Phase I Study of MetXia-P450 Gene Therapy and Oral Cyclophosphamide for Patients with Advanced Breast Cancer or Melanoma

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ABSTRACT

Purpose: MetXia-P450 is a novel recombinant retroviral vector that encodes the human cytochrome P450 type 2B6 gene (CYP2B6), Escherichia coli lacZ, and neomycin resistance marker genes. Cytochrome P450 enzymes are primarily expressed in the liver and convert the prodrug cyclophosphamide to an active phosphoramide mustard and acrolein. Gene-based delivery of CYP2B6 to the tumor site leads to local prodrug activation and higher concentrations of the active metabolites at the target site.

Experimental Design: MetXia-P450 was directly injected into metastatic cutaneous tumor nodules on days 1 and 2 and nodules biopsied on day 7. Oral cyclophosphamide (100 mg/m2) was administered between days 8 and 1 and 2 and nodules biopsied on day 7. Oral cyclophosphamide to an active phosphoramide mustard and acrolein. Gene-based delivery of CYP2B6 to the tumor site leads to local prodrug activation and higher concentrations of the active metabolites at the target site.

Results: Twelve patients with breast cancer (n = 9) and melanoma (n = 3) received three dose levels of MetXia-P450 (−8 × 105, −8 × 106, and −8 × 107 lacZ transferring units/mL). The product was safe and well tolerated. The lacZ transgene was detected in biopsy material by immunohistochemistry in 10 of 12 patients and integrated viral sequences by PCR in 3 of 6 patients. One (8%) patient with breast cancer had a partial response and received 7 months of oral cyclophosphamide. Four (33%) patients had stable disease for ≥3 months and the rest had progressive disease. Preliminary immunologic analyses were suggestive of an antitumor response in two patients (partial response in one patient and stable disease in one patient).

Conclusion: MetXia was safe and well tolerated. Gene transfer was detected at all dose levels, and the initial suggestion of an antitumor response indicates that MetXia-P450 should undergo further clinical assessment.

INTRODUCTION

Administration of cytotoxic chemotherapy is frequently limited by systemic toxicity. Local metabolism of a prodrug, within tumor cells, provides a potential mechanism for delivering high local concentrations of the active metabolites while minimizing systemic drug concentrations. MetXia-P450 (Oxford Biomedica, Oxford, United Kingdom) is a novel replication-deficient retroviral vector enabling the delivery and subsequent expression of the cytochrome P450 2B6 gene (CYP2B6) for the activation of the prodrug cyclophosphamide within cancer cells (reviewed in ref. 1).

After p.o. or i.v. administration, cyclophosphamide undergoes metabolism by cytochrome P450 enzymes (primarily in the liver and to a lesser extent in the lung and renal cortex) to 4-hydroxycyclophosphamide and aldophosphamide and then to phosphoramide mustard and acrolein (2, 3). Phosphoramide mustard is an alkylating agent that induces DNA cross-links and strand breaks. Most normal tissues are protected from the activation of cyclophosphamide by the detoxifying effects of aldehyde dehydrogenase and glutathione S-transferase that convert aldophosphamide to the inactive carboxyphosphamide. Aldehyde dehydrogenase is frequently absent from cancer cells but may be up-regulated in tumors resistant to cyclophosphamide (4–7). Experiments in rats showed that stable cell lines transfected with cytochrome P450 2B1 could be made sensitive to cyclophosphamide (8, 9). Studies with the human homologue CYP2B6 confirmed this to be the most efficient P450 isoform for induction of cyclophosphamide-mediated cytotoxicity (3, 10, 11). Direct delivery of cytochrome P450 enzymes to tumor cells should increase local activation of cyclophosphamide leading to greater cell kill and less normal tissue toxicity. Rapid cell death may cause release of tumor antigens and induction of a host immune response against tumor cells. Vile et al. (12) reported that, following the introduction of a thymidine kinase gene and administration of ganciclovir, there was a marked reduction in B16 melanoma lung metastases in immunocompetent but not immunodeficient mice. They and others propose that necrotic and perhaps apoptotic cell death leads to an inflammatory response and induction of antitumor immunity (13–15).
MetXia-P450 is derived from the Moloney murine leukemia virus–based retroviral vector. The genome is configured to express CYP2B6 from the Moloney murine leukemia virus long-term repeat along with coordinate expression of the Escherichia coli lacZ gene enabled by an IRES sequence (reviewed in ref. 1). All retroviral sequences, apart from those that are essential for packaging, reverse transcription, and integration are removed to prevent replication (16). The use of a human retroviral packaging cell line is used to extend the vectors biological half-life in human serum and to maximize the concentration of vector (17).

Kan et al. (18) showed that in vitro transduction of human HT29 (human colon cancer) and T47D (human breast cancer) cell lines with MetXia-P450 led to sensitization to cyclophosphamide. These observations were replicated in nude mice in vivo using HT29, MDA-MB-231 (human breast cancer), and MDA-MB-468 (human breast cancer) xenografts. MetXia-P450 was directly injected into tumors before administration of i.p. cyclophosphamide. Mice treated with both MetXia-P450 and cyclophosphamide had a significant delay in tumor growth compared with those treated with cyclophosphamide alone or an untreated control group. Histologic sections found <5% of tumor cells expressed lacZ (as an indication of CYP2B6 expression), suggesting a significant bystander effect. Alternatively, MetXia-P450 may induce an antitumor antibody response in addition to the direct cytotoxic activation of cyclophosphamide, thereby potentiating overall potency. The safety of MetXia-P450 was evaluated by i.v. and s.c. administration into mice. No adverse reactions were observed during or after administration, and no abnormalities were found in any organs at pathologic examination of the animals (18).

This phase I study was developed as the first trial of direct intratumoral injection of MetXia-P450 in patients with cutaneous tumor deposits from advanced breast cancer or melanoma. Low-dose oral cyclophosphamide was subsequently administered to provide an assessment of safety of MetXia in the context of cyclophosphamide as well as to provide the opportunity for efficacy. The primary aims of the study were to determine the safety of the vector and to assess the efficiency of gene transfer. Secondary aims evaluated clinical response and the possibility of induction of an antitumor immune response.

PATIENTS AND METHODS

MetXia-P450 Production

MetXia-P450 was introduced into a human cell line derived from the FLYA packaging cell lines as described previously (16). Briefly, cell lines FLYA13 (derived from human fibrosarcoma cell line HT1080) and TEFLYA (derived from human rhabdomyosarcoma cell line TE671) were transduced and produced clones characterized by β-galactosidase expression and ability to secrete retroviral vector. TEFLYA producer cell clone PTC6 showed greatest transducing power and was used for preclinical and clinical testing.

Producer cells were maintained at 80% confluence in growth medium, with vector-containing medium collected and replaced by fresh medium every 24 hours. Viral material was isolated by centrifugation and resuspended in TBS [19.75 mmol/L Tris (pH 7.0), 37.5 mmol/L NaCl, 40 mg/mL lactose, 1 mg/mL human serum albumin, 5 μg/mL protamine sulfate]. Average yield was $4 \times 10^7$ to $8 \times 10^7$ lacZ transferring units/mL. Formulated clinical product (1 mL) was injected into glass vials at three strengths $[8 \times 10^5$ transferring units/mL (1×), $8 \times 10^6$ transferring units/mL (10×), or $8 \times 10^7$ transferring units/mL (100×)] before freezing at $-80^\circ$C. Each batch was tested for identity, impurities, adventitious agents, in vitro potency, and titer. The product was found to be stable at $-80^\circ$C. MetXia-P450 for clinical trials was manufactured under contract by Q-One Biotech Ltd. (Glasgow, United Kingdom).

Patients

Patients with skin nodules from advanced breast cancer or malignant melanoma not suitable for other systemic treatments were entered into the study. Inclusion criteria included histologic confirmation of cancer with at least one cutaneous tumor nodule ≥0.5 cm in diameter, age ≥18 years, WHO performance status 0 to 2, expected survival ≥3 months, adequate hematologic and biochemical function, and no chemotherapy or radiotherapy within 4 weeks (6 weeks for nitrosourea or mitomycin C). Patients with clinical evidence of cerebral metastases or severe intercurrent infection were excluded. The study was approved by the Gene Therapy Advisory Committee and the Central Oxford Research Ethics Committee. All patients gave written informed consent and were treated at the Cancer Research UK Medical Oncology Unit (Oxford, United Kingdom).

Administration and Assessment

Before treatment, eligible patients underwent complete medical history and physical examination, full blood count and biochemical function, staging computed tomographic scan, clinical photographs, electrocardiogram, and, where applicable, pregnancy testing. This phase I study was designed to treat a minimum of three patients at each dose level, with dose escalation only permitted following full safety assessment of patients treated at the preceding dose level after one course of cyclophosphamide. Dose levels were determined pragmatically based on the maximum available dose of MetXia-P450 ($8 \times 10^7$ transferring units/mL), with initial cohorts being treated at substantially lower doses [either $8 \times 10^5$ transferring units/mL (1×) or $8 \times 10^6$ transferring units/mL (10×)]. This cautious approach was chosen to minimize inadvertent toxicity from first administration of a novel viral vector in humans.

MetXia-P450 was administered by two intratumoral injections 24 hours apart. This was an empirical decision based on a balance between patient intervention and preclinical studies, suggesting increased gene expression with increased injections. The vector was thawed for 1 minute in a 37°C water bath before direct injection (within 5 minutes) via a 25 gauge needle. The volume of MetXia-P450 injected depended on tumor size (nominally 1 mL for 0.5-1.5 cm, 2 mL for 1.6-2.5 cm, and 4 mL for >2.5 cm). All treatment was administered in a side room, and before injection, Emla cream (2.5% lidocaine, 2.5% prilocaine) was applied to the tumor nodule for at least 1 hour. Multiple tracts, via a single entry site, were used for injection to maximize distribution of MetXia-P450 within the tumor. After each treatment, the injection site was swabbed and swab tips were placed in a
sterile container containing 2 mL DMEM before storage at −80°C. Venous blood was taken into EDTA-containing tubes preinjection and at 1, 4, and 24 hours after the first injection and again at 24 hours after the second injection. Blood was separated into plasma and peripheral blood mononuclear cell fractions by centrifugation before storage at −80°C.

Further assessment was done in outpatients on day 7. Biopsy of the injected tumor nodules was performed under local anaesthetic. The biopsies were snap frozen in liquid nitrogen, sectioned, and fixed for histologic assessment. Day 7 was chosen as a time point at which the kinetics of vector transduction, reverse transcription, and integration and subsequent expression would have been completed while providing a window of recovery between nodule injection and nodule biopsy. Treatment with cyclophosphamide (100 mg/m² p.o.) was commenced for 14 of every 28 days. Patients were reviewed weekly for the first 8 weeks and at the end of study assessment, including tumor size and appropriate restaging computed tomographic scans done at 12 weeks. Response was assessed according to WHO criteria. Patients with stable or responding disease continued with cyclophosphamide at the discretion of the investigator. Toxicity was graded according to National Cancer Institute common toxicity criteria (version 2). Pain assessment at injection sites was self-rated by patients as none, mild, moderate, or severe. The surrogate tumor markers carcinoembryonic antigen (CEA) and CA15-3 were measured in the serum of patients while on study. Levels of both markers were determined using an ELISA-based methodology according to the manufacturer’s instructions (both Autogen Bioclear UK, Wiltshire, United Kingdom). In view of the study design and the small number of patients in each cohort, formal statistical analyses were not carried out.

Gene Transfer Assessment
Gene transfer was evaluated by histologic assessment of frozen biopsy material. Briefly, 5 μm sections were cut using a cryostat and stained for lacZ expression with X-gal. The transduction efficiency and level of therapeutic gene expression was scored according to the number of positively staining cells. Further analysis of gene transfer efficiency was done using lacZ quantitative PCR from DNA extracted from biopsy material. Data were normalized to the number of cell equivalents sampled with DNA mass determined by glyceraldehyde-3-phosphate dehydrogenase quantitative PCR.

Immunologic Assessment
Humoral Responses. A standard ELISA was used for measurement of both ST4 and CEA specific antibody titers. These surface expressed tumor antigens are commonly associated with breast cancer with preclinical models, indicating that antibodies to ST4 are key for antitumor responses (19). Briefly, 96-well plates (Immulon-4, Dynex, Worthing, West Sussex, United Kingdom) were coated overnight at 4°C in a humid environment with purified antigen (1 μg/mL) diluted in carbonate coating buffer (pH 9.6). Wells were then washed with PBS-Tween and incubated with PBS plus fetal bovine serum (10%) for 1 hour at room temperature to block nonspecific antibody binding. Primary human serum was diluted serially across the plate in PBS-Tween and incubated for 2 hours at room temperature. Wells were then washed five times in PBS-Tween and incubated with an anti-human IgG horseradish peroxidase secondary antibody (1:1,100, DAKO, Ely, Cambridgeshire, United Kingdom) for 2 hours at room temperature. Subsequently wells were washed five times with PBS-Tween and incubated with an OPD substrate (OPD-Fast, Sigma, Poole, Dorset, United Kingdom). The colorimetric change was monitored using a plate reader.

Immunohistochemistry. The expression of tumor-associated antigens on biopsies taken from each patient were determined by immunohistochemistry. Cryostat sections of biopsied tumors were cut at 5 to 7 μm using a Leica CM 3050 S cryostat and fixed in acetone. Endogenous peroxidase activity was quenched by incubating slides in 0.2% hydrogen peroxide in methanol for 10 minutes. Sections were incubated in 5% normal goat serum to block nonspecific protein binding sites before the addition of rabbit anti-CEA antibody (A0115, DAKO) used at a dilution of 1:300 or mouse anti-H8 (hST4) antibody (Oxford Biomedica) used at a dilution of 1:1,000 and incubated for 1 hour at room temperature. Binding of primary antibodies was detected using Vectorstain elite anti-rabbit ABC (PK-6101, Vector Laboratories, Burlingame, CA) or Vectorstain elite anti-mouse ABC (PK-6102, Vector Laboratories) detection kits according to the manufacturer’s instructions. Slides were washed in 10 mmol/L PBS (pH 7.4) containing 0.02% Tween before the application of each reagent. Staining was visualized using 3,3′-diaminobenzidine substrate kit (SK-4100, Vector Laboratories). Sections were counterstained using Mayer’s hematoxylin and mounted in DPX.

Safety Monitoring
Careful assessment of the safety of MetXia-P450 was undertaken. Skin swabs were taken as described 24 hours after each injection. Thawed samples were plated onto HT1080 indicator cells and after incubation stained with X-gal at 48 hours to test for the presence of infectious vector via expression of lacZ. Batched samples of peripheral blood mononuclear cells were thawed for RNA extraction and PCR analysis. The presence of free vector in plasma samples was determined by quantitative real-time reverse transcription-PCR of the lacZ gene. Temporal PCR survey for integrated proviral sequences (targeting lacZ sequences) was done on peripheral blood mononuclear cells from patients 101 to 106, whereas the presence or absence of replication competent retroviruses was assessed by PCR-based assays designed to detect Moloney murine leukemia virus gag-P30 sequences. An increase in signal with time in the samples from an individual patient was used to indicate that a replication competent retrovirus was present.

Further safety assessments evaluated the presence or absence of antibodies against the vector core (gag-P30) or the 4070A envelope protein of the vector. Venous blood samples were taken preinjection and at 3 and 12 weeks (or last time point available if patients came off study before 12 weeks). Clinical-grade MetXia-P450 vectors (105 LTU), tetanus toxin C fragment (100 ng, Quadratech, Epsom, Surrey, United Kingdom), and purified whole human IgG molecules (10 ng, Chemicon, Chandlersford, Hampshire, United Kingdom) were loaded individually onto a polycrylamide gel. After SDS-PAGE, these proteins were transferred onto Hybond enhanced chemiluminescence membrane (Amersham Bioscicences, Chalfont St., Giles, Buckinghamshire, United Kingdom) using a Novex Xcell II
mini-cell and blot module (Invitrogen, Paisley, United Kingdom). The membrane was blocked with TBST (TBS (pH 7.5) with 1% v/v Tween 20) containing 5% (w/v) fat-free dried milk powder. Blocked membrane was then incubated with 50 μL of patient’s serum sample followed by a horseradish peroxidase–conjugated goat anti-human IgG antibody (Chemicon). The presence of patient’s antibodies against the MetXia-P450 vector was visualized by incubation with enhanced chemiluminescence reagents and subsequent exposure to Hyperfilm enhanced chemiluminescence. For the detection of antibodies to the vector core, the same membrane was stripped of any residual antibodies and was reprobed with a rat anti-Moloney murine leukemia virus gag-P30 antibody followed by a horseradish peroxidase–conjugated goat anti-rat IgG antibody (DAKO). The presence of antibodies against vector core was visualized by incubation with enhanced chemiluminescence reagents and exposure to Hyperfilm enhanced chemiluminescence.

RESULTS

Patient Characteristics. Twelve patients were enrolled in the study. Demographic data are listed in Table 1. All patients had progressed after standard treatment. Two patients were treated at 1× dosage, four patients at 10×, and six patients at 100×. All patients received two consecutive daily injections of MetXia-P450 into at least one cutaneous nodule and at least one course of cyclophosphamide. Five patients completed the 12-week study period and seven patients were withdrawn (two patients at 1×, two patients at 10×, and two patients at 100×), with positive results observed in three patients (one at each dose level; Table 2). As a result of the small amount of material used for PCR, these results were at the limit of detection for the assay and the negative results were thought to be due to sampling from areas of tumor that had not been close to injection tracts.

Clinical Response. One (8%) patient with breast cancer (patient 104) had a clinical and radiological partial response. Four (33%) patients with breast cancer (patients 101, 107, 111, and 112) had stable disease for at least 12 weeks on study and the rest (59%) had progressive disease. The surrogate tumor markers CEA and CA15-3 were measured in pretreatment samples from all patients. Elevated pretreatment levels (>10 ng/mL) of CEA were found in patients 101, 104, and 111 and of CA15-3 (>25 units/mL) in six patients (patients 101, 104, 105, 107, 111, and 112). A significant decrease in serum CEA was observed in patient 104 and a decrease in CA15-3 in patients 101 and 104 (Fig. 2A-D). CA15-3 remained stable for patients 105, 107, 111, and 112 while on study.

Patient 104 with breast cancer had a partial response documented for 7 months in skin (Fig. 2E), nodal and hepatic metastases having previously been treated with cytotoxic chemotherapy that included cyclophosphamide, methotrexate, and 5-fluorouracil; mitozantrone and mitomycin C; 5-fluorouracil, epirubicin, and cyclophosphamide; docetaxel; capetcitabine; and vinorelbine. Response was observed at all sites. Interestingly, when her disease subsequently relapsed, she did not have disease progression at the site of the MetXia-P450 injection. Of the patients with stable disease, patient 101 (breast cancer) was noted to have a differential response with a 70% reduction in the size of her injected tumor nodules but no change in the size of her visceral metastases.

Immunologic Response. The potential for gene therapy strategies to induce an antitumor immune response has been shown in animal models (12–15). In this study, preliminary analyses were done to evaluate whether an immune response may have contributed to the observed tumor responses. For patients who completed at least 8 weeks of the study period, tumor biopsy material was stained to detect expression of CEA and hST4, and antibody titers against CEA and hST4 were measured in the serum. Strong expression of ST4 was observed on biopsy material from patients 101, 104, 107, and 111 and of CEA from patients 104, 111, and 112. Representative sections of tumor from patients 101 and 104 stained for CEA and hST4 are shown in Fig. 3. Interestingly, a significant increase in serum anti-CEA and anti-ST4 antibody titers was observed in patients 101 and 104 by 12 weeks but not in the patients who had no evidence of a clinical or tumor marker response (Table 3; Fig. 4).

Table 1  Patient characteristics

| No. patients | 12 |
| Female       | 10 |
| Male         | 2 |
| Tumor type   |    |
| Breast       | 9 |
| Melanoma     | 3 |
| Median age (range), y | 59 (34-74) |
| Eastern Cooperative Oncology Group |    |
| performance status |    |
| 0            | 2 |
| 1            | 8 |
| 2            | 2 |
| Sites of disease* |    |
| Skin         | 12 |
| Liver        | 3 |
| Lung         | 2 |
| Bone         | 6 |
| Nodes        | 5 |
| No. previous chemotherapy schedules |    |
| 1            | 3 |
| 2            | 4 |
| ≥3           | 5 |

*Some patients had more than one site.
mild) were observed at the injection site with symptoms resolving in all patients. The main toxicity observed in the study was attributed to oral cyclophosphamide with the most frequent nonhematologic events described as nausea (nine patients), alopecia (six patients), headache (five patients), anorexia (four patients), and fatigue (four patients). All of these events were grade 1 or 2 by National Cancer Institute common toxicity criteria, with the exception of one patient with grade 3 fatigue. Two patients had clinically significant hematologic toxicity with grade 3 leucopenia and grade 2

Table 2  Gene transfer efficiency

<table>
<thead>
<tr>
<th>Patient</th>
<th>MetXia-P450 dose level</th>
<th>Nodule size [maximum diameter (cm)]</th>
<th>Immunohistochemical detection of β-galactosidase</th>
<th>PCR detection of transduced cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Week 0</td>
<td>Week 12</td>
<td>Biopsy 1</td>
</tr>
<tr>
<td>101</td>
<td>1×</td>
<td>1.3</td>
<td>0.8</td>
<td>Positive</td>
</tr>
<tr>
<td>102</td>
<td>1×</td>
<td>1.2</td>
<td>NA</td>
<td>Negative</td>
</tr>
<tr>
<td>103</td>
<td>10×</td>
<td>1.7</td>
<td>NA</td>
<td>Negative</td>
</tr>
<tr>
<td>104</td>
<td>10×</td>
<td>0.9</td>
<td>0.9*</td>
<td>Positive</td>
</tr>
<tr>
<td>105</td>
<td>100×</td>
<td>2.1</td>
<td>NA</td>
<td>Positive</td>
</tr>
<tr>
<td>106</td>
<td>100×</td>
<td>2.0</td>
<td>NA</td>
<td>Positive</td>
</tr>
<tr>
<td>107</td>
<td>100×</td>
<td>1.0</td>
<td>1.2</td>
<td>Positive</td>
</tr>
<tr>
<td>108</td>
<td>100×</td>
<td>0.6</td>
<td>NA</td>
<td>Positive</td>
</tr>
<tr>
<td>109</td>
<td>10×</td>
<td>0.9</td>
<td>NA</td>
<td>Negative</td>
</tr>
<tr>
<td>110</td>
<td>10×</td>
<td>1.6</td>
<td>NA</td>
<td>Negative</td>
</tr>
<tr>
<td>111</td>
<td>100×</td>
<td>0.8</td>
<td>1.0</td>
<td>Positive</td>
</tr>
<tr>
<td>112</td>
<td>100×</td>
<td>0.8</td>
<td>0.9</td>
<td>Positive</td>
</tr>
</tbody>
</table>

NOTE. PCR values in parentheses are normalized values where the PCR is normalized to the number of cell equivalents. Biopsy 1 and 2 refer to two core biopsies from different areas of the same lesion.

Abbreviations: ND, not done; NA, not available; NQ, detected but not quantifiable.

*Outline of lesion still measured at week 12 but had become flat with no erythema. Subsequently the lesion resolved and this patient was therefore considered to have had a partial response.

Pt-101

Pt-104

Pt-105

Fig. 1  Histologic sections showing gene expression. Photomicrographs illustrating representative sections from biopsies taken from three patients after intratumoral injection of MetXia-P450. Cells are stained with X-gal for the expression of β-galactosidase as described in Patients and Methods. The presence of blue cells indicates gene expression from E. coli lacZ.
neutropenia. No patients were admitted with neutropenic fever. During the study, three patients with breast cancer experienced serious adverse events (patient 103 hypotension, dysarthria, and left-sided weakness thought to be due to cardiovascular disease; patient 104 trismus due to use of prochlorperazine; and patient 108 deep vein thrombosis, hypotension, and dyspnea following disease progression). None of the serious adverse events were considered related to MetXia-P450.

Safety Assessment. The presence of MetXia-P450 was assessed by skin swabs taken from the injection site, presence of vector in peripheral blood, and detection of anti-gag-P30 and 4070A envelope antibodies in serum. Low levels of residual vector (~2 transferring units/mL) were detected in swabs from one patient in the 100× group at 24 hours after each injection. Despite this being at the detection limit for the assay, modification to the disinfection method for patients 109 onward was made with the addition of a further ethanol wipe at the injection site. There was no subsequent detection of viable vector at the skin site. Free vector was only detected by reverse transcription-PCR in patients treated with 100× at 1 hour [4 of 6 (67%)] and 4 hours [1 of 6 (17%)] with no free vector detected in venous blood from any patient 24 hours after either injection. Antibodies against gag-P30 were detected in three patients at week 3. One of these patients also had antibodies detected in the pretreatment serum and 12-week serum. The other two patients were withdrawn from the study with progressive disease before

![Fig. 2](clincancerres.aacrjournals.org) Measurement of surrogate markers of tumor response (A-D). Circulating plasma levels of the surrogate markers CEA (A and C) and CA15-3 (B and D) in patients 101 (A and B) and 104 (C and D) are illustrated throughout the 12-week clinical follow-up period. Results are expressed as ng CEA/mL plasma or as units CA15-3/mL plasma. E, clinical photographs of skin response in patient 104.
further serum samples were taken. Western blots for the presence of antibodies to vector envelope protein were negative in all patients at each time point evaluated.

**DISCUSSION**

Conversion of a nontoxic prodrug to an active metabolite within a cancer cell provides a potential technique for delivery of high local concentrations of cytotoxic chemotherapy to the target tumor. In this phase I study, MetXia-P450 was directly injected into at least one cutaneous tumor deposit on 2 consecutive days with biopsy of the nodule 1 week later. Low-dose oral cyclophosphamide was subsequently administered. Direct injection was chosen to maximize local delivery to the tumor. Previous experiments showed that this technique did not impact on vector viability and could be used to transduce mouse xenograft models (18). Although accepting the limitations of this approach for the management of systemic metastases, the patients in this study all had accessible cutaneous metastases, with the principle end point for the study being the safety assessment of MetXia-P450. In this trial, expression of β-galactosidase as a surrogate indicator for CYP2B6 showed that gene transfer was achieved in most (10 of 12) patients. However, histologic staining with X-gal showed only low levels of transduction, with <1% of cells expressing lacZ. PCR techniques in three of six biopsies assessed confirmed this low but consistent level of lacZ expression. The reason that PCR seemed less sensitive is due to the level of expression being at the limit of detection of the assay for the small amount of biopsy material used. There was no evidence of increased transduction efficiency with higher dose levels, although the minimally invasive biopsy procedure was not likely to yield this level of qualitative analysis.

**Table 3** Summary of pretreatment serum CEA and CA15-3, immunohistochemical staining of CEA and h5T4 expression by tumor cells, and h5T4 and CEA antibody titers in patients completing at least 8 weeks of treatment

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Clinical response</th>
<th>Pretreatment serum†</th>
<th>Tumor biopsy‡</th>
<th>Antibody titers (wk postinjection)§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CA15-3</td>
<td>CEA</td>
<td>CEA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CEA</td>
<td></td>
</tr>
<tr>
<td>101</td>
<td>SD</td>
<td>+</td>
<td>+</td>
<td>--</td>
</tr>
<tr>
<td>104</td>
<td>PR</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>105</td>
<td>PD</td>
<td>+</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>107</td>
<td>SD</td>
<td>+</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>111</td>
<td>SD</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>112</td>
<td>SD</td>
<td>+</td>
<td>--</td>
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</tbody>
</table>

*SD, stable disease; PR, partial response; PD, progressive disease.
†+, elevated at baseline (CEA >10 ng/mL or CA15-3 >25 units/mL); --, within reference range.
‡+, positive staining; --, negative staining.
§−, negative; +, titer >1:20; ++, titer >1:40; ++++, titer >1:160; NA, not available.
Efficient gene transfer has been one of the main limiting factors in gene therapy development. Retroviral transduction of cancer cells is a multistep process dependent on diffusion and absorption of viral particles onto the cell surface, binding of the viral envelope to the plasma membrane, absorption into the cell nucleus, and integration of a DNA copy of the retroviral genes into the cancer cell genome. Failure of any one step in the process will prevent transduction and viral gene expression. Despite direct injection, the technique of administration is limited by the distribution of the vector along the needle tracts with incomplete coverage of the whole tumor nodule. The low levels of expression of \( h-\)galactosidase observed in this trial are comparable with those observed in a recently published study using adenovirus-mediated gene therapy directly injected into recurrent gliomas via an implantable catheter. Resection material from that trial only found transfected cells on average within 5 mm of the injection site (20).

Whereas a low level of transduction is disappointing, even small increases in metabolism of cyclophosphamide could lead to a significant increase in tumor cell kill because of diffusion of the metabolites to neighboring cells leading to a bystander effect (9, 21). Indeed, in mouse xenografts, a significant reduction in tumor growth rates were seen with MetXia-P450 plus cyclophosphamide when compared with cyclophosphamide alone, with a transduction efficiency of <5% (18). However, the relative contribution of MetXia-P450 CYP2B6 metabolism of cyclophosphamide compared with hepatic metabolism is not known for patients treated in this clinical trial.

Although primarily evaluating the efficacy of gene transfer, this study also looked at clinical and immunologic responses to MetXia-P450 in combination with low-dose oral cyclophosphamide. Tumor markers CA15-3 and CEA were measured to provide surrogate indicators of response, whereas tumor expression of and antibodies against CEA and hST4 were studied for evidence of immunologic activation. ST4 is an oncofetal antigen that is frequently overexpressed in tumor cells, including colon, gastric, and breast cancers, but rarely expressed in normal tissue. Overexpression has been associated with a worse prognosis in gastric tumors (19, 22, 23). In the current trial, one heavily pretreated patient with breast cancer (patient 104) had a documented partial response both at the injected lesion and at distant sites (Fig. 4C). Her previous treatment had included two schedules of chemotherapy that contained i.v. cyclophosphamide. Although not meeting formal WHO response criteria, another patient had evidence of tumor response at the site of the injected lesion only. In both of these patients, there was a decrease in serum CEA associated with an increase in anti-CEA and anti-ST4 antibody titers during the 12-week study period. It was noticeable that the tumor biopsy from patient 104 strongly expressed both CEA and ST4, whereas the biopsy from patient 101 strongly expressed ST4. The responses are most likely to be due to the activity of low-dose cyclophosphamide alone. However, other mechanisms of action are possible. Although the results are preliminary, the presence of antibody induction in one patient with a documented clinical response and in another with stable disease associated with a significant decrease in serum CA15-3 raises the possibility of an antitumor immune effect. This may be due to the actions of cyclophosphamide associated with tumor necrosis but may also be partially attributed to the potential increased cell kill and release of tumor-associated antigens at the site of MetXia-P450 injection. This phenomenon of antitumor immune bystander effects, following gene-directed enzyme prodrug therapy, has previously only been reported in animal studies (12, 13, 15) and requires further evaluation in clinical trials. A further possible mechanism for antitumor activity may relate to the possibility of antiangiogenic activity of low-dose cyclophosphamide. Although not directly comparable, a recent phase II clinical trial with low-dose cyclophosphamide and methotrexate in patients
with breast cancer found a response rate of 19% with a proposed antiangiogenic mechanism of action (24).

An important end point of this study was to determine the safety and toxicity of MetXia-P450. Extensive monitoring was done for the presence of viable vector at the injection sites as well as any systemic effects. Swabs from the injection site showed the presence of viable vector in one patient at 24 hours. For all subsequent patients, the procedure to disinfect the skin was modified to include a second ethanol wipe of the injection site. No viable virus was detected at the injection sites in the patients treated after this modification. The presence of vector in the plasma of patients treated was assessed by real-time PCR. Free vector could be detected in four of six patients at the 100× level 1 hour after injection and in one of these patients at 4 hours. No free vector was detected in any patient 24 hours after injection. Anti-gag-P30 antibodies were detected in three patients 3 weeks after injection (one of these patients had pretreatment antibodies that persisted at 12 weeks). These assessments suggest that low levels of MetXia-P450 reach the systemic circulation in patients treated by intratumoral injection at the 100× strength, with a small proportion of patients developing an immune response. Direct toxicity from MetXia-P450 was minimal. A few patients reported pain, bleeding, or inflammation at the injection site, but in all cases this had resolved within 48 hours. No systemic toxicity or serious adverse events were associated with MetXia-P450. The only toxicities observed were all attributed to oral cyclophosphamide, with two patients experiencing significant neutropenia. Nonhematologic toxicity was mild, with nausea, alopecia, headaches, fatigue, and anorexia reported.

In conclusion, this phase I study showed that intratumoral injection of MetXia-P450 is safe and well tolerated. Low but consistent levels of gene transfer were observed at all dose levels, suggesting that expression of CYP2B6 from MetXia-P450 can be achieved within cancer cells. Studies to optimize gene transfer are ongoing. The possibility of induction of an antitumor immune effect associated with clinical response is intriguing and the subject of further clinical trials.

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REFERENCES

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