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ZD1694 (Tomudex): A New Thymidylate Synthase Inhibitor with Activity in Colorectal Cancer

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ZD1694 (Tomudex) is a new antifolate which is a specific inhibitor of thymidylate synthase (TS). Evidence suggests that ZD1694 has a spectrum of activity that only partially overlaps with 5-fluorouracil (modulated with leucovorin) against colon tumours *in vitro*. Potent cytotoxic activity is dependent upon active uptake into cells via the reduced folate/methotrexate cell membrane carrier (RFC) and subsequent metabolism to polyglutamated forms (tri, tetra and pentaglutamates). These polyglutamates are approximately 60-fold more active as TS inhibitors and are not effluxed readily from cells. Extensive polyglutamation also occurs in various mouse tissues (e.g. small intestinal epithelium, liver and kidney), resulting in high tissue/plasma drug ratios which persist for a prolonged period. ZD1694 has antitumour activity in mice, although the high plasma thymidine in this species complicates: (1) the interpretation of therapeutic index; (2) tumour types in which activity is likely to be observed; and (3) translation of doses and schedules for clinical evaluation. ZD1694 entered clinical study and has completed Phase I and II evaluation, with activity observed in several tumour types. Appreciable activity in the Phase II colorectal study (29% objective response rate on interim analysis) led to the current Phase III study, randomised against 5-fluorouracil/leucovorin.

Key words: ZD1694, thymidylate synthase, polyglutamation
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INTRODUCTION

TMP (THYMIDINE 5'-MONOPHOSPHATE) is a pyrimidine deoxyribonucleoside monophosphate which, after metabolism to TTP (thymidine 5'-triphosphate), is essential for DNA replication and repair. An enzyme critical to the *de novo* synthesis of TMP is thymidylate synthase (TS) which makes it an attractive target for the development of anticancer therapeutic agents (Figure 1) [1]. The substrate for TS is dUMP and the co-factor for the reaction is 5,10-methylene tetrahydrofolate (5,10-CH_2FH_4). A thymidine (dThd) salvage pathway also exists, which, through the activity of thymidine kinase (TK), can utilise extracellular dThd to form intracellular TMP (Figure 1). The co-existence of these two pathways has important consequences for drug development, and will be discussed further below.

Fluorinated pyrimidine-based inhibitors of TS have been known for many years, but need to be delivered into the cell as a base (e.g. 5-fluorouracil; 5-FU) or nucleoside (5-fluorodeoxyuridine; FdUrd) after which metabolism occurs to the TS inhibitory species, FdUMP. However, 5-FU is extensively metabolised intracellularly to several anabolites other than FdUMP so that incorporation of 5-FU into RNA and possibly DNA may be important additional determinants of its antitumour activity and toxicity (reviewed in [1]). The relative importance of RNA versus TS effects in various tissues and in different drug administration schedules, particularly when modulated with leucovorin (LV; folinic acid), has been widely debated and reviewed [2]. The biochemical basis for LV modulation relates to the sub-optimal level of 5,10-CH_2FH_4 in many colon tumours,

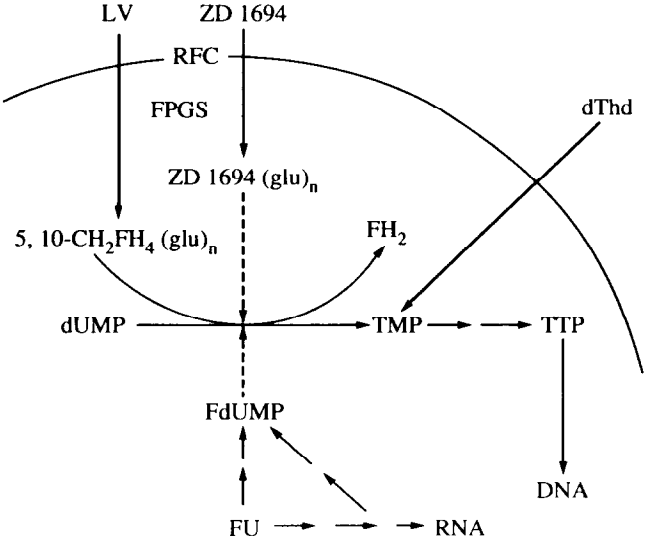

Figure 1. Thymidylate synthase and its position in folate metabolism.

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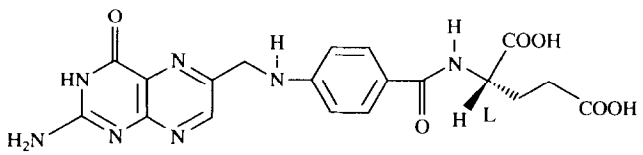
which can be elevated by co-administration of LV, thereby stabilising the enzyme-FdUMP-5,10-CH_2FH_4 ternary inhibitory complex [2]. However, the increased clinical toxicity seen when these agents are combined suggests that biomodulation of 5-FU also occurs in normal proliferating tissues. One further factor which may compromise optimal TS inhibition by FdUMP is the large expansion in the level of the competing substrate, dUMP, that occurs following TS inhibition (reviewed in [1]).

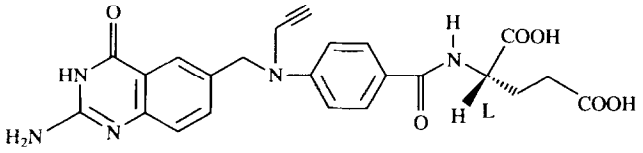
In randomised colorectal cancer trials, response rates to single agent 5-FU are in the region of 5–17% [3]. A number of trials have been conducted comparing the efficacy of LV modulated 5-FU with 5-FU alone [2]. One prospective randomised study is worth particular mention; the NCCTG study randomised patients to either 5-FU (500 mg/m^2 i.v. (intravenous) bolus daily for 5 days) or 5-FU (425 mg/m^2) plus 20 mg/m^2 LV or 5-FU (370 mg/m^2) plus 200 mg/m^2 LV and reported improved response rates and survival with modulated 5-FU [4]. Diarrhoea and mucositis were the most notable toxicities encountered with 5-FU + LV combinations, and severe diarrhoea with or without concomitant myelosuppression was responsible for a number of toxic deaths, especially in earlier studies when the importance of intensive support was not universally recognised [5]. There is *in vitro* evidence to suggest that the predominant cytotoxic action of bolus 5-FU administration is a RNA effect whereas continuous exposure works more via inhibition of TS [6]. One study, prospectively comparing conventional bolus dose 5-FU with continuous infusion, reported a 7 and 30% response rate, respectively, although overall survival was comparable [7]. Such clinical studies suggest that TS may be an important chemotherapeutic target in colorectal cancer.

The additional or secondary effects of 5-FU suggested that a more selective inhibitor of TS would be highly desirable [1]. The problems associated with incorporation into nucleic acids could be overcome by the design of analogues of the folate cofactor rather than the pyrimidine substrate [1]. Furthermore, it was argued that the increase in the dUMP level, which may compromise the binding of FdUMP to TS, could only be beneficial to ternary complex formation with a folate-based TS inhibitor. The primary medicinal chemistry problem was to synthesise antifolates that were specific for TS rather than dihydrofolate reductase (DHFR), as inhibition of the latter has effects on both the synthesis of TMP and purines (Figure 1). This led to the discovery of CB3717 (Figure 2) [1, 8], a potent inhibitor of TS (K_i approximately 3 nM) which has antitumour activity against a variety of tumour types including ovarian, liver and breast (reviewed in [9]). Unpredictable and life-threatening nephrotoxicity prevented further development of CB3717. The cause of this toxicity was identified as being the poor water-solubility of the drug and not a result of TS inhibition. This problem was rapidly solved, and new highly water-soluble, non-nephrotoxic experimental agents became available [10–13]. Further, structurally diverse compounds had different affinities for TS, folate transport proteins and the folate-metabolising enzyme, folylpolyglutamate synthetase (FPGS). The first new drug to emerge from this programme was ZD1694 (Tomudex), which is the subject of the rest of this paper.

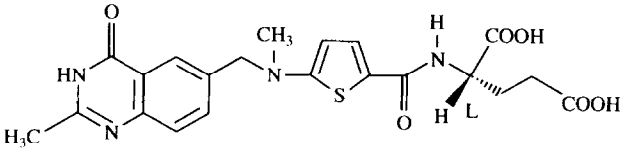
The design, synthesis and development of ZD1694 (Tomudex; Figure 2) has been a collaboration between the Institute of Cancer Research and Zeneca Pharmaceuticals. This paper is largely a review of published material relating to the preclinical and clinical development of ZD1694, but, where appropriate, new information is provided.



Folic acid



CB 3717



ZD 1694

Figure 2. The structure of CB3717 and ZD1694.

IN VITRO STUDIES

ZD1694 has a K_i for the inhibition of isolated murine or human TS of approximately 60 nM [14], 20-fold higher than that of CB3717 (K_i approximately 3 nM). However, this reduced activity is more than compensated for by its far superior activity as an inhibitor of cell growth. The IC_{50} (continuous exposure) for the growth of mouse or human cells in culture is in the 1–10 nM range and results for the mouse L1210 cell line are given in Table 1. The potency of ZD1694 is equivalent to that of methotrexate (MTX) but is 570, 94 and 56-fold improved over CB3717, 5-FU and 5-FU + 10 μM LV, respectively. The increased activity of ZD1694 over the structurally related CB3717 is the result of active cellular uptake via the reduced folate–methotrexate cell membrane carrier (RFC) (K_m ZD1694 approximately 2.5 μM; K_m CB3717 approximately 40 μM) and excellent substrate activity for FPGS (mouse liver K_m = 1.3 and 40 μM for ZD1694 and CB3717, respectively) [14]. The importance of both of these proteins for the activity of ZD1694 is best shown by its reduced cytotoxicity in cell lines where these proteins are underexpressed (Table 1). Polyglutamation enables ZD1694 to concentrate within cells (see below) and these polyglutamates are significantly more potent as TS inhibitors (K_i tetraglu = 1 nM) than the parent monoglutamated drug (60 nM) [14], which together explain the high cytotoxic potency of ZD1694 under continuous exposure conditions. MTX is polyglutamated much more slowly [15, 16] and once formed the polyglutamates are no more active against DHFR than the parent drug. This explains why MTX retains a good level of activity in the L1210:R^{D1694} cell line (polyglutamation defect) under continuous exposure conditions (Table 1) [15]. The different loci of action of ZD1694 and MTX are illustrated by the retention of activity of the former against the L1210:R7A cell line (amplification of the *DHFR* gene) (Table 1). Similarly, cell lines with raised TS levels are resistant to ZD1694 but sensitive to MTX [14, 15].

Polyglutamation of ZD1694 is so rapid that intracellular metabolites can be detected within a few minutes, such that after mouse L1210 or human W1L2 cells were incubated with 0.1 μM ZD1694 for 30 min, the majority of the intracellular drug was

Table 1. Activity of ZD1694 against the L1210 mouse leukaemia and its resistant sub-lines

	Inhibition of cell growth, IC_{50} (μM)			
	L1210	L1210:1565 ↓ RFC	L1210:R^{D1694} ↓ polyglu	L1210:R7A ↑ *DHFR*
ZD1694	0.0088 ± 0.0031	0.76 ± 0.19 (86)	>100 (>10000)	0.028 ± 0.0092 (3)
CB3717	5.0 ± 1.2	3.8 ± 0.57 (0.8)	13 ± 2.1 (3)	41 ± 6 (8)
MTX	0.011 ± 0.0035	0.95, 0.93 (85)	0.024 ± 0.003 (2)	5.3, 8.8 (640)
5-FU	0.93, 0.72	0.85, 0.70 (0.9)	1.5, 0.81 (1)	—
5-FU + 10 μM LV	0.60, 0.39	0.44, 0.33 (0.8)	0.52, 0.36 (0.9)	—

The L1210 sub-lines have the following resistance mechanisms; L1210:1565 = impaired RFC; L1210:R^{D1694} = impaired polyglutamation; L1210:R7A = amplification of the *DHFR* gene. Cells were incubated with drugs for 48 h and cell number determined by Coulter counting. The figures in parentheses indicate the resistance factor i.e. IC_{50} resistant cell line/IC_{50} sensitive cell line. MTX, methotrexate; LV, leucovorin; RFC, reduced folate/methotrexate carrier; DHFR, dihydrofolate reductase.

found as polyglutamates, and by 4 h parent drug represented <3% of the total drug pool (Table 2) [14, 17]. ZD1694 polyglutamation is considerably faster and more extensive than that of CB3717 or MTX [16]. Four other human tumour cell lines (two ovarian, one breast and one colon) were investigated for their relative ability to form polyglutamates (Table 2) [16]. Interestingly, the human HT29 colon line, after exposure to only 0.1 μM ZD1694, formed the highest intracellular level of polyglutamates of any cell line investigated (9.5 μM and 21 μM at 4 h and 24 h, respectively). Recently, a panel of human colon tumour cell lines has been used to assess the relative activity of ZD1694 and 5-FU and, using dThd protection experiments, their specificity for the *TS* locus (Table 3). Five of the six cell lines were highly sensitive to ZD1694 with IC_{50} values in a

Table 2. Polyglutamate formation in a range of cell lines

	Intracellular concentration of ZD1694 (μM)			
	Parent drug	Di + triglutamates	Tetra + pentaglutamates	Total drug
Human W1L2 Lymphoblastoid (n = 2)	0.041	0.75	2.3*	3.2
Human CH1 Ovarian tumour (n = 3)	0.30	1.4	0.26	2.0 ± 0.77
Human 41M Ovarian tumour (n = 3)	0.24	1.3	0.74	2.3 ± 0.6
Human MCF-7 Breast tumour (n = 1)	0.24	1.4	1.2	2.8
Human HT29 Colon tumour (n = 1)	0.34	1.6	7.5†	9.6
Mouse L1210 Leukaemia (n = 3)	0.07	0.40	2.4	2.9 ± 1.2
Mouse L1210 resistant to ZD1694 (L1210:R^{D1694})	0.077	–	–	0.078

Logarithmically growing cells were incubated with 0.1 μM 5-[^{3}H] ZD1694 for 4 h. ZD1694 and its polyglutamates were extracted and analysed by HPLC as described previously [16]. Values given are the mean of two or three separate experiments. Where n = 1 experiment, duplicate cultures were analysed. Total intracellular drug concentrations are given as mean ±S.D. when n = 3 separate experiments.

* 0.09 μM hexaglutamate also present; † 0.2 μM hexaglutamate also present.

Table 3. The activity of ZD1694, 5-FU and FdUrd against a panel of human colon tumour cell lines

	Inhibition of cell growth, IC_{50} (μM)					
	HT29	LoVo	SW480	SW620	MaWi	BE
ZD1694	0.0019 ± 0.0003	0.0013 ± 0.0002	0.0039 ± 0.0003	0.0016 ± 0.0004	0.0026 ± 0.0004	>1
ZD1694 + dThd	>100	>100	>100	20	>100	>100
5-FU	3.3 ± 0.78	0.65 ± 0.06	5.4 ± 1.3	6.1 ± 1.5	4.7 ± 2.2	11 ± 2.0
5-FU + dThd (10 μM)	2.4 ± 0.70	3.0 ± 0.61	4.8 ± 1.4	5.5 ± 0.5	3.3 ± 1.1	8.7 ± 1.5
5-FU + LV (10 μM)	2.2 ± 0.27*	0.33 ± 0.041*	5.8 ± 1.1	5.0 ± 1.2	3.9 ± 2.2	12 ± 1.5
FdUrd	0.0067 ± 0.0039	0.0034 ± 0.0007	0.029 ± 0.013	0.007 ± 0.0022	0.0084 ± 0.0018	~1 (n = 4)

* Activity significantly enhanced by LV ($P < 0.05$). Cells were incubated for 4/5 days with the above compounds. Growth inhibition was assessed by MTT and IC_{50} values derived. Results given as mean ±S.D. where $n > 3$ separate experiments.

narrow concentration range (0.0013–0.0039 μM). The fact that co-incubation with dThd prevented activity confirms TS as the single cytotoxic locus of action. 5-FU, even when combined with LV, was considerably less potent against these same cell lines, with IC_{50}s in a wider concentration range (5-FU + LV = 0.33–5.8 μM) and, with the exception of the LoVo line, dThd did not prevent activity. These facts, together with the broad spectrum of activity seen with FdUrd (a pure TS inhibitor after a one-step activation via TK), support the belief that factors other than TS inhibition are influencing 5-FU activity. One cell line (BE) was resistant to ZD1694 and FdUrd and to other TS inhibitors not included here. This line was also the least sensitive to 5-FU or 5-FU + LV. The mechanism of resistance has not been elucidated, but TS activity is within the same range as the other colon cell lines.

L1210 cells exposed to ZD1694 briefly (4 h) rather than continuously are only 10-fold less sensitive to the growth inhibitory effects of the drug (IC_{50} approximately 0.1 μM). This good level of activity is consistent with the very rapid formation and retention of intracellular polyglutamates [14, 16, 17]. In turn, this results in prolonged TS inhibition and low TTP pools in tumour cells for a prolonged period, even after cells are resuspended in drug-free medium [17, 18].

Cells in culture are in a low dThd environment which essentially means that dThd salvage is not operating at a level which significantly circumvents TS inhibition [19]. However, conditions for clonogenic assays have to be carefully controlled to prevent the low level of dThd in the medium circumventing TS inhibition. The use of TK mutant cell lines or dialysed serum has demonstrated that ZD1694 is extremely cytotoxic with several decades of cell kill being achieved with low drug concentrations. For example, 1 μM ZD1694 induces four decades of cell kill in the L5178Y TK-mouse lymphoma cell line after just a 4 h drug exposure period. The cytotoxic action of ZD1694 has also been demonstrated by Smith and associates where 0.04 μM gave three decades and 1 μM gave six decades of cell kill in the human WiDr colon cell line (24 h exposure) [20].

IN VIVO STUDIES

Pharmacology

After i.p. (intraperitoneal) or i.v. bolus administration to mice, ZD1694 is cleared rapidly from the plasma with a half-life of approximately 30 min [21]. However, a prolonged final elimination phase is apparent so that low levels may persist for some time. The significance of this is not clear. Rapid drug accumulation and retention occurs in certain mouse tissues due to extensive polyglutamation resulting in total levels in tissues much higher than that of the plasma. Mice were injected i.p. with 5 mg/kg [^{3}H]ZD1694 and tissues removed 24 h later. The concentration in the plasma was approximately 0.01 μM, but the drug concentration in the liver, kidney and small intestinal epithelium was approximately 50–100-fold higher [22]. The majority of the drug (approximately 80%) was present as polyglutamates (Figure 3). A relatively low level of total drug (approximately 0.03 nmol/g) was detected in the gastrocnemius muscle. All tissues had a substantially higher drug level after three daily injections of 5 mg/kg (data not shown). Thus, as was predicted by *in vitro* studies, polyglutamation is extensive in mouse tissues and serves both to concentrate and retain the drug intracellularly. Total tissue drug levels have also been estimated

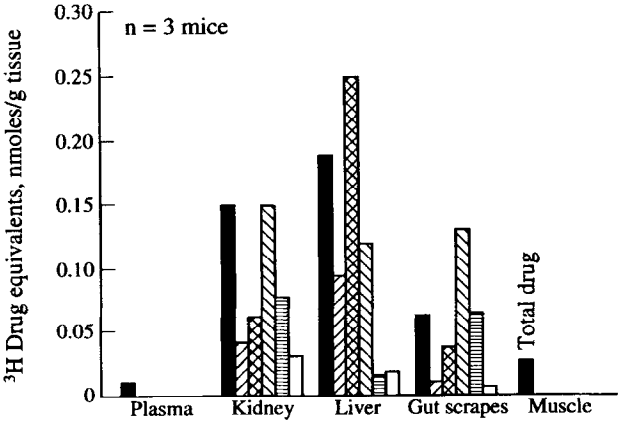


Figure 3. Mouse tissue levels of ZD1694 and its polyglutamate forms 24 h after a single i.p. dose of 5 mg/kg 5-[^{3}H] ZD1694. ■ parent drug; ▨ diglu; ▩ triglu; ▧ tetraglu; ▤ pentaglu; □ hexaglu. Mice were injected with 5 mg/kg of 5-[^{3}H] ZD1694 (1.1 Ci/mmole) and blood and tissues were removed 24 h later. Blood was immediately microfuged and the plasma removed and frozen on Cardice. Livers and kidneys were placed in 1 ml of 0.1 M Tris buffer, pH 10 and frozen. Hind legs were also quickly removed and frozen for later separation of the gastrocnemius muscle. Small intestine was removed and immediately flushed with ice-cold saline and one 5 cm section was taken between 10 and 15 cm below the stomach. This was scraped to remove the epithelium and then placed in 0.5 ml Tris buffer pH 10 and immediately frozen on Cardice. Later samples were thawed on ice, homogenised in pH 2 volumes (9 volumes for gut scrapes and muscle) of Tris buffer (pH 10). Polyglutamates were extracted with 1 volume of acetonitrile at −20°C followed by centrifugation and dilution with an equal volume of water. The extracts were stored at −20°C and later analysed by HPLC (ion pairing) as previously described [16]. Results are given as the mean of three mice.

using a radioimmunoassay (RIA) employing a specific ZD1694 sheep antibody that cross-reacts equally with the polyglutamate forms [23]. For example, after mice were injected with 100 mg/kg ZD1694 for 4 days and tissues analysed for total ZD1694 content on days 5, 7 and 15, the liver/plasma ratios were 55, 144 and 1000, respectively (25, 6.5 and 2.3 nmol/g liver). Similar persistence of ZD1694 in liver and other tissues has also been observed after a single dose of 10 or 100 mg/kg (data not shown).

Although the above studies still have to be performed in tumour-bearing mice, the formation of polyglutamates was shown indirectly to occur in the L1210 mouse ascitic leukaemia. After mice were given a single i.v. injection of 10 mg/kg and the tumour removed 24 h later, TS activity (rate of ^3H release from 5-[^3H]dUrd) was still substantially inhibited [14].

Antitumour activity and toxicity

In discussing the activity of ZD1694, it is essential to understand why the doses and schedules that give activity/toxicity in rodents, have little relationship to the dose and schedules used in the Phase I and II clinical studies. Probably the single most complicating issue is the high level of plasma dThd in mouse relative to man (approximately 1 and <0.1 μM, respectively) which, through the activity of TK, essentially circumvents TS inhibition [19]. Indications of putative antitumour efficacy can be obtained using TK negative tumours but this still leaves a perplexing problem, that is how to derive a therapeutic index when normal proliferating tissues are still able to salvage dThd. This problem is partially overcome using chronic administration of ZD1694 which gives both antitumour activity and toxicity to normal proliferating tissues in mice.

DBA2 mice bearing the subcutaneous L5178Y TK−/− mouse lymphoma were cured by a single dose (i.p.) of 10 mg/kg (30 mg/m^2) [24]. This same dose (i.v.) inhibited TS for >24 h in the ascitic L1210 tumour [14]. Normal proliferating tissue toxicity was not seen. A dose of 3.3 mg/kg/twice daily × 5 days of ZD1694 was active in the L5178Y TK+/− tumour (5 day growth delay) [24] and 10 mg/kg/daily × 5 days (i.v.) produced cures in mice bearing the L1210:ICR ascitic mouse tumour (grown in C57/DBA2 F1 hybrid mice) [14]. This dose gave some gastrointestinal toxicity which was manifested as weight loss (approximately 15%) and a fall in neutrophils and platelets [24]. Co-administration of either dThd or LV prevented both the antitumour activity and toxicity [14]. This is consistent with *in vitro* mechanistic studies that indicate that dThd acts by circumventing the TS block and LV prevents both cellular transport and polyglutamation [14, 17]. A range of human tumour xenografts was tested in mice using a daily × 14 days protocol and growth delays were observed, although often requiring doses as high as 100 mg/kg daily × 14 days [17, 25]. The most sensitive tumour tested was the HX62 human ovarian xenograft, and growth delays of 15 days were achieved with 1 mg/kg/day × 14 days [25]. Importantly, acute or chronic administration of ZD1694, unlike CB3717, did not induce nephrotoxicity [24].

Recently, we have reported that the Balb/c mouse strain is particularly susceptible to the gastrointestinal toxicity (diarrhoea and weight loss) of ZD1694 giving an MTD of approximately 10 mg/kg i.p. daily × 5 compared with >500 mg/kg daily × 5 in DBA2 mice (weight loss only) [26]. Balb/c mice have been used to address the question of whether the delayed administration of dThd (500 mg/kg thrice daily) or LV (30 or 200 mg/kg twice daily) to mice with overt gastrointestinal toxicity could aid recovery. If administration of either agent was started 24 h after a course of ZD1694 (100 mg/kg daily × 4) when diarrhoea was evident, and continued for 3–4 days, mice lost less weight and recovered more rapidly [27]. The mechanisms underlying LV rescue are being investigated, and preliminary data suggest that drug tissue levels may be reduced after LV is given [23]. This is possibly due to competition for FPGS which interrupts a cycle of hydrolysis (via folylpolyglutamate hydrolase) and re-polyglutamation. Therefore, it may be appropriate to give LV rescue to the small minority of patients presenting with severe combined grade IV gastrointestinal and haematological toxicities (see below). However, as LV prevents antitumour activity when given concurrently, it is currently recommended that LV rescue only be given in the presence of life-threatening toxicity.

CLINICAL EVALUATION

The rapid formation of retentive polyglutamates of ZD1694 in tumour cells and tissues *in vitro* and *in vivo*, when considered together with the curative activity against the L5178Y TK- mouse lymphoma, suggested that an infrequent bolus administration protocol should be considered for humans. Indeed, it was argued that too frequent dosing and the accumulation of polyglutamates in normal tissues might produce unacceptable toxicity. The choice of a safe starting dose was not easy to predict from rodent studies and, therefore, dogs, which have dThd levels similar to humans, were used to predict a safe dose for the Phase I study. The dose chosen was 0.1 mg/m^2 (20% of the toxic dose low in dogs) to be given via a 15 min infusion once every 3 weeks.

ZD1694 entered Phase I evaluation in Europe in February 1991 [9, 26, 28]. In all, 61 patients, with a range of tumours, were treated with a total of 161 courses (median of two courses per patient, range 1–11) [28]. 55 of these had received prior chemotherapy. Toxicity was encountered at doses of 1.6 mg/m^2 or greater with a maximum tolerated dose of 3.5 mg/m^2 being established. 28 patients received 74 courses at 3.0–3.5 mg/m^2. The most frequent toxicities at these doses included malaise, gastrointestinal (diarrhoea) and haematological (mainly neutropenia). Reversible rises in liver transaminases were also seen. As predicted, no drug-related nephrotoxicity was encountered. There were three objective partial responses (ovarian, breast and adenocarcinoma of unknown primary). Pharmacokinetic studies showed a triphasic elimination within a prolonged third phase of 50–100 h. A dose of 3.0 mg/m^2 once every three weeks was recommended for Phase II evaluation.

European Phase II studies of ZD1694 (3 mg/m^2) started in October 1992 and were conducted in a number of solid tumours, and interim analysis data have been published [29]. In a study of 31 cases of relapsed ovarian cancer after platinum-based therapy, the response rate was 9%, and in study of 46 women with advanced breast cancer it was 25%. Among the more chemoresistant tumours, ZD1694 had activity in advanced pancreatic cancer (14%) and non-small cell lung cancer (10%). Very encouraging results were seen in an interim analysis of 164 patients with advanced colorectal cancer [30]. The objective response rate was 29% (22–36% CI) (including two complete responses). A further 76 patients had stable disease. Two patients died of suspected drug-related toxicity with a combination of severe diarrhoea and myelosuppression. Toxicity profiles reported across all these studies were similar to those reported in the European Phase I study. The significant activity seen against colorectal cancer resulted in a Phase III study with ZD1694 given in the above dosage schedule and compared to 5-FU and LV given as five daily i.v. bolus injections repeated in weeks 4, 8 and then 5 weekly (20 mg/m^2 LV, 425 mg/m^2 5-FU).

DISCUSSION

ZD1694 (Tomudex) was designed as a non-nephrotoxic and highly active analogue of the folate-based TS inhibitor, CB3717. Rational design was based on an understanding of CB3717 physico-chemical properties and analogue interactions with transport proteins, FPGS and TS. Rapid and extensive intracellular polyglutamation occurs *in vitro* and *in vivo* accounting for both drug potency and retention. The latter feature predicted that infrequent bolus administration would be suitable for administration to humans. Indeed, a 15 min infusion once every 3 weeks has proved to be a very active protocol in clinical studies. A Phase II study in advanced, metastatic colorectal cancer suggested that the objective response rate at interim analysis (29%) was in the same order as LV modulated 5-FU. The randomised Phase III results are awaited. Clinical issues that need to be addressed include further investigation of potential toxicity rescue, other drug schedules, the sequence of possible drug combinations and the activity of ZD1694 in 5-FU-resistant disease. Some of these issues can be investigated in experimental systems, as can more fundamental questions regarding factors responsible for antitumour activity and toxicity, and cell death. The pharmacology of the drug in relation to transport proteins and the enzymes involved in polyglutamate homeostasis (FPGS and FPGH) requires particular attention. The synthesis and clinical evaluation of ZD1694, a drug which is apparently devoid of concomitant effects at other loci, has proved that TS is a good target for the development of new drugs active in the treatment of colorectal cancer.

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