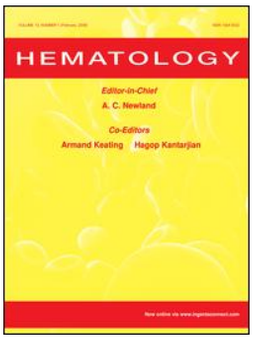

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Troxacitabine in acute leukemia

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Abstract
Troxacitabine (Troxatyl; BCH-4556; (−)-2′-deoxy-3′-oxacytadine) is the first synthetic L-nucleoside enantiomer to demonstrate broad spectrum cytotoxic activity. It was obtained by exchanging the sulphur endocyclic atom with oxygen in the structure of lamivudine, following the discovery that this agent had cytotoxic, as well as anti-viral activity. The unique "unnatural" stereochemistry of troxacitabine has produced impressive cytotoxic potency against a wide range of malignancies in the laboratory which led to its selection for clinical development. The initial trials with troxacitabine have established its efficacy in both solid and haematological malignancies, including those resistant to ara-C (cytarabine). This review will consider troxacitabine in terms of its pharmacology, mode of action, pharmacokinetics, tolerability and clinical efficacy.

Keywords: *Troxacitabine, nucleoside analogues, review, acute myeloid leukaemia*

Introduction

Nucleoside analogs remain the cornerstone of treatment for acute leukaemia. Naturally occurring nucleosides and their analogs, including cytosine arabinoside (Ara-C) and fludarabine, are approved as anti-neoplastic agents in the D-configuration [1]. Until recently, it was held that nucleoside enantiomers in the L-configuration were not effective inhibitors of cellular metabolic enzymes [2]. However, in the early nineties, several dioxolane nucleosides were evaluated as anti-viral agents including the cytosine derivative lamivudine (3TC). This agent displayed potent activity against HIV and Hepatits B virus [3–5], as well as being amongst the first nucleoside L-enantiomers to demonstrate cytotoxic activity [6]. Troxacitabine (Figure 1) was obtained from the substitution of the sulphur endocyclic atom with oxygen in the structure of lamivudine [6,7]. Despite similar stereochemistry, troxacitabine has broader cytotoxic activity by comparison to lamivudine [7–10].

Pharmacology and mechanism action

Troxacitabine, by having an unnatural β-L configuration, has distinct metabolic and pharmacokinetic properties. Early work on troxacitabine transport into cells focussed on equilibrative sensitive and insensitive transport systems [8], however, recent experiments have shown that its membrane permeation is largely non-carrier mediated. Tumour cells refractory to traditional nucleoside analogs, because of the absence of nucleoside transporters, have been shown to be sensitive to troxacitibne [11]. *In vitro* studies have shown that troxacitabine is a poor permeant to a range of molecularly characterised nucleoside specific membrane transporters (NSMT) which mediate plasma membrane permeation of many of the nucleoside analogs. [12]. Additionally, NSMT deficient variant CCRF-CEM leukaemia cell lines are resistant to ara-C (1150-fold) and gemcitabine (432-fold) but not to troxacitabine [7,11,13]. These data indicate that passive diffusion is likely to be the main mechanism of cellular uptake. It is not surprising

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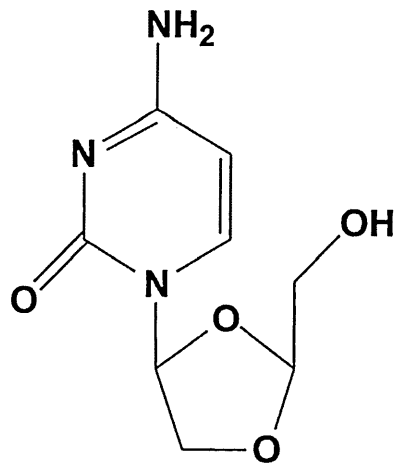

Figure 1. Chemical structure of the β-L-nucleoside analogue, troxacitabine.

then, that troxacitabine has displayed efficacy in multi-drug resistant cancers expressing *p*-glycoprotein [14]. Cellular NSMT content in leukaemia cell blasts correlate directly with sensitivity to ara-C *in vitro* [12], therefore troxacitabine may be effective in cases of ara-C resistance mediated by NSMT driven mechanisms.

Following uptake in to the cell, monophosphorylation of troxacitabine, similar to ara-C, is mediated by the enzyme deoxycytidine kinase (dCK), indicating that dCK lacks stereo-selectivity [7]. Resistance to troxacitibine *in vitro* has been simulated in DU145 prostate cancer cell lines by producing a single amino acid switch in the dCK gene resulting in reduced dCK activity [11]. Studies involving the CCR-CEM T-lymphoblastic human leukaemia cell line have demonstrated similar results [13]. Deoxycytidine kinase, therefore, is a critical rate limiting step and mediator of the anti-neoplastic effect of troxacitabine. Phosphorylation of di- to tri-phosphate is mediated by 3-phosphoglycerate kinase, unlike ara-C, where this step is facilitated by the enzyme nucleoside diphosphonate kinase [15,16]. Also, intracellular troxacitabine diphosphate concentrations increase in a linear fashion with the extracellular concentrations of the pro-drug [17]. In contrast, this linear relationship is not seen with ara-C and intracellular levels of ara-CTP.

After phosphorylation of the drug has occurred, it is incorporated into DNA, but not RNA [18]. Importantly, the cytotoxic effect of troxacitabine relates directly to the amount of mono-phosphorylated drug present in DNA terminals [8]. This produces degradation of DNA into large fragments at high concentrations [8], as well as causing chain termination, probably because its dioxolane ring lacks a hydroxyl moiety necessary for chain elongation. Once incorporated, the 3′–5′ exonuclease associated with DNA polymerases, by having chiral preference for nucleoside-D-enantiomers, do not effectively remove troxacitabine monophsphate from DNA. The human apurinic/apyrimidinic endonuclease (APE-1), otherwise known as HAP-1 or Ref-1, has been implicated in the removal of troxacitabine monophosphate from the DNA of leukaemic blasts [19]. Thus, the incorporation of phosphorylated troxacitabine into DNA and, to a lesser extent, its ability to inhibit replicative and repair DNA polymerases [18], represent the main mechanisms of action of the drug.

Grant et al. [20] have shown that resistance to ara-C, in some cases, is mediated by deamination by the enzyme deoxycytidine deaminase (dCD). Unlike dCK, dCD is stereo-selective and does not deaminate troxacitabine which led to work by Gordeau et al. [21] showing troxacitabine activity in ara-C resistant human leukaemia xenograft models that expressed increased levels of dCD. Other *in vitro* studies have confirmed this experience. Ara-C was shown to be more effective against CCRF-CEM leukaemia cell lines compared to HL60 cell lines which have high dCD activity, whilst troxacitabine showed similar anti-proliferative activity in both cell lines [21].

Pre-clinical development

Biological activity

Troxacitabine mediated cytotoxicity has been shown in a wide range of solid tumour types (Table I). *In vitro* studies have confirmed efficacy in cancer cell lines of hepatocellular (HepG2), prostate (PC3, DU145), non-small cell lung (NCI-H460, NCI-322M) colon (HT29), renal (CAK-1, A498, RXF-393, SN12-C) and pancreatic origin (Pnac-01, MiaPa Ca) [8–10,22]. Schwartz et al. [23] showed that troxacitabine caused cellular arrest of human keratinocytes with an IC50 of 50 nM, 1000 times more potent that 3′-azidothymidine. *In vivo*, complete regression was seen in mice with CAK-1, A498 and RXF393 renal cell carcinomas with time and dose dependant administration of troxacitabine [10]. Inhibition of visceral metastatic disease by troxacitabine was shown by Rabbani et al. [24] when rats were inoculated with murine prostate cancer cell lines. Troxacitabine was more effective than gemcitabine in mice bearing the highly resistant pancreatic cell line, line Panc-01 with tumour inhibition levels of 88.5 and 84.3% at 10 and 25 mg/kg, respectively, [21]. Kim et al. [22] have shown that troxacitabine and camtothecan have displayed synergy in KB and KB100 (camtothecan resistant) oropharyngeal carcinoma cell lines.

In vitro studies of troxacitabine in haematological malignancies have confirmed efficacy in HL60 and CCRF-CEM T-lymphoblastoid leukaemia cell lines [21,22]. Troxacitabine based combinations tested *in vitro* have revealed that the addition of ara-C was synergistic and prolonged the survival of CCRF-CEM cell lines [25], whilst the combination of troxacitabine

Table I. Troxacitabine efficacy against human tumour xenografts in nude mice.

Cell line	Origin/tumour histology	Troxacitabine % T/C
Myeloid malignancies		
CCRF-CEM	T-Lymphoblastoid leukaemia	152*
BKM5	Chronic myeloid leukaemia	171*
HL60	Acute myeloid leukaemia	422*
Solid tumour		
HT-29	Colon	5**
KBV	Head and neck	19
HepG2	Liver	26
A549	NSCLC	41
NCI-H460	NSCLC	46
NCI-H322M	NSCLC	62
Panc-01	Pancreas	16†
MiaPaCa	Pancreas	77†
PC-3	Prostate	4
DU-145	Prostate	7
SN-12C	Renal	8
RXF-393	Renal	13
CAKI-1	Renal	16
A498	Renal	27

Troxacitabine was given intraperitoneally twice daily at a dose of 25 mg/kg unless specified. T/C values resulted from dividing the mean treated tumour volume divided by the mean tumour control volume times 100. As per the NCI criteria, T/C $< 42\%$ indicates that the drug is active. NSCLC: Non-small cell lung carcinoma. *Troxacitabine was administered once daily. Results are expressed as the percentage of mean survival time of treated animals over the mean survival time of the control group. As per the NCI criteria, T/C $> 125\%$ indicates significant anti-tumour activity of troxacitabine. **Two cycles of troxacitabine were given. †Troxacitabine was administered once daily intravenously.

and imatinib showed synergy against imatinib—sensitive (KBM5, KBM7) and imatinib resistant (KBM5-R, KBM7-R) cell lines [26]. *In vivo* studies of SCID mice inoculated with HL60 promyelocytic leukaemia cell lines and treated with intra-peritoneal ara-C or troxacitabine revealed that troxacitabine prolonged survival, however, survival times were comparable for both drugs in SCID mice inoculated with CCRF-CEM cells [7,21]. These findings concur with other studies showing troxacitabine efficacy in ara-C resistant leukaemias *in vitro* and *in vivo* [21,22]. Troxacitabine with ara-C (TA) slowed the progression of leukaemia in SCID mice and the combination approach was more effective that either agent used alone without additional toxicity [25]. SCID mice inoculated with KBM5 and KBM5-R cell lines lived longer when treated with troxacitabine compared to treatment with imatinib and some KBM5 inoculated animals achieved long-term survival when treated with both agents together.

Metabolism

Moore et al. [17] administered troxacitabine intravenously and orally to adult Sprague-Dawley rats at doses of 10, 25 or 50 mg/kg with 6 day washout periods between doses. Plasma concentrations of troxacitabine fell rapidly within 8 h of dosing with average half life (T1/2) values of 1.65 ± 1.12 h. Non-renal clearance was the primary route of elimination with approximately 22% of unchanged drug appearing in the urine. An average steady state volume of distribution (V_{SS}) of 1.42 ± 0.66 l/kg implied an intracellular distribution of the drug. Oral absorption of troxacitabine was slow and unpredictable with wide variation in bioavailability occurring between individual rats indicating that intravenous infusion would be the most appropriate method to deliver the drug in the phase I setting.

Toxicology

The toxicity of troxacitabine at different doses and methods of administration has been evaluated in rats and cynomolgus monkeys [27]. Rats were relatively more tolerant, and single doses of 12 mg/m^2 did not produce any toxic effects. With cumulative dosing toxicity was generally haematological. Cynomolgus monkeys, however, experienced haematological toxicity at much lower doses and 12 mg/m^2 was considered as the toxic low dose (TLD). Higher doses of 36 mg/m^2 resulted in the death of some animals, whilst others experienced severe marrow failure, mucositis and hepatic toxicity. Species differences may have been due to slower clearance rates in monkeys compared to rats producing a relatively longer half life. These findings led to comparative myelosuppression studies of troxacitabine using monkey and human progenitor cells which concluded that human progenitors may be up to 50-fold more sensitive to troxacitabine than cynomolgus monkey progenitor cells [28].

Clinical pharmacokinetics

Three phase I trials involving a total of 60 patients with solid tumours evaluated the pharmacokinetics

of troxacitabine given in different dosing schedules: as a single 0.025–10.0 mg/m^2 dose, as 5 daily doses of 0.12–1.0 mg/m^2 every 3 weeks or as 0.4–3.2 mg/m^2 weekly doses for the first 3 weeks of a 4 week course [27,29–31]. Combined data from these studies revealed that following 1 dose, troxacitabine had a low protein binding (<3%) and 48 h later an average of 61% was excreted unchanged in the urine. V_C, V_{SS}, $T1/2$ and Cl_S values of 13 l, 107 l, 25 h and 135 ml/min were found in 25 of these patients and reflected lower than anticipated PK parameters compared to the PK studies of other nucleoside analogs. Higher AUC values were seen in patients experiencing grade 3–4 neutropenia. Troxacitabine was administered in escalating doses (0.12–1.8 mg/m^2/d) to 39 patients with advanced solid malignancies in another phase I trial [31]. Infusions of troxacitabine were given for 30 min daily for 5 days of a 4 weekly cycle. On day 1 V_{SS} and Cl_S values were 60 l and 161 ml/min, respectively. At day 5, mean $T1/2$ was 39 h and Cl_S had fallen by close to 20%. On average urinary excretion of unchanged troxacitabine was 50 and 61% of the total dose given during the first 24 h.

Giles et al. [32] carried out a phase I and PK study of troxacitabine given as a 30 min iv infusion daily for 5 days in doses ranging from 0.72 to 10 mg/m^2 in patients with acute leukaemia in the salvage setting. An increase in the plasma concentration curve was seen after 48 h indicating potential hepatobiliary recirculation. The assay limit of quantification (LOQ) was exceeded in all patients 72 h post infusion on day 5. This finding persisted in some patients beyond 21 days following completion of the 5 day course. C_{max} and AUC values increased proportionally with dose. V_{SS} and Cl_S values of 71 (±42) and 170 (±52) ml/min respectively were recorded on day 1. On day 5 the mean $T1/2$ was 82 (±44) hours and Cl_S had reduced by 20% to an average of 137 ml/min with corresponded with the glomerular filtration rate. Between 41 and 54% of drug was excreted in the urine in the first 24 h. An additional 15% was excreted between 24 and 48 h after the fifth dose was given. The AUC was higher (1.8- and 2.8-fold) in patients with grade 3 stomatitis or hand foot syndrome following administration on day 5.

Giles et al. [33] went on to conduct a phase II study of troxacitabine on 42 patients with haematological malignancies given in a similar dosing schedule. Safety and efficacy of troxacitabine were evaluated. Patients with day 5 AUC values in quartile 1–3 did not experience grade 3 stomatitis or hand foot syndrome. Grade 3 stomatitis (at doses of 10.0 mg/m^2) and hand foot syndrome (at doses of 8.0 mg/m^2) was seen in patients with day 5 AUC values in quartile 4 (2 of 4 patients). An anti-tumour effect was evident in 3 of 4 (75%), 3 of 5 (60%), 4 of 5 (80%) and 4 of 4 (100%) patients with AUC values in quartiles 1–4, respectively.

Lee et al. [34] retrospectively studied troxacitabine plasma concentration vs. time data from 111 patients recruited in 4 separate phase I clinical trials [27,29,32,35]. A population pharmacokinetic model was validated to characterise clinical co-variates that influenced troxacitabine pharmacokinetics. This model identified renal function and body surface area (BSA) as sources of pharmacokinetic variation and predicted a dosing regime for continuous infusion administration that achieved pre-determined plasma concentrations. The authors highlighted the value of population pharmacokinetics in optimising troxacitabine therapy since elevated troxacitiabine plasma exposure accounted for dose limiting toxicity.

Safety and tolerability

A phase I study of 39 patients with advanced solid malignancies treated with escalating doses of troxacitabine (0.12–1.8 mg/m^2) given as a 30 min infusion over 5 days (124 courses in total) revealed that myelosuppression was the principal toxicity observed [35]. Onset of neutropenia was seen on days 8–15 of treatment with a median time to haematological recovery of 7 days. Neutropenia worsened with subsequent cycles of treatment suggesting cumulative toxicity. Severe thrombocytopenia and anaemia were less common and generally seen concomitantly with neutropenia, usually in heavily pre-treated (HP) patients. Troxacitabine produced 3 patterns of skin toxicity: skin rash, hand foot syndrome (palmar–plantar erythrodysesthesia) and asymptomatic hyperpigmentation. Skin rash accounted for the most common non-haematological toxicity in 54% of patients and was generally asymptomatic however, some patients experienced pruritus and one patient developed bullous features. Four patients experienced the hand foot syndrome and complained of pain, tenderness, blistering and reduced function. These symptoms were maximal 8–15 days following treatment with imcomplete recovery by the next scheduled cycle. Two of four patients had grade 3 toxicity. Nausea and vomiting were easily managed and malaise and mucositis were also reported.

In patients with haematological malignancies, combined data from 73 patients (114 cycles) given 8 mg/m^2/d of troxacitabine as a 30 min infusion for 5 days revealed that the dose limiting toxicities (DLT) were prinicipally stomatitis and hand foot syndrome [32,36]. Grade 2–3 hand foot syndrome was seen in 33 of 73 assessable patients usually following subsequent courses of treatment. Symptoms and signs were more marked in the hands and patients developed erythema, peri-articular soft tissue swelling, pruritus and a sensation of skin tightening. These resolved after 5 days with skin desquamation. More severe grade 3 toxicity with blistering and loss of function took longer to resolve (5–7 days). Sixteen

(22%) patients experienced stomatitis with 9, 5 and 6 patients having grade 2–4 toxicity respectively. Thirty two (44%) patients reported a skin rash and 24 of those had grade 2 toxaicity with 8 suffering grade 3 toxicity. Two patients developed Sweets Syndome. Skin rashes were generally mild, asymptomatic and localised, with complete resolution following a 5 day course of prednisolone 20 mg daily. Fever occurred during the first course of therapy in 56 (77%) patients, 12 (16%) of these had pneumonia, 10(14%) had septicaemia, 5(17%) had skin cellulitis and in 17(23%) patients no cause was found. Rarer causes of fever were urinary and upper respiratory tract infections, peri-rectal abscess, sinusitis and herpes labialis. Other grade 3–4 extramedullary side effects included hepatitis (2 patients), elevated creatinine (1 patient), cardiotoxicity (2 patients), stroke (1 patient) and seizures (1 patient).

Clinical efficacy

Summary of clinical experience in solid tumours

A Phase I study indicated that the recommended dose for troxacitabine given as a 30 min infusion repeated every 3 weeks was 10.0 mg/m^2 in the solid tumour setting [27]. Neutropenia was the main DLT. A total of 45 patients with solid malignancies were enrolled onto this study. Two confirmed partial responses were reported in 1 patient with previously untreated renal cell carcinoma and in another with carcinoma of unknown primary. Eighteen patients had a best response of stable disease with a median duration of 5.1 months (range 2.1–18.7 months).

Another phase I trial [35] of troxacitabine given as a 30 min infusion daily for 5 days defined the maximum tolerable dose (MTD) separately for minimally pre-treated (MP) and HP patients if it seemed that HP patients were more susceptible to DLT. Thirty four patients with advanced solid malignancies were enrolled and toxicity was experienced by HP patients and MP patients at doses exceeding 1.2 and 1.5 mg/m^2/d, respectively. An unconfirmed response was reported in 1 patient with metastatic melanoma whilst 18 patients had a best response of stable disease.

Troxacitabine had modest efficacy in renal cell carcinoma when administered to 35 patients with advanced disease in escalating doses as a 30 min infusion repeated every 3 weeks [37]. Median age was 60 years. DLTs were skin rash and myelosuppression. Thirty three patients were evaluable for response, 2 had a confirmed PR (partial remission), 21 had stable disease (median duration of 4.1 months) and 10 patients progressed.

Summary of leukaemia phase I/II studies

The clinical development for troxacitabine for patients with acute leukaemia began in 1998 (Table II). In a phase I study by Giles et al. [32] escalating doses of troxacitabine (starting dose of 0.72 mg/m^2/d) over 30 min iv for 5 days were administered to 42 patients with relapsed or refractory acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL), myelodysplastic syndromes (MDS) and chronic myeloid leukaemia in blastic phase (CML-BP). Courses were given every 3–4 weeks according to toxicity and anti-leukaemic effect. The dose was escalated by 50% until grade 2 toxicity was observed and then by 30–35% until the DLT was defined. The MDT was established at 8 mg/m^2/d × 5 and DLTs were mucositis and hand foot syndrome. Of the 31 AML patients evaluated, 11, 3 and 2 patients received troxacitabine as second, third and fourth line therapy, respectively. Seventeen patients had never achieved a prior remission, 3 had relapsed post bone marrow transplant and all patients had received ara-C in intermediate or high doses as part of prior therapy. Three patients with AML achieved complete remission (CR) at doses of 3.28, 8 and 10 mg/m^2/d × 5, respectively. Twenty two (73%) AML patients had hypocellular marrows 2 weeks after the first course of troxacitabine was given. One MDS patient achieved a CR without haematological recovery. None of the 4 ALL patients responded. One patient with CML-BP was returned to chronic phase.

A subsequent phase II investigation by the same authors evaluated troxacitabine given as an intravenous infusion over 30 min for 5 days at a dose of 8 mg/m^2/d every 3–4 weeks (40 mg/m^2 per course) [33]. Forty two patients (AML: 18 patients; MDS; 1 patient; ALL: 6 patients; CML-BP: 17 patients) were treated. Mucositis and hand foot syndrome were the significant toxicities as with the phase I trial. Marrow hypoplasia occurred between days 14 and 28 in 12 of 16 (75%) evaluable AML patients. Responses were seen in 17 AML patients (2 CRs and 1 PR). Six of 16 evaluable CML-BP patients returned to chronic phase disease. These data led to a further phase II trial which was opened to evaluate the efficacy of troxacitabine specifically in CML-BP [36]. Thirtyone patients, 29 (93%) of whom had failed prior imatinib therapy, received troxacitabine in a similar dosing schedule (30 min infusions of 8 mg/m^2/d for 5 days 3 weeks apart). Overall responses of 13% were reported, 2 returned to chronic phase, 1 achieved CR and a fourth patient achieved a haematological improvement in counts.

Troxacitabine warrants further study in patients with granulocytic sarcoma. Alvarado et al. [38] evaluated 170 patients with refractory AML treated with troxacitabine-based regimens on Phase I/II studies, 10 (6%) had extramedullary AML either with or without bone marrow involvement. Six of these patients received single agent troxacitabine and the remainder got a combination of troxacitabine and ara-C (TA). Six of 10 (60%) patients got complete

Table II. Clinical studies of troxacitabine in patients with AML.

Study	Patients	Therapy	Response	Duration	Author and reference
Phase I	31 R/R AML	TXC 0.72–10 mg/m^2/d × 5d (30 min iv infusion) every 21–28d	3 CR, 1 PR. 22 (73% of 31 pts. had marrow hypocellularity beyond day 14 of cycle 1	CR durable for 12 +, 3 and 11 + months	Giles et al.
Phase II	18 R/R AML	TXC 8 mg/m^2/d × 5d (30 min iv infusion) every 21–28d	2 CR, I PR. 12 (75%) of 16 pts had marrow hypocellularity beyond day 14 of cycle 1	CR durable for 12 + and 18 + months	Giles et al.
Phase II	31 CML (93% imatinib resistant), 11 cp, 20 AP/BP	TXC 8 mg/m^2/d × 5d (30 min iv infusion) every 21–28d	1 CHR, 2 RCP, 1 HI	CHR for 39 + weeks; BCP durable for 12 and 20 weeks	Giles et al.
Randomised phase I/II	68 AML (41 R1, 10 RC, 7 R3, 10 R 4)	TXC 5 mg/m^2/d iv over 30 min × 5d + ara-C 1 mg/m^2/d over 2 h × 5d or ida 12 mg/m^2/d over 5 min × 3d or TT 1 mg/m^2/d iv × 5d	CR 7 (4 TXC + ara-C, 2 TXC + ida, 1 TXC + TT), HI 4 (all TXC + ara-C	CR durable for 43, 70,46 +, 21, 36 and 4 + weeks	Giles et al.
Adaptive randomized	34 AML > 50 years with advers karyotype	Ida 12 mg/m^2/d iv daily × 3d = ara-C 1.5 gm /m^2/d iv over 2 h daily × 3d or TXC 6 mg/m^2/d iv dialy × 5d + ara-C 1 mg/m^2/d iv over 2 h daily × 5d or TXC 4 mg/m^2/d iv daily × 5d + ida 9 mg/m^2/d iv daily × 3d	CR: 10 of 18 (55%) with ida + ara-C, 5 of 11 (45%) with TXC + ara-C and 1 of 5 (20%) with TXC + ida	Relapse of 7 of 10 (70%) with ida + ara-C, 4 of 5 (80%) with trox + ara-C, 1 of 1 (100%) with TXC + ida. Time to relapse: 6,10,11,12,25,32 and 52 weeks for ida + ara-C; 9,21,22 and 40 weeks for TXC + ara-C; 12 weeks for TXC + ida	Giles et al.
Phase I	32 AML (12 R1, 9 R2, 10 R3)	10.1 mg/m^2/d for 2,3,4,5 or 6 days iv by continuous infusion	4 CR, 2 CRp	NA	Roboz et al.

Abbreviations: R/R, relapsed/refractory; CML, chronic myeloid leukaemia; d, day; CP, chronic phase; AP, accelerated phase; BP, blast phase; R1, first relapse; R2, second relapse; R3, third relapse; R4, fourth relapse; TXC, troxacitabine; ida, TI; iv, intravenous; CR, complete response; PR, partial response; CHR, complete haematological response; RCP, return to chronic phase; HI, haematological improvement; CRp, CR without platelet recovery; NA, not available.

responses with regression of extramedullary lesions. Two of the 6 responding patients relapsed within 3 months, 2 patients had remissions of 8 and 9 months duration, respectively, 1 patient was in on-going remission at the time of publication, and 1 patient was lost to follow-up.

Giles et al. [39] evaluated combinations of TA, idarubicin (TI) or topotecan (TT) to determine phase II study doses in 87 patients with refractory AML (68 patients), MDS (8 patients) and CML-CP (11 patients). Patients were randomised to receive 30 min infusions of troxacitabine at doses of 5 mg/m^2/d on days 1–5 combined with either ara-C 1 Gmg/m^2/d on days 1–5, TI 12 mg/m^2/d on days 1–3 or TT 1 mg/m^2/d on days 1–5. DLTs in the TA combination were hepatitis and hand foot syndrome. The recommended phase II doses were 6 and 1 mg/m^2/d × 5, respectively. DLTs in the TT combination were diorrhoea, skin rash and mucositis and the recommended phase II doses were 4 and 0.75 mg/m^2/d × 5, respectively. Hand foot syndrome, rash and mucositis were the DLT in the TI combination and doses of 4 mg/m^2/d × 5 and 9 mg/m^2/d × 3, respectively were recommended for study in the phase II setting. Of the 74 evaluable patients with AML or MDS, 10 (13%) achieved a CR (6 with TA, 3 with TI and 1 with TT) with 4 (5%) achieving haematological improvement. Two (18%) of 11 of patients with CML-BP returned to chronic phase, thus, all three troxacitabine combinations had important anti-leukaemic activity.

Based on these findings Giles et al. [40] opened an adaptive randomised study of TI and ara-C (IA) vs. TA vs. troxacitabine and TI in 34 newly diagnosed AML patients over the age of 50 years. Thirtytwo of thirtyfour patients had an adverse karyotype. CR rates for IA were 55% (10 of 18 patients), 45% for TA (5 of 11 patients) and 20% for TI (1 in 5 patients). Troxacitabine based combinations therefore, cannot be recommended over IA for the treatment of elderly patients with poor prognosis AML.

Another phase II trial was conducted in elderly patients with AML using a combination of troxacitabine and the anti-CD33 monoclonal antibody gemtuzumab ozogamicin (Myelotarg). Troxacitabine was administered at starting doses of 4 mg/m^2/d for 5 days and repeated every 4 weeks. Myelotarg was given in 2 doses of 9 mg/m^2 2 weeks apart. The study was closed after 2 patients got VOD, directly attributable to troxacitabine [41].

Different dosing schedules for troxacitabine continue to be evaluated. Pre-clinical studies have shown that in human xenograft tumour models, prolonged dosing schedules of troxacitabine, especially by continuous intravenous infusion are the most active regimes [42]. A phase I/II study of study of troxacitabine administered by continuous infusion in patients with refractory AML conducted in the MDACC is now closed to further accrual and provisional reports have been published [43,44]. To date 48 patients have been treated. The MTD was established at 12 mg/m^2/d for 5 days and was administered by continuous infusion through a central venous catheter. Steady state plasma troxacitabine levels were reached by the third day of dosing and steady state levels were proportional to the dose administered (Figure 2). Troxacitabine levels were also found to be linearly and inversely proportional to creatinine clearance at doses of 10 and 12 mg/m^2/d (Figure 3). DLTs included mucositis and hand foot syndrome. Nine patients (19%) achieved a CR or CRp (CR with incomplete platelet recovery). Median duration of CR was 7 months. Responding patients achieved serum troxacitabine levels of greater than 80 ng/ml. In 27 patients achieving target drug concentrations the CR rate was 33%. These data indicate that continuous infusion of troxacitabine allows increased dose density compared with iv bolus regimens where the MTD is significantly less (12 vs. 8 mg/m^2/d × 5, respectively).

Conclusions and future directions

To date, over 700 patients with solid tumours and haematological malignancies have been exposed to troxacitabine. Its unique stereochemistry sets it apart from the commonly used nucleoside analogues. Its cellular membrane permeation is non-carrier mediated and it is resistant to deamination. Its phosphorylated from diphosphonate to triphosphonate by 3-phosphogylcerate kinase and is excised from DNA by human apurinic/apyrmidinic DNA exanuclease. Troxacitabine is a true chain terminator and a potent inhibitor of DNA polymerases. These properties have made troxacitabine an effective agent in ara-C resistant malignancies. Its cytotoxic and antiviral properties would make it a feasible option for use in post transplant lymphoproliferative disorders, however, this rationale has not been tested in clinical trials as yet. On its own, or in combination, the efficacy of troxacitabine in the myeloid malignancies and in advanced solid tumours is well established. To date, it has limited activity in multiple myeloma and lymphoid malignancies.

In the phase I/II leukaemia studies the most common DLT were myelosuppression and skin toxicity particularly with hand foot syndrome. Pyridoxine and topical DMSO have been used to alleviate the hand foot syndrome toxicity of other drugs [45,46]. These agents, together with the use of pharmacokinetic models to define optimally effective doses, should be considered in future troxacitabine studies. The role of troxacitabine in the upfront component of AML therapy as well as its place in the treatment of the lymphoid malignancies has yet to be elucidated, a continuous infusion dosing regimen

is likely to produce more effective results in these trials. Finally, the ultimate clinical activity of troxacitabine will be defined only in appropriate phase II/III studies and it seems likely, based on results so far, that troxacitabine will continue to have a sustained therapeutic role.

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