HIGH RATE OF CLINICAL AND MOLECULAR REMISSIONS IN FOLLICULAR LYMPHOMA PATIENTS RECEIVING HIGH-DOSE SEQUENTIAL CHEMOTHERAPY AND AUTOGRRAFTING AT DIAGNOSIS: A MULTICENTER, PROSPECTIVE STUDY BY THE GRUPPO ITALIANO TRAPIANTO MIDOLLO OSSEO (GITMO)

Running title: multicenter trial in high-risk follicular lymphoma

Headings: Clinical Observations, Interventions and Therapeutic Trials

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Abstract
Single-center experiences have shown that intensified treatments with autologous transplantation are a promising therapeutic strategy for patients with high-risk follicle-center lymphoma (FCL) at diagnosis, whereas data from prospective multicenter trials are still lacking. This paper describes the results of a prospective multicenter study of an intensified purging-free high-dose sequential (i-HDS) chemotherapy schedule with peripheral blood progenitor cell (PBPC) autografting. The main feature of this program is harvesting stem cells after intensified chemotherapeutic debulking, with no ex vivo manipulation of PBPC. Ninety-two previously untreated patients aged ≤ 60 with advanced stage FCL were enrolled by 20 Italian Centers and evaluated on an intention-to-treat basis. i-HDS proved feasible with limited toxicity (87% patients completed the planned treatment schedule). i-HDS led to a complete remission rate of 88%. The projected overall survival and disease-free survival (DFS) were respectively 84% and 67% at four years. Centralized molecular analysis showed that PCR-negative harvests could be collected in 47% of cases. Following autograft, 65% of molecularly evaluable patients achieved clinical and molecular remission. The projected DFS at four years of this subgroup is 85%. This result emphasizes the importance of achieving maximal tumor reduction in these patients. In conclusion, our data show that highly effective intensified treatments can now be routinely offered to young patients with poor risk FCL even at small Institutions, with no need for sophisticated and expensive cell manipulation procedures.
**Introduction**

Several studies have investigated the role of intensified chemotherapy followed by autologous transplantation in the management of relapsed follicle-center lymphoma (FCL) (1,2,3,4,5,6,7). Results were encouraging with high rates of complete (CR) and molecular remissions (1,2,3,4,5,6,7,8,9,10). The latest findings from the Dana Farber Cancer Institute show that molecular remission is associated with an extremely low relapse rate and a >80% projected freedom-from-relapse at 12 years (7). Autologous transplantation may thus possess a curative potential in this otherwise incurable disease (11,12). Similar approaches have been less frequently used at diagnosis (13,14,15,16). In fact, a recent retrospective study from Stanford University showed that patients treated with autologous transplantation as first-line treatment have a better outcome compared to those treated with conventional chemotherapy (16).

Three important issues, however, still need to be addressed in evaluation of the real role of intensified approaches in FCL. First, there have been no multicenter prospective trials. A single-center trial carries the risk of overestimation of outcomes due to selection biases, and only highly qualified clinical teams may be able to achieve similar results with high-dose programs. Second, most autografting programs require ex vivo purging procedures, which are cumbersome, expensive and difficult to reproduce (7,17,18,19,20). Third, the most promising results have been obtained only in small groups of patients (16).

Promising results have recently been provided by using an intensified high-dose sequential chemotherapy (i-HDS) program as front-line therapy for high-risk FCL patients (15,21). This involves the collection of peripheral blood progenitor cells (PBPC) following a prolonged chemotherapeutic debulking in order to obtain an in vivo purging effect (15). The i-HDS does not include any ex vivo purging procedure. In a single-center experience,
PCR-negative harvests were collected in 68% of patients and approximately half of them achieved persistent clinical and molecular remission following autologous transplantation (21,22).

A multicenter, prospective trial was therefore launched in 1996 by 20 hematological Centers affiliated to the Gruppo Italiano Trapianto Midollo Osseo (GiTMO) to evaluate applicability and efficacy of the i-HDS regimen in 92 FCL patients. Its results were similar to those observed in previous single-center pilot trials. They show that an ex vivo purging-free autografting procedure: i) is feasible with limited toxicity; ii) induces high rates of CR; iii) leads to persistent molecular remissions in a good proportion of patients. Thus, high-dose chemotherapy treatments aimed to maximally cytoreduce and possibly cure FCL patients can be easily performed at both small and large Institutions.
PATIENTS AND METHODS

Inclusion Criteria

Patients were eligible if they were aged between 18 and 60 and had Ann Arbor stage III or IV FCL as defined by the International Working Formulation (WF B, C or D) (23) or Revised European and American Lymphoma classification (REAL grade I, II or III) (24). Patients should have received no previous chemotherapy or extended-field radiotherapy and have one or more of the following adverse prognostic features: bulky disease (greater than 5 cm), high serum LDH, disease related compression symptoms, systemic “B” symptoms, Eastern Cooperative Oncology Group (ECOG) performance status ≥ 2 or bone marrow (BM) invasion greater than 20%. Absence of concurrent heart, kidney, lung and liver disease was also required, as well as HBs antigen and HCV antibody negativity. Informed consent was obtained and the Institutional Review Boards of all the participating Centers approved the study.

Patient Characteristics

Between December 1996 and February 1999, 92 patients (median age 46, range 28 to 60) were treated at 20 Italian hematological Centers affiliated to the GITMO. Patient characteristics are described in Table 1. Eighty-four percent had Ann Arbor stage IV disease. BM involvement was present in 80%, while extranodal sites of disease other than BM were present in 55%. Fifty-one percent had a bulky mass and 37% had an elevated serum LDH. “B” symptoms were present in 30% and leukemic disease (peripheral blood lymphocytes >12000/mm³) in 12%. Thirty-seven percent had an age adjusted International Prognostic Index (aaIPI) score ≥ 2 (25,26).

The median number of patients treated at each Center was three (range 1-15). The annual reports of the GITMO national registry show that the 20 Units performed a median number of 31 (range 8-94) autologous transplants per year in 1997-1998. Thirty-six patients (39%)
were treated at small Institutions performing 31 or less autografts per year for the treatment of hematological malignancies, while 56 (61%) were treated at larger Institutions.

Table 1. Patient characteristics at study entry

<table>
<thead>
<tr>
<th></th>
<th>N°</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>92</td>
<td>100</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>42/50</td>
<td></td>
</tr>
<tr>
<td>Median age (range)</td>
<td>46 (28-60)</td>
<td></td>
</tr>
<tr>
<td>Stage IV</td>
<td>77</td>
<td>84</td>
</tr>
<tr>
<td>Bulky mass (&gt; 5 cm)</td>
<td>47</td>
<td>51</td>
</tr>
<tr>
<td>High serum LDH</td>
<td>34</td>
<td>37</td>
</tr>
<tr>
<td>“B” symptoms</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>BM involvement</td>
<td>74</td>
<td>80</td>
</tr>
<tr>
<td>Extraneidal sites (other than BM)</td>
<td>51</td>
<td>55</td>
</tr>
<tr>
<td>Leukemic disease (lymphocytes&gt;12000/mm³)</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>ECOG PS ≥2</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>aalPI ≥2</td>
<td>34</td>
<td>37</td>
</tr>
</tbody>
</table>

BM indicates bone marrow; ECOG PS, Eastern Cooperative Oncology Group performance status; aalPI, age adjusted International Prognostic Index.

Treatment Schedule

The i-HDS regimen has already been described (15,27). Briefly, it consists of intensive debulking prior to the high-dose (hd) phase, including two complete, full-dose APO (Doxorubicin, Vincristine, Prednisone) courses, totaling of four 75 mg/m² Doxorubicin administrations (28). Patients not achieving CR following these courses received two additional DHAP (Ara-C, Cisplatin, Dexamethasone) courses (29). The hd-phase
consisted of Etoposide (VP16) 2g/m², followed by Methotrexate (MTX) 8g/m² and Cyclophosphamide (CTX) 7g/m². PBPC collection was scheduled after the last course to exploit the “in vivo purging effect” operated by hd-chemotherapy (15). A chemotherapy-free interval of 40 days was scheduled prior to hd-CTX 7g/m², to allow optimal PBPC mobilization (30). Three hd-Dexamethasone courses (Dexamethasone at 40 mg/day for four consecutive days) were administered every 10 days during this interval. A minimum of 5 × 10⁶ CD34⁺ cells/kg was required for autologous transplantation with PBPC only. Patients failing to meet this minimum were placed off therapy. The conditioning regimen for autologous transplantation consisted of Mitoxantrone (MITO) 60 mg/m² on day –5 and Melphalan (L-PAM) 180 mg/m² on day –2 (31). PBPC were reinfused on day 0. G-CSF (Filgrastim or Lenograstim) was given at 5 µg/kg daily following VP16, CTX, and autograft. Radiotherapy was scheduled on bulky sites or on residual masses approximately two months after autograft. The whole i-HDS program is summarized in Figure 1.

Figure 1
**Evaluation and Statistics**

Clinical response was assessed by complete restaging at two months after autograft, thereafter at 3-month intervals for the first year and then at 6-month intervals. According to the Cheson criteria (32) CR was defined by the absence of any clinical sign of disease, while partial remission (PR) was defined by a 50% or more tumor reduction. Patients achieving less than PR were considered as having stable disease (32). Progression was defined as a 50% or more tumor increase or by the appearance of new lesions (32). All patients started on treatment were considered evaluable for response and outcome on an intention-to-treat basis. Overall survival (OS) was measured from the start of therapy up to the date of death or last follow-up alive (32). Progression-free survival (PFS) for all patients was taken from the start of therapy until disease progression or death from lymphoma (32). Disease-free survival (DFS) for patients in CR was measured from the first recording of a CR to the date of progression (32). Event-free survival (EFS) was calculated from the start of therapy up to the first adverse event, i.e. relapse or progression, secondary malignancy, treatment-related death or last follow-up alive. The closing date for analysis was December 31, 2001. OS, DFS, PFS and EFS were calculated according to the Kaplan and Meier method (33). The log-rank test was used to compare survival curves (34).

**Minimal Residual Disease Assessment by Nested PCR**

All patients with an available tumor specimen were initially screened for the presence of the Bcl-2 translocation on diagnostic tissues (i.e. lymph node or BM). Nested polymerase chain reaction (PCR) amplification for both the major breakpoint region and minor cluster region was carried out as originally described by Gribben et al (8,21,35). When the Bcl-2 translocation could not be amplified, an alternative tumor marker was sought by amplifying and sequencing the immunoglobulin heavy-chain (IgH) gene rearrangement (36,37). This method gave a tumor specific forward primer derived from the second complementarity-
determining region and a reverse tumor specific primer derived from the third complementarity-determining region (37). PCR detection of minimal residual disease (MRD) was then performed as previously described (37).

Timepoints chosen for molecular analysis are shown in Figure 1. PCR analysis was performed at diagnosis, on PBPC and BM samples obtained before autologous transplantation and then at 6-month intervals following autologous transplantation. Patients were considered as having PCR-negative harvests if at least one PBPC or BM harvest was PCR-negative. Molecular remission was defined as absence of molecular disease in two consecutive BM samples (spaced by at least six months) in a patient showing evidence of CR by means of standard radiological and histological analysis.
RESULTS

Treatment Feasibility and Clinical Response.

Treatment feasibility and responses are illustrated in Tables 2 and 3. The regimen proved feasible at the multicenter level (Table 2). Eighty patients (87%) completed the program. Interruptions were due to: i) toxic deaths (2%), ii) disease progressions (3%), iii) grade IV toxicity (1%), iv) consent withdrawal (3%), and v) insufficient PBPC mobilization (3%). There was no difference in feasibility between small and large Institutions (data not shown) (p= 0.89).

Table 2. i-HDS feasibility

<table>
<thead>
<tr>
<th>Patients evaluable = 92 (100%)</th>
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<tr>
<td>Toxic deaths</td>
</tr>
<tr>
<td>Treatment withdrawals</td>
</tr>
<tr>
<td>Not transplanted:</td>
</tr>
<tr>
<td>due to low-mobilization</td>
</tr>
<tr>
<td>due to toxicity</td>
</tr>
<tr>
<td>Progressions</td>
</tr>
<tr>
<td>Patients successfully transplanted</td>
</tr>
<tr>
<td>Median CD34+ cells x10^6/kg mobilized (range)</td>
</tr>
</tbody>
</table>

The most frequent violations to the treatment schedule were delays due to shortage of hospital beds. The overall delay exceeded three months (range 2-6) in 12% of patients. In addition, nine patients eligible for post-graft radiotherapy did not receive it. One patient was switched to allogeneic transplantation while she was in PR at the end of the hd-phase. Follow-up for this patient was stopped at this time.

Eighty-one patients (88%) achieved CR (Table 3): 49 at the end of the hd-phase; 32 following autologous transplantation. In spite of the intensive program, three patients (3%)
had disease progression under treatment (Table 3). These three patients underwent salvage programs with multiple regimens including fludarabine and rituximab with poor response. The two patients (2%) who died of treatment related toxicity were in clinical remission when the fatal toxic episode occurred.

Table 3. Response to i-HDS

<table>
<thead>
<tr>
<th>Patients evaluable = 92 (100%)</th>
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<tbody>
<tr>
<td>Toxic deaths</td>
</tr>
<tr>
<td>Progressions</td>
</tr>
<tr>
<td>Partial responses</td>
</tr>
<tr>
<td>Complete responses</td>
</tr>
</tbody>
</table>

Early and Late Toxicity

Two toxic deaths were reported: one patient died of ventricular fibrillation associated with myocardial infarction on day +10 following autologous transplantation; the second developed severe cytomegalovirus pneumonia 15 days after hd-CTX and died of respiratory failure on day +21. Hematopoietic recovery and transfusion requirements following hd-VP16, hd-CTX and following MITO/L-PAM are summarized in Table 4. Grade III-IV extra-hematological early non-fatal toxicity (other than oral and gastrointestinal mucositis during the myeloablative phase) included: i) ischemic stroke at the end of the hd-phase (1%); ii) sepsis (2%); iii) pneumonia (3%); iv) hepatitis due to HBV reactivation (2%); v) gallbladder empyema (1%); vi) acute heart infarction (1%); vii) pulmonary embolism (1%); viii) gastric hemorrhage following the initial APO course in a patient with gastric localization (1%). Thirty-one per cent of these side effects were recorded during the debulking phase with conventional chemotherapy, 38% during the hd-phase and 31% during the final myeloablative phase. No difference in toxicity was observed between
patients treated at small and large Institutions (p=0.99) (data not shown). All patients recovered from these acute episodes except the patient experiencing ischemic stroke who had persistence of neurological defects. Since this patient was already in CR, the final autografting phase was omitted (Table 2).

**Table 4. Hematological toxicity and transfusional requirement following high-dose Etoposide, high-dose Cyclophosphamide and autograft**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>hd-VP16</th>
<th>hd-CTX</th>
<th>MITO/L-PAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days with WBC &lt; 0.5 x 10^9/l</td>
<td>3 (0-8)</td>
<td>5 (0-9)</td>
<td>8 (3-14)</td>
</tr>
<tr>
<td>Days with platelets &lt; 20 x 10^9/l</td>
<td>3 (0-7)</td>
<td>5 (0-10)</td>
<td>10 (5-20)</td>
</tr>
<tr>
<td>Median n°. of platelets transfusions (range)</td>
<td>0 (0-3)</td>
<td>1 (0-6)</td>
<td>3 (0-8)</td>
</tr>
<tr>
<td>Median n°. of RBC transfusions (range)</td>
<td>0 (0-4)</td>
<td>1 (0-9)</td>
<td>2 (0-8)</td>
</tr>
</tbody>
</table>

WBC indicates white blood cells; hd, high-dose; VP16, Etoposide; CTX, Cyclophosphamide; MITO, Mitoxantrone; L-PAM, Melphalan; RBC, red blood cells

With a median follow-up of 40 months, the following late toxic episodes were recorded: i) herpes zoster reactivation (3%) always responding to Acyclovir, ii) autoimmune thrombocytopenia (1%) that resolved spontaneously, iii) congestive heart failure (3%) (NYHA I and II) effectively controlled by therapy. Myelodysplastic syndrome (MDS) and secondary myeloid leukemia occurred in four patients (4%). One was in CR. The other events occurred following repeated courses of salvage chemotherapy due to relapsed or resistant FCL. Another patient developed T-cell acute lymphoblastic leukemia (T-ALL) while in CR at 48 months since autografting. Two of these five patients have already died (one with myeloid leukemia and one with T-ALL), while three are presently alive (two without treatment).
Clinical Outcome

The survival projections are shown in Figure 2. Among the 81 patients in CR at the end of the treatment, there have been 24 relapses; five relapses occurred among the six patients in PR. At present 56 patients are alive in continuous CR at a median follow-up of 43 months (range 24 to 61), one with secondary untreated myelodisplastic syndrome. The 4-year DFS and PFS projections are 67 and 60 % respectively (Figures 2B and 2C). Of the 29 patients who relapsed, 21 are alive at a median follow-up of 44.4 months, four with no need for additional treatment. Salvage treatments were heterogeneous: in most cases patients were treated with rituximab-containing conventional or intensified schedules. Twelve patients achieved a second CR, 11 by means of a rituximab-containing regimen and one by means of radiotherapy alone. Thus, at present 78 of 92 (85%) patients are alive. At a median follow-up of 43 months, the estimated 4-year OS projection is 84% (Figure 2A). Overall, 56 (55 in CR and one in PR) patients are alive, with no sign of disease progression and no severe late complications, with a 4-year EFS projection of 57% (Figure 2D).

Figure 2
The outcome has been also evaluated according to the aaIPI score (25,26). There were no significant differences in OS and DFS between patients with aaIPI 0-1 and those with aaIPI 2-3 (Figures 3A and 3B).

**Figure 3**

**A**

![Graph A](image)

**B**

![Graph B](image)

**PCR Analysis of Stem Cell Harvests**

As summarized in Table 5, a molecular marker was obtained from the diagnostic tissue in 42 of 55 patients tested molecularly (76%). The tumor marker was the Bcl-2/IgH translocation in 36 (65%) patients. In addition a molecular marker derived from the IgH sequence was obtained in six (31%) of 19 patients lacking a Bcl-2/IgH translocation (Table 5). A total of 126 pretransplant stem cell harvests were analyzed. Fifty-nine (47%) were PCR-negative. Twenty of 42 evaluable patients (48%) obtained one or more PCR-negative harvests: 18 are in continuous CR and only two had disease recurrence. Thirteen of the 22 patients (59%) collecting only PCR-positive harvests relapsed (p<0.01). DFS curves of the two populations are shown in Figure 4A. The outcome of the six patients collecting both
PCR-negative and PCR-positive harvests was similar to that of patients collecting only PCR-negative harvests (data not shown). Patients in which the diagnostic sample was not available had a similar clinical behavior in terms of OS, PFS, DFS, EFS compared to those studied molecularly (data not shown).

Table 5. Results on PCR-based analysis of minimal residual disease

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>Samples tested</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with a molecular marker</td>
<td>42</td>
<td>55</td>
<td>76</td>
</tr>
<tr>
<td>Bcl-2 positive</td>
<td>36</td>
<td>55</td>
<td>65</td>
</tr>
<tr>
<td>IgH positive</td>
<td>6</td>
<td>19</td>
<td>31</td>
</tr>
<tr>
<td>PCR-negative harvests*</td>
<td>59</td>
<td>126</td>
<td>47</td>
</tr>
<tr>
<td>Patients in molecular remission*</td>
<td>24</td>
<td>37</td>
<td>65</td>
</tr>
</tbody>
</table>

PCR indicates polymerase chain reaction; IgH, immunoglobulin heavy chain
*In some patients in which a molecular marker was available, follow-up samples were not available.

Figure 4
Molecular Follow-up

Molecular monitoring was performed on post-graft BM samples. Twenty-four (65%) out of 37 evaluable patients achieved molecular remission: 22 immediately following autologous transplantation; two at six and 12 months, following an initial detection of PCR-positive results on one or two samples. All these patients were also in CR. Six patients autografted with PCR-positive PBPC became PCR-negative during the molecular follow-up.

Only three (12%) relapses occurred among patients achieving post-graft molecular remission. One was a localized retro-orbital relapse with persistent PCR negativity at BM level. This patient achieved second CR with radiotherapy alone and he is in persistent molecular remission. The second occurred in a patient who displayed two consecutive PCR-negative results at six and 12 months from autografting. This relapse was heralded by recurrence of PCR positivity at 18 months from autografting while the patient still had no sign of clinical relapse. A third patient relapsed at 12 months from transplant as diffuse large cell lymphoma. Unfortunately we could not perform IgH sequencing on the relapse sample to rule out the occurrence of a second lymphoma as already reported (38). In contrast ten relapses were noted in the 13 patients who failed to achieve molecular remission (77%). DFS of patients achieving post-graft molecular remission compared to those remaining PCR-positive is shown in Figure 4B (p<0.001).
DISCUSSION

This paper illustrates the results of a multicenter prospective study using i-HDS, an ex vivo purging-free intensified approach with PBPC autografting, in a series of 92 previously untreated patients with advanced FCL aged \( \leq 60 \). Results show that i-HDS is a feasible approach that can be performed with acceptable toxicity at both small and large Institutions. Response and outcome were similar to those reported in previous single-center experiences and are promising, particularly for patients with aaIPI \( \geq 2 \) (14,15,16). Centralized molecular analysis showed that PCR-negative harvests can be collected using a chemotherapy-mediated in vivo purging approach. Finally, the observation of a high proportion of patients in prolonged clinical and molecular remission suggests that at least some of them might have been cured of their disease.

Feasibility is a major issue in the setting of intensified regimens in FCL, especially due to the need to obtain PCR-negative collections for autografting (7,8,9,10,11,12,21,22). This is critical in FCL as opposed to other neoplasms such as multiple myeloma, where transplantation is not delivered with curative intent (39,40), and diffuse large cell lymphoma, where tumor contamination of stem cell harvests is infrequent. Conventional autografting approaches such as those employed by the Dana Farber (7,8,14) and the Stanford University (16) groups successfully clear MRD from stem cell harvests by ex vivo manipulation. However, this strategy is expensive, time-consuming and too sophisticated for the small and medium-sized Institutions that currently treat most FCL patients. This probably explains why no multicenter trial has been so far published using these strategies. Indeed, most Centers participating to our study (16 of 20) do not currently perform ex vivo manipulation procedures. Nevertheless, all Centers were able to perform the whole schedule. The chemotherapy program was completed in most patients enrolled and no differences were observed in terms of feasibility between small and large Centers.
Toxicity is another important issue for FCL patients treated with autografting programs. Early toxicity was not excessive, although two toxic deaths were reported. This is in line with the treatment related mortality (TRM) expected with the use of intensive chemotherapy with autograft (41,42). The TRM of 2% is, in fact, analogous or even lower than that reported in single-center experiences with autograft in FCL at diagnosis (14,15,16). Additional major toxic episodes were successfully managed with appropriate treatment and did not show evidence of clustering in any treatment phase. Thirty-one per cent occurred during the early conventional phase, suggesting that a significant proportion of them would also have occurred if patients had only received a CHOP-like regimen.

The occurrence of four cases of secondary MDS is of some concern, particularly because it cannot be excluded that additional episodes will occur during the long-term follow-up. However, it should be noted that three out of four MDS occurred in patients who received additional treatment due to relapse. Although our treatment is already TBI-free, additional steps should probably be undertaken in order to reduce the risk of second tumors. One possibility would be to replace hd-VP16 with a less leukemogenic drug such as Ara-C (43,44). A more intensified etoposide-free program has proved feasible and effective for patients with mantle cell lymphoma and relapsed FCL (45,46,47). In addition, new non-chemotherapeutic drugs, such as anti-CD20 rituximab, are suitable for inclusion in the i-HDS schedule to reduce the risk of recurrence (45,46). This might reduce the need for salvage chemo-radiotherapy and lower the risk of secondary neoplasms.

The efficacy of i-HDS in FCL was confirmed in this multicenter study. The 88% CR rate is analogous to that reported in the previous single-center pilot study (15). Thus, the promising results observed at the single-center level do not reflect selection biases or
availability of particularly experienced teams. In addition, results of centralized PCR-based analysis were consistent with a potent anti-lymphoma activity of i-HDS. Approximately 60% of patients evaluable for MRD reached a persistent PCR-negative status following autologous transplantation. These patients had an extremely low risk of relapse. Thus, a good proportion of FCL patients undergoing i-HDS at diagnosis experiences a prolonged clinical and molecular remission. It is conceivable that these patients might have been cured of their disease, as already suggested in previous experiences using intensive approaches (7,12,21).

The most significant results with the use of high-dose chemoradiotherapy and autograft in FCL patients at diagnosis have been obtained at the Dana Farber Cancer Institute and at Stanford University (14,16). Our patient characteristics were quite similar. They were selected for age ≤ 60, advanced disease and one or more adverse prognostic features, according to the criteria available at the time of the study. We observed a 84% survival projection at four years. This is lower than the OS reported by the two American groups. It should be noted that in their studies only patients responsive to conventional induction therapy were considered for the high-dose program, whereas our analysis was made on an intention-to-treat basis and the outcome of all enrolled patients was evaluated (14,16). In addition, the differences in OS may in part reflect a better handling of disease recurrence for patients enrolled in single-center compared to multicenter programs. In fact, our PFS and DFS projections were comparable to those reported by the Stanford and Dana Farber groups (14,16). Our results demonstrate that approximately 60% of patients are disease-free survivors as in the single-center studies.

Our study was not designed to demonstrate the superiority of i-HDS compared to conventional chemotherapy and thus any conclusion on this issue should be suspended,
until the results of currently ongoing prospective randomized trials are available. However, the observation that following i-HDS we failed to see any difference in outcome between patients with aaIPI ≥ 2 and those with aaIPI < 2 is particularly intriguing. Indeed, these results suggest that an intensified treatment might be beneficial for patients with poor prognosis according to the aaIPI score, while any benefit for patients with less aggressive disease would be extremely difficult to prove, even in large randomized trials.

We are witnessing a very exciting age in the treatment of FCL as novel treatment approaches are dramatically changing its natural history. Several new molecularly targeted therapeutic approaches are now entering the clinical arena, such as naked and radiolabeled monoclonal antibodies, vaccination strategies and antisense oligonucleotides (48,49,50,51,52,53,54,55). There is little doubt that intensified chemotherapies may appear rather obsolete by comparison. Nevertheless, it should be noted that autografting-containing regimens were one of the most effective in the pre-monoclonal antibody era. This treatment was the first proving able to modify the natural evolution of FCL as outlined by the high incidence of prolonged clinical and molecular remission observed in a high proportion of patients (7,12,15,16). It is now clear that rituximab and perhaps other innovative drugs can be easily integrated within autografting-containing regimens (45,46). Thus intensified treatments should still be considered as effective therapeutic weapons worthwhile of being evaluated in combination with novel drugs. To verify this hypothesis a randomized trial comparing rituximab-supplemented i-HDS vs. rituximab-supplemented CHOP has been recently launched by the GITMO group for FCL patients with aaIPI score ≥ 2.
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**FIGURE LEGEND**

**Figure 1:** Schematic representation of the treatment schedule employed in this patient series. APO course consisted of Doxorubicin 75 mg/m^2^ days 1 and 22, Vincristine 1.2 mg/m^2^ days 1,8 and 22, Prednisone 50 mg/m^2^ days 1-22. DHAP course consisted of Cisplatin 100 mg/m^2^ day 1, Ara-C 4 g/m^2^ day 2, Dexamethasone 40 mg days 1-4. Abbreviations: VP16, Etoposide; MTX, Metotrexate; CTX, Cyclophosphamide; MITO, Mitoxantrone; L-PAM, Melphalan; PBPC, peripheral blood progenitor cells; MRD, minimal residual disease.

**Figure 2:** Kaplan-Meyer estimate of probability of overall survival (A), disease-free survival (B), progression-free survival (C) and event-free survival (D) for the 92 patients evaluated in the study. Data were evaluated on an intention-to-treat basis.

**Figure 3:** Kaplan-Meyer estimate of probability of overall survival (A) and disease-free survival (B) according to aaIPI score. (A) Overall survival and (B) disease-free survival for patients with low (0,1) aaIPI score (n=58, solid line) versus patients with high (2,3) aaIPI score (n=34, dotted line); p=NS.

**Figure 4:** Kaplan-Meyer estimate of probability of disease-free survival according to PCR status of harvests (A) and molecular follow-up (B). (A) Disease-free survival for patients whose harvests were PCR-negative (n=20, solid line) versus patients whose harvests were PCR-positive (n=22, dotted line); p<0.01. (B) Disease-free survival for patients achieving a molecular remission (n=24, solid line) versus patients with PCR-positive follow-up (n=13, dotted line); p<0.001.
High rate of clinical and molecular remissions in follicular lymphoma patients receiving high-dose sequential chemotherapy and autografting at diagnosis: a multicenter, prospective study by the Gruppo Italiano Trapianto Midolla Osseco (GITMO)

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