Evidence for Microtubule Target Engagement in Tumors of Patients Receiving Ixabepilone

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Abstract

Purpose: Microtubule-stabilizing agents, such as taxanes, have been shown to be effective anticancer drugs. α-Tubulin, a basic unit of microtubules, can undergo several posttranslational modifications after assembly into stabilized microtubules, including acetylation and detyrosination. These modifications have been observed in cell cultures after exposure to microtubule stabilizers. Our objective was to develop a straightforward and dependable assay to show tubulin target engagement in tumor tissue after treatment of patients with ixabepilone (BMS-247550; Ixempra).

Experimental Design: Levels of posttranslationally modified α-tubulin were assessed in lysates of cultured malignant cell lines, as well as in both tumor tissue and peripheral blood mononuclear cells derived from patients before and after treatment with ixabepilone. Modification-specific antibodies permitted quantitative Western blot analysis.

Results: In cultured cell lines, the levels of detyrosinated (glu-terminated) and acetylated α-tubulin increased after microtubule stabilization induced by ixabepilone. Ixabepilone treatment also induced a 2-fold to 25-fold increase in detyrosinated α-tubulin levels in 11 of 13 serial biopsies and a 2-fold to 100-fold increase in acetylated α-tubulin in 11 of 12 serial biopsies obtained from patients receiving ixabepilone. Overall, little or no difference in tubulin modifications were observed between the before and after ixabepilone treatment in lysates from their peripheral blood mononuclear cells at the time point examined.

Conclusion: Assessing the levels of detyrosinated and/or acetylated α-tubulin seems to provide a simple and reliable assay to show target engagement by the microtubule-stabilizing agent ixabepilone. Such analyses may provide further understanding of therapeutic success or failure of microtubule-stabilizing agents in cancer therapy.

Vincristine, introduced in the 1950s, was the first drug to target microtubules in clinical oncology. Since then, the successful introduction of similar agents has established microtubules as one of the most effective targets in cancer chemotherapy (1, 2). Whereas most agents initially used in clinical oncology resulted in depolymerization of microtubules, the success of the taxanes, evidenced by their widespread use, has led to the search for additional agents with similar mechanisms of action. Several new classes of microtubule-stabilizing agents have been identified and are at various stages of clinical development (7–14). These include the epothilones, discodermolide, and others. These microtubule-stabilizing agents are more potent than paclitaxel, are effective in paclitaxel-resistant tumor models (7–9, 15), and are poor substrates of P-glycoprotein, a mediator of drug resistance encoded by the MDR-1 gene. It is foreseeable that microtubule-stabilizing agents will continue to be important cancer chemotherapeutic agents, and new agents in this class will continue to be introduced.

Microtubules are dynamic polymers composed of α-tubulin and β-tubulin heterodimers. Research has shown that α-tubulin undergoes several different posttranslational modifications after assembly into stable microtubules (16–23). These modifications include (a) detyrosination or removal of the C-terminal tyrosine residue exposing glutamic acid as the new C-terminal residue (glu-terminated α-tubulin; ref. 18), (b) acetylation at Lys40 (19), (c) phosphorylation (20), (d) palmitoylation (21), (e) polyglutamylation (22), and (f) polyglycylation (23). Although the physiologic role of these modifications remains largely unclear, detyrosination and acetylation occurs on α-tubulin in microtubules, rather than on α-tubulin monomers or αβ-tubulin heterodimers, and are
thought to be indicators of stable microtubules (16, 17, 24, 25). These modifications are not the cause of microtubule stabilization, but rather, they occur on tubulin after microtubule assembly (26–29).

Whereas microtubule-stabilizing agents for the treatment of cancer have been developed without clinical evidence of microtubule stabilization, a simple, sensitive, and reliable assay to monitor their pharmacodynamic effect would be of value in the future development of these agents. Such an assay could help answer a critically important question that arises during their clinical development: does the drug reach the tumor and exert its microtubule-stabilizing effects? In turn, this would provide us with greater insight into why these agents that are otherwise very active in cell culture fail to benefit a large proportion of patients.

Ixabepilone (BMS-247550; Ixempra) an epothilone B analogue and microtubule-stabilizing agent, is currently undergoing clinical evaluation as a cancer chemotherapeutic agent. As a part of an ongoing phase II clinical trial to evaluate the activity of ixabepilone in metastatic renal cell cancer, when possible, we have obtained tumor biopsies before the start of therapy and after the fifth dose of ixabepilone. We sought to determine the presence of posttranslationally modified α-tubulin as a marker of a pharmacodynamic effect of ixabepilone and reassurance that its molecular target was engaged. We conclude that glutermated and/or acetylated α-tubulin levels are simple and reliable markers for the pharmacodynamic effects of ixabepilone. As microtubule stabilization continues to be an important mechanism of action in new cancer drug development, we believe that assessing posttranslationally modified tubulin levels may provide a simple and reliable assay of the pharmacodynamic effects of other microtubule-stabilizing agents.

**Materials and Methods**

**Materials.** BMS-247550 was obtained from Bristol-Meyers Squibb Company. Mouse monoclonal anti–α-tubulin (clone DM1A), mouse monoclonal antiacetylated α-tubulin (clone 6-11B-1), and rabbit antianticlin antibody were purchased from Sigma. Mouse monoclonal anti–glyceraldehyde-3-phosphate dehydrogenase, affinity purified rabbit anti–glyceraldehyde-3-phosphate dehydrogenase, affinity purified rabbit antifractin, and rabbit antifractin polyclonal antibodies were obtained from Chemicon/Millipore.

**Cell lines and culture.** The renal cell cancer cell line #181 was established by L. Marston Linehan (Urologic Oncology Branch, National Cancer Institute) and is maintained in IMEM medium (Biosoftware International) supplemented with 10% fetal bovine serum, 2 mM/L glutamine, 50 units/mL penicillin, and 50 μg/mL streptomycin. A2780 was obtained from the American Type Culture Collection. A2780(1A9), a single-cell clone derived from the A2780 ovarian cancer cell line, is maintained in RPMI 1640 (Biosource Collection). A2780(1A9), a single-cell clone derived from the A2780 ovarian cancer cell line, is maintained in RPMI 1640 (Biosource International) supplemented as described above. The renal carcinoma cell line 786-0 was obtained from Robbie Burk (Albert Einstein College of Medicine).

**Microtubule stabilization assay.** Exponentially growing cells were exposed to different concentrations of ixabepilone for 4 h and then harvested in hypotonic lysis buffer [1 mmol/L MgCl$_2$, 2 mmol/L EGTA, 30% glycerol, 5% DMGSO$_4$, 5 mmol/L TTP, 1% Nonidet P-40, 0.1 mmol/L Pipes (pH 6.9), and protease inhibitors] and in the centrifugation conditions (180,000 × g in a TLA55 rotor at 37°C for 1 h; the 37°C temperature avoids the depolymerization of microtubules). The bands detected on Western blots using antibody specific for total α-tubulin were quantified by densitometry using IPLabgel software. The extent of microtubule stabilization was determined as the percentage of polymerized tubulin using this formula: % polymerized tubulin = pellet tubulin / (soluble tubulin + pellet tubulin) × 100. An advantage of this assay is that the amount of total protein loaded for each sample is irrelevant because the polymerized and soluble fractions are equalized for each pair, and it is the proportion of the polymerized to the soluble tubulin fraction that is measured.

**Determination of the levels of modified tubulin.** Exponentially dividing cells were harvested after 4 h of exposure to different concentrations of ixabepilone in a 3:1 mixture of room temperature hypotonic lysis buffer and 4× SDS-PAGE sample buffer. Equal aliquots (~50 μg) of each sample were loaded on gels, and Western blots were probed with antibodies specific for total α-tubulin, glu-terminated, or acetylated α-tubulin. For patient samples, tumor biopsies were obtained before treatment and after the fifth dose of ixabepilone (protocol guidelines called for administering ixabepilone on five consecutive days at a dose of 6 mg/m$^2$/day). All of the patients were enrolled in an ongoing Institutional Review Board–approved phase II clinical trial at the Clinical Center of NIH. All biopsies were acquired after informed consent had been obtained from the patients. Fresh patient core biopsies were Dounce homogenized at 22°C in hypotonic lysis buffer [1 mmol/L MgCl$_2$, 2 mmol/L EGTA, 0.5% Nonidet P-40, 20 mmol/L Tris-HCl (pH 6.8), and containing protease inhibitors] and vortexed vigorously. The lysates were passed through 18 and 21 gauge needles before centrifugation for 1 min at 1,000 rpm, removing unlysed debris. Total protein concentrations in the lysates were determined using the Bio-Rad assay, and equal amounts of protein were loaded on SDS-PAGE gels for each pair of pretreatment and posttreatment samples. The Western blot was sequentially probed with antibodies to total and modified α-tubulins and with an antibody to actin, the latter as a loading control for each pair. Equally loaded pretreatment and posttreatment lysate pairs were also separated by SDS-PAGE on 15% gradient gels, alongside a renal cell carcinoma cell line control pair that was either treated or not with 400 nmol/L ixabepilone for 75 h to induce cell death. The bands were sequentially probed with antibodies to actin and glyceraldehyde-3-phosphate dehydrogenase. For assessment of posttranslational tubulin alterations in peripheral blood mononuclear cells (PBMC), blood samples were collected from patients in cell preparation tube vacutainers with sodium citrate (Becton Dickinson) either before or 24 h after treatment with ixabepilone and centrifuged at 2,000 rpm for 15 min to obtain the PBMC layer. PBMCs were then washed twice in PBS and stored frozen at -80°C before further processing as noted above. Lysates were analyzed by SDS-PAGE on four identical loaded gels that were probed either with antibodies to total or modified α-tubulins or with an antibody to actin. We would note that posttreatment biopsies were obtained anywhere from 1 to 4 h after the fifth dose. In practical terms, clinically, this was the best that could be done. The variables of when the dose was given on the infusion ward and when the slot becomes available in the radiology suite necessitated a range of times. Because the posttranslational effects usually occur rapidly and last for a long time, we were not concerned about this variability. Also note that we chose this because it is 1 to 4 h after the fifth dose but 24 h after the fourth day dose, 48 h after the third day dose, 72 h after the second day dose, and 96 h after the first day dose. Thus, we were covering a range of times in this way.

*Modified Tubulin Indicates Tumor Target Engagement*
of α-tubulin, exposing the penultimate glutamic acid (glu-terminated α-tubulin). Figure 1A presents the results obtained when cellular tubulin from #181 renal cancer cells was fractionated into soluble and polymerized fractions. As shown previously, with the assay conditions used, the majority of tubulin in untreated cell fractions into the soluble fraction (30). Incubation with increasing concentrations of ixabepilone results in a dose-dependent shift of tubulin from the soluble into the more stable polymerized form in the pellet (Fig. 1A). Ixabepilone also increased the levels of detyrosinated (glu-terminated α-tubulin) and acetylated α-tubulin. As shown in Fig. 1B, the effect of ixabepilone on microtubule stabilization correlated well with the levels of the detyrosinated and glu-terminated α-tubulin. This is depicted graphically in Fig. 1C. Similar results were obtained using other cancer cell lines, including 1A9 cells, an ovarian cancer cell line, as shown in Fig. 1D.

Because the substrate for the tubulin acetyl transferase and the carboxypeptidase is polymerized tubulin, we would expect that acetylated and glu-terminated tubulin would fractionate with polymerized or oligomerized tubulin. The results of experiments designed to address this question are shown in Fig. 2. Figure 2A shows the results obtained with our routine fractionation procedure that separates polymerized and soluble tubulin fractions by centrifugation at 15,000×g at 22°C for 10 min. These experimental conditions were also used for the data shown in Fig. 1. When the distribution of modified forms of tubulin was examined, glu-terminated and acetylated tubulins were found almost exclusively in the pellet or polymerized fraction at higher concentrations of ixabepilone (Fig. 2A). However, at lower drug concentrations, a significant proportion of the tubulin with either of these modifications was also observed in the soluble fraction. Because we recognized that the 15,000×g centrifugation was likely insufficient to optimally pellet tubulin oligomers that are also substrates for the two modifying enzymes or those arising as

- Exponentially dividing RCC #181 cells were treated with different concentrations of ixabepilone (BMS-247550) for 4 h before lysis. Polymerized and soluble tubulins were separated by centrifugation at ~15,000×g for 10 min at 22°C. The pellets (P) were resuspended in a volume of lysis buffer equal to that of the supernatant (S). Equal aliquots of polymerized and soluble fractions were loaded on SDS-PAGE and subjected to Western blotting with antibody specific for α-tubulin.
- Exponentially dividing RCC #181 cells were treated with different concentrations of ixabepilone (BMS-247550) for 4 h, then harvested in a 3:1 mixture of lysis buffer and 4× SDS-PAGE loading buffer. Equal aliquots were evaluated by Western blotting using the indicated antibodies.
- Quantitation of A and B. Percentage of polymerized tubulin after analysis of the appropriate bands by densitometry was calculated using this formula: percentage of polymerized tubulin = [pellet tubulin / (soluble tubulin + pellet tubulin)] × 100. The relative glu-tubulin levels were determined by quantifying bands of the upper panel of B and setting the glu-tubulin level in the control arbitrarily as 1. D, the same experiment described in B was done using the ovarian cancer cell line A2780(1A9).

Results

Initially, we investigated whether microtubule stabilization induced by ixabepilone correlated with the levels of posttranslationally modified α-tubulin in cultured cell lines. Specifically, we sought the extent of correlation between microtubule stabilization and either acetylation of Lys40 in α-tubulin (catalyzed by a tubulin acetyl transferase) or the appearance of α-tubulin with a C-terminal glutamic acid residue (catalyzed by tubulin carboxypeptidase). The latter arises as a result of a carboxypeptidase-mediated removal of the C-terminal residue of α-tubulin, exposing the penultimate glutamic acid (glu-terminated α-tubulin). Figure 1A presents the results obtained when cellular tubulin from #181 renal cancer cells was fractionated into soluble and polymerized fractions. As shown previously, with the assay conditions used, the majority of tubulin in untreated cell fractions into the soluble fraction (30). Incubation with increasing concentrations of ixabepilone results in a dose-dependent shift of tubulin from the soluble into the more stable polymerized form in the pellet (Fig. 1A). Ixabepilone also increased the levels of detyrosinated (glu-terminated α-tubulin) and acetylated α-tubulin. As shown in Fig. 1B, the effect of ixabepilone on microtubule stabilization correlated well with the levels of the detyrosinated and glu-terminated α-tubulin. This is depicted graphically in Fig. 1C. Similar results were obtained using other cancer cell lines, including 1A9 cells, an ovarian cancer cell line, as shown in Fig. 1D.

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polymerized tubulin is initially disassembled, we altered the experimental conditions. When the centrifugation was done at 37°C at 180,000 x g for 1 h, conditions sufficient to precipitate tubulin oligomers, the modified tubulins were only present in the pellet or polymerized fraction (Fig. 2B).

The evidence thus indicated that the amounts of glutermated and acetylated α-tubulin reflect the extent of microtubule stabilization in cell culture. Given this result, we examined tumor samples obtained from patients with renal cell carcinoma who were receiving the microtubule-stabilizing agent ixabepilone in a phase II clinical trial. Samples were obtained before the start of treatment and after the completion of a cycle of therapy. As shown in Fig. 3, a majority of patients treated with ixabepilone had increased levels of total tubulin, glutermated, and acetylated α-tubulin. For these blots, equal amounts of total protein from each patient's pretreatment and posttreatment biopsy lysates were loaded in adjacent wells. The comparability of each pair is confirmed by the immunoblot using actin as a loading control. Normalizing to actin levels, glutermated α-tubulin increased 2-fold to 25-fold in 11 of 13 serial tumor samples whereas acetylated α-tubulin increased 2-fold to 100-fold in 11 of 12 serial tumor biopsies (Fig. 3). Total α-tubulin levels also increased 2-fold to 8-fold in 10 of 13 patients after treatment with ixabepilone when normalized to actin (Fig. 3B). Whereas the increase in total α-tubulin is part of the cellular response to ixabepilone, even when normalizing to total α-tubulin, there were still increases in one or both posttranslational modifications in the majority of patients, as shown in Supplementary Fig. S1. These data show that the microtubule target in these tumors has been engaged as evidenced by increased levels of posttranslationally modified forms of α-tubulin. When the extents of these posttranslational modifications were correlated with responses as measured by increase or decrease in tumor size determined using RECIST criteria, no correlation was observed (see Supplementary Figs. S2 and S3). We also noted that except for an increase in acetylated tubulin in the PBMCs obtained from one patient after administration of ixabepilone (Fig. 4), this patient and three others showed no differences in glu-terminated tubulin between samples obtained before and after ixabepilone treatment at this time of harvest.

### Discussion

Microtubule stabilization is the mechanism of action for paclitaxel (Taxol) and docetaxel (Taxotere), two of the most widely used cancer chemotherapeutic agents (2, 4, 5). The success of the taxanes catalyzed the search for additional classes...
of drugs able to stabilize microtubules. Agents from two of these classes, the epothilones [ixabepilone (BMS-247550); Epo906 and EpoD] and discodermolide, are currently in different phases of clinical development (2, 7–14). The present study was carried out to determine whether we could find evidence that ixabepilone reached tumor cells and exerted its microtubule-stabilizing effect in patients receiving ixabepilone, an epothilone B analogue. We first showed that in vitro microtubule stabilization induced by ixabepilone correlates with the levels of detyrosinated (glu-terminated) and acetylated α-tubulin, suggesting these two modifications can be used to monitor microtubule stabilization. Furthermore, we found that after treatment with ixabepilone, the levels of glu-terminated α-tubulin increased 2-fold to 25-fold in 11 of 13 serial tumor samples, whereas acetylated α-tubulin increased 2-fold to 100-fold in 11 of 12 serial tumor biopsies (Fig. 3B). These data indicate that ixabepilone reached the tumors and engaged the microtubule target, leading to microtubule stabilization. We propose that the levels of detyrosinated (glu-terminated) and acetylated α-tubulin can be used as simple and reliable pharmacodynamic markers of microtubule stabilization.

Despite the widespread use of the microtubule-stabilizing taxanes, in vivo demonstration of drug-target interaction has been very limited. A study conducted early in the clinical development of paclitaxel examined the effects of adding paclitaxel ex vivo to blasts obtained from patients with refractory leukemias before therapy (31). Paclitaxel-induced microtubule bundles were observed in the blasts of 8 of 12 patients who also had cytoreduction of tumor, and sensitivity to bundle formation was related to the magnitude of antitumor activity. Furthermore, paclitaxel failed to induce microtubule bundles ex vivo in the blasts of the other four patients, and none of these experienced any evidence of antitumor activity. More recently, tubulin bundle formation was observed in tumor cells and PBMCs (32), or PBMCs obtained from patients receiving ixabepilone (33). PBMCs exhibited dramatic microtubule bundle formation 1 h after the drug infusion, and this declined by 24 h (33). A similar pattern of tubulin bundle formation was also observed in breast tumor cells from one patient who exhibited a partial response to ixabepilone. Given the paucity of clinical data, we were especially interested in examining this phenomenon in our patients for several reasons. Firstly, taxanes have been found to be ineffective in patients with renal cell cancer, and P-glycoprotein has been implicated as a possible cause for this lack of activity. Like other epothilones in clinical development, ixabepilone is a poor P-glycoprotein substrate. We therefore expected that it would be more likely to reach its target and sought to show this. However, we also anticipated that, like all chemotherapeutic agents, ixabepilone would have only limited activity in renal cell carcinoma. Hence, we wanted, if possible, to begin to develop assays that would allow us to discriminate between patients with a clinical response from those without clinical benefit. We wanted to know where the drug failed in patients, whether it had reached its target and successfully engaged it. Our data show a range of increased levels of postranslationally modified α-tubulin in all tumor tissues examined, indicating engagement of its microtubule target by ixabepilone. This observation is consistent with the drug’s ability to avert P-glycoprotein and reach its target. However, because a majority of the patients did not have evidence of clinical benefit, it is clear that microtubule engagement is insufficient to bring about a clinical response. At least four possibilities can be advanced for this observation. The first is that microtubule engagement is not sufficient to bring about cell death. Death pathways must be activated, and this may not occur despite a shown drug-target interaction. This pathway, often envisioned as a “final common pathway” to cell death, may be more refractory in renal cell cancer and may explain the widespread resistance to chemotherapy observed in this tumor (34–36). Our current efforts are directed at identifying more “downstream targets” that may be reliable markers of this cell death pathway in a solid tumor. A second possibility is that cell death and in turn clinical response may depend on the extent of microtubule stabilization. Whereas the current study lacks sufficient numbers to draw this conclusion, we may find, as additional patients are examined with a variety of malignancies, that the extent of microtubule stabilization, as evidenced by postranslational modification of α-tubulin, can help to predict response. We cannot exclude the possibility that a threshold level of microtubule stabilization as evidenced by postranslationally modified α-tubulin needs to be reached for the tumor to respond clinically to the treatment. A third possibility is that despite microtubule stabilization, cell death did not occur because by the time the vulnerable phase of the cell cycle has been reached in these often slow growing tumors, the effects of the drug had been repaired. Finally, it is possible that the timing of the posttreatment biopsies is important. In the present study, the tumor biopsies were obtained before treatment and after the fifth dose of ixabepilone given for five consecutive days. We empirically chose this schedule for its feasibility and patient convenience. A more optimal result may have been obtained with a different biopsy schedule. Poly (ADP-ribose) polymerase and caspase cleavage have been difficult to show in our tumor samples, and cell death is likely to be an event occurring later than the early microtubule target engagement we are presently able to document. We show, in Supplementary Fig. S4, preliminary results, using a fractin antibody that recognizes a fragment of actin seen in dying cells, on four pretreatment and posttreatment patient samples, as well as a renal carcinoma cell line (786-O), the latter treated with 400 nmol/L ixabepilone for 75 h to induce cell death.

It is interesting that total α-tubulin levels, normalized to actin levels, also increased in 10 of 13 patients after 5 days of
treatment with ixabepilone (Fig. 3B). We would note that in vitro studies have previously shown increased nuclear runoff in cells after treatment with paclitaxel, a phenomenon ascribed to a reduced pool of soluble α-tubulin (37, 38). It is possible that ixabepilone–induced stabilization might have caused increased tubulin production and/or protected tubulin from degradation. Ideally, it would have been interesting to examine the α-tubulin mRNA levels in these patients’ samples to determine whether the increases in α-tubulin protein were due to an increase in transcription or a decrease in degradation. However, we are limited by the amounts of the tissue obtained from the patients. Moreover, in the present study, the tumor biopsies were obtained before treatment and after the 5th dose of ixabepilone given for five consecutive days. We do not know whether a biopsy obtained after a different interval post–ixabepilone treatment would change this observation. However, despite the increase in the total α-tubulin levels in most patients, we showed that, after adjusting for total α-tubulin, one or more of the modified α-tubulin levels increased 2-fold to 18-fold in 9 of 13 patients receiving ixabepilone (Supplementary Fig. S1).

The extent and kinetics of ixabepilone–induced changes in the levels of glu-terminated α-tubulin and those of acetylated α-tubulin were found to be very different in the cultured cell lines. The levels of glu-terminated α-tubulin levels were very low and showed a dose-dependent relationship with ixabepilone treatment, whereas basal acetylated α-tubulin levels were higher and increased only 2-fold to 3-fold after treatment with ixabepilone (Fig. 1). Although one would predict, based on these observations in cultured cell lines, that glu-terminated α-tubulin levels would be a better pharmacodynamic marker for ixabepilone, the clinical data do not support this. The pretreatment levels of acetylated α-tubulin in tumor samples were low and, in 11 of the 12 patients, showed 2-fold to 10-fold increases after ixabepilone treatment, suggesting that, in patient samples, acetylated α-tubulin levels may be at least as valuable as glu-terminated α-tubulin levels. Further studies will help to clarify this. We would note that at a minimum four different enzymes are involved in these modifications. Two enzymes are involved in the acetylation of tubulin (tubulin acetylase and tubulin deacetylase) with net acetylation, a balance between these two activities. Two enzymes are also involved in the decarboxylation (tubulin carboxypeptidase and tubulin tyrosine ligase). Here again, net decarboxylation depends on the balance between these two activities. The activities of these enzymes may vary among different tumor types.

Finally, we did not observe as marked an effect on tubulin obtained from circulating mononuclear cells. Figure 4 shows the results in four of the patients for whom tumor samples were available. Similar results were observed in study subjects as a group. This observation casts some doubt on the value of nondividing mononuclear cells as a surrogate. The apparent discrepancy might be explained by the fact that the mononuclear cell samples were obtained after only one dose of ixabepilone, whereas the tumor biopsies were processed after the final dose. Further evaluation is required before recommending these cells as suitable surrogates.

In summary, we have shown that the amount of glu-terminated α-tubulin and acetylated α-tubulin, two postranslational modifications previously shown to be markers of stable microtubules, correlate with microtubule stabilization after treatment with a microtubule-stabilizing agent. In clinical samples, increased levels of both postranslational modifications can be shown after treatment with the novel epothilone agent ixabepilone. This study shows for the first time, in a group of patients, evidence of microtubule engagement in the tumor by a microtubule-stabilizing agent. In this era of targeted therapies, wherein demonstration of this phenomenon by a drug is recognized as essential, the present study indicates that, with agents that stabilize microtubules, such an evaluation is possible. This assessment may lead to a better understanding of microtubule agents and why they fail to provide benefit to patients as often as one would hope.

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