Can We Cure Indolent Lymphomas?

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Abstract

The current consensus is that indolent lymphomas are incurable disorders. There are some indications that these malignancies are potentially curable. Indeed, not all indolent lymphomas are currently incurable. For example, patients with Ann Arbor stage I-II indolent lymphomas can experience long-term disease-free survival and probable cure. Also, from the available literature data, it seems that the achievement of a molecular complete remission is a desirable yet understood, this group might actually constitute a prognostically different subset with a more aggressive and perhaps more curable lymphoma. Whether the plateau observed in their failure-free survival curve will be maintained with more follow-up and whether they might be a curable subset remain to be determined.

Introduction

One of the most difficult and challenging problems in the field of lymphomas is the management of the stage IV indolent cell types. Although most standard treatment regimens achieve CR§ rates in the range of 60–80%, it is clear that none of them produce cures. In spite of their great chemosensitivity, these cell types show a persistent trend for a continuous relapse pattern. Because of their indolent behavior and apparent incurability, it was logical for a policy of watch and wait to evolve and to be quickly adopted by most clinicians (1). In spite of the fact that this watch and wait approach seems reasonable in elderly patients or in those with serious comorbid conditions, there are two serious problems associated with this strategy:

(a) It is a circular argument as well as a self-fulfilling prophecy: the disease is declared incurable, so the patients are not treated on a timely basis; ergo, the disease is never cured.
(b) The median survival of patients with stage IV indolent lymphomas managed with this strategy is 7–9 years. Young patients understandably have considerable difficulty accepting this.

Can Indolent Lymphomas Be Cured?

The current consensus is that indolent lymphomas are incurable disorders. Are there any indications that these malignancies are potentially curable? Indeed, not all indolent lymphomas are currently incurable. Frequently, clinicians fail to appreciate that indolent lymphomas presenting with early disease (stage I-II) are curable with either radiation alone or with combined modality therapy (2–7). Most of the literature on this subject describes a plateau in the FFS curves after approximately 10 years. The literature on stage III is more scarce but also points in the same direction (8, 9). The main problem really lies with the stage IV presentations, which unfortunately represent approximately 66–75% of all cases.

Even in the stage IV cases, however, there is a small fraction consisting of approximately 10% of patients who continue in CR for periods of time >10 years (10). A plateau in the FFS of these stage IV presentations is not as clearly evident, but there is no question that a change in the hazard ratio occurs at approximately 7 years (10). This raises an important question: how do we define cures? A cure should probably be defined on the basis of the hazard ratio dropping below a certain level, even if it never approaches zero. There is a need for consensus regarding what the hazard ratio that defines a cure should be. It is a well-known fact that even in diffuse large cell lymphoma and childhood acute lymphoblastic leukemia, neoplasms we all accept as potentially curable, there are late relapses, and the FFS


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3 The abbreviations used are: CR, complete remission; ATT, alternating triple therapy; FFS, failure-free survival; CHOP-Bleo, cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin; BMT, bone marrow transplant; mbr, major breakpoint region; mcr, minor cluster region.

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During the past decade, a number of developments have taken place that could represent the first steps toward developing a strategy to cure a larger number of stage IV indolent lymphomas. These will be described in the next paragraphs.

IFN Improves FFS

In 1982, we devised a treatment regimen that used IFN in a maintenance schedule for 24 months for patients achieving a CR after chemotherapy. From 1982–1987, we treated 127 patients with stage IV low-grade lymphoma with CHOP-Bleo for an average of 12 months (10). Maintenance IFN consisted of 3 × 10^6 units/M2 s.c. three times per week given for a total of 24 months. With a median follow-up of 9 years, the 10-year survival was 50%, and the FFS was 24% for all cases treated with CHOP-Bleo alone. This represents an improvement for FFS (P = 0.01) and for overall survival (P = 0.07).

These results have been confirmed by four independent randomized trials (11–14). In summary, IFN, when given either as maintenance or simultaneously with chemotherapy, is capable of significantly prolonging remission duration and time to treatment failure in patients with advanced low-grade lymphoma. In at least one randomized study, it was also associated with a significant prolongation of overall survival, and in all other studies, the survival trend, although not significant yet, also favored IFN. Longer follow-up is likely to show a significant improvement in survival in the other studies; in the study we conducted in 1982, it took over 10 years to show an improvement. In spite of this improvement in FFS, it is not yet clear that IFN has completely altered the relapse pattern to the extent that we can claim that a large fraction of patients are being cured.

A Powerful New Tool: PCR Detection of Minimal Residual Disease

The major obstacle that has hindered progress in treating indolent lymphomas is the need for very long follow-up times before any conclusions can be drawn about the effectiveness of therapy. Inability to eradicate minimal residual disease is the most likely reason that treatment usually fails to cure stage IV indolent lymphomas, despite the consistent achievement of clinical CRs. A surrogate measure of therapeutic success, such as a sensitive and specific tumor marker, would be immensely helpful in assessing the results early during the conduct of a clinical trial. Until recently, there was no ideal marker to follow the clinical course of patients with indolent lymphoma. The Southern blot has been tested and found to be too insensitive because of its finite ability to detect only 1–5% of cells with JH or bcl-2 rearrangement.

Approximately 85% of cases of indolent follicular lymphomas carry a cytogenetically and molecularly detectable t(14;18) chromosomal marker (15). This makes follicular lymphomas a prime target for applying the powerful PCR technique to detect this chromosomal abnormality. In 1987, we developed the methodology to apply PCR to the detection of minimal numbers of cells (1/100,000) with bcl-2-JH-rearranged sequences (16). We then decided to ask a number of questions that this powerful technique could answer.

Do Long-Term Complete Remitters Become PCR Negative? To determine whether the long-term complete responders showed any trend to become PCR negative, we examined the peripheral blood of 28 patients treated with either CHOP-Bleo and IFN maintenance or any other standard treatment regimen who had been in continuous CR for more than 2 years (range, 2–19 years). Of these 28 patients, only 15 (54%) were found to be negative by PCR. The expected rate of molecular breakpoints that fall in the mbr region (and would thus

Fig. 1 Survival of 127 patients with stage IV indolent lymphoma treated with CHOP-Bleo + IFN maintenance for 2 years (study group, ○) as compared with 96 historical controls with the same stage and diagnosis but treated with CHOP-Bleo alone (control group, △). Reproduced from P. McLaughlin, Biomed. Pharmacother., 50: 140–148, 1996 with permission.

Fig. 2 FFS of 127 patients with stage IV indolent lymphoma treated with CHOP-Bleo + IFN maintenance for 2 years (study group, ○) as compared with 96 historical controls with the same stage and diagnosis but treated with CHOP-Bleo alone (control group, △). Reproduced from P. McLaughlin, Biomed. Pharmacother., 50: 140–148, 1996 with permission.
be expected to be PCR negative just because of the breakpoint not being amplifiable with the mbr probe. These findings suggest that only 21% (54% minus 33%) of this highly selected group of clinical complete responders might be true molecular complete responders after standard therapy. These data show that the PCR technique is a much more sensitive procedure for detecting minimal residual disease than the clinical methods currently in use. Our findings are compatible with the known fact that patients with stage IV indolent lymphoma can have prolonged remissions, but eventually most of them relapse. These results have been confirmed in part by Price et al. (17).

Can Standard Therapy Consistently Induce Molecular CRs? In a recent study, Gribben et al. (18) examined the bone marrow aspirates and biopsies of 25 previously untreated stage III-IV follicular lymphoma cases before treatment with cyclophosphamide, doxorubicin, vincristine, and prednisone. Twenty-two presented with bcl-2 rearrangements when studied by PCR; of the 3 who were negative, none had an amplifiable breakpoint in their tumor. After these 22 patients received 6 courses of standard-dose CHOP-Bleo, none of them had become PCR negative when 6–9 simultaneous bone marrow samples were analyzed.

When one bone marrow aspirate was analyzed by PCR, 20 of the 25 cases were still positive after 6 courses of CHOP-Bleo. These findings are comparable with our observation that the majority of peripheral blood samples of patients treated with standard chemotherapy with CHOP-Bleo or cyclophosphamide, vincristine, and prednisone were still positive even after prolonged disease-free survival. It became doubtful that standard CHOP-Bleo-type therapy was capable of inducing molecular complete responses in indolent stage III-IV follicular lymphoma patients with rearranged bcl-2 sequences.

At that point, it became clear that most of the time, CHOP-Bleo is incapable of inducing molecular complete responses, but it was unclear whether that was an essential requisite to produce long-term disease-free survival. We questioned whether the rearranged bcl-2 sequences in these PCR-positive long-term responders represented truly malignant circulating cells or whether they consisted of precursor cells with the abnormal DNA sequence but without the invasive capacity of tumor cells. The latter possibility was strengthened by the study by Limpens et al. (19) that suggested that nonmalignant cells derived from hyperplastic but otherwise normal tonsils can have rearranged bcl-2 sequences.

Does PCR Positivity Directly Reflect the Presence of Circulating Lymphoma Cells? All this led to initial pessimism about the potential clinical application of the PCR test. We as well as others suspected that this technique was either too sensitive or perhaps artifactual and that it might not be absolutely necessary to obtain a molecular CR to cure these patients. However, the study by Price et al. (17) proved that circulating blood cells with rearranged bcl-2 sequences in long-term complete responders with follicular lymphoma had the same fragment size as the original neoplasm in two cases tested, thus diminishing but not totally dispelling concerns that the blood results were either artifactual or unrelated to the disease. More conclusive evidence would require sequencing the DNA breakpoints of both blood and direct tumor samples. We have tested the blood of 50 patients with chronic myelogenous leukemia as well as that of 10 normal donors and found none of them to be amplifiable by PCR for bcl-2.4 In total, of 130 cases thus far tested, not a single false positive bcl-2 PCR has been recorded in blood or bone marrow specimens. However, a recent study has shown that if a large amount of DNA derived from mononuclear peripheral blood cells is used in the PCR reaction, almost 50% of the normal population will have bcl-2 detectable sequences (20). Thus it is important that the amount of DNA loaded as well as the source of cells be specified when reporting the results of this technique.

Can New Treatment Strategies Induce Molecular CR? In a recent study, Gribben et al. from the Dana-Farber Cancer Institute used the PCR technique to test the pre- and posttreatment bone marrows of patients with follicular lymphoma (both previously untreated as well as relapsed lymphoma) who received high-dose chemotherapy and total body irradiation (21). All of these patients were known to have an amplifiable bcl-2 breakpoint in their pretreatment bone marrow sample.

After high-dose chemotherapy and BMT, 77 of 134 cases (57%) became PCR negative. Of these 77, none had relapsed within follow-up times as long as 6 years. Of those 35 who were persistently PCR positive after BMT, 71% had relapsed. An additional 22 who had mixed PCR results after BMT behaved as an intermediate category, with 36% of them relapsing. In summary, 57% of 134 PCR-positive cases attained a PCR-negative state after high-dose chemotherapy, whereas none of them were able to reach such a state with standard chemotherapy. This study strongly suggests that intensive chemotherapy is capable of producing PCR-negative status in a group of patients that consisted mostly of previously treated recurrent lymphomas. Unfortunately, high-dose chemotherapy is not an option for many patients, either because of their age, persistent disease in bone marrow, or other considerations. In addition, this study does not address the issue of whether other standard-dose chemotherapy regimens other than CHOP-Bleo can produce the same results in previously untreated cases and whether the positive correlation of a molecular CR with favorable clinical outcome also holds for previously untreated patients.

Nevertheless, the studies by Gribben et al. (21–24) have also shown that the PCR test can be used as an early surrogate end point that objectively predicts the clinical outcome in a group of patients treated with high-dose chemotherapy and BMT mostly for recurrent indolent lymphomas. In spite of those data, a recent study by Johnson et al. (25) in 76 patients who underwent BMT for recurrent follicular indolent lymphoma used the PCR test for bcl-2 before and after treatment. Their purged marrows also were studied by PCR. The blood and bone marrow specimens studied consisted of mononuclear cells separated by density gradient centrifugation. Seventy-five had baseline material available for PCR study, and 50 (67%) had an amplifiable breakpoint within the mbr or mcr. Only one patient achieved a negative PCR during follow-up; he was one of the four transfused with PCR-negative marrow and relapsed 4 months after treatment at a site of previous disease (only one

4 Unpublished observations.
sample was available for study during follow-up). In summary, this study did not find that any significant number of cases achieved a PCR-negative state. The authors conclude that the role of PCR in monitoring indolent follicular lymphomas is not clear. However, the major difference between this study and that of Gribben is that neither the pattern nor the timing of the PCR results was studied. There is no indication as to how many samples were negative in a given patient or at a given time point. It seems that the samples were not drawn at specific time points in a systematic fashion. Another possible source of difference is the PCR technique used in the paper by Johnson, which differs from that of Gribben and is potentially more sensitive.

In a study by McLaughlin et al. (26) from the M. D. Anderson Cancer Center, 138 patients with indolent lymphoma were treated with an intensive ATT consisting of three different standard-dose chemotherapy combinations plus IFN maintenance for 1 year. The three combinations consisted of CHOP-Bleo; etoposide, solumedrol, high-dose 1-β-d-araibinofuranosylcytosome, and platinum; and novantrone, Oncovin, procarbazine, and prednisone. Nineteen patients had serial PCR tests monitored for bcl-2, and 65% achieved a clinical CR. Of the 19 monitored by PCR, 13 achieved a negative PCR state at some point during therapy, much higher than that seen with standard therapy such as CHOP. Thus far, no relapses have been seen in the 13 patients who achieved a PCR-negative state. At 5 years, 55% of all patients entered are projected to be free of recurrent disease (for follicular lymphomas, it is 67%), and no relapses have been seen beyond 42 months; however, it is still too early to draw final conclusions because the follow-up is still short. Interestingly, at 58 months, 94% of patients remain alive. More recently we have introduced a new treatment strategy for indolent lymphomas. We tested a regimen consisting of fludarabine, mitoxantrone (Novantrone), and dexamethasone as a salvage regimen (27). The high response rate observed in this setting has prompted us to compare it with the ATT regimen as frontline therapy for indolent lymphomas. We have found that this powerful regimen is capable of inducing molecular responses in a significant proportion of cases.

**Does Achievement of a Molecular CR Have Any Clinical Significance?** We have studied patients with indolent follicular lymphomas whose baseline PCR before therapy was positive. These patients were treated according to Ann Arbor stage (all stages were included in this study). Treatment varied from radiotherapy alone for early stages to the ATT regimen described above. In this group, those who converted to a negative PCR during the first year of treatment had a significantly longer FFS than those who did not (4-year FFS, 76 versus 38%, respectively; \( P < 0.001 \); Ref. 28). By multivariate analysis, \( \beta_2 \)-microglobulin (\( P < 0.01 \)) and molecular response (\( P < 0.001 \)) were the most important variables associated with outcome. Hence, we conclude that serial PCR analysis during the first year of treatment correlates well with clinical outcome.

**Is There Any Clinical Significance to the Variability in Breakpoint Site in Follicular Lymphomas?** Approximately 70% of the bcl-2 rearrangements in follicular lymphomas occur at the mbr, whereas in another 10% of cases, they take place in the mcr (29, 30). They occur in other sites, such as the variant cluster region, in a very small proportion of cases. Consequently, in 10–15% of patients, no t(14;18) translocation can be detected by either cytogenetic, Southern blot, or PCR techniques. Up to now, the significance of these different bcl-2 rearrangement sites in follicular lymphomas has remained unclear. Because most patients with follicular lymphoma, including those with early stage presentations, have circulating cells with the bcl-2 rearrangement, we took advantage of this unique opportunity to determine what type of rearrangement was present in each one. We analyzed the blood or bone marrow tissues of 247 patients by PCR. Our results indicate that 71% had the breakpoint at mbr, 11% had the breakpoint at mcr, and another 18% were germ-line for both mbr and mcr (31).

The mcr-rearranged patients have experienced an excellent FFS, with only 1 of 27 having relapsed thus far at a median follow-up of 27 months (31). On the other end of the spectrum are those patients with germ-line bcl-2, whose prognosis has been unfavorable. Although their relapse rate is the highest of all, their FFS curve tends to plateau early, similar to that of large cell lymphoma; in contrast, the mbr-rearranged cases seem to display the typical FFS curve of indolent lymphomas.

**Conclusions**

From the available literature data, it seems that the achievement of a molecular CR is a desirable objective. Patients who achieve a persistently negative PCR state seldom relapse, whereas the opposite is true for persistently positive cases. In view of its excellent correlation with disease-free survival when examined serially in multiple blood or marrow samples, the PCR technique has the potential of providing a tumor marker that can be used as an early end point for clinical trials. Because of their slow-growing nature, indolent lymphomas require a long follow-up before the efficacy of a given therapeutic regimen can be resolved. By serving as an early surrogate end point, the PCR could play an important role in expediting the development of new treatment strategies. If the PCR is confirmed to be a suitable end point, we could reduce the duration of clinical trials in these disorders from decades to 2–3 years, and new drug combinations and novel strategies could be evaluated much faster.

Whether IFN is capable of increasing the molecular CR rate as measured by PCR is not known. However, it is clear that from the clinical standpoint, IFN has been able to increase 2-fold the length of remission in patients with advanced indolent lymphomas. In at least two studies, this has been associated with prolongation of survival. More intensive regimens such as ATT, when used in combination with IFN, seem to have improved the quality of remissions as judged by the PCR assay.

Finally, the site where the bcl-2 breakpoint occurs seems to have clinical significance. Those follicular lymphomas with germ-line bcl-2, in our experience, have behaved more aggressively than the others, and their FFS seems different from that of the usual indolent lymphomas. Although the biological significance of this observation is not yet understood, this group might actually constitute a prognostically different subset. Whether the plateau observed in their FFS curve will be maintained with more follow-up and whether they might be a curable subset remain to be determined.
References


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