ZD9331: discovery to clinical development

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Thymidylate synthase (TS) has been targeted in cancer therapy for many years. As a result of a prolonged and extensive drug development program specific TS inhibitors have come into clinical practice. Following on from the development of the polyglutamatable TS inhibitor raltitrexed (Tomudex, ZD1694), ZD9331 is a rationally designed third-generation specific inhibitor of TS that does not require polyglutamation for its activity. Its development was based on the dual rationale of increased efficacy, by overcoming the potential for resistance due to reduced expression of folylpolyglutamate synthetase (FPGS), whilst potentially reducing the toxicities associated with polyglutamation and drug retention in normal tissues. Preclinical studies have shown it to be transported by the ubiquitously expressed reduced folate carrier as well as the α-folate receptor which is overexpressed in some cancers, especially ovarian. *In vivo* studies demonstrated a broad range of activity, leading to an extensive phase I program with several administration schedules. Whilst not being targeted to any individual tumor type, a large number of phase I, II, monotherapy and combination studies have been undertaken, and overall activity has been most promising, particularly in platinum-refractory relapsed ovarian, pancreatic and gastric cancers. Its role in the treatment of these diseases may be important, especially if patients were to be selected on the basis of their folate transport and FPGS status. The true potential of the drug remains to be determined. *Anti-Cancer Drugs* 16:1–9

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Introduction

Thymidylate synthase (TS) inhibitors have been used in clinical practice for over 50 years. Methotrexate (MTX), although principally an inhibitor of dihydrofolate reductase (DHFR), has a complex mode of action including inhibition of TS. The pyrimidine analog 5-fluorouracil (5-FU) is widely used, and again has a range of effects that include inhibition both of TS and of purine biosynthesis. It has been suggested that the latter results in unwanted side-effects. A rational drug design programme was initiated at the Institute of Cancer Research (ICR) 25 years ago to develop folate-based selective TS inhibitors. Three specific TS inhibitors have entered clinical trials, CB3717 [1], raltitrexed (RTX, Tomudex) [2] and ZD9331 [3].

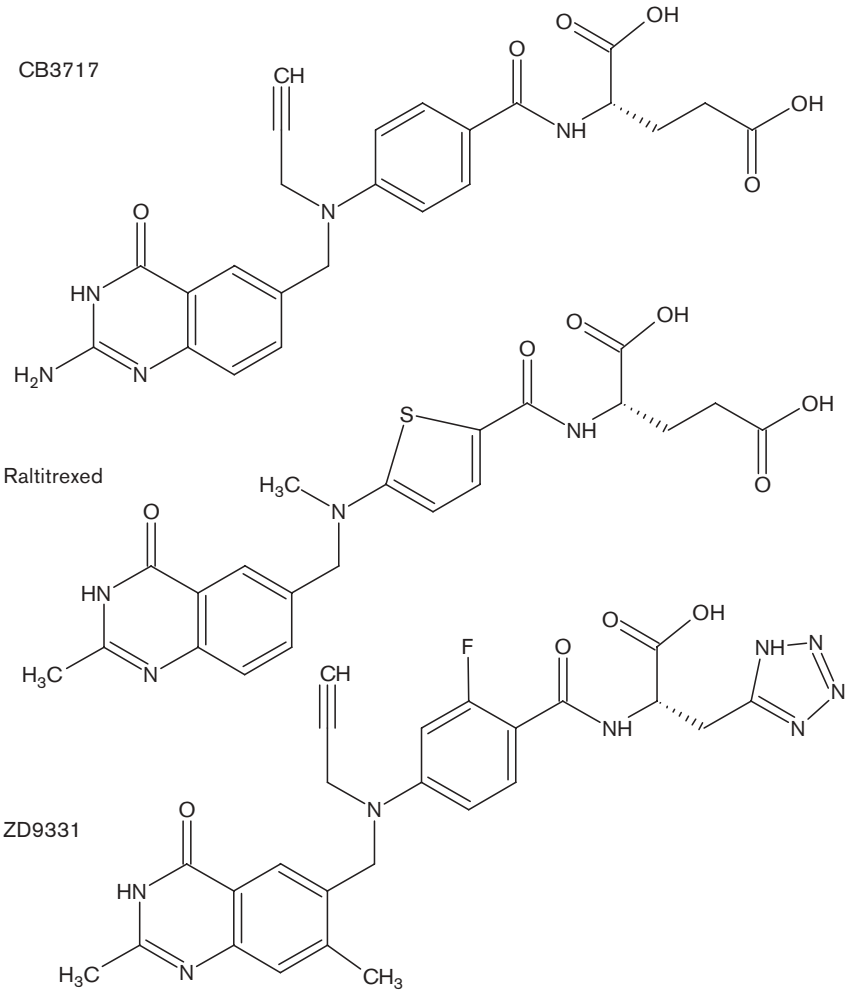
TS binds both deoxyuridine monophosphate (dUMP) and 5,10-methylene tetrahydrofolate. There was therefore a choice of model in the initial development of a specific TS inhibitor. The folate substrate was considered better. First, because with the exception of polyglutamation it would not require metabolic activation (unlike pyrimidine analogs). Second, in contrast to pyrimidines, there are no specific enzymes for folate catabolism. Third, folate analogs cannot be incorporated into nucleic acids, thus circumventing the side-effects associated with this property of nucleoside analogs. Finally, as the natural folate co-substrate for TS was also a substrate for three additional enzymes and therefore lay at a metabolic branch point, a selective folate-based TS inhibitor would potentially limit accumulation of the displaced substrate as it was not a substrate for these other enzymes. With pyrimidine-based inhibitors, accumulation of dUMP can compete with the inhibitor.

The first such compounds to show specific TS inhibitory activity were 2,4-diamonoquinazoline analogs and were synthesized in the early 1970s [4]; however, it was found that these compounds also bound very tightly to DHFR [5]. In the 2-amino-4-hydroxy series a methyl group substitution in the 5 position impaired TS inhibition [6], but introduction in the 10 position was found to enhance TS inhibition. This prompted further systematic structure–activity investigation evaluating the effect of substitution at the N^{10} position on cytotoxicity and TS inhibition. Initially, a propyl group substitution (CB3715) generated a compound with an I_{50} for L1210-derived TS of 170 nM. Subsequent N^{10} allyl substitution (CB3716) improved TS inhibition 3-fold compared to CB3715. CB3717 had an N^{10}-propargyl substitution (Fig. 1) with a 30-fold improved I_{50} for TS compared to CB3715 [1] and was the first of the class to enter clinical trials.

The reduced folate carrier (RFC) is a low-affinity, high-capacity system that bi-directionally transfers folates and anti-folates across the plasma membrane (Fig. 2). CB3717

0959-4973

Fig. 1

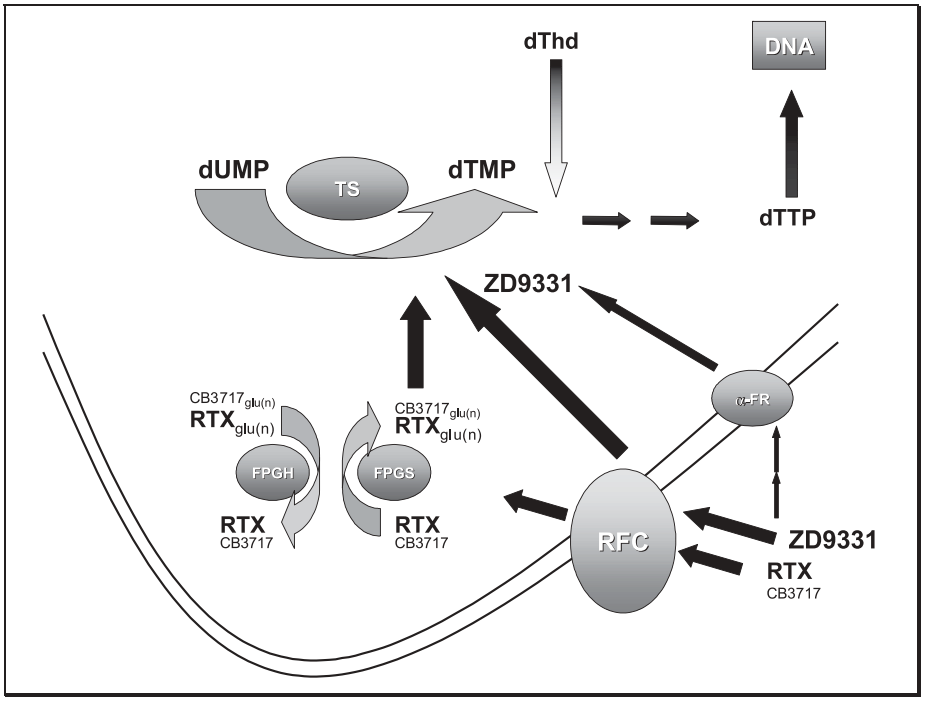


The structures of CB3717, RTX and ZD9331.

has low affinity for the RFC and is therefore transported slowly into cells. However, it is efficiently polyglutamated by the enzyme folylpolyglutamate synthetase (FPGS) [7] and the polyglutamates are very potent TS inhibitors, up to 2 orders of magnitude more effective than the parent drug. Phase I evaluation of CB3717 demonstrated activity in relapsed ovarian cancer with a response rate of 29% [8]. Phase II activity was greatest in mesothelioma (43%) and ovarian cancer (18%) [9]. Unfortunately, unpredictable nephrotoxicity (attributed to poor aqueous solubility at the acid pH of urine), when combined with myelosuppression, was potentially life threatening and prevented its further clinical development. However, the activity demonstrated supported further development of folate-based TS inhibitors.

Jones *et al.* proposed that the poor aqueous solubility was due to a physiochemical event, thought to be due to inter-molecular hydrogen bonding revolving around the 2-amino-3,4-dihydro-4-oxopyrimidine moiety of the drug [10]. Indeed 2-desamino CB3717 reduced the ability of the molecule to form inter-molecular hydrogen bonds and was thus more water soluble. ICI pharmaceuticals then commenced a joint pharmaceutical program which led to the synthesis of 2-substituted analogs as TS inhibitors [11]. Substitution at the C^2 position of these molecules with hydrogen resulted in a slight loss of TS inhibition, but with an increase in cytotoxic potency [10,12]. The 2-desamino-2-methyl analog (ICI198583) was the most promising candidate. It was found to be a 3-fold less potent TS inhibitor than CB3717 (10 versus 3 nM), an equal substrate for FPGS, but a better substrate for the RFC [2]. Further modification by replacement of the N^{10} propargyl group with a methyl and the PABA ring with a thiophene ring resulted in a compound with promising preclinical activity. This drug is now known as RTX

Fig. 2



The site of uptake and action of CB3717, RTX and ZD9331. FPGS = folylpolyglutamate synthetase, FPGH = folylpolyglutamate hydrolase, TS = thymidylate synthase, RFC = reduced folate carrier, α-FR = α-folate receptor, dUMP = deoxyuridine monophosphate, dThd = thymidine, dTMP = deoxythymidine monophosphate, dTTP = deoxythymidine triphosphate, RTX = raltitrexed. CB3717, RTX and ZD9331 are taken up by the RFC. RTX and, to a lesser extent, CB3717 are substrates for FPGS, and the polyglutamates inhibit TS. FPGH degrades polyglutamates and its relative activity is a potential mechanism of resistance to polyglutamatable antifolates.

(ICI1694, Fig. 1). The monoglutamate has a K_i against isolated TS from L1210 mouse leukemia cells of 62 nM; however it is a good substrate for FPGS and the predominant tetraglutamate metabolite has a K_i of 1 nM [2]. Although polyglutamation has the twin advantages of enhanced TS inhibition and retention in cells, if tumors express low levels of FPGS this may confer resistance [13,14]. In phase I trials RTX demonstrated mainly gastrointestinal and hematological toxicities, with no renal toxicity observed [15]. A number of single-agent phase II studies have been performed. The most clinical activity as monotherapy was seen in breast cancer [16] and colorectal cancer (CRC) [17] for which RTX has now been registered as first-line palliative treatment in Europe for patients in whom bolus or infusional 5-fluorouracil schedules are inappropriate or not tolerated.

ZD9331: a non-polyglutamatable, specific TS inhibitor

In vitro studies

ZD9331 was developed as an analog of ICI198583. It differs from this molecule by virtue of the presence of a tetrazole ring in the γ-carboxyl position of the glutamate residue (Fig. 1) [3] which prevents it being a substrate for FPGS. It was designed to overcome resistance to RTX due to the presence of low FPGS expression and has been demonstrated to do so in *in vitro* studies, as well as potentially providing a different, more tolerated toxicity profile [3]. It was essential to enhance the potency of the parent compound against TS rather than relying on polyglutamation and the compound has a K_i for TS of approximately 0.4 nM [3]. It is transported via the RFC, having displayed a K_i for the inhibition of [^{3}H]MTX transport of 0.7–1.5 μM [3]. In addition, recent evidence suggests that it may be transported by the α-folate receptor (α-FR) under certain conditions *in vitro* [18], which may have therapeutic implications for tumors that overexpress this receptor, such as ovarian cancer [19].

In vivo studies

Initial *in vivo* experiments using a thymidine salvage incompetent mouse lymphoma demonstrated 100% tumor inhibitory activity at a dose of 3 mg/m^2 by a 24-h continuous infusion. A single i.p. injection of 10 mg/kg cured 60% of mice and 25 mg/kg cured all mice [3]. This is attributable to the long terminal phase of plasma elimination seen in DBA2 mice following a single bolus injection ($t_{1/2}$ = 5–6 h) [20]. Chronic administration by

continuous infusion (7 days) in thymidine salvage competent mice resulted in nine out of 16 cures at a dose of 100 mg/kg/day [3].

A number of xenografts in nude mice were treated with oral and infusional ZD9331. At an infusional dose of 80 mg/kg/day the most sensitive xenograft was Colo 205, a human colorectal tumor with a median growth delay of 56.2 days. The least sensitive was the A549 human non-small cell lung cancer xenograft, with a mean growth delay of 2.5 days. The ovarian HX-62 xenograft had an intermediate median growth delay of 23.1 days. This *in vivo* activity led to the expectation of broad range clinical activity (Table 1).

Toxicity studies in mice showed 5–25% body weight loss with 25–200 mg/kg/day continuous s.c. infusion over 7 days. Concomitant treatment with thymidine (dThy) reduced this toxicity indicating that this was related to inhibition of TS [21]. Further mouse studies showed ZD9331 to cause mainly hematological and intestinal toxicity, in a different pattern from that of ZD1694, which was attributed to the lack of polyglutamation.

Dog studies differed in that, unlike rodents, they do not have high circulating plasma thymidine levels which can provide an effective salvage pathway following TS inhibition. A single bolus dose of 50 mg/kg was not tolerated in beagles. In low-dose toxicity studies using a 5-day bolus or 5-day continuous infusion of ZD9331, the lowest doses causing minimal reversible toxic effects were 0.06 and 0.04 mg/kg/day, respectively (the main side-effects observed were reduced food intake and lymphopenia) [21].

Rodent pharmacokinetic (PK) studies reported plasma concentrations 30% higher in males compared to females. The distribution half-life ($dt_{1/2}$) was deemed to be short (0.2 h), which was confirmed by the absence of accumulation after multiple dosing. In dogs the $dt_{1/2}$ was 0.3 h with a terminal elimination half-life ($t_{1/2}$) of 5 h and, like rodents, no accumulation was seen after multiple dosing. Elimination of the drug was predominantly fecal in dogs and rats, indicating biliary excretion as the major route of elimination. Metabolism accounted for less than 20% of the administered dose and the drug was found to be highly protein bound in all species [21].

Table 1 Activity of ZD9331 in a panel of human tumor xenografts (adapted from [21])

Tumor	Dose[a] (mg/kg/day)	Median growth delay (days)
Colo 205 (colorectal)	80	56.2
N592 (SCLC)	80	34.8
HX62 (ovarian)	80	23.1
Lovo (colorectal)	80	20.5
N417A (SCLC)	80	20.4
MKN45 (gastric)	80	20.1
HT29 (colorectal)	80	16.9
HX147 (NSCLC)	80	9.1
A549 (NSCLC)	80	2.5

[a]ZD9331 administered as a 14-day bolus or s.c. infusion. SCLC = small cell lung cancer; NSCLC = non-small cell lung cancer.

Encouraging preclinical activity in certain tumor types led to an extensive phase I program. There were five i.v. monotherapy phase I studies of ZD9331 (Table 2). The first two trials involved prolonged dosing based on animal PK data. In trial 0001, ZD9331 was administered as an i.v. bolus daily for 5 days every 21 days. Following a starting dose of 0.4 mg/m^2/day a total of 74 patients were enrolled. The maximum tolerated dose (MTD) was 16 mg/m^2/day. Myelosuppression and fatigue were the worst grade 3 or 4 toxicities in this study. Clearance of ZD9331 in this study was triphasic and the mean elimination half-life ($t_{1/2}$) was 71 h, considerably longer than predicted from animal studies. One tumor response was seen in a patient with CRC [22].

In trial 0002 (45 patients), ZD9331 was administered as a 5-day continuous infusion every 3 weeks and the MTD was established at 6 mg/m^2/day. The mean plasma elimination $t_{1/2}$ in this study was similar at 75 h. Two patients showed evidence of tumor response (one breast and one ovarian) [23]. The subsequent two i.v. trials had short administration schedules. Trial 0003 (58 patients) administered ZD9331 as a single i.v. bolus on day 1 every 21 days and the MTD was 654 mg/m^2/day. The elimination $t_{1/2}$ in this study was slightly longer at 92 h [24].

Trial 0004 (71 patients) administered ZD9331 as a single i.v. bolus on days 1 and 8 every 21 days, and the MTD was established at 130 mg/m^2/day. The elimination $t_{1/2}$ in this study was 32 h at the MTD. Two-thirds of the patients recruited had either colorectal or ovarian cancer. This dose and schedule was put forward as the recommended dose for phase II evaluation. The toxicities seen in these studies were similar and the dose-limiting toxicity was hematological, consisting of thrombocytopenia and neutropenia. Nausea, vomiting, fatigue, diarrhea and rash were the commonest non-hematological toxicities, but were not dose limiting. One patient with CRC had a partial response and five patients with ovarian cancer had a greater than 50% reduction in their CA-125 (one of whom had a 40% reduction in the size of their marker

Table 2 Phase I i.v. single-agent trials of ZD9331

Study	Administration	No. patients	Reference
0001	i.v. bolus days 1–5 q 21	74 (44 CRC)	[22]
0002	5-day continuous infusion q 21	45	[23]
0003	i.v. bolus day 1 q 21	58	[24]
0004	i.v. bolus days 1 and 8 q 21	71	[25]
Japanese study	i.v. bolus days 1 and 8 q 21	18	[26]

lesion) [25]. The Japanese study enrolled 18 patients with predominantly gastrointestinal malignancies. ZD9331 was administered on a days 1 and day 8 schedule (at doses of 69, 108 and 130 mg/m^2/day). Although no responses were seen, one-third of patients achieved disease stabilization. The toxicity profile was similar [26].

Oral studies were also undertaken due to a perceived need for prolonged administration of ZD9331. Anti-tumor activity was observed following oral administration of ZD9331 in rodents (AstraZeneca, personal communication) and a similar toxicity profile was observed with oral administration as with i.v.

Three oral phase I studies enrolling 130 patients were undertaken (Table 3), two of which have been reported [27,28]. In the de Jonge study, 42 patients were enrolled, with CRC being the most common tumor type. Dosing was initially for 5 days on a 21-day cycle (ranging from 2.5 to a maximum of 40 mg/day). Although DLTs were only seen at doses of 40 mg/day, PK data demonstrated no increased plasma exposure to ZD9331 above 20 mg/day. The oral preparation was rapidly absorbed, and peak concentrations were achieved between 1 and 2 h after dosing. The mean elimination $t_{1/2}$ was relatively constant in all subjects at 84 h. As a result, 7- and 10-day dosing schedules were investigated. In humans the toxicity reported was slightly different, with skin rash becoming the prominent non-hematological toxicity. Overall, limited toxicity was observed and one patient with gastric cancer achieved a partial response.

In the other published study 55 patients were enrolled and ZD9331 was administered in doses from 0.5 to 4.0 mg/day, for 28 consecutive days out of a 42-day cycle. The majority of patients enrolled had upper or lower gastrointestinal malignancies, with eight patients with ovarian or endometrial cancer. The dose-limiting toxicities were thrombocytopenia and neutropenia, with nausea and rash being the predominant non-hematological toxicities. The mean $t_{1/2}$ at 3.0 mg/day was 25 h ($\pm$ 22 h). There were no objective tumor responses, but 14 patients experienced disease stabilization [28].

Three clinical pharmacology/metabolic studies have been performed with ZD9331 (Table 4). The study by de Jonge *et al.* assessed the effects of renal impairment on ZD9331 PKs. Twenty-three patients were enrolled in the study. Patients received ZD9331 as an i.v. infusion on day 1 of a 28-day cycle for the first cycle, followed by standard days 1 and 8 dosing every 21 days for subsequent cycles. Analysis of plasma clearance of ZD9331 in these patients showed no relationship between degree of renal impairment, as determined from creatinine clearance, and plasma free drug concentration. However, myelotoxicity was proportionately increased in patients with renal dysfunction independent of plasma PK which may necessitate dose reduction of the compound in patients with an impaired glomerular filtration rate [29].

The other published metabolic study looked at metabolism excretion and PK of [^{14}C]ZD9331. The major route of elimination of the drug was fecal, 70.5%, with urinary excretion accounting for 23.6%. Recovery of the dosed radiolabeled dose was essentially complete by day 7 [30].

Single-agent phase II studies of ZD9331

There have been four single-agent phase II trials of ZD9331 (Table 5). The most promising clinical activity demonstrated has been in the first-line treatment of advanced, relapsed or inoperable gastric cancer [31]. A total of 34 patients were recruited to the study. The first 10 patients were treated with ZD9331 at 130 mg/m^2 days 1 and 8 on a 21-day cycle. However, following a protocol amendment, the remaining 24 patients were treated at a reduced dose of 65 mg/m^2 on the same schedule. The toxicity profile was similar to other studies. The most

Table 3 Oral phase I studies of ZD9331

Study	Administration	No. patients	Activity	Reference
0010	oral ZD9331 for 14, 21 or 28 days every 6 weeks	55	25% (SD)	[28]
0011	oral ZD9331 for 5, 7 or 10 days every 3 weeks	42	2% (PR); 43% (SD)	[27]
0012	oral ZD9331 continuously	33	21%	AstraZeneca, personal communication

Table 5 Phase II single-agent studies of ZD9331

Tumor type	First line/ second line	No. patients	Response rate (%)	Reference
Gastric	first	29	17	[31]
Ovarian	third	46	7	[47]
NSCLC	second	46	2	[34]
Colorectal	second/third	100	2	[36]

Table 4 Clinical pharmacology/metabolic studies of ZD9331

Study	Administration	No. patients	Reference
0018 (to assess the effects of renal impairment on ZD9331 PK)	ZD9331 i.v. day 1 then days 1 and 8 from cycle 2	23	[29]
0020 (to assess the effects of hepatic impairment on ZD9331 PK)	Oral ZD9331 for 5, 7 or 10 days every 3 weeks	14	AstraZeneca, personal communication
0021 (to assess the metabolism and excretion of [^{14}C]ZD9331)	Oral ZD9331 continuously	7	[30]

frequently reported toxicities were neutropenia (62%), diarrhea (38%), anemia (35%) and abdominal pain (32%). Of 29 assessable patients there was an overall response rate of 17%. Interestingly all responders were in the 65 mg/m^2 treatment subgroup. This response rate, although generally lower than that seen with combination therapies in gastric cancer [32], is comparable to single-agent therapy with 5-FU [33].

In a study in non-small cell lung cancer as second-line therapy, a response rate of only 2% was seen in 46 patients [34]. This may be a reflection of the poor activity seen against xenografts in this tumor type *in vivo* [21] or just due to the poor chemosensitivity seen with TS inhibitors in this disease [35]. In a study of ZD9331 as second or third line in CRC, of 100 patients treated (45 second line and 55 third line), a response rate of only 4% was seen in the patients treated third line [36] (AstraZeneca, personal communication). This compares unfavorably with other agents in relapsed CRC [17].

ZD9331 combination and randomized studies (non-ovarian)

A number of combination studies with ZD9331 in different tumor types have been undertaken (Table 6). On the basis of preclinical synergy between ZD9331 and SN38 the active metabolite of irinotecan, a phase I dose escalation study of ZD9331 followed by irinotecan at a fixed dose was undertaken in patients with relapsed CRC. Twenty-one patients were enrolled. DLTs were seen at 90 mg/m^2 ZD9331 in 50% of patients. One patient achieved a partial response and 12 achieved disease stabilization [37].

A phase I study in combination with cisplatin enrolled 16 patients at three dose levels (ZD9331 100 or 130 mg/m^2 on days 1 and 8 with 50 mg/m^2 of cisplatin on day 1 or 130 mg/m^2 ZD9331 on days 1 and 8 with 75 mg/m^2 cisplatin on day 1). Dose-limiting toxicities were seen at the highest dose level, and were neutropenia, thrombocytopenia and fatigue. Of 15 assessable patients, two responded to treatment, with six achieving disease stabilization [38]. Preclinical synergy was the rationale for combining ZD9331 with gemcitabine. Although toxic to normal tissues, no anti-tumor synergism was demonstrated (AstraZeneca, personal communication).

A phase I trial of ZD9331 and docetaxel was reported in 2002 with 16 patients enrolled with predominantly refractory colorectal and non-small cell lung cancer. The most prominent toxicities were neutropenia, thrombocytopenia and fatigue [39].

A second/third-line randomized phase II study in breast cancer is now complete. A total of 65 patients were randomized, of which 63 were evaluable. Patients received ZD9331 at 3 mg/day by mouth for 28 days every 6 weeks or 130 mg/m^2 ZD9331 i.v. days 1 and 8 every 3 weeks. The oral schedule was better tolerated and response rates were 9.7 (oral) versus 12.5% (i.v.) [34].

A randomized study in pancreatic cancer is also complete. A total of 55 patients were randomized to receive i.v. ZD9331 at 130 mg/m^2 on days 1 and 8 every 3 weeks or i.v. gemcitabine 1000 mg/m^2 weekly for 7 out of 8 weeks then subsequently 4-weekly on days 1, 8 and 15. Objective and clinical and benefit response rates were

Table 6 Phase I/II combination/randomized studies of ZD9331

Predominant tumor type	Combined with	First line/second line	No. patients	Response rate (%)	Reference
Combination studies					
colorectal	topotecan		16	9	[41]
NSCLC					
pancreas					
colorectal	irinotecan	second/third	17	0	[37]
mesothelioma	cisplatin		16	12.5	[38]
colorectal					
pancreas	gemcitabine		27		AstraZeneca, personal communication
colorectal	docetaxel		16		[39]
NSCLC					
ovarian	carboplatin	second/third/fourth	14	28	[48]
Randomized phase II studies					
ovarian	ZD9331 135 mg/m^2		2		AstraZeneca, personal communication
	topotecan 1.5 mg/m^2 5/21 days				
ovarian (second)	ZD9331 65 mg/m^2		40	2.5	[34,41]
	ZD9331 130 mg/m^2		40	10	
	ZD9331 65 mg/m^2 and topotecan 0.5 mg/m^2		41	14.6	
pancreatic	ZD9331 65 mg/m^2		30	3	[40]
	gemcitabine		25	8	
breast	ZD9331 (oral)		65	12.5	[34]; AstraZeneca, personal communication
	ZD9331 (i.v.)			9.7	

similar; however, a greater proportion of patients were alive in the ZD9331 arm at cut-off compared to the gemcitabine arm (13 versus 8%). The time to progression and the median duration of survival also favored the ZD9331 arm [40].

A phase I combination study with topotecan was also undertaken. Sixteen patients with predominantly refractory colorectal, non-small cell and pancreatic cancer were enrolled. ZD9331 was administered days 1 and 8, with topotecan as a 30-min infusion on days 1–5 of a 21-day cycle. Fatigue, nausea and thrombocytopenia were the most common toxicities [41].

ZD9331 studies in ovarian cancer—rationale for investigation

Whilst ZD9331 was investigated in many tumor types, initial evidence from a phase I study [25] suggested that ZD9331 may be active in ovarian cancer. In this trial of heavily pre-treated patients, one patient with ovarian cancer had a radiologically confirmed response to treatment and a further five patients had more than 50% reductions of their CA-125 levels on treatment. Targeting TS in the form of 5-FU or RTX in relapsed ovarian cancer has previously resulted in disappointing clinical activity, i.e. a 6% response rate for RTX [42] and 13% for 5-FU [43]. However, there were a number of other reasons for wanting to investigate ZD9331 in this disease.

Pre -clinical studies demonstrated a high incidence of low FPGS expression in a limited panel of six human ovarian tumor cell lines as compared to a panel of human CRC cell lines and ZD9331 overcame the associated resistance to RTX in these cells [44]. It was hypothesized that this may be the reason for the poor clinical activity of RTX.

CB3717, RTX [45] and ZD9331 [44] demonstrated non-cross-resistance to platinum agents in human ovarian tumor cell lines.

The first specific folate-based TS inhibitor to be developed clinically, CB3717, demonstrated considerable phase I [8] and phase II [9] activity in platinum-resistant ovarian cancer. This drug is a relatively poor substrate for FPGS [46].

RTX and ZD9331 enter the cell via the RFC, but under certain conditions may be taken up into the cell via the α-FR, which is overexpressed in approximately 90% of epithelial ovarian cancers [19]. Recently, it has been shown *in vitro* that when drug exposure times are short, α-FR-mediated uptake may be more important for ZD9331 than for RTX [18].

As well as demonstrating activity at phase I, ZD9331 also demonstrated encouraging activity in a phase II study in relapsed ovarian cancer [47].

There have been four clinical studies of ZD9331 in ovarian cancer. A dose-finding phase I combination PK and pharmacodynamic study, in combination with carboplatin in platinum-sensitive relapsed ovarian cancer [48], demonstrated tolerability of the combination, anti-tumor activity in four of 14 patients enrolled and pharmacodynamic evidence of TS inhibition.

A third-line single agent study in ovarian cancer enrolled 44 patients who received ZD9331 130 mg/m^2 days 1 and 8 every 21 days as monotherapy. All patients had received at least carboplatin and taxol, and subsequently failed topotecan second-line. A 7% response rate was seen in this study [47].

The first randomized study of ZD9331 versus topotecan was stopped due to poor patient accrual (AstraZeneca, personal communication) and the subsequent study was a triple arm randomization of ZD9331 at 65 versus 130 mg/m^2 versus ZD9331 65 mg/m^2 plus topotecan 0.5 mg/m^2. A total of 121 patients were recruited. Drug-related serious adverse events were more frequent in the combination arm. Overall response rates and response rates in platinum refractory patients are shown in Table 7. In comparison to other agents in relapsed ovarian cancer, these response rates are poor as most agents approved for this indication have single-agent activity above 10%. However, when patients with primary platinum refractory disease (relapse within 3 months of completion of last treatment) are identified, the ZD9331 single-agent response rate at 130 mg/m^2 is comparable to that of other agents.

Conclusion

ZD9331 is a third-generation specific TS inhibitor that was developed through an extensive program of rational drug development over many years. Its development was based on the rationale of increased efficacy, by overcoming the potential for resistance due to reduced expression of FPGS, whilst potentially reducing the toxicities associated with polyglutamation and retention in normal tissues. While the drug was not targeted to any individual tumor type, a large number of phase I, II, monotherapy and combination studies have been undertaken, and overall activity has been most promising in

Table 7 Overall and platinum-refractory response rates in trial 0024 ([34,41] and AstraZeneca, personal communication)

Arm	Dose ZD9331 (mg/m^2)	Total patient no.	Overall response rate (%)	Platinum-refractory patients	Platinum-refractory response rate (%)
ZD9331	65	40	2.5	19/40	5.3
ZD9331	130	40	10	12/40	16.7
ZD9331 and topotecan	65	41	14.6	1/41	7.1

ovarian, pancreatic and gastric cancers. It could play an important role in the treatment of these diseases, especially if patients were to be selected on the basis of their folate transport and FPGS status. The true potential of the drug remains to be determined.

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