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Questions Raised by the Benelux CML Study Group: Results From the Randomized Study With Hydroxyurea Alone Versus Hydroxyurea Combined With Low-Dose Interferon-α 2b for Chronic Myeloid Leukemia

To the Editor:

The recently reported Benelux study1 raised several issues, some of them regarding current and future investigational studies in chronic myeloid leukemia (CML).

Dose-intensity schedule of interferon-α (IFN-A) therapy and outcome. Our studies and several others,2-10 including a randomized study by Alimena et al,11 have confirmed the association between IFN-A dose schedule and response (Table 1). The Cancer and Leukemia Group B (CALGB) study9 was also initiated at a lower dose schedule of IFN-A at 2 MU 5 times per week, which was abandoned after the first 16 patients on study because of low response rates in favor of a higher dose schedule. There has also been an association between the IFN-A dose delivered and response (Table 2).2,4,11-14 Our studies using the maximally tolerated individual IFN-A dose have shown better rates of cytogenetic response than studies delivering lower dose schedules, even after accounting for the risk group distribution (Table 3).3

Despite these data, and relying on the results from a small study in 27 patients in early chronic phase,10 in which the investigators concluded that lower dose schedules were as effective as higher dose schedules, many investigators adopted the lower dose schedules of IFN-A, perhaps understandably, because they are less toxic and less expensive. When the results with the lower dose schedules were found to be less effective, as in the Benelux study, they concluded that IFN-A, and not low-dose IFN-A (which was emphasized in the title, but not in the Discussion), may not be effective. The Benelux study, in our opinion, confirms what has already been well-known, ie, that low-dose IFN-A schedules increase the cost and toxicity of therapy without providing benefit. The benefit of IFN-A therapy is primarily observed in patients who achieve a major cytogenetic response. If this rate is reasonable, a survival benefit would be possibly reflected in the study group. Otherwise, it may not be, but would be observed only among patients achieving a cytogenetic response. Predictably, with a complete cytogenetic response of 9% and a partial cytogenetic response of 7% (major cytogenetic response, 16%) with IFN-A, a survival advantage was observed only among patients who obtained a cytogenetic response (Fig 5 in the report by The Benelux CML Study Group).

Although it may be reasonable to conduct further randomized trials of lower versus higher IFN-A dose schedules in CML, investigators reporting on lack of IFN-A efficacy, as in the Benelux study, should emphasize that the study results apply only to low-dose IFN-A schedules, not to IFN-A therapy in general. In studies combining IFN-A with low-dose cytosine arabinoside (ara-C) or homoharringtonine (HHT), in which myelosuppression may limit dose delivery, the outcome may be less dependent on IFN-A dose intensity but more related to the incidence and degree of the targeted endpoint, namely the rate of significant and durable suppression of the Philadelphia chromosome (Ph)-positive clones.

Intended study design versus treatment actually delivered. The study intended to accrue 100 patients in each treatment group (200 total). However, 24 patients (25%) discontinued IFN-A for side-effects, 8 (8%) for other reasons, and 16 (17%) to undergo transplant in chronic phase. The median treatment time on study was 25 months. Thus, the investigators were left with about only half of the IFN-A study group (52 patients) whom they were treating with a low-dose IFN-A schedule (3 MU 5 times per week; 2.2 MU daily) and still expected to observe a benefit, a difficult proposition at best. To further confound the results, 10 of 95 patients in the hydroxyurea arm were switched to IFN-A therapy, 14 were taken off for other reasons, and 7 underwent transplant, leaving 64 patients to be evaluated for hydrea therapy. A similar problem of intended versus delivered therapy was also discussed in the German trial,15 which concluded that a beneficial effect of IFN-A therapy would have been observed if patients who actually received IFN-A therapy were analyzed. After all, it is only logical to assume that, if there is any benefit to be expected from a particular therapy, the treatment should actually be administered to the patient.

Study design and statistical considerations. With the small number of patients projected to be entered on study and the expectation that a particular proportion (based on the investigators' historical experience) may not be able to receive the intended therapy, the study design must have assumed that a large difference in outcome had been anticipated between the low-dose IFN-A arm and the control arm. This may often not be the case. The recently published study of Guilhot et al16 was able to detect a small but significant difference in survival outcome with the addition of low-dose ara-C to IFN-A (3-year survival rate of 86% with IFN-A plus ara-C v 79% with IFN-A alone; P = .02) only because of the large number of patients treated (~700 patients). Thus, future randomized studies should clearly delineate the statistical design and the expectations (which should be reasonable) and empower the study with enough patient numbers to answer the questions posed.

The updated results of the Medical Research Council (MRC) Study. The investigators quoted an earlier,17 but not a subsequent18 update of the MRC study to support their suggestion that low-dose IFN-A therapy is not superior to hydroxyurea therapy. In fact, the subsequent update18 reports a superior survival with IFN-A versus hydroxyurea therapy.

### Table 1. Dose-Response With IFN-A Therapy in CML

<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Dose Schedule</th>
<th>No. % CHR</th>
<th>% Cytogenetic Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDACC (2)</td>
<td>5 MU/m²/d</td>
<td>274</td>
<td>80 58 38 26</td>
</tr>
<tr>
<td>Gastl (7)</td>
<td>0.6-2.5 MU/m²/d</td>
<td>10</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>Anger (5)</td>
<td>3 MU TIW</td>
<td>9</td>
<td>22 0 0 0</td>
</tr>
<tr>
<td>Freund (6)</td>
<td>3 MU/m² (5 MU) TIW</td>
<td>27</td>
<td>37 16 0 0</td>
</tr>
<tr>
<td>Alimena (4)</td>
<td>2 MU/m² TIW</td>
<td>33</td>
<td>24 — —</td>
</tr>
<tr>
<td>Thaler (8)</td>
<td>5 MU/m² TIW</td>
<td>30</td>
<td>63 — —</td>
</tr>
<tr>
<td>Schofield (10)</td>
<td>2 MU/m²/d (3.5 MU/d)</td>
<td>77</td>
<td>39 28 13 8</td>
</tr>
</tbody>
</table>

Abbreviation: TIW, 3 times weekly.
trials show an advantage of IFN-A therapy over hydroxyurea, as do hydroxyurea versus busulfan therapy. Thus, the Italian and update MRC (12, 34, 31, 7) studies of recombinant interferon alpha 2a and hydroxyurea for chronic myelogenous leukemia after cytogenetic response to interferon-

(P = .05), whereas no difference in outcome was observed with hydroxyurea versus busulfan therapy. Thus, the Italian and update MRC trials show an advantage of IFN-A therapy over hydroxyurea, as do the metaanalysis results (19) and even the German trial (15) when patients actually treated with IFN-A therapy were analyzed.

In summary, the present Benelux study, using a low-dose IFN-A schedule in a small number of patients treated, can only conclude that the benefit was not observed within the limitations of their particular study. This has been observed in many studies of various treatments in different cancers, in which contradictory studies are often explained by different treatment schedules, suboptimal treatment deliveries, a small number of patients, and unachievable statistical considerations. The results of the Benelux study cannot be considered as representing the relative benefit of IFN-A therapy in CML.

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18. Allan NC, Richards SM, Shepherd PCA: Interferon-α therapy with busulfan or hydroxyurea compared with either BU or HU alone in treatment of chronic phase CML. Results from MRC CML III trial. Int J Hematol 64:S68, 1996 (abstr 258, suppl 1)

Response

We thank Drs Kantarjian and Talpaz for their extensive comments on our report.1 Above all, we would like to underline that we did not compare low-dose IFN-α with hydroxyurea. Instead, as outlined in the title of the manuscript, our study compared low-dose IFN plus hydroxyurea with hydroxyurea alone. From the remarks made by the MD Anderson Cancer Center, it again becomes clear that only randomized trials analyzed on an intention to treat basis provide the best way to evaluate the benefit of a therapy.2,3

Dose intensity of IFN-α and risk profile. Drs Kantarjian and Talpaz say that the interferon dose in the Benelux study was ineffective. They appear to ignore the data presented in Table 4 of our report. Thus, the major/complete cytogenetic response rate to IFN therapy was 16% in the low-dose Benelux randomized trial and 19% in the high-dose Italian trial,4 the difference being predictable on the basis of the lower percentage high-risk Sokal patients in the Italian trial. We do not accept that the lower dose schedules increase cost and toxicity without benefit; rather, they diminish cost and toxicity while achieving benefit equivalent to that seen in high-dose randomized, multicenter trials.

As was extensively discussed by us, it is still not known which dose of IFN is required to obtain the best results in CML. Of note, all studies cited in Table 1 of the MDACC letter are either nonrandomized or very small. Although Drs Kantarjian and Talpaz always stated that “more is better,” their Table 2 illustrates quite the opposite: the cytogenetic responses and survival duration do not differ between the multicenter studies that used high doses and those that applied much lower doses. Moreover, tables such as this should be accompanied by risk profiles, because these correlate very well with survival: patients with a low Sokal risk score having a median survival of 96 months5 and with the new IFN-based prognostic score6 also 96 months. The percentage of patients with a low Sokal risk score in the MDACC letter are either nonrandomized or very small. Although Drs Kantarjian and Talpaz always stated that “more is better,” their Table 2 illustrates quite the opposite: the cytogenetic responses and survival duration do not differ between the multicenter studies that used high doses and those that applied much lower doses. Moreover, tables such as this should be accompanied by risk profiles, because these correlate very well with survival: patients with a low Sokal risk score having a median survival of 96 months6 and with the new IFN-based prognostic score7 also 96 months. The percentage of patients with a low Sokal risk score in the MDACC letter are either nonrandomized or very small. Although Drs Kantarjian and Talpaz always stated that “more is better,” their Table 2 illustrates quite the opposite: the cytogenetic responses and survival duration do not differ between the multicenter studies that used high doses and those that applied much lower doses.

Concluding remarks. As shown in the meta-analysis,10 IFN-α therapy for newly diagnosed CML patients offers a 13% survival advantage at 5 years when compared with chemotherapy alone. The low dose IFN-α + hydroxyurea results of the Benelux study are in line with the results from three other randomized trials as far as hematologic responses, cytogenetic responses, and median survival are concerned.8,11 What is remarkable about the Benelux data is that the group treated with hydroxyurea did so well. In addition, the Benelux study suggests that hydroxyurea alone, administered at a dose aiming at strict control of the white blood cell counts, can result comparatively in a very good median survival of 68 months. A joint analysis of all hydroxyurea-treated patients in the Benelux study, Italian study,4 and German trial11 will be undertaken to see whether the Benelux results obtained by hydroxyurea are indeed better, and if so, to find explanations. Because the median age of CML patients is around 60 years, insight may be obtained from that analysis and may help those (frequently elderly) patients who do not tolerate IFN.

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FGFR3 Gene Mutations Associated With Human Skeletal Disorders Occur Rarely in Multiple Myeloma

To the Editor:

Fibroblast growth factor receptor 3 (FGFR3) is one of four distinct tyrosine-kinase receptors (FGFR1-4) that are capable of binding a repertoire of at least nine related mitogenic fibroblast growth factors (FGFs). FGFRs encode proteins that all contain three glycosylated extracellular Ig-like domains, a transmembrane domain (TM), and a split cytoplasmic tyrosine-kinase domain. Point mutations in distinct domains of the FGFR3 gene are associated with autosomal dominant human skeletal disorders, such as achondroplasia, thanatophoric dysplasia types I and II, and hypochondroplasia. Recent reports indicate that the point mutations associated with these disorders produce constitutively activated FGFR3, which shows autophosphorylation in the absence of ligand and is no longer regulated by FGF binding.

We and others have recently provided the first evidence of FGFR3 gene involvement in human cancer. In particular, the FGFR3 gene located at 4p16.3 is translocated to chromosome 14q32 as a result of a novel and karyotypically undetectable t(4;14)(p16.3;q32): in particular, the Y373C mutation in the KMS-11 cell line, the K650E mutation in the OPM2 cell line, and the K650M mutation in a patient with multiple myeloma (MM), a malignant proliferation of plasma cells. Molecular studies have shown this lesion in five MM-derived cell lines and in four primary tumors. Although the breakpoints on 4p16.3 are located approximately 50 to 120 kb centromeric to FGFR3, the gene is overexpressed in these cases, but absent or barely detectable in cell lines without the translocation. Interestingly, FGFR3 gene mutations associated with distinct human skeletal disorders have also been identified in some MM tumors carrying the t(4;14)(p16.3;q32): in particular, the Y373C mutation in the KMS-11 cell line, the K650E mutation in the OPM2 cell line, and the K650M mutation in a primary MM tumor.

These findings prompted us to look for FGFR3 mutations known to be associated with skeletal disorders in a representative panel of MM, including 80 primary cases (60 patients at first diagnosis, 12 at relapse, and 8 affected by plasma cell leukemia) and 10 MM-derived cell lines (including the KMS-11 and OPM2 cell lines). The analysis was performed by means of the polymerase chain reaction–single-strand conformation polymorphism (PCR-SSCP) direct sequencing of genomic DNA. We amplified five distinct genomic FGFR3 fragments containing codons affected by mutations: codon 248, the entire TM domain of FGFR3 (exon 6), 5'-CTGAGCTTCCTCGGC-3', and 248R (exon 7), 5'-CATTGATCCTCCACAAGGC-3'; TDS (exon 10), 5'-AAGAGATGGGAGGCTGCA-3', and TD3 (exon 10), 5'-GGAGATGGGAGGCTGCA-3'; 540F (exon 13), 5'-ACTGACACAGCGCTTGACC-3', and 540R (exon 13), 5'-GCCCTCGTGAGGCAGCGCC-3'; 650F (exon 15), 5'-GATCCAGACAGGACCTGG-3', and 650R (exon 15), 5'-AGGGCCGGTGTTGGCGCAG-3'; 807F (exon 19), 5'-AGGGCCGGTGTTGGCGCAG-3', and 807R (exon 19), 5'-ACAGCGAGGGTGTCGGCTGAG-3'.

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**Fig 1.** Schematic representation of the primers from the human FGFR3 gene used in the study. The FGFR3 exons are indicated by white boxes, and the introns are indicated by lines. The 3' untranslated region is indicated by the dashed box. The approximate locations of the primers, the length of the amplified fragments, and the approximate positions of codons 248, 540, 650, and 807 are indicated. The nucleotide sequence of FGFR3 cDNA and the intron-exon organization of the gene have been previously reported. The sequences of the primers are as follows: 248F (exon 6), 5'-CTGAGCTTCCTCGGC-3', and 248R (exon 7), 5'-CATTGATCCTCCACAAGGC-3'; TDS (exon 10), 5'-AAGAGATGGGAGGCTGCA-3', and TD3 (exon 10), 5'-GGAGATGGGAGGCTGCA-3'; 540F (exon 13), 5'-ACTGACACAGCGCTTGACC-3', and 540R (exon 13), 5'-GCCCTCGTGAGGCAGCGCC-3'; 650F (exon 15), 5'-GATCCAGACAGGACCTGG-3', and 650R (exon 15), 5'-AGGGCCGGTGTTGGCGCAG-3'; 807F (exon 19), 5'-AGGGCCGGTGTTGGCGCAG-3', and 807R (exon 19), 5'-ACAGCGAGGGTGTCGGCTGAG-3'.

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8. Allan NC, Richards SM, Shepherd PCA: Interferon-α therapy with busulphan or hydroxyurea compared with either BU or HU alone in treatment of chronic phase CML. Results from the MRC III trial. Int J Hematol 64:S68, 1996 (abstr 258, suppl 1).


Questions Raised by the Benelux CML Study Group: Results From the Randomized Study With Hydroxyurea Alone Versus Hydroxyurea Combined With Low-Dose Interferon-? 2b for Chronic Myeloid Leukemia

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