

## CORRESPONDENCE

### Phase I Trial of Adoptive Immunotherapy With Cytolytic T Lymphocytes Immunized Against a Tyrosinase Epitope

*To the Editor:* In the February 15, 2002, issue of the *Journal of Clinical Oncology*, Mitchell et al<sup>1</sup> reported the results of a phase I trial in which melanoma patients were infused with autologous T cells that had been stimulated in vitro against peptide YMNGTMSQVN. Although this is the amino acid sequence predicted from the tyrosinase DNA sequence for codons 369 to 377, it is well established that the peptide actually expressed by melanoma cells is YMDGTMSQVN, having undergone posttranslational deamination at the third amino acid (asparagine → aspartic acid).<sup>2</sup> We are aware of no evidence that the YMN peptide used in this article is actually expressed by melanoma cells.

Work by Skipper et al<sup>2</sup> showed that T cells against the YMN peptide do not cross-react with melanoma expressing the YMD peptide (although both bind to HLA-A2.1), suggesting that the third amino acid of this peptide is critical for interaction with the T-cell receptor. This may explain why the T cells expanded by the YMN peptide showed limited cytotoxicity against melanoma cells (Mitchell et al,<sup>1</sup> Fig 1). This is also sufficient to explain why the investigators were unable to detect circulating cytotoxic T lymphocytes against melanoma or demonstrable targeting of indium-111-labeled T cells in patients after reinfusion of the expanded T cells. We find the clinical responses described in Fig 3 difficult to discern, which may possibly be explained by the inability of the infused cells to bind the native peptide.

A typographical error appears to occur in the Introduction that could cause confusion and errors in clinical or laboratory experiments using this article as a reference. The first amino acid of the posttranslationally modified peptide is incorrectly identified as a threonine (T), rather than tyrosine (Y).

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*In Reply:* We are grateful for the opportunity to reply to the comments of Drs Wolchok and Chapman about the relevance and importance of the encoded epitope sequence and their interpretation of our published data.<sup>1</sup> Also, although it was not called into question, we would like to clarify our numbering of the tyrosinase epitope as 369 to 377 rather than as 368 to 376, as it is often presented.

The first important point is that we conducted preliminary experiments to determine whether immunization with the unmodified epitope led to cross-reactive cytotoxic T lymphocytes (CTLs) capable of lysing melanoma cells. As shown in Fig 1, which was typical of several different experiments, immunization in vitro with YMNGTMSQV led to CTLs cross-reactive against the other epitope but not vice versa. This is what prompted us to test YMNGTMSQV in the study, with full knowledge of the article by Skipper et al,<sup>2</sup> especially because in their report only a single CTL clone was evaluated, whereas our cell product was a polyclonal "bulk" culture.

Lysis of melanoma cells in our hands, and probably in most others', is always less than that of heavily peptide-loaded T2 cells. However, that lysis is specific, as shown by far lower lysis of control HLA-matched target cells lacking the epitope (MALME 3) (Figs 2 and 3).

Perhaps most relevant to this discussion, Visseren et al<sup>3</sup> obtained tyrosinase-specific CTL bulk cultures from an HLA-A2 donor against YMNGTMSQV-sensitized target cells. The bulk cultures had strong CTL activity against JY cells loaded with YMNGTMSQV peptide and melanoma cells SK-23 and 453 at low effector-to-target ratios without the addition of exogenous tyrosinase peptide. Ten CTL clones were isolated from the bulk culture that recognized YMNGTMSQV peptide,

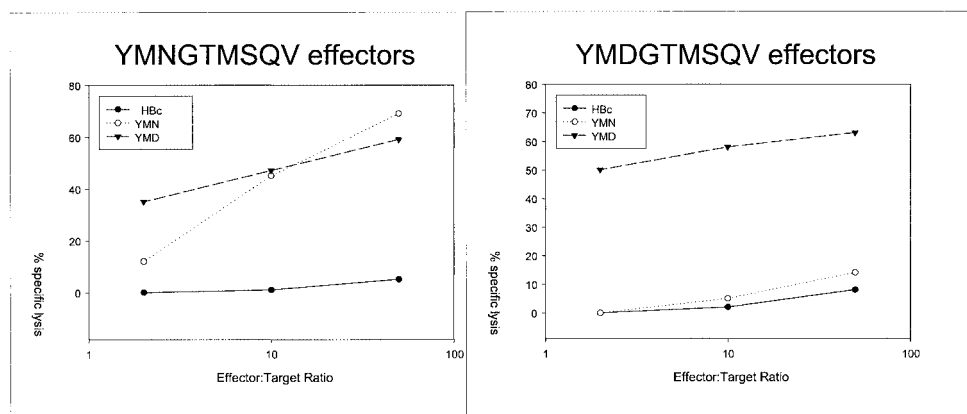


Fig 1. YMN effectors recognize both Tyr peptides.

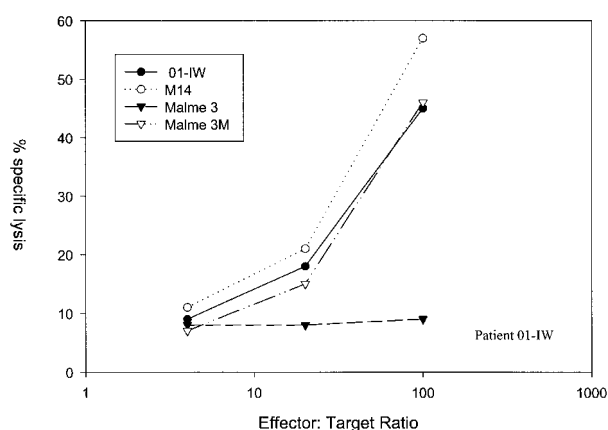
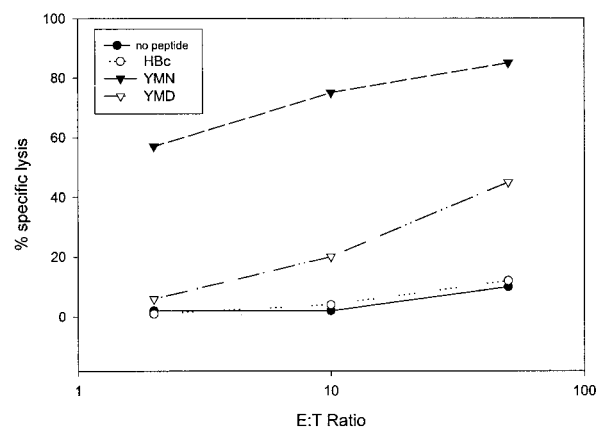


Fig 2. Lysis of autologous melanoma cells with effectors generated to tyrosinase peptide 369-377 (YMNGTMSQV).

and eight of the 10 clones cross-reacted with melanoma cell lines SK-23, 453, and Mi 3046/2, in a tumor necrosis factor release assay. Lytic activity of three clones was tested further on the melanoma tumor panel and shown to be identical with that of the bulk culture. The melanoma cell panel was also lysed efficiently by CTL from five further bulk cultures generated from normal donors. The authors concluded that the YMNGTMSQV peptide was naturally processed and presented on melanoma cells, but the reactivity could equally well have been explained by cross-reactivity to YMDGTMSQV on the melanomas.

Reduction of the size of the computed tomography scans in the final publication, which was a decision made by the *Journal*, undoubtedly made it difficult for Wolchok and Chapman to fully appreciate the change in the lesions, which was also observed on physical examination. The mediastinal nodes disappeared after treatment as we noted in the text, and the inguinal and iliac nodes dramatically shrank and became more discrete masses. The scans were read officially by disinterested university radiologists, not by the investigators. We would be pleased to provide either the full-size photographs or a copy of the original computed tomography scans to be certain Wolchok and Chapman see the changes we described.



With regard to the numbering of the peptide, the original article by Brichard et al<sup>4</sup> describing the cloning of the tyrosinase cDNA (clone 123.B2) presents a cDNA whose amino acid residues 369 to 377 are given as YMNGTMSQV in the direct submission sequence provided to Genbank. The authors note that in the sequence reported by Kwon et al<sup>5</sup> in 1987, there is a three-base pair deletion beginning at base 124, which accounts for the numbering of the peptide beginning at 368 rather than 369 in some publications.

Finally, we thank Wolchok and Chapman for correcting the typographical error substituting T for Y in the sequence of the YMDGTMSQV peptide in the Introduction.

We believe that further refinement of our approach and the addition of other elements such as cytokines to the regimen may well yield more convincing results than our phase I study. Nevertheless, if the article stimulated in other readers the same degree of enthusiasm as we generated in Wolchok and Chapman, we may have achieved one of our aims.

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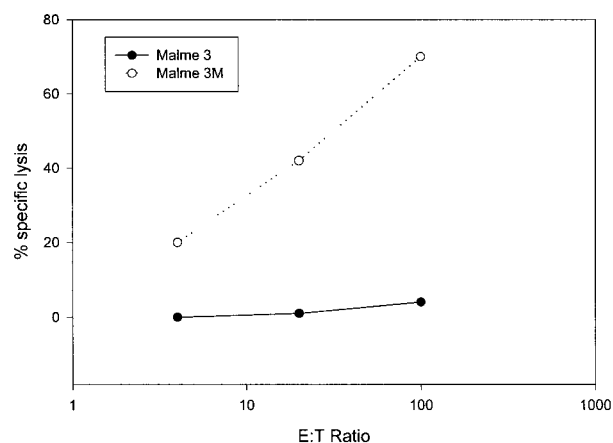


Fig 3. Antipeptide and antimelanoma response (patient no. 05-RE).

## Missense Mismatch Repair Gene Alterations, Microsatellite Instability, and Hereditary Nonpolyposis Colorectal Cancer

*To the Editor:* The conclusion of Scartozzi et al<sup>1</sup> that “germ-line mutations of the MMR [mismatch repair] gene can occur in people with MSI [microsatellite instability]-negative tumors” is valid only if the missense mutations they identified are pathogenic; as the authors acknowledge, these missense changes could represent rare polymorphisms. The determination of whether or not a missense mismatch repair gene alteration is disease-causing is one of the most challenging aspects of the genetics of hereditary nonpolyposis colorectal cancer (HNPCC); however, we would point out that two of the four supposedly pathogenic missense changes reported in this study (*hMLH1*: Lys618Ala and *hMSH2*: Asp167His) were reported by us in controls without colon cancer.<sup>2</sup> It is also of concern that none of the five MSI-high tumors reported by the authors were associated with loss of expression of mismatch repair proteins, whereas six tumors without MSI-high were associated with loss of expression. This total discordance between MSI-high and loss of protein expression is inconsistent with previous studies<sup>3,4</sup> and raises questions regarding the authors’ methodology. Furthermore, the fact that only one of 25 tumors exhibited instability with BAT-26, a microsatellite repeat marker with 100% sensitivity for HNPCC-associated tumors in a recent study,<sup>5</sup> suggests that few if any of these individuals had HNPCC, regardless of their age or family history. We feel that a more reasonable interpretation of the authors’ results is that germline missense mutations that do not significantly impair the mismatch repair mechanism are not associated with MSI and are not related to HNPCC.

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*In Reply:* We thank Drs Samowitz and Slattery for their comments to our article.<sup>1</sup> We are aware that assessing the role of missense mutations of mismatch repair (MMR) genes represents a challenging but critical aspect of the hereditary non-polyposis colorectal cancer (HNPCC) syndrome. Unfortunately, the criteria currently used to predict the pathogenicity of such genetic abnormalities are often inconclusive, and in vitro assays to test the functional consequences of

single amino acid substitutions are not widely available. The K618A *hMLH1* mutation, which was found by Samowitz et al<sup>2</sup> in a healthy control and by us in a patient with suspected HNPCC,<sup>1</sup> was shown by other authors to segregate with the HNPCC phenotype<sup>3-5</sup> and to impair the interaction of *hMLH1* with *hPMS2* in vitro.<sup>6</sup> In addition, codon 618 of *hMLH1* is the target of other pathogenic mutations, such as K618T, 618 deletion of lysine, which are often found in HNPCC.<sup>3</sup> Taken together, these data strongly suggest that the K618A mutation belongs to the group of genetic abnormalities implicated in the pathogenesis of the HNPCC syndrome. The *hMSH2* A167H mutation, which we found in a case of early-onset colon cancer,<sup>1</sup> may represent a rare polymorphism, because it was also found in one healthy control<sup>2</sup>; however, we feel that the data available are not sufficient to conclusively establish its role.

Drs Samowitz and Slattery argue that the germ line mutations we found may be unrelated to HNPCC because they are not associated with high microsatellite instability (MSI). The likelihood that “such a low level of MSI may suggest that these mutations were not implicated in the familial susceptibility to colon cancer” is clearly stated in our article.<sup>1</sup> Nevertheless, it should be re-emphasized that certain missense mutations of MMR genes do not correlate with MSI, although they segregate with the disease and inactivate the MMR machinery in vitro; these include the K618A,<sup>5,6</sup> the V326A,<sup>7,8</sup> and the E578G *hMLH1* mutations.<sup>5,6</sup> Discrepancies between the occurrence of MSI and immunohistochemical abnormalities of MMR gene expression have been previously reported, as well as germ line mutations of *hMLH1* or *hMSH2* not correlating with alterations of the BAT 26 microsatellite marker.<sup>7,9,10</sup>

Most population-based studies dealing with the HNPCC syndrome analyze large cohorts of colon cancer patients for MSI and search for MMR gene mutation in individuals with MSI positive tumors.<sup>2</sup> This study design ensures a high correlation between germ line mutations and occurrence of MSI and allows the identification of patients with mutations that are mostly chain-terminating and are associated with a strong phenotype. Therefore, it is likely that these studies will miss patients harboring germ line mutations associated with an attenuated phenotype and low or no MSI. The identification of individuals with suspected HNPCC should be primarily clinical, based on the personal and familial cancer history.<sup>11</sup> Recognizing the clinical features of HNPCC is critical to assess the risk of colon cancer, to counsel the at-risk individuals, and to recommend the appropriate surveillance program. In these patients, genetic testing represents a useful complement, which could contribute to establish the diagnosis. However, the clinician should not disregard the suspicion of HNPCC if the test results are negative or uncertain. In our article, we studied the genetics of a series of patients with the clinical features of HNPCC, to formulate a selection procedure for individuals who should be offered genetic testing.<sup>1</sup> The fact that some of the mutations we found do not have a certain biologic significance and that some of our observations do not meet the current consensus, may depend on the clinical criteria we used to carry out our study. As with our patients, many individuals with suspected HNPCC often display an attenuated phenotype and show mutations of MMR genes whose biologic consequences are uncertain.<sup>4,5</sup> More clinically oriented research is warranted to clarify the role of these genetic abnormalities and to understand their relationship with the HNPCC syndrome.

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### Treatment of Newly Diagnosed Glioblastoma Multiforme

*To the Editor:* Treatment of glioblastoma multiforme (GBM) has been among the most challenging fields in oncology for more than 20 years. Despite considerable effort and notable advances in the surgical, radiotherapeutic, and chemotherapeutic fields, the trend toward continuous improvement in outcome that could be observed for many other neoplasms seemed to be almost completely absent. Patients' prognoses are mainly determined by several tumor- and patient-related factors, whereas changes in treatment protocols so far have contributed little to avoid death from uncontrolled local disease.<sup>1</sup>

Since the 1970s, many cytotoxic drugs, most often nitrosoureas, have been added to surgery and radiotherapy for newly diagnosed high-grade glioma. However, none has unequivocally prolonged tumor control or patient survival. A meta-analysis of 16 randomized clinical trials from a 17-year period suggested a moderate increase of survival of 8.6% at 2 years by adding chemotherapy.<sup>2</sup> Median survival in-

Table 1. Phase II Studies of GBM

| Parameter   | Reardon et al <sup>4</sup><br>(N = 33) | Stupp et al <sup>5</sup><br>(N = 64) |
|---|--|--------------------------------------|
| Glioblastoma, %                                       | 82%                                    | 100%                                 |
| Age, years  |  |                                      |
| Median  | 50                                     | 52                                   |
| Range   | 19-68                                  | 24-70                                |
| Karnofsky performance status, %                       |  |                                      |
| 90-100  | 88                                     | 64                                   |
| ≤80   | 12                                     | 36                                   |
| Surgical resection v biopsy, %                        | 100 v none                             | 76 v 23                              |
| Median time from diagnosis to study treatment, months | 1.3                                    | 0.8                                  |
| Median follow-up of surviving patients, months        | 21                                     | 10                                   |
| Median survival, months                               | 18 for GBM                             | 16                                   |
| 95% Confidence interval, months                       | 14-∞                                   | 11-21                                |
| Prognostic factors                                    | Not analyzed                           | Age, resection, RPA class            |

Abbreviation: RPA, recursive partitioning analysis.

creased from 9.4 to 12 months. The Medical Research Council recently published the results of a randomized study in which 674 patients were treated with surgery plus radiotherapy or additional procarbazine, lomustine, and vincristine.<sup>3</sup> There was no statistically significant difference in median survival (9.5 v 10 months). Further statistical interpretation excluded a survival benefit of more than 7% to 8% at 2 years. Such results were not convincing enough to make chemotherapy a generally accepted part of standard first-line treatment on both sides of the Atlantic Ocean, especially for GBM and/or unselected groups of patients with high-grade glioma.

In the light of these data, two phase II studies published in the March 1, 2002, issue of the *Journal of Clinical Oncology* merit further comment. Their most important features are summarized in Table 1. The study by Reardon et al<sup>4</sup> included 33 patients with newly diagnosed glioma treated with surgical resection plus antineoplastic monoclonal antibodies. One month later, radiotherapy was given (6 weeks, dose not specified, 88% of all patients). Afterward, 91% of the patients received 1 year of systemic alkylator-based chemotherapy. The study by Stupp et al<sup>5</sup> included 64 patients with newly diagnosed GBM treated with radiotherapy (60 Gy) plus concomitant temozolomide (75 mg/m<sup>2</sup>/d × 7 d/wk) for 6 to 7 weeks after resection or biopsy. Afterward, temozolomide was continued for six cycles of 200 mg/m<sup>2</sup>/d for 5 days every 28 days. Neither of the articles describes further treatment in case of tumor progression.

In direct comparison, both strategies seem to improve median survival to a comparable extent, considering the fact that prognostic factors were more favorable in the study by Reardon et al.<sup>4</sup> Acute toxicity and side effects were acceptable. Detailed data on quality of life and long-term toxicity are not available yet. The ultimate impact on survival will be determined in phase III studies. Phase III data will soon become available for the temozolomide strategy. An interesting point that needs to be emphasized is that comparable results were obtained with different treatment strategies, ie, addition of systemic treatment to surgery plus radiation versus further intensification of local treatment by monoclonal antibodies delivering an average cumulative radiation dose (external beam plus labeled antibodies) to the 2-cm-thick interface

resection cavity/brain of 102 Gy. From these results, it might be interesting to repeat the antibody study with addition of temozolomide instead of conventional alkylator-based chemotherapy.

The study by Stupp et al<sup>5</sup> unfortunately confirmed the unfavorable prognosis of patients with unresectable tumors. Their median survival was only 5 months and therefore not appreciably longer than after radiotherapy alone. Obviously, systemic treatment still does not provide the answer for this group of patients, while the outlook might improve for those with residual small tumor masses after resection.

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## Promising Survival and Concomitant Radiation Plus Temozolomide Followed by Adjuvant Temozolomide

*To the Editor:* Stupp et al<sup>1</sup> have reported a phase II study of concomitant radiation therapy plus temozolomide therapy followed by adjuvant temozolomide therapy in the treatment of patients with newly diagnosed glioblastoma multiforme. At the time of study design, cranial radiation therapy followed by adjuvant chemotherapy as a nitrosourea was considered to be potentially the best standard of care for patients with malignant glioma.<sup>2,3</sup> This clinical study indicated that this type of concomitant regimen could prolong survival in patients with malignant glioma. The authors conclude that this "is a promising regimen . . . . This regimen is currently being compared with standard [radiation therapy] alone in an international randomized trial . . . ." We would like to downplay the authors' enthusiasm.

As is well known, the specific prognostic factors such as age, performance status, histologic grade, and extent of tumor resection predicted significantly the survival of patients with malignant glioma.<sup>4,5</sup> A younger age, a high performance status, an anaplastic astrocytoma, and a complete tumor resection are considered the best prognostic factors and authorize a prolonged survival for patients, whatever the type of oncologic treatment used.<sup>4,5</sup> Of particular interest in the study by Stupp et al<sup>1</sup> is that their population was younger (median age, 52 years) than we could expect for this type of study.

Indeed, the incidence peak of malignant gliomas is 65 years old.<sup>3</sup> The authors said, "Two thirds . . . of the patients were  $\geq$  50 years old, and 20% of the patients were between 40 and 50 years old." From the statistical analysis used and data given, we could maintain that the second quartile,  $\leq$  52 years old, is more important than other quartiles and explain why this population is too young compared with the others reported in the literature.<sup>3</sup> Moreover, this included population was unfortunately selected, which should minimize the promising efficacy of this study.

Regarding trial entry criteria, we also regret that there was a high percentage of included patients in whom there was the possibility that chemotherapy would be too beneficial: "The majority (77%) of patients had undergone prior debulking surgery, with 42% being considered macroscopically complete resections. . . . Fifteen patients (23%) had a stereotactic biopsy only." Two thirds (64%) of the patients had a high Karnofsky score,  $\geq$  90. Again, we could consider that this population was selected. Due to the presence of these favorable prognostic factors, ie, young age, high performance status, and complete tumor resection, the median survival (16 months) found in this study could be expected. So any clinical argument supporting the potential efficacy of this combination temozolomide plus radiation therapy was given by the authors. To support our position, the authors state, "In patients  $\geq$  50 years old, the median survival was only 11 months. . . . [F]or patients who did not undergo debulking surgery, survival time was 5 months." These results confirm the dismal prognosis of patients with malignant glioma and are unfortunately classic and expected for this disease.<sup>2,3</sup>

Finally, we congratulate the authors for demonstrating that a concurrent combination of radiation therapy and chemotherapy was feasible and safe. Toxicity was acceptable, and no late toxicity was seen. However, the rationale for combining temozolomide with radiation therapy is poor, and preclinical data did not clearly demonstrate additive or perhaps synergistic activity. Currently, the best radiosensitizers are cisplatin, etoposide, and paclitaxel.<sup>6</sup> In our clinical study testing a concomitant-to-sequential use of etoposide and radiotherapy for newly diagnosed malignant gliomas, the median survival for patients with glioblastoma multiforme was 13.9 months.<sup>6</sup> However, our population included a high percentage of patients with unfavorable prognostic factors, such as residual tumor (41.1% of the population had stereotactic biopsy and 25.4% had incomplete tumor resection, as shown by postsurgery computed tomography scans) and age (median age was 61 years).<sup>6</sup>

However, the results of this study, in our opinion, do not justify the development of a phase III study, and do not offer a beneficial effect for patients with malignant glioma.

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*Reply 1, to Nieder and Beauchesne:* Dr Nieder compares the results of two novel strategies in the treatment of malignant glioma, both published in the March 1, 2002, issue of the *Journal of Clinical Oncology*.<sup>1,2</sup> However, comparison of the results should be cautioned. Treatment with <sup>131</sup>I-labeled antitenascin antibodies<sup>1</sup> requires a surgical resection, whereas chemoradiotherapy with temozolomide<sup>2</sup> is applicable to the majority of patients. The median survival of the 49 patients in our study who had some type of surgical debulking was 17 months; more importantly, our patients had an encouraging 2-year survival rate of 42%.<sup>2</sup> It is unlikely that this improvement is due only to second-line treatment at recurrence. In our series, only one third of patients received second-line therapy, which was most frequently re-treatment with temozolomide with a continuous administration schedule. As stated by Nieder, for the patients who did not undergo surgical resection, the results with concomitant chemoradiotherapy were not appreciably improved over historical results. However, the heterogeneity of this disease and the small number of patients (n = 15) in this subgroup do not allow for any conclusive statement. Individual patients with an initially good performance status may still benefit from this treatment.

Dr Beauchesne warns of a possible selection bias in our study.<sup>2</sup> The age distribution of our patient population is comparable to that of a recent meta-analysis: 63% of our patients and 54% in the meta-analysis were 40 to 60 years old, and 23% of our patients were more than 60 years old, compared with 28% in the meta-analysis.<sup>3</sup> The Karnofsky performance status in our study was indeed higher than what is commonly reported and may have contributed to the favorable results. However, we compared our results with the Radiation Therapy Oncology Group prognostic classes and could demonstrate a prolonged median and 2-year survival (data shown in the article).<sup>4</sup> Beauchesne criticizes the rationale for using temozolomide and concomitant radiation therapy. The goal of combining temozolomide with radiation was to use an intrinsically active agent (spatial cooperation) that has a different toxicity profile (toxicity independence) and has shown in vitro additive or synergistic activity (radiosensitization).<sup>5-7</sup> Temozolomide is also thought to inhibit signaling of radiation-triggered migration and invasiveness<sup>8</sup> and to decrease tumor repopulation.

Because we are aware of the limitations of any phase II study, we proceeded to confirm (or infirm) our results in a large, prospective, randomized trial. This European Organization for Research and Treatment of Cancer/National Cancer Institute of Canada trial has now completed accrual of more than 550 patients and will provide an answer regarding the true benefit of this approach.

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*Reply 2:* We appreciate the comments of Dr Nieder on our article presenting a phase II study of patients with newly diagnosed malignant glioma treated with <sup>131</sup>I-radiolabeled antitenascin monoclonal antibody 81C6.<sup>1</sup> As summarized by Nieder, the outcome for most patients with malignant glioma remains dismal despite aggressive multimodality therapeutic strategies incorporating systemic chemotherapy and external-beam radiotherapy. Innovative approaches, including the application of therapeutic agents directed against tumor-associated targets, offer a promising means of more effectively treating these challenging tumors. Furthermore, the administration of such agents directly into the tumor bed maximizes delivery to regions at greatest risk for recurrence<sup>2</sup> while minimizing systemic exposure and toxicity. The results of our current phase II trial confirm that the administration of <sup>131</sup>I-radiolabeled 81C6 into the surgically created resection cavity clearly provides a significant survival advantage to patients with these tumors.

As pointed out by Nieder, patients treated in our study represent a favorable prognostic subgroup of patients with glioblastoma multiforme (GBM) in that all had undergone a gross total resection and had a Karnofsky performance status of 70% or greater. According to a recursive partitioning analysis of prognostic factors among 1,578 patients with newly diagnosed malignant glioma treated on three consecutive Radiation Therapy Oncology Group trials, patients with newly diagnosed GBM over age 50, who also have a Karnofsky performance status greater than 70% and have undergone either a gross total or partial resection, have a median survival of 37 to 46 weeks.<sup>3</sup> In comparison, patients in our study with similar prognostic factors had a median survival of 65 weeks. The survival advantage conferred by the administration of <sup>131</sup>I-radiolabeled 81C6 was equally significant in patients younger than 50 with newly diagnosed GBM. In the analysis by Curran et al,<sup>3</sup> such patients had a median survival of 44 to 72 weeks

after the administration of external-beam radiotherapy with or without chemotherapy. In our study, such patients had a median survival of 87 weeks.

A further point for emphasis not raised by Nieder is that the survival advantage conferred by  $^{131}\text{I}$ -radiolabeled 81C6 was not obtained at the expense of additional morbidity. Only one patient (3%) treated on our study required debulking surgery for radiation necrosis, which is a much lower rate than that associated with either stereotactic radiosurgery or  $^{125}\text{I}$  brachytherapy.<sup>4</sup>

For all of these reasons, we wholeheartedly concur with Nieder that a phase III study incorporating  $^{131}\text{I}$ -radiolabeled antitenascin monoclonal antibody 81C6 is warranted.

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## More Concern About Transfusion Requirement When Evaluating Quality of Life in Anemic Patients

*To the Editor:* The report from Gabrilove et al,<sup>1</sup> as published in the June 1, 2001, issue of the *Journal of Clinical Oncology* last year, did contribute important results from a large multicenter trial on the effects of once-weekly dosing of 40,000 units of epoetin alfa by subcutaneous injection in patients with nonmyeloid malignancies. The authors also described improvements in hemoglobin (Hb) level, transfusion requirements and quality of life (QOL), though the results concerning QOL assessment need further clarification.

First of all, did the authors test the reliability (internal validity) of the questionnaires in their own study population? Second, there was a positive correlation between changes in Hb levels and changes in QOL according to the linear analog scale assessment and the Functional Assessment of Cancer Therapy-Anemia (FACT-An) anemia subscale scores.<sup>1</sup> Did the changes in transfusion requirements, mainly dictated by Hb levels, have an impact on QOL? If so, should the authors have controlled for the influence of transfusion requirements on the changes in QOL at increasing Hb levels?

Our concerns arise from the implicit nature of health-related QOL<sup>2</sup> and our own experience in 17 adult patients with primary myelodysplastic syndrome (MDS). In a QOL study in MDS patients endorsed by the Associazione Italiana Leucemie section in Reggio Calabria, Italy,

**Table 1. Internal Validity of QOL-E Subscales on Test-Retest Administration in 39 Patients With MDS**

| QOL-E Subscale (type, no. of items) | Cronbach's Standardized Coefficient Alpha |        |
|-------------------------------------|---|--------|
|                                     | Test                                      | Retest |
| QOL-E-FIS (physical, 6 items)       | 0.83                                      | 0.75   |
| QOL-E-FUN (functional, 4 items)     | 0.77                                      | 0.73   |
| QOL-E-SOC (social, 4 items)         | 0.77                                      | 0.67   |
| QOL-E-SEX (sexual, 3 items)         | 0.88                                      | 0.92   |
| QOL-E-FAT (fatigue, 8 items)        | 0.73                                      | 0.75   |
| QOL-E-MDSS (MDS-specific, 11 items) | 0.78                                      | 0.75   |

the translated and validated Italian version of FACT-An questionnaire<sup>3</sup> was applied to all patients with the intention to evaluate QOL, according to the severity of anemia. Only physical, functional, and fatigue components of the questionnaire showed acceptable levels of internal consistency (Cronbach's standardized reliability coefficient alpha of 0.74, 0.90, and 0.83, respectively). Most importantly, the seven-item nonfatigue component of the anemia subscale was not reliable in our patients (alpha = 0.57).

The lack of a specific questionnaire for the evaluation of health-related QOL in patients with MDS<sup>2</sup> motivated the development and introduction of a new tool, QOL-E, in which four general dimensions (physical, functional, social, and sexual), one nearly specific dimension (fatigue) and one disease-related dimension (ie, MDS-related symptoms) were explored. QOL-E was assessed in a pilot epidemiologic cross-sectional survey in 52 consecutive MDS patients followed regularly in our hematology department in Reggio Calabria, Italy.<sup>4</sup> The mean Hb level was 10.0 g/dL (SD, 2.4 g/dL), and Hb levels ranged from 6.8 to 16.4 g/dL. Fourteen patients (27%) were receiving supportive care with one to four transfusions per month.

The questionnaire was administered initially in all 52 patients and retested in 39 of them after at least 2 months. All QOL-E dimensions demonstrated good internal validity on test-retest administration in our patients with MDS (Table 1).

Single Hb levels, measured on the day of survey administration at baseline, were significantly associated with different QOL dimensions (eg, physical, social, fatigue, MDS-specific, treatment outcome index, general, and total scores). At the second administration (retest) in 39 patients, the associations with all but the fatigue scores were confirmed. In addition to the baseline findings, a correlation with functional well-being also emerged. Noteworthy, at baseline, the transfusion requirement (mean value of the preceding 2 months, expressed as a categorical attribute with three levels) also correlated inversely with physical ( $r = -.33$ ,  $P = .02$ ), social ( $r = -.34$ ,  $P = .01$ ), fatigue ( $r = -.36$ ,  $P = .009$ ), MDS-specific ( $r = -.51$ ,  $P < .0001$ ), treatment outcome index ( $r = -.48$ ,  $P < .0001$ ), general ( $r = -.36$ ,  $P = .009$ ), and total ( $r = -.44$ ,  $P = .001$ ) QOL-E components. At retest, the associations of transfusion requirement with physical, MDS-specific, and treatment outcome index scores persisted.

In a multifactorial stepwise regression model at baseline, when controlling for covariate effects, the single Hb level appeared as an independent predictor of physical well-being ( $r = .40$ ,  $P = .005$ ), fatigue ( $r = .29$ ,  $P = .050$ ), and general well-being ( $r = .38$ ,  $P = .006$ ), while the associations with social, MDS-specific and treatment outcome index scores were not significant. Instead, the transfusion requirement maintained independent effects on social ( $r = -.36$ ,  $P = .01$ ), MDS-specific ( $r = -.48$ ,  $P = .001$ ), treatment outcome index

( $r = -.45, P = .001$ ), and total ( $r = -.41, P = .003$ ) scores. At retest, Hb levels were not associated with QOL-E measures, whereas the transfusion requirement was an independent marker of treatment outcome index and physical and MDS-specific well-beings.

It is of note that even small, pilot, and inexpensive trials<sup>4,5</sup> could provide reliable results on QOL which could be later extended and generalized across time and various settings. However, to properly evaluate the clinical effectiveness of therapeutic agents in MDS patients, apart from Hb level improvements as shown, concomitant changes in the transfusion requirement must be taken into prime consideration. This is justified also by the assumption that supportive care acts on the self-perception of well-being in a multidimensional context (eg, understanding of the severity of anemia, frequent day hospital admissions and laboratory tests, worries about availability of compatible RBC units, fear about adverse events, and so on), and in such patients, it may eventually introduce confounding to each of the QOL component measures. We suppose this could hold true in the case of nonmyeloid malignancies as well.

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*In Reply:* Drs Oliva et al have raised two relevant issues that pertain to our community-based study of epoetin alfa in anemic cancer

**Table 1. Internal Consistency of FACT-An Analysis**

|                                | FACT-An<br>(20 items) | Fatigue<br>(13 items) | Nonfatigue<br>(7 items) |
|--------------------------------|-----------------------|-----------------------|-------------------------|
| Cronbach's alpha at baseline   | 0.920                 | 0.929                 | 0.627                   |
| Cronbach's alpha at month 2    | 0.933                 | 0.940                 | 0.661                   |
| Cronbach's alpha at completion | 0.940                 | 0.947                 | 0.685                   |

patients, providing further insight into the interpretation of the published results.<sup>1</sup> First, they point out that establishing the internal validity of quality-of-life (QOL) questionnaires is important in determining the reliability of the QOL analyses. Indeed, while such consistency results were not reported in the original article because of space constraints, we had performed a Cronbach's alpha calculation for the 20-item anemia subscale of the Functional Assessment of Cancer Therapy-Anemia (FACT-An) scale. The FACT-An questions were grouped into two domains: 13 fatigue-related items and seven items not pertaining to fatigue. Overall and fatigue-specific Cronbach's alpha values were very high ( $> 0.9$ ), as shown in Table 1. This finding suggests that the FACT-An was reliable and provided stable and consistent responses across individual items within the questionnaire. Furthermore, the correlation between the linear analog scale assessment (LASA) QOL instrument and the FACT-An subscale was high ( $r = .66, P < .05$ ).

Oliva et al also suggested that the reported relationship between hemoglobin (Hb) and QOL may have stemmed from a change in transfusion use. In other words, it is possible that patients with higher Hb values reported increased QOL simply because they received fewer, if any, transfusions. To examine the merit of this assertion, we conducted two analyses, reported in Tables 2 and 3. In the first multivariate analysis, we compared the relationship between Hb and overall QOL scores from the LASA for clinic visits during which no transfusion was received with visits during which at least one unit of blood was transfused. Patients' overall QOL scores during each visit were regressed with age, sex, and Hb as covariates. If transfusion was the mechanism through which Hb affected QOL, we should have observed (1) a significant and large coefficient on the Hb variable among patients who received transfusions and (2) a small and insignificant coefficient among patients who did not receive transfusions. However, the coefficients on the Hb variable were similar in size and not statistically significantly different from each other. Similar results were observed for the energy and activity QOL parameters (data not shown).

A second analysis based on multivariate analysis of all patients is reported in Table 3 and confirmed the first results. In this analysis, both

**Table 2. Comparative Panel Regression Analysis**

| Factor    | Patient Visits With No Transfusion |      |         | Patient Visits With Transfusion |      |         |
|-----------|------------------------------------|------|---------|---------------------------------|------|---------|
|           | Estimate                           | SD   | P       | Estimate                        | SD   | P       |
| Intercept | 31.46                              | 3.24 | < .0001 | 24.50                           | 5.27 | < .0001 |
| Time      | 0.43                               | 0.06 | < .0001 | 0.54                            | 0.10 | < .0001 |
| Age       | -0.11                              | 0.03 | .0002   | -0.11                           | 0.06 | .0884   |
| Female    | 0.84                               | 0.83 | .3146   | 0.35                            | 1.56 | .8227   |
| Hb        | 2.40                               | 0.25 | < .0001 | 2.56                            | 0.38 | < .0001 |

NOTE. A *t* test showed that the regression coefficients associated with Hb were not statistically significantly different between the two models ( $P = .7188$ ).

**Table 3. Multivariate Linear Regression Model on the Relationship Between Transfusion, Hb, and Overall QOL**

| Factor         | Estimate | SD   | P       |
|----------------|----------|------|---------|
| Intercept      | 32.19    | 3.04 | < .0001 |
| Time           | 0.47     | 0.05 | < .0001 |
| Age            | -0.11    | 0.03 | < .0001 |
| Female         | 0.68     | 0.75 | .36     |
| Hb             | 2.31     | 0.24 | < .0001 |
| Transfusion    | -8.49    | 4.00 | .03     |
| Transfusion*Hb | 0.46     | 0.38 | .22     |

transfusions and the interaction of transfusions with Hb were included as additional covariates. If QOL is more sensitive to changes in Hb among patients with low Hb who receive transfusions, then the interaction term should be highly significant (thereby indicating that the relationship between QOL and Hb is greatest among patients with low Hb who received transfusions). However, the interaction variable between Hb and transfusion use is small and insignificant, thereby confirming that the effect of Hb on QOL was the same regardless of whether patients received transfusions.

Taken together, these results indicate that the direct relationship between Hb and QOL described in our article was present regardless of RBC transfusion.

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## ERRATUM

In the February 15, 2002 issue of the *Journal of Clinical Oncology*, the article by Mitchell et al, entitled "Phase I Trial of Adoptive Immunotherapy With Cytolytic T Lymphocytes Immunized Against a Tyrosinase Epitope" (*J Clin Oncol* 20:1075-1086), contained an error.

A typographical error appears in the amino acid sequence in the introduction. The first amino acid of the posttranslationally modified peptide is incorrectly identified as a threonine (T), rather than tyrosine (Y). The correct sequence is YMDGTMSQV.