Stem cell-based treatments for gynecological solid tumors

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Abstract. – Background and objective: We have recently assisted to an increasing scientific interest and a new research effort in the field of stem cell-based therapy. Since the late 1980s hematopoietic stem cells (HSC) have been used to set up therapeutic strategies for the treatment of solid tumors such as gynecological cancers. In this context, different approaches have been suggested and clinically investigated.

State of the art: In the autologous setting we can describe the well-known use of HSC as hematologic support to high-dose chemotherapy regimens, and the use of HSC as a source of dendritic cells for cancer vaccination protocols. In our institution a long-term experience has been developed in high-dose chemotherapy with autologous HSC transplantation as first-line treatment of advanced ovarian cancer, and in the use of cytokines both for HSC collection and for post-transplantation hematopoietic recovery and immune reconstitution. An alternative approach consists of allogenic HSC transplantation following either myeloablative/standard or non-myeloablative/reduced conditioning regimens, which have been proposed as new adoptive immunotherapeutic treatments for different non-hematologic malignancies.

Perspectives: Future strategies in the use of HSC in oncology comprise the possibility of HSC ex-vivo expansion, the use of umbilical cord blood HSC, and the development of HSC-based gene-therapy programs. Further investigations are expected in the new field of cancer stem cells.

Key words: Stem cells, Progenitor cell transplantation, Gynecological tumors.

Introduction: biological considerations and therapeutic applications of hematopoietic stem cells

Human hematopoietic stem cells (HSC) are defined by their functional property of self-renewal with a concomitant ability to differentiate into specialized and active lympho-myeloid cells. Therefore HSC are able to reproduce themselves with long-term survival and to generate progenitor cells which, in turn, loose their self-renewal capacity, giving rise to mature cells. In fact, the concept of progenitor identifies cells with limited survival, poor self-renewal capacity, high proliferative capacity in response to stress signals, and differentiation capacity in a limited repertoire of maturative lineages. HSC are characterized by a hierarchical organization and several classes of HSC have been very recently described which express or do not express the CD34 protein that characterizes the “bulk” of active hematopoietic progenitor cells, or showing a certain degree of differentiative “plasticity”, i.e. the ability to transdifferentiate across their specific embryonic germ layer of origin. Current clinical applications of stem/progenitor CD34+ cells consist of both allogenic (in hematopoietic malignancies, immunodeficiencies, bone marrow aplasias, storage diseases, hemoglobinopathies) and autologous (in acute leukemias, myelomas, lymphomas, breast and ovarian cancer, germ cell tumors, autoimmune diseases) transplantation. Suitable sources of human non-embryonic HSC are fetal liver and cord blood or adult bone marrow and mobilized peripheral blood. Future applications of adult HSC could contribute to establish regenerative treatments through the use of organ-specific cell therapies for the prevention of organ failures or progressive diseases due to inherited or degenerative disorders.

The role of autologous HSC for therapeutic strategies in gynecological tumors

High-dose chemotherapy (HDCT): rationale and clinical results

Epithelial ovarian cancer still causes more deaths than any other tumor of the female re-
productive system. Two thirds of women with ovarian carcinoma present with advanced stage disease. Although some advances have been made in first and second-line chemotherapy development, few patients with stage III and IV disease are long-term survivors as, inevitably, chemotherapy drug resistance leads to progression of disease. HDCT treatment has been developed based on the observation that a step dose-response curve exists for certain tumors. In ovarian cancer, a large randomized trial demonstrated that, if modest, increases in chemotherapy doses, which do not require HSC transplantation, do not improve survival and lead to increased toxicity. HDCT with HSC support, allowing much greater increases in drug delivery, has been proposed as a way to overcome drug-resistance and prevent recurrences in ovarian cancer. Most early trials enrolled patients with refractory, bulky disease, and used bone marrow as the HSC source. Few conclusions were drawn from these initial experiences: HDCT could lead to impressive response rates even in refractory patients, but responses were transient, morbidity and mortality were high, and response rates were obtained at great cost to the patients' quality of life. The use of peripheral blood progenitor cells (PBPC), as source of hematopoietic rescue along with improved supportive care has led to a decrease in treatment-related morbidity. Moreover, the observation that relapsing chemotherapy-resistant ovarian cancer patients do not show a clinical benefit from the HDCT procedure, supported the idea of moving HDCT earlier in the course of treatment, perhaps yielding more durable effects. Limited data are available on HDCT and hematopoietic support as a first line treatment in advanced ovarian cancer patients, and the ideal patient population as well as the best treatment regimen still remain to be established. Rapidly sequenced HDCT with PBPC transplantation in the front-line has been proposed in previously untreated ovarian cancer patients. However, results of these trials were disappointing showing the failure of repeated courses of front-line HDCT to improve the outcome of patients. Other groups treated a large number of patients with a course of single high-dose chemotherapy as a consolidation approach following a few cycles of induction chemotherapy. These phase II trials contributed to define which patient population could benefit from the HDCT procedure. In most studies of HDCT as a consolidation approach, a low tumor burden and the presence of chemosensitive disease predict patient outcome. Other more recent phase II studies have been designed to define the better HDCT regimen, or to evaluate the use of new anticancer drugs such as topotecan in HDCT treatments.

In this context, the “GINECO trial”, which represents the first phase III randomized study of HDCT for stage III/IV ovarian cancer patients following one platinum-based induction regimen, has randomized 110 advanced ovarian cancer patients showing tumor responsiveness at second-look operation, to receive either three cycles of standard-dose cyclophosphamide and carboplatin or one high-dose cycle of the same drugs. With a median follow-up of 60 months, both the progression-free survival (12.2 months vs. 17.5 months) and the overall survival (42.5 months vs. 49.7 months) were not significantly different between the conventional-dose arm and the high-dose arm respectively. According to the results of this small-sized trial, HDCT shows no significant advantages in ovarian cancer consolidation. However, we are waiting for more data for HDCT in first-line (OVCAT trial) or in relapse (EBMT trial).

**High-dose chemotherapy as first-line treatment: the Catholic University of Rome experience**

**Study design and patient outcome in ovarian cancer.** In our institution we assessed the long-term impact of HDCT with hematopoietic PBPC support as a consolidation approach in a phase II study enrolling fifty-five patients with advanced chemosensitive ovarian cancer (stage IIIc or IV, G2-G3 tumors). These patients were optimally cytoreduced at the first surgery or at the interval debulking surgery (IDS) and received HDCT as first-line chemotherapy. HDCT was administered after the administration of 2-3 courses of cisplatin-based nonmyeloablative chemotherapy and consisted of carboplatin 1,200 mg/mq, etoposide 900 mg/mq and melphalan 100 mg/mq (CEM). HSC support consisted of autologous bone marrow infusion in
The efficacy and toxicity of the regimen containing multiple cycles of moderately high dose carboplatin plus paclitaxel and epirubicin with PBPC and growth factor (GF) support was explored as neoadjuvant treatment in locally advanced cervical cancer (LACC) patients with bulky metastatic lymph node disease\textsuperscript{22}. FIGO stage IIB-IVA cervical cancer patients with bulky pelvic and/or paraaortic node involvement were included. Mobilization of PBPC was accomplished by rh-G-CSF (10 mg/kg/d) for 4 days. PBPC aliquot containing a fixed dose of 1.5 $\times$ 10$^6$/kg CD34+ cells was infused. Neoadjuvant chemotherapy containing carboplatin (AUC = 8), paclitaxel (175 mg/m$^2$), epirubicin (75 mg/m$^2$) intravenously was administered on day 1 of a 4-weekly cycle for 3 cycles. PBPC were administered on day 3 and rh-G-CSF (10 mg/kg/d) was administered from day 4 to day 13. The primary end-point of the study was the overall response rate (OR). Between April 2000 and February 2003, 12 patients entered the study. A total of 30 courses of therapy were administered. Severe (grade 3,4) leukopenia, neutropenia, anemia and thrombocytopenia occurred in 30%, 36.7%, 23.3%, and 40% of the cycles, respectively. Two patients developed sepsis recovered with antibiotics. No case of treatment related death was observed. Objective response was observed in two cases (18.2%) including a complete and a partial response. Due to the toxicity, the study was stopped prematurely. Our data do not favour the use of regimens leading to the delivery of high total dose platinum compounds plus PBPC and GF support in LACC patients with poor prognosis features.
creased PBPC mobilization and collection as compared with that of G-CSF-treated patients ($p=0.0009$ and $p=0.0026$, respectively), who required a significantly higher number of leukaphereses than G-CSF plus EPO treated patients ($p=0.0076$) to obtain the planned dose of PBPC. Qualitative analysis by cloning assay of PBPC collected in both arms revealed that G-CSF- and G-CSF plus EPO-mobilized PBPC have comparable in vitro functional properties. In light of these results, we also tested the mobilization capacity of EPO in combination with sequential GM-CSF/G-CSF. This new EPO-based cytokine regimen improved the PBPC collection efficiency after platinum-based intensive polychemotherapy, associating high PBPC mobilization to high collection efficiency during apheresis. Furthermore, in a recent experience we evaluated the use of cytokines alone (without chemotherapy) for PBPC collection in patients with gynecological malignancies. In our series EPO addition to G-CSF in different schedules did not seem to improve PBPC mobilization/collection, suggesting that potentiation of PBPC mobilization induced by EPO in chemotherapy-primed patients cannot be reproduced in steady-state conditions.

The use of cytokines in high-dose chemotherapy treatment: hematopoietic recovery phase and clinical outcome

Autologous PBPC transplantation (PBPCt) represents a supportive measure to rapidly reconstitute hematopoiesis following the administration of myeloablative high-dose chemotherapy. Hematopoietic growth factors such as G-CSF or GM-CSF accelerate neutrophil recovery with a consequent reduction in the number of days with fever and antibiotic treatment, without relevant effects on platelet and erythroid recovery. Theoretically, the clinical use of exogenous cytokines after PBPCt may cause a selective expansion of hematopoiesis in which a single lineage might expand at the expense of the others, via a mechanism of progenitor cell competition. This point assumes particular interest if we consider that post-chemotherapy immune-hematopoietic reconstitution probably contributes to the control of the residual disease. In reference to this, recent data suggest that reconstitution of the immune response could eradicate tumor cells that escaped the cytotoxic damage produced by chemotherapy. In this context, we analysed the clinical outcome according to myeloid/lymphoid recovery of patients randomized to receive G-CSF or GM-CSF after PBPCt. Thirty seven ovarian cancer and 34 breast cancer patients ranging in age from 24 to 60 years were treated with CEM high-dose chemotherapy and subsequently randomized to receive G-CSF (5 µg/kg subcutaneously) or GM-CSF (5 µg/kg subcutaneously) until day + 13 post-PBPCt. Hematopoietic recovery and post-transplant clinical management were comparable in both the G-CSF and GM-CSF series. Conversely, significantly higher T lymphocyte counts were observed in G-CSF patients during the early and late post-transplant follow up. Patients who received G-CSF showed a significantly longer median TTP. A parallel analysis revealed that patients who obtained a higher CD3+ count had a significantly longer OS and TTP. The enhancement in post-PBPCt T cell recovery observed in G-CSF patients encourages the use of G-CSF to ameliorate immune recovery which seems to play a role in the post-PBPCt disease control of cancer patients. GM-CSF might be administered to prolong immunosuppression after auto-PBPCt for autoimmune diseases or allogeneic PBPCt. Conceivably, the early administration of a growth factor involved in T-cell development such as IL-2 might produce a lymphoid-oriented differentiating stimulus on transplanted stem cells and activation on de-novo generated lymphoid cells. To verify the role of post-PBPCT low-dose IL-2 administration, we successively carried out a prospective non-randomized study where G-CSF/EPO-treated patients were compared with G-CSF/EPO plus IL-2-treated patients at several time points from PBPCt. To this end, two consecutive series of patients were prospectively assigned to distinct post-PBPCt cytokine regimens (from day +1 to day +12) which consisted of G-CSF (5 µg/kg/day) plus EPO (150 IU/kg/every other day) in 17 patients (13 BrCa and 4 OvCa) or G-CSF/EPO plus IL-2 ($2 \times 10^6$ IU/m²/day) in 15 patients (10 BrCa and 5 OvCa). Hematopoietic recovery and post-transplantation clinical care were comparable in G-CSF/EPO- and in G-CSF/EPO plus IL-2-treated patients without significant side-eff-
Effects attributable to IL-2 administration. In the early and late post-transplant period a significantly higher PMN count was observed in the G-CSF/EPO plus IL-2-treated patients \((p=0.034\) and \(p=0.040\) on day +20 and +100, respectively). No significant differences were found between the two groups of patients in the kinetics of most lymphocyte subsets except naive CD45RA+ T cells which had a delayed recovery in G-CSF/EPO plus IL-2 patients \((p=0.021\) on day +100). No significant difference was observed between NK activity in the two different groups, albeit a significantly higher NK count was observed in G-CSF/EPO plus IL-2 series on day +20 \((p=0.020)\). These results demonstrate that low-dose IL-2 can be safely administered in combination with G-CSF/EPO early after PBPTC and that it exerts favourable effects on post-PBPTC myeloid reconstitution but not on immune recovery.

Furthermore, we investigated 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\(^{34}\). Thirty-eight patients with stages II–IB–IV epithelial ovarian cancer were studied. Lymphocyte immunophenotyping for the identification of CD3(+), CD4(+), CD8(+), and CD3(−)/CD16(+)CD56(+) natural killer T cells and CD19 B cells was performed. Twenty-three patients (60%) had a CD3(+) cell count <850 cells/µl. Multivariate logistic regression showed that tumor grading \((p=0.010)\) and type of growth factor \((p=0.042)\) 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% in cases with high CD3(+) cell count with respect to a 3-year TTP of 23% in cases with low CD3(+) cell count \((p=0.0026)\). The absolute number of CD3(+) cells was shown to be inversely associated with the risk of progression \((p=0.028)\), as assessed by the Cox univariate analysis using the CD3(+) cell count as a continuous covariate. In multivariate analysis only the residual tumor and the status of CD3(+) cell counts retained an independent association with shorter TTP. Similar results were obtained for overall survival. 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.

**Hematopoietic stem cells as a source of specialized cells for the vaccine-therapy of malignancies**

For some tumors, the use of surgery and conventional chemo-/radio-therapy treatments cannot achieve a good therapeutic efficacy. For this reason, in the last ten years, oncological research has been applied to the study of the interactions between the immune system and the neoplasm, in order to identify biological mechanisms that could be clinically used to specifically eliminate neoplastic cells which express immunogenic antigens on their surface. Although it has been clearly established that tumour progression takes place despite the control of the immune system, it is also clear that neoplastic cells can select biological mechanisms able to escape this immune control\(^{35}\), such as the secretion of immunosuppressive factors, the selection of resistant neoplastic clones, and the induction of the immune tolerance. Dendritic cells are the most efficient cells of the immune system in presenting an antigen to the helper/cytotoxic T-lymphocytes. In the last few years, dendritic cells have been used to try to break the tumor-induced immunological tolerance on specific cytotoxic T-cells. Various experimental and clinical data have demonstrated that it is possible to use dendritic cells to induce a protective and therapeutic antineoplastic immunity\(^{36-38}\). In particular, a tumour regression has been observed in patients with non-Hodgkin lymphoma or melanoma using ex-vivo generated dendritic cells pulsed with purified tumor antigens or with tumor cell lysates. Dendritic cells can be obtained ex-vivo by a differentiation from both hematopoietic stem cells and peripheral blood monocytes. Experimental conditions able to obtain such differentiation have been extensively investigated\(^{39}\). The research and the clinical use of vaccination protocols with dendritic cells open new perspectives for the development of efficient immunotherapeutic strategies for the treatment of malignant tumors.
The role of allogenic HSC for therapeutic strategies in solid tumors

Allogenic stem cell transplantation: background and rationale

Earlier phase II trials suggested that high-dose treatments can increase the overall survival in ovarian cancer patients without obtaining the complete eradication of the neoplastic clone. In this context, a complementary treatment could ameliorate the control of the minimal residual disease after the success of a significant cytoteruction with high-dose chemotherapy. Different observations suggest the possibility of inducing an immune autologous response against ovarian cancer obtaining the control of the minimal residual disease through immunological mechanisms. At present allogenic transplantation with donor lymphocyte infusion (DLI) is the only kind of adoptive immunotherapy whose efficacy has been demonstrated in haematological malignancies. More recent studies have also demonstrated an alloreactive antitumoral effect of graft-versus-host (GVT) in solid tumours such as breast and ovarian cancer. Over the last few years a significant reduction of the high toxicity and mortality-rate related to allogenic transplantation has been achieved through the use of reduced-conditioning regimens (i.e. “mini-allografts”), followed by the DLI after the achievement of bone marrow chimerism. For this reason the use of allogenic transplantation procedures in solid tumours is now both scientifically and ethically warranted, even if efficient palliative treatments are available.

Nonmyeloablative HSC transplantation

Since a GVT effect had been documented in solid tumors, several transplant groups sought to test the hypothesis of whether allogenic transplants could be effective at eliminating malignancy almost solely through the allograft effect rather than the preparative regimen. This approach requires the use of reduced-intensity conditioning regimens with an only immunosuppressive purpose to prepare the recipient for transplantation, and the administration of short immunosuppression to prevent graft-versus-host disease (GVHD) in order to permit rapid and complete immune recovery and a GVT effect. Donor lymphocyte infusions are given to provide further GVT effects by converting the immune system to 100% donor. A nonmyeloablative strategy has obvious advantages over the standard transplant approach using intensive preparative regimens in metastatic cancer patients who are typically resistant to chemo/radiotherapy and do not respond to dose intensification. Furthermore patients with widespread disease are debilitated and tolerate intensive preparative regimens poorly. Thus, the lower toxicity of nonmyeloablative allogenic transplant justifies, also from an ethical point of view, the use of this procedure in solid tumors. While data of Childs et al. and those of other groups show clear evidence of the existence of a GVT effect in renal cell carcinoma, few data are available on other solid tumors such as ovarian cancer. Different small pilot studies have been recently published exploring the clinical role of nonmyeloablative allograft both in metastatic breast cancer and chemoresistant ovarian cancer. In summary, preliminary results reported in chemoresistant ovarian cancer patients (Table I) confirm the feasibility of a reduced-intensity allogenic transplant procedure and suggest that response rate and survival might be increased compared to the outcome after conventional chemotherapy. However, it is too early to determine whether allogenic transplantation in ovarian cancer is a good alternative because additional patients and more follow-up time are required. Furthermore, it is important to emphasize that the risk of life-threatening regimen-related complications (i.e. uncontrolled acute GVHD) remains high. For these reasons, allograft procedure for solid tumors still has to remain under investigation in specialist units. Future strategies should aim at achieving a wider therapeutic window through the enhancement of the anti-tumor effect and the decrease of the GVHD.

Perspectives in the clinical use of hematopoietic stem cells in gynecological oncology

The recent biological acquisitions on hematopoietic stem cells disclose the possibility of using these cells as vectors for gene-therapy strategies. The transduction of
After in-vivo transplantation in NOD/SCID mice, the evaluation of the “stemness” in solid tumors has been performed by means of a phenotypic analysis of suitable antigens such as CD133 which is also expressed by hematopoietic stem cells. This new stem cell model for cancer may have implications for both the biological carcinogenesis and the treatment of cancer. As a matter of fact, the main goal of an effective anticancer therapy should be the elimination of the cancer stem cells which drive the abnormal tumor growth and represent the tumorigenic cell fraction. Therefore, anticancer drugs which target the deregulated self-renewal pathways in cancer stem cells have to be identified and used to improve outcomes in the treatment of solid tumors.

**The hypothesis of cancer stem cells**

In the last years, the idea of the existence of cancer stem cells has been proposed and investigated in solid tumors. It is based on two experimental observations: (I) the tumor growth is not supported by all the cells within the tumor, (II) large numbers of tumor cells are needed to transplant a tumor in xenograft models. Moreover, the clonal origin of most cancers has been criticized: cancer may consist of heterogeneous cell populations imitating the hierarchical tree of stem cell lineages. A long-lived stem cell could be hypothesized at the origin of tumor initial proliferation and progression towards malignancy through accumulation of mutations and epigenetic changes. However, the isolation of cancer stem cells has been performed only recently in breast, prostate, nervous system and blood tumors, by applying techniques used in the stem cell field. Self-renewing cell populations have been identified which are capable of giving rise to heterogeneous primary and secondary tumors after in-vivo transplantation in NOD/SCID mice. In this context, the evaluation of the “stemness” in solid tumors has been performed by means of a phenotypic analysis of suitable antigens such as CD133 which is also expressed by hematopoietic stem cells.

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Acknowledgements

This research was supported by Cord Blood Stem Cell Project “Fondazione Cassa di Risparmio”, Rome (Italy).