Intensive Cytotoxic Therapy Followed by Autologous Bone Marrow Transplantation for Non-Hodgkin’s Lymphoma of High-Grade Malignancy

By Leo F. Verdonck, Adriaan W. Dekker, M. Loes van Kempen, Kees Punt, Jan A.M. van Unnik, Henny A. van Peperzeel, and Gijsbert C. de Gast

Fourteen patients with non-Hodgkin’s lymphoma (NHL) of high-grade malignancy were treated with cyclophosphamide and total body irradiation followed by autologous bone marrow transplantation (ABMT). All patients were pretreated with conventional chemotherapy. Three of four patients with drug-resistant disease achieved complete remission (CR), but relapse occurred within six months. Four patients in partial remission (PR) achieved CR; one died because of sepsis, two relapsed within six months, and one is still in CR 28 + months later. Six were treated in CR, five in first CR, and one in second CR. From these six patients (who received this treatment as consolidation therapy), five are in unmaintained CR seven to 31 + months after ABMT (one patient died of a secondary illness). There were two therapy-related deaths, both in patients with a poor clinical condition. Toxicity of this treatment was mild for those receiving transplants who were in better condition. These preliminary results suggest that intensive cytoreductive therapy followed by ABMT may improve disease-free survival in patients in NHL of high-grade malignancy in CR.

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MATERIALS AND METHODS

Patients

Fourteen patients with an NHL of high-grade malignancy, according to the Kiel classification and “Working Formulation,” were studied between Nov 1, 1981, and Aug 1, 1984. Details of the patients are summarized in Table 1. The stage of the disease, according to the Ann Arbor, Mich, classification, was determined at diagnosis and again just before the ABMT procedure. Twelve of 14 patients had bulky disease (>10 cm) at presentation. Bone marrow involvement was assessed by bone marrow biopsies from both posterior iliac crests. Bone marrow involvement at presentation was only found in patient No. 8 and disappeared rapidly after second chemotherapy with hydroxydaunorubicin, Oncovin (Lilly, Indianapolis), and cytosine arabinoside (HOC). All patients received cyclophosphamide, hydroxydaunorubicin, Oncovin, and prednisone (CHOP) as initial treatment except patients No. 1 and 7. Additional treatment (Table 1) was given when no response or progression occurred. Apart from patients No. 1, 3, and 5, all received CNS prophylaxis with three injections of 15 mg methotrexate intrathecally. One patient (No. 4) showed CNS disease at the first relapse, simultaneously with a leukemic phase. Meningeal and probably also bone marrow involvement disappeared after therapy with HOC and a PR was achieved.

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Supported by a grant from the Stichting Koningin Wilhelmina Fonds, Nederlandse Organisatie voor de Kankerbestrijding (UUKC-Haem 81).

Submitted June 12, 1984; accepted Sept 29, 1984.

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0006-4971/85/0604-0004$03.00/0
Bone marrow was used as described by Appelbaum et al.²¹ with slight modifications. Briefly, bone marrow was obtained by multiple aspirations from both iliac crests under general anesthesia and were anticoagulated (preservative-free heparin). The suspension was filtered and centrifuged (Haemotetics 30 blood cell separator) to obtain the buffy coat. This was placed in plastic blood bags (50 mL, medium) and cooled to 100°C in a programmed freezer (Cryocon) at a rate of 1 to 2°C/min. The marrow was then stored in the vapor phase of a nitrogen freezer. The bone marrow was aspirated and cryopreserved nine days to 14 months prior to transplantation, marrow biopsies not being negative for lymphomatous infiltration at that time (bone marrow aspiration in patient No. 4 was done early in first CR). The bone marrow aspirates contained a mean of 1.8 x 10⁶ nucleated cells per kilogram (range, 1.2 to 2.3 x 10⁶/kg), which contained a mean of 1.1 x 10⁴ CFU-GM per kilogram (range, 0.4 to 2.4 x 10⁵/kg).

Cytoreductive Regimen

All patients received cyclophosphamide (Cy) 60 mg/kg; days -4 and -3) and total body irradiation (TBI) 800 rad, 16 to 18

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Time of Diagnosis to BMT (mo)</th>
<th>Status at BMT</th>
<th>Response</th>
<th>Duration of CR (mo)</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>Drug resistant</td>
<td>CR</td>
<td>2</td>
<td>CMV-IP (relapse)</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>1st CR</td>
<td>CR</td>
<td>31-</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>PR</td>
<td>CR</td>
<td>28-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>PR</td>
<td>CR</td>
<td>21 d</td>
<td>Sepsis (no relapse)</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>Drug resistant</td>
<td>CR</td>
<td>4</td>
<td>Relapse</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>Drug resistant</td>
<td>CR</td>
<td>5</td>
<td>Relapse</td>
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<tr>
<td>7</td>
<td>12</td>
<td>Drug resistant</td>
<td>PR</td>
<td>7</td>
<td>Progression</td>
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<tr>
<td>8</td>
<td>5</td>
<td>1st CR</td>
<td>CR</td>
<td>7</td>
<td>ANLL (secondary?; no relapse)</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>PR</td>
<td>CR</td>
<td>5</td>
<td>Relapse</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>PR</td>
<td>CR</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>7</td>
<td>1st CR</td>
<td>CR</td>
<td>14</td>
<td></td>
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<tr>
<td>12</td>
<td>6</td>
<td>1st CR</td>
<td>CR</td>
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<td>7</td>
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<td>CR</td>
<td>10</td>
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</tr>
<tr>
<td>14</td>
<td>6</td>
<td>2nd CR</td>
<td>CR</td>
<td>7-</td>
<td></td>
</tr>
</tbody>
</table>

CMV-IP, cytomegalovirus-induced interstitial pneumonitis; ANLL, acute nonlymphocytic leukemia.

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex/Age</th>
<th>Histology (Initial Stage, Symptoms)</th>
<th>Site of Initial 'Bulky' Disease*</th>
<th>Prior Therapy†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/53</td>
<td>B-centroblastic (III, B): diffuse, large cell, noncleaved</td>
<td>Mediastinum 6 x COP; 7 x CHOP; 1 x MOPP</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M/20</td>
<td>T-lymphoblastic (IV, B): lymphoblastic, convoluted cell</td>
<td>Mediastinum 3 x CHOP</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F/35</td>
<td>B-centroblastic (III, A): diffuse, large cell, noncleaved</td>
<td>Abdominal 8 x CHOP</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>F/19</td>
<td>T-lymphoblastic (IV, B): lymphoblastic, convoluted cell</td>
<td>Mediastinum 9 x CHOP; 2 x HOC</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M/39</td>
<td>B-immunoblastic (II, A): large cell, immunoblastic</td>
<td>Mediastinum 3 x CHOP; 1 x MOPP</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M/33</td>
<td>T-lymphoblastic (IV, B): lymphoblastic, nonconvoluted cell</td>
<td>Mediastinum 4 x CHOP; 1 x MOPP</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>M/15</td>
<td>T-lymphoblastic (IV, B): lymphoblastic, convoluted cell</td>
<td>Mediastinum 1 x CHOP; 1 x ID-ARA-C</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>M/23</td>
<td>T-lymphoblastic (IV, A); lymphoblastic, nonconvoluted cell</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>M/38</td>
<td>True histiocytic (IV, B); histiocytic</td>
<td>Abdominal 7 x CHOP</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>M/27</td>
<td>T-immunoblastic (II, B): large cell, immunoblastic</td>
<td>Mediastinum 4 x CHOP</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>M/22</td>
<td>T-lymphoblastic (IV, B); lymphoblastic, nonconvoluted cell</td>
<td>Mediastinum 6 x CHOP</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>M/24</td>
<td>B-lymphoblastic (IIE, A); small cell, noncleaved, Burkitt's type</td>
<td>—</td>
<td>5 x CHOP</td>
</tr>
<tr>
<td>13</td>
<td>F/48</td>
<td>B-centroblastic (IV, A); diffuse, large cell, noncleaved</td>
<td>Mediastinum 6 x CHOP</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>M/19</td>
<td>T-immunoblastic (III, A); large cell, immunoblastic</td>
<td>Mediastinum 3 x CHOP; 1 x VAMP</td>
<td></td>
</tr>
</tbody>
</table>

Histology was determined by Kiel classification,²⁹ and by working formulation.³⁰

*Mass greater than 10 cm in diameter.

†COP, cyclophosphamide, Oncovin, and prednisone;²⁰ CHOP, cyclophosphamide, hydroxydaunorubicin, Oncovin, and prednisone;²¹ MOPP, nitrogen mustard, Oncovin, procarbazine, and prednisone;²² HOC, hydroxydaunorubicin, Oncovin, and cytosine arabinoside;²³ BACOP, bleomycin, Adriamycin, cyclophosphamide, Oncovin, and prednisone (modified from Skarin et al²⁴; ID-ARA-C, intermediate-dose cytosine arabinoside, 500 mg/m², twice daily for seven days; VAMP, Oncovin 2 mg × 6, Adriamycin 250 mg/m² × 3, prednisone 60 mg/d for six weeks.

‡E, extranodal disease.

Status of the Disease at ABMT

The characteristics of the patients are detailed in Tables 1 and 2. Four patients were drug resistant (ie, no response or progression on previous polychemotherapeutic course[s]). Five patients were in first CR after CHOP (No. 2, 11, 12, and 13) or after HOC (No. 8; the patient with marrow involvement at presentation, which showed progression after the first CHOP course and achieved CR after HOC therapy). One patient was in second CR (No. 14). This patient relapsed after the third CHOP course, but achieved a CR with one course of Oncovin, Adriamycin, methotrexate, and prednisone (VAMP) (Table 1). Four patients (No. 3, 4, 9, and 10) were in PR (reduction of measurable tumor size of more than 50%) without further tumor reduction with the last chemotherapy course.

Bone Marrow Aspiration and Cryopreservation

Techniques for procurement and cryopreservation of the bone marrow were used as described by Appelbaum et al with slight modifications. Briefly, bone marrow was obtained by multiple aspirations from both iliac crests under general anesthesia and were anticoagulated (preservative-free heparin). The suspension was filtered and centrifuged (Haemotetics 30 blood cell separator) to obtain the buffy coat. This was placed in plastic blood bags (50 mL, medium) and cooled to 100°C in a programmed freezer (Cryocon) at a rate of 1 to 2°C/min. The marrow was then stored in the vapor phase of a nitrogen freezer. The bone marrow was aspirated and cryopreserved nine days to 14 months prior to transplantation, marrow biopsies not being negative for lymphomatous infiltration at that time (bone marrow aspiration in patient No. 4 was done early in first CR). The bone marrow aspirates contained a mean of 1.8 x 10⁶ nucleated cells per kilogram (range, 1.2 to 2.3 x 10⁶/kg), which contained a mean of 1.1 x 10⁴ CFU-GM per kilogram (range, 0.4 to 2.4 x 10⁵/kg).
was found. Two patients relapsed three and five months, respectively, after ABMT and both died of relapse. Only one patient (No. 3) is still in unmaintained CR 28+ months after ABMT.

Six patients received transplants in CR, one in second CR and five in first CR. The patient who received a transplant in second CR (No. 14) is in unmaintained CR over seven months after ABMT. Of the five patients who received transplants in first CR, one patient (No. 8) with a T cell lymphoblastic NHL developed acute nonlymphocytic leukemia (ANLL) six months after ABMT and died during remission induction therapy. No evidence of recurrence of lymphoma was present at that time. Marker tests of his T cell NHL at diagnosis were positive for OKT11a, Leu-1, Leu-4, Leu-5, Leu-3a, and HLA-DR, and negative for slg, clg, OKT6, Leu-2a, and TdT. Later, the peripheral blood blast cells were Sudan black positive in 30% of the cells and marker tests were positive for VIM-D5 and negative for OKT11a, Leu-1, Leu-4, Leu-3a, HLA-DR, slg, clg, OKT6, and TdT. The remaining four patients are still in unmaintained CR ten to 31+ months after ABMT. Figure 1 shows the survival for those who did and did not receive transplants in CR (P < .05; Mantel-Cox test). For the time being, no CNS involvement after ABMT is observed.

Engraftment
All patients had leukocyte counts below 0.1 x 10^9/L following Cy and TBI therapies. Marrow engraftment occurred in all (including patient No. 4, in whom regeneration of the hematopoiesis was found at autopsy). The median number of days following ABMT to ≥0.5 x 10^9/L granulocytes was 20 (range, 11 to 40 days). The median number of days to self-sustaining platelet counts of ≥50 x 10^9/L was 27 (range, 13 to 42 days).

RESULTS

Antitumor Effect
The results are summarized in Table 2. Four patients were drug resistant at the time of transplantation. Three achieved a CR that lasted only 2, 4, and 5 months, respectively. One died two months after ABMT of a primary cytomegalovirus (CMV) interstitial pneumonitis (IP) and also had recurrence of lymphoma at autopsy (two months after ABMT fever and interstitial pneumonitis developed; cultures of lung material [bronchoscopy] yielded CMV. Autopsy revealed typical cytomegalic cells in both lungs38); both others died of relapse. The fourth patient achieved a PR and died two months later of progression.

Four patients received transplants in PR and all achieved CR, which lasted in three patients for 3, 5, and 28+ months, respectively. One patient (No. 4) died in the aplastic phase within one month after ABMT because of bacterial sepsis and associated complications. At autopsy, no evidence of lymphoma was found. Two patients relapsed three and five months, respectively, after ABMT and both died of relapse. Only one patient (No. 3) is still in unmaintained CR 28+ months after ABMT.

Supportive Care
In all patients, a Hickman catheter was implanted. Patients were treated in single rooms with reversed isolation, received colistin, trimethoprim-sulfamethoxazole, amphotericin B, and nystatin suspension for selective decontamination of the gut, and were given semisterile food until the granulocyte counts were above 0.5 x 10^9/L. Some patients needed parenteral hyperalimentation. Antibiotics (mostly an aminoglycoside and a cephalosporin) were given intravenously for febrile episodes associated with granulocytopenia. No granulocyte transfusions were given. Platelets from single donors and leukocyte-free red cell concentrates were administered routinely to maintain platelet counts above 20 x 10^9/L and hemoglobin (Hb) above 10 g/dL. All allogeneic blood cells were irradiated (2,000 rad).

Statistical Methods
Survival duration curves were estimated by the method of Kaplan and Meier26 and differences between the curves were assessed according to the Mantel-Cox test.27

Engraftment
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Fig 1. Kaplan-Meier analysis of survival in six patients who received transplants during CR (----) and in eight patients who received transplants while not in CR (-----).
**Toxicity of the Cytoreductive Treatment**

Characteristics of the nonhematologic toxicity are summarized in Table 3. All patients experienced nausea, vomiting, and diarrhea. Mucositis was found in all patients, mostly of a mild degree. Five patients needed parenteral hyperalimentation because of this moderate mucositis and/or persistent vomiting. Abnormal liver function tests were observed in four patients, consisting of a slight and transient elevation of liver enzymes. Fever (>38 °C) developed in 11 patients and was of unknown origin in five patients. Proven infections were seen in seven patients, localized (oral) herpes simplex was seen in two patients, a fatal CMV interstitial pneumonitis was seen in one patient (No. 1), and bacterial sepsis was noted in five patients, which responded to parenteral antibiotics in four. One patient (No. 4), who was in poor clinical condition and unable to take the drugs for selective decontamination of the gut, died of an enterococcus sepsis combined with extensive mucositis and renal failure. Cumulative toxicity of the previous HOC course and the Cy plus TBI treatment probably played a role too. Five patients developed a transient skin rash, probably related to antibiotics. Atrial fibrillation with hypotension occurred in one patient (No. 1), which responded to digitalis and was possibly a sign of treatment-related cardiotoxicity. No other (acute) toxicity was observed.

**DISCUSSION**

Despite modern chemotherapy, the prognosis of patients with NHL of high-grade malignancy is still rather poor. Only 20% to 40% of them will become long-term survivors and those patients who fail to achieve CR or who relapse while on chemotherapy have an extremely poor outlook. Recently, it has been postulated that aggressive alternating sequences of chemotherapy may improve survival. In the last few years, a small number of studies has been published in which patients with refractory NHL were treated with high-dose (radio)chemotherapy followed by ABMT. A few patients achieved prolonged disease-free survival, showing the value of this approach. However, the grades of malignancy and treatment in these studies were heterogenous, and in the majority of cases, the necessity for marrow transplantation was not clear because the treatment did not include the marrow-lethal TBI.

A recent multicenter report supports the efficacy of high-dose chemotherapy followed by ABMT in NHL, but the disease-free survival of refractory patients at one year post-ABMT was only 5%. This contrasted with the results in patients who were in PR after conventional therapy. About 80% of these are projected to be in CR at one year post-ABMT. Again, however, grades of malignancy and therapy were heterogeneous and no TBI was used.

Another very recent study of 24 patients with relapsing NHL treated with Cy and TBI followed by ABMT resulted in CR in 14 patients and five were in continuous remission 19 to 71 months after ABMT. Most of the relapses after ABMT occurred within the first six months. The fact that 15 patients had experienced CRs before is important. The five long-term disease-free survivors (>19 months) had experienced previous CRs and four of these five patients had disease progression before ABMT without receiving chemotherapy, so probably only one patient was truly refractory before ABMT.

A study of high-dose chemotherapy and TBI followed by syngeneic BMT in eight patients with refractory NHL of various grades of malignancy resulted in CR in seven patients and three are in unmaintained CR with a follow-up of 45 to 150 months (updated January 1983). This study is of importance because this provides a yardstick to measure the success of high-dose radiochemotherapy followed by (normal) BMT in refractory NHL.

All of our patients had lymphoma of high-grade malignancy and 12 of 14 had bulky disease (>10 cm), an additional risk factor associated with poor survival and they were treated uniformly with Cy and TBI followed by ABMT. Four patients had a drug-resistant lymphoma, of whom three achieved CRs and one achieved PR. However, the CRs were short-lasting and all relapsed within six months. Four patients had PRs and all achieved CR, but two relapsed within six months, one died of bacterial sepsis in the aplastic phase, and only one patient has become a long-term survivor (28+ months). Although the number of CRs in those patients who had failed to achieve CR after conventional therapy was remarkably high (seven of eight), only one patient has become a long-term survivor. Five of six patients treated in CR after conven-

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### Table 3. Nonhematologic Toxicity of 14 Patients

<table>
<thead>
<tr>
<th>Type of Toxicity</th>
<th>Grade</th>
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<th>4</th>
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<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mucositis</td>
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<td>0</td>
<td>10</td>
<td>0</td>
<td>4</td>
<td>0</td>
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<td>Nausea/vomiting</td>
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<td>0</td>
<td>1</td>
<td>3</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Diarrhea</td>
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<td>8</td>
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<td>SGOT/SGPT</td>
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<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Infection</td>
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<tr>
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<td></td>
</tr>
<tr>
<td>Pneumonitis (1)</td>
<td></td>
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<td></td>
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<td></td>
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</tbody>
</table>

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tional therapy are still in unmaintained CR seven to 31 + months after the ABMT procedure. The number of patients and length of follow-up are still limited but the preliminary results are promising in this group. One patient in this group died of ANLL (secondary?) without evidence of recurrence of the lymphoma.

The overall toxicity of the ABMT procedure was acceptable, with two therapy-related deaths (CMV-IP and sepsis) that occurred in patients in poor clinical condition. Sepsis related to granulocytopenia occurred in five of 14 patients, and was the cause of death in one case. Apart from one patient with CMV-IP, no IP was found. In the patients in good clinical condition (especially in first CR), toxicity was mild.

Although the number of patients and the length of follow-up is limited, the following conclusions may be drawn: (1) high-dose chemotherapy followed by ABMT is not successful in achieving long-term survival in patients with refractory NHL; (2) as a consolidation strategy, this therapeutic program may improve the long-term prognosis of patients who achieved CR of their disease; (3) in cases of recurrences after ABMT, no involvement was found in the bone marrow; (4) no relapse was found in the CNS, which occurs not infrequently in patients with NHL of high-grade malignancy; and (5) toxicity is acceptable, especially for those patients who received transplants in good clinical condition (and CR).

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Intensive cytotoxic therapy followed by autologous bone marrow transplantation for non-Hodgkin’s lymphoma of high-grade malignancy

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