Antitumor Activity of ZD1694 (Tomudex) against Human Head and Neck Cancer in Nude Mouse Models: Role of Dosing Schedule and Plasma Thymidine

Shousong Cao, John J. McGuire, and Youcef M. Rustum
Grace Cancer Drug Center, Roswell Park Cancer Institute, Buffalo, New York 14263

ABSTRACT
We studied the antitumor activity and toxicity of ZD1694 (tomudex), a specific inhibitor of thymidylate synthase (TS), in nude mice bearing human head and neck squamous cell carcinoma A253 and FaDu xenografts. Mice were treated by single i.v. push (i.v. × 1), i.v. push once a week for 3 weeks (weekly × 3), and i.v. push once a day for 5 days (daily × 5), and the maximum tolerated doses (MTDs) of ZD1694 were 300 mg/kg, 60 mg/kg/week, and 30 mg/kg/day, respectively. ZD1694 was moderately active against both A253 and FaDu xenografts. Antitumor activity was schedule-dependent in both tumors: weekly × 3 ≥ i.v. × 1 >> daily × 5. In contrast, the rank order of toxicity was daily × 5 >> weekly × 3 ≥ i.v. × 1. ZD1694 at the MTD produced 20% complete tumor regression and 20% partial tumor regression (PR) with i.v. × 1 and weekly × 3 schedules and 12-day tumor growth delay with daily × 5 schedule against FaDu xenografts. No complete tumor regression was observed with i.v. × 1, weekly × 3, and daily × 5 schedules against A253. Of interest and potential clinical importance was the observation that ZD1694 was slightly more effective against FaDu than against A253. Of interest and potential clinical importance was the observation that ZD1694 was still active at doses lower than the MTD (MTD), which showed a high therapeutic index and wide safety margin. Study of ZD1694 compared with 5-fluorouracil and 5-fluoro-2′-deoxyuridine at the MTD revealed that the antitumor activity of ZD1694 was comparable with or superior to 5-fluorouracil and 5-fluoro-2′-deoxyuridine against both A253 and FaDu xenografts, with less toxicity. High plasma thymidine in mouse relative to human (1.3 μM and <0.1 μM, respectively) may complicate the study of antitumor activity and toxicity of TS inhibitors with human tumor xenografts grown in the mouse. To test this hypothesis, we preadministered methoxypolyethylene glycol-conjugated thymidine phosphorylase (MPEG-TPase; 2500 units/kg/dose) to reduce mouse plasma thymidine, then treated with various doses of ZD1694 using the daily × 5 or i.v. × 1 schedules in the A253 tumor model. MPEG-TPase significantly increased the toxicity of ZD1694; the MTD of ZD1694 plus MPEG-TPase was reduced 3- and 10-fold compared with ZD1694 alone for i.v. × 1 and daily × 5 schedules, respectively. However, preadministration of MPEG-TPase did not potentiate the antitumor activity of ZD1694 with either schedule. The data indicate that the study of TS inhibitors in rodent models may not be suitable for predicting a safe dose for clinical study. However, rodent models, particularly human tumor xenografts, are still useful models for evaluation of antitumor activity and schedule selection for TS inhibitors.

INTRODUCTION
Head and neck cancer, especially squamous cell cancer (which accounts for 80–85% of tumors of the upper aerodigestive tract) continues to present a major therapeutic challenge in clinical settings. Standard therapy for head and neck cancer is focused on local and regional approaches, such as surgery or radiation therapy, or both (1, 2). However, most patients present with metastatic or locally recurrent disease and cannot be treated with surgery or radiation therapy; they generally receive chemotherapies (3). In this setting, several agents were identified as active with 10–30% response rates, but were not curative (3, 4). Use of drug combinations (e.g., cisplatin/FUra3, cisplatin/paclitaxel) results in significantly higher response rates (40–50%); however, the response duration is short, and overall survival is unchanged (5, 6) and is often associated with increased toxicities (3). As the role of chemotherapy in the treatment of head and neck cancer expands, the identification of new active agents remains a high priority.

ZD1694 is a quinazoline antifolate that is a highly specific TS inhibitor (7). Cellular uptake of ZD1694 is via the reduced-folate carrier and it subsequently undergoes rapid and extensive intracellular polyglutamylation (8). Polyglutamylation increases

Received 3/11/99; revised 4/14/99; accepted 4/16/99.
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.
1 Supported in part by Grants CA65761, CA65755, and Institute Core Grant CA16056 from the National Cancer Institute.
2 To whom requests for reprints should be addressed, at Grace Cancer Drug Center, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263. Phone: (716) 845-1638; Fax: (716) 845-8857; E-mail: scao@sc3101.med.buffalo.edu.

3 The abbreviations used are: FUra, 5-fluorouracil; HNSCC, head and neck squamous cell carcinoma; TS, thymidylate synthase; ZD1694, tomudex (raltitrexed); FaDu, 5-fluoro-2′-deoxyuridine; dThd, thymidine; MPEG-TPase, methoxypolyethylene glycol-conjugated thymidine phosphorylase; CR, complete tumor regression; PR, partial tumor regression; TGI, tumor growth inhibition; TDT, tumor doubling time; MTD, maximum tolerated dose; MWL, maximum weight loss; LV, leucovorin.

Downloaded from clincancerres.aacrjournals.org on December 31, 2017. © 1999 American Association for Cancer Research.
Role of Schedule and dThd on ZD1694 Selectivity

IC50 in the 1–10 nM range for continuous exposure (8). This is the growth of both mouse and human tumor cell lines, with an occurs in various tissues, resulting in a high tissue:plasma drug
venient infrequent dosing schedule (8). Polyglutamylation also
drug potency and intracellular retention, which allows a con-
venient infrequent dosing schedule (8). Polyglutamylation also
occurs in various tissues, resulting in a high tissue:plasma drug
ratio (8).

In vitro studies showed ZD1694 to be a potent inhibitor of
the growth of both mouse and human tumor cell lines, with an
IC50 in the 1–10 nM range for continuous exposure (8). This is
equivalent in potency to methotrexate, but 94- and 56-fold more
potent than FUra alone and FUra plus 10 μM LV, respectively
(8). In vivo studies with ZD1694 showed it was curative in mice
bearing LS178Y TK −/− lymphoma and L1210:ICR ascitic
tumors (8, 9). It was also active against human colon, gastric,
lung, and ovarian cancers and was superior to FUra and meth-
otrexate against these xenografts (10).

Clinical trials with ZD1694 demonstrated that it is active in
a number of solid tumors, including colorectal (11–15), breast
(11, 16), head and neck (17), ovarian (11), non-small-cell lung
(11), and pancreatic (11) cancers. ZD1694 is currently being
investigated in Phase I and II studies in patients with hormone-
resistant prostate cancer, soft tissue sarcoma, and pediatric and
adult leukemias (15). ZD1694 had similar efficacy to FUra/LV
therapy, with a 25–30% response rate in patients with advanced
colorectal cancer, but induced less mucositis and leucopenia. In
addition, patients who received ZD1694 spent a substantially
shorter time in the hospital for drug administration than those
who received FUra/LV (13–15). ZD1694 treatment also offered
more frequent palliative benefits and a more convenient admin-
istration schedule (13–15). ZD1694 is now available in more
than 10 countries for first-line treatment of advanced colorectal
cancer (15). In a Phase II study of advanced breast cancer,
ZD1694 achieved a 26% overall response rate and a 44%
response rate in patients with measurable liver lesions (16).

Cytotoxicity of ZD1694 is completely reversed by coinoc-
ubation with dThd or LV in vitro (9, 18, 19). The toxicities and
antitumor activity of ZD1694 can be abrogated by coadminis-
tration of dThd or LV in vivo (9, 20). These results demonstrate
that TS is the locus of action of ZD1694 for both antitumor
effect and toxicity. High circulating levels of dThd in rodents
relative to humans (≈1.3 and <0.1 μM, respectively) may
complicate the study of toxicity and antitumor activity of TS
inhibitors (7, 21). Wilson et al. (22) reported that administration
of 2500 units/kg of MPEG-TPase could reduce mouse plasma
dThd levels from 1–2 μM to undetectable levels (<0.2 μM);
coadministration of MPEG-TPase with 1843U89, a benzoquina-
zoline TS inhibitor, potentiated the in vivo activity of 1843U89
in a tumor-specific manner by reduction of dThd.

The principal objectives of this study were to determine:
(a) the antitumor efficacy of ZD1694 against human HNSCC
established in nude mice; (b) the role of the dosing schedule of
ZD1694; and (c) the effect of plasma dThd on the antitumor
activity and toxicity of ZD1694 in nude mice. The human
HNSCC A253 and FaDu models in athymic nude mice were used
to test the antitumor efficacy and determine the role of the
dosing schedule of ZD1694 with three clinically relevant sched-
ules [single i.v. push (i.v. × 1) versus i.v. push once a week
for 3 weeks (weekly × 3) versus i.v. push once a day for 5 days
(daily × 5)] and to compare the effect of ZD1694 with FUra and
FdUrd on the weekly schedule. We also investigated the anti-
tumor activity and toxicity of ZD1694 after preadministration
of MPEG-TPase in the A253 model.

MATERIALS AND METHODS

Chemicals. ZD1694 was kindly supplied by Zeneca
Pharmaceuticals (Macclesfield, United Kingdom). FUra and
FdUrd were obtained from Hoffmann-La Roche, Inc. (Nutley,
NJ).

Mice. Female athymic nude mice (nu/nu, body weight
20–25 g), 8–12 weeks of age, were obtained from Harlan
Sprague Dawley, Inc. (Indianapolis, IN). They were housed five
mice/cage under specific pathogen-free conditions with water
and food ad libitum, according to an institutionally approved
protocol.

Tumors. The HNSCC cell lines A253 and FaDu were
purchased from American Type Culture Collection (Manassas,
VA) and maintained as a monolayer in RPMI 1640 supple-
mented with 10% fetal bovine serum (Atlanta Biologicals,
Norcross, GA). Xenografts were initially established by im-
planting s.c. 106 cultured A253 or FaDu cells and passed several
generations by transplanting ~50 mg necrotic tumor tissues
before treatment.

Preparation of MPEG-TPase. MPEG-TPase was pre-
pared essentially as described (22, 23). Briefly, TPase (Sigma
T-3678; 12,500 units, 370 mg total protein), supplied in 0.5 M
K-phosphate (pH 7.0), 2 mM uracil, 0.02% NaN3, and 2.5%
(w/v) BSA was dialyzed for 24 h at 4°C against 150 mM
Na-phosphate (pH 7.0), containing 2.5 mM thymine, with three
changes of 1 liter each. To the dialyzed TPase, solution (30.5
ml) was added slowly with stirring, 3.5 g of solid MPEG-
succinimidyl succinate (Sigma M3152; ~75% pure, molecular
weight (MW) ~ 5000). The mixture was allowed to stir at 4°C

Fig. 1 Determination of the MTD of ZD1694 in nude mice with or
without HNSCC A253 or FaDu xenografts. All treatments were admin-
istered by i.v. push. ■, i.v. × 1 (day 0); ▲, weekly × 3 (days 0, 7, and
14); ○, daily × 5 (days 0–4). Animals were observed for up to 3 months
after treatment. Each group represents 10–30 animals of two to six
independent experiments.
Table 1  Antitumor activity and toxicity of ZD1694 in nude mice bearing human HNSCC A253 and FaDu xenografts: role of dose and schedulea

<table>
<thead>
<tr>
<th>ZD1694 (mg/kg)</th>
<th>Schedule</th>
<th>TGI (%)</th>
<th>TDT (day)</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>MWL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 i.v. × 1</td>
<td>A253</td>
<td>68 ± 16</td>
<td>3.4 ± 0.5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FaDu</td>
<td>74 ± 19</td>
<td>3.2 ± 0.6</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>200 i.v. × 1</td>
<td>A253</td>
<td>72 ± 14</td>
<td>8.0 ± 2.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>FaDu</td>
<td>77 ± 15</td>
<td>8.2 ± 2.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>300 i.v. × 1</td>
<td>A253</td>
<td>78 ± 14</td>
<td>9.6 ± 1.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>FaDu</td>
<td>82 ± 16</td>
<td>10.2 ± 2.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20 Weekly × 3</td>
<td>A253</td>
<td>58 ± 10</td>
<td>7.2 ± 1.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>FaDu</td>
<td>62 ± 12</td>
<td>7.6 ± 2.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40 Weekly × 3</td>
<td>A253</td>
<td>70 ± 12</td>
<td>8.8 ± 2.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>FaDu</td>
<td>73 ± 15</td>
<td>9.5 ± 2.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>60 Weekly × 3</td>
<td>A253</td>
<td>80 ± 15</td>
<td>10.8 ± 3.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>FaDu</td>
<td>86 ± 16</td>
<td>12.2 ± 2.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10 Daily × 5</td>
<td>A253</td>
<td>44 ± 6</td>
<td>5.0 ± 1.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>FaDu</td>
<td>48 ± 8</td>
<td>5.2 ± 1.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20 Daily × 5</td>
<td>A253</td>
<td>52 ± 9</td>
<td>5.8 ± 1.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>FaDu</td>
<td>56 ± 10</td>
<td>6.0 ± 1.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30 Daily × 5</td>
<td>A253</td>
<td>58 ± 12</td>
<td>6.6 ± 1.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>FaDu</td>
<td>65 ± 11</td>
<td>6.8 ± 1.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*The data are combined from two to six independent experiments with 10–30 animals for each group.

Fig. 2  Antitumor activity (A) and weight loss (B) produced by ZD1694 at the MTD with different schedules: role of dosing schedule. Bars, SE; ○, control; ●, 300 mg/kg ZD1694, i.v. × 1 (day 0); ▲, 60 mg/kg/week ZD1694, weekly × 3 (days 0, 7, and 14); ■, 30 mg/kg/day ZD1694, daily × 5 (days 0–4). Each group represents 10–30 animals of two to six independent experiments.
for 3.5 h, and the reaction was quenched by the addition of 0.4 ml of 1 M glycine-NaOH (pH 7.0) and further stirring for 30 min. The MPEG-TPase was dialyzed for 24 h at 4°C against 10 mM K-phosphate (pH 7.0), with three changes of 1 liter each, and concentrated by nitrogen pressure over an Amicon PM-30 membrane to 20.5 ml. This material was flash-frozen in dry-ice acetone and stored below −80°C, where it was stable for months. Overall recovery of activity was about 40%, and no unmodified protein was observed on SDS-PAGE analysis (data not shown).

TPase activity was measured spectrophotometrically (24); one unit of activity hydrolyzes 1 µM TdR/min. Protein concentrations were determined colorimetrically using the Bio-Rad Protein Assay (Bio-Rad, Hercules, CA), according to manufacturer’s instructions, using BSA as the standard.

**Drug Solutions.** ZD1694 was dissolved in sterile saline, and the solution was adjusted to pH 7.4 with NaOH immediately before use.

**Drug Doses and Schedules.** ZD1694 was administered by i.v. injection via the tail vein of animals using three sched-

**Table 2** Antitumor activity and toxicity of ZD1694, FUra, and FdUrd administered by i.v. push weekly × 3 in nude mice bearing human HNSCC A253 and FaDu xenografts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TGI (%)</th>
<th>TDT (%)</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>MLW (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A253</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.4 ± 0.5</td>
<td></td>
<td>0</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>ZD1694 (60)</td>
<td>80.2 ± 15.4</td>
<td>10.8 ± 3.2</td>
<td>0</td>
<td>40</td>
<td>14.2 ± 1.6</td>
</tr>
<tr>
<td>FUra (100)</td>
<td>77.8 ± 11.8</td>
<td>10.5 ± 1.8</td>
<td>0</td>
<td>40</td>
<td>12.4 ± 2.0</td>
</tr>
<tr>
<td>FdUrd (400)</td>
<td>61.6 ± 10.2</td>
<td>7.2 ± 2.0</td>
<td>0</td>
<td>0</td>
<td>12.4 ± 2.0</td>
</tr>
<tr>
<td>FaDu</td>
<td>3.2 ± 0.6</td>
<td></td>
<td>20</td>
<td>20</td>
<td>10.2 ± 3.2</td>
</tr>
<tr>
<td>Control</td>
<td>85.8 ± 14.2</td>
<td>12.2 ± 2.8</td>
<td>20</td>
<td>20</td>
<td>14.5 ± 2.2</td>
</tr>
<tr>
<td>ZD1694 (60)</td>
<td>83.6 ± 10.2</td>
<td>12.2 ± 1.6</td>
<td>0</td>
<td>40</td>
<td>12.4 ± 2.0</td>
</tr>
<tr>
<td>FUra (100)</td>
<td>86.2 ± 9.6</td>
<td>14.8 ± 2.2</td>
<td>0</td>
<td>0</td>
<td>12.8 ± 2.6</td>
</tr>
</tbody>
</table>

*The data are combined from two to four independent experiments with 10–20 animals for each group.

*mg/kg/week.
tumor was undetectable by palpation for 90 days after treatment, at which time the mouse was sacrificed. The response rate was expressed as a percentage of animals in the group. In general, tumor in mice with PRs regrew within 2 weeks after therapy. However, tumors rarely (<5%) regrew after CRs occurred (cure). As a general policy, animals were sacrificed when the tumor weight exceeded 2000 mg. Each experimental group had five mice, and each experiment was repeated at least once.

**Statistical Analysis.** Differences between the mean values were analyzed for significance using the unpaired two-tailed Student’s *t* test for independent samples; *P* ≤ 0.05 was considered to be statistically significant.

**RESULTS**

**Determination of the MTD of ZD1694.** To identify the MTD of ZD1694, nude mice with or without A253 or FaDu xenografts were treated with various drug doses, administered by three different schedules. The MTDs of ZD1694 were 300 mg/kg, 60 mg/kg/week (180 mg/kg/course), and 30 mg/kg/day (150 mg/kg/course) for i.v. × 1, weekly × 3, and daily × 5 schedules, respectively (Fig. 1). We also compared i.v. to i.p. injection; the MTD of i.p. ZD1694 was the same as for i.v. injection with single and weekly × 3 schedules, but the MTD for i.p. ZD1694 was lower (20 mg/kg/day) with the daily × 5 schedule. In general, lethality was observed in a significant number of animals when body weight loss was >20% of their original weight.

**Antitumor Activity and Toxicity of ZD1694 with Different Schedules: Role of Dose and Schedule.** Table 1 summarizes the antitumor activity and toxicity of ZD1694 with three different schedules at various doses (MTD). ZD1694 at the MTD produced a 20% CR and a 20% PR with i.v. × 1 or weekly × 3 schedules, and 12-day tumor growth delay (Fig. 2) were observed with the daily × 5 schedule against FaDu xenografts. ZD1694 did not achieve CR against A253; however, 20% PR, 40% PR, and a 10-day tumor growth delay (Fig. 2) were observed with i.v. × 1, weekly × 3, and daily × 5, respectively. Of note, ZD1694 was still active at doses lower than the MTD against both tumors (Table 1), demonstrating its significant chemotherapeutic index and safety margin. The tumor growth inhibitory curves and the animals’ body weight loss produced by ZD1694 at the MTD with three different schedules are illustrated in Fig. 2. The data clearly demonstrated that single and weekly schedules were more active and less toxic than the daily schedule (Fig. 2). ZD1694 administered i.p. also showed a similar pattern of antitumor activity and toxicity with the three schedules (data not shown).

**Comparative Antitumor Activity and Toxicity of ZD1694 with FUra and FdUrd.** We chose the weekly × 3 schedule to compare the antitumor activity and toxicity of ZD1694 with FUra and FdUrd based on our previous results, which showed both FUra and FdUrd were much more active with a weekly schedule than with single and daily schedules (26, 27). The data in Table 2 showed that the antitumor activity of

---

4 S. Cao and Y. M. Rustum, unpublished data.
ZD1694 was comparable with FUra, but superior to FdUrd, against A253 xenografts and was more active than both FUra and FdUrd, in terms of tumor regression against FaDu xenografts. The animals experienced less toxicity (as weight loss) when treated with ZD1694 than with FUra or FdUrd at the MTD (Table 2). However, ZD1694 showed wider deviation of tumor response in individual animals than did FUra and FdUrd in both models (Fig. 3).

The Effect of dThd on the Antitumor Activity and Toxicity of ZD1694. High levels of plasma dThd in the nude mouse could affect the antitumor activity and toxicity of a TS inhibitor, such as ZD1694. To test this hypothesis, we used MPEG-TPase to reduce plasma dThd levels (22), to see whether and how profoundly the antitumor activity and toxicity of ZD1694 were affected at low plasma dThd in the A253 model.

With the daily \( \times 5 \) schedule of ZD1694 ± MPEG-TPase, MPEG-TPase was administered i.p. at 2500 units/kg, 1 h before the first, third, and fifth doses of ZD1694 (three doses in total). MPEG-TPase shifted the dose-response curve to the left, the MTD of ZD1694 with MPEG-TPase was reduced about 10-fold compared with ZD1694 alone (Fig. 4). However, MPEG-TPase did not alter the pattern of antitumor activity or toxicity of ZD1694; TGI, TDT, and tumor response and weight loss at the MTD of ZD1694, with or without MPEG-TPase, were comparable (\( P > 0.05 \); Fig. 5 and Table 3).

We also studied ZD1694 ± MPEG-TPase on the i.v. \( \times 1 \) schedule in the same tumor model. MPEG-TPase was administered i.p. at 2500 units/kg 1 h before ZD1694. The data in Fig. 6 and Table 4 shows no significant difference in TGI, TDT, and tumor response between ZD1694 plus MPEG-TPase and...
ZD1694 alone ($P > 0.05$; previous experiments with ZD1694 alone were excluded). The data indicate MPEG-TPase did not augment the antitumor activity of ZD1694 at an equitoxic dose, but a 3-fold dose reduction was evidenced.

**DISCUSSION**

We used human head and neck xenograft models in nude mice to study the antitumor activity and toxicity of ZD1694 and to determine the role of schedule of administration. Moreover, we studied the effect of plasma dThd on the antitumor activity and toxicity of ZD1694 in one model. We have tried to answer three questions in this investigation: (a) Is ZD1694 active against human head and neck cancer in these animal models? (b) What is the optimal schedule for ZD1694 in these animal models? and (c) Does plasma dThd affect the antitumor activity and toxicity of ZD1694 in the mouse model system? and, if so, how profoundly? The answer to the latter question can determine whether nude mice bearing human tumor xenografts are appropriate tumor models to evaluate the antitumor activity and toxicity of TS inhibitors.

ZD1694 is a direct and specific TS inhibitor that lacks the nonspecific effects on RNA and protein synthesis of FUra; it was hypothesized that ZD1694 would have similar antitumor efficacy, but fewer side effects than FUra (28–31). Indeed, in clinical Phase III trials of patients with advanced colorectal cancer, ZD1694 showed similar efficacy compared with FUra/LV therapy, but induced less toxicities and improved palliative benefits (13–15). Our data herein also demonstrate that ZD1694 had a better therapeutic index than FUra and FdUrd in human HNSCC models (Table 2).

Earlier preclinical studies demonstrated that ZD1694 is active against a range of human solid tumors including colon, ovarian, lung, gastric, bladder, and breast xenografts (8). Preliminary data from Phase I trials showed ZD1696 was moderately active in patients with head and neck cancer; two of five patients achieved minor responses (15). ZD1694 as monotherapy with or without radiotherapy in patients with head and neck cancer has entered a Phase II trial in Europe and the United States (32,33). To our knowledge, no preclinical in vivo data with ZD1694 against head and neck tumor has been reported to date, although it was active against a wide panel of murine and human tumors (8–10). Our data indicate that ZD1694 is moderately active against human HNSCC FaDu and A253 xenografts in a schedule-dependent manner (Table 1 and Fig. 2). Of interest and potential clinical importance was the finding that ZD1694, unlike FUra with which optimal antitumor activity is achieved only with doses at or near the MTD (34), was active at doses lower than the MTD, particularly with weekly and single schedules (Table 1). The data indicate that ZD1694 had a large chemotherapeutic index and safety margin and may not need to be given at the MTD in the clinic. The comparative study of ZD1694 with FUra and FdUrd showed the antitumor activity of ZD1694 was similar to FUra, but superior to FdUrd, against A253 and was more active than both FUra and FdUrd, in terms of CR against FaDu with less toxicity (Table 2). However, ZD1694 showed wider deviation of tumor response in an individual animal than FUra and FdUrd in both models (Fig. 3); this may relate to different individual plasma dThd levels. The in vivo data also indicate that A253 is slightly less sensitive than FaDu to ZD1694 (Table 1 and Fig. 2); this is consistent with early findings with the same tumor cell lines in vitro (18). Because ZD1694 is not highly active in these models, to achieve higher response rate, it may need to be combined with other chemotherapeutic agents with different mechanisms of action, such as a topoisomerase I inhibitors (CPT-11), platinum compounds (cisplatin, carboplatin), and/or radiotherapy. The study of antitumor efficacy and role of sequence of ZD1694 in combination with CPT-11 or cisplatin is under way in this laboratory.

The data presented herein demonstrated that the antitumor activity and toxicity of ZD1694 was highly schedule-dependent. Single-dose and weekly schedules were more active and less toxic than the daily schedule (Table 1 and Fig. 2). Higher toxicity was observed with the daily × 5 schedule, probably from rapid drug accumulation and retention in mouse tissues due to extensive polyglutamylation, resulting in high drug levels (50–100-fold higher than plasma) associated with prolonged TS inhibition in tissues (8). This confirms the argument that frequent dosing schedules with ZD1694 may produce unacceptable toxicity (8). Unlike drugs with different mechanisms of action,

---

**Table 3** Antitumor activity and toxicity of ZD1694 ± MPEG-TPase administered by i.v. push daily × 5 in nude mice bearing human HNSCC A253 xenografts

<table>
<thead>
<tr>
<th>ZD1694 (mg/kg)</th>
<th>MPEG-Tpase* (2500 U/kg)</th>
<th>Antitumor activity</th>
<th>Toxicity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>–</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>54.8 ± 10.5</td>
<td>13.4 ± 1.8</td>
</tr>
<tr>
<td>30</td>
<td>−</td>
<td>76.2 ± 4.6</td>
<td>28.0 ± 3.4</td>
</tr>
<tr>
<td>15</td>
<td>+</td>
<td>70.0 ± 5.2</td>
<td>24.1 ± 2.8</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>58.8 ± 8.0</td>
<td>17.6 ± 4.2</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>55.2 ± 5.2</td>
<td>15.6 ± 3.2</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>51.6 ± 6.6</td>
<td>11.8 ± 1.6</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>42.6 ± 5.2</td>
<td>6.6 ± 1.8</td>
</tr>
</tbody>
</table>

* MPEG-TPase was administered i.p. 1 h before the first, third, and fifth doses of ZD1694 in a total of three doses.

† The actual TDT could not be determined since 80–100% of treated animals died on days 6 or 7.

Death occurred on days 6 or 7 after the initiation of the treatment (2–3 days after treatment). The data are combined from two to four independent experiments with 10–20 animals for each group.
such as CPT-11, with which higher antitumor activity is usually associated with prolonged drug administration (35), ZD1694 showed higher antitumor efficacy with an infrequent dosing schedule (single or weekly). A possible explanation is that ZD1694 was rapidly and efficiently polyglutamylated with resultant high intracellular drug levels and more potent, prolonged inhibition of TS (36). However, other mechanisms must be involved because the dose difference in MTD is small between

---

**Fig. 6** Antitumor activity (A) and weight loss (B) produced by ZD1694 ± MPEG-TPase with i.v. × 1 (day 0) schedule in nude mice bearing HNSCC A253 xenografts. Bars, SE; ○, control; ●, 2500 units/kg MPEC-TPase; △, 300 mg/kg ZD1694; ■, 50 mg/kg ZD1694 + 2500 units/kg MPEG-TPase; ●, 100 mg/kg ZD1694 + 2500 units/kg MPEG-TPase. MPEG-TPase was administered by a single i.p. 1 h before ZD1694. Each group represents 10 animals of two independent experiments.

---

**Table 4** Antitumor activity and toxicity of ZD1694 ± MPEG-TPase administered by i.v. push × 1 in nude mice bearing human HNSCC A253 xenografts

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Antitumor activity</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TGI (%)</td>
<td>TDT (day)</td>
</tr>
<tr>
<td>Control</td>
<td>3.6 ± 0.4</td>
<td>0</td>
</tr>
<tr>
<td>ZD1694 (300)</td>
<td>68.5 ± 9.2</td>
<td>7.5 ± 0.8</td>
</tr>
<tr>
<td>ZD1694 (50) + MPEG-TPase</td>
<td>68.6 ± 5.0</td>
<td>7.3 ± 0.5</td>
</tr>
<tr>
<td>ZD1694 (100) + MPEG-TPase</td>
<td>71.8 ± 4.8</td>
<td>7.8 ± 0.4</td>
</tr>
</tbody>
</table>

*The data are combined from two independent experiments with 10 animals for each group.

* MPEG-TPase was administered by a single dose at 2500 units/kg 1 h before ZD1694.
the weekly and the daily schedule (60 versus 30 mg/kg). More importantly, with the weekly schedule, lower drug doses (20–40 mg/kg) produced higher antitumor activity than the daily schedule at the MTD (Table 1). We hypothesize that host immune function may be decreased with the daily schedule because of high toxicity. The mechanism of action of ZD1694 with different schedules should be further investigated. Nevertheless, these data demonstrate that current clinical trials with infrequent administration of ZD1694 are appropriate.

It was believed that the high level of plasma dThd in mouse relative to human might affect the toxicity and antitumor activity of TS inhibitors (8, 21). Indirect supporting evidence was that the cytotoxicity of ZD1694 was completely reversed by coinubcation with dThd in vitro (9, 18, 19) and the toxicity and antitumor activity of ZD1694 can be abrogated by coadministration of dThd in vivo (9, 20). We preadministered MPEG-TPase to reduce plasma dThd in the A253 xenograft model, then treated with various doses of ZD1694. The results showed that MPEG-TPase significantly increased the toxicity of ZD1694, produced a 3–10-fold dose reduction in MTD with the two tested schedules (Fig. 4; Tables 3 and 4). However, MPEG-TPase did not potentiate the antitumor activity of ZD1694 (Tables 3 and 4; Figs. 5 and 6). The data indicate that low plasma dThd would profoundly affect toxicity, but has little effect on the antitumor activity of ZD1694. We agree that the MTD of a TS inhibitor obtained from rodent models should not be used to predict a safe dose for clinical Phase I study. It is better to choose other models with low plasma dThd level similar to humans, such as dogs, to determine the clinical starting dose. However, we believe that rodent models, particularly human tumor xenografts, are still useful models for predicting the outcome of antitumor activity of TS inhibitors and for schedule selection for clinical trials.

In conclusion, these studies with human HNSCC models show ZD1694 was moderately active against human HNSCC xenografts. Antitumor efficacy of ZD1694 is comparable with or superior to FUra andFdUrd, with a better chemotherapeutic index and safety margin in these model systems. The antitumor efficacy and toxicity of ZD1694 is schedule-dependent. It is less active and more toxic with frequent daily dosing. The data provide direct evidence for the rationale of current clinical studies with ZD1694. Animal models with high plasma dThd are not the most suitable models for assessment of the toxicity of and for selecting a safe dose for clinical trial of TS inhibitors. However, these models are useful for in vivo study of antitumor activity and schedule selection with TS inhibitors.

ACKNOWLEDGMENTS

We thank Drs. G. W. Koszalka and Robert Ferone of Glaxo Wellcome for helpful discussions of the preparation of MPEG-TPase and Jill Canestri for its preparation and assay.

REFERENCES

Antitumor Activity of ZD1694 (Tomudex) against Human Head and Neck Cancer in Nude Mouse Models: Role of Dosing Schedule and Plasma Thymidine

Shousong Cao, John J. McGuire and Youcef M. Rustum