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New ways to optimize breast cancer treatment

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The impact of hematopoietic growth factors on supportive care in clinical oncology: with special attention to potential tumor cell contamination in the stem cell harvest

A review

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Hematopoietic growth factors (HGFs) are now for a number of years available for use in oncological patients. Drugs currently registered are: granulocyte colony stimulating factor (G-CSF) and granulocyte macrophage colony stimulating factor (GM-CSF) for neutrophil stimulation and erythropoietin for stimulation of erythropoiesis. With several other HGFs phase I, II and III studies have been performed or are ongoing. The new as yet unregistered compounds are often under investigation for their capacity to stimulate thrombopoiesis.

Indications for the use of neutrophil stimulating growth factor are: 1) prevention of neutropenia and its complications following chemotherapy, 2) treatment of neutropenic fever, 3) prevention or abbreviation of neutropenia during chemotherapy requiring bone marrow reconstitution and 4) peripheral stem cell harvest.

1. The prevention of neutropenia and its complications following chemotherapy

Bacterial and fungal infection is a considerable cause of death in cancer patients (2). Leucopenia due to multi-agent chemotherapy regimens, is associated with substantial febrile morbidity (3, 4). Infection rates increase when the peripheral blood granulocyte count falls below $0.5x10^9$/L, and especially when it is less than $0.1x10^9$/L or when duration of leucopenia is prolonged (5). Haematopoietic toxicity can be decreased by chemotherapy dose reduction. However, this may have a negative effect on treatment outcome. Therefore, other means of reducing and preventing febrile leucopenia have been studied (6). Prophylactic haematopoietic growth factors are used to reduce the incidence of febrile neutropenia, by reducing the duration of neutropenia (1, 7).

In a number of phase III studies in which chemotherapy was used that induced a neutropenic fever of at least 40%, G-CSF was found to reduce the incidence of severe neutropenia, it ameliorated neutrophil nadir, reduced neutropenia duration and

reduced the incidence of neutropenic fever (50%), culture positive infections and the use of antibiotics (1).

Another approach might be the use of chemoprophylaxis by the use of prophylactic antibiotics. Also, prophylactic antibiotics (so called chemoprophylaxis) have shown to reduce the risk of febrile morbidity (8). Various antibiotics have been used for this purpose (9, 10). Chemoprophylaxis by quinolone-based treatment was found to be particularly effective for intestinal decontamination (11). This way, infections with Gram negative bowel organisms, a major cause of morbidity and mortality in the leucopenic patient, can be substantially reduced (12). Next to bacterial infections, fungi also constitute a major problem in neutropenic patients, requiring specific approaches for prevention and therapy. A number of anti-fungal agents, for instance amphotericin B, can be used for chemoprophylaxis (13, 14).

In two studies comparing the use of granulocyte-stimulating growth factor (G-CSF) to G-CSF plus antibiotics (15, 16), an additional, beneficial effect of antibiotics was found. Furthermore, a few placebo-controlled reports on prophylactic norfloxacin or ofloxacin also clearly indicated a positive contribution in this setting (17-19). In a retrospective study, prophylactic G-CSF was compared to prophylactic oral ciprofloxacin in ovarian cancer patients with paclitaxel induced leucopenia (20). No difference between fever rates was observed when prophylactic G-CSF was compared to oral ciprofloxacin, but comparison with a historical control group not receiving any prophylactic agent, showed a clear benefit from prophylaxis in these patients with relatively short but deep leucopenia. In a non-randomized dose-finding study (21), early stage breast cancer patients received 5-fluorouracil (5-FU, 500 mg/m2 intravenously), epirubicin (60 mg/m2 IV) and cyclophosphamide (75 mg/m2 for 14 days). Co-trimoxazol was administered prophylactically to a group of 89 consecutive

patients, and fever rates decreased significantly compared to the control group not receiving prophylaxis. Currently for example in our center a study to compare G-CSF versus quinolones is analyzed. A possible disadvantage of prophylactic quinolones may be the development of resistant organisms (22). Although most data actually contradict this (23), a possible way to circumvent the risk of developing infection in these immunocompromised patients would be to evaluate the efficiency of intestinal decontamination by monitoring of faecal organisms during prophylactic treatment. This way, antibiotic treatment can be altered if necessary to provide the best prophylaxis. Another option would be to limit the use of prophylaxis to those patients who have actually developed grade IV leucopenia. If chemoprophylaxis induces resistance at all, it may be limited by these precautions.

Therefore, chemoprophylaxis can be viewed as a reasonable alternative for G-CSF in preventing febrile leucopenia. However, in future studies placebo-controlled assessment of chemoprophylaxis in high risk patients would be useful.

2. Treatment of neutropenic fever

Randomized trials have not conclusively demonstrated a clinical benefit when G-CSF and GM-CSF are given for uncomplicated febrile neutropenia. The routine use of these factors in this setting can not be recommended. We performed a study to determine whether GM-CSF used in addition to standard inpatient antibiotic therapy shortens the period of hospitalization due to chemotherapy induced neutropenic fever. Patients with a hematologic (n=47) or solid tumor (n=87) who had severe neutropenia and fever were randomly assigned to receive GM-CSF 5 μg/kg/d (n=65) or placebo (n=69) in conjunction with broad spectrum antibiotics for a minimum of 4 days and a maximum of 14 days. GM-CSF/placebo and antibiotics were stopped if the neutrophil count was

greater than $1.0x10^9/L$ and temperature less than 37.5°C during 2 consecutive days, or for a leucocyte count ($10x10^9/L$, both followed by a 24 hour observation period (hospitalization period). Compared with placebo, GM-CSF enhanced neutrophil recovery. Median neutrophil counts at day 4 were $2.5x10^9/L$ (range, 0-25) in the GM-CSF arm and $1.3x10^9/L$ (range 0- 9) in the placebo arm. No significant difference was observed with regard to median number of days with less than $1.0x10^9/L$ neutrophils or days of fever. The median number of days patients were hospitalized while on study was comparable in the GM-CSF and placebo groups at 6 (range 3-14) versus 7 (range 4-14). Quality of life scores in 90 patients demonstrated differences in favor of the placebo group. Hospital costs were higher for GM-CSF treated patients if GM-CSF was included in the price. These results indicate that GM-CSF did not affect the number of days for resolution of fever or the hospitalization period for this patient group, although a significant effect of GM-CSF was observed on neutrophil recovery (24).

3. Reduction of neutropenic period during chemotherapy requiring bone marrow reconstitution

Patients receiving high-dose chemotherapy followed by bone marrow or peripheral stem cell reinfusion have a slightly faster neutrophil recovery if stem cell reinfusion is combined with G-CSF or GM-CSF administration (1).

4. Peripheral stem cell harvest

The most important role of CSFs is in the phase of peripheral blood stem cell (PBSC)

mobilization. CSFs facilitate mobilization of hematopoietic stem cells. The advantage of PBSC transplantation following high-dose chemotherapy is that it reduces the duration of not only neutropenia but also thrombocytopenia compared to autologous bone marrow transplantation. It has been shown to shorten the duration of neutropenia and thrombocytopenia, and it reduces incidence of infections and hospital stay. Stem cell harvest before high-dose chemotherapy can also collect tumor cells from the circulation. The exact relevance for the clinic of the presence of tumor cells in the stem cell harvest is as yet unknown. Several purging methods are developed and currently in clinical trial. Ex-vivo culturing of hematopoietic stem cells in the presence of CSFs is an other way to eliminate tumor cells. Therefore, the role of tumor cell contamination is more extensively explained in this review.

4.1 Tumor cell detection: introduction

The increased potential clinical relevance of adjuvant high-dose chemotherapy in solid tumors has raised the relevance of tumor cell contamination in bone marrow. There is increasing evidence that not only in bone marrow but also, although studies do suggest less likely, in peripheral blood stem cell harvest, tumor cell infiltration may be involved (25, 26). Early papers reported on sporadic findings of tumor contamination of solid tumors in the peripheral blood. In other studies blood was collected from cancer patients during or just after surgery and these samples often contained significant numbers of tumor cells, yet these patients do not always develop metastatic disease. Often the clinical follow up in these studies was not long (27). It may be that the circulating tumor cells are not always viable or able to form metastases. In the animal model, however, it is shown that many viable tumor cells are shed into the circulation. So, the significance of presence of tumor cells in the circulation is as yet

undetermined. The process of metastasis involves multiple host-tumor interactions and it is thought that only a few of all the circulating cells are successful in establishing metastatic colonies. Tumor cells are likely to acquire the ability to metastasize as a result of cumulative genetic changes that provide the cells with progressive metastatic capability through alterations in cell regulatory mechanisms, secretion of proteases, induction of angiogenesis, increased cellular motility and altered expression of cell adhesion molecules (28). The ability to detect very small numbers of tumor cells may provide the clinician with an important predictive tool with respect to recurrence and might help in a better selection for adjuvant therapy.

4.2 Bone marrow micrometastases

Recently, with the availability of multiple antibodies directed against epithelial cells, it became clear that in breast carcinoma patients without signs of metastatic disease tumor cells can often be detected in the bone marrow. In breast carcinoma patients without evidence of distant metastases. Redding et al. reported already in 1983 a study in 110 patients (29). They performed immunocytochemical analysis on bone marrow smears and detected tumor cells in 28% of the samples. Bone marrow was positive in 24% of the patients who had no lymph node involvement. In a larger, identical diagnostic group of 285 patients, 27% had positive bone marrow with an antiserum raised against the epithelial membrane antigen EMA (30). In following studies also the effect on prognosis was analyzed. Cote et al. studied 49 patients with stage I and II operable breast cancer (31). With a 30 months median follow up time there was an association between early recurrence and tumor infiltration of the bone marrow. Also in this study, the importance of quantifying the number of cancer cells was shown. Multivariate analysis indicated the ratio cancer cells: nucleated cells was the only

significant variable for the prediction of early recurrence (>10 cells per $4x10^6$ nucleated cells). Diel et al. reported in 1992 on a much larger study. In 260 patients with primary breast carcinoma bone marrow aspirates obtained from six sites of the skeleton were analyzed for tumor cells (32). After density centrifugation, cells in interphase were smeared and stained. For the immunocytologic reaction the monoclonal antibody TAG12 was used. Tumor cells could be detected in 115 (44%) of the bone marrow samples. The presence of tumor cells correlated with tumor stage, nodal stage and tumor grading. Relapses occurred especially in those with positive bone marrow. The highest prediction for distant metastases was obtained in this study by combining the nodal status, negative progesteron receptor and tumor cell presence in the bone marrow. Pantel et al. reported on the immunological detection of various markers associated with tumor progression. In cytokeratin 18 positive bone marrow from patients with cancer from the breast, gastrointestinal tract or the colon, proliferation markers Ki-67 and p120, and erb2 oncogene expression was studied. Only few cells labeled with these markers, but CK18-erb2 double labeling was associated with increased clinical stage in breast and gastrointestinal carcinoma (33). The role of bone marrow metastases in cancer of the breast, lung, stomach and colon have been investigated in various studies (29-32, 34-38). In general, it has been shown that detection of bone marrow metastases predicts for recurrent disease, and that results have correlated with clinicopathological staging parameters. A recent study, in which a large number (n=552) of bone marrow aspirates from stage I, II and III beast cancer patients were analyzed by means of cytokeratin directed antibody A45-B/B3, described micrometastic bone marrow disease, unrelated to the presence or absence of lymph-node metastases (39). Thirty-six percent of all patients showed bone marrow micrometastases, and of patients with node-negative disease this percentage was 33%.

It appears conceivable therefore, that there may be alternative metastatic routes, other than the classical sequence of tumor-lymph node-hematogenous metastases (reflected in the TNM classification for breast cancer staging, Union International Contre le Cancer 1997). The apparent ability of tumor cells to expand hematogenously to the bone marrow is independent of their ability to metastasize to axillary lymph nodes, which indicates that sampling of bone marrow in addition to sampling of axillary nodes could possibly lead to more accurate staging in breast cancer. It should be reminded though, that the percentage of bone marrow micrometastases in this study is higher than the percentage of patients that are at risk for relapse (for instance: in tumors <1 cm: 35% bone marrow micrometastases, whereas long-term survival in these patients is 95%). Therefore, one might suggest that this additional staging method is useful preferably to identify those patients without bone marrow or lymph node metastases as very-low risk patients, who do not require systemic treatment.

4.3 Peripheral blood

The presence of tumor cell contamination in the peripheral blood is considered relevant because peripheral stem cell harvest and reinfusion after high-dose chemotherapy is increasingly thought to be a useful treatment for patients with high risk of tumor relapse. Also, tumor cell contamination in peripheral blood can be considered a potentially useful diagnostic tool, allowing a better selection of patients that may benefit from adjuvant chemotherapy. If a simple blood test could have prognostic value, this might be a clinically very interesting alternative to other staging methods. The detection of single tumor cells in peripheral blood or peripheral blood stem cells (PBSC) is a particular challenge. Techniques to detect these rare circulating cells should be both highly sensitive and specific. The markers used should be

indicators of tumor cell presence in the blood, not expressed by haematopoietic cells, and not shed from the tumor into the circulation. In haematological malignancies, tumor-specific qualities are available for this purpose. Gene translocations, such as t(14;18) in follicular lymphpoma, were found to be accessable for molecular detection, using polymerase chain reaction (PCR) amplification (40). The presence of t(14;18) bearing cells in bone marrow, peripheral blood stem cells and possibly peripheral blood was found to be associated with early recurrences (41-43). Also in other haematological malignancies, specific gene translocation could be detected at a cellular level (44). However, the cytogenetics of solid tumors are considerably more complex and less well defined than those of haematological malignancies. Mutations of either oncogenes or tumor suppressor genes have been studied, but a common problem is the lack of consistency within tumor types, and the number of different mutations. For instance, p53 tumor suppressor gene mutations are found throughout the open reading frame, and although 'hotspots' have been identified, even these extend over four exons. Thus, other targets have been sought. Instead of tumor specific qualities, the detection of tissue specific antigens or enzymes by reverse transcriptase-PCR (RT-PCR) has been evaluated. This approach is based on the fact that malignant cells continue to express specific marker characteristics of their tissue of origin. RT-PCR was found to be an extremely sensitive technique, allowing the detection of one tumor cell in $1x10^6$-$1x10^7$ normal cells (45, 46). Thus, cells expressing prostatic specific antigen (PSA) were found in the peripheral blood of prostatic cancer patients without metastases (46). Tyrosinase expression was used as a marker for melanoma cells, showing a correlation between clinical disease stage and a positive tyrosinase RT-PCR (47). A similar result was obtained in neuroblastoma patients (48). For breast cancer cells, specific epithelial markers were studied. Datta et al. developed a RT-PCR for keratin 19 (K19) transcripts

to identify breast carcinoma cells in the peripheral blood and bone marrow of patients with breast cancer. K19 mRNA is a marker for the intermediate filament protein which is found in all normal and malignant breast cells and also in a number of simple epithelial cells and their malignant counterparts (49). In their experiment the K19 RT-PCR reliably detected 10 breast cancer cells in $1x10^8$ normal peripheral blood mononuclear cells (49). A similar experiment using also keratin filament transcripts, was earlier performed by Traweek et al. (50). In contrast to Datta et al. they detected presence of keratin 19 activity in stromal cells (fibroblasts and endothelial cells). These stromal cells could be a possible source of keratin 19 transcripts.

Immunocytochemistry is also a widely applied technique to detect single tumor cells from solid malignancies. The detection of micrometastases in bone marrow, using antibodies against cytokeratins, has proven to be feasible and of prognostic value in breast, gastric, colorectal and lung cancer (30-33, 35-40). Immunocytochemistry is considered to be less sensitive, but more specific than molecular PCR detection (51) (although also a detection level of one tumor cell in $1x10^6$ normal cells has been described (52). Certainly, the morphological tumor cell assessment increases the specificity of this method. Sensitivity is perhaps hampered by the sample size to analyze. However, molecular detection of cellular expression of epithelial markers has recently been shown to harbor its own risks (53), particularly regarding specificity. Fifty-three control bone marrow samples were compared to 63 samples of patients with breast or prostate cancer. Commonly used markers as CK 18, CEA and PSA were evaluated. Only PSA mRNA was not detected in any of the control samples, but the other markers were (CK18: 5 out of 7 control samples positive). It was stated that limiting factors in the detection of micrometastatic tumor cells by RT-PCR are: the illegitimate transcription of epithelial genes in haematological cells and the varying

expression of the marker gene in micrometastatic tumor cells. Also (low level) expression of cytokeratin 19 (commonly used in the detection of micrometastatic breast cancer), was described to be detected in control tissues (54, 55). Possibly, low background expression of these markers in bone marrow, PBSC or peripheral blood can presumably be circumvented by quantifying the signal of the analyzed sample. In our center, a quantitative RT-PCR assay was developed for the gene encoding for the epithelial related membrane antigen EGP-2 (56). EGP-2 is one of the most tissue specific epithelial markers known so far, and is widely expressed on almost all carcinomas derived from simple epithelia (57). The EGP-2 molecule is not shed into the circulation. There is a monoclonal antibody available against the antigen for which this gene is encoding (58). With the quantitative RT-PCR for EGP-2, a base-line for background signals can be established, allowing a more meaningful interpretation of RT-PCR results.

Considering the technical challenges of detecting vary rare tumor cells in peripheral blood, it is not surprising that the data on this subject are limited. In one study, the specificity issue of RT-PCR appeared to have been overcome (59). With the detection of the maspin-transcript, tumor cell contamination was found in peripheral blood of 3 out of 9 stage IV breast cancer patients, particularly during systemic treatment. However, the role of this unusual marker remains unclear; no other research groups have validated this method so far. Recent data in breast cancer patients have all employed the classical CK19 marker(60-63): some have used an RT-PCR method (60), while most have combined PCR methods with immunostaining (61-63). In patients without distant metastases, an incidence of patients with positive peripheral blood samples of 5 to 9% with immunostaining, and 13 to 36% with (q)RT-PCR was reported (60-62). In one study, RT-PCR positive blood samples was associated

with distant metastatic versus node-negative or node-positive disease (60), but these results were not quantified or related to immunostaining. In metastatic breast cancer patients, the reported incidence of blood samples positive for CK19 mRNA expression by qRT-PCR was up to 50%, and decreased with disease response (63).

Concluding, it can be said that evaluation of the possibilities to measure micrometastatic disease in peripheral blood is ongoing. The low incidence of these cells and technical issues render this issue particularly challenging. When the prognostic value of peripheral blood contamination is further evaluated, this could be a helpful tool in the allocation of therapy to poor prognostic groups. Possibly, also a disease stage amendable for immunotherapy can be distinguished. The use of cellular and molecular detection may thus be used in allocating and evaluating new clinical approaches.

4.4 Peripheral blood stem cells

Tumor cell contamination of peripheral stem cell harvest has gained considerable interest, as the number of cancer patients treated with high-dose chemotherapy and stem cell support has increased steadily over the past decade. In breast cancer patients, Ross et al. reported on a study in which paired samples of bone marrow and peripheral stem cell harvest from 48 patients were analyzed with an immunocytochemical technique (25). In cell seeding experiments with a cocktail of monoclonal antibodies, one tumor cell per $5x10^5$ mononuclear cells was detected in bone marrow or peripheral stem cell harvest. Immunostained tumor cells were detected in 9.8% (13/133) peripheral stem cell specimens from 9/48 patients obtained after chemotherapy and a haematopoietic growth factor and in 62.3% (38/61) bone marrow specimens from 32/48 (66.7%) patients. It was concluded that peripheral stem

cells contain fewer tumor cells than paired bone marrow specimens from patients with advanced disease and that these cells appear, based on the clonogenic assay, to be capable of clonogenic tumor growth. Brügger et al. described a study in which a small number of patients was analyzed. In their immunocytochemical assay they also use a panel of monoclonal antibodies with detection of one tumor cell per $4x10^5$ normal cells. They found that there was a difference in appearance of the tumor cells in the circulation after chemotherapy and haematopoietic growth factor treatment between patients without bone marrow infiltration and with bone marrow involvement. In those without tumor involvement in the bone marrow, tumor cells appeared earlier in the circulation after chemotherapy and growth factor than in those with tumor contamination of the bone marrow (26). From this study it was clear that chemotherapy and growth factors resulted in a higher frequency of tumor cells in the circulation than without these compounds. The appearance of tumor cells in the circulation in patients with bone marrow contamination with tumor coincided with the appearance of peripheral stem cells. It was suggested that, possibly, tumor cells that metastasize to bone marrow may share some of the characteristics of haematopoietic progenitor cells, such as homing receptors/adhesion molecules. It may well be that there is a downregulation of adhesion molecules of stem cells by chemotherapy and growth factors, similar as occurs in normal maturation, but now happening at an immature stage. The fact that tumor cells can appear in the circulation without tumor cell contamination in the bone marrow suggests that either the assay to detect tumor contamination of the bone marrow was insensitive or that chemotherapy plus haematopoietic growth factors also mobilize tumor cells from other spots in the body. This raised the question whether this would also be the case in tumor types that do in general not metastasize early to the bone marrow, but in which high-dose

chemotherapy is considered to be potentially useful, such as in ovarian carcinoma. In a study addressing this issue, bone marrow and PBSC samples from 22 ovarian cancer patients were analyzed. No tumor cells were found in PBSC, but 47% of bone marrow samples stained positive. The exact influence of mobilizing regimes on stem cell contamination in this setting, remains to be established (64). In breast cancer, the influence of mobilizing stem cells with growth factor was compared to growth factor combined with chemotherapy (65). Immunocytochemical detection and clonogenic assays were used. Of stage IIIb or IV breast cancer patients receiving only G-CSF, 1 of 37 peripheral blood samples, 4 of 36 bone marrow samples and 2 of 38 PBSC samples were positive. Results were similar in the group receiving GM-CSF and cyclophosphamide, implying no additional role for chemotherapy in clearing tumor cells. Recently however, a number of reports contradicting this finding were presented. In 329 breast cancer patients, mobilization of peripheral blood stem cells with cytokine plus chemotherapy resulted in less tumor cell contamination (11.7%), than mobilization with cytokines alone (27.8%) (66). Also, a significant reduction of tumor cell contamination was seen when harvesting stem cells after the third course of chemotherapy for stage IV breast cancer was compared to harvesting after the first ($p=0.0052$) (67). The prognostic value of immunocytochemical detection of tumor cells in bone marrow and PBSC was evaluated in a fairly large number of stage IV breast cancer patients (68). A cocktail of antiepithelial antibodies was used. Patients without bone marrow and PBSC contamination had a significantly longer disease free survival than others with positive bone marrow and/or PBSC (471 days vs 339 days). It was concluded that immunocytochemical staining is a useful prognostic marker for autologous stem cell transplant. Although the significance and prognostic value of the detection of tumor cells in PBSC still remains to be clarified (69), it seems conceivable

that reinfusing tumor cells into the patient will affect the clinical outcome. Indeed, two studies (70, 71), strongly support the assumption that these malignant cells reinfused after high-dose chemotherapy might contribute to relapse. In both studies, grafts were marked by retroviral vectors encoding neomycin phosphotransferase or other foreign genes. These marker genes could be detected in the malignant cells in a majority of patients at relapse. These data have reinforced the need for efficient techniques for purging tumor cells from stem cell material, to reduce the risk of relapse after transplantation.

4.5 Purging

Measures to eliminate malignant cells from the graft are generally referred to as 'purging' (72). Ex vivo elimination of tumor cells is reasonable if there are no adverse effects on engraftment, haematopoietic and immune reconstitution, or other treatment outcomes. Evaluation of the efficacy of purging is difficult, as relapse may originate from residual disease in the patient as well as from malignant cells reinfused with the transplant. It is not known yet to what extent tumor cells should be depleted from the autograft, which is presumably strongly depending on the clonogenic ability of these single tumor cells. Basically, two methods for purging have been studied: depletion of tumor cells and selection of stem cells from the graft (73). Depletion was first studied using chemotherapy, mainly 4-hydroperoxycyclophosphamide (4-HC) (74, 75). Treatment with 4-HC clearly reduced in vitro tumor colony growth, but also colony formation and engraftment (76). In searching for more specific purging methods, immunotherapy using monoclonal antibodies were studied. For this approach to be effective, the antibody should be specifically reactive with tumor cells. Similar as with immunocytochemical staining, this is a difficulty in solid tumors. After reacting with

the antibody, additional steps are necessary to eliminate the tumor cells from the graft, either through cytotoxicity, immunotoxicity or immuno (magnetic) separation. When lymphoma cells were purged from bone marrow through an antibody-complement combination (41), a 3 to 6-log destruction was obtained. Disease free survival was increased in patients who received purged bone marrow, compared to those who did not. In a study by Mykleburst et al., the efficiency of immunotoxins and immunobeads for purging breast cancer cells from bone marrow were compared (77). The use of three monoclonal antibodies and immunomagnetic beads removed up to 6-log units of tumor cells. Immunotoxin efficacy was more variable, but both methods only slightly affected colony formation in bone marrow. Especially, a combination of antibodies on precoated immunobeads and two treatment cycles appears effective (72), but in these experimental settings still very high effector:target ratios are being employed. Also, these studies are performed with cell lines which are immunophenotypically well characterized. The efficacy in clinical settings with presumably a less homogeneic tumor cell population, remains to be established. Furthermore, an immunoselection method that eliminates 100% of tumor cells predictably, has not yet been described. To increase cytotoxicity, the use of cytokines has been studied. Especially the use of interleukin-2 (IL-2) in this setting seems promising. Whether IL-2 incubation of PBSC could induce tumor cell kill was studied by Verma et al. (78). Cytotoxicity was obtained with up to 50% tumor cell kill. No adverse effect was seen on colony formation of the PBSC. If this effect can be obtained with IL-2 alone, it is tempting to speculate on an increased effect with additional monoclonal antibodies. Future possibilities also include stimulation of the graft with GM-CSF, to induce monocyte-mediated cytotoxicity, and enhance cellular cytotoxicity (79). Apart from increasing purging efficacy in vitro, these approaches may also contribute to a possible graft versus host

effect. Residual tumor cells in the patient may thus be attacked. Post-autologous bone marrow transplantation administration of GM-CSF in vivo was shown to result in increased cytotoxicity, evaluated in vitro (80). In a similar setting, post-transplant administration of IL-2 is currently being evaluated (81). It will be very interesting to see whether from future studies an optimal time schedule (during mobilization in vivo, culturing of the graft in vitro, post transplantation in vivo) and an optimal combination of cytokines and perhaps antibodies can be determined, to increase cytotoxic efficiency in this particularly interesting clinical setting. The second approach for obtaining purified stem cells is to actually select out stem cells. Systems for selecting CD34 expressing haematopoietic progenitor cells, have been evaluated and are now available for large scale purification (82, 83). Engraftment of progenitor cells is not affected by this selection procedure (83, 84). Although the CD34 antigen is not detected on tumor cells from patients with most solid tumors (82, 83), a recent report indicated that tumor cells were still detectable after CD34 enrichment (85). Therefore, it is conceivable that a combination of purging strategies including a positive selection (of CD34 positive cells) and a negative selection (of tumor cells) would be the best approach to eliminate tumor cells from the transplant. This approach however, is time- and resources consuming, and through randomized trials more insight should be gained as to the actual benefits. To date, no randomized trials have addressed the long-term effects of CD34 selection in the adjuvant breast cancer setting. In metastatic breast cancer, the available data do either not allow conclusions on long-term impact of CD34 selection (because of too short follow-up, in a randomized trial, 86), or they suggest no beneficial effect (87). However, it should be reminded that a fundamentally different residual disease status between transplantation in the metastatic setting or in the adjuvant setting exists, and that therefore the metastatic setting may not have

predictive value for adjuvant treatment. One other important point about CD34 selection is that natural killer cells and T cells will be absent from the graft (73). Whether or not this will affect clinical outcome as a result of regrowth of residual cancer cells is not known. However, it is clear that immunological methods to increase cytotoxic effects as a graft versus host (e.g. residual disease) may be greatly hampered by this depletion. The impact of residual disease in the patient after high-dose chemotherapy and stem cell support, is as yet unknown (88). If the patient is the main source of residual tumor cells that cause relapse, this may be a rationale for adjuvant treatment with immunotherapy. The stage of minimal residual disease seems well suited for this treatment modality, as was shown by Riethmüller et al., for colorectal patients (89). How, in the setting of stem cell transplantation this can be combined with pretreatment of the graft to increase immunological efficiency, is of particular interest, but remains to be clarified.

4.6 Conclusion

Tumor cell detection is of particular interest in the setting of high-dose chemotherapy and peripheral blood stem cell support. It is well conceivable that reinfused tumor cells of the graft contribute to relapse, but the impact of residual disease in the patient in this setting remains to be established. A number of strategies are currently employed to purge tumor cells from stem cells. Presumably, a combination of these will be most effective in eliminating tumor cells. Short term effects of these purging procedures regarding engraftment, appear not harmful. Whether long term adverse effects will also be negligible, remains to be clarified.

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