

Unexpected Association between Induction of Immunity to the Universal Tumor Antigen CYP1B1 and Response to Next Therapy

John G. Gribben,¹ David P. Ryan,³ Richard Boyajian,¹ Robert G. Urban,⁴ Mary L. Hedley,⁴ Kathleen Beach,⁴ Patrick Nealon,⁴ Ursula Matulonis,¹ Susana Campos,¹ Timothy D. Gilligan,¹ Paul G. Richardson,¹ Blossom Marshall,¹ Donna Neuberg,² and Lee M. Nadler¹

Abstract **Purpose:** The carcinogen activator cytochrome P450 1B1 (CYP1B1) is expressed on almost all human tumors with rare expression on normal tissues. Anti-CYP1B1 – specific T cells kill CYP1B1-expressing tumors, providing the rationale to examine CYP1B1 as a target for immunotherapy. **Experimental Design:** ZYC300, a plasmid DNA of CYP1B1 encapsulated in biodegradable poly-DL-lactide-coglycolide microparticles, was used in a phase I clinical trial to treat 17 patients with advanced stage, progressive cancer. ZYC300 was administered i.m. at a fixed dose of 400 µg every other week for up to 12 doses. **Results:** Thirteen patients received six vaccinations and five received all 12 doses. No significant adverse events were observed. Six patients developed immunity to CYP1B1, three of whom developed disease stabilization. All but 1 of 11 patients who did not develop immunity to CYP1B1 progressed and did not respond to salvage therapy. Five patients who developed immunity to CYP1B1 required salvage therapy for progressive metastatic disease and showed marked response to their next treatment regimen, most of which lasted longer than 1 year. **Conclusions:** The association between immunity to CYP1B1 and response to next salvage therapy was not expected. Because six of the seven patients who had clinical benefit regardless of the nature of salvage therapy had developed immunity to CYP1B1, it seems highly unlikely that this occurred by chance alone. Regardless of the mechanism(s) that induced tumor regression, these findings force us to rethink how the generation of antitumor immunity might be integrated into the treatment of cancer.

The human immune system can be manipulated to recognize and kill tumor cells, suggesting that cancer vaccines have the potential to become a clinically significant treatment modality (1, 2). As a targeted therapy, cancer vaccines should not only kill cancer cells with limited toxicity, but also generate ongoing antitumor responses secondary to development of immunologic memory and eradicate residual tumor. Because tumor antigens are largely autoantigens, the most significant obstacle is the requirement to overcome immune tolerance (3, 4) and emergence of antigen-negative tumor variants might further limit utility (5). In addition, despite demonstration of development of immunologic responses, the clinical responses

to vaccination trials have often been disappointing (6–8). Potential reasons for this include suppressive mechanisms within the tumor-bearing patient so that within the tumor microenvironment both soluble and cell-based immunosuppressive mechanisms seem to dampen or prevent the function of antitumor effector cells (9, 10). Strategies to overcome these obstacles are necessary before cancer vaccines become a clinical reality (2).

The carcinogen activator cytochrome P450 1B1 (CYP1B1) is an extrahepatic xenobiotic drug-metabolizing member of the cytochrome P450 family of monooxygenases that has been implicated in carcinogenesis (11, 12). In contrast to other cytochromes, CYP1B1 is not expressed in human adult liver and does not have a major role in hepatic metabolism (13). CYP1B1 protein is expressed by virtually all human tumors and little heterogeneity of expression is observed within individual tumors. In contrast, protein expression in adult normal tissues is rare, but there is low-level expression in ureter, fallopian tube, breast, and uterus (14), as well as in monocytes and short-term cultured fibroblasts (15, 16). Mass spectrometric analysis has shown that CYP1B1 peptides are presented by HLA-A*0201 and these peptides serve as targets for antigen-specific cytotoxic T cells (14). Cytotoxicity was achieved *in vitro* against a wide variety of tumor cell lines and tumor cells, whereas there was no cytotoxicity against a panel of normal cells, including

Authors' Affiliations: Departments of ¹Medical Oncology, and ²Biostatistics and Computational Biology, Dana-Farber Cancer Institute and Division of Medical Oncology, Brigham and Women's Hospital; ³Massachusetts General Hospital, Department of Medicine, Harvard Medical School, Boston, Massachusetts; and ⁴MGI Pharma Biologics, Lexington, Massachusetts
Received 10/15/04; revised 3/6/05; accepted 3/18/05.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Lee M. Nadler, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115. Phone: 617-632-4939; Fax: 617-632-3541; E-mail: lee_nadler@dfci.harvard.edu.

©2005 American Association for Cancer Research.

monocytes and immortalized fibroblasts (14). Taken together, these data suggest that CYP1B1 can function as a nearly universal tumor antigen and supported investigation of CYP1B1-directed vaccination for the treatment of human cancer.

In the results reported below, we describe a safety and feasibility study of vaccination with ZYC300, a plasmid encoding an inactivated form of the CYP1B1 DNA formulated within biodegradable poly-DL-lactide-coglycolide microparticles, a strategy previously shown to elicit T-cell-mediated responses (17, 18). Seventeen patients with advanced-stage cancer were vaccinated for up to 12 vaccinations. We show that this approach is safe and although T-cell-mediated responses were generated, no autoimmunity was observed. Intriguingly, all patients who increased anti-CYP1B1 immunity had significantly improved response to their next therapy and this seems to be associated with clinical benefit.

Materials and Methods

Study population

Adult patients with advanced-stage cancer with an estimated life expectancy of at least 6 months were eligible for inclusion. Histologic diagnosis was confirmed in all cases. All patients had measurable disease and good performance status with Eastern Cooperative Oncology Group status 0 (14 patients) or 1 (3 patients). All patients had progressive disease at the time of study entry. Five were male and 12 were female. The median age was 56 years with an age range from 37 to 73 years. The Institutional Review Board approved the protocol and written informed consent was obtained from all subjects. An independent Data and Safety Monitoring Board monitored the study.

Study design

The primary objective of the study was to determine the safety, tolerability, and feasibility of administering at least 6 and up to 12 doses of ZYC300 every other week. The secondary objectives were to determine the immunologic response to CYP1B1 antigen before and after treatment and to associate any clinical activity with the number of doses given. The study was conducted as an open-label, fixed-dose, single-arm phase 1 trial. The investigational agent was given i.m. in alternating lateral quadriceps at 400 μ g DNA/dose once every 2 weeks.

Clinical assessments

Dose-limiting toxicity was defined as grade 2 allergic or autoimmune reactions or any grade 3 or higher hematologic or nonhematologic toxicities. Initially, the study was open to enrollment of 15 subjects. To ensure safety, accrual was constrained by the number of dose-limiting toxicity observed within the first six injections given to the first cohort of 15 subjects. Patients were evaluated regularly for local tolerability, adverse events, and laboratory evidence of toxicity. Following the initial injection, immunologic testing for anti-CYP1B1 reactivity was done at weeks 2, 6, 10, 14, 18, and 22. At a posttreatment visit, all safety and immunology studies, tumor markers, tumor staging, and, if feasible, tumor biopsy was to be repeated.

Immunologic monitoring

IFN- γ ELISPOT was used to monitor T-cell activity. All assays were done in triplicate and run with appropriate positive [phytohemagglutinin (PHA); cytomegalovirus, Epstein Barr virus, influenza virus (CEF)] and negative (media alone) controls.

Peptides. Synthetic peptides based on the full-length sequence of CYP1B1 were used as reagents in immune assays. The peptides were 30 amino acids in length and prepared by Boc/benzyl-based chemistry. The peptides were purified by reversed-phase high-performance liquid chromatography and verified by mass spectral analysis. After purification,

the peptides were mixed and used as a pool. The CEF peptides were obtained from the NIH AIDS Research and Reference Reagent Program (<http://www.aidsreagent.org/>).

Isolation of peripheral blood mononuclear cells. Approximately 50 mL of heparinized venous blood samples were drawn during each subject visit. Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation on a Ficoll-Paque gradient (Amersham Pharmacia Biotech AB, Uppsala, Sweden) and stored in a freezing medium containing 90% FCS (JRH Biosciences, Lenexa, KS), 10% DMSO (Sigma Aldrich, St. Louis, MO). All PBMC samples were aliquoted at a concentration of 5×10^6 /mL and stored frozen overnight at -80°C before transfer to liquid nitrogen where they were maintained in the vapor phase.

ELISPOT assay. To limit interassay variability, the entire set of patient-derived samples taken at all visits were thawed and run simultaneously in single large format assay on the same day. On the day of the assay, sufficient numbers of PBMCs were thawed and washed twice with PBMC wash medium [RPMI (JRH Lifescience, Lenexa, KS) containing 1% penicillin-streptomycin (Life Technologies, Carlsbad, CA) and 1% HEPES buffer (Life Technologies)]. PBMC samples were then counted in trypan blue (Life Technologies). The samples were adjusted to the appropriate viable cell concentration in PBMC medium and with no prior *in vitro* sensitization step, 4×10^5 viable PBMCs per well were added directly for 24 hours in the ELISPOT assay (R&D Systems, Inc., Minneapolis, MN). All steps involving plate development were done according to the manufacturer's instructions. Once developed, the plates were dried at room temperature and then shipped to Zellnet Consulting, Inc. (New York, NY), for counting via the ELISPOT reader system (Carl Zeiss Vision, Germany) with KS ELISPOT 4.0 software. Results were reported as spot-forming cells per 10^6 PBMCs. All spot counting was done without knowledge of the experimental design or patient status. The criteria to define ELISPOT responses were as defined by Russell et al. (19), but based on the intra-assay and inter-assay variability was determined to be 20 spot-forming cells per 10^6 PBMCs after subtraction of negative control and a >2-fold change from baseline was required to be considered significant. The standard operating procedure established for the assay rejected any PBMC sample with >100 spot-forming cells per 10^6 PBMCs in the negative control wells. The upper range for PHA-positive controls were set to 2,000 spot-forming cells per 10^6 PBMCs and samples with >100 spot-forming cells per 10^6 PBMCs in media alone tests were disqualified.

Statistical considerations

To protect subject safety, safety rules were established such that the trial was to be terminated if the estimated rate of dose-limiting toxicity assessed as grade 2 autoimmune, or other grade 3 toxicity was 33% or higher, or if any grade 4 toxicities determined to be related to ZYC300 were observed. It was anticipated from this population of the advanced-stage cancer patients that 50% would have progressive disease during treatment and that only half of the subjects entered would complete at least six injections. Therefore, the design called for closing the study to new subject entry when 15 subjects had completed the sixth injection. All quantitative measures were assessed in triplicate. Results are presented descriptively, with exact binomial confidence intervals for binary outcomes and with median and range of changes from baseline for quantitative outcomes. Clinical response was addressed descriptively. Immune responses were assessed as secondary end points in this study. For quantitatively measured outcomes, a positive response was determined by comparing posttreatment measurements with baseline measurements.

Results

Patient demographics at study entry. Of the 17 patients enrolled, 14 had metastatic solid tumors (six ovarian, three

colorectal, three renal, one prostate, and one breast cancer). One patient with metastatic renal cell carcinoma was previously untreated, two patients had failed one prior regimen, and eleven patients had received at least two prior regimens (median 3, range 2-6) for metastatic disease. Two patients had multiple myeloma (two and three prior regimens) and one had multiple relapsed follicular non-Hodgkin's lymphoma (four prior regimens). At the time of study entry, all patients had progressive metastatic disease. The demographics of the 17 subjects enrolled are shown in Table 1.

Vaccination and toxicity. The number of cycles of CYP1B1 administered to each patient is shown in Table 1. All but four patients received the planned six vaccinations and five patients received all 12 doses (4.8 mg DNA cumulative) over 24 weeks, confirming the feasibility of the cumulative dose escalation approach. No evidence of autoimmune toxicity was observed. One patient had a transient borderline antinuclear antibody observed at single visit (fifth dose) but continued for full 12 doses without incident. The most common adverse events were injection site pain, induration, and discomfort that affected 59%, 41%, and 41% of patients respectively. All were grades 1 to 2 and did not worsen after repeated vaccinations. Four patients were reported to have serious adverse events while on protocol. One patient had injection site abscesses with no evidence of infection, which occurred following injections 7 and 8 and discontinued the study. Three patients had worsening pleural effusions and/or ascites, all considered related to disease progression. Ten patients discontinued the protocol because of progressive disease. No patients died on study and none discontinued due to dose-limiting toxicities.

Development of immune response to CYP1B1. We assessed baseline immunity and response to vaccination to CYP1B1 by measuring IFN- γ ELISPOT responses to nonoverlapping CYP1B1 peptides spanning the entire molecule. Patients were

assessed as responders if at any time point they had ≥ 2 -fold increase from their pretreatment baseline levels as defined in the Materials and Methods. Six patients developed an immune response to CYP1B1, whereas the remaining 11 patients did not (Table 1). The patterns of the immune response following vaccinations of these six patients and representative results from four patients who did not develop immunity to CYP1B1 are shown in Fig. 1. As shown, there was significant heterogeneity in the number of cycles required to induce peak CYP1B1 immunity, ranging from two to six cycles. Immunity to CYP1B1 in the peripheral blood did not persist but largely returned to baseline from the sixth cycle onwards.

Factors that predict immune response to CYP1B1 vaccination. We sought to determine the patient characteristics at study entry that might predict for the induction of an immune response to CYP1B1 (Table 2). The responding patients received significantly fewer prior regimens (median 2, range 0-3) compared with the nonresponders (median 4, range 1-6; $P = 0.01$). We also assessed immune response throughout the study to PHA, to pooled pathogen-specific peptides to CEF. ELISPOT responses to PHA were significantly increased in responders compared nonresponders ($P = 0.04$), but there were no significant differences between the responders and nonresponders in the response to CEF ($P = 0.40$) and CYP1B1 at baseline ($P = 0.90$). Although not a characteristic at study entry, responders also received a higher number of vaccinations (median 12, range 7-12) compared with nonresponders (median 6, range 1-12; $P = 0.008$).

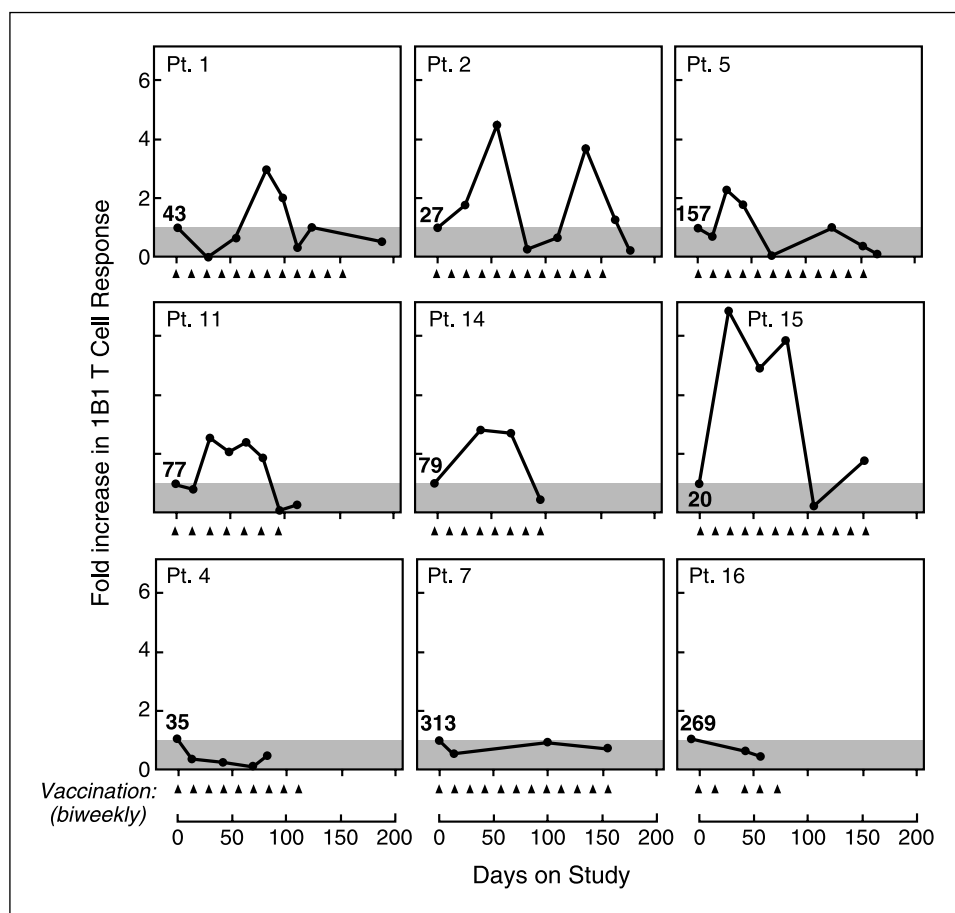
Clinical outcome after vaccination to CYP1B1. The outcome after vaccination is shown in Table 3. Although all patients had progressive metastatic disease at the time of study entry, by the time of first restaging after six vaccinations five patients had disease stabilization and two patients remained stable at second restaging after completion of 12 vaccinations. One of these

Table 1. Patient characteristics

| Patient no. | Disease | Age | Sex | No. prior regimens | No. cycles | Immune response to vaccination |
|-------------|------------|-----|-----|--------------------|------------|--------------------------------|
| 1 | Renal | 59 | M | 1 | 12 | + |
| 2 | Renal | 73 | F | 0 | 12 | + |
| 3 | Colorectal | 46 | M | 1 | 5 | - |
| 4 | Ovarian | 56 | F | 3 | 9 | - |
| 5 | Ovarian | 69 | F | 2 | 12 | + |
| 6 | Ovarian | 46 | F | 2 | 11 | - |
| 7 | Breast | 67 | F | 6 | 12 | - |
| 8 | Ovarian | 50 | F | 5 | 3 | - |
| 9 | Myeloma | 54 | F | 3 | 6 | - |
| 10 | Colorectal | 57 | F | 4 | 2 | - |
| 11 | Myeloma | 55 | F | 2 | 7 | + |
| 12 | NHL | 36 | F | 4 | 1 | - |
| 13 | Ovarian | 56 | F | 3 | 6 | - |
| 14 | Colorectal | 68 | M | 2 | 8 | + |
| 15 | Prostate | 71 | M | 3 | 12 | + |
| 16 | Ovarian | 55 | F | 5 | 6 | - |
| 17 | Renal | 70 | M | 4 | 6 | - |

Abbreviation: NHL, non-Hodgkin's lymphoma.

Fig. 1. Immune response during vaccination in representative patients. Immune responses are shown as fold increase in specific CYP1B1 responses compared with response at baseline. The baseline ELISPOT responses for each patient are shown. Each vaccination is shown as an arrow.



patients (patient 1) remains stable, with continued decreasing disease 16 months after last vaccination and has not required any further therapy. All other patients subsequently progressed.

Assessment of these patients at their most recent follow-up provides additional support to the hypothesis that induction of an immune response to CYP1B1 may have contributed to clinical benefit to these patients. We assessed the response to the first salvage regimen given for disease progression after completion of vaccination. Of the 11 patients who did not develop immunity to CYP1B1, three died before receiving salvage therapy, seven progressed through their next salvage therapy, and one had a complete remission (CR) following surgical removal of tumor and a subsequent autologous vaccine experimental product. All five patients who responded to vaccination and required salvage therapy had clinically significant responses to the next therapeutic regimen (Table 3), in only one of whom (patient 15) the type and duration of response was not considered highly unlikely to have occurred by their treating physician. A comparison among the 13 patients who received salvage therapy shows a highly significant advantage among the immune responders compared with the nonresponders ($P = 0.005$). Patients 6 and 14 attained a CR to their first postvaccination treatment and remain in continuous CR at 17 and 20 months postvaccination. Patient 6 entered the trial with elevated levels of CYP1B1-specific lymphocytes ($239 \text{ spots}/10^6 \text{ cells}$) and this level did not statistically increase following vaccination. Patient 11 attained a partial remission (PR) following salvage therapy and after 4

months elected to undergo autologous hematopoietic stem cell transplant and is currently in CR. Patient 15 attained a PR to first salvage therapy that lasted for 12 months, but has now again progressed and is currently receiving further therapy. Of the two patients who developed stable disease, after their next therapy patient 2 had disease stabilization that persisted for 15 months before showing evidence of disease progression, whereas patient 5 progressed after 4 months of stable disease and is currently receiving further salvage therapy. Patient 1 still has no evidence of disease progression and at last restaging 16 months after completion of vaccination had continued evidence of disease reduction without the need for any treatment >1 year after last vaccination. Presently, among the six patients who developed an immune response to CYP1B1, all are alive with excellent performance status (Eastern Cooperative Oncology Group status 0). Among the 11 patients who did not develop an immune response to CYP1B1, six have died of progressive disease.

Discussion

In the present report, we have established the feasibility and safety of treating cancer patients with the immunogen encoding the universal tumor antigen, CYP1B1. Eligible patients with advanced-stage, progressive metastatic cancer were vaccinated with a fixed dose of CYP1B1 DNA vaccine every 2 weeks for up to 12 vaccinations. Although 13 of 17 patients received the planned six or more vaccinations, only six developed a

measurable immune response to CYP1B1. No patient experienced significant toxicity. In this phase I study, stabilization of progressive metastatic disease occurred after six vaccinations in five patients and persisted after 12 vaccinations in two patients, both of whom developed immunity to CYP1B1. One of these patients has not required any further therapy and shows continued disease regression 16 months after vaccination. The unexpected clinical benefit was observed when we examined the response of these patients with progressive metastatic disease to their next treatment. Four of the five patients who developed immunity to CYP1B1 and required treatment for progressive cancer showed clinically significant responses to their next treatment and the other two had disease stabilization. In contrast, only one of the eight patients who failed to develop immunity to CYP1B1 and survived to receive additional therapy derived clinical benefit from their next treatment.

In view of the association between the development of immunity to CYP1B1 and response to salvage therapy, we sought to determine why some patients developed CYP1B1 immunity whereas others did not. We had predetermined successful immunity to CYP1B1 as a doubling over prevacci-

nation values of the ELISPOT IFN- γ responses to the pool of CYP1B1 peptides and six patients achieved this standard. Neither age, sex, type of malignancy, performance status, nor sites of metastatic disease were predictive for induction of immunity. The only prevaccination demographic that predicted for the development of immunity to CYP1B1 was the number of prior treatment regimens (median of 2 for responders versus 4 for nonresponders). Baseline response to CYP1B1 was not predictive for response to CYP1B1 vaccination. However, the wide variability in baseline immunity to CYP1B1 was striking. All five patients who had >100 spots/ 10^6 cells had either ovarian ($n = 4$) or breast ($n = 1$) cancer. Whether this represents reactivity against antigen-positive normal tissue or evidence of a prior immune response to their tumors is not known. Although this level of response could potentially be due to gender, women who did not have ovarian or breast cancer did not have elevated baseline CYP1B1 responses. We expected that those patients who had received more treatment regimens before vaccination would be less immunocompetent and indeed these patients had decreased PHA responses. However, we cannot conclude that patients who had received greater number of

Table 2. Immune responsiveness of patients

| Immune response to vaccination | Patient no. | No. prior regimens | No. vaccinations | Immune response | | |
|--------------------------------|----------------|--------------------|------------------|-----------------|----------|--------|
| | | | | PHA | CEF | Cyp1B1 |
| + | 1 | 1 | 12 | 1,244 | 481 | 43 |
| + | 2 | 0 | 12 | 1,038 | 779 | 27 |
| + | 5 | 2 | 12 | 1,679 | 149 | 157 |
| + | 11 | 2 | 7 | 1,699 | 555 | 77 |
| + | 14 | 2 | 8 | 1,438 | 131 | 79 |
| + | 15 | 3 | 12 | 548 | 559 | 20 |
| | Medians | 2.0 | 12.0 | | | |
| - | 3 | 1 | 5 | 464 | 155 | 13 |
| - | 4 | 3 | 9 | 807 | 150 | 35 |
| - | 6 | 2 | 11 | 989 | 192 | 239 |
| - | 7 | 6 | 12 | 528 | 328 | 313 |
| - | 8 | 5 | 3 | 1,759 | 139 | 201 |
| - | 9 | 3 | 6 | 1,122 | 376 | 30 |
| - | 10 | 4 | 2 | 742 | 660 | 12 |
| - | 12 | 4 | 1 | 239 | 35 | 0 |
| - | 13 | 3 | 6 | 919 | 1,018 | 62 |
| - | 16 | 5 | 6 | 338 | 167 | 269 |
| - | 17 | 4 | 6 | 314 | 100 | 20 |
| | Medians | 4.0 | 6.0 | | | |
| | Healthy donors | | | | | |
| | Z25 | | | 1,998 | 135 | 29 |
| | Z26 | | | 1,030 | 111 | 13 |
| | Z27 | | | 1,156 | 303 | 32 |
| | Z34 | | | 662 | 58 | 12 |
| | Z38 | | | 1,286 | Not done | 27 |
| | Z44 | | | 1,296 | 215 | 6 |
| | Z39 | | | Out of range | 5 | 3 |
| | Mean | | | 1,238 | 138 | 17 |

NOTE: Immune response measured as spots on IFN- γ ELISPOT in response to PHA, CEF, and CYP1B1.

Abbreviations: PHA, phytohemagglutinin; CEF, pooled peptides from cytomegalovirus, Epstein Barr virus, and influenza virus; CYP1B1, nonoverlapping peptides from full-length CYP1B1, measured as baseline response.

treatment regimens lacked the ability to mount a T-cell-mediated response because they had normal T-cell responses to the pool of CEF peptides. The number of vaccinations received was associated with development of immunity to CYP1B1. This might suggest that the administration of more vaccinations progressively boosts anti-CYP1B1 immunity. However, this was not what we observed. All patients who developed immunity to CYP1B1 had done so between two and six vaccinations and in no patient did the level of immunity increase with further vaccination. It should be stressed that we are only measuring immunity in the peripheral blood and it is possible that antigen-specific lymphocytes traffic to normal lymphoid organs

and/or tumor sites. In addition, 7 of the 10 patients who did not develop immunity received between six or more vaccinations. Similarly, we cannot conclude that the nonresponders were immunologically tolerant to CYP1B1 because the number of patients with high baseline immunity to CYP1B1 was comparable in the responder and nonresponder groups. One possibility is that those patients who responded had biologically less aggressive disease and that active cancer growth inhibits the ability of the host to develop an immune response to vaccination. Conversely, induction of CYP1B1 immunity may inhibit tumor growth, rather than tumor growth inhibiting immunity. Taken together, these observations

Table 3. Patient outcome

| No. | Immune response to vaccine | Disease | Metastatic disease at study entry | Response to vaccination | | Next therapy | Outcome after next therapy | Duration of response to first salvage after vaccine (mo) |
|-----|----------------------------|------------|-----------------------------------|-------------------------|-----------------|--|----------------------------|--|
| | | | | 1st staging | 2nd staging | | | |
| 1 | + | Renal | Liver | SD | SD | No further therapy, lesions continue to decrease | | 24+* |
| 2 | + | Renal | Lung | SD | SD | IFN- α | SD | 15 |
| 5 | + | Ovarian | Liver, bulky peritoneal | PD | PD | Gemcitabine | SD | 4 |
| 11 | + | Myeloma | Bone | PD | | Thalidomide/dexamethasone, ASCT | PR | 4 \rightarrow ABMT |
| 14 | + | Colorectal | Lymph node | SD | | Oxaliplatin/5-FU | CR | 22+ |
| 15 | + | Prostate | Lymph node | PD | PD | Ketoconazole | PR | 12 |
| 3 | - | Colorectal | Liver, spleen, lymph node | PD* | | Capecitabine, 5-FU/Oxaliplatin | PD | PD (died) |
| 4 | - | Ovarian | Liver, pleura | SD | PD [†] | Gemcitabine, Taxol/Carboplatin, Topotecan | PD | PD (died) |
| 6 | - | Ovarian | Bulky peritoneal | PD | PD [†] | Surgery, autologous tumor vaccine | CR | 17+ |
| 7 | - | Breast | Liver, bone | SD | PD* | Doxil/Pamidronate | PD | PD |
| 8 | - | Ovarian | Peritoneal | PD* | | | Died without therapy | |
| 9 | - | Myeloma | Bone | PD | | Revimid, Pamidronate, Velcade, ASCT | PD | PD |
| 10 | - | Colorectal | Thyroid, lung | PD* | | | Died without therapy | |
| 12 | - | NHL | Skin, bone marrow | PD* | | ICE, DHAP, Zevalin, ASCT | PD | PD |
| 13 | - | Ovarian | Liver | PD | | Radioablation | PD | PD |
| 16 | - | Ovarian | Lung, peritoneal | PD | | Radiotherapy, Topotecan, Cisplatin | PD | PD (died) |
| 17 | - | Renal | Lung, lymph node | PD | | | Died without therapy | |

Abbreviations: SD, stable disease; PD, progressive disease; ASCT, autologous stem cell transplantation; 5-FU, 5-fluorouracil; ICE, ifosfamide, carboplatin, etoposide; DHAP, dexamethasone, cisplatin, cytarabine.

*Patients progressed before planned restaging at cycle 6 (see Table 2).

[†]Patients progressed before planned restaging at cycle 12 (see Table 2).

suggest that additional mechanisms, potentially immunosuppressive in nature, are operative in selecting which patients will develop immunity to CYP1B1. This hypothesis will be evaluated in subsequent trials to analyze the number and types of regulatory T cells and/or suppressive cytokines before and after vaccination.

The most intriguing finding is the association between induction of immunity and response to next salvage therapy. As stated above, patients who did or did not develop immunity to CYP1B1 seemed comparable with regard to clinical characteristics except for the numbers of previous treatment regimens and vaccinations. The most trivial explanation of these data is that development of immunity to CYP1B1 is simply a surrogate that selects those patients who would respond to their next treatment regimen. The most compelling argument against this is that we rarely see patients respond dramatically with such durable clinically significant responses to third line therapy, although it must be stressed that these

patients are heterogeneous and the numbers small. The fact that all six patients who developed immunity seemed to derive clinical benefit argues that this is unlikely to be due to chance alone. Rather than concluding that immunity to CYP1B1 is merely a surrogate marker, we hypothesize that immunity to CYP1B1 primes for response to salvage therapy. Whether the mechanism of response is immunologically mediated or whether the generation of anti-CYP1B1 immunity has biologically altered tumor cell resistance or the microenvironment, we believe that these results are of considerable interest and should be verified and further explored. We, therefore, present these data as a hypothesis to be addressed in ongoing and future tumor vaccine studies. Such studies should determine not only which patients develop immunity but also whether patients who develop immunity can be given additional therapy, including conventional antitumor drugs or agents that either enhance immunity or reverse immune suppression to induce clinically beneficial responses.

References

- Rosenberg SA. Shedding light on immunotherapy for cancer. *N Engl J Med* 2004;350:1461–3.
- Finn OJ. Cancer vaccines: between the idea and the reality. *Nat Rev Immunol* 2003;3:630–41.
- Pardoll D. Does the immune system see tumors as foreign or self? *Annu Rev Immunol* 2003;21:807–39.
- Mapara MY, Sykes M. Tolerance and cancer: mechanisms of tumor evasion and strategies for breaking tolerance. *J Clin Oncol* 2004;22:1136–51.
- Yee C, Thompson JA, Byrd D, et al. Adoptive T cell therapy using antigen-specific CD8⁺ T cell clones for the treatment of patients with metastatic melanoma: *in vivo* persistence, migration, and antitumor effect of transferred T cells. *Proc Natl Acad Sci U S A* 2002;99:16168–73.
- Jager E, Jager D, Knuth A. Antigen-specific immunotherapy and cancer vaccines. *Int J Cancer* 2003;106:817–20.
- Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. *Nat Med* 2004;10:909–15.
- Belardelli F, Ferrantini M, Parmiani G, Schlom J, Garaci E. International meeting on cancer vaccines: how can we enhance efficacy of therapeutic vaccines? *Cancer Res* 2004;64:6827–30.
- Liyanage UK, Moore TT, Joo HG, et al. Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma. *J Immunol* 2002;169:2756–61.
- Somasundaram R, Jacob L, Swoboda R, et al. Inhibition of cytolytic T lymphocyte proliferation by autologous CD4⁺/CD25⁺ regulatory T cells in a colorectal carcinoma patient is mediated by transforming growth factor- β . *Cancer Res* 2002;62:5267–72.
- Kaminsky LS, Spivack SD. Cytochromes P450 and cancer. *Mol Aspects Med* 1999;20:70–84, 137.
- Estabrook R. An introduction to the cytochrome P450s. *Mol Aspects Med* 1999;20:5–12, 13–137.
- Edwards RJ, Adams DA, Watts PS, Davies DS, Boobis AR. Development of a comprehensive panel of antibodies against the major xenobiotic metabolizing forms of cytochrome P450 in humans. *Biochem Pharmacol* 1998;56:377–87.
- Maecker B, Sherr DH, Vonderheide RH, et al. The shared tumor-associated antigen cytochrome P450 1B1 is recognized by specific cytotoxic T cells. *Blood* 2003;102:3287–3294.
- Baron JM, Zwaldlo-Klarwasser G, Jugert F, et al. Cytochrome P450 1B1: a major P450 isoenzyme in human blood monocytes and macrophage subsets. *Biochem Pharmacol* 1998;56:1105–10.
- Eltom SE, Larsen MC, Jefcoate CR. Expression of CYP1B1 but not CYP1A1 by primary cultured human mammary stromal fibroblasts constitutively and in response to dioxin exposure: role of the Ah receptor. *Carcinogenesis* 1998;19:1437–44.
- Hedley ML, Curley J, Urban R. Microspheres containing plasmid-encoded antigens elicit cytotoxic T-cell responses. *Nat Med* 1998;4:365–8.
- Garcia F, Petry KU, Munderspach L, et al. ZYC101a for treatment of high-grade cervical intraepithelial neoplasia: a randomized controlled trial. *Obstet Gynecol* 2004;103:317–26.
- Russell ND, Hudgens MG, Ha R, Havenar-Daughton C, McElrath MJ. Moving to human immunodeficiency virus type 1 vaccine efficacy trials: defining T cell responses as potential correlates of immunity. *J Infect Dis* 2003;187:226–42.

Clinical Cancer Research

Unexpected Association between Induction of Immunity to the Universal Tumor Antigen CYP1B1 and Response to Next Therapy

John G. Gribben, David P. Ryan, Richard Boyajian, et al.

Clin Cancer Res 2005;11:4430-4436.

Updated version Access the most recent version of this article at:
<http://clincancerres.aacrjournals.org/content/11/12/4430>

Cited articles This article cites 19 articles, 6 of which you can access for free at:
<http://clincancerres.aacrjournals.org/content/11/12/4430.full#ref-list-1>

Citing articles This article has been cited by 23 HighWire-hosted articles. Access the articles at:
<http://clincancerres.aacrjournals.org/content/11/12/4430.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://clincancerres.aacrjournals.org/content/11/12/4430>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.