Response and Determinants of Sensitivity to Paclitaxel in Human Non-Small Cell Lung Cancer Tumors Heterotransplanted in Nude Mice

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ABSTRACT
The lack of tumor models that can reliably predict for response to anticancer agents remains a major deficiency in the field of experimental cancer therapy. Although heterotransplants of certain human solid tumors can be successfully grown in nude mice, they have never been appropriately explored for prediction of in vivo chemosensitivity to anticancer agents. We determined the tumor response rate and studied the influence of several biological and molecular tumor parameters on the in vivo sensitivity to paclitaxel in a series of heterotransplanted human non-small cell lung cancer (NSCLC) tumors. One hundred consecutive resected NSCLC tumors were heterotransplanted s.c. in nude mice. The in vivo sensitivity to i.v. paclitaxel (60 mg/kg every 3 weeks) was studied in 34 successfully grown heterotransplants. Treatment started when the tumors reached a size of 5 mm in diameter, and strict standard clinical criteria (>50% shrinkage in tumor weight or cross-sectional surface) were used to define tumor response. Baseline multidrug resistance protein (MRP), Her-2/neu, and epidermal growth factor receptor (EGFR) expression, and pre- and posttherapy bax and bcl-2 expression were determined by Western blot analysis. p53 status was determined by sequencing. The overall take rate was 46% (95% confidence interval, 36–56%) and was significantly higher (P < 0.05) for squamous carcinoma tumors (75%) than for adenocarcinoma tumors (30%) and bronchoalveolar tumors (23%). The heterotransplants were morphologically very similar to the original tumors. The response rate to paclitaxel was 21% (95% confidence interval, 9–38%). Baseline tumor parameters associated with response were no Her-2/neu expression (none of the responding tumors expressed Her-2/neu versus 48% of the nonresponding tumors, P = 0.05) and baseline bcl-2 expression (all responding tumors expressed bcl-2 versus only 43% of the nonresponding tumors, P = 0.02). There was a trend toward a higher response rate in bax-positive tumors, and MRP- and EGFR-negative tumors, but it was not statistically significant. The response was independent of baseline p53 status and baseline mitotic index. Responding tumors had a higher bax/bcl-2 ratio 24 h after therapy, but the difference was only marginally significant (2.8 for responding tumors versus 1.1 for nonresponding tumors, P = 0.07). The extent of mitotic arrest at 24 h after therapy was not associated with response. Human NSCLC heterotransplants are morphologically identical to the original tumors and have a response rate to paclitaxel that is equivalent to that reported in Phase II studies in patients with advanced NSCLC treated with single-agent paclitaxel. NSCLC heterotransplants deserve to be explored to evaluate new agents for lung cancer and to predict clinical response on an individual basis in selected groups of patients.

INTRODUCTION
The lack of clinically relevant tumor models of the different human cancers in general or of a specific individual tumor from a given patient is a major impediment to the development of new effective anticancer therapies in general and in selecting the most appropriate therapy for individual patients.

Most anticancer agents introduced in clinical trials are never approved for use, and in the majority of cases their development is aborted by insufficient antitumor activity in Phase II clinical studies rather than intolerable and/or unpredictable toxicity. The current in vitro drug screening panels used by the NIH (1) and most pharmaceutical companies are composed of human tumor cell lines derived from multiple sequential in vitro subcultures of human tumor explants. These cell line panels are well characterized from a molecular standpoint and are useful in identifying molecular determinants of in vitro sensitivity or confirming putative molecular mechanisms of action of the compounds screened. However, because most human tumors have many accumulated genetic and molecular abnormalities (2) and display a high degree of phenotypic heterogeneity, their relevance in predicting in vivo clinical activity remains to be established.

New anticancer agents are routinely screened in vivo in human tumor xenografts grown s.c. in nude mice before initiation of clinical trials using mostly tumor growth inhibition, not tumor shrinkage, as sufficient evidence of antitumor activity. These xenografts are not representative of the heterogeneous population of tumor cells of the human tumor from which they were derived. In addition, the vascularity and stroma of these
xenografts is exclusively from murine origin. In many cases, xenografts are selected to suit the putative molecular mechanism of the agent tested, the approach being one of proof of principle in an in vivo model rather than screening the new agent in a panel of clinically relevant and predictive models. If panels of in vivo experimental tumor models clinically representative of each major human cancer type were available, the selection criteria for pursuing the clinical development of new antitumor agents would be more restrictive, but the success in identifying new active agents for particular tumors much higher, thus expediting and reducing the cost and patient resources needed for anticancer drug development.

Similarly, if individualized models of human cancers were available, they would also greatly facilitate selecting the best therapy for each individual patient. Ex vivo sensitivity tests using tumor cells obtained from fresh tumor specimens have been explored extensively and have been, in general, more useful in confirming resistance to agents that are already known to be inactive against a particular tumor rather than in helping select the most active agent (3). In addition, and as in the case of all in vitro systems, these assays cannot account for the in vivo pharmacological determinants of antitumor activity.

Many human tumors can be grown s.c. in immunodeficient mice. It is well known that the morphological and cell kinetic characteristics of the tumors change with subsequent passages (4). The determinants of successful heterotransplantation are mostly unknown, and in general, successful heterotransplantation has been found to be associated with a poor clinical prognosis (5). The potential use of first mouse-to-mouse transplants of human tumors as tumor models of potential clinical relevance to predict the antitumor activity of different antitumor agents or to optimize therapy for an individual patient has never been explored. As a first step toward that goal, we decided to develop and molecularly characterize a large panel of human NSCLC3 heterotransplants, to study their sensitivity to paclitaxel on first mouse to mouse passage, and to investigate different biological and molecular determinants and correlates of sensitivity. The results indicate that the response rate to paclitaxel of these tumors is identical to that of human NSCLC tumors. We report here the results of this study.

MATERIALS AND METHODS

Patients and Tumors. Between December 1995 and February 1998, 100 fresh NSCLC tumor samples were obtained from the Pathology Department of the University of Texas M. D. Anderson Cancer Center. These samples were taken from 100 consecutive patients who underwent surgical resection for stages I to IIA primary NSCLCs. None of these patients had undergone preoperative radiation therapy or preoperative chemotherapy.

Tumor Implantation in Nude Mice. The fresh tumor samples were cut into 1–2 mm³ pieces in sterile saline. Three or four pieces of nonneoplastic tissue were inoculated s.c. into the lower back and anterior chest of female Nu/Nu mice, 6–8 weeks of age, using a biomedical stainless steel needle (implant needle). Nude mice were maintained under standardized sterile conditions. Animals transplanted with NSCLC tumors were checked for tumor growth for a maximum of 36 weeks. Tumor formation measuring at least 5 mm in diameter was considered a positive take. Tumor formation was confirmed histologically in all cases. Temporary growth was defined as tumor formation followed by spontaneous regression before reaching a diameter of 5 mm. All other incidences were considered no growth.

Paclitaxel Treatment and Assessment of Response. The therapeutic experiments were designed as a standard human Phase II clinical study with a target response rate of 20%, the only difference being that tumor response was assessed as the average of two animals rather than in a single subject as is the case in a human Phase II trial.

The tumor grown after implantation from human to mouse was resected and cut into small pieces as described above for the original tumor and transplanted s.c. into several animals. At the time of first mouse-to-mouse transplantation, tumor aliquots for storage in liquid nitrogen and for paraffin embedding and H&E staining were taken. This tissue was used to determine p53 status by sequencing and morphological comparison with the tumor originally resected from the patient. Tumors from the first mouse-to-mouse passage were allowed to grow until reaching 5 mm in diameter, at which time two groups were established: a control group of two animals and a treatment group of three animals. The proliferation rate of the heterotransplants was assessed in the control group animals by calculating: (a) the tumor doubling time defined as the average number of days needed for the tumor to grow from 0.5 to 1 g in weight (which corresponds to a change in size from approximately 10–12 mm); and (b) the time for the tumor to reach a size of 10 mm in diameter from the day of transplantation. When the tumors reached a size of 5 mm in diameter, animals in the treatment group received 60 mg/kg paclitaxel i.v. as a bolus in the tail vein. Two of the animals in the treatment group were followed to determine tumor response. Tumor measurements were performed twice a week, and the tumor volume was calculated using the formula: \(a \times b^2/2\), where \(a\) is the longest diameter and \(b\) is the shortest diameter. Partial tumor response was defined as an average reduction in tumor weight of at least 50% in the two animals of the treatment group for a period of at least 3 weeks. Complete tumor response was defined as complete disappearance of the palpable tumor in both animals of the treatment group. One animal in the control group and the third animal in the treatment group were sacrificed at 24 h. The tumors were resected; an aliquot was taken for paraffin embedding and H&E staining, and another aliquot was frozen for Western blot analysis. The tumor sample from the control animal was used to determine the baseline mitotic index, the expression of Her-2/neu and EGFR by immunohistochemistry and Western blot, and the expression of MRP, Bax, and Bcl-2 by Western blot. The tumor sample from the treated animal was used to determine the extent of mitotic arrest and induction of bax and bcl-2 after therapy.

In 16 cases, animals in the treatment group were treated again with a second dose of 60 mg/kg paclitaxel on day 21 to assess the effect of a second dose of therapy. The therapeutic experiments were also performed in 21 cases using second mouse-to-mouse transplants to assess whether sequential passages alters the in vivo chemosensitivity to paclitaxel.

3The abbreviations used are: NSCLC, non-small cell lung cancer; EGFR, epidermal growth factor receptor; MRP, multidrug resistance protein; CI, confidence interval.
Histological Studies: Mitotic Index and Immunohistochemistry. The mitotic index was assessed in the H&E stained slides by determining the number of mitotic figures per high power field. The baseline and posttherapy mitotic index was assessed in tumor samples from control animals and taken 24 h after therapy. In a few cases, it was also assessed at 72 h after therapy. The ratio between the mitotic index at 24 or 72 h and the baseline mitotic index was calculated and used as the index of paclitaxel-induced mitotic arrest.

Baseline Her-2/neu and EGFR expression was assessed by immunohistochemistry in formalin-fixed, paraffin-embedded tissue using commercially available monoclonal antibodies (Her-2, Ventana Medical Systems, Tucson, AZ; EGFR, Zymed Laboratories, San Francisco, CA), an automatic immunostainer (NexES, Ventana Medical Systems), and a diaminobenzene chromogen. Several positive controls were used including the breast cancer cell line MCF-7 and clinical specimens of invasive ductal breast carcinoma for Her-2/neu and lung squamous carcinoma for EGFR. Appropriate antigen retrieval was performed for each tissue specimen. Positive staining for Her-2/neu and EGFR was considered only for membranous staining. The following grading scale was established according to the number of cells staining positively: 0, 0%; 1, 1–25%; 2, 26–50%; and 3, >50%.

Western Blot Analysis. Her-2/neu, EGFR, MRP, bax, and bcl-2 were assessed by Western blot analysis. Frozen tumor samples from the control animals were used for these studies. In addition, bax and bcl-2 were also determined in samples obtained at 24 h after therapy as indicated above. A small piece of tumor measuring ~3 mm diameter was taken from the frozen heterotransplant NSCLC mass, washed three times with cold PBS solution, and homogenized with a glass homogenizer fitted with a Teflon pestle with a clearance of 0.4 mm. After centrifugation at 1500 rpm at 4°C, the homogenate was lysed with lysis buffer containing 50 mM Tris-HCl (pH 7.4), 1% Triton X-100, 250 mM NaCl, 25 mM NaF, 15 mM MgCl₂, 20 mM EDTA, 20 mM EGTA, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 20 μg/ml aprotinin, 20 μg/ml leupeptin, and 1% SDS. After centrifugation at 15,000 rpm at 4°C for 10 min, the supernatants were collected, and the protein amount was measured with a Bio-Rad DC protein assay kit (Hercules, CA). Aliquots containing 50 μg of protein were subjected to SDS-PAGE. After electrophoresis, the protein bands were transferred to a nitrocellulose membrane. After blocking with 5% nonfat milk in TBST buffer for 1 h, the membrane was incubated with 1:1000 dilution of corresponding antibodies for 1 h at room temperature. All antibodies were obtained from Calbiochem (Cambridge, MA). After washing twice with TBST buffer, the membrane was incubated with a 1:5000 dilution of antimouse IgG-conjugated horseradish peroxidase as a second antibody in TBST buffer for 30 min at room temperature. The specific protein bands were detected by a chemiluminescence reaction conducted following the manufacturer’s recommendation (Amersham, Arlington Heights, IL). The protein signal was quantitated by laser scanning densitometry (Molecular Dynamics, Sunnyvale, CA).

DNA Analysis for p53 Mutations. p53 status was assessed by sequencing. Briefly, tumor cells were microdissected from formalin-fixed, paraffin-embedded tissue sections after light hematoxylin staining and digested in 300 μl of 50 mM Tris-HCl (pH 8.0) containing 1% SDS-proteinase K and incubated at 42°C for 12–24 h. Digested samples were purified, and DNA was precipitated as described previously (6). Exons 5–6, exon 7, and exons 8–9 of the human p53 gene was PCR amplified separately using the following primer pairs to ensure successful amplification: 4S (sense), 5′-TTCATCTGTGCCTTGACCTTG-3′ and Int 6A (antisense), 5′-CCATCGACAACACCCTTT-3′ for exons 5 and 6; 6S (sense), 5′-CCAAGGCCACTGGCCCTC-3′ and X7 (antisense), 5′-AGGGGCGAGCAAGGGCTG-3′ for exon 7S (sense), 5′-CTTACTGCTCTTGTCTG-3′ and 9AS (antisense), 5′-CTTGAAGACTTCCACTTGTGAT-3′ for exons 8 and 9.

PCR reactions were carried out in a 25-μl volume containing 50 ng of genomic DNA, 1% DMSO, 200 μM deoxynucleotide triphosphates, 1.5 mM MgCl₂, 100 ng of each primer, and 1.25 units of Taq DNA polymerase (Life Technologies, Inc., Gaithersburg, MD). DNA was amplified for 40 cycles at 95°C for 30 s, 52–56°C for 60 s, and 70°C for 60 s in a temperature cycler (Hybaid; Ommigene, Eooodbridge, NJ) in 500-μl plastic tubes after a 2-min initial denaturation at 95°C and followed by a 5-min extension at 70°C. After purifying the amplified DNA fragment using phenol/chloroform extraction and ethanol precipitation, one-fourth to one-third of amplified DNA and a sequencing primer labeled with [γ-32P]ATP as described below were subjected to PCR amplification for 35 cycles using the AmpliCycle sequencing kit (Perkin-Elmer, Branchburg, NJ), according to the manufacturer’s protocol. Each amplified product (3 μl) was run on a 6% Long-Ranger gel (FMC BioProducts, Rockland, ME) and exposed to film. Each mutation identified has been confirmed by a repeat sequence analysis. Sequencing primers used for p53 mutation analysis are as follows: 5AS, 5′-AACCAGCCCTGTCGCTC-3′ for exon 5; X6, 5′-GAGCAGAGGGCTGTT-3′ for exon 6; 7AS, 5′-CACAGCAGCCGTCTGCG-3′ for exon 7; 8AS, 5′-TGAATCTCGAGGCATAACCTGC-3′ for exon 8; 8S, 5′-TTATGCCTCAGATTCACTTT-3′ for exon 9.

Statistical Analysis. The χ² test was used to test the hypothesis of equality of growth success proportions in the different histological groups. The neuroendocrine and sarcomatoid tumors were not included in this analysis. Because the overall test was significant, pairwise comparisons were conducted. Following angular transformation of the growth success proportions, a Tukey-type multiple comparison test was conducted (7). The Mann-Whitney test was used to compare continuous variables in responders and nonresponders, and the Fisher’s exact test was used for categorical variables. All tests were two-sided. P < 0.05 was considered significant.

RESULTS

Characteristics of Patients and Tumor Formation Rates. Table 1 shows the take rate observed in the 100 heterotransplanted tumors. The overall tumor take rate was 46% (95% CI, 36–56%). The take rate was significantly higher (P < 0.05) for squamous cell carcinomas (75%; 95% CI, 57–89%) than for adenocarcinomas (30%; 95% CI, 17–45%) and bronchoalveolar carcinomas (23%; 95% CI, 0–54%). Large cell undifferentiated carcinoma had an intermediate take rate (5 of 9; 55%). Tumor take was independent of site of implantation. In 10 cases, a palpable tumor was initially observed but spontaneously...
disappeared. These cases are considered temporary growths and not counted as successful tumor takes.

**Morphological and Growth Kinetics Characteristics.** The histological morphology of all successfully heterotransplanted tumors was compared with that of the resected original tumors by one of the authors (B. K.). There were no significant morphological differences between the tumors resected from the patients and the initial successful implants, although the median mitotic index was slightly higher in the heterotransplants (9 initial successful implants, although the median mitotic index was 10.9 per high power field versus 10.2 in the resected tumors; 26% in MRP-positive tumors; 0.05; 0% in bax-negative tumors versus 24% in Bax-positive tumors; 33% in EGFR-negative tumors versus 6% in EGFR-positive tumors; 26% in MRP-negative tumors versus 11% in MRP-positive tumors). p53 status as assessed by sequencing was not associated with response (response rate of 16% for wild-type tumors versus 25% for mutated tumors).

Interestingly, there was a discrepancy between Her-2/neu expression by Western blot and expression of bcl-2 were the only two parameters significantly associated with a higher response rate (response rate of 33% in Her-2/neu-negative tumors versus 0% in Her-2/neu-positive tumors, 0.05; 0% in bcl-2-negative tumors versus 37% in bcl-2-positive tumors, 0.02; Table 4). Bax expression, lack of EGFR expression by Western blot, and lack of MRP expression were associated with a higher response rate, but these differences were not statistically significant (response rate of 0% in bax-negative tumors versus 24% in Bax-positive tumors; 33% in EGFR-negative tumors versus 6% in EGFR-positive tumors; 26% in MRP-negative tumors versus 11% in MRP-positive tumors). p53 status as assessed by sequencing was not associated with response (response rate of 16% for wild-type tumors versus 25% for mutated tumors).

**Posttherapy Correlates of Sensitivity.** The correlation between tumor response and posttherapy mitotic arrest and bax:bcl-2 ratio was analyzed. Results are shown in Table 5. A 2.1–8.7-fold increase in mitotic index after paclitaxel therapy was observed. The extent of mitotic arrest was not significantly different in responders and nonresponders, both at 24 and 72 h, thus confirming prior studies (8, 9) that mitotic arrest is required but not sufficient for paclitaxel-induced antitumor activity. A higher bax:bcl-2 ratio after therapy was marginally associated with response (2.8 in responders versus 1.1 in nonresponders, 0.07).

**DISCUSSION.**

The results of this study indicate that heterotransplants of NSCLCs have an in vivo sensitivity to paclitaxel that is similar to that observed in clinical studies using paclitaxel alone in chemotherapy-naive NSCLC patients. The reported overall response rate to single-agent paclitaxel in four Phase II studies that enrolled a total of 160 previously untreated patients with NSCLCs is 24% (10, 21, 24, and 38%; Refs. 10–13). The response rate in our series of

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**Table 1 Human NSCLC heterotransplants: take rate by histology**

<table>
<thead>
<tr>
<th>Histology</th>
<th>Number</th>
<th>Successful growth (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma</td>
<td>44</td>
<td>13 (30)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Squamous</td>
<td>32</td>
<td>24 (75)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Bronchioalveolar</td>
<td>13</td>
<td>3 (23)</td>
<td></td>
</tr>
<tr>
<td>Large cell</td>
<td>9</td>
<td>5 (55)</td>
<td></td>
</tr>
<tr>
<td>Neuroendocrine</td>
<td>1</td>
<td>1 (100)</td>
<td></td>
</tr>
<tr>
<td>Sarcomatoid</td>
<td>1</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>46 (46)</td>
<td></td>
</tr>
</tbody>
</table>

In 21 heterotransplants tested both on first and second mouse-to-mouse passages, the response rate were 24% (95% CI, 8–47%) and 29% (95% CI, 11–52%), respectively.

**Baseline Determinants of Sensitivity.** As shown in Table 2, there were no differences in response rate between tumors of different histological subtype, although the small number of responses observed and the great predominance of squamous cell carcinoma tumors do not allow to draw conclusions in that respect.

Tables 3 and 4 show the distribution of tumor responses according to different baseline parameters. There were no significant differences in doubling time, time for the tumor to reach 10 mm in diameter on first mouse to mouse passage, and baseline mitotic index between responders and nonresponders (Table 3).

Lack of Her-2/neu expression by Western blot and expression of bcl-2 were the only two parameters significantly associated with a higher response rate (response rate of 33% in Her-2/neu-negative tumors versus 0% in Her-2/neu-positive tumors, 0.05; 0% in bcl-2-negative tumors versus 37% in bcl-2-positive tumors, 0.02; Table 4). Bax expression, lack of EGFR expression by Western blot, and lack of MRP expression were associated with a higher response rate, but these differences were not statistically significant (response rate of 0% in bax-negative tumors versus 24% in Bax-positive tumors; 33% in EGFR-negative tumors versus 6% in EGFR-positive tumors; 26% in MRP-negative tumors versus 11% in MRP-positive tumors). p53 status as assessed by sequencing was not associated with response (response rate of 16% for wild-type tumors versus 25% for mutated tumors).
The heterotransplantability of human NSCLC tumors in nude or SCID mice has been the subject of several previous studies (6, 14–16). The take rate of 47% observed in our study is very similar to the take rate reported by these studies and is significantly higher than that reported for other common solid tumors. In particular, the take rate of hormone-dependent tumors like breast and prostate cancer is only around 10% (5). Also, the higher take rate of squamous carcinomas of the lung compared with adenocarcinomas of the lung has also been reported previously (6, 15). Except for histological type, the determinants of successful heterotransplantability of NSCLC tumors remain undefined. There were no differences in tumor take according to stage or p53 status in our study (data not shown). Previous studies have suggested that myc expression (17) and the ability of the human tumor-infiltrating lymphocytes to engraft and produce human immunoglobulin (18) might be a positive and negative determinant, respectively, of successful transplantation. In another study, VEGF expression by tumor cells was not found to be a determinant of successful transplantation (19). Identification of determinants of transplantability should help in devising methods to increase the take rate, which will be important if these models are found to be predictive of chemosensitivity in individual patients.

The potential prognostic implications of successful heterotransplantation have been studied previously in many tumors including leukemia (20), renal cell carcinoma (21), breast carcinoma (5), medulloblastoma (22), head and neck cancer (23), colorectal cancer (24), ovarian carcinoma (25), osteosarcoma (26), and lung cancer (14). In general, these studies have shown that, within tumors of the same histology, successful transplantation is a sign of more aggressive biology and is associated with a worse prognosis or more advanced disease. In the case of lung cancer, Volm and Mattern (14) initially reported that patients whose tumors could be established in nude mice had significantly shorter survival rates than those whose tumors could not be established. In subsequent studies, they were not able to confirm a relationship between take rate and prognosis (15) but showed a relationship between Fos, Jun, EGFR, Ras expression, and take rate (16). In our series, it is too early to analyze the impact of successful heterotransplantation on time to progression and survival.

We analyzed the relationship between tumor response and several baseline and posttherapy molecular markers that have been shown to play a role in paclitaxel sensitivity. The results

### Table 2: Human NSCLC heterotransplants: response to paclitaxel

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number</th>
<th>PR (%)</th>
<th>CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>First mouse-to-mouse passage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous carcinoma</td>
<td>20</td>
<td>4 (20%)</td>
<td>0</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>11</td>
<td>1 (9%)</td>
<td>0</td>
</tr>
<tr>
<td>Undifferentiated cancer</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Neuroendocrine cancer</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>7 (21%)</td>
<td>0</td>
</tr>
<tr>
<td>First mouse-to-mouse passage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After one dose of paclitaxel</td>
<td>16</td>
<td>4 (24%)</td>
<td>0</td>
</tr>
<tr>
<td>After two doses of paclitaxel</td>
<td>16</td>
<td>1 (6%)</td>
<td>3 (18%)</td>
</tr>
<tr>
<td>Second mouse-to-mouse passage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First passage, one dose</td>
<td>21</td>
<td>5 (24%)</td>
<td>0</td>
</tr>
<tr>
<td>Second passage, one dose</td>
<td>21</td>
<td>6 (29%)</td>
<td>0</td>
</tr>
</tbody>
</table>

* PR, partial response; CR, complete response.

34 NSCLC heterotransplants was 21% (95% CI, 9–38%). These findings and the morphological similarity between the heterotransplants and the original tumors justify studying further their potential use to predict chemosensitivity.

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We analyzed the relationship between tumor response and several baseline and posttherapy molecular markers that have been shown to play a role in paclitaxel sensitivity. The results

### Table 3: Human NSCLC heterotransplants: Baseline determinants of sensitivity to paclitaxel

| Parameter               | Responders | Nonresponders | P
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Doubling time (days)</td>
<td>15 (11–40)</td>
<td>14 (10–35)</td>
<td>NS</td>
</tr>
<tr>
<td>Time to reach 1 cm (wk)</td>
<td>18 (8–28)</td>
<td>13 (4–37)</td>
<td>NS</td>
</tr>
<tr>
<td>Mitotic index</td>
<td>11 (3.6–26)</td>
<td>8.4 (1–17.4)</td>
<td>NS</td>
</tr>
</tbody>
</table>

* PR, partial response; CR, complete response.
are mostly confirmatory of studies reported previously of sensitivity to paclitaxel in different preclinical tumor systems. Lack of Her-2/neu expression by Western blot was associated with a higher response rate. This observation is in accordance with previous reports that Her-2/neu expression is associated with chemoresistance in NSCLC and breast cancer cell lines (27, 28). Baseline bcl-2 expression was also associated with a higher response rate, which is surprising in view of the antiapoptotic function of bcl-2 and recent reports of prevention of paclitaxel-induced apoptosis by bcl-2 (29). Interestingly, bcl-2 expression has been associated previously with a good prognosis in NSCLC (30). Bax expression and lack of MRP and EGFR expression has been associated previously with a good prognosis in NSCLC and breast cancer cell lines (27, 28).

Baseline mitotic index and p53 status were not associated with response. The differences did not reach statistical significance, maybe because of the small number of responses observed. Baseline mitotic index and p53 status were not associated with response. The lack of association between p53 mutations and sensitivity to paclitaxel has been suggested by several prior studies (31), although there are studies that have reported the opposite (32–34). With respect to posttherapy molecular markers of response, extent of mitotic arrest was not associated with response, which confirms data published previously in both in vitro and in vivo systems (9, 35), and a higher bax:bcl-2 ratio was only marginally associated with response. In summary, these studies suggest in a clinically relevant model that Her-2/neu, EGFR, and MRP expression may predict for lack of response, whereas mitotic index, extent of posttherapy mitotic arrest, and p53 status are less related to response. Other described determinants of sensitivity, such as tubulin isotypes and tubulin mutations, were not analyzed (36, 37).

The possibility of using panels of human NSCLC heterotransplants to perform Phase II-like studies with new agents potentially effective against NSCLC has not been explored before. Ideally, tumors harvested from first mouse-to-mouse passages should be used, which is possible because tumor fragments from a successful first take preserved frozen in 10% DMSO retain their ability to grow when reimplanted. The importance of orthotopic implantation versus s.c. implantation has probably been overemphasized. Although it has been reported that s.c. implanted human xenografts may have a different chemosensitivity profile than orthotopically implanted xenografts, this observation is not supported by the rarity of mixed responses in patients with metastatic NSCLCs who have s.c. metastatic disease. In addition, assessing tumor response in an orthotopically implanted NSCLC in a nude mouse would require expensive and time-consuming imaging techniques.

If the response rates using standard clinical criteria in Phase II-like studies conducted with the heterotransplants were similar to those observed in human Phase II studies with the same agents, as we have demonstrated with paclitaxel in this study, this observation would help in validating the clinical relevance of these models and justify their use to select new antitumor agents for clinical development. Obviously, such relevance would still be limited to drugs whose metabolism in humans and mice are similar, and at doses that result in a similar AUC both in humans at the maximum tolerated dose and the dose used in mice. This might exclude a few agents from being amenable to this type of screening.

A greater challenge is to demonstrate that these tumors can serve as models representative of individual patient NSCLC tumors and therefore, a powerful tool for individualized selection of therapy. The slow growth of the heterotransplants, their suboptimal take rate, the scarcity of patients with metastatic disease easily accessible to biopsy, and the need to start therapy as soon as possible in many patients for symptom palliation make that demonstration difficult in patients with metastatic disease. As a result, we are planning studies of individual correlation in asymptomatic patients with metastatic disease and slow-growing tumors, because timing of onset of chemotherapy does not affect survival, and we are planning studies in patients with stages I-II who relapse after surgical resection, thus allowing a correlation between response to chemotherapy upon relapse and sensitivity of the heterotransplant derived from the resected primary tumor.

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Response and Determinants of Sensitivity to Paclitaxel in Human Non-Small Cell Lung Cancer Tumors Heterotransplanted in Nude Mice

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