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First experiences and perspectives for the use of cytokines in the treatment of non-Hodgkin's lymphoma

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Numerous clinical trials have been carried out to investigate the therapeutic potential of cytokines in the management of non-Hodgkin's lymphoma (NHL). Relevant interferon-α activity is restricted to low-grade malignant NHL of both B and T cell subtypes, provided the tumor mass is low. Granulocyte-macrophage colony stimulating factor (GM-CSF) accelerates hematopoietic recovery following myelosuppressive therapy, and reduces the risk and severity of infection. Thus, GM-CSF support makes dose intensification feasible and may thereby contribute to an improvement of response rates and long-term survival. Among interleukins (IL) studied, IL-2, with or without lymphocyte activated killer cells, so far has only achieved stabilization of refractory NHL but this approach may still require further refinement. IL-3 has already been successfully applied in the treatment of NHL, augmenting neutrophil and platelet recovery after conventional salvage therapy or following autologous bone marrow transplantation. On the basis of these findings promising applications for the use of cytokines in the treatment of NHL can be envisaged.

Key words: Granulocyte-macrophage colony stimulating factor, interferon-α, interleukin-2, interleukin-3, non-Hodgkin's lymphoma

Introduction

Cytokines exert fundamental regulatory activities within the complex mechanisms controlling hematopoiesis and mature leucocyte function. Acting via specific membrane receptors, they produce either direct stimulation of defined target cells resulting in proliferation or differentiation, or indirect induction of effector cells releasing promoter molecules or activating special cell functions, or both. A number of cytokines, including hematopoietic growth factors such as granulocyte-macrophage colony stimulating factor (GM-CSF), interleukins such as interleukin (IL)-2 and IL-3, and interferons such as interferon-α (IFN-α), are now available in recombinant form. Thus, broader scale investigations of the *in vivo* activities of these species have become feasible.

In this context, hematopoietic and lymphoid malignancies may be considered model systems for investigation in two respects. Firstly, the study of these disorders may help to increase the knowledge about the functions of various growth factors (colony stimulating factor, CSF) which in turn, could lead to an improved understanding of the mechanisms by which malignant clones evade proliferation restriction control. Secondly, the myelotoxicity associated with all effective treatments of these diseases can lead to severe granulocytopenia, and this can often be reversed by administration of appropriate CSFs. Considering the biological heterogeneity of non-Hodgkin's lymphomas (NHL), it may be hypothesized that the diversity of cytokine functions could lead to the development of new therapeutic approaches.

Following the identification of separate entities of NHL according to immunohistological criteria in the Kiel classification, a series of clinical trials have established the characteristic clinical features of each of these entities. Thus, the distinction between the two categories of low- or high-grade malignant NHL by morphological criteria, relates to differences in proliferative activity rather than automatically implying prognosis. However, this subdivision does correlate very well with other clinical features, such as relapse behavior and the incurability typical of low grade, as opposed to high-grade, malignant NHL.

In the treatment of these lymphomas radiotherapy has been successfully applied to localized disease, whereas in advanced stages of NHL systemic polychemotherapy is required. In the international experience of the past two decades several cytotoxic drugs have proven to be highly effective in the control of tumor growth usually as multidrug combinations. However, myelotoxicity is a major and often dose-limiting side effect of all regimens and it often precludes the use of the full doses of polychemotherapy and within the planned schedule. The high percentage of unresponsive cases (approximately 30% in high-grade malignant NHL) also suggests that procedures other than conventional radio- or chemotherapy are

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required if improvements in the therapeutic response rates are to be achieved.

With the recent discovery of the influence of cytokines on hematopoietic progenitor cell amplification, differentiation and proliferation control, the possible clinical relevance of growth factors and immunomodulators was quickly recognized.[1] The therapeutic potential of hematopoietic growth factors and interleukins in the management of lymphoma[2–4] became evident and resulted in numerous clinical trials designed to investigate their modes of application and spectra of activity.

IFN-α

The first lymphokine shown to have antiproliferative activity in clinical use was IFN-α. Although high expectations for the successful treatment of malignomas by IFN-α were anticipated, they were not fulfilled and details of the functional mechanisms involved still remain obscure. In the meantime, however, the role of IFN as a potent biological response modifier has been established.[4]

In NHL, IFN-α is virtually inactive in patients with high-grade malignant tumors[3,4] while it has proved to be effective in various entities of low-grade malignant NHL.[3,5–7] The potential synergism between conventional chemotherapy and interferon has initiated several studies in the follicular lymphomas—the centroblastic-centrocytic (CB-CC) or centrocytic (CC) entities.[5,6] However, the lack of clear dose–response relationships still makes comparative evaluations of these studies difficult.

The preliminary results of current trials have demonstrated the therapeutic benefits of IFN-α in low-grade NHL where it has been shown to be quite active in early stage disease. In previously untreated chronic lymphocytic leukemia (CLL) IFN-α monotherapy induced remissions[7] but was not convincingly effective in advanced stages.[3] In other B cell low-grade NHL, such as CB-CC and CC lymphomas, IFN-α was recently evaluated in randomized trials, both in combination with induction chemotherapy and[5]/or[6] in the form of low-dose maintenance after achievement of partial or complete remissions by conventional polychemotherapy. Similar complete or good partial remission rates were obtained by chlorambucil irrespective of simultaneous IFN-α in the induction phase of therapy. However, IFN given additionally during a maintenance period of 18 weeks reduced the relapse rates from 40 to 20% and improved survival rates compared with patients who did not receive further therapy.[5] The prognostic relevance of maintenance IFN-α may be confirmed in the near future in a trial currently in progress.[6]

In low-grade malignant T cell lymphoma, particularly in the cutaneous tumors such as mycosis fungoides and Sézary syndrome, and in peripheral T cell lymphoma, (lymphogranulomatosis X and Lennert's lymphoma) low-dose IFN-α seems to exhibit anti-proliferative activity and to be well tolerated. This compares favorably with the disappointing results often gained with the application of systemic polychemotherapy, especially in the latter group.

The experience obtained to date implies that there is a role for IFN-α in the treatment of low-grade malignant NHL of both B and T cell subtypes provided the tumor mass is low as in early stage disease, or when minimal residual disease is present after conventional treatment.

GM-CSF

The colony-stimulating factors G-CSF and GM-CSF have proven to be strong promoters of hematopoietic progenitor cell proliferation in both *in vitro* studies and various clinical studies of therapy-induced myelosuppression, and occasionally in other forms of myeloaplasia.

In low-grade malignant NHL the invariably fatal outcome following years of slow progression has prompted the introduction of more aggressive therapeutic approaches especially in younger patients in an attempt to ultimately achieve a cure.

In high-grade malignant NHL, the minority of patients with strictly localized disease are treated quite effectively with local or regional radiotherapy, often resulting in 80–90% long-term survivors. The vast majority of patients with advanced stage II to IV disease need intensive systemic polychemotherapy for which several highly effective cytostatic drug combination regimens have been developed and evaluated in large clinical trials. Careful comparative analysis of the international trials has resulted in the identification of initial parameters which influence therapeutic outcome, as determined by multivariate risk factor analysis performed in several trials. According to results recently obtained from a large meta-analysis (International NHL Prognostic Factors Project), these prognostic risk factors are predominantly age, performance status, serum LDH, stage and number of extranodal manifestations. The early identifica-

tion of patient subgroups likely to be treated inadequately by conventional approaches thus seems to be possible. In the context of treatment failure rates, evaluations of dose intensity have attracted considerable interest since adequate dose intensity may be relevant for prognosis. Deviations from protocol, dosage and schedule are frequently caused by myelotoxicity and subsequent infectious complications, resulting in a loss of dose intensity and thus possibly of response rate. The individual dynamics of hematopoietic recovery may thus constitute a crucial factor determining therapeutic success. The potential role of GM-CSF in NHL has already been evaluated in several studies, and it has been shown to produce beneficial effects.

The ultimate test for cytokine activity is provided by cases of severe myeloaplasia, as observed after ultra-high dose, myeloablative treatment followed by peripheral blood progenitor cell or bone marrow transplantation rescue. The efficacy of GM-CSF in accelerating neutrophil recovery in these conditions has recently been reported[8] and the role of GM-CSF as an adjunct to bone marrow transplantation will be discussed in detail in the report by S. Gulati.[9] Two methods of using GM-CSF have proven to be effective in these experimental treatment approaches. When given prior to the collection of progenitor cells, GM-CSF expands the circulating progenitor cell pool and therefore amplifies the autotransplant cell harvest. When given after transplantation, GM-CSF accelerates the kinetics of engraftment, and it has even been used in place of bone marrow transplantation in some patients.[10]

Myelosuppression induced by conventional chemotherapy is a much more frequent condition. In high-grade malignant NHL the activity of GM-CSF has been evaluated predominantly in patients with refractory or relapsing disease, compared with a few non-pretreated patients, who received high dose salvage therapy. These studies have shown that GM-CSF significantly accelerates the recovery of peripheral leucocyte counts, thereby reducing the risk of infection.[2,11,12]

In the first randomized, double-blind and placebo-controlled trial of GM-CSF as an adjunct to induction chemotherapy it could be demonstrated that GM-CSF significantly reduces the incidence of fever and infections, the length of neutropenia and the days of hospitalization and intravenous antibiotic demands.[13]

Even in HIV-infected patients, who are increasingly at risk of developing high-grade NHL of B cell type, GM-CSF support has been shown to improve the hematopoietic recovery after CHOP treatment.[14] Thus, in conditions analogous to those seen after autologous bone marrow transplantation, GM-CSF was active, even though the progenitor cell pool was impaired. However, a temporary increase of circulating HIV 1-P24 antigen during GM-CSF cycles was observed in this study, and this will require particular attention in future trials.

A definitive judgement on further effects of GM-CSF, particularly on the complete response (CR) rates and/or survival in NHL patients, is not yet possible. Preliminary data implied a moderate advantage in CR rates[13] and survival[12] in favor of the GM-CSF treated patients. Whether this is a real consequence of direct tumor growth control, or a coincidental phenomenon related to indirect or entirely different mechanisms remains to be clarified. Preliminary results from clinical trials performed with conventional chemotherapy in the absence of GM-CSF have indicated that early achievement of CR may be predictive of long-term survival.[15] A possible explanation for this could be that a rapid response might reduce the risk of the development of resistance to chemotherapy. A recent study has shown that GM-CSF support makes an increase in dose intensity feasible.[16] The final analysis of this particular study and of randomized trials currently in progress should show whether an increased dose intensity will actually improve response rates and long-term survival. GM-CSF support in treatment regimens might additionally help to maintain autologous immunological tumor surveillance sources by reducing the impact of severe infections in the immunocompromised patient. A model of these potential mechanisms of GM-CSF activity is given in Figure 1.

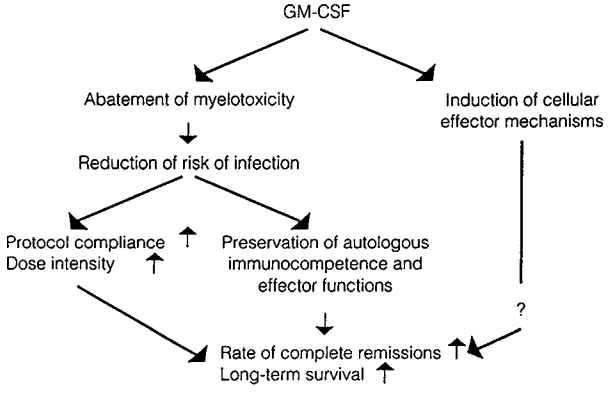

Figure 1. Potential mechanisms of GM-CSF activity in the treatment of lymphoma.

In the complex network of interleukin and cytokine interactions, further indirect and, as yet undefined, effects of GM-CSF may be operative.

IL-2

The discovery of the specificity of the first interleukins immediately prompted speculations about their possible clinical applications. However, initial high expectations were not fulfilled. *In vitro* studies of IL-2 suggested it to be a prime candidate for the immunotherapy of lymphoid malignancies because of its activation of monocyte, T-lymphocyte and natural killer (NK) cell-mediated cytotoxic mechanisms, including lymphocyte activated (LAK) cell cytotoxicity. In the early investigations[4] autologous IL-2-activated and re-infused LAK cells did indeed evoke some anti-tumor response in metastatic cancer. However, in subsequent trials designed to enhance the control of tumor growth by the use of IL-2 alone or in combination with autologous LAK cells[4,17,18] in patients with refractory or relapsing lymphoma, only minimal responses were induced, mostly limited to NHL of low-grade malignancy.

Optimal indications for this immunotherapy agent may still be found, but some reservations concerning the activity of IL-2 indicate that it should be used with caution, since a proliferative influence on the lymphoma cells cannot be entirely excluded.

IL-3

Although this cytokine has been reported to exert some stimulatory activity on follicular lymphoma cell isolates *in vitro*[19] (similar to the lymphocyte activation by GM-CSF observed in one report[20]) it has recently been used very successfully in clinical applications.[21–22]

With the increase in dose intensity permitted by the use of GM-CSF (as a result of the reliable abatement of leucocytopenia) the problem of thrombocytopenia, with the inherent danger of bleeding complications arises, and may become the dose-limiting factor. In inducation chemotherapy this generally occurs only when experimental high-dose protocols are used. In cases of relapsed or refractory lymphoma however, thrombocytopenia-related therapy delays must be expected. In these patients, the introduction of more efficient regimens, made feasible by the availability of IL-3, may contribute to a significant improvement in the otherwise poor prognosis. In addition to its stimulatory activity on granulocyte and macrophage differentiation which is comparable with that of GM-CSF, IL-3 has been found to stimulate the regeneration of thrombocytes and also to influence erythropoiesis. This was shown quite convincingly in the setting of myeloaplasia following autologous bone marrow transplantation in lymphoma, where in a phase I/II trial neutrophil, platelet and reticulocyte recovery was accelerated by IL-3 application.[21] Under conventional treatment conditions IL-3 has recently been studied in patients with refractory or relapsed lymphoma undergoing cyclic salvage therapy with IEV[22] or DHAP.[23] Direct comparison of these phase I/II trials with different chemotherapy regimens and varying dosages of IL-3, applied either as continuous infusion or subcutaneously over different periods of time is limited. However, improvement of neutrophil recovery by IL-3 accompanied by a better platelet recovery was demonstrated in both studies. While these observations still need to be confirmed by the ongoing phase III trials, further interesting possibilities are suggested. Sequential application of both GM-CSF and IL-3, adapted to the particular dynamics of hematopoietic toxicity and recovery may help to alleviate short-term risks that would otherwise reduce the likelihood of a successful outcome to therapy.

Conclusion

In the treatment of advanced stage NHL, the beneficial activity of GM-CSF in accelerating the hemopoietic recovery from chemotherapy-induced myelosuppression has been established by several independent studies under various treatment conditions. This is associated with a considerable reduction of some of the toxic side effects of chemotherapy, in particular the incidence and severity of infectious complications. These short-term effects will increase the tolerability of chemotherapeutic regimens and will facilitate protocol modifications that may improve response rates and long-term survival. These results obtained predominantly from phase I/II studies, are confirmed by the first controlled randomized trial conducted in an induction phase treatment protocol for NHL.

Promising applications for the use of cytokines in the treatment of NHL can be envisaged on the basis of the favorable effects observed with the use

of GM-CSF. In addition, the combined application of different cytokines appears to be feasible. This approach should involve the use of substances that are active at two different levels of progenitor cell maturation, administered in a time schedule which will meet the particular demands of chosen treatment regimens. This could lead to the development of novel treatment approaches which may involve the use of cytokines as inducers of tumoricidal molecule release or other tumor cell control functions.

References

1. Oettgen HF. Cytokines in clinical cancer therapy. *Current Opin Immunol* 1991; **3**: 699–795.
2. Cicco NA, Lübbert M, Oster W, *et al.* Cytokines in the pathogenesis and management of non-Hodgkin's lymphomas. *Hematol Oncol Clin North Am* 1991; **5**: 1053–66.
3. Gaynor ER, Fisher RI. Clinical trials of α-interferon in the treatment of non-Hodgkin's lymphoma. *Semin Oncol* 1991; **18**(Suppl 7): 12–17.
4. Gilewski TA, Richards JM. Biologic response modifiers in non-Hodgkin's lymphomas. *Semin Oncol* 1990; **17**: 74–87.
5. Price CG, Rohatiner AZ, Steward W, *et al.* Interferon-alpha 2b in the treatment of follicular lymphoma: preliminary results of a trial in progress. *Ann Oncol* 1991; **2**(Suppl 2): 141–5.
6. Unterhalt M, Koch P, Nahler M, *et al.* Comparison of prednimustine.mitoxantrone (PmN) and cyclophosphamide/vincristine/prednisone (COP) in patients with low-grade non-Hodgkin's lymphomas followed by interferon maintenance. *Ann Hematol* 1992; **62**: A74 (abstr).
7. Ziegler-Heitbrock HWL, Schlag R, Flieger D, *et al.* Favourable response of early stage B-CLL patients to treatment with IFN-α_2. *Blood* 1989; **73**: 1426–30.
8. Advani R, Chao NJ, Horning SJ, *et al.* Granulocyte-macrophage colony-stimulating factor (GM-CSF) as an adjunct to autologous hemopoietic stem cell transplantation for lymphoma. *Ann Intern Med* 1992; **116**: 183–9.
9. Gulati S, Bennett C, Toia, *et al.* Role of granulocyte-macrophage colony stimulating factor (GM-CSF) after autologous bone marrow transplantation forHodgkin's disease. *Anti-Cancer Drugs* 1993; **4**: 13–16.
10. Laporte JPh, Fouillard L, Douay L, *et al.* GM-CSF instead of autologous bone marrow transplantation after the BEAM regimen. *Lancet* 1991; **338**: 601–2.
11. Gianni AM, Bregni M, Siena S, *et al.* Recombinant human granulocyte-macrophage colony-stimulating factor reduces hematologic toxicity and widens clinical applicability of high-dose cyclophosphamide treatment in breast cancer and non-Hodgkin's lymphoma. *J Clin Oncol* 1990; **8**: 768–78.
12. Ho AD, Del Vallue F, Haas R, *et al.* Sequential studies on the role of mitoxantrone, high-dose cytarabine and recombinant human granulocyte-macrophage colony-stimulating factor in the treatment of refractory non-Hodgkin's lymphoma. *Semin Oncol* 1990; **17**(Suppl 10): 14–9.
13. Gerhartz HH, Engelhard M, Brittinger G, *et al.* Randomized double-blind placebo-controlled phase III study of recombinant human granulocyte/macrophage colony stimulating factor (rhGM-CSF) as adjunct to induction-treatment of aggressive non-Hodgkin's lymphomas. *Blood* 1992; **80**(Suppl 1): 73a (abstr).
14. Kaplan LD, Kahn JO, Crowe S, *et al.* Clinical and virologic effects of recombinant human granulocyte macrophage colony-stimulating factor in patients receiving chemotherapy for human immunodeficiency virus-associated non-Hodgkin's lymphoma: results of a randomized trial. *J Clin Oncol* 1991; **9**: 929–40.
15. Engelhard M, Meusers P, Brittinger G, *et al.* Prospective multicenter trial for the response-adapted treatment of high-grade malignant non-Hodgkin's lymphomas: updated results of the COP-BLAM/IMVP-16 protocol with randomized adjuvant radiotherapy. *Ann Oncol* 1991; **2**(Suppl 2): 177–80.
16. Gordon LI, Andersen J, Habermann T, *et al.* Phase I trial of ProMACE-Cyta BOM and growth factor (rhGM-CSF) in previously untreated patients with diffuse aggressive lymphoma (DAL): analysis of dose intensity. *Blood* 1991; **78**(Suppl 1): 126a (abstr).
17. Margolin KA, Aronson FR, Sznol M, *et al.* Phase II trial of high-dose interleukin-2 and lymphokine-activated killer cells in Hodgkin's disease and non-Hodgkin's lymphoma. *J Immunother* 1991; **10**: 214–20.
18. Tourani JM, Levy V, Briere J, *et al.* Interleukin-2 therapy for refractory and relapsing lymphomas. *Eur J Cancer* 1991; **27**: 1676–80.
19. Clayberger C, Luna-Fineman S, Lee JE, *et al.* Interleukin 3 is a growth factor for human follicular B cell lymphoma. *J Exp Med* 1992; **175**: 371–6.
20. Ho AD, Haas R, Wulf G, *et al.* Activation of granulocytes induced by recombinant human granulocyte-macrophage colony-stimulating factor in patients with malignant lymphoma. *Blood* 1990; **75**: 203–12.
21. Fibbe WE, Raemaekers J, Verdonck LF, *et al.* Recombinant human interleukin-3 after autologous bone marrow transplantation for malignant lymphoma: a phase I/II multicenter study. *Blood* 1991; **78**(Suppl 1): 163a (Abstr).
22. Gerhartz HH, Walther J, Bunica O, *et al.* Clinical-hematological-, and cytokine-response to interleukin-3 (IL-3) supported chemotherapy in resistant lymphomas: a phase II study. *Proc Am Soc Clin Oncol* 1992; **11**: 329 (abstr).
23. Raemaekers J, van Imhoff G, Fibbe W, *et al* (introdution by De Witte T). A randomized double-blind placebo-controlled phase I/II study on human recombinant interleukin-3 after DHAP chemotherapy in patients with relapsed malignant lymphoma. *Blood* 1991; **78**(Suppl 1): 162a (abstr).

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