicate culture plates for each experiment were set up and their mean values are shown under respective experiments. The mean values are shown under respective experiments. The mean ± SD of these three experiments are shown.

respectively. These results indicate that LPRT-induced toxicity is preferentially directed toward the leukemic cells. The effect of LPRT on CFU-GM, GEMM, CFU-C, and BFU-E colony formation was also examined. The survival data of normal marrow progenitors are shown in Tables 1 and 2. Results show that BFU-E were the most sensitive whereas the CFU-GM were least sensitive to LPRT. In liquid culture system the LPRT-treated cells show a 20% and 220% increase in CFU-GM generation at seven and 21 days, respectively (Fig 2). Similar results have been reported with lymphoma cells since the viability of Daudi cells was small as compared with the complete abrogation after LPRT. To increase the sensitivity of the clonogenic assay, 20 x 10⁶ phototreated cells were plated. Again, tumor cell colonies were not detected, suggesting that LPRT completely inhibits the growth of clonogenic leukemia and lymphoma cells.

The effect of rIFN on LPRT was tested. Results show (Table 4) that in the presence of 30, 300, and 3,000 U/mL of rIFN, the viability of HL-60 cells increased from 0% to 1.45%, 0.55%, and 0.22%, respectively. However, in the presence of 6,000 and 12,000 units of rIFN, this increase in viability was not observed. Normal marrow cells were treated in an identical manner and the results show that in the presence of 30, 300, and 3,000 U/mL of rIFN the viability of normal marrow cells increased from 75.9% to 94.9%, 96.3%, and 95.6%, respectively. These results suggest that colony-forming normal marrow progenitor cells are significantly less sensitive to LPRT than HL-60, Daudi, and U-937 cells.

In simulated marrow experiments containing a mixture of gamma-irradiated normal marrow cells with leukemia or lymphoma cells (1:1 and 10:1), there was no regrowth of tumor cells for a period of 35 days following LPRT. Untreated cells grew rapidly, indicating that the presence of normal cells did not interfere with the effective killing of tumor cells.

Clonogenic tumor stem cell assay. Results show (Table 3) that there were 254 tumor cell colonies per 10⁶ cells plated in untreated controls for lymphoma cell lines and 261 colonies for leukemia cell line. However, after LPRT, tumor cell colonies were not detected. The reduction in clonogenic tumor stem cells on exposure to MC540, and laser light alone was small as compared with the complete abrogation after LPRT.

Table 2. Effect of LPRT on Survival of CFU-GEMM, BFU-E, and CFU-C Colony Forming Cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CFU-GEMM</th>
<th>BFU-E</th>
<th>CFU-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>MC540 (20 µg/mL)</td>
<td>88.87 ± 13.32</td>
<td>93.04 ± 8.14</td>
<td>61.39 ± 8.98</td>
</tr>
<tr>
<td>MC540 (25 µg/mL)</td>
<td>81.35 ± 10.55</td>
<td>85.09 ± 13.30</td>
<td>61.78 ± 5.04</td>
</tr>
<tr>
<td>MC540 (20 µg/mL + 31.2 J/cm²)</td>
<td>40.09 ± 8.39</td>
<td>33.42 ± 26.86</td>
<td>16.20 ± 15.74</td>
</tr>
<tr>
<td>MC540 (20 µg/mL + 93.6 J/cm²)</td>
<td>20.13 ± 7.63</td>
<td>17.84 ± 2.37</td>
<td>9.05 ± 4.73</td>
</tr>
<tr>
<td>MC540 (25 µg/mL + 93.6 J/cm²)</td>
<td>11.48 ± 3.98</td>
<td>3.86 ± 6.51</td>
<td>1.78 ± 1.47</td>
</tr>
</tbody>
</table>

CFU-GEMM, BFU-E, and CFU-C assays were set up as described in Materials and Methods. Colonies consisting of 30 or more cells were counted on day 14 for CFU-GEMM, and on day 10 for both BFU-E and CFU-C. The mean ± SD of three separate experiments is reported. The results shown are calculated percentages of untreated controls.
of 50 or more cells were counted on day seven (mean ± SD). Cells treated with MC540 and laser light show complete inhibition of colony formation in both cell lines.

**Table 3. Effect of LPRT on Clonogenic Lymphoma and Leukemia Stem Cell Colony Formation**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cells/mL</th>
<th>MC540 (µg/mL)</th>
<th>Laser Light (J/cm²)</th>
<th>No. of Colonies (Mean ± SD)</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-937</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 x 10⁵</td>
<td>—</td>
<td>25</td>
<td>25.24 ± 34.02</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>1 x 10⁶</td>
<td>25</td>
<td>161.00 ± 9.59</td>
<td>63.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 x 10⁷</td>
<td>93.6</td>
<td>204.75 ± 18.03</td>
<td>80.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 x 10⁷</td>
<td>25</td>
<td>93.6</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>20 x 10⁶</td>
<td>25</td>
<td>93.6</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Daudi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 x 10⁵</td>
<td>—</td>
<td>25</td>
<td>25.75 ± 29.30</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>1 x 10⁶</td>
<td>20</td>
<td>156.50 ± 17.84</td>
<td>61.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 x 10⁷</td>
<td>31.2</td>
<td>217.00 ± 26.19</td>
<td>85.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 x 10⁷</td>
<td>20</td>
<td>31.2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>20 x 10⁶</td>
<td>20</td>
<td>31.2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HL-60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 x 10⁵</td>
<td>—</td>
<td>261.00 ± 49.24</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 x 10⁶</td>
<td>20</td>
<td>166.00 ± 25.53</td>
<td>63.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 x 10⁷</td>
<td>93.6</td>
<td>209.00 ± 23.26</td>
<td>80.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 x 10⁶</td>
<td>20</td>
<td>93.6</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

For clonogenic lymphoma and leukemia stem cell assays, treated and untreated cells were plated in 0.8% methylcellulose. Colonies consisting of 50 or more cells were counted on day seven (mean ± SD). Cells treated with MC540 and laser light show complete inhibition of colony formation in both cell lines.

**Table 4. Effect of rIFN-α on Laser Photoradiation of Leukemia, Lymphoma, and Normal Bone Marrow Cells**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cell Type</th>
<th>% Survival (± SD)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC540</td>
<td>BM</td>
<td>75.9 ± 5.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HL-60</td>
<td>0 ± 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>U-937</td>
<td>0 ± 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Daudi</td>
<td>0 ± 0</td>
<td></td>
</tr>
<tr>
<td>MC540 + 30 units rIFN-α</td>
<td>BM</td>
<td>87.2 ± 7.8</td>
<td>.05</td>
</tr>
<tr>
<td></td>
<td>HL-60</td>
<td>1.45 ± 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>U-937</td>
<td>0 ± 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Daudi</td>
<td>0 ± 0</td>
<td></td>
</tr>
<tr>
<td>MC540 + 300 units rIFN-α</td>
<td>BM</td>
<td>90.2 ± 6.1</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>HL-60</td>
<td>0.56 ± 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>U-937</td>
<td>0 ± 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Daudi</td>
<td>0 ± 0</td>
<td></td>
</tr>
<tr>
<td>MC540 + 3,000 units rIFN-α</td>
<td>BM</td>
<td>88.5 ± 7.1</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>HL-60</td>
<td>0.20 ± 0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>U-937</td>
<td>0 ± 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Daudi</td>
<td>0 ± 0</td>
<td></td>
</tr>
<tr>
<td>MC540 + 12,000 units rIFN-α</td>
<td>BM</td>
<td>87.4 ± 2.5</td>
<td>.05</td>
</tr>
<tr>
<td></td>
<td>HL-60</td>
<td>0.000001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>U-937</td>
<td>0 ± 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Daudi</td>
<td>0 ± 0</td>
<td></td>
</tr>
</tbody>
</table>

Laser light dose of 93.6 J/cm² was used for HL-60 and U-937 cells. For Daudi cells the light dose was 31.2 J/cm². 1 x 10⁵ leukemia, lymphoma, and normal cells were treated with rIFN and incubated at 37°C. After overnight incubation, cells were washed and exposed to laser photoradiation therapy as described in Materials and Methods. For HL-60 and U-937 cells, 93.6 J/cm² of laser light was used. For Daudi cells, 31.2 J/cm² of laser light was used. Viability was determined by trypan blue dye exclusion method. Mean ± SD of 4 separate experiments is shown.

were done to determine the membrane fluidity of rIFN-treated lymphoma and leukemia cells. Fluidity-sensitive probe DPH, capable of sensing molecular motions in the hydrocarbon core of bilayer, was used in these experiments. Our results (Table 5) show that in the rIFN treated HL-60 cells (30 to 300 U/mL) and normal marrow cells, the polarization values decreased as compared with the untreated controls. This decrease in fluidity correlated with increase in viability of HL-60 cells and marrow cells. In the presence of 3,000 and 6,000 units of rIFN, the polarization values were essentially unchanged and correlated with no change in viability of HL-60 cells. The polarization values for lymphoma cells remained unchanged or increased (results not shown). These results suggest that rIFN influences the membrane fluidity of leukemic and normal marrow cells, thereby altering the interaction of MC540 with the membrane, resulting in an increased viability.

**DISCUSSION**

Merocyanine 540 is a lipophilic dye that has been used to measure the membrane potential of a variety of cells.35-37 In the presence of serum it preferentially binds to leukemia, lymphoma, neuroblastoma, and certain classes of immature...
daylight sources is virtually eliminated when laser light is

PHOTORADIATION IN BONE MARROW PURGING

compared with 25 μg/mL + 93.6 i/cm² for maximum

study. Our results show that Burkitt's lymphoma (Daudi)

lymphoma cell lines (Daudi and U-937) and a leukemia cell

were used as a model for LPRT purging in this

lymphoma cell lines (Daudi and U-937) and a leukemia cell

to lymphoma cells for their selective killing by LPRT. Two

used.9 We have exploited the preferential binding of MC540

blood cells.38 The nonspecific cytotoxicity observed with
daylight sources is virtually eliminated when laser light is

used.29 We have exploited the preferential binding of MC540

to lymphoma cells for their selective killing by LPRT. Two

lymphoma cell lines (Daudi and U-937) and leukemia cell

line (HL-60) were used as a model for LPRT purging in this

study. Our results show that Burkitt’s lymphoma (Daudi)
cells were more sensitive to LPRT than the histiocytic

lymphoma (U-937) cells because they required a lower dose

of laser light energy and MC540 (20 μg/mL + 31.2 J/cm²

compared with 25 μg/mL + 93.6 J/cm²) for maximum

tumor cell kill. The sensitivity of a cutaneous T-cell lym-

phoma cell line (HUT-102) to LPRT was identical to HL-60

leukemia cell line (unpublished results). The laser light
dose-response curve for all cell lines was biphasic, indicating

more than one population of cells with different sensitivities
to LPRT. The LPRT response data were evaluated by trypan
blue dye exclusion method, which is not a very sensitive

assay. Therefore, in these experiments, LPRT-treated cells

were placed in long-term culture so that living cells that

may have escaped detection, can grow back for ease of detection.

In limiting dilution assays, as few as 10 or 15 living cells grew
back to an easily detectable number in 4 weeks. This
procedure increases the accuracy of cell detection as
described earlier.34 Photoration of lymphoma cells in the

presence of 20 μg/mL or 25 μg/mL of MC540 resulted in > 5

log reduction of clonogenic tumor cells because no colony

formation was observed with 2 x 10⁷ photoirradiated cells.

Photoration of normal marrow cells in the presence of 20

μg/mL and 25 μg/mL MC540 resulted in 87% and 76%

survival of marrow cells and 45.9% and 17.5% survival of

CFU-GM cells, respectively. The long-term liquid culture

with the LPRT-treated cells shows that the regenerating

capacity of the survivors remained intact. Phototoxicity

against leukemia and lymphoma cells was not affected when

they were treated in the presence of gamma ray-irradiated

normal marrow cells. The data obtained in this study show a

significant improvement in normal cell survival when com-

pared with the chemotherapeutic or monoclonal antibody

(MoAb) approach. For example, with 24.6 μg/mL of etopos-

do, only 7.4% CFU-GM survival was reported39 and a

complete inhibition of CFU-GM was obtained with 80 μg/

mL of 4 hydroxycyclophosphamide in phase I clinical trials.40

Bone marrow purging by cytotoxic antibodies or antibody-

toxin conjugates has severe limitations due to tumor

associated antigen modulation and an unpredictability of
developing a tumor cell-specific antibody for each patient.

Therefore, results with the use of MoAbs are short-term at

best, due to a lack of complete elimination of tumor cells.41

Hence, LPRT may provide an improved alternative for

autologous bone marrow purging.

Since many leukemia and lymphoma patients are treated

with rIFN, its effect on LPRT was investigated. The interac-
tion between protein and lipids in the plasma membrane

depends on the microenvironment.42,43 There is a limited

range of membrane fluidity over which the proper physio-

logic functions can occur.44,45 The fluidity measurements

have proven to be of value in the understanding of plasma

membrane functions.44,45 Alterations in membrane fluidity

are known to cause dramatic effects on cellular physiology.

For example, alterations in fluidity affect the functions of

chemotactic receptors of neutrophils.46 The small decrease in

fluidity of 3T3 mouse fibroblast by incorporation of cholest-
yl hemisuccinate increases the passive and the insulin-

stimulated transport of glucose and amino acids, whereas

further decrease in fluidity reverses this process.47 These

reports document the small physiologic range of membrane

fluidity that is essential for its optimal function. Results

show that the presence of low doses of rIFN in the irradiation

mixture interfere with the complete elimination of leukemia

cells. The fluorescence polarization data indicate that rIFN

also increases the membrane fluidity of these cells. Similar

effects were observed in normal marrow cells; however, the

lymphoma cells remained unaffected at all doses of rIFN

tested. While the increase in viability of normal cells by itself

could be considered a plus, the increase in viability of

leukemia cells is a major concern, since very small numbers

of residual tumor cells can grow back and cause relapse.

Although the evidence presented in this paper suggests that

rIFN does influence the membrane fluidity of leukemia cells,

the mechanism of change in membrane fluidity remains a

complex unanswered question, especially when one consid-

ers the differential response of leukemia and lymphoma cells.
The two most important objectives for successful marrow

purging are (1) 100% tumor cell kill, as incomplete purging

is associated with high relapse rates42; and (2) survival of

maximum number of normal cells, as reconstitution of the

marrow and the recovery rate of myelosuppressed patients

are primarily dependent upon high survival rates of normal

cells. These objectives are accomplished by LPRT. The

presence of rIFN could inhibit the complete elimination of

leukemia cells by LPRT; however, we believe that this laser

photoration therapy may be useful in eliminating occult
tumor cells from contaminated marrow grafts.

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Table 5. Fluorescence Polarization of rIFN-α Treated Normal
Bone Marrow Cells and Leukemic Cells

<table>
<thead>
<tr>
<th>rIFN-α (IU/mL)</th>
<th>Bone Marrow Cells Polarization Value ± SD</th>
<th>HL-60 Cells Polarization Value ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.4031 ± 0.0010</td>
<td>0.3646 ± 0.0036</td>
</tr>
<tr>
<td>30</td>
<td>0.3986 ± 0.0027</td>
<td>0.3569 ± 0.0017</td>
</tr>
<tr>
<td>300</td>
<td>0.3986 ± 0.0027</td>
<td>0.3602 ± 0.0017</td>
</tr>
<tr>
<td>3,000</td>
<td>0.3945 ± 0.0046</td>
<td>0.3647 ± 0.0012</td>
</tr>
<tr>
<td>6,000</td>
<td>0.3986 ± 0.0026</td>
<td>0.3642 ± 0.0011</td>
</tr>
<tr>
<td>12,000</td>
<td>0.3969 ± 0.0022</td>
<td>0.3647 ± 0.0016</td>
</tr>
</tbody>
</table>

1 x 10⁶ cells/mL were incubated with 0, 30, 300, 3,000, 6,000, and
12,000 units of rIFN-α for 24 hours. Cells were then washed with
RPMI-1640, and 1,6-diphenyl-1,3,5 hexatriene probe (5 μmol/L final
conc.) was added. After 30 minutes of incubation at 37°C, fluorescence
polarization measurements were conducted at room temperature. Correc-
ted polarization values of a typical experiment (obtained after subtracting
the background signal from cells only) ± SD are shown.
REFERENCES


Elimination of clonogenic tumor cells from HL-60, Daudi, and U-937 cell lines by laser photoradiation therapy: implications for autologous bone marrow purging

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