review
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PERIPHERAL BLOOD STEM CELL TRANSPLANTATION FOR THE TREATMENT OF MULTIPLE MYELOMA: BIOLOGICAL AND CLINICAL IMPLICATIONS

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The aim of this review is to define the role of peripheral blood stem cell transplantation for the treatment of multiple myeloma. Therefore, we first review our present knowledge of this disease and then analyze the clinical trials based on the use of autologous bone marrow or peripheral stem cell transplantation. Optimal methods for peripheral blood stem cell transplantation will also be discussed.

Myelomagenesis

Multiple myeloma (MM), the prototype plasma cell malignancy, is characterized by the uncontrolled accumulation of plasma cells that replace normal bone marrow (BM) and by the overproduction of monoclonal immunoglobulins (Ig) and cytokines. A number of observations provided both by basic sciences and by clinical investigation allow us to place the disease and its unusual features in a more coherent perspective and to discuss new therapeutic options properly.

Epidemiology

The reported incidence of MM is available for the years up to 1982 and varies substantially in different countries.[1] A striking increase in the incidence of MM has been noticed in the last thirty years and is only partially[2] explained by amelioration of diagnostic capabilities.[3] Between 1973 and 1990 an increase of 40% among people over 65 and of almost 15% among people under 65 has been recorded in US Cancer Death rates.[4] Ethnic differences are apparent: the incidence is twice as high and the mortality rate has quadrupled in blacks, while doubling in whites.[4] By contrast, rates among Asians are lower than those of whites living in the same geographic area.[5]

Both genetic and environmental factors can be invoked to explain these ethnic differences. A significant increase has been detected in first-degree relatives of patients.[5] Moreover, an increased risk has been observed to be associated with occupational and environmental elements that include farming exposure to pesticides, exposure to ionizing radiations, petroleum and rubber processing, as well as persistent (viral) infections.[3] The main conclusion that can be drawn from a large body of observations is the necessity of discriminating the genetic roots from the environmental links of the disease. As a corollary, it may be asked which elements (genetic vs. environmental) are associated with the development of monoclonal gammopathy of undetermined significance (MGUS) and how they relate to the progression of MGUS to overt MM.

Cytogenetics and molecular biology

Two major pieces of information have emerged from cytogenetic studies. The first is that no consistent (yet not random) chromosome abnormalities have been detected in MM.[6] The second is that numeric chromosome abnormalities are shared by MGUS and MM.[7,8] Both

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facts lead us to ask what the prerequisite is and what the additional events are in the development of plasma cell malignancies. We still do not know the prerequisite events that lead to MGUS, to MM or to the evolution of MGUS into MM, or how they differ from collateral elements that simply favor the malignant process. Along the same vein, it is interesting that no known specific oncogene has yet been related to the development of MM or to the transition from MGUS to overt MM. The genes most commonly implicated in MM, like N-RAS, P53 and retinoblastoma gene (RB), are all involved in the late stages of the disease.[9]

If the same cytogenetic abnormalities are shared by two clinical situations as different as MGUS and overt MM, a patrolling role for the immune system can be envisaged in the natural history of plasma cell disorders. It is not unreasonable to suspect that if the immune system is able to keep a malignant clone under control, a benign MGUS is the resulting disease; the breakdown of this control would lead to MM. Little direct, but much indirect evidence is available in murine models to suggest the immunomodulation of myeloma cell growth by host effector cells.[10]

Immunochemistry and B cell differentiation studies

Three major findings have been obtained through immunochemistry and by a more proper understanding of the differentiation processes of B lineage cells. First, MM paraproteins may be directed against a wide variety of infectious agents, suggesting that MM development and antigen (Ag) stimulation may be causally related.[11-13] Second, the Ig isotype of MM plasma cells is generally IgG or IgA, demonstrating that the predominant phenotype of MM tumor cells is post-switch.[9] Third, clonal proliferation involves a cell population that has already passed through the stage of Ig genes somatic hypermutation.[14,15] Since this process occurs in the germinal centers (GC) of secondary follicles,[16] its presence is a clear marker of the differentiative and functional level reached by the cell population being analyzed.

By and large, the observation that MM is a neoplasm of plasma cells that have a post-switch phenotype, show somatic mutations and may produce monoclonal Ig with targeted antibody (Ab) activity leads to the conclusion that MM is an Ag-driven process, even if the specific causal Ag is generally unknown. This assumption has to be confronted with the simple, though basic, lesson from clinical medicine that MM is a BM disorder. In contrast with the distribution of normal plasma cells, MM plasma cells localize uniquely within the BM.[9] Although the lamina propria of the intestine contains more Ig-producing cells than all other tissues in the body, it is never a site where MM develops, not even IgA1- and IgA2-producing MM.[17] Likewise, involvement of the spleen and/or lymph nodes, though typical of Waldenström's macroglobulinemia, is very unusual in MM.[17] The exclusive BM localization of MM plasma cells appears to conflict with the extensive somatic hypermutations of the Ig they produce, which indicate a peripheral origin of malignant cells. However, while the steps of Ag processing and presentation that lead to the generation of somatically

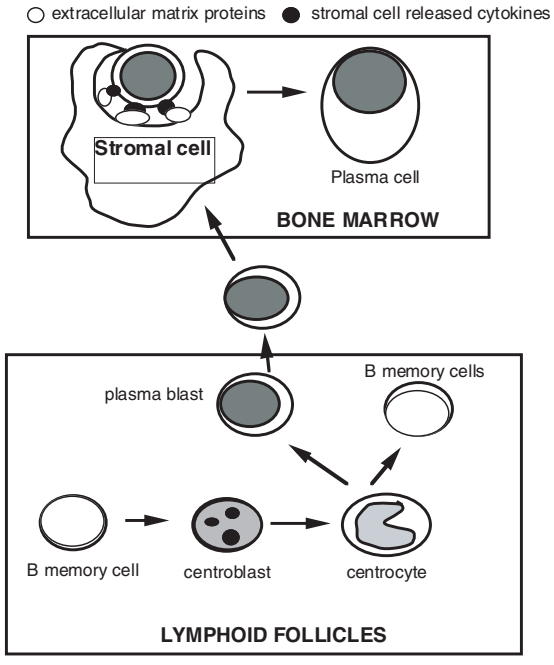

Figure 1. Plasma cell precursors generated in peripheral lymphoid organs differentiate in contact with bone marrow stromal cells.

mutated IgG and IgA plasma cells occur only in secondary lymphoid follicles, the BM is a major site of IgG and IgA production in T-cell-dependent secondary immune responses.[18-20] Plasma cell precursors with specific traffic commitments originate from secondary lymphoid organs and migrate to the BM a few days after the Ag challenge (Figure 1).[20,21]

The issue whether MM plasma cell precursors are early BM stem cells or late peripheral B cells is misleading. The cell whose original transformation has ultimately generated the malignant plasma cell progeny that we see in MM cannot be equated with the B cell population that disseminates the disease throughout the axial skeleton.[21] The identity of the hypothetical MM stem cell is unknown, i.e. we do not know either the cellular target of the primary transforming event or where, when and how the unknown cellular target was hit by the transforming event. By contrast, the information available on the B cell population that feeds the downstream compartment of plasma cells and disseminates the disease indicates that this population has been generated in peripheral lymphoid organs during secondary T-cell-dependent Ab response, is programmed to home to the BM, and is committed to differentiate in close association with the BM microenvironment (Figure 2).[21,22] On the basis of existing data, the most likely candidate for the physiological B lymphocyte equivalent of the MM plasma cell precursor is either a B memory cell or a plasma blast (Figure 1).[14,15,23,24]

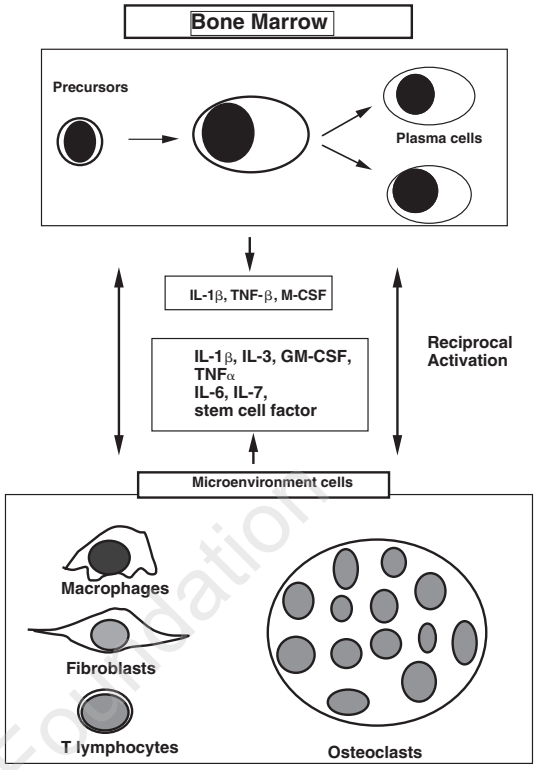

Figure 2. Model of multiple myeloma growth and progression based upon a series of mutual interactions between the B-cell clone and the bone marrow microenvironment.

Microenvironment and cytokines

It is assumed that BM-seeking plasma cell precursors receive a differentiation signal after contact with the BM stromal microenvironment (Figure 2).[25,26] Microenvironmental stromal cells play an essential role in the growth of plasma cell tumors both in mice[27] and in humans.[28] MM BM stromal cells are well equipped with a large series of adhesion and extracellular matrix molecules that mediate homotypic and heterotypic interactions and provide anchorage sites to cells selectively exposed to locally released growth factors.[22,29,30] MM BM stromal cells produce cytokines like IL-6 known to play a crucial role in the evolution of the disease both in experimental systems, including IL-6 transgenic mice, and *in vivo*.[31-34] High levels of IL-6 are observed in the sera of patients with aggressive or progressive MM,[35] and infusion of anti-IL-6 antibodies in patients with plasma cell leukemia or MM refractory to therapy has decreased the size of the plasma cell pool and hampered the proliferative activity of plasma cells.[36]

Malignant MM plasma cells are not inert vehicles of monoclonal Ig. They also produce a number of cytokines, including interleukin (IL)-1b, tumor necrosis factor (TNF)-β and monocyte-macrophage colony stimulating factor (M-CSF), that activate stromal and accessory cells, aa well as having significant osteoclast activating factor (OAF) activity.[22,37] A minority of human MM cell lines autonomously produce small amounts of IL-6, but it is unclear whether fresh MM plasma cells can also produce IL-6.[34] IL-6, besides promoting B cell proliferation and dif-

ferentiation, has recently been shown to have important OAF activity.[32,33]

These experimental findings linked to clinical observations lead to the attractive hypothesis (Figure 2) that a self-maintaining series of mutual interactions between the malignant B cell clone and the BM microenvironment may explain the progression of MM[22] through the production of ever-increasing amounts of cytokines capable of recruiting and activating several microenvironmental cells, including osteoclasts.

The role of autologous transplantation in the treatment of multiple myeloma

Investigations into the use of myeloablative therapy for the management of MM were pioneered in the mid-1980s and were stimulated by a persistent lack of progress in prognosis with conventional chemotherapy.[38,39] As is the case with any experimental approach, initial trials were restricted to the treatment of patients with advanced refractory or relapsing disease and were focused mainly on defining the feasibility and toxicity of the procedure. These preliminary experiences were performed without the support of hemopoietic stem cells and demonstrated that high-dose melphalan (HDM), given intravenously (i.v.) at doses ranging between 100 and 140 mg/m^2, yielded an increase in the complete remission (CR) rate, albeit at the expense of prolonged marrow aplasia and an unacceptably high early mortality rate.[40-42] On the basis of these observations later studies with chemotherapeutic agents administered at myeloablative doses, and possibly added total body irradiation (TBI), were carried out with the support of autologous BM and/or peripheral blood hemopoietic stem cells (PBSC).[43] Demonstration of the safety and relative efficacy of autotransplants in refractory MM[41,44-46] encouraged subsequent application of this procedure in earlier phases of the disease[45,46] and, more recently, in newly diagnosed patients as well.[47,48] Over the past decade interest in this new treatment strategy has progressively grown, and the number of reported patients receiving autologous hemopoietic stem cell-supported myeloablative therapy is now approximately one thousand worldwide.

What lessons have we learned from this collective experience? It is difficult to draw firm conclusions from published trials since none of them were controlled and patient populations were different, as were the preparative treatments and the criteria used for evaluating tumor response. In addition, the bias introduced by patient selection and, in most of the cases, the lack of an adequate follow-up also helped complicate correct interpretation of the data. As a consequence, the exact role of autotransplantation in the management of MM still remains poorly defined and could be properly addressed only in controlled clinical studies comparing autografting and conventional chemotherapy. There are at least several such trials in progress at the moment in Europe and the United States. Data reported at the last ASH meeting in Seattle (1995) by the *Intergroupe Français du Myelome* are promising and suggest an advantage for autografted patients in terms of increased CR rate and extended survival duration.[49]

Obviously, these results warrant confirmation in larger independent series. For this reason, similar investigations are currently being conducted in the United States under the auspices of the National Cancer Institute. While the conclusions of these studies are being awaited, analyses of available transplant data have provided the following important information.

Transplant-related mortality

Transplantation of autologous hemopoietic stem cells following myeloablative therapy has greatly improved the tolerance to this modality of treatment and reduced the frequency of procedure-related mortality to less than 5-10%[50-52] (Tables 1, 2). More recently, with the combined support of BM and PBSC followed by post-transplant administration of hemopoietic growth factors, early mortality was further decreased to approximately 1-2%.[53]

Tumor response and overall survival

Increased tumor response, as recognized by an increase in the CR rate, has been reported by many groups following myeloablative treatments (Table 1).[45-48,54,55] Basically, criteria for CR includ-

Group	No. pts.	% sens.	Source % BM	% PB	% ED	% CR	Median mos. PFS	Surv.
EBMT	130	68	63	25	6	48	17	27
Univ. Arkansas (USA)	287	60	unknown		<5	27 (IF)	22	35
French Registry	133	77	61	38	4	37	33	46

Table 1. Results of autotransplants for multiple myeloma.

Abbreviations: EBMT, European Group for Blood and Marrow Transplantation; Sens., responsive to conventional chemotherapy; BM, bone marrow; PB, peripheral blood; ED, early death; CR, complete remission; IF, immunofixation analysis; PFS, progression-free survival.

Author	No. pts.	Median mos to transpl.	BM/PB	TBI	% ED	% CR	IFN-α	Median mos. PFS	Surv.
Jagannath	14	<12	+/-	+	0	36 (IF)	–	16	33+ (86%)
Attal	35	9	+/-	+	3	43	+	33+ (53%)	41+ (81%)
Cunningham	53	<12	+/-	–	2	75	–	23	54+ (63%)
Harosseau	103	7.5	+/+	+	4	33	±	37	54
Barlogie	89	<12	+/+	+/-	0	46 (IF)	+	37	71+

Table 2. Results of autotransplants for recently diagnosed MM patients with chemosensitive disease.

Abbreviations: BM, bone marrow; PB, peripheral blood; ED, early death; CR, complete remission; IF, immunofixation analysis; IFN-α, interferon-α; PFS, progression-free survival.

ed both the disappearance of monoclonal plasma cells in the bone marrow, as evaluated on cytological smear examination or on flow cytometric analysis of DNA and cytoplasmic immunoglobulins, and no detectable M component by routine electrophoresis (later immunofixation was added). As would be logically expected, the CR rate varied in different studies, with a range between 20% and 80%, mainly depending on the use of more or less stringent definition criteria and the status of the disease at transplant (Tables 1 and 2). Moreover, the length of survival was generally extended after autotransplant, up to a median of approximately 3 to 5 years (Tables 1 and 2).[48,50-52]

Choice of myeloablative therapy

Historically, the autotransplant experience in MM can be divided into two groups of studies: the ones using and those not using TBI as part of the conditioning regimen. With few exceptions,[55] HDM, administered at doses ranging between 140 and 200 mg/m^2 has been the mainstay of both chemo-radiotherapy[45,47,49,50,52,56] and radiation-free regimens[48,53,54,57] for the following reasons: it shows a close dose relationship, is not cross-resistant with other alkylating agents and compared to cyclophosphamide, seems to offer a better chance of overcoming chemotherapy resistance.[58] In the absence of controlled clinical studies comparing different preparative treatments in specific subgroups of patients, it is hard to draw any meaningful conclusion concerning the best conditioning treatment. The impression from the data available in the literature is that no particular regimen demonstrated clear-cut superiority over the others. Therefore the choice of treatment to be used as preparation for autotransplant should ultimately take into account the ability to perform TBI, patient eligibility for TBI (those previously irradiated on the spine cannot, in fact, be candidates for radiation), and the expected toxicity. HDM at 200 mg/m^2 probably has less acute extrahematological toxicity than regimens including TBI, a finding that formed the basis for exploring repeated administrations of this drug with tandem (or double) autotransplant programs.[53,59]

Remission duration

As previously emphasized, myeloablative therapy requiring autologous hemopoietic stem cell support provides substantial antitumor response, especially in patients with good prognosis (see below). However, even in this favorable condition, a considerable relapse rate, approaching 60% at 3 years, is reported after autotransplant and no *plateau* is yet apparent on relapse-free survival curves.[50-52] These results contrast with the 30% probability of long-term unmaintained remissions (and possible cures) reported by several groups for patients receiving allogeneic transplantation.[60] It has been suggested that the lack of an immunological effect by the donor's marrow T lymphocytes on the residual myeloma cells (i.e. graft-versus myeloma)[61,62] and/or possible tumor reseeding may account for the apparently less durable duration of disease control following autologous as opposed to allogeneic transplantation. For this reason, important issues currently under clinical investigation in the autografting setting include further increases in the cytotoxic dose intensity level and depletion of tumor cells from the graft (see below).

Prognostic variables

Several important variables affecting the outcome of autologous transplantation have been identified (Table 3), including β_2-microglobulin (β_2-M) levels,[45,47,50-52,56] pre-transplant disease status,[45,51,52] age,[45,51,52] performance status,[45] Ig isotype[45,51,52] and response to myeloablative therapy (e.g. attainment or non-attainment of CR).[47,48] In particular, at multivariate regression analysis early mortality was reported to be highest among resistant relapsing patients, who also had the poorest response to myeloablative therapy and the shortest relapse-free survival duration.[45] In contrast, low serum β_2-M levels, both at diagnosis and before autografting, and prior responsiveness to conventional chemotherapy conferred the highest CR rate, as well as prolonged relapse-free and overall survival durations.[45,47,50-52,56] In addition, the timing of autotransplant also emerged as an important and independent prognostic parameter.[56,64] This observation, on the one hand, was related to the generally reported improved outcome of patients transplanted earlier and, on the other hand, reflected the acquisition of multiple biological abnormalities in advanced phases of the disease[63] that ultimately led to refractoriness even to high-dose therapy.[64] Conversely, retaining sensitivity to high-dose therapy in earlier phases of MM assured better results, even in patients with primary refractory disease.[45,47,65]

New perspectives under clinical investigation

Based on the assumption that the failure of the conditioning regimen to eradicate the myeloma clone contributes most to post-transplant relapse, attempts to increase the intensity, and possibly the efficacy, of treatment by means of repeated courses of myeloablative therapy have recently been undertaken.[46,53] The more rapid recovery of hemopoiesis assured by the combined use of PBSC and post-transplant administration of hemopoietic growth factors[59] made the double transplant strategy feasible for approximately 60% of patients within one year.[53] Results of pilot trials in primary refractory MM indicated that such an approach provided superior antitumor effect with improved event-free and overall survival durations with respect to a single transplant.[53]

A controlled clinical study comparing in a randomized fashion single vs. double autografting in newly diagnosed patients is currently

Table 3. Variables affecting the outcome of autotransplants for multiple myeloma.[@]

Variable	Disease status					
	Refractory			Refractory + Responsive		
	CR	RFS	Surv.	CR	RFS	Surv.
Low β_2M	–	+*	+*	+	+*	+*
Early transplant	+	+	+	+	+*	+*
CR achievement					+*	+*
Double transplant		+*	+*			
CT responsiveness			+	+	+	
Younger age	–	+	+	+	+	+
Non IgA isotype	–	+	+	–	+	+

**in multivariate analyses.*
Abbreviations: CT, conventional chemotherapy; RFS, relapse-free survival.
[@]Ref.: 45,47,48,50,51,52,56,63,64,65.

underway in France. A similar trial is already in the early accrual stage in Italy. These studies will clarify in the next several years whether double transplant is associated with better prognosis. Alternatively, efforts to improve the clinical impact of autotransplant have been carried out by several groups and have included depletion of tumor cells from autografts by both negative selection of myeloma cells and positive selection of $CD34^+$ hemopoietic stem cells,[66,67] as well as post-transplant immunomodulation with interferon-α (IFN-α).[47,49,68]

In summary, hemopoietic stem cell-supported myeloablative therapy holds the promise of being a safe and effective treatment modality for MM. It yields better overall response and CR rates than conventional chemotherapy and may prolong the duration of survival.[49]

These conclusions, while encouraging, have been drawn mainly from uncontrolled studies carried out in select groups of patients and obviously warrant confirmation in controlled clinical trials which are currently under way. Therefore the next several years will clarify whether newly diagnosed patients with symptomatic MM can be routinely offered a single or double autotransplant as first-line or early salvage therapy for their disease.

While the results of these studies are being awaited, wider application of myeloablative therapy should probably be encouraged. Less heavily pretreated patients who did not respond to prior conventional chemotherapy are more likely to benefit primarily from autotransplant. In addition, data available from the literature do suggest that a superior outcome of this procedure can be anticipated in patients with chemosensitive disease and low tumor burden at diagnosis. Hence, ongoing clinical trials aimed at comparing conventional versus myeloablative therapy will also address the important issue of the role of autotransplant as early consolidation therapy in patients with intrinsically good prognosis. However, even in this favorable situation, recurrence of the underlying malignant disease remains a major problem and is the most common cause of treatment failure. For this reason, attempts to improve the clinical impact of autografting are under active clinical investigation.

In addition, many other problems regarding autologous transplantation for MM are still unresolved and should be formally addressed in future clinical trials. The most important of these issues include the choice of the best conditioning regimen, the optimal source of hemopoietic stem cells, the nature of relapse after autografting, the benefit from purging techniques and, finally, the likelihood of long-term disease control, especially for patients with molecularly defined CR.[69]

Advantages offered by the use of PBSCs in the treatment of multiple myeloma

The use of PBSC in support of high-dose chemoradiotherapy (peripheral blood stem cell transplantation) (PBSCT) is a valid alternative to autologous bone marrow transplantation (ABMT) in the treatment of both hematologic and non-hematologic neoplastic disorders.[70-72] The growing interest in this procedure can be explained by: i) the possibility of mobilizing and collecting large amounts of hemopoietic progenitors,[73,74] and ii) the rapid hemopoietic recovery observed following PBSCT.[70,71,74-78]

Progenitor collection represents the critical step in the procedure. Daily monitoring of circulating $CD34^+$ cells is an essential assay in predicting the number and timing of leukaphereses.[79,80] Under proper conditions, only a few leukapheresis procedures are required to collect enough progenitor cells for marrow reconstitution after myeloablative treatments. Indeed, when circulating $CD34^+$ cells rise to >50/μL, 1-2 leukaphereses may yield more than 50×10^4/CFU-GM/kg or 8×10^6/$CD34^+$ cells/kg, which are considered the ideal values for optimal engraftment.[80-82] In addition, it has been shown that large quantities of very immature elements, identified as long-term culture-initiating cells (LTC-IC), are mobilized as well.[83-85]

Inclusion in the harvested material of very immature elements is responsible for the stable and durable marrow reconstitution observed in patients autografted with circulating progenitors.[77,83] Thus the term PBSC, now commonly employed to identify mobilized hemopoietic progenitors, relies on both biological and clinical

Intensified treatments*	Time to recovery from° leukopenia (days)	thrombocytopenia (days)	Treatment-related deaths (%)	References
Without autograft	28#	27	17	42,46,54,93-95
With BMT	20	26	7	44,45,47,48,96
With PBSCT	14	18	3.7	52,56,97,98

Table 4. Toxicity of intensified treatments with or without autologous stem cell support in multiple myeloma patients.

**intensified treatments consisted of HDM (60-200 mg/sqm) in most studies; the association of TBI/HDM was also used in some programs with autograft; °time to recovery from leukopenia and thrombocytopenia was reported as days to reach >0.5x10^9 ANC/μL and > 25x10^9 platelets/L, respectively, in nearly all studies; #table data have been calculated as medians from median values of hemopoietic recovery and from percentages of treatment-related deaths reported in each quoted study.*

observations. As previously emphasized, the rapidity of engraftment is the major advantage offered by PBSC. Nevertheless, some authors argued that BM cells stimulated by growth factor administration might be at least as efficient as mobilized progenitors in ensuring rapid engraftment following myeloablative treatment.[87-89] However, it has recently been shown that both committed and early progenitors are by far more frequent in PB than in BM during maximal mobilization.[90] This conclusive observation points toward the preferential use of PBSC as the hemopoietic cell source for grafting purposes.

Since its introduction into clinical practice, PBSCT has been considered a promising approach for MM patients.[91,92] Several studies have been designed in the last few years.

Reported results have shown a significant decrease in hemopoietic toxicity following this procedure as compared to ABMT, with recovery of granulocytes $> 0.5\times10^9$/L and plateles > 25-30×10^9/L within approximately 2 weeks after autograft (Table 4)[42,44-48,52,54,56,93-98] This was paralleled by rather good tolerability with rare early fatal events.[52,56,97,98]

In addition, hemopoietic reconstitution by PBSC seems to be long lasting. MM patients may require repeated exposure to high-dose cytotoxic therapy. Reducing hemopoietic toxicity might be critical for the ultimate treatment outcome. Therefore, also for its long-term effect, PBSCT may have a positive impact on the life expectancy of those patients who are suitable for intensified chemo-radiotherapy treatments.[99]

PBSC mobilization and collection in multiple myeloma

PBSC mobilization in myeloma patients

PBSC collection presents specific problems in patients with MM, where a decrease of progenitors in the bone marrow is due in part to a defect of the monocyte/macrophage activation pathway. In fact, CD34$^+$ cells from MM patients grow normal numbers of colonies when stimulated by normal monocytes, while normal CD34$^+$ cells have a reduced growth rate with MM monocytes.[100] Another aspect is prior treatment. Repeated courses of chemo-radiotherapy are able to exhaust the pool of pluripotent stem cells,[101] resulting in insufficient progenitor cell harvests.[59,102-105] Studies specifically addressed at MM patients show that melphalan[106] and treatment-free interval prior to PBSC mobilization[107] also have an influence on the release of progenitors into the peripheral blood, while the value of BM plasmacytosis as an independent factor is more questionable.[108,109] As a consequence of these and other unknown factors, progenitor yields in MM are often unpredictable and lower than those observed in other malignant disorders.[110] Nonetheless, cell harvests sufficient for one or two subsequent autografts are usually obtained,[59,97,108,111-113] even in patients with markedly infiltrated marrow or primary resistant disease.[109] To avoid the adverse influence of pre-mobilization treatment, PBSC collection in MM patients should be planned as early as possible in the course of disease, and alkylating drugs should be omitted in the primary treatment. It should also be kept in mind that heavily

Issue	Related aspects
Dysregulated or suppressed hematopoiesis	Decreased rate of progenitors,* defective monocyte activation,* prior chemotherapy
Methods for mobilization	Type (and doses) of chemotherapy, use of growth factors
Toxicity of mobilization therapy	Fever, allergy, infections, thrombosis
Kinetics of recovery after mobilization	Timed and asynchronous use of WBC, monocytes and platelets
Prediction of harvest	Prior chemotherapy, G-CSF test
Progenitor cell assays	$CD34^+$ cells, CFU-C
Target of collections	Need for $>5\times10^6$/kg $CD34^+$ cells in heavily pre-treated patients*
Apheresis method	Cell separator, volume processed, schedule of aphereses
Tumor contamination of harvest	Purging technique

Table 5. Key issues in mobilization and collection of PBSCs.
*Note. Most aspects are shared with other malignant disorders, and only a few may specifically affect multiple myeloma. These latter are marked with an *.*

pre-treated patients require more leukaphereses and show slower platelet recovery after autograft.[109] The key issues in the apheretic harvest of PBSCs in MM are presented in Table 5.

PBSC also may be collected from patients with malignancies in steady state conditions;[114] however, multiple aphereses are required with this method. Mobilization of progenitors with cytotoxic chemotherapy, hemopoietic growth factors, or a combination of the two is therefore generally preferred. The hematopoietic recovery that occurs after cytotoxic chemotherapy is accompanied by a PBSC rise that is proportional to the intensity of myelosuppression.[102, 108]

In MM, chemotherapy alone with either HDM,[115] or CHOP-like regimens[112,113,116] or intermediate- to high-dose cyclophosphamide (Cy)[97,117,118] has been used to mobilize PBSC. However, the failure rate, defined as the percentage of patients with a low progenitor cell peak in the blood or poor collections at the end of the apheresis program, was relatively high, ranging from 20 to 30%. Moreover, when using high-dose therapy protocols without growth factor support, one should consider that this implies an undue risk of severe toxicity.[118]

G-CSF[73,119-121] and GM-CSF,[75,112] as well as other cytokines are able to promote a dramatic rise of progenitors in the circulation. In a study of MM patients, administration of G-CSF at 10 μg/kg alone for six days induced a considerable increase in CFU-GM and $CD34^+$ cells,[111] with rapid recovery of counts after autograft. However, the use of growth factors alone in patients with neoplastic disorders produces little enthusiasm among hematologists. In fact, the spike of progenitor cells can be further amplified by combining growth factors with chemotherapy.[71] Together with the demonstration that tumor cells are also mobilized by growth factors,[123] this fact makes the combination of chemotherapy with G-CSF or GM-CSF the most reliable approach.[86,109,112,113]

In MM as in other diseases,[74,77] the use of growth factors following cytotoxic treatment proves to be superior to chemotherapy alone in terms of progenitor cell yield,[108,112] and significantly contributes to minimizing treatment toxicity.[112,124] High progenitor peak levels are reported[108] with high-dose chemotherapy, namely Cy at 7 g/m^2 or etoposide (VP16) at 2 g/m^2 followed by G-CSF or GM-CSF, and results seem to compare favorably with intermediate-dose Cy with or without G-CSF or GM-CSF. In conclusion, the optimal schedule for PBSC mobilization in MM has not yet been defined, though the most experience is with Cy at 7 g/m^2 followed by G-CSF or GM-CSF. A review of the mobilization schedules reported so far in MM patients is presented in Table 6.

Target of collections and cell monitoring

$CD34^+$ cell number and CFU-GM dose are both reliable predictors of engraftment time.[125-129] The amount of PBSC necessary for engraftment is not clearly defined, but values of 10 to 20×10^4/kg CFU-GM represent a reasonable minimal dose.[110,120] Irrespective of disease, rapid neutrophil engraftment has been reported with 20×10^4/kg CFU-GM or 2×10^6/kg $CD34^+$ cells.[125,130,131] However, a higher dose may be nec-

Table 6. PBSC mobilization schedules in multiple myeloma.

Authors	*No. pts*	*Treatment*	*Growth factor*	*Day of progenitor peak*	*Peaked CD34⁺/μL*	*Peaked CFU-GM/mL*	*Notes*
Reiffers[117]	15	Cy 7 g/m^2	no	nr	nr	nr	5/13 failures
Jagannath[97]	36	Cy 6 g/m^2	no	nr	nr	nr	better with GM-CSF
	39	Cy 6 g/m^2	GM-CSF	17	nr	nr	
Tarella[108]	11	Cy 7 g/m^2 or VP16 2 g/m^2	GM-CSF	15 (13-16)	126	6432	
	4	Cy 2×1.2 g/m^2	no	16 (16-18)	31	462	
	4	Cy 2×1.2 g/m^2	GM-CSF	14 (14-15)	77	2588	
Ossenkoppele[111]	6	no	G-CSF×6 gg	6		845	
Majolino[112]	7	VCAD	no	20 (17-30)		622	
	7	VCAD	G-CSF	13 (9-17)	22	893	
Vasta[113]	6	VCED	G-CSF	13 (12-15)	70	2391	

Legend. Cy: cyclophosphamide; VCAD: vincristine 1 mg, cyclophosphamide 4x500 mg/m^2, adriamycin 2x50 mg/m^2, dexamethasone 4x40 mg. VCED was identical to VCAD except that epirubicin 2x60 to 80 mg/m^2 was substituted for adriamycin. nr: not reported.

essary for rapid and full platelet engraftment.[105,132] In a recent study of MM autografts, Tricot *et al.*[59] found that platelet engraftment is influenced by previous history and cell dose. In patients with more than 24 months of chemotherapy before the autograft, they found a dose ≥5×10^6/kg to be required for rapid and full platelet recovery post graft. This number of CD34$^+$ cells may be obtained with 1 or 2 apheretic runs, and only a minority of patients, namely those with prolonged pre-mobilization treatment, need a higher number of apheretic procedures. The number of cells needed is obviously greater when a double autograft is planned. When this is the case, since recovery after a second autograft is influenced by the same factors as the first,[59] the number of CD34$^+$ cells to be collected simply has to be doubled.

CD34$^+$ cell monitoring in blood and collection products is undoubtely the most reliable and rapid method for apheresis planning,[131,133-135] though the assay requires skillful personnel and carries a substantial cost. The issue has been reviewed extensively by Rowley.[136] Siena *et al.*[133] initially suggested starting the collection program as soon as CD34$^+$ cells were detectable in the peripheral blood. However, in terms of efficiency, the best collections are performed when CD34$^+$ cells are at their peak. In practice, aphereses should be started as soon as the CD34$^+$ cells in the blood exceed a given level. We suggest a value of 20 CD34$^+$ cells/μL combined with a WBC level >1.0×10^9/L and a platelet count >30×10^9/L before starting collections.[82,133] Mononuclear cells (MNC) in DNA synthesis also predict a good yield when their level in the blood is >5% (or >250/μL).[137]

Few studies report detailed data on apheretic PBSC collection in MM. Dimopoulos *et al.*[109] began the aphereses when the MNC count went above 0.3×10^9/L, having as target the collection of > 2×10^6/kg CD34$^+$ cells. They were able to collect > 3.0×10^6/kg CD34$^+$ cells daily in patients with ≤4 months of prior chemotherapy, but the mean daily yield was uniformly lower (< 1×10^6 CD34$^+$ cells/kg) in patients with more than 12 months of chemo-radiotherapy. Tricot *et al.*[59] initiated collections upon recovery of a WBC count > 0.5×10^9/L, and assumed a target of > 63,108/kg MNC to support two autografts. In a recent study[113] aphereses were started as soon as the WBC count exceeded 5×10^9/L after a CHOP-like regimen followed by G-CSF, and > 6×10^6/kg CD34$^+$ cells were collected from all patients in 1 to 3 aphereses.

A predictive test with G-CSF, a single dose of 10 mcg/kg, followed by CD34$^+$ cell monitoring on days 4 and 5 has been proposed.[138] The study included patients with MM, but the sample was too small to draw any conclusions. Steady-state CD34$^+$ cell counts seem to predict the yield of PBSCs after mobilization with chemotherapy and G-CSF,[139] but not after G-CSF alone.[140] Table 7 shows the first apheresis day reported with dif-

Regimen	Day of progenitor peak	Day of first apheresis	No. apheresis	References
G-CSF 10 mcg/kg/day × 6 d	6	6	phlebotomy × 2	112
Cy 7 g/m^2	nr	20	6	117
Cy 7 g/m^2 + GM-CSF	15	nr	4	98-108
Cy 7 g/m^2 + G-CSF	15	14	2-3	141
VCAD	20	14	6	112
VCAD + G-CSF	13	12	2-3	112

Legend: nr: not reported.

Table 7. Day of cell peak and of first apheresis after PBSC mobilization in patients with MM. The addition of G-CSF or GM-CSF shortens the time to progenitor peak and consequently the time to apheresis. Mean number of apheretic procedures was lower when growth factors were employed.

ferent mobilization methods.[98,108,111,112,117,141] It is clear that the CD34$^+$ cell peak occurs very early (approximately day 5 or 6) during mobilization with growth factors alone. When chemotherapy is included in the mobilization schedule, the CD34$^+$ cell peak day occurs later (approximately day 20), but the subsequent use of growth factors will shorten it by a week or so.

To conclude, we suggest (Table 8) mobilizing PBSC with the combination of chemotherapy and growth factors (G-CSF or GM-CSF), and performing serial determinations of CD34$^+$ cells in the blood. Aphereses should be started as soon as the level of CD34$^+$ cells exceeds 20/μL, and collections should be performed daily with twice the blood volume processed each time. Continuous-flow separators are to be preferred. As target for collections, the figure of 2×10^6/kg CD34$^+$ cells per single autograft should be adopted for patients with < 24 months of prior chemotherapy, while a greater number (> 5×10^6/kg) should be collected in patients with a longer treatment history.

Assessment of myeloma cells in the peripheral blood and role of ex-vivo purging

PBSC collections are generally believed to have lower tumor cell contamination than BM harvests in cancer patients eligible for autografting. Moreover, the use of circulating progenitor cells has shown more rapid hematopoietic reconstitution than reinfusion of BM-derived cells, thus reducing the incidence of serious infections and virtually eliminating mortality.[142] Consequently, PBSCT is widely used after myeloablative therapy for the treatment of myeloma patients.[53,56,59] However, myeloma-related B-cells bearing the same idiotypic determinant as the neoplastic plasma cells have been identified in the blood of MM patients under steady-state conditions,[143-149] and they may play a crucial role in the pathogenesis of the disease.[144,147] Therefore in this chapter we will review the published data concerning: i) the presence of MM elements in PB and their kinetics in response to mobilization protocols; ii) methods for myeloma cell assessment; iii) methods for *ex vivo* removal of contaminating tumor cells and the role of purging with respect to disease relapse.

Identification of circulating myeloma cells

Circulating B-cells belonging to the malignant clone were originally thought to be pre-B-cells on the basis of the surface expression of the CD10 (CALLA) Ag,[150] an endopeptidase present on all fetal pre-B and B-cells, on adult pre-B-cells and their neoplastic counterparts.[151] However, the CD10 Ag has also been found on activated B-cells[151] and does not seem to be restricted to the early stages of B-lineage differentiation. Moreover, PB abnormal B-lymphocytes express plasma cell markers such as PCA-1 and PC-1 and the CD45RO Ag isoform, which is typical of late B-cells.[145] Thus phenotypic analysis

Table 8. Recommendations for PBSC mobilization and their apheretic harvest in patients with multiple myeloma.

- Mobilization with chemotherapy + growth factors (G-CSF or GM-CSF)
- Serial CD34$^+$ determinations according to institutional protocol
- Start apheresis when CD34$^+$ cells in blood >20×10^6/L
- Continuous flow separator, volume processed × 2 blood volume per run
- Collect at least 2×10^6/kg CD34$^+$ cells in patients with < 24 months prior chemothrapy, at least 5×10^6/kg CD34$^+$ cells in patients with > 24 months prior chemotherapy

of circulating CD19+ cells indicates a heterogeneous, continuously differentiating B-lineage.[145] By physical parameters, CD19+ cells include a small and a large subset that are mainly late B-cells (pre-plasma cells) coexpressing CD20, CD10, PCA-1, CD45RO and CD24 Ag.[148] The majority of large B-cells also express the CD56 Ag and high density CD38, whereas small lymphocytes show only minor expression of these 2 antigenic determinants. This phenotypic profile (i.e. CD19+ CD20+ CD38++ CD56+) is not found in normal resting B-cells. Interestingly, malignant cells were detected at diagnosis, irrespective of tumor burden and stage of disease,[148] and treatment had no detectable effect on the large B-cell subset. Conversely, a significant decrease in the number of small B-lymphocytes followed chemotherapy, although these cells returned to baseline value once the therapy was discontinued. In this regard, it was previously shown that circulating CD19+ cells in MM express the functional multidrug transporter p-glycoprotein,[147,169] thus suggesting that blood B-cells include a highly drug-resistant subset capable of inducing disease recurrence in myeloma patients. However, it should be noted that mature plasma cells do not always express the CD19 Ag, whereas the presence of the CD56 Ag discriminates clonal plasma cells from normal ones.[152] In addition, the recently described monoclonal antibody B-B4[152] seems to be highly specific for BM and circulating terminal plasma cells.

More recently, the issue of myeloma cell contamination in leukapheresis products and the kinetics of circulating tumor cells in response to mobilization protocols have been addressed.[67,69,153-155] These studies have consistently shown that the majority of PBSC collections, if not all, are contaminated by myeloma cells, which represent up to 10% of PB mononuclear cells by immunophenotyping and molecular analysis using polymerase chain reaction (PCR) with consensus oligonucleotides to the Ig heavy chain complementary determining region III (CDR III) (see below).[155] The same pattern of contamination has been shown following high-dose Cy and either G- or GM-CSF,[67,69,155] as well as after G-CSF alone,[154] suggesting that growth factors for stem cell mobilization, regardless of the use of chemotherapy, may influence the expression of adhesion molecules associated with the myeloma cell membrane. Notably, kinetic analysis has demonstrated that following high-dose Cy and G-CSF, the concomitant mobilization of plasma cells and hematopoietic progenitor cells in the PB takes place with the maximum peak of neoplastic elements occurring within the optimal time period for collection of circulating CD34+ cells.[67] Conversely, GM-CSF seems to reduce asynchronous mobilization of neoplastic elements and hematopoietic stem cells into PB, so that the contamination of actively proliferating myeloma cells is minimal in the first two days of apheresis.[156]

Methods for assessment of minimal residual disease

A number of methods have been proposed to detect malignant cells in the blood of myeloma patients, including immunologic assessment by monoclonal antibodies, flow cytometry analysis of DNA and cytoplasmic Ig, studies on gene rearrangement. Each of these techniques has limitations in sensitivity and, in some cases, specificity. For instance, analysis of the hypervariable region of the Ig heavy chain (IgH) gene using a set of family-specific primers (IgH fingerprinting) requires 0.1% monoclonal cells[157] and may produce false positive results. Conversely, dual-parameter flow cytometric

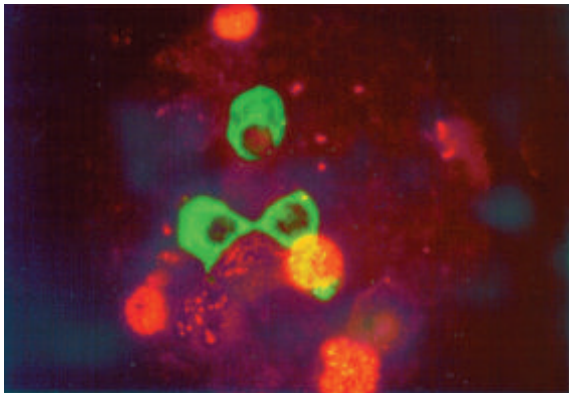

Figure 3. Circulating monoclonal B-lymphocytes and plasma cells assessed by double fluorescence immunostaining: intracytoplasmic Ig (green)/nuclear BRDU (red). Bromodeoxyuridine (BRDU) is incorporated in actively proliferating cells.

analysis (e.g. CD19/monoclonal light chain) and evaluation of intracytoplasmic monoclonal heavy or light chain are highly specific and allow detection as low as 0.1%[67] (Figure 3); however, they only assess mature Ig^+ B-cells. Recently, several laboratories have described applications of PCR techniques to increase significantly the sensitivity and specificity of detection of minimal residual disease (MRD). Both consensus oligonucleotides (ODN)[146] and family-specific primers[69] have been used to amplify the CDRIII of rearranged heavy chain alleles (Figure 4) from myeloma samples. From the sequence of the amplified products, allele-specific (tumor-specific) oligonucleotides (ASO) were synthesized and used directly in PCR amplification reactions (ASO-PCR) for each patient sample to detect the malignant clone. The sensitivity of this method is $1:10^5$ normal cells and a quantitative analysis can be performed by generating titrations curves of tumor cells. Alternatively, direct fingerprinting of CDRIII IgH gene rearrangement may be used, although the sensitivity is $1:10^4$ normal cells.[67]

The biological and prognostic significance of cancer cells present in autologous grafts is still unknown and circulating myeloma cells may only reflect advanced stages of the disease; therefore relapse may be caused by regrowth of residual clonogenic cells *in vivo*. However, considering that MM is a disease intrinsic to BM and recent studies clearly show that reseeding of reinfused malignant cells contributes to relapse,[158] several attempts have been made to remove myeloma cells from BM or PBSC autografts using different strategies.

Ex vivo purging of myeloma cells

Of the purging methods proposed for the elimination of MRD, the cyclophosphamide derivative 4-hydroperoxycyclophosphamide (4-HC) was the first used,[159] on the basis of *in vitro* mod-

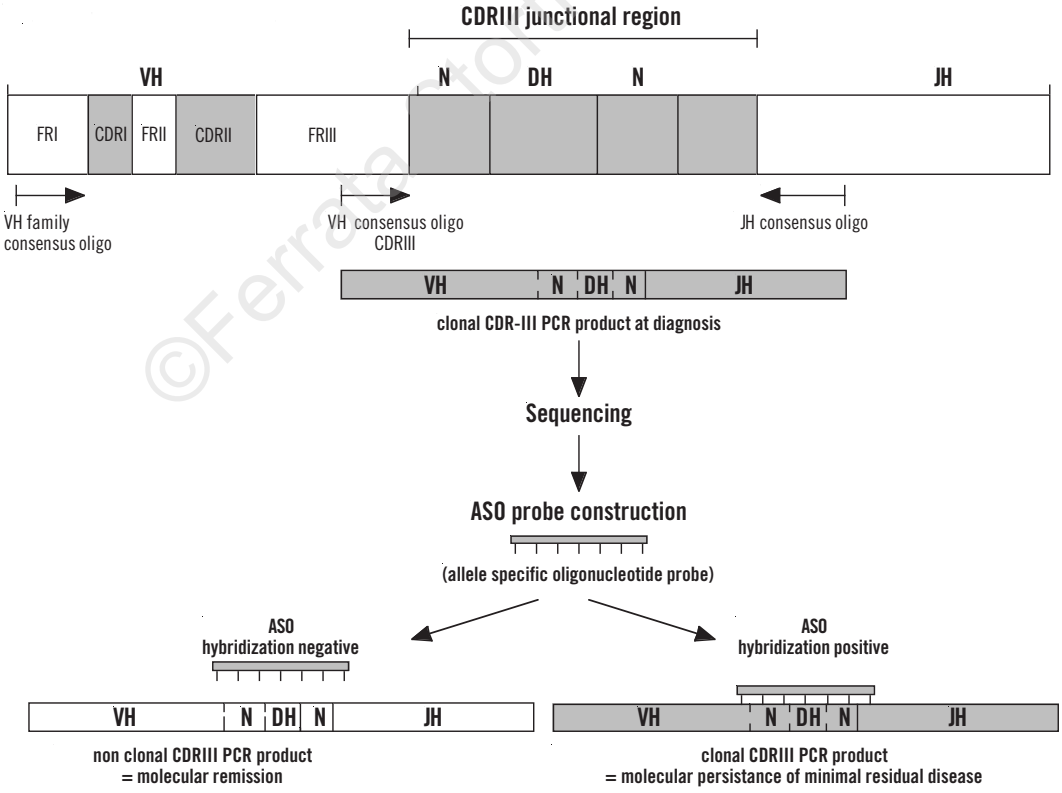

Figure 4. Schematic representation of the genomic region of rearranged CDRIII of IgH gene and further utilization of the PCR product for detection of MRD. For further details see text.

els demonstrating that this compound was able to eliminate BM-infiltrating MM cell lines.[160] The main mechanism of action of 4-HC is based on a marked inhibition of myeloma cell growth, whereas it spares normal primitive hematopoietic cells.[161] Moreover, this alkylating agent seems to induce the apoptotic death of tumor cells[162] as well as activate immune mechanisms capable of controlling malignant cell proliferation.[163] Because 4-HC does not affect surface antigen expression of myeloma cells, it is also a potential candidate for combined treatment with monoclonal antibodies (MoAbs), and preliminary *in vitro* data confirm the additive effect of these two purging techniques.[160] Several MoAbs directed against tumor-associated or cell differentiation antigens not expressed by primitive cells responsible for hematopoietic engraftment have been selected for clinical trials after *in vitro* studies demonstrated high purging efficacy with the use of complement,[164,165] toxins[166,167] or immunoaffinity columns.[168] Gobbi *et al*. developed a series of MoAbs that recognize mature plasma cells as well as B-cell precursors. One of them (8A) was conjugated with the ribosome-inactivating toxin momordin and clinically tested in 8 advanced stage MM patients to eliminate, *ex vivo*, contaminating myeloma cells prior to ABMT.[166] Although a marked tumor reduction was observed in all evaluable patients, none of them achieved CR and hematopoietic reconstitution following the myeloablative conditioning therapy was significantly delayed in 3 patients. These preliminary results showed the feasibility of this purging approach despite the poor selection of patients.

The same MoAbs were also employed *in vitro* to remove myeloma cells through the avidin-biotin immunoabsorption technique, and the result was a greater than 3 log reduction in tumor cells with acceptable recovery of BM progenitors.[168] More recently, Goldmacher *et al.*[167] reported the development of an anti-CD38 immunotoxin capable of killing 4-6 logs of human myeloma and lymphoma cell lines. The immunotoxin was composed of an anti-CD38 antibody conjugated to a chemically modified ricin molecule (blocked ricin). However, the CD38 Ag may not be the proper target for purging because it is strongly expressed on myeloma plasma cells (see above) and on committed hematopoietic progenitor cells,[169] which are thought to be essential for rapid BM reconstitution. More specific antibodies directed either toward B-cells (anti-CD10 and CD20) or mature plasma cells (PCA-1) and complement were used to deplete tumor cells from the graft before ABMT by Anderson *et al.*[165] Following a TBI-containing conditioning regimen, a neutrophil count greater than 0.5×10^9/L and an unsupported platelet count greater than 20×10^9/L were reached at a median of 21 days (range 12-46) and 23 days (range 12-53), respectively. Similarly, immunologic reconstitution was not different from that commonly observed in cancer patients receiving unmanipulated autograft. This study documented that high-dose chemo-radiotherapy can produce a high response rate in pretreated patients with sensitive disease, and MoAb-based purging methods do not prevent rapid and sustained engraftment. However, the occurrence of relapses post-ABMT and partial responses will not define the need, if any, for marrow purging until more effective ablative strategies are developed. Taken together, these data demonstrate that the heterogeneity of Ag expression on neoplastic cells and the lack of true tumor-specific determinants may greatly influence the efficacy of antibody-based strategies for the depletion of myeloma cells. Alternatively, long-term Dexter-type marrow cultures have been used to select normal myeloid progenitors from heavily infiltrated myeloma BM, on the basis of the selective growth advantage of benign cells over malignant cells in this system.[170]

Enrichment of hematopoietic $CD34^+$ cells has lately been shown to be an alternative approach to myeloma cell removal with a limited loss of normal stem cells. The CD34 Ag is a 110-120 kD glycoprotein that is mainly expressed on the earliest identifiable precursor cells and committed myeloid progenitors.[169] In normal individuals, $CD34^+$ cells represent 1% to 4% of the mononuclear cell fraction in the BM, whereas they are barely detectable in the PB.[169] In addition, the CD34 Ag is not expressed on the surface of mature plasma cells in MM, although the possibility that this glycoprotein may be present on clonally less differentiated B-lymphocytes is still

a matter of debate. As reported above, recent data support the hypothesis that MM originates in the later stages of B-cell differentiation when B lymphocytes have lost the CD34 Ag,[171] whereas other studies have found $CD34^+$ cells to be part of the neoplastic clone.[172,173] It should be underlined, however, that reverse transcription-PCR, which was used to detect MRD in those studies, is an extremely sensitive technique, and the potential contamination of the $CD34^+$ cell fraction by unwanted cells should be carefully avoided.

In this respect, Vescio *et al.*[171] did not find IgH gene clonal rearrangement in collections of 99.99% pure $CD34^+$ cells obtained after using a combination of two methods of stem cell purification. Schiller *et al.*[66] and Lemoli *et al.*[67] reported the first studies on purified, CD34-selected PBSCT conducted in patients with advanced MM. A median of 4.65 and 4×10^6 $CD34^+$ cells/kg were reinfused in the two trials with a median purity of 77% and 88.5%, respectively. The median time to neutrophil and platelet recovery was 12 days and 10 and 11 days, respectively, with no difference with respect to a group of patients receiving unmanipulated PBSCs.[67]

Both reports utilized rigorously quantitative immunofluorescence and/or IgH gene rearrangement analysis, and tumor cell depletion ranging from 2.5 to 4.5 logs was achieved. However, the persistence of myeloma cells in the $CD34^+$ cell fraction was documented by sensitive PCR assay in all cases heavily contaminated before positive selection of $CD34^+$ cells. Thus an additional purging step may be necessary to achieve a virtually tumor-free autograft.

In this regard, studies aimed at optimizing myeloma cell depletion by positive selection of primitive $CD34^+Lin^-Thy^+$ cells have already been performed[155] and clinical trials are currently in progress.

In summary, all these studies show the capacity of purging techniques to eliminate a substantial proportion of the myeloma cells from autologous grafts without affecting their engraftment potential. The clinical impact of purging on disease relapse remains to be determined in future randomized trials.

Post-transplant (immuno)therapy

In MM as well as in other hematologic malignancies, the primary objective of high-dose therapy with hemopoietic stem cell support is to prolong survival and possibly to cure an otherwise incurable disease. The aim of post-transplant therapy is to prevent recurrence of the disease while assuring good quality of life. From this latter point of view, there is no room for additional chemotherapy as a preventive means. In addition, high-dose chemotherapy itself involves a risk of secondary myelodysplastic syndrome or acute myeloid leukemia. This risk is apparently related to prolonged alkylating agent therapy prior to transplantation and would undoubtedly increase with additional post-transplant chemotherapy.

In the past few years interferon-α (IFN-α) has been extensively evaluated in the management of MM, either as part of the induction program or as maintenance therapy.[173] Although controversial findings were frequently reported, several clinical trials showed a prolongation of the remission phase, and even of the survival duration, for patients receiving IFN-α after a favorable response to conventional chemotherapy.[174,175] These results suggested that IFN-α might be particularly useful in patients with low tumor burden or minimal residual disease, and led to clinical investigations of this agent in the autograft setting.

The *European Group for Blood and Marrow Transplantation* (EBMT) has recently presented a retrospective study of a large series of MM patients treated with autologous stem cell transplantation.[50] Interestingly, post-transplant treatment with IFN-α was independently associated with extended survival of responding patients, i.e. those achieving either CR or partial remission. Moreover, Powels *et al.*[176] designed a randomized clinical trial aimed at comparing maintenance IFN-α therapy with no maintenance after HDM and ABMT.[175] The authors found that IFN-α prolonged remission and improved the survival after autotransplant, and that this effect was particularly marked in the group of patients achieving CR.

Maintenance IFN-α is usually started three

months after transplant and is given sc at a dosage of 3×10^6 U/m^2, 3 times weekly. This dose usually induces mild hematological and non-hematological toxicity, thus allowing good quality of life. Available data indicate that about 50% of the MM patients who achieve CR and are then treated with IFN-α remain in remission four years after transplantation.

Alternatively, maintenance treatments aimed at prolonging the duration of disease control after transplantation may also include the administration of interleukin 2 (as nonspecific immunotherapy)[177] or humanized anti-idiotype monoclonal antibodies, which could allow selective killing of myeloma cells and might be particularly useful for controlling minimal residual disease.

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