Lymphocyte Recovery in Advanced Ovarian Cancer Patients after High-Dose Chemotherapy and Peripheral Blood Stem Cell Plus Growth Factor Support: Clinical Implications

Gabriella Ferrandina, Luca Pierelli, Alessandro Perillo, Sergio Rutella, Manuela Ludovisi, Giuseppe Leone, Salvatore Mancuso, and Giovanni Scambia

Departments of Gynecology [G. F., S. M., G. S.] and Hematology [L. P., A. P. S. R., M. L., G. L.], Catholic University of Rome, 00168 Rome, Italy

ABSTRACT

Purpose: The purpose of this study was to investigate the clinical role of immunological recovery together with selected biological parameters on long-term survival in a series of ovarian cancer administered high-dose chemotherapy with peripheral blood stem cell and growth factor support.

Experimental Design: Thirty-eight patients with stages IIIb–IV epithelial ovarian cancer were studied. Lymphocyte immunophenotyping for the identification of CD3(+) cells, CD4(+), CD8(+), and CD3(-)/CD16(+)/CD56(+) natural killer T cells and CD19 B cells was performed.

Results: Twenty-three patients (60%) had a CD3(+) cell count <850 cells/μl. Multivariate logistic regression showed that tumor grading (χ² = 6.6, P = 0.010) and type of growth factor (χ² = 4.1, P = 0.042) retained an independent role in predicting T-cell recovery above the value of 850 cells/μl. The 3-year time to progression (TTP) rate was 86% (95% confidence intervals, 70, 102) in cases with high CD3(+) cell count with respect to a 3-year TTP of 23% (95% confidence intervals, 8, 38) in cases with low CD3(+) cell count (P = 0.0026). The absolute number of CD3(+) cells was shown to be inversely associated with risk of progression (χ² = 4.8; P = 0.028), as assessed by Cox univariate analysis using CD3(+) cell count as continuous covariate. In multivariate analysis only residual tumor and status of CD3(+) cell counts retained an independent association with shorter TTP. Similar results were obtained for overall survival.

Conclusions: Long-term immune reconstitution and particularly the recovery of adequate counts of CD3(+), CD4(+), and CD8(+) T cells are independent markers of longer TTP and overall survival in ovarian cancer patients receiving high-dose chemotherapy with peripheral blood stem cell and growth factor support.

INTRODUCTION

PBSCT represents an effective supportive strategy for rapid reconstitution of hematopoiesis after HDC regimens (1, 2). Efforts are ongoing to set up the best conditions for an earlier and possibly cell lineage selective growth factor-induced hematopoietic cell recovery (3–5). Evidence has been reported that, besides the advantages of earlier hematopoietic reconstitution and easier clinical management, the use of specific growth factors can play a role in improving immunological recovery: in particular, experiences with allogeneic transplant recipients suggest that restoration and/or maintenance of the immune response might be as effective in controlling microscopic/minimal residual tumor cells that escaped chemotherapy (6, 7). In a different clinical setting, we recently reported (5) the results from a randomized comparison between G-CSF and GM-CSF in the hematopoietic and immune recovery in ovarian and breast cancer patients administered intensive, myeloablative cancer chemotherapy with PBSCT. We showed that although hematopoietic recovery and posttransplant clinical management were comparable in G-CSF–versus GM-CSF-treated patients, significantly higher T cell counts could be found in G-CSF patients during the early and late posttransplant follow-up. Moreover, we reported for the first time in human solid tumors that patients achieving high CD3(+) cell count at long-term follow-up showed a longer TTP, suggesting that growth factor-driven improvement in immunological recovery could play a role in post-PBSCT control of disease and result in a survival benefit (5).

Although a more in-depth analysis of the association between enhanced recovery of T cells in the post-PBSCT period and a more favorable prognosis needs to be carried out, the possibility that biological characteristics of the tumor can also play a role in influencing tumor/host interactions and patient outcome cannot be ruled out. In particular, there is evidence that qualitative and/or quantitative alterations of tumor suppressor genes (such as p53; Ref. 8) and/or oncogenes (like members of the erbB family; Ref. 9) can identify ovarian cancer patients...
immune status during the late posttransplant follow-up, blood
parameters on long-term survival in a series of advanced ovarian
cancer patients with minimal chemosensitive disease, adminis-
tered HDC with PBSC and growth factor support (11).

PATIENTS AND METHODS
Thirty-eight patients with histologically confirmed, advanced
(International Federation of Gynecology and Obstetrics stages III–IV) epithelial ovarian cancer, residual tumor ≤2 cm achieved at primary cytoreductive surgery, or interval
debulking surgery without signs of progression after induction chemotherapy were enrolled into a Phase II study investigat-
ing G-CSF versus GM-CSF effects after HDC with
PBSC and growth factor support (11). The length of median time to study entry for mobilization chemotherapy was 3
weeks (range, 2–4; SD, 0.80) and 3 weeks (range, 2–4; SD, 0.87) for patients submitted to primary debulking surgery or interval debulking surgery, respectively. Other eligibility cri-
tera were: age younger than 60 years; performance status of
0–1 (WHO scale); adequate bone marrow reserve (WBC
count, >4000 × 10⁹/liter; platelet count, >100 × 10⁹/liter); and adequate pulmonary, cardiac, hepatic, and renal func-
tions, as described previously (11).

Treatment Plan and Supportive Care. Breifly, the in-
duction phase consisted of only one cycle of cisplatin (100
mg/m²), epirubicin (110 mg/m²), and paclitaxel (175 mg/m²), followed by rh-G-CSF (5 μg/kg/day) s.c. as PBSC mobilizing
median (12). Leukaphereses were performed using the Frese-
nius AS104 blood cell separator (Fresenius, St. Wendel, Ger-
y). A minimum of 4 × 10⁹ peripheral blood mononuclear
cells/kg or 2.5 × 10⁹/kg CD34(+) cells were collected per
patient (13). An additional two cycles of the same regimen were
administered. The intensification regimen consisted of carbo-
platin (600 mg/m², days 1 and 2), etoposide (450 mg/m², days 1 and 2), and melphalan (50 mg/m², days 3 and 4). PBSCs were
reinfused on day 5. Twenty-four hours later patients received
rh-erythropoietin at a dose of 150 IU/kg s.c. every 48 h until day +11, plus 5 μg/kg/day rh-G-CSF s.c. until day +12, or rh-GM-
CSF (5 μg/kg/day) s.c. until day +12. In particular, the dose of
GM-CSF was selected on the basis of the range of doses commonly used and reported in the literature specifically in setting of autologous transplantation (5, 14, 15). Hematopoietic
engraftment was defined as the number of days necessary to
reach WBCs >1,000 per μl, polymorphonuclear leukocytes
>500 per μl, and platelets >50,000 per μl (11).

Long-Term Hematological and Immunological Follow-up.
As previously reported (5), to evaluate the hematological and
immune status during the late posttransplant follow-up, blood
cell counts and circulating lymphocyte immunophenotyping
were monitored after an interval from PBSC of 12 months in all
evaluable patients receiving either G-CSF or GM-CSF. Circulating lymphocyte immunophenotyping for the identifi-
cation of CD3(+), CD4(+), CD8(+), and CD3(−)/
CD16(+)CD56(+) NK T cells and CD19 B cells was per-
formed as described previously (5).

Immunohistochemistry. For p53 immunohistochemical
assessment, the DO7 monoclonal antibody (diluted 1:100;
DAKO, Carpiniteria, CA) was used. For Her-2/neu assay, we
used the high-affinity murine monoclonal antibody 300G9
(Ig2α; 50 μg/ml), recognizing an epitope of the Her-2/neu
extracellular domain, which has an 80.3% concordance rate with
protein expression (16). EGFR staining was performed by using
the monoclonal antibody 108 (used as culture supernatant di-
luted 1:4) directed to the extracellular domain of the receptor. Immunohistochemical analysis of p53, Her-2/neu, and EGFR
was performed as described previously (17, 18).

For p53 analysis, cases were scored on the basis of the
intensity of staining and the proportion of cells stained, and
judged as negative in the absence of any staining and positive in
cases of staining in >1% of cells (corresponding to the median
value). Scoring for HER-2/neu was assigned according to the
intensity of staining and graded from 0, 1+, 2+, 3+. Strong
immunohistochemical reaction (3+) was considered as Her-2/
neu positivity. EGFR immunostaining was scored on the basis
of the fraction of stained tumor cells: negative (fraction of
stained cells <20%) or positive (fraction of stained cells
>20%).

The analysis of all tissue sections was done without any
prior knowledge of the clinical parameters or other immunohis-
tochemical results, by two different pathologists by light mi-
icroscopy. In case of disagreement, consensus was reached by a
joint reevaluation using a multi-head microscope.

Statistical Analysis. Mann-Whitney nonparametric test
was used to analyze the distribution of CD3(+) cells according
to several variables. The χ² test and Fisher’s exact test for
proportion were used to analyze the distribution of clinicopath-
ological parameters according to different patient populations.
Multiple logistic regression (19) was used to analyze the role of
clinicopathological parameters as predictors of CD3(+) cell
recovery. OS and TTP were calculated from the date of diag-
osis to the date of death/progression or date last seen. Medians
and life tables were computed using the product-limit estimate
by the Kaplan and Meier method (20), and the log rank test was
used to assess the statistical significance (21). To reduce the
possible bias related to the use of an arbitrary cutoff required in
the Kaplan and Meier analysis, we also analyzed the prognostic
role of CD3(+) cell count as a continuous variable, by the Cox
Mantel method (22). Statistical analysis was carried out using
SOLO (BMDP Statistical Software, Los Angeles, CA). Median
follow-up was 38 months (range, 13–107). Analysis was as of

RESULTS
During the late posttransplant follow-up, erythrocyte, gran-
uocyte, and platelet counts were comparable in G-CSF-
versus GM-CSF-treated patients whereas WBC and circulating
lymphocytes were significantly higher in the G-CSF series (Table
1). Cytokine release into blood of cytokines significantly higher in
the cytokine series.
Table 1  Patients’ blood cell counts after a 12-month follow-up in GM-CSF- versus G-CSF-treated patients

<table>
<thead>
<tr>
<th></th>
<th>GM-CSF</th>
<th>G-CSF</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Range</td>
<td>Median</td>
</tr>
<tr>
<td>WBC (µl)</td>
<td>3,350</td>
<td>2,000–6,180</td>
<td>4,500</td>
</tr>
<tr>
<td>Granulocytes (µl)</td>
<td>2,000</td>
<td>1,100–3,600</td>
<td>2,800</td>
</tr>
<tr>
<td>Lymphocytes (µl)</td>
<td>950</td>
<td>280–2,000</td>
<td>1,600</td>
</tr>
<tr>
<td>Platelets (µl)</td>
<td>190,000</td>
<td>125,000–355,000</td>
<td>185,000</td>
</tr>
</tbody>
</table>

* Calculated by Mann-Whitney nonparametric test. n.s., not significant.

Table 2  Distribution of CD3+ cell counts according to clinicopathological characteristics and type of growth factors

<table>
<thead>
<tr>
<th></th>
<th>CD3+ cell count (mean ± SE)</th>
<th>P*</th>
<th>Cases with &lt;850 CD3+ cells/µl n (%)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Range</td>
<td>P*</td>
<td>Median</td>
</tr>
<tr>
<td>All</td>
<td>38</td>
<td>835 ± 59</td>
<td>0.13</td>
<td>11</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>23</td>
<td>891 ± 73</td>
<td>0.14</td>
<td>3</td>
</tr>
<tr>
<td>FIGO* stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>35</td>
<td>857 ± 62</td>
<td>20 (57)</td>
<td>857 ± 98</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>583 ± 98</td>
<td>21 (75)</td>
<td>583 ± 98</td>
</tr>
<tr>
<td>Grading</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1/G2</td>
<td>10</td>
<td>1093 ± 116</td>
<td>2 (20)</td>
<td>1093 ± 116</td>
</tr>
<tr>
<td>G3</td>
<td>28</td>
<td>743 ± 61</td>
<td>21 (75)</td>
<td>743 ± 61</td>
</tr>
<tr>
<td>Residual tumor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2 cm</td>
<td>28</td>
<td>828 ± 71</td>
<td>6 (60)</td>
<td>828 ± 71</td>
</tr>
<tr>
<td>&gt;2 cm</td>
<td>10</td>
<td>857 ± 111</td>
<td>17 (61)</td>
<td>857 ± 111</td>
</tr>
<tr>
<td>Ascites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>15</td>
<td>964 ± 108</td>
<td>7 (47)</td>
<td>964 ± 108</td>
</tr>
<tr>
<td>Yes</td>
<td>23</td>
<td>751 ± 64</td>
<td>16 (69)</td>
<td>751 ± 64</td>
</tr>
<tr>
<td>Histotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>30</td>
<td>850 ± 72</td>
<td>18 (60)</td>
<td>850 ± 72</td>
</tr>
<tr>
<td>Other</td>
<td>8</td>
<td>781 ± 77</td>
<td>5 (62)</td>
<td>781 ± 77</td>
</tr>
<tr>
<td>Growth factor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-CSF</td>
<td>21</td>
<td>966 ± 91</td>
<td>9 (43)</td>
<td>966 ± 91</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>14</td>
<td>674 ± 48</td>
<td>14 (82)</td>
<td>674 ± 48</td>
</tr>
</tbody>
</table>

* Calculated by Mann-Whitney nonparametric test.
  a Calculated by Fisher’s exact test for proportion.
  * FIGO, International Federation of Gynecology and Obstetrics.

CD4(+), CD8(+), but not CD3(-)/CD16(+)/CD56(+) were higher in G-CSF-treated versus GM-CSF-treated patients (data not shown). No difference in the counts of B lymphocytes was observed (data not shown). GM-CSF (+) cell counts at 12-month follow-up ranged from 350-1850 cells/µl (mean ± SE values, 835 ± 59 cells/µl). The cutoff of 850 GM-CSF (+) cells/µl was chosen a priori without any prior knowledge of the clinical parameters or patient clinical outcome to define patients with low versus high T-cell recovery at the 12-month follow-up period. Twenty-three patients (60%) had a CD3(+) cell counts <850 cells/µl.

There was no difference in the distribution of the absolute CD3+ cell counts according to age, stage, residual tumor, ascites, and histotypes (Table 2). However, patients with poorly differentiated tumors showed a lower CD3(+) cell count with mean ± SE values of 743 ± 61 cells/µl with respect to 1093 ± 116 cells/µl in cases with well/moderately differentiated tumors (P = 0.0059). Similar results were found when considering the percentage of cases with low CD3+ cell counts (20% versus 75% in G3 versus G1–2 tumors, respectively, P = 0.0037). Patients with G1–2 tumors were found to be older than patients with G3 tumors (mean ± SE, 42.1 ± 2.8 versus 49.0 ± 1.2; P = 0.027).

We failed to find any correlation between the CD3(+) cell count and graft characteristics such as the number of PBSC or CD3(+) cells infused. Moreover, we did not find any association between the number of lymphocyte count at diagnosis and CD3(+) cell recovery at the 12-month posttransplant (r = 0.1, P = 0.6). Finally, no correlation between CD3(+) cell count and the time from diagnosis to PBSC collection (P = 0.5) or transplant (P = 0.5) was found.

Twenty-one of 38 (55.2%) cases showed positive immunoreaction for p53, whereas 8 of 38 (21.0%) and 18 of 38 (47.3%) showed positive immunostaining. No association between the status of biological parameters and final CD3(+) cell count at the 12-month interval was observed. Interestingly, multivariate logistic regression showed that tumor grading (χ2 = 6.6, P = 0.010) and type of growth factor (χ2 = 4.1, P = 0.042) retained an independent role in predicting T-cell recovery above the value of 850 cells/µl.

Survival Analysis. During the follow-up period, progression and death from disease occurred in 27 of 38 (71%) and 19 of 38 (50%) cases, respectively.

Median TTP was 57 months with a 3-year rate of 86% (95% CI: 70, 102) in cases with high CD3(+) cell count with respect to median TTP of 20 months with a 3-year TTP of 23%
(95% CI: 8, 38) in cases with low CD3(+) cell count ($P = 0.00026$; Fig. 1A). The median OS was 107 months with a 3-year rate of 93% (95% CI: 81, 105) in cases with high CD3(+) cell count with respect to median OS of 49 months and a the 3-year OS rate of 62% (95% CI: 42, 82; $P = 0.0015$; data not shown). The plot of the relative risk of progression as a function of CD3(+) cell number is shown in Fig. 1B. By using CD3(+) cell count as continuous covariate, CD3(+) cell number was shown to be inversely associated with risk of progression ($\chi^2 = 4.8; P = 0.028$) Similar results were obtained when analyzing OS ($\chi^2 = 8.5; P = 0.0036$) as assessed by Cox univariate analysis.

In univariate analysis large residual disease at first surgery, low CD3(+) cell status, stage IV disease, and use of GM-CSF proved to be associated with a higher risk of progression (Table 3). In multivariate analysis only residual tumor and status of CD3(+) cell counts retained an independent association with shorter TTP (Table 3). Similar results were obtained when conducting multivariate analysis for OS, even when using CD3(+) cell count as a continuous variable (data not shown). Superimposable results in terms of TTP and OS were obtained when patients were dichotomized as having more or less than 400 CD4(+)CD8(+)NK cells/$\mu$L (data not shown). On the contrary, no difference in the clinical outcome was observed for patients with more or less than 150 (median value) CD3(−)/CD16(−)/CD56(−) NK cells/$\mu$L (data not shown).

DISCUSSION

This is the first study demonstrating that the absolute CD3(+) cell count after the 12-month follow-up after PBSCT is an independent prognostic factor for both TTP and OS in advanced ovarian cancer patients administered HDC with PBSC and growth factor support. Similar results documenting a correlation between the achievement of high lymphocyte count and favorable prognosis have been recently reported in hematological malignancies and metastatic breast cancer (10, 23) and may be related to the well known graft versus tumor effect occurring in allogeneic bone marrow recipients, in which the donor immune system is supposed to contribute to the eradication of residual disease in the host (24, 25). In the autologous setting these results could be explained by a more effective control of residual tumor cells through the action of an increased number of effector T cells, as suggested by our previous reports and by other investigators (5, 10).
It is, therefore, conceivable that the favorable clinical outcome in terms of TTP and OS observed in patients with high CD3(+) cell count could be related to a more effective control of residual tumor cells that survived HDC regimens.

We recently showed that G-CSF supports a better lymphocyte recovery than GM-CSF in patients receiving HDC, confirming data previously reported in other clinical settings (26). In this context, the availability of growth factors displaying different potential in enhancing T-cell recovery or hematopoietic rescue is of clinical relevance, although a more in-depth analysis of the kinetics of recovery of T subpopulations as well as their functionality is needed. Particularly, the role of specific stem/progenitor subsets present in the graft in determining the speed of CD3(+) recovery must be further clarified, even though our analysis and other reports excluded that counts of recovered CD3(+) cells are influenced by the dose of CD34(+) or CD3(+) cells in the graft (23, 27). In the current study, we first showed that besides the use of G-CSF, among clinicopathological parameters tumor grade is significantly associated with higher CD3 + cell counts. Whether this association reflects the ability of poorly differentiated ovarian tumors to interfere with growth factor-driven immune cell reconstitution or whether other unknown factors associated with tumor grading could be causally linked to earlier T-cell recovery is a major issue that needs to be addressed considering also that tumor grading failed to be associated with clinical outcome. On the other hand, preexisting (before HDC) low CD3(+) counts in patients with G3 tumors may be hypothesized as a tumor-host biological association because of unknown factors that could also contribute to delayed CD3(+) recovery after PBSC infusion. Specifically, a contribution of age-related thymic hypoplasia to poor release of thymic emigrants [namely circulating CD3(+) cells] could be taken into account (28), because in our series patients with G1/G2 tumors were shown to be significantly older than patients with G3 tumors. It is worth noting that the only study addressing the role of posttransplant T-cell recovery in breast cancer reported more aggressive biological features (prevalence of estrogen/progesterone receptor negativity) in patients with delayed lymphocyte recovery (10).

Interestingly enough, the prognostic role of the absolute CD3(+) cell counts was retained in multivariate analysis irrespective of the type of growth factor given after PBSC infusion. In the same way, additional statistical analysis revealed that a CD4(+) or CD8(+) cell predicted a significantly different TTP and OS rate as did the CD3(+) cell status, indicating the clinical relevance of adequate counts of both T-cell subsets. Conversely, no differences in TTP and OS were observed according to CD3(−)/CD16(+)CD56(+) NK cells, thus minimizing the role of late NK cell reconstitution. In reference to this, it has been considered that NK cells had a very prompt recovery in most patients and the range of NK cell count at 1 year of posttransplant follow-up was so narrow to prevent the identification of distinct clinical outcome by statistical analysis.

All patients in this series received autologous PBSC differently from previous published results in which several sources of progenitor cells were used (12, 23). Therefore, it is conceivable that besides the specific activity of different growth factors, individual repopulating potential or activities of T-cell subpopulations may influence long-term immune reconstitution. In this context, the analysis of thymic rearrangement circles of CD3(+) cells, which are a useful marker to establish the ontogenic proximity of posttransplant T cells to the thymus (28), could be clinically relevant, as reported in other clinical settings of immune reconstitution (29). Similarly, it is tempting to speculate that the recovery of low-affinity CD3(+) cells, which are considered more likely to recognize tumor cells (30), could possibly translate into antitumor-specific cell response. Our data would, therefore, be considered as potential surrogate markers for immunological antitumor response only when a thorough functional characterization of CD3(+) cell subtypes recovered after a long time from transplant will be available.

In conclusion, we showed that long-term immune reconstitution and particularly the recovery of adequate counts of CD3(+), CD4(+), and CD8(+) cells are independent markers of longer TTP and OS in ovarian cancer patients receiving HDC with PBSC and growth factor support.

Indeed, the possibility to predict the repopulating potentiality of each patient on the basis of her own tumor characteristics as well as source, number and subsets of progenitors infused, and mixtures of growth factors, would be clinically relevant to select patients who might benefit from HDC versus patients unlikely to take advantage of intensified regimen, who can be spared the related toxicity. This issue warrants further investigations in a larger series of cases.

REFERENCES


Lymphocyte Recovery in Advanced Ovarian Cancer Patients after High-Dose Chemotherapy and Peripheral Blood Stem Cell Plus Growth Factor Support: Clinical Implications

Gabriella Ferrandina, Luca Pierelli, Alessandro Perillo, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/9/1/195

Cited articles
This article cites 29 articles, 9 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/9/1/195.full.html#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
/content/9/1/195.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.