

# Population-Based Molecular Detection of Hereditary Nonpolyposis Colorectal Cancer

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**Purpose:** Cancer morbidity and mortality can be dramatically reduced by colonoscopic screening of individuals with the hereditary nonpolyposis colorectal cancer (HNPCC) syndrome, creating a need to identify HNPCC. We studied how HNPCC identification should be carried out on a large scale in a sensitive and efficient manner.

**Patients and Methods:** Colorectal cancer specimens from consecutive newly diagnosed patients were studied for microsatellite instability (MSI). Germline mutations in the *MLH1* and *MSH2* genes were searched for in MSI(+) individuals.

**Results:** Among 535 colorectal cancer patients, 66 (12%) were MSI(+). Among these, 18 (3.4% of the total) had disease-causing germline mutations in *MLH1* or *MSH2*. Among these 18 patients, five were less than 50 years old, seven had a previous or synchronous colorectal or endometrial cancer, and 15 had at least one

first-degree relative with colorectal or endometrial cancer. Notably, 17 (94%) of 18 patients had at least one of these three features, which were present in 22% of all 535 patients. Combining these data with a previous study of 509 patients, mutation-positive HNPCC accounts for 28 (2.7%) of 1,044 cases of colorectal cancer, predicting a greater than one in 740 incidence of mutation-positive individuals in this population.

**Conclusion:** Large-scale molecular screening for HNPCC can be done by the described two-stage procedure of MSI determination followed by mutation analysis. Efficiency can be greatly improved by using three high-risk features to select 22% of all patients for MSI analysis, whereby only 6% need to have mutation analysis. Sensitivity is only slightly impaired by this procedure.

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GERMLINE DEFECTS IN several DNA mismatch repair genes predispose people to colorectal and endometrial cancer as well as to some other cancer types, a syndrome named hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome.<sup>1-3</sup> Tumors associated with HNPCC are often poorly differentiated, with mucinous features and peritumoral lymphocytic infiltration, yet have no distinct or unique features that could be used for diagnosis of the disease. Instead, the definition of HNPCC has been based on its genetic transmission and occurrence at a young age. The widely used Amsterdam criteria<sup>4</sup> require that colorectal cancer occurs in at least three closely related individuals in at least two generations. More recently, other sets of criteria have been proposed to accommodate the fact that other cancers, mainly endometrial cancer, are common manifestations of HNPCC and that family size is shrinking.<sup>5</sup> However, clearly, many HNPCC cases are not identified by these criteria, prime examples being individuals with small families or limited knowledge about their families and individuals whose parents and/or siblings died at an early age without acquiring cancer. Whether a family fulfills the clinical criteria or not, a definitive diagnosis of HNPCC can only be established by demonstrating a germline mutation.<sup>6</sup>

Mutation detection using patient DNA or RNA can be done by several different methods, none of which are 100% sensitive.<sup>7</sup> We and others have favored direct exon-by-exon genomic sequencing,<sup>8</sup> but this may have to be supplemented

by Southern hybridization to detect large deletions.<sup>9</sup> By far, most HNPCC cases diagnosed to date are caused by mutations in either *MLH1* or *MSH2*, so it is presently reasonable to limit clinical testing to these two genes,<sup>10</sup> perhaps with the addition of *MSH6*.<sup>11-13</sup>

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Screening for cancer is now available and desirable.<sup>14</sup> The need to diagnose HNPCC is presently becoming an increasingly important issue because of recent successes in cancer prevention by colonoscopic screening and the prospect of chemoprevention.<sup>15</sup> In a recently completed 15-year prophylactic screening project comprising 252 individuals, colonoscopy at 3-year intervals more than halved colorectal cancer risk and decreased overall mortality by approximately 65% in at-risk and mutation-positive members of HNPCC families (Järvinen et al, manuscript submitted for publication).<sup>16,17</sup> In mutation-positive families, there is a need to determine who has and who does not have the mutation. Thus, there is a challenge to diagnose as many HNPCC individuals as possible in an efficient and cost-effective manner. Indeed, HNPCC may be one of a handful of genetic conditions in which large-scale mutation screening is meaningful.<sup>18</sup> Because mutational analysis of *MLH1* and *MSH2* is a work-intensive and expensive undertaking, it is highly desirable to prescreen by a simpler method followed by mutational analysis in a high-risk subset. A formalized approach to the problem was recently published.<sup>19</sup> A logistic model for patient selection was proposed. The parameters were composed of the following clinical data: young age at diagnosis of colorectal cancer, fulfillment of the Amsterdam criteria, and the presence of endometrial cancer in the kindred. Patients scoring high-risk values would be scrutinized by molecular methods, mainly germline mutation analysis. We previously tested this formula in a series of colorectal cancer probands. If only first-degree relatives and their cancer status was known, the value of the formula was limited.<sup>20</sup> If extensive pedigree data were used, the formula<sup>19</sup> was able to identify most, but not all, mutation carriers in our series.<sup>20</sup> Unfortunately, extensive pedigrees and family histories are not usually available in clinical practice.

Microsatellite instability (MSI) is characteristic of HNPCC tumors<sup>21</sup> but occurs in a 10% to 15% subset of sporadic colorectal cancers as well.<sup>22</sup> Thus, MSI is a relatively sensitive but nonspecific marker for HNPCC. We proposed the following criteria for primary selection of colorectal cancer patients for MSI testing.<sup>8</sup> Colorectal tumors should be tested for MSI when the patient is under 50 years of age, or has multiple primary cancers of the colorectum and/or endometrium, or has a first-degree relative with colorectal or endometrial cancer. Mutation analysis should then be performed in individuals whose tumors score MSI(+). These criteria were designed based on the results of a molecular and genealogic analysis of 509 consecutive colorectal cancer probands.<sup>8</sup> In the present study, we prospectively tested these criteria in a series of 535 newly diagnosed colorectal cancer patients. Moreover,

by combining the results of both series, we were able to assess the frequency of HNPCC based on a total of 1,044 colorectal cancer patients. We propose a model for the population-based molecular screening for HNPCC targeted at all newly diagnosed colorectal cancer patients.

## PATIENTS AND METHODS

### *Patients and Tissue Preparation*

The study was approved by the appropriate ethics review committees. Cancer and normal tissue specimens were derived from 535 consecutive consenting colorectal cancer patients treated at nine large regional hospitals in southeastern Finland (Table 1). The consent rate was greater than 90%. The individuals ranged in age from 29 to 91 years, with a mean age of 67 years. The fresh-frozen samples were collected between March 1996 and June 1998. All lesions were histologically evaluated before DNA extraction to document proportion of tumor tissue. Out of 535 samples, 520 (97%) contained 50% or more carcinoma tissue. The specimens representing normal mucosa were always derived from a separate site, not from tumor margins.

All first-degree relatives (parents, siblings, and children) of each of the 535 patients were identified through official population registries. On average, eight first-degree relatives were identified. Data on patients and relatives were cross-linked with the Finnish HNPCC registry and the Finnish Cancer Registry to reveal possible connections to previously identified HNPCC families and to derive data on occurrence of cancer in the kindreds. The Finnish Cancer Registry has been functioning since 1953 and has almost complete coverage of histologically or cytologically verified cancer.<sup>23-25</sup> All individuals whose tumors were MSI(+) were offered a genetic counseling session. Individuals with germline mutations and their families were offered one or two more counseling sessions.

### *Analysis of MSI*

A flow diagram of the molecular analyses is shown in Fig 1A. DNA extracted from the carcinoma tissue was studied for MSI using the BAT26 and transforming growth factor (TGF)- $\beta$ RII mononucleotide (poly-A) markers by fluorescence-based polymerase chain reaction (PCR). Our previous experience and that of others encouraged us to prominently rely on BAT26.<sup>26-30</sup> All results were evaluated by two independent reviewers. The forward (F) and reverse (R) primers used were: BAT26F: TGA CTA CTT TTG ACT TCA GCC; BAT26R: AAC CAT TCA ACA TTT TTA ACC; TGF- $\beta$ RIIF: CTT TAT TCT GGA AGA TGC TG; TGF- $\beta$ RIIR: GAA GAA AGT CTC ACC AGG C. PCR-reactions were carried out in 10- $\mu$ L reaction volume containing 100 ng of genomic DNA, 1  $\times$  PCR buffer (Perkin Elmer Applied Biosystems [PE/ABI], Foster City, CA), 200 mmol/L of each diethylnitrophenyl thiophosphate ([dNTP] Finnzymes, Espoo, Finland), 0.3  $\mu$ mol/L (TGF- $\beta$ RII) or 0.6  $\mu$ mol/L (BAT26) of each primer, and 1.5 units of AmpliTaqGOLD polymerase (PE/ABI). The MgCl<sub>2</sub> concentration was 1.5 mmol/L. The following PCR cycles were used for amplification: BAT26: 95 degrees 10 minutes, 30 cycles of 95 degrees 45 seconds, 55 degrees 1 minute, and 72 degrees 30 seconds; and TGF- $\beta$ RII: 94 degrees 10 minutes, 28 cycles of 94 degrees 30 seconds, 55 degrees 75 seconds, and 72 degrees 20 seconds. Final extension was 72 degrees 10 minutes. PCR products were loaded on a 6% polyacrylamide 8-M urea gel and run in an ABI PRISM 377DNA Sequencer (PE/ABI) according to manufacturer's instructions. The data were

Table 1. Characteristics of the 535 Colorectal Cancer Probands

Characteristic	MSI(-) Patients (n = 469)		MSI(+) Patients (n = 66)		Patients With Germline Mutation (n = 18)		All Patients (n = 535)	
	No.	%	No.	%	No.	%	No.	%
Site of tumor								
Proximal to splenic flexure	113	24	45	68	7	39	158	29
Distal to splenic flexure	354	75	21	32	11	61	375	70
Dukes' stage								
A	94	20	9	14	3	17	103	19
B	173	37	35	53	11	61	208	39
C	129	27	20	30	3	17	149	28
D	61	27	2	3	1	6	63	13
Type of carcinoma								
Adenocarcinoma intestinal type	443	94	49	65	12	67	492	92
Partially mucinous adenocarcinoma*	9	2	5	8	2	11	14	3
Mucinous adenocarcinoma†	16	3	12	18	4	22	28	5
Signet-ring-cell tumor	1	0.2	0		0		1	0.1
Average age at onset, years		67		66		56		67
First-degree relative with colorectal or endometrial cancer	53	11	24	36	15	83	77	14
History of colorectal or endometrial cancer	4	1	9	19	7	39	13	2
Age at diagnosis < 50 years	34	7	11	17	5	28	45	8
Any of the three preceding characteristics	83	18	34	52	17	94	117	22
Average no. of first-degree relatives verified for cancer		7.9		7.3		7.3		7.8

NOTE. Because data were missing in some cases, the numbers shown do not equal the totals in all groups.

\*Mucinous cells 25% to 50%.

†Mucinous cells > 50%.

collected automatically and analyzed by the GeneScan 3.1 (PE/ABI) software. In all patients where the tumor showed loss of adenosines in the BAT26 tract, the analysis was repeated by comparing paired normal/carcinoma DNA to confirm the somatic origin of the aberrant alleles and to exclude polymorphisms.<sup>31,32</sup>

#### Detection of Germline Mutations

All 535 patients were scrutinized for the two most common mismatch repair gene mutations in Finland. Founder mutation 1 is a 3.5-kilobase genomic deletion of *MLH1* comprising exon 16, and founder mutation 2 is *MLH1* exon 6 splice site mutation IVS5-1G→A at 454 - 1. Together, these two mutations account for more than half (45 out of 82) of mutation-positive HNPCC families so far diagnosed in Finland.<sup>33</sup> Mutation 1 was detected by a PCR-based method that has been described previously.<sup>34</sup> Mutation 2 was detected by allele-specific oligonucleotide hybridization, as previously described,<sup>34</sup> with the following modifications. PCR-products were run in 2% NuSieve agarose gel (FMC Bioproducts, Rockland, ME) to verify the amplification, thus avoiding the need for hybridization with a wild-type probe. PCR-products from three individuals were pooled together onto the filter. Filters were hybridized with a probe containing the mutant sequence (5' CTT CTG TTC AAG TGG AGG AC 3'). If a positive signal was obtained, the respective samples were rehybridized separately on a new filter.

If neither of the founder mutations were detected but the patient's tumor had displayed MSI, mutation analysis of *MLH1* and *MSH2* was performed by direct genomic sequencing of the coding exons, including

the flanking intronic regions and promoter region, as previously described.<sup>8</sup>

The significance of a previously unreported missense variant, *MSH2* exon 12 D603N (1808C→A), was evaluated in 90 healthy individuals to eliminate the possibility that it was a polymorphism. For allele-specific oligonucleotide hybridization, the forward (F) and reverse (R) primers used were: (F): TTT TAG GTG GGT TCC TTT GA, and (R): CTC CAA AAT GGC TGG TCG TA. PCR reactions were carried out in 20  $\mu$ L of reaction volume containing 50 ng of genomic DNA, 1  $\times$  PCR buffer, 300 mmol/L of each dNTP, 0.6  $\mu$ mol/L of each primer, and 1 unit of AmpliTaqGOLD polymerase. The MgCl<sub>2</sub> concentration was 2.1 mmol/L. The following PCR cycles were used for amplification: 95 degrees 10 minutes, 40 cycles of 95 degrees 1 minute, 58 degrees 1 minute, and 72 degrees 1 minute. Final extension was 72 degrees 10 minutes. PCR products were run in 2% agarose gel to verify the amplification, thus avoiding the need for hybridization with a wild-type probe. PCR-products from three individuals were pooled together onto the filter. Probe was labeled with  $\gamma$ -P<sup>32</sup>ATP using T4-polynucleotide kinase (New England Biolabs Inc, Beverly, MA). Filters were hybridized with a probe containing the mutant sequence (5' CTC AGC TAA ATG CTG TTG TC 3'). If a positive signal was obtained, the respective samples were rehybridized separately on a new filter.

Missense change G322D (G→A) in *MSH2* exon 6 has been reported both as a pathogenic mutation<sup>35</sup> and as a polymorphism<sup>36</sup> (<http://www.nfdht.nl/database/mdbchoice.htm>). We evaluated the presence of this change in 89 cancer-free individuals by *HinfI* (New England Biolabs) digestion. The F and R primers used were: (F): TGA GCT

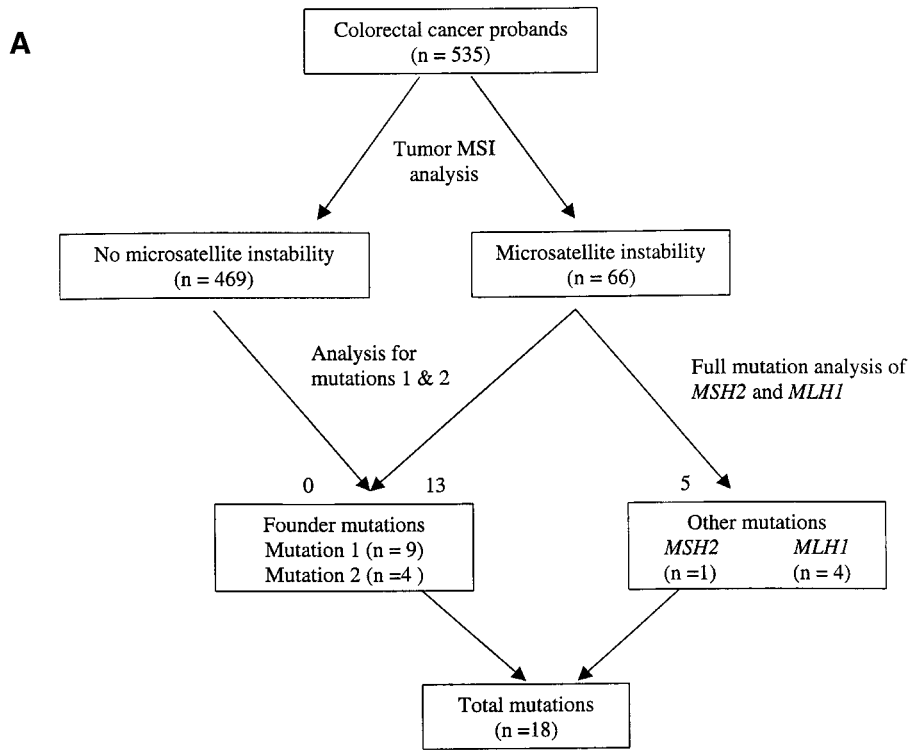
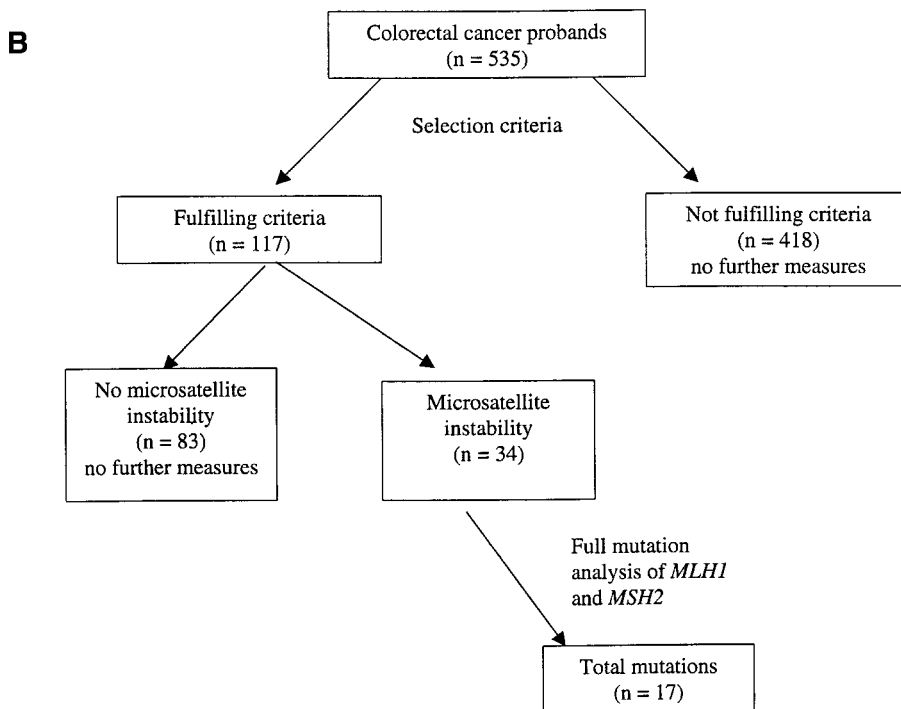


Fig 1. (A) Flow chart and results of the study. (B) Calculated flow chart and results of the study if the proposed selection criteria had been applied.



TGC CAT TCT TTC TAT T, and (R): TGG TAT AAT CAT GTG GGT AAC TGC. PCR reactions were carried out in 20  $\mu$ L of reaction volume containing 50 ng of genomic DNA, 1  $\times$  PCR buffer, 300

mmol/L of each dNTP, 0.6 mmol/L of each primer, and 1 unit of AmpliTaqGOLD polymerase. The  $MgCl_2$  concentration was 2.1 mmol/L. The following PCR cycles were used for amplification: 95

**Table 2. Data on the 18 Patients With Germline MSH2 and MLH1 Mutations**

Patient No.	Age at Onset (years)	No. of First-Degree Relatives Identified	No. of First-Degree Relatives With Colorectal or Endometrial Cancer	No. of Synchronous or Previous Colorectal or Endometrial Cancer	No. of First-Degree Relatives With Other Cancers	Mutation Detected
138	39	5	0	1	3	MSH2 exon 13, D603N (G→A)
275	72	9	1	1	4	3.5-kb genomic deletion of MLH1 exon 16
587	45	10	1	0	0	MLH1 exon 6, 454-1G→A (splice acceptor)
614	75	6	3	2	0	MLH1 exon 4, I107R (T→G)
615	39	5	2	0	0	MLH1 exon 6, 454-1G→A (splice acceptor)
661	54	6	1	0	1	MLH1 exon 4, I107R (T→G)
676	41	7	1	0	0	3.5-kb genomic deletion of MLH1 exon 16
698	43	4	1	0	0	3.5-kb genomic deletion of MLH1 exon 16
700	61	6	0	0	2	MLH1 exon 6, 454-1G→A (splice acceptor)
811	58	14	1	1	2	MLH1 exon 4, I107R (T→G)
870	61	5	1	0	1	MLH1 exon 4, Y126X (C→G)
899	59	6	1	0	0	MLH1 exon 6, 454-1G→A (splice acceptor)
952	66	10	2	2	1	3.5-kb genomic deletion of MLH1 exon 16
953	64	9	6	0	0	3.5-kb genomic deletion of MLH1 exon 16
955	65	7	1	0	1	3.5-kb genomic deletion of MLH1 exon 16
1027	57	8	0	1	0	3.5-kb genomic deletion of MLH1 exon 16
1077	56	6	2	1	0	3.5-kb genomic deletion of MLH1 exon 16
1095	56	8	2	0	1	3.5-kb genomic deletion of MLH1 exon 16

degrees 10 minutes, 40 cycles of 95 degrees 1 minute, 58 degrees 1 minute, and 72 degrees 1 minute. Final extension was 72 degrees 10 minutes. PCR-products were run in 2% agarose gel to verify the amplification. *HinfI* cuts the PCR fragment (239 base pairs [bp]), which contains the substitution into two fragments (approximately 60 bp and 180 bp), whereas the wild-type PCR fragment lacks the restriction site and is not digested. The digestion was performed in 1 × NEBuffer (New England BioLabs) at +37°C overnight. After digestion the PCR products were electrophoresed through 3% agarose gel.

## RESULTS

### Sampling

On the basis of Finnish Cancer Registry data on regional cancer incidence,<sup>25</sup> we estimate that the sample collection covered approximately 60% of all colorectal carcinomas removed between March 1996 and June 1998 in the collaborating hospitals. The patients provide a representative sampling of the entire population in the area because almost no operations for colorectal cancer are performed in other hospitals in the region. We discuss below (see Discussion) why the patients we accrued were unlikely to be biased for or against HNPCC.

### MSI

Of the 535 cancer samples, 66 (12%) displayed MSI (Table 1). All 66 had instability at the BAT26 locus; the analysis of the TGF-βRII poly A tract displayed a deletion in 58 of the MSI(+) samples (88%) and in two additional cases. These two tumors were considered MSI(−) after

analysis of a set of five markers<sup>22</sup> that gave no evidence of MSI.

### Mutation Analysis

Founder mutations 1 and 2 were screened in all 535 probands. No cases were found among the 469 MSI(−) cases, whereas, of the 66 MSI(+) cases, 13 displayed either mutation 1 (nine cases) or mutation 2 (four cases) (Table 2). Genomic sequencing of the remaining 53 MSI(+) cases revealed three cases of a third recurrent mutation, a missense mutation in exon 4 of the *MLH1* gene (I107R 320[T→G]). The mutation has been studied functionally and shown to be pathogenic.<sup>37</sup> Further previously unreported changes were detected in each of two MSI(+) probands, nonsense mutation *MLH1* exon 4 (Y126X [378C→G]) and missense mutation *MSH2* exon 12 (D603N [1808G→A]). The latter was absent in 90 healthy control individuals. Both patients had a family history compatible with HNPCC (Table 2), so these changes are likely to be pathogenic.

A previously reported change, *MSH2* exon 6 G322D (G→A),<sup>35,36</sup> was present in one of the MSI(+) patients but also in six (6.7%) out of 89 healthy controls. This change seems to be a neutral variant. Thus, altogether 18 mutations were found among the 535 probands (3.4%).

### Fulfillment of Risk Criteria

*Entire series.* Forty-five probands (8.1%) were under 50 years of age (Table 1). Thirteen patients (2.4%) had a

history of synchronous or previous primary colorectal and/or endometrial tumors. On average, eight first-degree relatives were identified for each proband (Table 1). In 77 cases (14.3%), the proband was found to have at least one first-degree relative with colorectal and/or endometrial cancer. In total, 117 probands (21.8%) fulfilled at least one of the three criteria.

**Mutation-positive patients.** Among the 18 new HNPCC patients, only five were less than 50 years, seven had a previous or synchronous colorectal or endometrial cancer, and 15 fulfilled the criterion that one first-degree relative had colorectal or endometrial cancer (Table 2). Notably, 17 patients (94%) fulfilled at least one of the three criteria. One patient, a 61-year-old woman, did not (patient no. 700, Table 2). None of her six first-degree relatives had colorectal or endometrial cancer, nor had she had cancer previously. However, when her pedigree was extended, typical HNPCC features became apparent because there were distant genealogically related relatives with early-onset colorectal cancer. This patient would not have been diagnosed if the described criteria had been applied. Based on typical family histories in the entire series of patients, only three fulfilled the original Amsterdam criteria,<sup>4</sup> and two additional patients fulfilled the more relaxed Amsterdam criteria.<sup>5</sup> Among these five patients, one (C576) was MSI(-). Mutations in *MLH1* and *MSH2* were sought in this patient but not found. The remaining four patients were MSI(+) and mutation-positive. These data illustrate the relatively high specificity but low sensitivity of the Amsterdam criteria. Even when the population registries were used to enlarge the pedigrees of all 18 HNPCC patients to comprise an average of 38 relatives, six did not fulfill the Amsterdam criteria. None of the 469 patients whose tumors were MSI(-) fulfilled the Amsterdam criteria.

#### Frequency of HNPCC

Combining the results of this study with those of a previous investigation on the same geographically defined population,<sup>8</sup> 28 mutation-positive patients were detected in a cohort of 1,044 patients newly diagnosed with colorectal cancer. Thus, the proportion of mutation-positive HNPCC in this combined series is 2.7%. The 95% confidence interval is 1.7% to 3.2%, calculated as a normal approximation to the binomial.<sup>38</sup> Assuming a 5% lifetime risk of colorectal cancer, an incidence of gene carriers calculated as 2.7% of 5%, or 0.135%, or one carrier in 740 individuals, is suggested in this population. This is an underestimate because the mutations are not 100% penetrant.<sup>1</sup> Furthermore, these figures pertain only to HNPCC caused by mutations in *MLH1* and *MSH2*. These genes account for the great majority of all HNPCC mutations in genes that are known today.<sup>6,10</sup> However, if the proportion of all HNPCC is between 5% and 10% of all colorectal cancers,<sup>1</sup>

then only approximately half to one third of all HNPCC can be molecularly diagnosed at present.

#### DISCUSSION

Our results allow us to calculate the outcome if this study had been conducted by selecting patients for MSI analysis based on age, previous tumor, and positive family history (Fig 1b). Only 117 patients (22%) would have been selected for MSI analysis, and only 34 of them (6% of the total) would have been MSI(+) and subjected to mutation analysis. Among these 34 patients, 17, or half, would have been diagnosed with a germline mutation, ie, HNPCC. One patient (6%) would have been missed. Figures of a similar nature need to be obtained from other populations and circumstances. For instance, it is not clear whether the existence of founder mutations, such as the ones predisposing to HNPCC in Finland, increases the overall HNPCC incidence. It is also possible, at least in principle, that mismatch repair gene mutations show different penetrances in different populations and circumstances. Nevertheless, the results presented here can be tentatively extrapolated into a true population-based diagnostic endeavor, for instance as follows. In the state of Ohio (population, 10 million) colorectal cancer is diagnosed in 6,100 individuals annually.<sup>39</sup> The requirement for MSI testing being 22%, only 1,342 such tests would need to be performed and would disclose 6% of the total or 366 MSI(+) results. Thus, only 366 germline mutational analyses would be needed to diagnose the approximately 165 new mutation-positive HNPCC patients (2.7%) that would occur. Our results suggest that the rate of missed mutations would be low. This scenario, although hypothetical at present, illustrates the general feasibility of population-based approaches to the practice of human cancer genetics in a clinical and population setting. In this scenario, account is not taken of several factors that are likely to reduce its feasibility, such as noncompliance of patients,<sup>40</sup> inability to provide even first-degree relative family histories and to assess personal risk,<sup>41</sup> lack of interest on the part of physicians, other health personnel, and the society,<sup>18</sup> fear of discrimination,<sup>42</sup> and cost issues.<sup>43</sup>

The model we propose relies heavily on the MSI test as a primary screen. A high sensitivity is suggested by the fact that 80% to 95% of HNPCC tumors have been shown in the past to be MSI(+).<sup>44,45</sup> However, these tumors emanated from members of previously diagnosed typical HNPCC families. It does not automatically follow that the sensitivity of MSI is equally high in tumors from newly diagnosed, apparently sporadic colorectal cancer patients. Our study provided an excellent test of this in that we screened all 1,044 patients for two founder mutations that are so wide-

spread that they account for over half of all HNPCC in the country. Among the total of 128 MSI(+) patients, 19 were found to have one of these mutations, whereas among 916 MSI(-) patients, no such mutation was found. We consider this to indicate that MSI, when adequately determined, shows high sensitivity for mutation-positive HNPCC tumors. These data also confirm that BAT26 alone is a sensitive indicator of MSI as suggested.<sup>29-31</sup> However, the specificity of MSI is low mainly because a large proportion of all MSI(+) tumors are caused by epigenetic silencing of the *MLH1* gene, a somatic event caused by promoter methylation.<sup>46</sup> We show here that the specificity can be enhanced to approximately 50% by selecting patients fulfilling certain clinical criteria. Only two thirds of the mutation-positive individuals fulfilled the Amsterdam criteria for HNPCC<sup>4</sup> even when extensive pedigree information was available, ie, when an average of 38 relatives per proband were identified and their cancer status ascertained (data not shown). We do not presently have enough evidence to evaluate whether the cancer penetrance is lower in HNPCC families identified as described in this article compared with data from previously studied large typical HNPCC families.<sup>47</sup> Such data will presumably be forthcoming when further studies become available.

Is the 3.4% frequency of detectable HNPCC among 535 unselected colorectal cancer patients a reliable estimate? There could have been biases inflating the figure. For instance, even though the protocol called for accrual of every patient, as many as 40% were missed for a variety of reasons, the most common one being vacation of the surgeon or protocol nurse. We considered whether the patients accrued might have been inadvertently enriched for individuals at higher risk of HNPCC. To evaluate this, we compared the proportion of patients under the age of 50 in

our series with the proportion of all patients in this age category in Finland<sup>25</sup> and found both to be 8%. Thus, a bias in favor of young age, a major predictor of HNPCC<sup>48</sup> did not occur in this series, and we are not aware of other biases either.

In contrast, there are reasons to suggest that 3.4% is an underestimate. MSI could show false-negative results either for technical reasons or because the specimen did not contain enough cancer cell nuclei. The sensitivity of sequencing to detect heterozygous mutations in these genes is difficult to evaluate, but it certainly is not 100%.<sup>36</sup> Moreover, large deletions that are not detectable by most methods except Southern hybridization exist in *MSH2* and could account for up to 10% of all mutations.<sup>19</sup> Finally, HNPCC (albeit perhaps with somewhat unusual phenotypic features) has recently been shown to be caused by mutations in *MSH6*,<sup>11-13</sup> but how commonly this occurs is unclear. Thus, the 2.7% figure obtained in this study when combined with a previous figure of ours,<sup>8</sup> is in all likelihood an underestimate in the population we studied. If HNPCC accounts for 5% to 10% of all colorectal cancer,<sup>1</sup> and as the 2.7% proportion found by us is considerably lower, it is increasingly important to recognize the existence of both mutation-positive and mutation-negative HNPCC.<sup>49</sup> In this study, only one patient clearly had mutation-negative [and MSI(-)] HNPCC. Such patients may have yet unknown predisposing genes. We do recommend that they and their relevant family members be offered clinical cancer surveillance.

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## CORRESPONDENCE

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### Myelodysplasia After Autotransplantation

*To the Editor:* We share the point that secondary myelodysplasia and secondary acute myelogenous leukemia (MDS/AML) rates play an important role in the evaluation and judgment of treatment modalities in high-dose therapy (HDT) with autologous hematopoietic progenitor-cell support for lymphoid malignancies. Two recent reports on low-grade lymphoma with reverse Kaplan-Meier cumulative incidences of greater than 15% are compelling.<sup>1,2</sup> This incidence is higher than what was reported in most series or registry data on HDT with autologous stem-cell support.<sup>3</sup> Several factors have been incriminated: conditioning regimen, type of prior chemotherapy (with a special emphasis on fludarabine), and bone marrow in vitro treatment. In the study reported by Micallef et al<sup>2</sup> from the St Bartholomew's Hospital, total-body irradiation (TBI) was suspected as the main factor responsible for this unusual high incidence of secondary MDS. In our institution, in 395 patients with lymphoid malignancies submitted to autotransplantation, the 7-year cumulative incidence was 6.3% (11 patients; 95% confidence interval, 2.3% to 10.3%; crude rate, 2.8%), and one half of them had received TBI. But before eliminating radiation therapy from preparative regimens for patients with lymphoma, a discussion of the discrepancy induced by the application of different statistical approaches seems indicated, with special emphasis on the interpretation of resulting estimates.

First, all published reports are retrospective analyses and few of them have control populations. We designed with Andre et al<sup>4</sup> a study on Hodgkin's disease (HD), with an important goal of eliminating this type of bias. Three ungrafted Hodgkin's patients treated with conventional chemotherapy and radiation therapy were randomly matched with one grafted patient treated with a chemotherapy-only conditioning regimen. The different parameters used for matching were primary sensitive disease, primary refractory disease (PRD), or first relapse; age at HD diagnosis; sex; clinical stage (I-II or III-IV); presence or absence of "B" symptoms; and follow-up at least as long as the interval between the end of first therapy and the date of graft for the reference case. Overall, 1,179 ungrafted patients were matched with the 393 grafted cases: 258 in the PRD group, 300 in the primary sensitive disease group, and 621 in the first relapse group. Six cases of MDS/AML occurred in the grafted group (crude rate, 1.5%) and 18 occurred in the conventionally treated group (crude rate, 1.5%). The 5-year cumulative incidence of MDS/AML was 4.3% for the grafted group, in which 80% of patients received radiation therapy during the disease course. In multivariate analysis, only four factors were significantly correlated with an increased risk of second malignancy in the whole population: age over 40 years ( $P < .001$ ), relapse ( $P = .006$ ), PRD ( $P = .033$ ), HDT ( $P = .024$ ). This work clearly demonstrated an independent risk owing to HDT with the drug-only conditioning regimen and raises the question of the interpretation of rate estimates in the presence of competing events.

Second, the methodology proposed by Kaplan and Meier to estimate survival curves is perfectly suitable when the end point is death or a combined end point is event-free survival. However, if the aim of the study is to estimate the probability of a particular event in the presence of other competing events, then this methodology fails, because the resulting incidence curves can only be

interpreted as probabilities, in the hypothetical situation that the competing risks could be eliminated. If the event is MDS/AML, then the Kaplan-Meier method would produce estimated probabilities of MDS/AML in the hypothetical situation that the risk for death has been completely removed. The crude rate is also statistically misleading, because it does not account for the censoring within the years of follow-up. To allow for censoring, the MDS/AML rate must be weighted with the overall survival rate, which leads to a better estimate of the cumulative incidence. Details about calculation and corresponding SEs can be found in Marubini et al.<sup>5</sup> In particular, the usefulness of this cumulative incidence estimate has been illustrated in articles on local recurrence of breast cancer.<sup>6</sup> In the case of no censoring, the cumulative incidence rate is equal to the crude rate. In our institution, in the presence of strong censoring (10-year survival rate, 58.0%; 95% confidence interval, 52% to 64.0%, the 7-year cumulative incidence rate of MDS/AML was 4.0%; the crude rate (2.8%) leads to an underestimation, whereas the Kaplan-Meier method (6.3%) leads to an overestimation.

So, before making definitive decisions in the absence of a randomized trial comparing TBI versus non-TBI conditioning regimens, we suggest that MDS/AML cumulative incidence and multivariate analyses should be computed with an approach that accounts for competing risks.

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*In Reply:* We agree with Drs Mounier and Gisselbrecht that it may be premature to eliminate all radiation therapy from transplantation-preparative regimens for patients with non-Hodgkin's lymphoma solely

on the basis of recently published retrospective analyses.<sup>1-3</sup> However, there are important distinctions between our study on bone marrow transplantation for non-Hodgkin's lymphoma and the cited study by Andre et al<sup>4</sup> on second malignancies after autologous stem-cell transplantation for Hodgkin's disease. In the Hodgkin's disease study, the majority of grafted patients and conventionally treated control patients received mechlorethamine, vincristine, procarbazine, and prednisone (MOPP) chemotherapy, a regimen that is independently associated with secondary acute myelogenous leukemia (AML), particularly when combined with radiotherapy.<sup>5</sup> A control group of patients is critical in this situation, because it is important to distinguish the effects of MOPP from the effects of the chemotherapy-only conditioning regimen. Very few of our patients received MOPP-like regimens, and 10 of our patients who subsequently developed myelodysplastic syndrome (MDS) were treated only with cyclophosphamide, doxorubicin, vincristine, and prednisone before autologous stem-cell transplantation, a well-studied regimen not historically associated with MDS in standard dosing.<sup>6</sup> Moreover, the series by Andre et al<sup>4</sup> may have underestimated MDS risk by evaluating cumulative incidence of MDS at 5 years, which in our series was significantly shorter than the reported 10-year incidence.

Mounier and Gisselbrecht are correct in noting that the cumulative incidence provides an unbiased estimate of the rate of MDS/AML in the setting in which patients who die of disease or other causes are censored for incidence of MDS/AML.<sup>7</sup> We have reanalyzed our data and find that the cumulative incidence of MDS/AML in our series is 14.5%. This is, as expected, lower than the rate of 19.8%, which we obtained by inverting the Kaplan-Meier estimator. Nevertheless, 14.5% remains an unacceptably high rate of MDS after autologous transplantation for non-Hodgkin's lymphoma, and novel strategies must be developed to prevent this devastating complication.

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## Problematic WHO Reclassification of Myelodysplastic Syndromes

*To the Editor:* A World Health Organization (WHO) panel recently issued a report in the *Journal of Clinical Oncology* with proposals for reclassifying myelodysplastic syndromes (MDS).<sup>1</sup> In this report, suggestions were made to modify the French-American-British (FAB) definitions<sup>2</sup> of MDS. An important goal for characterizing patients with MDS is to further improve methods that enhance our ability to better risk-stratify patients according to their morphologic and biologic features. Such approaches could improve prognostication and treatment for these individuals, but any changes should be evidence-based. Many of the proposals of the WHO panel are controversial, lack supportive data, and should not be adopted without much wider discussion.

1. The WHO panel proposed excluding refractory anemia with excess blasts in transformation (RAEBT) patients from MDS, proposing acute myeloid leukemia (AML) to now include patients with 20% marrow blasts rather than the previously used 30% value.<sup>3</sup> We recognize that the blast percentage alone is an arbitrary feature. However, MDS is distinguished from de novo AML not merely by marrow blast count, but by a more indolent pace of disease associated with distinctive biologic features.<sup>4,5</sup>

For a number of reasons, we believe that RAEBT is not AML, and its inclusion as AML would be problematic. In contrast to AML, the differing features of RAEBT include the following: (a) the pace of disease is more indolent (thus requiring MDS to have clinical stability for 1 to 2 months, in contrast to the relatively acute course of AML)<sup>4</sup>; (b) some early MDS patients evolve into RAEBT, generally with an indolent course; (c) morphologic dysplasia is evident in RAEBT, not in all AML patients; (d) the biology of RAEBT stem cells differs from that of standard AML, with an increase in the former entity of complex/poor-risk cytogenetics, early stem-cell phenotype, and multi-drug resistance overexpression<sup>5,6</sup>; and (e) RAEBT patients (diagnosed with characteristics excluding true de novo AML) have poorer response to standard chemotherapy.<sup>7,8</sup> This feature is associated with the inherent biology of the MDS-related neoplastic clone rather than solely with the blast percentage per se.

Given these disparate features, we are concerned that the WHO proposal merging RAEBT and AML risks losing these fundamental distinctions. Such approaches have implications for patient management as well as for accurate design and interpretation of clinical trials.

2. The WHO panel also proposed that refractory anemia (RA, or refractory anemia with ringed sideroblasts [RARS]) have abnormalities solely involving the erythroid line. However, as clinically characterized by most MDS investigators, MDS requires dysplasia in at least two hematopoietic cell lines.<sup>4</sup> It is well recognized that RARS (a subgroup of MDS) differs clinically from unilineage erythroid dysplasia, such as is present in pure sideroblastic anemia (also termed idiopathic sideroblastic ineffective erythropoiesis).<sup>9</sup> The latter has a much improved natural history, essentially lacking evolution to AML. These unilineage erythroid dysplastic changes may also occur in a number of reactive

nonneoplastic conditions. Merging these distinctive entities would problematically blur relevant clinical distinctions.

3. The WHO panel added a new category of "refractory cytopenia with multilineage dysplasia (RCMD)". Although many patients with these findings have poorer prognoses than those lacking extensive dysplasia,<sup>10</sup> data have not demonstrated independent prognostic value of these abnormalities (ie, independent of marrow cytogenetic abnormalities and blasts, degree and number of cytopenias). Such weighting of clinical and biologic variables has been shown by the International MDS Risk Analysis Workshop to be important for classifying MDS, generating the International Prognostic Scoring System (IPSS).<sup>4</sup> In addition, RARS patients with multilineage dysplasia would be merged with other RCMDs, despite data indicating differences for RARS regarding certain therapeutic responses.<sup>11</sup>

These features highlight two important issues not addressed by the WHO proposals: (a) the lack of minimum essential criteria for defining MDS, and (b) the lack of morphologic criteria for defining dysplastic involvement of a particular hematopoietic cell line (as needed for the diagnosis of RCMD and RA).

4. The WHO group proposed another new category: "MDS, unclassifiable." This entity is not further defined. Such a category is confusing and would include a miscellany of poorly characterized cases. It would not add clarity to communication about diagnostic classification. In contrast, the use of FAB criteria has permitted nearly all MDS cases to be classified, with relative consistency of definitions worldwide.

5. The WHO proposals retain refractory anemia with excess blasts (RAEB) as a category. It is reasonable to retain this category. However, although this category was initially used by FAB, it has become well recognized that RAEB patients with  $\geq 10\%$  blasts had poorer prognoses than those with less than 10% blasts.<sup>4,12</sup> New and germane classifications would best provide good opportunity for segregating RAEB into two subcategories.

6. The WHO proposals include 5q- syndrome as a separate entity. Although a classical syndrome with 5q- alone has been described,<sup>13</sup> many patients with the 5q- abnormality lack consistent clinical features, particularly if they have additional cytogenetic abnormalities or higher marrow blast percentages (including AML patients with 5q-).<sup>4,14,15</sup> Multivariate analyses have failed to show 5q- cytogenetic features in MDS to have independent prognostic significance. Similarly, although other cytogenetic subgroups may have somewhat specific clinical features (chromosome 7, 17p abnormalities), they also have not been shown to be distinctive in multivariate analysis. Rather, incorporating MDS patients' specific cytogenetic abnormalities with other clinical features (eg, blasts, cytopenias), as in IPSS categorization,<sup>4</sup> more usefully defines the natural history of their disease.

7. The WHO proposals suggest that all patients with chronic myelomonocytic leukemia (CMML) be placed into another new subgroup, that of "MDS/myeloproliferative diseases (MPD)." Although cellular dysplasia may be present, CMML is predominantly an MPD, with dominant clinical characteristics of excessive myeloproliferation.<sup>16-18</sup> Despite marrow blast percentage having major influence on clinical outcomes, it seems confusing to consider (for example) leukopenic RAEB patients possessing monocytosis as having CMML and to merge such patients with atypical chronic myeloid leukemia (as included in the MDS/MPD category by the WHO proposal). MDS patients with relatively low WBCs who have monocytosis seem to be best categorized as an MDS subgroup defined by their marrow blast percentage using FAB (or as nonproliferative CMML<sup>4,19</sup>), rather than as standard CMML. Even this is not an entirely satisfactory characterization, as some patients with nonproliferative CMML become proliferative in the course of time.

8. The WHO proposals suggest excluding from MDS the "low blast count" leukemias with "AML-type" cytogenetic abnormalities, eg, t(8;21), inv(16), t(15;17).<sup>20</sup> We concur with this suggestion. In fact, a problem with some prior studies of MDS patients that suggested that RAEBT and AML were similar was their inclusion of a number of patients with these cytogenetic findings within the RAEBT subgroup.<sup>21</sup>

Thus although further morphologic advances (eg, degree of dysplasia, fibrosis, cellularity) could provide additive information for characterizing MDS, we believe that such proposals should be evidence-based and built upon well-established forms of MDS categorization (eg, FAB, IPSS)<sup>2,4</sup> unless clear data indicate otherwise. Such an approach should demonstrate the independent nature of new criteria by multivariate statistical analysis. Regarding biologic advances, as new understanding of critical molecular, immunologic, and cytogenetic features of MDS emerge, addition of these parameters to currently accepted methods of characterization will likely further improve prognostication methods for MDS. For the reasons given above, we do not believe the WHO proposals for reclassifying MDS provide useful new information for either prognostic or morphologic assessment of these patients.

Members of the International MDS Study Group\*

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*Reply 1:* We appreciate the comments of Dr Greenberg et al regarding the classification of the myelodysplastic syndromes (MDS) proposed by the World Health Organization (WHO) Committees.<sup>1</sup> The letter is timely, because it has engendered a re-evaluation of the proposals before their final publication, and because our response allows an opportunity to provide the rationale for some of the suggested modifications to the French-American-British (FAB) scheme. It is important to note that the WHO proposals were not instigated by a small number of individuals. Rather, they represent the consensus opinion of an international panel of nearly 100 clinicians, pathologists, and scientists who met at Airlie House, VA, in 1997, where many of the same issues raised in the letter from Greenberg et al were debated at length. Although complete unanimity was not achieved on every issue, it was the majority view that there are sufficient data in the literature to indicate that changes to the FAB classification for MDS<sup>2</sup> and acute

myeloid leukemia (AML)<sup>3</sup> should be made to permit better identification of biologically distinct subgroups of patients. The following paragraphs address the major concerns of the International MDS Study Group (IMDSSG) and reflect the discussions of the Airlie House Conference participants.

1. *Elimination of the category of refractory anemia with excess blasts in transformation (RAEBT) by lowering the blast requirement for a diagnosis of AML from 30% to 20% risks losing the fundamental difference between AML and RAEBT.* In their letter, the members of the IMDSSG argue that RAEBT is biologically different from AML, and that it has a more indolent pace. In part, we agree. But the comparison that must be made is whether there are significant biologic and clinical differences between RAEBT and the proposed WHO category into which such cases would be reclassified, ie, "AML with multilineage dysplasia (with or without a preceding history of MDS)." The literature indicates that RAEBT and cases of AML that arise from a previous MDS or that have a background of multilineage dysplasia share important biologic and clinical features, including poor-risk cytogenetic abnormalities, increased expression of MDR-1, and poor response to chemotherapy.<sup>4-6</sup> Some investigators have also reported that when matched for similar disease features, patients with RAEBT and AML who are treated with identical therapy have nearly identical outcome and survival times.<sup>7-9</sup> Furthermore, our interpretation of the data generated by the International MDS Risk Analysis Workshop is that RAEBT is not necessarily more indolent than AML; 25% of patients with  $\geq 20\%$  marrow blasts evolved to AML in 2 to 3 months, 50% in 3 months, and more than 60% within 1 year, with an overall median survival time less than 1 year.<sup>10</sup> The sum of such data indicates to us that patients with MDS and 20% blasts in the marrow have essentially the same disease as patients with AML with multilineage dysplasia and 30% blasts in the marrow.

We agree with Greenberg et al that MDS and AML cannot be separated by an arbitrary blast count, and we urge that treatment decisions, in particular, be made in the context of the clinical findings, knowledge of the cytogenetic and molecular genetic data, and the rate of disease progression, regardless of whether the marrow blast count is 20% or 30%. But we believe that the WHO proposal to eliminate RAEBT as a disease category and to classify such patients as AML with multilineage dysplasia does not diminish any fundamental differences between AML and MDS, but instead emphasizes the continuity of the MDS disease process with a unique subtype of AML. Perhaps just as importantly, however, is that elimination of RAEBT will also eliminate a common practice that is a disservice to some patients: that of classifying a patient with true AML de novo, ie, a patient with no myelodysplastic features who has good-risk cytogenetic findings, as RAEBT only because there were less than 30% blasts on a single determination. The IMDSSG does accept this latter group as AML when blasts are less than 30% in the marrow, and thus they evidently suggest that two different thresholds of blast percentages be used to establish the diagnosis of AML. The WHO committee proposes that there is a rationale for one threshold.

In summary, the WHO committee believes that the blast count is a useful but inexact marker for distinguishing one disease process from another. In this case, we believe there is evidence that the current distinction between RAEBT and the AML with myelodysplastic features is indeed arbitrary.

2. *All MDS categories require dysplasia in at least two hematopoietic cell lineages, and the WHO proposals for refractory anemia (RA), refractory anemia with ringed sideroblasts (RARS), and refractory cytopenia with multilineage dysplasia (RCMD) "would problematically blur relevant clinical distinctions."* We acknowledge, as asserted

by the IMDSSG, that some investigators require dysplasia in at least two cell lineages to make a diagnosis of MDS, including RA or RARS. Although the FAB guidelines for RA and RARS do state that "morphological abnormalities in the granulocytic and megakaryocytic series identical to those present in the other subtypes of MDS may occasionally be found in varying degrees," they also indicate that the erythroid series is mainly affected, and that "the granulocytic and megakaryocytic series almost always appear normal."<sup>2</sup> However narrowly or loosely one interprets these criteria, in practice RA and RARS include a heterogeneous population of patients, ranging from those with only erythroid dysplasia to those with significant dysplasia in the other lineages as well. We believe there are data that indicate patients with RA and RARS can be further assigned to clinically relevant groups based on whether the erythroid series is principally involved or whether there is prominent dysplasia (more than 10% dysplastic cells) in the granulocytic and megakaryocytic series as well. As noted by Greenberg et al, numerous investigators (see review in Germing et al<sup>11</sup>) have shown that patients with pure sideroblastic anemia (PSA) in whom there is unilineage dysplasia in only the erythroid series enjoy a longer survival and have a much lower incidence of transformation to AML than those who have RARS with multilineage dysplasia. The WHO proposal (admittedly not made clear in the initially published outline) does recognize these two types of sideroblastic anemia, ie, RARS with unilineage dysplasia (or PSA) and RARS with multilineage dysplasia. The WHO committee believes that RA as defined by the FAB guidelines is similarly heterogeneous and has proposed that patients who demonstrate mainly unilineage erythroid dysplasia be designated as RA, whereas those with prominent multilineage dysplasia be classified as RCMD. There are data from four recent studies to support this view.<sup>12-15</sup> Patients with RCMD have been reported to have a higher incidence of pancytopenia and of cytogenetic abnormalities, more frequent progression to AML, and a worse survival than patients with RA in whom only the erythroid series is affected. Whether there are major clinical or biologic differences between RCMD and RARS with multilineage dysplasia is not yet clear, although preliminary data from Germing et al,<sup>15</sup> in a study of 284 patients with RCMD, show no significant difference in survival or progression to AML between RCMD and RARS with multilineage dysplasia.

We concur with Greenberg et al that minimum essential criteria should be established for defining MDS. This is particularly true for RA, because as defined in the FAB classification, there is considerable leeway for inclusion of cases, including some that may not be myelodysplastic in nature. In the WHO proposal, the definition of RA is sufficiently restrictive to exclude most nonclonal cases of refractory anemia, thus further reducing the heterogeneity of the RA group and allowing for more appropriate patient management.

Overall, it is the aim of the WHO committee to refine the definition of the low-grade myelodysplastic disorders so that the subgroups are more homogeneous. This should permit clinicians and investigators to more accurately compare the results of future biologic and clinical studies.

3. "*Chronic myelomonocytic leukemia (CMML) is predominantly a myeloproliferative disorder.*" Although we respect the opinion of the IMDSSG that CMML is primarily a myeloproliferative disorder, there is considerable controversy among a number of other investigators regarding whether CMML is mainly myeloproliferative or myelodysplastic in nature, or both.<sup>16-18</sup> Recent studies that divide CMML into MPD and MDS types according to the leukocyte count have concluded that this parameter alone is likely not sufficient to recognize subgroups of CMML with major biologic or prognostic differences,<sup>19</sup> and to our knowledge, there are no specific cytogenetic or molecular differences

between CMML with myelodysplastic features and CMML with myeloproliferative features. The WHO committee understands the rationale for including CMML with the chronic myeloproliferative disorders and discussed raising the threshold for the monocyte count required for defining CMML so that it could be considered in that group of diseases. Cases with lower monocyte counts might then be assigned to the appropriate myelodysplastic categories, as suggested by the IMDSSG. However, there are no studies that provide another value that has been proven to be more clinically or biologically relevant, so any change in the monocyte requirement would be arbitrary. Furthermore, Greenberg et al correctly acknowledge that some patients who start out as having nonproliferative CMML may eventually have quite proliferative CMML. It may be that, at present, there is no satisfactory definition for this disease, but it was the overwhelming consensus of the Airlie House Conference participants that arbitrarily subdividing CMML into two subtypes would not help to understand it any better. The WHO proposal does not make any significant changes in the criteria for the diagnosis of CMML. We have merely placed it in a category that acknowledges its multifaceted features. This category provides a less restrictive view of CMML than does the FAB classification, and permits clinicians, if they wish, to view the condition of those patients with low or normal leukocyte counts and monocytosis as being more like MDS, and those with higher counts as myeloproliferative in nature, and to manage them accordingly. We believe that atypical chronic myeloid leukemia also fits into this category, as it too has both myeloproliferative and myelodysplastic features, and may at times be difficult if not impossible to separate from CMML on the basis of morphologic and clinical features.<sup>16-18</sup> If future studies provide more definitive evidence that CMML or atypical chronic myeloid leukemia are more accurately classified as myelodysplastic or myeloproliferative processes, then appropriate changes in their classification will be warranted. In our opinion, such evidence is not available at the present time.

We believe that most of the other concerns raised in the letter of Greenberg et al are addressed in the details of the classification, which will be published shortly. In particular, refractory anemia with excess blasts (RAEB) is divided into two subgroups, RAEB-1 and RAEB-2, based mainly on the finding of less than or more than 10% marrow blasts, respectively. A similar subdivision is proposed for CMML. The 5q- syndrome is defined narrowly and includes only those patients with a de novo, isolated del(5q) abnormality, refractory anemia, hypolobated megakaryocytes, and less than 5% medullary blasts.<sup>20,21</sup> We do believe, however, that the category of "MDS, unclassifiable" should remain. The frequency with which this category is used will vary with the experience of the morphologist and clinician, but all members of the WHO committee have encountered cases that defy accurate classification into one of the FAB categories, and we acknowledge that will also be the case with the WHO classification.

Finally, we agree with Greenberg et al that the best prognostic classification scheme will incorporate a number of genetic, immunologic, and biologic markers. But one must first accurately classify the patient into homogeneous groups in order to accurately interpret the results of studies using these techniques, and we believe that the WHO classification system does provide a useful framework for achieving this latter goal. Recently, Germing et al<sup>15</sup> applied the WHO proposals to 1,600 MDS patients for whom long-term follow-up was available. In their study, multivariate analysis of the data demonstrated that the WHO proposals provided more homogeneous categories, fewer unclassifiable cases, and more precise prognostic information than did the FAB classification.

We hope that the members of the IMDSSG will consider evaluating the WHO modifications in their studies of patients with MDS. Thank you for giving us the opportunity to respond.

Members of the WHO Myeloid Disease Writing Committees and the WHO Clinical Advisory Committee\*

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*Reply 2:* The letter from the International MDS Study Group (IMDSSG) and the World Health Organization (WHO) committee response raise thoughtful issues. An analysis of differences persisting between the two groups follows:

1. *Separation of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML).* Both groups agree that a marrow blast percentage threshold is irrelevant in one subset of AML, true de novo AML with recurring cytogenetic translocations. This AML subset should be eliminated from discussion. The other major subset, myelodysplasia-related or MDR-AML (WHO classification: AML with multilineage dysplasia), is clearly related to MDS. Both the WHO and IMDSSG groups agree that any marrow blast percentage is imprecise for separating MDS and MDR-AML; when possible, serial marrows and clinical parameters should be used. They further agree that the blast threshold is a surrogate for poorly understood changes that result in transformation of stem cells in MDS and progression to AML. The remaining difference between the groups is which arbitrary blast percentage threshold should be used in making this distinction in the absence of serial marrows or more definitive criteria. A low blast percentage threshold will misclassify as AML more cases that will continue to behave as MDS, whereas a high threshold risks the opposite result. Neither is satisfactory, and we must strive to find a better means to recognize transformation to AML in this setting.

2. *Definitions and minimal diagnostic criteria for low-grade MDS.* Disagreement here is philosophical. Should we still consider MDS a syndrome, as it was historically and as still implied by its name, or rather should we now consider it a specific set of diseases sharing

common biologic and clinical parameters, genetics, and pathogenesis and requiring common treatments? The WHO group has retained the syndrome approach, with more inclusive but less specific diagnostic criteria (unilineage dysplasia) for low-grade MDS. With this approach low-grade MDS will be heterogeneous, including diseases with no acquired genetic abnormality or risk for progression to high-grade MDS or MDR-AML. The IMDSSG has adopted the disease approach, attempting to restrict MDS to a specific set of diseases united at least by involvement of a multipotential hematopoietic progenitor, hence the requirement for bilineage dysplasia. Neither approach is inherently incorrect. However, for clinical protocols and for studies of the biology and pathogenesis of the specific disease group MDS, the disease approach seems preferable, with its more stringent diagnostic criteria (bilineage dysplasia).

3. *Definition and placement of chronic myelomonocytic leukemia (CMML).* CMML comprises a heterogeneous spectrum of disease, on one end predominantly hypoproliferative with minor monocytosis, on the other predominantly hyperproliferative and sharing features with myeloproliferative diseases, with a continuous spectrum between. The entire spectrum shares cytogenetic and dysplastic features with MDS. Current data do not indicate how to subdivide CMML, although intuitively the hypoproliferative end of the spectrum belongs in MDS and the hyperproliferative end in a separate group with a major proliferative component. Placing the entire group in MDS (French-American-British classification) seems to err in placement of the hyperproliferative high end, whereas removing all CMML from MDS (WHO classification) may err in removing the hypoproliferative end from MDS. Although available data are not helpful, a compromise is obviously possible for use in clinical trials to allow study of hypoproliferative CMML cases in common protocols with other cases of MDS, pending clarification of this issue.

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### Radiation Therapy or Surgery for Base-of-Tongue Tumors?

*To the Editor:* I read with interest the article by Mendenhall et al<sup>1</sup> in the January 2000 issue of the *Journal of Clinical Oncology*. In this retrospective nonrandomized study, the authors analyzed their single-center experience of radiation therapy alone in a series of 217 patients with previously untreated squamous cell carcinoma of the base of tongue treated between 1964 and 1996. They concluded that local-regional control rates and survival rates after radiation therapy were comparable to those after surgery, and the morbidity associated with radiation therapy was less.

I do not agree with their conclusion concerning morbidity in patients with T1 and T2, N0 or N1 tumors. In their article, Mendenhall et al<sup>1</sup> did not mention any data concerning xerostomia, a major late complication observed in almost all patients with base-of-tongue cancer treated with external radiation therapy with or without concomitant chemotherapy. Xerostomia is not observed after surgical treatment when no postoperative radiation therapy is indicated. The major complication after surgical treatment is the risk of permanent gastrostomy, which is rarely observed in smaller tumors treated with the transpharyngeal approach.<sup>2</sup>

Xerostomia is not a fatal complication, but the quality of life with a fully preserved salivary function is obviously better than being without.

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*In Reply:* We appreciate Dr Ozsahin's comments and agree that xerostomia adversely affects quality of life. Essentially, all of our patients were treated with parallel-opposed fields for some or all of their irradiation and had at least some degree of xerostomia.<sup>1</sup> We disagree with Ozsahin's characterization of xerostomia as a major complication. The great majority of patients in our series speak normally and eat a regular diet.

There are several additional issues that should be addressed. The first is the proportion of patients who have tumors that are suitable for treatment with surgery alone. Eighteen (8%) of our unselected series of 217 patients had clinical stage T1N0 and T2N0 cancers.<sup>1</sup> A significant subset of these patients would have subclinical disease in the neck nodes and would likely require adjuvant irradiation. Eleven (85%) of 13 patients with T1 and T2 cancers operated on at the University of Pittsburgh received irradiation.<sup>2</sup> Twenty-four (77%) of 31 patients operated on at the University of California at Los Angeles had T1 and T2 tumors, and 24 of 31 patients received adjuvant irradiation.<sup>3</sup> At most centers, the subset of patients who would be suitable for treatment with surgery alone is relatively small. Patients who undergo adjuvant radiation therapy receive doses greater than 45 Gy (usually 60 to 65 Gy at 1.8 to 2.0 Gy per fraction) and have xerostomia that is as pronounced as one would anticipate after radiation therapy alone.

A second issue is whether the likelihood of major complications is lower after surgery for patients with T1 and T2 cancers, compared with radiation therapy. Three (23%) of 13 patients reported by Weber et al<sup>2</sup> experienced complications that consisted of fistulae (two patients) and pneumonia (one patient). Both patients with fistulae healed with conservation therapy. No patient was feeding-tube dependent. In contrast, nine (38%) of 24 patients with T1 and T2 cancers reported by Nasri et al<sup>3</sup> experienced complications that included swallowing disorders necessitating a permanent gastrostomy (three patients) or nasogastric tube feeding (one patient), fistulae (four patients), infected miniplate (one patient), and neck web (one patient). The incidence of severe late complications in our unselected series of 217 patients was 4%.<sup>1</sup> Thus the likelihood of major complications after surgery is higher than that observed after radiation therapy alone. Additionally, the vast majority of patients who undergo surgery also receive adjuvant irradiation and thus experience xerostomia in addition to their other problems.

A third question is whether quality of life after surgery is better than that observed after radiation therapy. Data pertaining to quality of life after treatment for base-of-tongue cancer are limited. Harrison et al<sup>4</sup> reported 30 patients who underwent radiation therapy and compared them with 10 patients treated surgically. They concluded that quality of

life was better after radiation therapy for patients with early-stage disease as well as for those with advanced lesions.

Because our goal is to maximize tumor cure and quality of life, it has been, and remains, our practice to treat essentially all patients with squamous cell carcinoma of the base of tongue with primary radiation therapy. New technologies, such as intensity-modulated radiation therapy, allow treatment of the primary lesion and both sides of the neck while limiting the dose to the parotid glands to reduce the severity of xerostomia. However, these techniques should be applied cautiously, because reduction of the treatment volume may result in an increased likelihood of patients with marginal recurrences, for whom salvage treatment will most likely be unsuccessful.

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## Thalidomide in the Treatment of High-Grade Gliomas

*To the Editor:* A phase II trial of thalidomide in the treatment of recurrent high-grade gliomas was published in the second February issue of the *Journal of Clinical Oncology*.<sup>1</sup> Important outcomes included objective partial responses in two of 36 assessable patients, a median survival of 28 weeks, and a greater than 1-year survival in eight of 36 patients. On the basis of this experience, the authors concluded that thalidomide had sufficient activity to recommend future study, with radiation therapy and other chemotherapy, in newly diagnosed, high-grade glioma patients. Are these conclusions justified?

High-grade gliomas include glioblastoma multiforme (GBM) and anaplastic astrocytoma (AA). These two histologic diagnoses are associated with very different prognoses. Curran et al<sup>2</sup> analyzed the survival outcome of 1,578 patients entered onto three Radiation Therapy Oncology Group malignant glioma trials. For specific prognostic groups of GBM patients, median survival ranged from 4.3 months to 17.6 months and 2-year survival ranged from 4% to 35%. For comparable AA patients, median survival ranged from 11.2 months to 58.6 months and 2-year survival ranged from 15% to 76%. In the current thalidomide report, neither responders nor survivors are identified by histology.

In addition to histology, other prognostic factors for response and survival include performance status, age (patients  $\leq$  50 years old have

a better prognosis than those  $>$  50), symptom duration, extent of surgical resection, and response to primary therapy.<sup>3,4</sup> Given these variables, the thalidomide study population was relatively favorable, with a median age of 49, a performance status of 80 to 100 in 77% of patients, and no prior chemotherapy in 49%.

Recently, a phase II trial was submitted to the United States Food and Drug Administration in support of an indication for the use of temozolomide (Temodar; Schering-Plough Corp, Kenilworth, NJ) for the treatment of relapsed, chemotherapy-refractory (carmustine [BCNU] and procarbazine) AA patients. Among 54 such patients, the objective response rate was 22%, with five patients (9%) having a complete response. The median duration of complete response was 348 days (range, 112 to 797 days). These results seem superior to those of the thalidomide study, in which no complete responses were noted among the 14 patients with AA or anaplastic mixed gliomas and in which response durations were, almost certainly, less than 1 year.

The modest thalidomide efficacy results in recurrent disease seem insufficient to warrant a recommendation that the drug be incorporated into first-line therapy. Perhaps additional studies might be done in the recurrent disease population to determine whether thalidomide is additive or synergistic when used with other Food and Drug Administration-approved glioma chemotherapies, such as Gliadel, a carmustine (BCNU)-impregnated biodegradable polyanhydride (poly[bis(p-carboxyphenoxy)] propane-sebacic acid) copolymer that, in wafer form, is applied directly to residual tumor after surgical resection.<sup>5</sup>

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## Paclitaxel for Limited-Stage Small-Cell Lung Cancer

*To the Editor:* Levitan et al<sup>1</sup> report an overall response rate of 96% with 39% complete responses for patients with limited-stage small-cell lung cancer using paclitaxel, cisplatin, and etoposide with concurrent radiation therapy. In their discussion, they did not mention a 1997 publication in the *Journal of Clinical Oncology*<sup>2</sup> that has relevance to their data. They did discuss a 1995 publication by Hainsworth et al<sup>3</sup> and pointed out that there were only 15 patients with limited disease in that trial. However, the 1997 trial<sup>2</sup> contained data on 41 patients with

limited-stage disease who received the three-drug combination of paclitaxel, carboplatin, and oral etoposide plus concurrent radiation. Twenty-nine (71%) of these patients obtained a complete response to therapy. Combining the patients from the first study<sup>3</sup> and the second study<sup>2</sup> shows that a total of 56 limited-stage patients had a median survival time of 26 months (95% confidence interval, 17 to 39.2 months) and an estimated 2-year survival rate of 45% (95% confidence interval, 24% to 68%). Growth factors were not administered in these studies,<sup>2,3</sup> and grade III and IV hematologic toxicities were similar to those seen by Levitan et al,<sup>1</sup> who did use growth factors. We agree that the addition of paclitaxel to a platinum agent and etoposide seems to offer promise in patients with limited-stage small-cell lung cancer and that prospective randomized comparisons are indicated.

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*In Reply:* The letter from Drs Greco and Hainsworth suggests that a comparison of the results of their trial of paclitaxel-based therapy<sup>1</sup> with ours<sup>2</sup> would be of interest. Hainsworth et al treated patients with both limited and extensive small-cell lung cancer. Their higher-dose regimen consisted of paclitaxel (200 mg/m<sup>2</sup>), carboplatin (area under the concentration-time curve, 6), and etoposide (50 mg alternating with 100 mg orally every day for 21 days). Only two patients received granulocyte colony-stimulating factor. Radiotherapy was administered concurrently with cycles 3 and 4 to the subset of patients with limited-stage disease. The radiation field included the tumor volume that was present after the completion of chemotherapy cycle 2. For patients with limited-stage disease, the overall and complete response rates were 98% and 71%, respectively. Grade 3 or 4 leukopenia occurred in 38% of treatment courses. Although the authors display the toxicity data for their regimen without stratifying between those patients who did or did not receive thoracic irradiation, they comment that "the concurrent administration of two courses of chemotherapy with radiation therapy produced no unexpected or severe toxicity." Grade 3 or 4 esophagitis occurred during concurrent chemoradiotherapy in 23% of patients with limited-stage disease.

Our trial involved only patients with limited-stage disease. The regimen used in the phase II component of our trial differed from that of Hainsworth et al in that cisplatin (60 mg/m<sup>2</sup>) was used instead of carboplatin; paclitaxel was administered at a dose of 135 mg/m<sup>2</sup> during cycles 1 and 2 (with concurrent radiotherapy) and at a dose of 170 mg/m<sup>2</sup> during cycles 3 and 4. Etoposide was administered at a dose of 60 mg/m<sup>2</sup> intravenously daily for 3 days during cycles 1 and 2 and at

a dose of 80 mg/m<sup>2</sup> intravenously daily for 3 days during cycles 3 and 4. Granulocyte colony-stimulating factor was used after cycles 3 and 4. The overall and complete response rates were 96 and 39%, respectively. Grade 3 or 4 leukopenia occurred in 48% of treatment courses. Grade 3 or 4 esophagitis occurred in less than 10% of patients.

What can we conclude from a comparison of these data? A principal difference between the two regimens pertains to the timing of radiotherapy. Hainsworth et al<sup>1</sup> used radiotherapy during chemotherapy cycles 3 and 4, whereas our regimen incorporated radiotherapy during cycles 1 and 2. Patients treated by Hainsworth et al received radiation to a considerably smaller lung volume. There exist conflicting data pertaining to the importance of "early" versus "late" thoracic irradiation.<sup>3,4</sup> The contrasting radiotherapy schedules may explain why Hainsworth et al were able to administer higher doses of chemotherapy without the use of growth factors. A higher incidence of esophagitis was observed in association with the Hainsworth regimen compared with ours. It is likely that patients who have already received two cycles of chemotherapy are more prone to the development of esophagitis when radiotherapy accompanies cycles 3 and 4. Evaluation of the paclitaxel/platinum/etoposide regimen for patients with limited-stage small-cell lung cancer in the phase III setting would more reliably determine the efficacy and toxicity of this regimen in comparison to conventional therapy.

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## Prophylactic Surgery: Oophorectomy or Adnexectomy?

*To the Editor:* In a recent article, Eisen et al<sup>1</sup> presented a comprehensive updated review, together with management recommendations, for prophylactic surgery in women from breast/ovarian cancer families. Although we agree with most of the proposed attitudes, we would like to emphasize the need for reflection regarding the extent of the surgical procedure of oophorectomy. Indeed, several reports have recently described the occurrence of fallopian tube carcinomas among *BRCA1/BRCA2* mutation carriers.<sup>2-5</sup> One of them clearly demonstrated a molecular link between the two tumors studied and *BRCA1* germline

mutations.<sup>4</sup> In our series, among 44 *BRCA1* and 17 *BRCA2* families identified to date, in which 23 and four ovarian carcinomas, respectively, have been diagnosed in carriers of deleterious mutations, one fallopian tube carcinoma occurred in a 33-year-old woman carrying a germline *BRCA1* mutation (3660del16). Fallopian tubes, like the ovaries and peritoneum, are derived from the coelomic epithelium. Although the residual risk of primary peritoneal carcinoma in prophylactically oophorectomized patients has been studied extensively,<sup>6</sup> little is known regarding the risk of fallopian tube cancer in the same context. Interestingly, a recent case report described a fallopian tube carcinoma discovered fortuitously during the pathologic examination of adnexa that had been removed prophylactically in a *BRCA1* carrier.<sup>7</sup> Since laparoscopic adnexectomy has been demonstrated to be as safe and as simple as oophorectomy using the same technique,<sup>8,9</sup> we currently recommend the prophylactic removal of both ovaries and fallopian tubes, together with systematic peritoneal biopsies, in women with a hereditary risk of ovarian cancer.

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*In Reply:* Drs Delalogue, Morice, Chompret, and Lhommé suggest that *BRCA1* and *BRCA2* mutation carriers should have their fallopian tubes removed at the time of prophylactic oophorectomy in order to prevent the

development of fallopian tube carcinoma. For technical reasons, the fallopian tubes are routinely removed at the time of oophorectomy at our institutions. Although we agree that case reports have suggested an association between germline *BRCA1* and *BRCA2* mutations and fallopian tube carcinoma,<sup>1-5</sup> this has not been confirmed in large series.<sup>6,7</sup>

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#### Stage Shift and Complementary/Alternative Medicine

*To the Editor:* I read with interest the two articles<sup>1,2</sup> and accompanying editorial<sup>3</sup> in the first July 2000 issue of the *Journal of Clinical Oncology* describing the widespread use of complementary and alternative medicine (CAM) interventions by patients surveyed both in Texas and in Ontario, Canada. However, I am concerned by some of the definitions that are now being used. As the authors point out,<sup>1-3</sup> CAM is no longer a term reserved just for questionable therapies, such as laetrile, but may also include many other more acceptable forms of nontraditional therapy. Although use of CAM in the United States is certainly increasing,<sup>4</sup> one must wonder how much of the increase described in the current articles is due to an increase in volume of use and how much is the result of expanded definitions. Richardson et al

provide figures for overall use of CAM and also for use of CAM excluding psychotherapy and spiritual practices. However, even this limited definition keeps "exercise" (a subtype of movement and physical therapies) within the CAM camp. If pressed, would not virtually all oncologists admit to advocating maintenance of performance status through activity and exercise for virtually all of our patients? Do all oncologists then become CAM practitioners?

This is not just a matter of semantics, since the changing definition of CAM also results in a stage shift<sup>5</sup> that may itself be counterproductive. On the one hand, dedicated evidence-based physicians can now feel increased pride as standard oncology becomes even more scientific by limiting itself to remedies with a strong experimental and molecular basis. On the other hand, CAM also becomes more respectable and scientific as standard humanistic practices of medicine are defined as falling into this alternative category. By these definitions, for example, the line begins to blur between strategies that might be discussed in The Art of Oncology section of the *Journal of Clinical Oncology*<sup>6</sup> and techniques such as spiritual and psychologic support that might now be classified as falling within CAM.

Is it therefore reasonable to include compassionate nonmedicinal interventions, folk remedies that may have pharmacologic value, and cancer quackery all under the single heading of CAM? It is true that these distinctions are not always easy to make. However, perhaps it is now necessary to once again sharpen our definitions and to recognize these different subtypes of nontraditional care so that the truly helpful

are not tainted and the truly harmful are not obscured by overly broad characterization.

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## ERRATUM

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The June 2000 article by Salovaara et al, entitled "Population-Based Molecular Detection of Hereditary Nonpolyposis Colorectal Cancer" (*J Clin Oncol* 18:2193-2200, 2000), should have listed two authors for reprint requests. Reprint requests for this article can be addressed to either Albert de la Chapelle, MD, PhD, Division of Human Cancer Genetics, 646 Medical Research Facility, 420 W 12th Ave, Columbus, OH 43210, email delachapelle-1@medctr.osu.edu, or Lauri A. Aaltonen, MD, PhD, Department of Medical Genetics, University of Helsinki, PO Box 21 (Haartmaninkatu 3), FIN-00014 Helsinki, Finland, email lauri.aaltonen@helsinki.fi.