Genetic susceptibility to Hodgkin’s disease and secondary neoplasias: FISH analysis reveals patients at high risk of developing secondary neoplasia

D. M. Lillington1*, I. N. M. Micallef1, E. Carpenter1, M. J. Neat1, J. A. L. Amess2, J. Matthews1, N. J. Foot1, T. A. Lister1, B. D. Young1 & A. Z. S. Rohatiner1

1Cancer Research UK, Department of Medical Oncology and 2Department of Hematology, St Bartholomew’s Hospital, London, UK

Background: Cytotoxic drugs administered before high-dose therapy (HDT) represent a significant factor in the development of leukemic complications in patients with lymphoid malignancies. This retrospective study was used to detect evidence of abnormal therapy-related myelodysplasia/secondary acute myeloid leukaemia (tMDS/sAML) clones before HDT in a subset of patients who subsequently developed secondary neoplasia.

Patients and methods: 230 patients with non-Hodgkin’s lymphoma (NHL) underwent HDT comprising cyclophosphamide and total body irradiation (TBI) with autologous hematopoietic progenitor-cell support. Thirty-three patients have developed tMDS/sAML and 20 of these were screened for the presence of emerging therapy-related abnormalities before HDT. A further 24 patients without evidence of secondary neoplasia were screened using fluorescence in situ hybridisation (FISH).

Results: Significant levels of abnormal cells were identified in 20/20 patients screened who have developed secondary neoplasia compared with only three of 24 patients in the HDT control group who have not. The latter three patients have since died.

Conclusions: The triple FISH assay was developed to detect loss of chromosomal material from 5q31, 7q22 and 13q14. It can potentially identify those patients at risk of alkylating agent-induced leukaemia before they proceed to HDT. Used in a prospective manner, the triple FISH assay could permit more informed clinical management.

Key words: cytogenetics, FISH, lymphoma, secondary acute myeloid leukaemia, secondary myelodysplasia, susceptibility

Introduction

The incidence of therapy-related myelodysplasia/secondary acute myeloid leukaemia (tMDS/sAML) in patients with a primary diagnosis of non-Hodgkin’s lymphoma (NHL) is between 5 and 15% following high-dose therapy (HDT). Factors thought to be associated with the development of secondary neoplasia include prior cytotoxic therapy, in particular, alkylating agents and topoisomerase II inhibitors [1–6], older age [7–9], total body irradiation (TBI) [10–12], number of hematopoietic stem cells infused, chemotherapy priming of peripheral blood stem cells [8, 9, 13, 14] and an extended interval from diagnosis to HDT [15, 16]. Alkylating agent related leukaemias are typically associated with abnormalities of chromosomes 5 and/or 7, whilst those occurring following exposure to topoisomerase II inhibitors frequently involve the mixed lineage leukemia gene at 11q23 [1–6]. Patients who have had multiple courses of therapy are at increased risk of secondary leukaemia following HDT [17] compared with those patients with minimal prior therapy [18].

All 230 NHL patients treated at St Bartholomew’s Hospital had received alkylating agents before HDT. Twenty of the 33 patients who developed secondary neoplasia were screened for evidence of emerging tMDS/sAML clones before HDT. A further 24 patients who had undergone HDT but had not developed tMDS/sAML were also screened using a fluorescence in situ hybridisation (FISH) assay designed to detect loss of 5q31, 7q22 and 13q14, which are the abnormalities commonly seen at diagnosis of tMDS/sAML.

Materials and methods

Between January 1985 and November 1996 [7, 19–22], 230 patients with NHL received HDT comprising cyclophosphamide and TBI with autologous hematopoietic progenitor cell support as consolidation of remission. Thirty-three of these patients developed tMDS/sAML at a median
time of 28 months from HDT. Diagnosis of MDS was made according to French, American and British criteria [23] and the clinical details of these patients have been previously reported [7, 22]. Standard G-banded karyotype analysis [24] was performed on bone marrow aspirates in 19 of 33 patients before HDT, and at diagnosis of secondary leukaemia in 30 of 33 patients. Bone marrow samples taken ‘pre-HDT’ or at the time of harvest, which had been cryopreserved in dimethyl sulphoxide and stored in liquid nitrogen, were available in 20 of 33 patients and were screened using FISH. FISH probes were thoroughly tested on normal control samples (peripheral blood and bone marrow from individuals with no evidence of a haematological malignancy) and the mean cut-off level for a false-positive result ascertained. The number of abnormal cells was determined to be significant when the number exceeded the mean + 3 standard deviations (SD). The results were also analysed using the binomial distribution, taking the binomial (mean) as the cut-off point. The triple FISH assay for 5q31 (D5S721/D5S23; Vysis Inc., Maurens-Scopont, France), 7q22 (H_DJ0138M12/H_NH0132A01, kindly provided by Professor Scherer, The Hospital for Sick Children, Toronto, Canada) and 13q14 (RB1; Vysis Inc.) was applied to bone marrow taken at harvest from one patient in whom no cytogenetic analysis was performed at diagnosis of tMDS/sAML, and a further 24 patients who had undergone HDT but had not developed tMDS/sAML (Figure 1).

**Results**

Twenty-two of the 30 patients analysed by G-banding at diagnosis of tMDS/sAML displayed complex karyotypes with three or more chromosomal abnormalities. The karyotypes of these patients have been reported previously [22]. The most common chromosomal change seen in 20 cases was loss of material from chromosome 7, including loss of 7q22. Loss of 5q was also prevalent (18 patients) and loss of 13q was seen in five of 30 patients. All 20 patients screened showed statistically significant levels of cells containing tMDS/sAML-related abnormalities before HDT. The median level of interphase cells demonstrating abnormalities consistent with those detected at diagnosis of tMDS/sAML was 7%. Using the binomial distribution as the statistical method, 19 of 20 patients had statistically significant levels of abnormal cells. FISH
excluded the possibility, in two patients, that the abnormalities seen at tMDS/sAML also existed as part of the lymphoma clone, as neither showed these abnormalities at diagnosis of NHL. Another patient was previously karyotyped when NHL was diagnosed, and this confirmed a difference between lymphoma and tMDS/sAML abnormalities.

In the control group of 24 patients who had undergone HDT but without evidence of tMDS/sAML, three patients showed statistically significant numbers of abnormal cells pre-transplant using the triple FISH assay (Figure 1). Essentially the clinical characteristics of the control group and the tMDS/sAML group were similar before HDT in terms of the number of previous therapies, complete remission (CR) status, lymphoma subtype, stage at diagnosis and patient age. Of the three patients with abnormal cells detected pre-HDT but without confirmed morphological evidence of MDS, one patient displayed loss of an RB1 allele in 47% of cells and died of recurrent lymphoma 22 months post-bone marrow transplantation with evidence of mild dyserythropoeisis. The other two patients also died; one of recurrent lymphoma and the other of an unrelated cause. FISH and/or G-banded analysis confirmed that in at least two out of three of these triple FISH-positive patients, the abnormal cells were not part of the lymphoma clone.

**Discussion**

Strategies to reduce the incidence of secondary neoplasia whilst permitting increased event free survival following treatment for a primary disease are clearly desirable. The median survival of the 33 patients with tMDS/sAML in this study was 11.9 months, consistent with previous reports detailing the unsuccessful management of such patients [12, 25], although it has been proposed that allogeneic bone marrow transplantation in the younger patients may result in a better outcome [26]. Four of the 33 tMDS/sAML patients presented here received a non-myeloablative allograft (two from a sibling donor and two from a matched unrelated donor), and currently three of four are alive and well at between 2.5 and 25 months post-treatment.

As a consequence of the increasing incidence of secondary leukaemia in this cohort of patients, the cyclophosphamide and TBI was replaced by BEAM (carmustine, etoposide, cytarabine and methotrexate) chemotherapy as the myeloablative regimen, and since that time only one patient has developed a leukaemic complication, although follow-up, at a median of 21 months, is clearly short.

Cytotoxic therapy before HDT appears to play a major role in the development of secondary neoplasia and recently, FISH was used to demonstrate MDS-related abnormalities in cells before HDT in nine of 12 patients with Hodgkin’s disease (HD), NHL, breast cancer or multiple myeloma [27]. Abnormalities affecting chromosomes 5 and/or 7 are typically associated with prior alkylating agent therapy, even in the absence of HDT [13]. The ability to identify the patients who might be predisposed to the development of tMDS/sAML as a consequence of prior cytotoxic therapy is critical to the management of future patients being considered for HDT. However, the role of TBI in the development of tMDS/sAML cannot be excluded in this cohort, since in patients with follicular lymphoma who have not received HDT, the incidence of tMDS/sAML is very low [28, 29].

The triple FISH assay was designed to identify the chromosomal abnormalities typically seen following alkylating agent exposure. Used prospectively, it has the ability to identify abnormal tMDS/sAML clones before clinical or morphological manifestation of secondary leukaemia. The assay is being evaluated in the current HDT programme to ascertain its predictive power of identifying an ‘at risk’ population of patients with a view to allowing more informed or perhaps alternative management.

**Acknowledgements**

The Triple FISH assay has been reproduced with kind permission of the authors and publisher (© Lippincott Williams & Wilkins). The original data was first published in the paper by Lillington et al., Journal of Clinical Oncology, 2001; 19: 2472–2481.

**References**

10. Darrington DL, Vose JM, Anderson JR et al. Incidence and characterization of secondary myelodysplastic syndrome and acute myelo-