Tumor Efficacy and Bone Marrow-sparing Properties of TER286, a Cytotoxin Activated by Glutathione S-Transferase

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ABSTRACT
TER286 is a latent drug activated by human glutathione S-transferase (GST) isoforms P1-1 and A1-1 to produce a nitrogen mustard alkylating agent. M7609 human colon carcinoma, selected for resistance to doxorubicin, and MCF-7 human breast carcinoma, selected for resistance to cyclophosphamide, both showed increased sensitivity to TER286 over their parental lines in parallel with increased expression of GST P1-1. In primary human tumor clonogenic assays, the spectrum of cytotoxic activity observed for TER286 was both broad and unusual when compared to a variety of current drugs. In murine xenografts of M7609 engineered to have high, medium, or low GST P1-1, responses to TER286 were positively correlated with the level of P1-1. Cytotoxicity was also observed in several other cell culture and xenograft models. In xenografts of the MX-1 human breast carcinoma, tumor growth inhibition or regression was observed in nearly all of the animals treated with an aggressive regimen of five daily doses. This schedule resulted in a 24-h posttreatment decline in bone marrow progenitors to 60% of control and was no worse than for a single dose of TER286. These studies have motivated election of TER286 as a clinical candidate.

INTRODUCTION
Despite problems with short-term and long-term toxic side effects, cytotoxic chemotherapy continues to be the mainstay of curative treatment for metastatic malignancies, whether prescribed as adjuvant therapy for local disease or for advanced malignancies, and is also an important component of regimens to palliate symptoms of late-stage disease. The narrow therapeutic index of current agents has precluded substantive dose intensification, exacerbated by the development of drug resistance. Attempts at circumventing prominent toxicities by use of antiemetics (2) and bone marrow-rescuing agents (3) have resulted in better-tolerated drug regimens but have not improved cure rates significantly (4). Improving the absolute therapeutic index of cytotoxins requires more effective protection for sensitive tissues such as bone marrow (5) or identification of tumor phenotypes susceptible to selective targeting (6–8). GST is a promising target in this regard, because it is elevated in many human tumors, is associated with drug resistance and poor prognosis, and can be selectively targeted via a latent drug activated by the catalytic activity of GST.

Over the past few years, overexpression of GST has become well established as a tumor phenotype. There are more than 10 human isoforms, present as dimers of subunits from within one of several distinct families (9); the most prevalent form in tumors is GST isoform P1-1. Following up early research (10–12), elevated GST in general, and P1-1 in particular, has been shown by several lines of evidence to be a strongly associated tumor phenotype for most major tumor types. Methods used have included a sensitive high-resolution chromatographic analysis of a comprehensive set of human tumor specimens compared to matched normal tissue (13), immunohistochemical staining of tumor specimens (14, 15), GST expression monitoring in the NCI cell line panel (16), and analysis of gene amplification in breast tumor specimens (17).

GST can detoxify certain chemotherapeutics via conjugation to the abundant intracellular peptide glutathione (18–20). Furthermore, overexpression of GST through gene transfer can induce drug resistance in some instances (21–23), and GST inhibition can sensitize cells to a modest degree (24, 25). Correlation of elevated GST with poor prognosis has been established in several studies (15, 26–30), although it is not clear whether this effect is mediated via a direct link to drug detoxification or via modulation of signal transduction pathways implicated in apoptosis (31). Regardless of mechanism, however, elevated GST P1-1 level is a robust tumor-associated phenotype and thus offers an opportunity for tumor-specific targeting.

TER286 exemplifies a class of compounds activated by GST, which differ in their isozyme selectivity and in the active agent released (32, 33). In the normal catalytic mechanism of GST P1-1, tyrosine-7 stabilizes the thiolate form of the glutathione cosubstrate, making it more nucleophilic and thus able to detoxify electrophiles of mitochondrial or exogenous origin (34–36). For compounds such as TER286, in which the sulfhydryl of a glutathione conjugate has been oxidized to a sulfone, tyrosine-7 promotes a β-elimination reaction that cleaves the compound. The cleavage products are a glutathione analogue and a phosphorodiamidate, which in turn spontaneously forms aziridinium species, the actual alkylating moieties (Fig. 1). In addition to phosphorodiamidate-containing compounds such as TER286, other active agents can also be liberated following a β-elimination if linked to the sulfone via a urethane bridge (33).

For TER286, the major activating isozymes are GST P1-1 and A1-1. The former is the most commonly elevated tumor GST, and the latter is representative of a family of GSTs that show strong in vitro detoxification activity against common alkylating agents. The aziridinium ring formed from the released nitrogen mustard is the same proximal alkylating species that is generated by known clinically active antitumor drugs, such as melphalan and cyclophosphamide. Previous work has shown that the appropriate cleavage products are generated by GST under physiological conditions (32, 33). In the present report, the antitumor and myelosuppressive effects of the compound are described.

MATERIALS AND METHODS

GST-activated Latent Drug. The synthesis and biochemical characterization of TER286 are fully described elsewhere (33). For cell culture testing, the drug was dissolved in DMSO (final DMSO concentration, <1%). For most in
cells were suspended at 1 × 10^5 cells/ml in RPMI 1640 containing 10% PCS; and chlorodinitrobenzene-conjugating activity were assayed by standard chromatography column (VWR Scientific; Brisbane, CA). Protein concentration was measured at 650 nm in a V_max plate reader (Molecular Devices; Menlo Park, CA). For the cyclophosphamide-resistant and wild-type MCF-7 experiments, cells were treated in suspension for 2 h in the presence of varying amounts of drug, diluted, and plated at clonaling density in quadruplicate. Plates were incubated for 2 weeks, and colonies were fixed in 95% ethanol and stained with crystal violet for colony counting.

Drug sensitivity of NIH-3T3 variants was evaluated in a soft agar clonogenic assay as described previously (39). Briefly, cells were suspended in 0.8% agar in DMEM plus drug and plated over a nutrient feeder layer of 0.5% agar in 35-mm flat-bottomed wells in the presence of drug at 0–150 μM. Colonies of >50 μm diameter were counted at the end of 10 days.

**Biopsied Tumor Clonogenic Assay.** Primary breast and lung tumor biopsies were initiated into culture using a two-layer soft agar protocol (42). Drugs were added at the initiation of the culture and were normally removed after 1 h. For standard chemotherapeutic drugs, cell culture dose levels were 1 or 2 times the clinical peak plasma level. TER286 was tested at 50 μM, well below the plasma concentration of 75 μM achieved in normal mice 10 min after a single 200 mg/kg i.p. dose and in the range for which activity was seen in culture with established cell lines. Because TER286 has an in vitro half-life of ~50 min in human blood or plasma and only ~3-fold longer in pH 7.5 buffer in the absence of cells (33), the compound was simply left for the duration of the culture period for most studies; the cellular exposure to active drug (area under the curve) was estimated to be equivalent to providing the drug at 60 μM for 1 h. The number of colonies was scored at the end of 2 weeks in culture. Treatment conditions that resulted in ≤50% control colony numbers were considered positive results. All but one of the lung samples were non-small cell tumors. All 20 breast specimens were confirmed carcinomas of varying types, including primarily infiltrating ductal carcinoma (n = 8) and adenocarcinoma (n = 7).

For clinical leukemia samples, specimens were obtained from bone marrow or peripheral blood. In the former instance, 5–10 ml of bone marrow were collected in RPMI 1640 with preservative-free heparin; peripheral blood was collected in heparinized tubes. As previously described (43), the leukemic cells were purified to >90% by centrifugation on sodium metrizoate/Ficoll, washed once, and then suspended with RPMI 1640. TER286, daunorubicin, or melphalan stock solutions were diluted at least 10-fold and incubated in sealed polypropylene tubes in a shaking water bath at 37°C with leukemic cells at 1.1 × 10^6 cells/ml in RPMI 1640 Glutamax with 10% FCS for a continuous incubation for TER286 and for 1 h for the others followed by centrifugation and suspension in fresh medium. As described (44), 2-ml aliquots of cells were cultured at 37°C in 5% CO2 humidified atmosphere for 4–5 days, and the toxic effects of the different drugs were determined by measuring the levels of ATP. Briefly, cellular ATP was extracted and ATP-degrading enzymes were inactivated by treatment with 2.5% trichloroacetic acid, followed by quantitation against standards using a 96-well microplate luminescence assay (45). The majority of the patients (n = 32) had AML; four had acute lymphocytic leukemia, and two had myelodysplastic syndrome. Not all of the samples were evaluated for each drug tested (n = 27–39).

**Tumor Xenographs in Mice.** BALB/c nude mice were implanted s.c. with tumors from donor mice. When tumors reached 20–25 mm³, mice were randomized into treatment groups. M7609 xenographs were treated with a single i.v. injection of 150 mg/kg TER286. Other xenografted mice were treated with a single i.p. dose, 400 mg/kg TER286 or 300 mg/kg cyclophosphamide, or with five daily i.p. doses of 200 mg/kg TER286 or 125 mg/kg cyclophosphamide. Mice were monitored for weight changes, and tumor volumes were determined by measurement with calipers. The experiment was terminated when the average size of the tumor in the control group reached 1000–2000 mm³, the timing of which varied depending on the tumor line. Following sacrifice, tumors were excised; for M7609 cells, tumor volume was calculated as ½πr^2h where r and h are the major and minor axes of the lump (46); the others were simply weighed. Percentage tumor growth inhibition was...
calculated as follows:

\[
\text{% tumor growth inhibition} = \frac{\text{Tumor weight of treated animals}}{\text{Tumor weight of control animals}} \times 100
\]

**Bone Marrow GM-CFC.** Femoral bone marrow specimens from male BDF1 mice (three mice/treatment group) were extracted at various times following i.p. treatment with TER286 or control vehicle as specified. Viable nucleated cells were plated in quadruplicate at \(4 \times 10^6\) cells/ml, 1 ml/35-mm plate, using conditioned medium from pokeweed mitogen-stimulated mouse spleen cells to select for growth of GM progenitors. Colonies containing \(\geq 50\) cells were counted on day 7.

**Peripheral Blood Analysis.** At various times after treatment, five mice/treatment group were anesthetized with CO\(_2\) and blood was collected from the retroorbital sinus into EDTA-containing tubes. Hematology analyses were performed the same day using a Technicon H1 analyzer (Bayer Diagnostics, Laguna Hills, CA).

**RESULTS**

**Cultured Cell Lines.** Under physiological buffer conditions and enzyme concentration, recombinant human GST P1-1 and A1-1 cleave the latent drug TER286 with a half-life of 50 and 67 min, respectively, compared to 175 min for either GST M1-1 or spontaneous decomposition in the absence of any GST (33). To test the hypothesis that increased cytotoxic activity of TER286 would be seen in cells with higher levels of GST P1-1, a variety of cell culture studies were conducted.

The first two experiments examined the effect of TER286 on GST P1-1-transfected MCF-7 and NIH-3T3 cells. MCF-7 cells have background GST activity ~10% of that seen in most cell lines, and the MCF-7-\(\pi\) transfects express a 4-fold increase in P1-1 compared to MCF-7-neo (vector control; Ref. 21). The only other GST detected in this pair of cell lines was GST M3-3, and the levels were equivalent in the two cell lines. The LD\(_{50}\) for a 2-h exposure to TER286 was 4 times lower in the MCF-7-\(\pi\) cells than the control cells (Fig. 2A). In the case of the NIH-3T3 cells (Fig. 2B), a ~5-fold increase in GST P1-1 over a low baseline was accompanied by a ~2-fold drop in LD\(_{50}\) (39).

In the next two experiments, TER286 was tested in cells selected for drug resistance. MCF-7/OAP is 9-fold more resistant than the parental MCF-7 human breast tumor line to the cyclophosphamide analogue oxazaphosphorine (37) but is 1.8-fold more sensitive to elevated GST; drug was never removed, with colonies >50 \(\mu\)m in diameter counted after 10 days. c, MCF-7 parental line compared to subline selected for resistance to a cyclophosphamide analogue; drug was removed after 2 h followed by 5 days of growth before viable cell counting. d, M7609 parental line compared to subline selected for resistance to doxorubicin; drug was never removed, with viable cell counting after 48 h. Data are means; bars, SE.

**Clonogenic Assays.** The cell culture data establish that TER286 is a cytotoxic agent that is active against a number of different human tumor cells established in long-term culture. Previous work established that the primary activating enzyme, GST P1-1, is elevated compared to adjacent normal tissue in a substantial majority of human tumor specimens from several major tumor types (10-15). To further evaluate the likelihood that TER286 would have clinical activity, both lung and breast tumor specimens were examined using a primary tumor biopsy clonogenic assay (49). A total of 41 samples (21 from lung and 20 from breast) have been tested with TER286, derived equally from patients who had received no prior chemotherapy or at least one regimen of chemotherapy. As described in more detail elsewhere (50), the results indicate that TER286 is an active antitumor agent. Overall, TER286 produced a positive response in ~50% of the samples, whether taken altogether or classified by tumor type and treatment history.

These data were collected for tumor specimens being evaluated as part of the treatment decisionmaking process, and not all of the specimens were treated with all of the drugs (Table 1). Nonetheless, comparing TER286 to the other drugs under consideration suggests that the new compound has a novel spectrum of activity (Fig. 3), with several striking comparisons. For example, only 1 of 16 lung tumor specimens tested with cisplatin showed a response, whereas 11 of

\[4\text{ A. Monks, personal communication.}\]
**Table 1** TER286 cytotoxicity in clonogenic assays on primary human tumor specimens

Data are shown as percentage survival of treated specimens compared to untreated controls. Specimens are arranged in order of responsiveness to TER286. See Fig. 3 for a graphical comparison to representative current drugs.

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<th>TER286 50 μM 1 h</th>
<th>TER286 50 μM 2 weeks</th>
<th>Taxol 0.2 μM 1 h</th>
<th>Cisplatin 0.7 μM 1 h</th>
<th>Etoposide 5 μM 1 h</th>
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*Blank entries, not tested; NSC, non-small cell lung carcinoma; SC, small cell lung carcinoma; A, lung adenocarcinoma; S, lung squamous cell carcinoma; B, breast carcinoma.

**Positive responses (<50% survival).**

These 16 responded to TER286. Of 13 breast tumor specimens tested with both Taxol and TER286, 6 responded to each; of the 6 responding to TER286, however, only 1 also responded to Taxol. Of 12 breast tumor specimens tested with both TER286 and cyclophosphamide, the response rate for TER286, 6 of 12, was twice that achieved by cyclophosphamide.

For these studies, standard drugs were tested at clinically relevant levels, 1 or 2 times the peak plasma levels with a 1-h treatment regimen. TER286 has not yet been tested in humans, and therefore attainable plasma levels are not known. To obtain an estimate, mice were given a single 200 mg/kg dose i.p., which was well tolerated (see below). The peak plasma level was 75 μM at 10 min later, dropping to 20 μM by 30 min. To generate an exposure (area under the curve) equivalent to this murine model for the clonogenic assays, the concentration of TER286 needed in the culture was estimated to be 125 μM. The normal concentration actually used for the clonogenic studies was conservatively chosen to be 50 μM, which is also near the LD₅₀ averaged over all cell lines in culture. In light of its limited half-life, no effort was made to remove TER286 during testing for most of the work. The reduced efficacy seen in some samples treated with a strict 1-h drug incubation format (Table 1) may reflect delayed cell permeation, perhaps due to adsorption to serum proteins.

Additional insight into the dose-response relationship was obtained in a more extensive dose comparison series of experiments in which TER286 was examined for cytotoxicity to tumor cells from a set of 40 patients with leukemia, mainly AML. As for most other tumor types, a high level of GST P₁-1 is common in leukemia samples (28). Fig. 4 shows that a strong response to TER286 was obtained at the normal 50 μM level. Even 10 μM TER286 resulted in growth suppression that exceeded that obtained in a parallel treatment with melphalan or daunorubicin; for the latter, the dose used in vitro yielded the same intracellular drug concentration as during infusion in vivo (44).

**Xenografted Tumor Models.** The M7609 parental line and its two variants studied in culture were also transplanted into nude mice. Once the tumors were 5 mm in diameter, the mice were treated with a single i.v. dose of 150 mg/kg TER286. The resulting tumor growth rates were inversely related to the level of P₁-1 expressed in the tumor. M7609/ADR (which has P₁-1 increased ~2-fold) was the most sensitive to TER286, whereas M7609/anti (which has P₁-1 decreased by ~2-fold), was the least sensitive (Fig. 5). There was no mortality from TER286, and the animals appeared lively, without weight loss.

Because the released toxic moiety of TER286 is similar to the active species generated from cyclophosphamide, efficacy was examined in human tumor xenografts that vary in responsiveness to this agent (Table 2). Both drugs were used at their approximate maximum tolerated dose. All tumor models showed a dose-dependent response.
Breast tumor treated with an aggressive regimen (daily for 5 days), n = 39.

HT-29 human colon carcinoma, which is known to have high levels inhibited or substantially regressed. The worst response was for the MX-1 human
AML (50 Î¼M TER286, n = 33; 10 Î¼M TER286, n = 27; melphalan, n = 36; daunorubicin.

Indicated doses. Means (bars, SE) from a total of 40 patients, the majority diagnosed as

Positive response. 

Quantitation and tumor type codes. Survival of <50% of control colonies is deemed a
to TER286. The best response to TER286 was for the MX-1 human breast tumor treated with an aggressive regimen (daily for 5 days), under which nearly all of the tumors were either severely growth inhibited or substantially regressed. The worst response was for HT-29 human colon carcinoma, which is known to have high levels of

A variety of detoxification enzymes (51). Given the different mechanisms of activation for TER286 and cyclophosphamide and the tetra-functional versus bifunctional presentation of the alkylating species, precise analysis of the comparative dose-response profiles of the two drugs is not possible without more extensive pharmacokinetic data. In addition, the background GST composition of mice is different from that of humans, with mice having higher specific activity per gram of tissue. Moreover, although the tumor lines had similar levels of GST P1-1 protein, representing ≥70% of total GST, they had distinctive patterns of various other GSTs, and they presumably differ in a variety of other aspects as well.

Myelosuppression. The xenograft experiments confirmed expectations from cell culture of a dose-response relationship for TER286, as is true for other nitrogen mustard alkylating agents (1). In addition, more frequent treatment with TER286 was more effective. Accordingly, toxicity of this compound to bone marrow, which is dose limiting for alkylating agents (52), was examined following both single and multiple doses. Freshly isolated mouse bone marrow cells were treated with varying doses of TER286, yielding a LD50 of 333 ± 10 Î¼M for the GM lineage progenitors. As noted above, i.p. treatment of mice at 200 mg/kg yields a peak plasma concentration of 75 Î¼M.

With a single 200 mg/kg i.p. dose of TER286, a transient decrease in bone marrow GM-CFC to 60% of normal was observed at 24 h, followed by a modest compensatory increase peaking on day 5 at 65% above control levels, with a return to control levels by day 10 (Fig. 6a). After the same dose was given daily for 5 days, GM-CFC levels at 24 h after the last dose were again 60% of normal; the daily for 5 days regimen with lower doses produced less of an effect (Fig. 6b). Prior reports indicate that a single dose of cyclophosphamide, at 200 mg/kg, can induce a similar compensatory increase (53), although the 24-h nadir is much lower (13% of control) and unlike TER286, the overshoot is followed by a steep decrease to subnormal values (54).

Peripheral blood counts were measured for 20 days following a single i.p. treatment with 400 mg/kg TER286 or 300 mg/kg cyclophosphamide, which are approximately equipotent in xenograft mod-
BONE MARROW-SPARING CYTOTOXIN

Table 2 GST isozyme profiles of cell lines tested as xenografts in nude mice shown along with tumor growth (% of control) under different treatment regimens

TER286 was given in Cremaphor vehicle, cyclophosphamide in saline. Data are means ± SE of excised tumor weight compared to untreated control for 10 mice/group.

<table>
<thead>
<tr>
<th>Tumor line</th>
<th>GST profile</th>
<th>Tumor growth (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Content (% of total)</td>
<td>TER286 (400 mg/kg, single dose)</td>
</tr>
<tr>
<td>MX-1</td>
<td>P1 91</td>
<td>32 ± 4</td>
</tr>
<tr>
<td></td>
<td>A1 9</td>
<td></td>
</tr>
<tr>
<td>SK-MES</td>
<td>P1 85</td>
<td>66 ± 9</td>
</tr>
<tr>
<td></td>
<td>A2 11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M1 4</td>
<td></td>
</tr>
<tr>
<td>DLD-2</td>
<td>P1 68</td>
<td>47 ± 10</td>
</tr>
<tr>
<td></td>
<td>A1 13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M1 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M2 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other 4</td>
<td></td>
</tr>
<tr>
<td>MV-522</td>
<td>P1 89</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>A1 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A2 5</td>
<td></td>
</tr>
<tr>
<td>HT-29</td>
<td>P1 80</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>A2 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M1 12</td>
<td></td>
</tr>
</tbody>
</table>

Ter 

Half of the animals showed some regression; figure given is percentage of control for the others.

NT, not tested.

e. As shown in Fig. 7, TER286 is more sparing to the WBC compartment, particularly to lymphocytes and to a lesser degree to neutrophils. Platelets and RBCs were essentially unaffected by either drug. Given i.p. in an aqueous citrate formulation, TER286 at 400 mg/kg produced 11% mortality, but the effects on peripheral blood were similar to that seen with 200 mg/kg (data not shown), suggesting the cause of death was not due to myelosuppression. In a Cremaphor vehicle, 400 mg/kg TER286 has shown variable mortality (0–11%) as well as variable collateral toxicity. More detailed pharmacokinetic studies will be needed to find the optimal regimen.

DISCUSSION

A major stimulus for studies of tumor GST expression was its postulated role in mediating drug resistance to a wide range of DNA-damaging electrophilic drugs (18, 55). The data accumulated over the past few years have revealed a more complex role for this abundant cellular protein, which can account for 5% of soluble protein in some tumors. Although GST can detoxify certain chemotherapeutics, inhibition only potentiates drug efficacy by 2–3-fold (24, 25, 55, 56). Despite this modest effect, elevated levels of GST Pl-1 are at least as well correlated with poor prognosis as a number of other prominent tumor phenotypes (26–30). There is also a high incidence of elevated GST at primary presentation with no prior treatment. The reasons for this strong tumor association are as yet unclear, but it may reflect a response to the necrotic environment of solid tumors as part of a coordinated regulation of stress response genes (31).

Irrespective of the reasons for GST elevation, the common occurrence of the phenomenon provides an opportunity for preferentially targeting a cytotoxin to cancer cells in an approach that may be applicable to a majority of patients for many important tumor types. Prostate cancer is the only notable exception to the general finding of elevated GST Pl-1 in tumors (57). Because the overexpression of GST Pl-1 creates an increased catalytic function in tumors, it seemed appropriate to turn this property against the tumor by means of a latent drug, TER286, which is activated by GST to produce a cytotoxic nitrogen mustard.

Of the major isozymes, TER286 is activated by GST Pl-1 and A1-1 but not M1a-1a. Pl-1 is the most prevalent GST isozyme in tumors, and α-class GSTs have been shown to directly inactivate a number of drugs (19). Other active agents can also be released via GST activation, which may be advantageous in certain circumstances; similarly, selectivity with regard to GST isozymes can also be enhanced if necessary (33).

The evidence presented here that TER286 is an active antitumor agent comes from studies on established cell lines, clonogenic assays
on primary tumor biopsies, and xenograft models. In all cases, the compound behaves as expected for a nitrogen mustard alkylating agent, with tumor cytotoxicity increasing with dose. Unlike other nitrogen mustards, for which elevated GST is associated with reduced efficacy, the antitumor activity of TER286 is generally increased in proportion to the GST level for paired cell lines. Particularly encouraging are the results with two different cell lines selected for resistance to a conventional DNA-damaging agent, each with an accompanying increase in GST level. In both cases, TER286 is more effective against the resistant line than the parental line.

Clonogenic studies on primary tumor specimens are perhaps the most indicative of what might be expected for TER286 clinically because there can be substantial changes in GST profile during adaptation as long-term stable cultured lines. Meta-analysis of more than 2000 uses of clonogenic assays in conjunction with clinical treatment has shown that failure of a drug in this assay is associated with clinical failure 90% of the time; conversely, success in the assay is associated with clinical success 70% of the time (49). These retrospective results on known drugs provide an incentive for testing TER286 clinically, because it performed at least as well in these assays as existing drugs.

The expectation of novel activity is reasonable in light of the evidence for a novel mechanism of TER286, which is particularly well illuminated by studies on variants of M7609 colon carcinoma cells. A doxorubicin-resistant subclone has elevated levels of GST, whereas an antisense-transfected subclone has decreased levels of GST. The efficacy of TER286 is directly related to the GST level in these three lines that are otherwise quite comparable.

A common dose-limiting side effect of alkylating agents is bone marrow toxicity, primarily affecting the GM lineage, which results in potentially life-threatening neutropenia, along with less critical but still serious thrombocytopenic effects (52). Compared to cyclophosphamide, which is among the least myelosuppressive alkylators, TER286 is only mildly toxic to the bone marrow compartment of mice. Similarly, peripheral blood analyses in mice indicate moderate toxicity to circulating WBCs. At higher doses, variable but still low levels of mortality and collateral organ toxicity appear, influenced in part by the vehicle used. Optimization of the dose regime will benefit from more detailed pharmacokinetic studies. Given the species-specific nature of the activation mechanism, however, it is likely that definitive information on this issue will require clinical data.

In summary, TER286 is the first clinical candidate from a novel class of latent drugs activated by GST (58). It is selectively more toxic in cells with elevated levels of the P1-1 isozyme, a strongly tumor-associated protein that shows moderate correlation to drug resistance. TER286 is quite active in clonogenic assays of primary human tumor specimens and produces significant tumor growth inhibition in human xenograft models, especially in those known to be alkylator sensitive. It produces mild bone marrow toxicity in mice at doses that are efficacious in xenograft models. Efforts are currently underway to scale up synthesis and perform formal toxicity testing in preparation for a clinical trial.

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