Autologous Transplantation of Bone Marrow Purged In Vitro With Anti-CD7-(WT1-) Ricin A Immunotoxin in T-Cell Lymphoblastic Leukemia and Lymphoma


Seven patients with high-risk acute T-cell lymphoblastic leukemia (T-ALL) and six with T cell lymphoma (T-LL) were treated with autologous bone marrow transplantation (ABMT) after in vitro purging of their bone marrow with WT1 (CD7)-ricin A-chain immunotoxin. CD7 expression on the tumor cells showed large variations between the individual patients and was highly related to the specific cytotoxicity of WT1-ricin A. Incubation of bone marrow with up to 10^-5 mol/L WT1-ricin A in the presence of 6 mmol/L NH4Cl did not compromise the growth potential of the hematopoietic progenitors CFU-GM, CFU-GEMM, and BFU-E. Hematologic engraftment (>10^8 leukocytes/L) occurred in normal time periods (median, 17 days). Seven patients are alive and in complete remission (CR) at 48+, 44+, 40+, 26+, 11+, 7+, and 6+ months after ABMT. Two of them had the lowest CD7 expression on their tumor cells, the other two were transplanted in CR2 and CR3. Two patients died from transplantation related infections. The immunologic reconstitution was delayed, although the numbers of T cells reached normal levels within 1 month. The number of CD7^+ cells remained low up to 1 year after transplantation. The T4/T8-ratio was increased for at least 6 months. The T-cell response to mitogens recovered to normal levels after 1 year. This study shows that ABMT with WT1-ricin A purged bone marrow in high-risk T-cell malignancies results in a complete hematopoietic and a delayed immunologic reconstitution. The actuarial relapse free survival is 61% at 3 years.

A UTLOGOUS BONE MARROW transplantation (ABMT) is increasingly applied as alternative treatment for patients with acute T-cell lymphoblastic leukemia (T-ALL) or lymphoma (T-LL) who lack an HLA-compatible donor. Bone marrow, even when aspirated during complete remission (CR), is probably contaminated with residual malignant T cells, and these may be responsible for relapse after ABMT. Various techniques have been developed to eliminate in vitro the malignant cells from the graft. Purging of the bone marrow with ricin A-chain immunotoxins (ITs) specifically directed against the malignant T cells appeared to be a promising approach to improve the therapeutic efficacy of ABMT. Recently we showed that CD7 is a suitable antigen as target for ITs since CD7 is expressed in most T-cell malignancies. The IT WT1-ricin A (anti-CD7) appeared to eliminate very effectively malignant T cells from the bone marrow, in part due to continuous internalization of the antigen-IT complex by the cells, provided that the CD7 density on the cell surface was high. We demonstrated that WT1-ricin A in the presence of 6 mmol/L ammonium chloride induces more than 6-logs kill of the malignant T-cell line "CEM".

In this report we give clinical and experimental data of ABMT, using bone marrow purged in vitro with WT1-ricin A, in 13 patients suffering from T-ALL and T-LL in CR. All patients with one or more bad risk factors were assigned to transplantation.

MATERIALS, METHODS, AND PATIENTS

Immunotoxin

Ricin A was kindly provided by Dr F.K. Jansen, Centre de Recherches Clin Midy, Montpellier, France. The murine anti-human T cell monoclonal antibody (MoAb): WT1, anti-CD7 (kindly provided by Dr W. Tax), was conjugated to ricin A by means of succinimidyl 3-(2-pyridyldithio) propionate (SPDP; Pharmacia Ltd, Uppsala, Sweden) as described previously.

Antigen Density

WT1 was labeled with 125Iodine (Amersham International, Amersham Bucks, UK) using the chloramine T method. The concentration and specific activity of the 125I-labeled MoAb were determined respectively radioimmunoassay and binding assay with limiting quantities of labeled antibody. The absolute number of binding sites per cell was determined as described previously.

Cytotoxicity of WT1-Ricin A

Fresh or thawed cryopreserved leukemic cells were incubated in Eagle's modified Minimum Essential Medium (EMEM) without leucine (Flow Labs, Irvine, Scotland) supplemented with 5% fetal calf serum (FCS), 2 mmol/L glutamine, 1 mmol/L sodium pyruvate and gentamycin (50 μg/mL). Each well of U-bottomed plates (Costar, Cambridge, MA) was filled with 10^6 cells supplemented with varying concentrations of IT in the absence or presence of 6 mmol/L NH4Cl to a final volume of 200 μL. Cells were incubated for 24 hours at 37°C in a humidified incubator with 5% CO2 in air, followed by 24 hours incubation with 0.5 μCi 3H leucine (TRK510, Amersham). Cells were harvested and radioactivity was counted. The cytotoxicity was expressed as percentage inhibition of 3H leucine incorporation of untreated cells corrected for the background value determined in the presence of 1 mmol/L cycloheximide.

Cytotoxicity to Bone Marrow Progenitor Cells

Bone marrow was aspirated from patients undergoing cardiac surgery after informed consent was obtained and anticoagulated.
with acid citrate dextrose (ACD). Erythrocytes and mature granulocytes were removed by gradient centrifugation on Ficoll (1.077 g/mL). Cells (10^6/mL) were incubated with ITs (10^-6 mol/L) with or without 6 mmol/L ammonium chloride for 24 hours. Cells were washed and the decrease of bone marrow progenitors was determined in clonogenic assays of granulocyte/macrophage and granulocyte/erythocyte/macrophage/megakaryocyte colony-forming cells (CFU-GM and CFU-GEMM, respectively), and erythroid burst-forming cells (BFU-E) as described previously.22

Patients

ABMT was performed in 13 patients with high-risk T-cell malignancies: seven T-ALL and six T-LL. Reasons for assignment to transplantation included one or more of the following risk factors: for T-ALL age of 30 years or older, leukocyte counts at diagnosis > 30 x 10^9/L, hepato/splenomegaly, CNS involvement and status of disease; for T-LL stage 4 of disease with > 300 U/L lactic dehydrogenase (LDH) > 300 U/L and hepato/splenomegaly. Patients no. 8, 9, 11, and 13 with T-LL had no CNS localization but bone marrow involvement and either splenomegaly or LDH > 300 U/L. Before ABMT nine patients were in CR1, one in CR2, and three in CR3 (Table 1). None of these patients had an HLA-identical sibling donor. The mean age was 21 years (range, 10 to 35 years). Data of phenotype of the malignant T cells and clinical status determined in blood count analysis and cell numbers were published during hypoplasia to define the day of engraftment (leukocyte count > 1 x 10^9/L). Platelet levels were maintained above 20 x 10^9/L with irradiated (20 Gy) platelet transfusions.

Follow-up of T-cell recovery. Heparinized venous blood and bone marrow aspirates (sternum) were obtained from every patient 1, 3, 6, and 12 months posttransplantation. Hematologic reconstitution and cell numbers were determined in blood count analysis and morphology of bone and marrow smears.

The reconstitution of lymphocyte subpopulations was determined by flow cytometry of the lymphocyte scatter area in relation to indirect immunofluorescence of bound MoAbs. The following anti-T-cell MoAbs were used: WT32 (CD3),24 WT1 (CD7),25 and WT82 (CD8)25 provided by Dr W. Tax; OKT6 (CD1),25 OKT4 (CD4),25 OKT3 (CD3),25 OKT1 (CD5),25 from ORTHO Diagnostic Systems Inc (Raritan, NJ). Additionally the following were used: OKB2, identifying B-cells, pre-B cells, and granulocytes; OKB7, identifying B cells expressing the 175 Kd antigen; OKM1 (CD11)27 and OKM5,
recognizing monocytes and platelets expressing the 88 Kd antigen. After the initial incubation with MoAbs, cells were incubated with FITC-labelled goat anti-mouse IgG (Fab')2 fragment (GAM-FITC; American Qualex International Inc, La Mirada, CA).

The functional reconstitution of the T lymphocytes was determined in stimulation assays carried out in 96-well U-bottom plates. Each well was filled with 10^6 cells in culture medium in the absence or the presence of 40 µg/mL phytohaemagglutinin (PHA) or 15 µg/mL concanavalin A (Con A) to a final volume of 200 µL and incubated at 37°C in a humidified atmosphere with 5% CO₂ in air for 48 hours, followed by a labeling with 0.5 µCi ^3H thymidine (TRK 61, Amersham) for 24 hours. Thereafter cells were harvested and radioactivity was counted. Stimulation was expressed as percentage ^3H thymidine incorporation by PHA or Con A stimulated lymphocytes of normal individuals, all corrected for background incorporation of unstimulated cells.

Statistical analysis. Differences in leukocyte number and hematopoietic progenitors before and after IT treatment were analyzed by the matched-pair t test.

After ABMT patients with no evidence of relapse were classified as being in CR. The overall survival and the probability of relapse posttransplant were evaluated by the Kaplan-Meier method.

RESULTS

Cytotoxicity of WTI-Ricin A to Malignant T Cells In Vitro in Relation to Antigen Density

The cytotoxic potency of WTI-ricin A in the presence of 6 mmol/L NH4Cl was tested on the malignant cells of 12 of 13 patients and expressed as dose required for 50% reduction of protein synthesis (ID50) (Table 2). Malignant cells before initial therapy of patient no. 4 were not available for cytotoxicity studies. Protein synthesis was inhibited mainly depending on antigen density of CD7 on the cell surface. The average antigen density varied individually from 9,000 to 250,000 molecules per cell. As described previously, a strong correlation was found between cytotoxicity and antigen density of CD7. Malignant cells from patients with C-ALL and B-ALL without CD7 expression were used as controls.

Effect of WTI-Ricin A on Hematopoietic Progenitors

The cytotoxicity of WTI-ricin A with or without NH4Cl to hematopoietic progenitors was determined by means of clonogenic assays. Cells were incubated with 10^{-4} mol/L WTI-ricin A, the dose used to treat large amounts of bone marrow. In comparison with untreated cells no influence of IT treatment in the presence of 6 mmol/L NH4Cl was found on the plating efficiency of CFU-GM (97 ± 11%), CFU-GEMM (101 ± 11%), and BFU-E (94 ± 10%) as determined in five independent experiments.

Purging of Bone Marrow

Average 5.4 x 10^8 (range, 2.8 to 9.8 x 10^8) low density marrow cells (d < 1.070 g/mL) were incubated with 10^{-8} mol/L WTI-ricin A in the presence of 6 mmol/L NH4Cl. This resulted in a 10% reduction of nucleated cells, and 26% reduction of CFU-GM and BFU-E, that was probably not caused by cytotoxicity of the IT but largely due to the procedure itself. Patients received a mean cell dose per kg of 7.3 ± 2.7 x 10^7 (range, 4.5 to 13.4 x 10^7) nucleated cells, 10.5 ± 9.5 x 10^9 (range, 1.9 to 37.6 x 10^9) CFU-GM, and 17.2 ± 11.2 x 10^9 (range, 3.7 to 42.9 x 10^9) BFU-E.

Hematologic Engraftment

All patients engrafted after reinfusion of WTI-ricin A purged bone marrow. The mean day of recovery of leukocyte count to >10^9/L was day 17.3 ± 5.0 (median, 17; range, 9 to 25 days) (Table 3). After recovery of leukocytes to >10^9/L patient no. 6 was treated with ganciclovir, fluoronazole, and flucytosine because of the suspicion of cytomegalovirus (CMV) infection and a proven Candidemia. Subsequently leukocyte count dropped and the patient died in aplasia. Platelet recovery (>50 x 10^9/L) was delayed in three patients (patients no. 8, 10, and 11) and was not evaluable in two patients (no. 6 and 9). The median recovery occurred on day 40 (range, days 14 through 270). No relation was found between the state of disease, the number of reinfused hematopoietic progenitor cells (CFU-GM and BFU-E), or the day of engraftment.

Survival

Clinical data of survival after ABMT are shown in Table 3. Nine of the 13 patients are alive; two of them (patients no. 5 and 7) relapsed within 5 months after ABMT in CR2 and CR3. Seven patients are still in CR (6+, 7+, 11+, 26+, 40+, 44+, 48+ months). Four patients have died; two patients died 1 month after recurrence of leukemia, one patient died by a Candida and CMV infection (patient no. 6), and one patient by Streptococcus viridans and CMV infection (patient no. 9).

The calculated overall survival at 3 years is 66.6% and the probability of relapse-free survival is 61.3% (Fig 1). The four relapses were found in patients treated for T-ALL: two of these patients (nos. 1 and 4) had a low CD7 expression on their malignant cells and the other two patients were transplanted in CR2 and CR3, respectively. In the group of T-LL patients, five of the six patients are alive and disease free 6, 7,

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Ag-density (mol/cell x 10^{-3})</th>
<th>ID50 (mol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>10^{-4}</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>4 x 10^{-10}</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>10^{-11}</td>
</tr>
<tr>
<td>4</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>4 x 10^{-11}</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>2 x 10^{-10}</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>3 x 10^{-11}</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>3 x 10^{-10}</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>1.5 x 10^{-11}</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td>10^{-11}</td>
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<tr>
<td>11</td>
<td>11</td>
<td>7 x 10^{-12}</td>
</tr>
<tr>
<td>12</td>
<td>30</td>
<td>10^{-10}</td>
</tr>
<tr>
<td>13</td>
<td>48</td>
<td>6 x 10^{-11}</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not determined.

*Antigen density of CD7 on the malignant cells of the patients as measured by ^125I-WTI expressed in average number of molecules per cell.

†ID50 of WTI-ricin A in the presence of 6 mmol/L NH4Cl.
Table 3. Clinical Data After ABMT

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnosis Before ABMT</th>
<th>Engraftment* (days)</th>
<th>Status After ABMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T-ALL; CR1</td>
<td>14</td>
<td>Died after 7 mo in relapse (6 mo post-ABMT).</td>
</tr>
<tr>
<td>2</td>
<td>T-ALL; CR1</td>
<td>17</td>
<td>Alive and CR, &gt;48 mo</td>
</tr>
<tr>
<td>3</td>
<td>T-ALL; CR1</td>
<td>14</td>
<td>Alive and CR, &gt;40 mo</td>
</tr>
<tr>
<td>4</td>
<td>T-ALL; CR1</td>
<td>9</td>
<td>Died after 7 mo in relapse (6 mo post-ABMT).</td>
</tr>
<tr>
<td>5</td>
<td>T-ALL; CR2</td>
<td>21</td>
<td>Alive and relapse (5 mo post-ABMT), &gt;15 mo</td>
</tr>
<tr>
<td>6</td>
<td>T-ALL; CR1</td>
<td>13</td>
<td>Died after 51 days by Candida and CMV.</td>
</tr>
<tr>
<td>7</td>
<td>T-ALL; CR3</td>
<td>14</td>
<td>Alive and relapse (5 mo post-ABMT), &gt;12 mo</td>
</tr>
<tr>
<td>8</td>
<td>T-LL 4A; CR1</td>
<td>13</td>
<td>Alive and CR, &gt;44 mo</td>
</tr>
<tr>
<td>9</td>
<td>T-LL 4A; CR3</td>
<td>20</td>
<td>Died after 61 days by Streptococcus viridans and CMV.</td>
</tr>
<tr>
<td>10</td>
<td>T-LL 4A; CR3</td>
<td>25</td>
<td>Alive and CR, &gt;26 mo</td>
</tr>
<tr>
<td>11</td>
<td>T-LL 4A; CR1</td>
<td>21</td>
<td>Alive and CR, &gt;11 mo</td>
</tr>
<tr>
<td>12</td>
<td>T-LL 4A; CR1</td>
<td>25</td>
<td>Alive and CR, &gt;7 mo</td>
</tr>
<tr>
<td>13</td>
<td>T-LL 4A; CR1</td>
<td>19</td>
<td>Alive and CR, &gt;6 mo</td>
</tr>
</tbody>
</table>

Abbreviations: ABMT, autologous bone marrow transplantation; CR, complete remission; T-ALL, acute T-cell lymphoblastic leukemia; T-LL, T-cell lymphoblastic lymphoma; NE, not evaluable.

*Recovery of leukocyte (WBC) counts to >10⁹/L and platelet counts to >50 × 10⁹/L in days after ABMT.

Immunologic Recovery

The patients who survived more than 1 year were followed for their hematologic and immunologic recovery (Table 4). Leukocyte counts normalized more than 6 months after ABMT, partly due to a longlasting granulocytopenia, whereas lymphocytes and monocytes achieved normal levels within 1 month. The immunologic recovery was delayed for at least 1 year as determined by flow cytometric analysis of the lymphocyte phenotypes. CD3⁺ and CD5⁺ cells returned to normal levels within 1 month after ABMT. However, the T4/T8-ratio was reversed for more than 6 months and did not return to normal levels for at least 1 year. This decreased T4/T8-ratio was due to a low number of CD4⁺ cells, whereas CD8⁺ cells returned to normal levels within 1 month. The number of CD7⁺ cells did not reach normal values for at least 1 year. The functional recovery was tested by responsiveness to mitogens compared with normal individuals (Table 5). T cells hardly responded to mitogens in the first 3 months, but returned to normal within 1 year after ABMT.

DISCUSSION

Bad risk criteria in patients with ALL and lymphoma are not precisely defined. Age of 30 years or older, leukocytosis of 30 × 10⁹/L, hepato/splenomegaly, CNS involvement, and LDH level of >300 U/L can be considered bad prognostic signs at time of presentation according to Hoelzer et al., Herzig et al., and Coleman et al. Therefore these patients and patients with more advanced disease can be considered as candidates for ABMT with or without in vitro purging. MoAbs with complement lysis are most commonly used for purging; however, ITs often appear more efficient in cell killing. The cytotoxicity of WT1-ricin A is highly related to the CD7 density on malignant T cells. Previously we demonstrated that WT1-ricin A in the presence of 6 mmol/L ammonium chloride induces more than 6-logs kill of the malignant T cell line CEM. CEM expresses intermediate amounts of CD7 comparable with malignant T cells of most patients, which may imply effective reduction of the residual leukemic cells in the bone marrow.

A prolonged purging procedure of bone marrow with IT may be harmful to the hematopoietic stem cells. We have chosen an incubation period of 16 hours based on kinetics of cytotoxicity and internalization of cell bound IT. We showed that WT1-ricin A is continuously internalized coinciding with increasing cytotoxicity. In the present and in other studies, no cytotoxicity of IT to hematopoietic precursors.
The delayed immunologic recovery appeared not to be 1 156
occurred rapidly compared with reports of hematopoietic precursors with the effects of cytostatic drugs. 1433 The slight reduction sors otherwise purged autologous induced by WT1-ricin A because other investigators have bone marrow (median, day 17). The marrow recoveries caused by intensive manipulation of the marrow. probably the platelet recovery to trast precursor cells were comparable with syngeneic marrow engraftment.36
three patients. After 1 month, functional recovery, detected of T-cell subsets is mainly tion by cells of normal individuals ±
response to mitogens, remained low for almost 1 year. CD7 cells remained low for at least 6 months. Data of de Gast et al.37
although the number of T lymphocytes attained normal
response to mitogens, remained low for almost 1 year. CD7 cells remained low for at least 6 months. Data of de Gast et al.37 suggesting that the recovery of T-cell subsets is mainly due to the proliferation of mature T cells in the graft, may be an explanation for this observation. No T cells with an immature phenotype (CD1) were detected after 1 month. Functional recovery, as measured with response to mitogens, remained low for almost 1 year. This may be due to a defective T-helper function as observed by Armitage et al38 resulting in a decreased T4/T8-ratio. The delayed immunologic recovery appeared not to be induced by WT1-ricin A because other investigators have found similar results in ABMT with other purging procedures or even without purging43,39 and in allogeneic BMT.40
Relapse is at date only seen in a group of patients with T-ALL. Whether the recurrence of leukemia was caused by residual leukemic cells in the patient or in the bone marrow graft cannot be determined. It may be of interest that two of the relapsed patients had initially a low CD7 expression on the malignant cells. The susceptibility to WT1-ricin A appeared not to be changed in relapse (data not shown). Our results suggest that only leukemias with high antigen density on the cell surface should be selected for purging with ITs in order to improve the results of ABMT with immunologically purified bone marrow.41,42
Statistical evaluation of survival after ABMT is virtually impossible because of the low number of patients and the heterogeneous group of diseases. Though the results of survival are preliminary, 43% of patients transplanted for T-ALL (seven patients) remained in CR, which is comparable with data of Gorin et al.1 In the group of T-LL none of the patients relapsed. Although this group is too small for statistical analysis our results seem in accordance with those of others obtained in lymphoblastic lymphoma.3,4
The advantage of bone marrow purging is difficult to assess due to the various methods used for marrow purging and the small number of patients studied. Gorin et al49 demonstrated by analysis of 125 patients with ALL in first CR that, although no statistically significant advantage for purging exists, patients who received purged bone marrow had a higher percentage of disease-free survival in 3 years (58%) than patients with nonpurged bone marrow (30%).
We conclude that purging of bone marrow with WT1-ricin A before ABMT is a safe therapeutic option. It may be the treatment of choice for patients with high risk T-cell malignancies.

Table 4. Hematologic and Immunologic Recovery in Patients Who Survived More Than 1 Year After ABMT

<table>
<thead>
<tr>
<th>Months After ABMT</th>
<th>1</th>
<th>3</th>
<th>6</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte</td>
<td>1.8 ± 1.0</td>
<td>2.5 ± 1.3</td>
<td>3.8 ± 1.4</td>
<td>4.8 ± 1.4</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>0.73 ± 0.30</td>
<td>0.96 ± 0.56</td>
<td>0.95 ± 0.57</td>
<td>1.37 ± 0.68</td>
</tr>
<tr>
<td>CD1</td>
<td>0.02 ± 0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CD3</td>
<td>0.68 ± 0.35</td>
<td>0.52 ± 0.30</td>
<td>0.64 ± 0.38</td>
<td>0.88 ± 0.50</td>
</tr>
<tr>
<td>CD4</td>
<td>0.28 ± 0.09</td>
<td>0.18 ± 0.06</td>
<td>0.21 ± 0.04</td>
<td>0.45 ± 0.23</td>
</tr>
<tr>
<td>CD5</td>
<td>0.52 ± 0.29</td>
<td>0.41 ± 0.25</td>
<td>0.64 ± 0.38</td>
<td>0.85 ± 0.50</td>
</tr>
<tr>
<td>CD7</td>
<td>0.12 ± 0.01</td>
<td>0.23 ± 0.12</td>
<td>0.29 ± 0.19</td>
<td>0.67 ± 0.70</td>
</tr>
<tr>
<td>CD8</td>
<td>0.43 ± 0.28</td>
<td>0.35 ± 0.21</td>
<td>0.34 ± 0.21</td>
<td>0.42 ± 0.25</td>
</tr>
<tr>
<td>Anti-B cell</td>
<td>0.08 ± 0.06</td>
<td>0.05 ± 0.03</td>
<td>0.09 ± 0.06</td>
<td>0.15 ± 0.08</td>
</tr>
</tbody>
</table>

Values are expressed as mean percentage of cells from normal individuals ± SD. Percentage of cells is given in parentheses.

Table 5. Functional Recovery of Lymphocytes in Patients After ABMT

<table>
<thead>
<tr>
<th>Months After ABMT</th>
<th>1</th>
<th>3</th>
<th>6</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHA</td>
<td>3 ± 3</td>
<td>14 ± 2</td>
<td>42 ± 10</td>
<td>101 ± 11</td>
</tr>
<tr>
<td>Con A</td>
<td>2 ± 3</td>
<td>8 ± 6</td>
<td>41 ± 27</td>
<td>97 ± 9</td>
</tr>
</tbody>
</table>

Values are expressed as mean percentage of 3H thymidine incorporation by cells of normal individuals ± SD of five measurements. Average incorporation by lymphocytes of five normal individuals was 37,104 ± 1,441 cpm after PHA stimulation and 26,691 ± 2,608 cpm after Con A stimulation.
REFERENCES


4. Philip T, Armitage JO, Spitzer G, Chauvin F, Jagannath S, Cahn JY, Colombat P, Goldstone AH, Gorin NC, Flesh M, Laporte SF, deoxyadenosine, 3’-1 monoclonal antibody, cells from human bone marrow using 2’-deoxycoformyly-


Autologous transplantation of bone marrow purged in vitro with anti-CD7-(WT1-) ricin A immunotoxin in T-cell lymphoblastic leukemia and lymphoma

FW Preijers, T De Witte, JM Wessels, GC De Gast, E Van Leeuwen, PJ Capel and C Haanen