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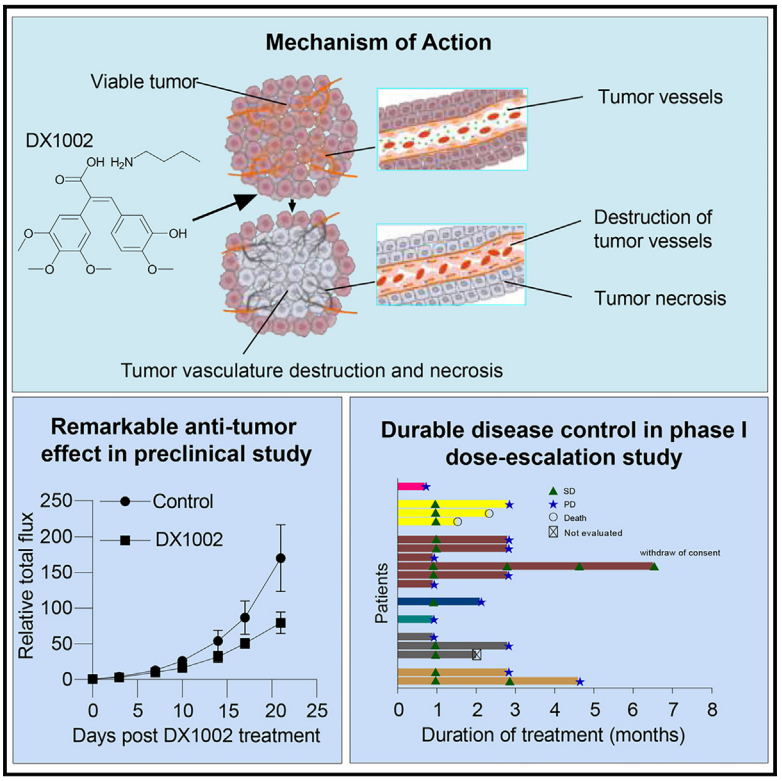
First-in-human phase 1 study of an orally bioavailable vascular-disrupting agent DX1002 in patients with advanced solid tumors

Graphical abstract



Highlights

- DX1002 is an orally bioavailable vascular-disrupting agent
- DX1002 leads to tumor vasculature destruction and necrosis in various animal models
- DX1002 shows manageable safety and preliminary anti-tumor efficacy in phase 1 study

Authors

Xiao-Li Wei, Hao-Xiang Wu, Dan-Yun Ruan, ..., Zhi-Quan Yong, Rui-Hua Xu, Hong-Yun Zhao

Correspondence

xurh@sysucc.org.cn (R.-H.X.), zhaohy@sysucc.org.cn (H.-Y.Z.)

In brief

Wei et al. present the preclinical results and phase 1 dose-escalation study of DX1002, an orally bioavailable vascular-disrupting agent. DX1002 can lead to tumor vasculature destruction and xenografted tumor necrosis in various animal models and shows manageable safety and preliminary anti-tumor efficacy in this phase 1 study.

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First-in-human phase 1 study of an orally bioavailable vascular-disrupting agent DX1002 in patients with advanced solid tumors

Xiao-Li Wei,[1,6] Hao-Xiang Wu,[2,6] Dan-Yun Ruan,[2,6] Feng Wang,[1] Li Xu,[3] Yu-Hong Li,[1] Yu-Xiang Ma,[2] Zhi-Qiang Wang,[1] Yun-Peng Yang,[1] Liang-Wei Tang,[4] Bao-Lin Chen,[4] Zhi-Quan Yong,[4] Rui-Hua Xu,[1,5,*] and Hong-Yun Zhao[2,7,*]

[1]Department of Medical Oncology, Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Guangdong Provincial Clinical Research Center for Cancer, Sun Yat-sen University, Guangzhou 510060, P.R. China
[2]Department of Clinical Research, Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Guangdong Provincial Clinical Research Center for Cancer, Sun Yat-sen University, Guangzhou 510060, P.R. China
[3]Department of Liver Surgery, Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Guangdong Provincial Clinical Research Center for Cancer, Sun Yat-sen University, Guangzhou 510060, P.R. China
[4]Guangzhou Anhao Pharmaceuticals Co., Ltd., Guangzhou 510060, P.R. China
[5]Research Unit of Precision Diagnosis and Treatment for Gastrointestinal Cancer, Chinese Academy of Medical Sciences, Guangzhou 510060, P.R. China
[6]These authors contributed equally
[7]Lead contact
*Correspondence: xurh@sysucc.org.cn (R.-H.X.), zhaohy@sysucc.org.cn (H.-Y.Z.)
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SUMMARY

DX1002 is an oral vascular-disrupting agent and exhibits promising results in preclinical studies, leading to tumor vasculature destruction and xenografted tumor necrosis in various animal models. In the phase 1 trial, 17 patients with solid tumors receive DX1002 ranging from 50 to 1,100 mg. The maximum tolerated dose and recommended phase 2 dose of DX1002 are determined as 600 mg once daily. The most common treatment-related adverse events are nausea (23.5%), vomiting (17.6%), and fatigue (11.8%). All patients are evaluable for anti-tumor response, 12 of which achieve stable disease as best response. One patient with non-small cell lung cancer achieves a stable disease duration of 6.5 months. The median time to progression (TTP) is 2.70 months (95% confidence interval [CI], 0.90–4.60). Interestingly, reduced blood perfusion is observed by contrast-enhanced ultrasound in a patient with colon cancer. In conclusion, DX1002 is well tolerated and exhibits preliminary anti-tumor efficacy in patients with solid tumors. This study was registered at chictr.org.cn (ChiCTR2400080298).

INTRODUCTION

In the late 1970s, researchers found a series of styrene active ingredients isolated from *Combretum caffrum*, which had strong anti-tumor activity.[1,2] The representative drug was combretastatin A4 phosphate (CA4P), which can not only kill tumor cells directly, but also interfere with the mitosis of tumor vascular endothelial cells by inhibiting the tubulin polymerization. It is reported to have the property of anti-vascular effect, inhibition of tubulin aggregation and tumor proliferation, and enhancement of the sensitivity of chemoradiotherapy.[3–5] In 1990s, the use of vascular-targeting therapies (VTTs) had become established as a viable possibility, with two major types of VTTs, i.e., vascular-disrupting agents (VDAs) and angiogenesis-inhibiting agents (AIAs). AIAs can inhibit the formation of tumor vessels, while VDAs can cause rapid and selective closure of existing tumor blood vessels and lead to ischemia and extensive hemorrhagic necrosis.[6,7] Tumor selectivity is determined by the pathophysiological differences between tumor and normal tissue blood vessels.[6] Previous early-stage clinical trials had shown a manageable safety profile and preliminary anti-tumor efficacy of VDAs.[8–12] VDAs encompass two primary categories, i.e., monoclonal antibodies, such as Tarvacin, and small-molecule VDAs. Small-molecule VDAs can be further categorized based on their molecular structure into colchicine, CA4 analogs (such as CA4P, ombrabulin, ZD6126, OXi4503, BNC105P, DX1002, and C118P),[13] and flavonoid drugs (e.g., DMXAA and MN029).[14,15] In animal models, these agents have demonstrated promising anti-tumor effects. Several of them, including CA4P, ombrabulin, and DMXAA (ASA404), have advanced to phase 3 trials. However, progress in other studies has been hindered, with many remaining in early clinical stages or being terminated. Consequently, despite their potential, no VDAs have yet gained approval for anti-tumor therapy. CA4P is a one of the most representative VDAs, but defect of CA4P such as unstable molecular structure, poor selectivity of targeted action on tumor blood vessels, and toxicity to cardiovascular system and peripheral nerve had substantially limited its application.[16–19]

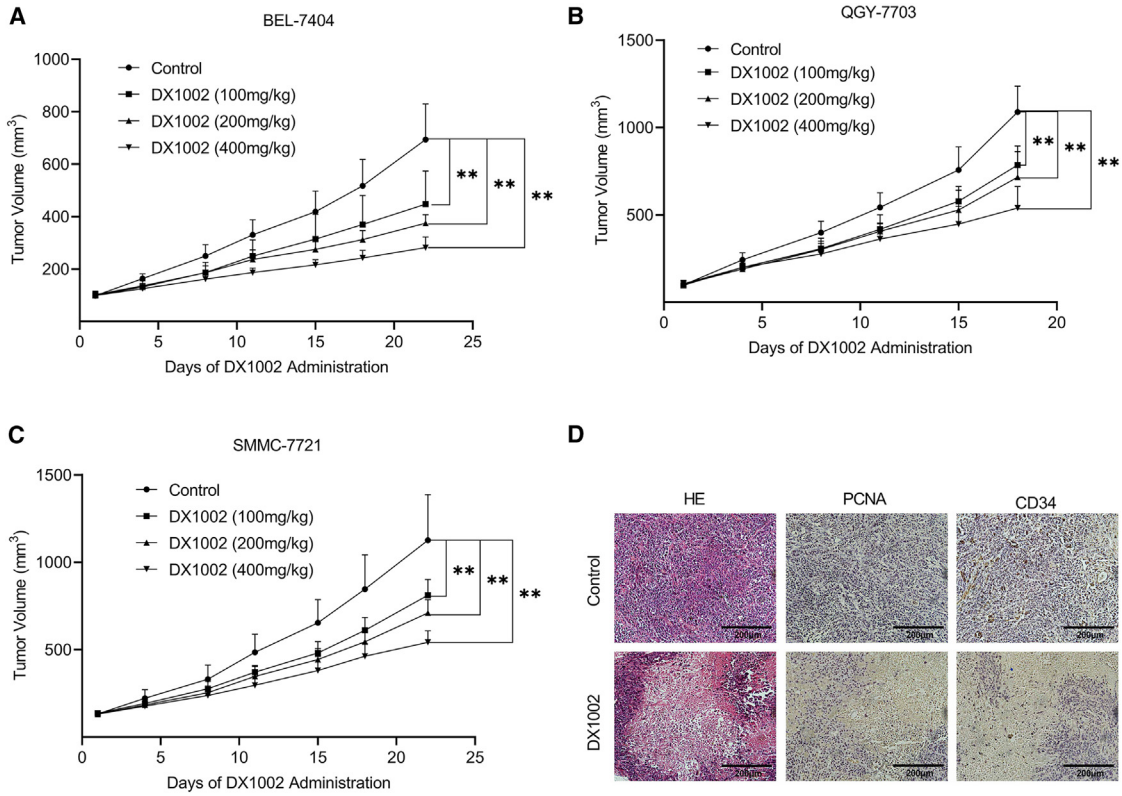


Figure 1. Preclinical characteristics of DX1002

(A–C) DX1002 showed strong inhibitory effects on three human cancer cell lines (BEL-7404 (A), QGY-7703 (B), SMMC-7721 (C)) transplanted in nude mice, and the inhibitory effect of DX1002 increased as dose strength increased from 100 mg/kg, 200 mg/kg, to 400 mg/kg (the *p* values were calculated by two-way ANOVA, $^{**}p < 0.01$).

(D) Representative images (100×) of hematoxylin-eosin (HE) and immunohistochemical staining with anti-PCNA and anti-CD34 in BEL-7404-transplanted tumors in nude mice, which were dissected 24 h after DX1002 administration (scale bar, 200 μm).

Therefore, we developed DX1002, an n-butyl amine salt with acrylic acid similar to CA4P; the carboxyl group is creatively introduced in the double-bond position of the CA4 stilbene structure, which enhances the stability of the double-bond plane structure, and forms a salt with n-butylamine, which greatly improves the solubility of the patent drug (China National Intellectual Property Administration, CN101074189B) and can be used orally that could overcome the chemical defects of CA4P substance.[20] In the preclinical study, anti-tumor signals were observed, thus we launched a first-in-human phase 1 study in patients with advanced solid tumors to evaluate the safety profile and preliminary anti-tumor efficacy of DX1002.

RESULTS

Preclinical characteristics of DX1002

Anti-tumor effect of DX1002 in animal models

Firstly, we assessed the effect of DX1002 on tumor proliferation *in vivo*. After 14 consecutive days of DX1002 gavage administration with DX1002, strong inhibitory effects were observed in three human liver cancer cell lines (BEL-7404, QGY-7703, and SMMC-7721) and three human lung cancer cell lines (A-549, NCI-H23, and 95-D) transplanted in nude mice. The inhibitory effect of DX1002 magnified as dose strength increased from 100 to 400 mg/kg (Figures 1A–1C and S1A–S1C). After 24 h of DX1002 administration, we dissected the tumors and performed hematoxylin-eosin and immunohistochemical staining, which showed that necrosis occurred in a large area of tumor tissues and the destruction of blood vessels evidenced by the decreased expression of proliferating cell nuclear antigen (PCNA) and CD34 (Figures 1D and S1D). Moreover, additive anti-tumor effect was observed in the combinational therapy of DX1002 with chemotherapy, including 5-fluorouracil and carboplatin (Figure S2). Furthermore, DX1002 demonstrated a significant ability to inhibit the growth of human gastric cancer SNU-5-luc cells orthotopically transplanted in B-NDG mice, thereby reinforcing its anti-proliferative effects *in vivo* (Figure S3).

Inhibitory effect of DX1002 on tumor vasculature

We further assessed the inhibitory effects of DX1002 on tubulin polymerization, and the results showed that DX1002 had a comparable inhibitory effect on the assembly and polymerization of tubulin to CA4P, and this inhibitory effect of DX1002 was dose dependent (Figure S4). Also, DX1002 had a comparable inhibitory effect on the cell mobility of human umbilical vein endothelial

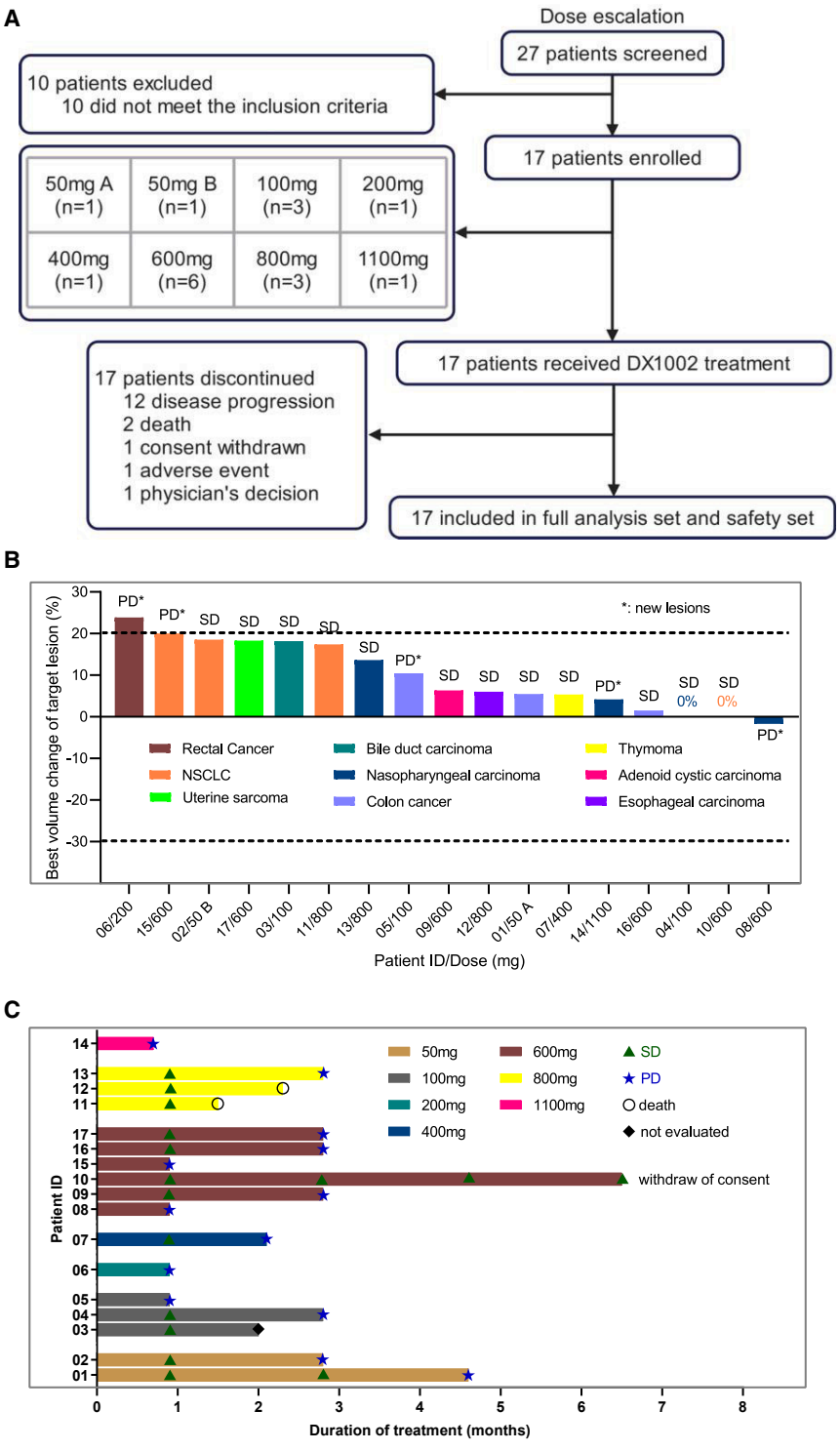


Figure 2. The anti-tumor efficacy of DX1002 in patients with advanced solid tumors

(A) Research flow chart.

(B) Waterfall plot depicting the best change from baseline in tumor burden.

(C) Swimming plot depicting the duration of treatment in the DX1002-treated patients.

cells (HUVECs) to that of CA4P (Table S1). Moreover, we assessed the inhibitory effects of DX1002 on non-confluent HUVECs (mimicking tumor blood vessels) and confluent HUVECs (mimicking normal blood vessels) and found that the half-maximal inhibitory concentration of DX1002 on normal blood vessels was much higher than that on tumor blood vessels, indicating that DX1002 could destroy tumor blood vessels without harming normal counterparts as compared to CA4P (Table S2).[3,21,22]

Pharmacokinetic and toxicity profile of DX1002

DX1002 was absorbed and eliminated quickly in both rats and beagle dogs, and the absorption degree was moderate (Tables S3–S6). General safety pharmacological tests showed that DX1002 at a dose of ≥600 mg/kg could reduce the voluntary activity of Institute of Cancer Research mice. A dose of 1,000 mg/kg of DX1002 decreased heart rate and increased blood pressure in beagle dogs. The maximum tolerated dose (MTD) for rats was 2,000 mg/kg, while it was >2,000 mg/kg for beagle dogs. The highest non-severe toxic dose for rats was 1,500 mg/kg, while it was 1,000 mg/kg for beagle dogs (Table S7). Based on preclinical pharmacology studies, the starting dose of DX1002 in humans was determined to be 50 mg.

Patient characteristics and disposition

A total of 17 patients were enrolled in the dose-escalation phase from October 2018 to September 2020 (Figure 2A). All patients were diagnosed with advanced solid tumors, including 4 patients with colorectal cancer (23.5%), 3 patients with lung cancer (17.6%), 4 patients with nasopharyngeal carcinoma (23.5%), and 6 patients with other cancer types. At baseline, the median age was 57 years old; a total of 10 (58.8%) patients had an Eastern Cooperative Oncology Group (ECOG) score of 0, and 7 (41.2%) patients had a score of 1 (Table 1).

DX1002 was taken orally once a day at doses ranging from 50 to 1,100 mg. The average duration of drug exposure was 49.8 ± 30.22 days. Sixteen patients (94.1%) had medication compliance of 80%–120%, and one case (5.9%) had medication compliance of <80% in the 1,100 mg dose group (Table 2).

Table 1. Baseline demographic and clinical characteristics of the 17 enrolled patients with advanced solid tumors

Characteristics	*N* (%)
Age (years; median [range])	57 (28–72)
Gender	
Female	7 (41.2)
Male	10 (58.8)
ECOG performance	
0	10 (58.8)
1	7 (41.2)
Time from diagnosis to enrollment (years; median [range])	4.0 (0.9–9.0)
Primary site of the tumor	
Colon	3 (17.6)
Rectum	1 (5.9)
Lung	4 (23.5)
liver (bile duct)	1 (5.9)
Nasopharynx	4 (23.5)
Thymus	1 (5.9)
Ear	1 (5.9)
Uterus	1 (5.9)
Esophagus	1 (5.9)
Number of metastatic sites	
≤2	10 (58.8)
>2	7 (41.2)
Sum of target lesions (RECIST v.1.1)	
<5 cm	5 (29.4)
≥5 cm; <10 cm	11 (64.7)
≥10 cm	1 (5.9)

MTD determination and safety

In this study, no dose-limiting toxicity (DLT) reaction occurred in all groups during the first 28-day treatment cycle. However, two patients in the 800 mg group died of bleeding (grade 5) during the second and third cycle of treatment, respectively, which was determined to be possibly related to DX1002 by the investigators. One of them with non-small cell lung cancer (NSCLC), who has tumor metastasis in lungs, lymph nodes, and bilateral adrenals, had grade 1 hemoptysis on day 13 of cycle 1 and achieved stable disease (SD) on day 28 of cycle 1 (C1D28); but this patient had massive hemoptysis on day 19 of cycle 2 and died unfortunately. We retrospectively reviewed the clinical courses of this patient and found that there was slight stenosis of the right main bronchus caused by metastatic lesions before DX1002 treatment, which became significant on C1D28. This might be the reason that related to the death of this patient. Another patient with metastatic esophageal squamous cell carcinoma, who has tumor metastasis in lymph nodes and adrenal glands, also achieved SD on C1D28. However, on day 12 of cycle 3, drastic hematemesis occurred, and the patient died eventually. We retrospectively reviewed the clinical courses of this patient and found that the patient's esophageal lesion was huge and enveloped large blood vessels, which might be the reason that related to the death of this patient.

Although these events occurred beyond the DLT observation period, and acknowledging the inherent tumor-related bleeding risks, a potential association with bleeding could not be disregarded given DX1002's nature as a VDA. Following extensive discussion between the sponsors and investigators, it was determined that 600 mg should be considered as the MTD. Besides, considering that no DLT was observed in three additional patients enrolled in the 600 mg group, it was determined that 600 mg should be designated as the recommended phase 2 dose (RP2D), and the dosage and administration method of RP2D were recommended as follows: in a 28-day treatment cycle, 600 mg DX1002 should be taken orally once a day for 3 consecutive weeks (21 days) and then stopped for the rest 7 days.

All patients were evaluable for safety. Ten out of 17 patients had 29 treatment-related adverse events (TRAEs) with an incidence of 58.8%, and grade 3–5 TRAEs were 17.6% (Table 3). All TRAEs were classified by systematic organ classification, with the highest to lowest incidence as follows: gastrointestinal system diseases (35.3%), systemic diseases and reactions at the site of administration (11.8%), routine inspection (11.8%), respiratory system, chest and mediastinum diseases (11.8%), skin and subcutaneous tissue diseases (11.8%) etc (Table 3).

Pharmacokinetic analysis

All 17 patients in this study are included in the pharmacokinetic (PK) set. DX1002 was rapidly absorbed and eliminated, with time to reach maximum plasma concentration ranging between 0.5 and 2.0 h and terminal elimination half-life ($t_{1/2}$) between 0.84 and 4.04 h after the first administration. DX1002 showed approximate dose-proportional PK on day 1 of cycle 1 (C1D1) as measured by maximum plasma concentration (C_{max}), area under the plasma concentration-time curve from time 0 to 24 h ($AUC_{0–24h}$), and area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0-\infty}$) across dose groups (Table 4). After the first dosing cycle, the C_{max}, $AUC_{0–24h}$, and $AUC_{0-\infty}$ increased in a dose-dependent manner. The accumulation ratio based on C_{max} and accumulation ratio based on area under the concentration-time curve between different dose groups were 0.81–2.18 and 0.84–1.14, respectively, indicating that there was no obvious accumulation after repeated dose of DX1002 (Table S8).

The relationship between the main PK parameters and dose in the range of 50–800 mg were analyzed using the Power model and dose normalization linear analysis. The $AUC_{0–24h}$, $AUC_{0-\infty}$, and C_{max} showed a linear relationship with the dose strength on C1D1; while for the first treatment cycle, the $AUC_{0–24h}$ and $AUC_{0-\infty}$ showed a linear relationship with the dose strength, but C_{max} did not.

Anti-tumor efficacy

All 17 patients enrolled were evaluable for anti-tumor response. Among them, 12 patients achieved SD, and the other 5 patients were primarily resistant to DX1002, with an overall disease control rate (DCR) of 70.6%. Neither complete nor partial response was observed; thus, the objective response rate was 0%, and the median time to progression (TTP) was 2.70 months (95% confidence interval, 0.90–4.60) (Figures 2B and 2C). Interestingly, one patient (patient 10) with NSCLC in the 600 mg group

Table 2. Patient disposition, DLTs, duration of exposure, and dose intensity

Item	DX1002 50 mg A	DX1002 50 mg B	DX1002 100 mg	DX1002 200 mg	DX1002 400 mg	DX1002 600 mg	DX1002 800 mg	DX1002 1,100 mg	All patients
Patient disposition (FAS)	*n* = 1	*n* = 1	*n* = 3	*n* = 1	*n* = 1	*n* = 6	*n* = 3	*n* = 1	17
Patients treated, *n* (%)	1	1	3	1	1	6	3	1	17 (100)
Treatment discontinued, *n* (%)	1	1	3	1	1	6	3	1	17 (100)
Primary reason for treatment discontinuation, *n* (%)									
Adverse event	0	0	1	0	0	0	2	0	3 (17.6)
Disease progression	1	1	2	1	1	5	1	0	12 (70.6)
Withdrawal of consent	0	0	0	0	0	1	0	0	1 (5.9)
Physician's decision	0	0	0	0	0	0	0	1	1 (5.9)
Duration of exposure, days; median (ranges)	70	63	42 (21–63)	21	41	60.5 (21–140)	54 (21–63)	21	54.0 (21–140)
DLTs occurring in the first cycle	0	0	0	0	0	0	0	0	0
Total DLTs, *n* (%)	0	0	0	0	0	0	2	0	2 (11.8)
Hemorrhage, *n* (%)	0	0	0	0	0	0	2	0	2 (11.8)

DLT, dose-limiting toxicity; FAS, full analysis set; DX1002 50 mg A and DX1002 50 mg B, the initial starting dose was 50 mg, 2 dosing regimens A and B were studied, dose regimen A is once daily (QD) for the first 2 weeks in a 28-day treatment cycle, dose regimen B is once daily (QD) for the first 3 weeks in a 28-day treatment cycle.

achieved SD and lasted for 6.5 months until withdrawal of informed consent and stopped treatment. Besides, there was only a 4.1% increase in the sum of target lesions for this patient (Figures 2B and 2C). Moreover, we selected one patient (patient 01) with a tumor lesion suitable to be observed with contrast-enhanced ultrasound (CEUS) and recorded the change of blood supply. This patient achieved a TTP of 4.5 months, and it was observed that cavities and necrosis emerged as DX1002 administration, while blood supply was significantly reduced (Figure 3).

DISCUSSION

In this first-in-human phase 1 clinical trial, we demonstrated that once-daily DX1002 within the 50–600 mg dose range is safe and well tolerated in patients with solid tumors, and the MTD is 600 mg once daily. Furthermore, our study provides early signals of the anti-tumor activity of DX1002 in a range of solid tumors. We also provide the description of the PK profile of DX1002, with $t_{1/2}$ of around 0.84–4.04 h. The AUC_{0-24h} and C_{max} increased in a dose-dependent manner, indicating that DX1002 had a linear PK profile.

DX1002 creatively introduced the carboxyl group in the double-bond position of the CA4 stilbene structure to form an n-butyl amine salt with acrylic acid, which had a similar mechanism of action of CA4P, but possessed enhanced stability. DX1002 has comparable inhibitory effects on tubulin polymerization and cell mobility of HUVECs to those of CA4P, while DX1002 could destroy tumor blood vessels without harming normal counterparts as compared with CA4P, indicating its better safety profile. In the preclinical study, tumor necrosis and destruction of tumor blood vessels were observed; reduced blood supply was also observed in one of the patient's tumor lesions in the phase 1 trial. Significantly better targeting of DX1002 to tumor blood vessels over normal blood vessels was observed as compared to CA4P, which was evidenced by the facts that toxicity to the cardiovascular system and peripheral nerve, which restricted the application of CA4P, was not observed in both Sprague-Dawley rats and beagle dogs receiving DX1002. These results indicate that DX1002 may possess a better safety profile and feasible administration compared to CA4P. During the observation period of DLT in this phase 1 trial, no DLT reaction was observed in all dose groups of 50–1,100 mg; besides, DX1002 did not cause cardiac and peripheral neurotoxicity compared to other VDAs, including CA4P and DMXAA,[23–25] which further supported the better safety profile of DX1002.

The incidence of TRAEs was 58.8% with grade 3–5 TRAEs of 17.3%, which is mainly because of the unexpected situation that two patients in the 800 mg dose group experienced death during continued DX1002 treatment though no DLT occurred during the observation period of DLT. We retrospectively reviewed the clinical courses of these two patients and found that they both had risk of bleeding before the initiation of DX1002 treatment, while no TRAEs related with bleeding were observed in the other patients with the longest DX1002 exposure of more than 6 months. Considering that DX1002 is a kind of VDA, patients with risk of bleeding should be carefully evaluated to determine whether DX1002 would significantly increase the risk of massive bleeding in further studies.

In terms of anti-tumor activity, although no complete response or partial response (PR) to DX1002 was observed, 12 patients achieved SD with an overall DCR of 70.6%, including one metastatic NSCLC patient who had sustained disease control (>6 months) until withdrawal of consent. In our preclinical study, DX1002 could destroy the blood vessels inside tumors and result in tumor tissue necrosis. However, at the same time, residual areas with live cells around the tumor could also be observed, suggesting that DX1002 did not overcome the shortcomings of the VDAs.[6,26–31] Tumors treated with VDAs can regrow from the characteristic edges of residual living cells around the tumor,[6] which may restrain the anti-tumor activity of DX1002 as

Table 3. Adverse events related to DX1002 with an incidence of ≥5%

		Affected/at risk (%)	
		All grade	Grade ≥3
Adverse events		10/17 (58.8)	3/17 (17.6)
Gastrointestinal diseases	nausea	4/17 (23.5)	0
	vomiting	3/17 (17.6)	0
	gastrointestinal bleeding	1/17 (5.9)	1/17 (5.9)[b]
	melena	1/17 (5.9)	0
Systemic disease and various reactions at administration site	fatigue	2/17 (11.8)	0
Routine inspection	urine protein detected	1/17 (5.9)	0
	white blood cell count decreased	1/17 (5.9)	0
Respiratory system, chest and mediastinal diseases	dyspnea	1/17 (5.9)	0
	cough	1/17 (5.9)	0
	bronchial hemorrhage	1/17 (5.9)	1/17 (5.9)[b]
Diseases of skin and subcutaneous tissue	maculopapule	1/17 (5.9)	0
	pruritus	1/17 (5.9)	0
	rash	1/17 (5.9)	0
	atopic dermatitis	1/17 (5.9)	0
Various neurological diseases	abnormal sensation	1/17 (5.9)	0
	hypoesthesia	1/17 (5.9)	0
Renal and urinary diseases	proteinuria	1/17 (5.9)	0
Vascular and lymphatic diseases	embolism	1/17 (5.9)	1/17 (5.9)[a]

[a]Grade 3.
[b]Grade 5.

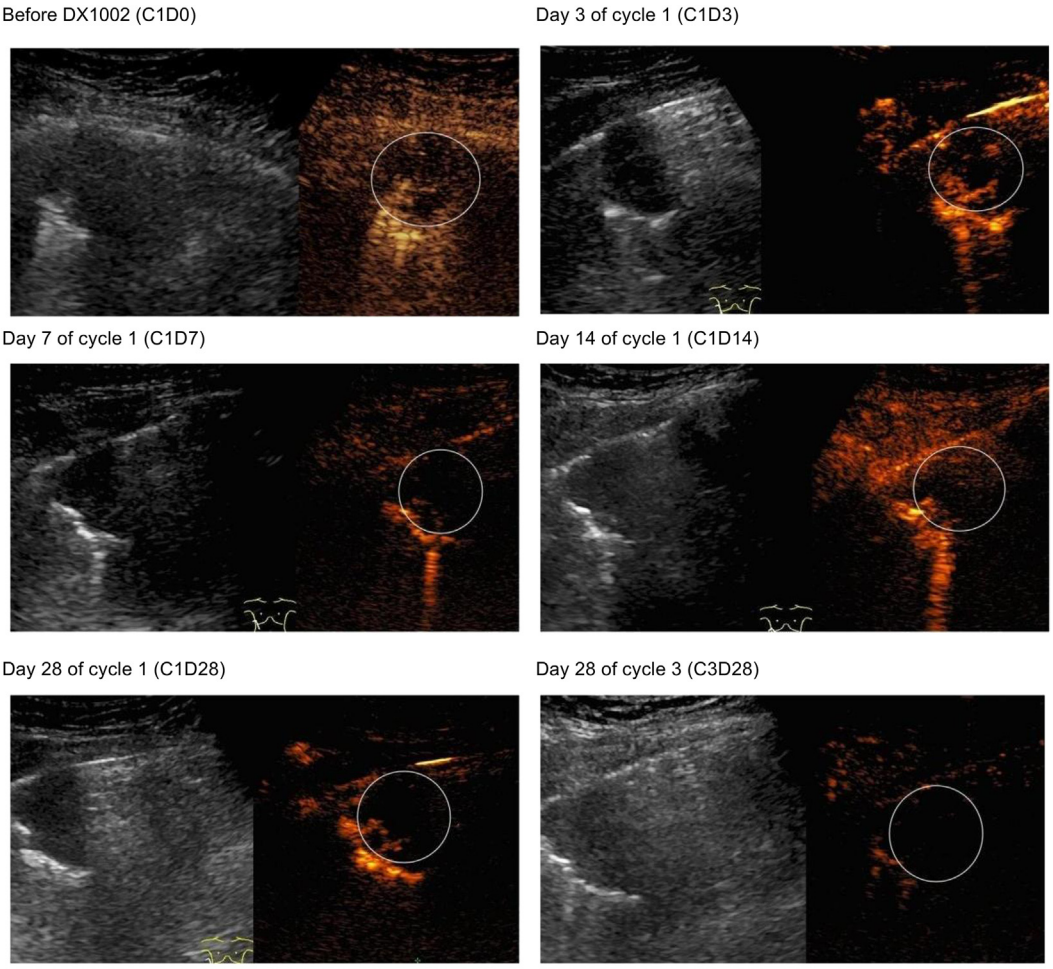
monotherapy. This phenomenon was also observed in other VTTs such as bevacizumab, with most of the patients achieving SD but few achieving PR; however, bevacizumab was later found to have an additive benefit in multiple cancers when combined with other treatment such as chemotherapy.[32–34] The combination of VDAs and chemotherapy had also been investigated and enhanced response was observed, which was most likely attributable to the VDA and cytotoxic drugs targeting two distinct cell populations to get a synergic effect.[7,35–39] In our preclinical study, an additive anti-tumor effect was observed in the combinational therapy of DX1002 with chemotherapy.

Among all enrolled patients, five had previously received anti-vascular therapy. This included four patients with colorectal cancer and one with intrahepatic cholangiocarcinoma. Three of these patients achieved SD, while two experienced progressive disease after DX1002 treatment. There was no significant difference in efficacy between patients with or without prior anti-vascular therapy. Due to the limited sample size of this phase 1 trial, it is not possible to establish a correlation between previous anti-vascular therapy and the response to DX1002. Interestingly, three patients who underwent later-line therapy after DX1002 treatment exhibited encouraging outcomes, with the best response being PR. These patients received capecitabine,

Table 4. Primary PK parameters of DX1002 at cycle 1 day 1 (*N* = 17)

Dose level (*n*)	T_{max} (h)	C_{max} (ng/mL)	$t_{1/2}$ (h)	AUC_{0-24} (h*ng/mL)	$AUC_{0-\infty}$ (h*ng/mL)
50 mg A (*n* = 1)	2.00	1,053.99	1.96	3,121.11	3,136.42
50 mg B (*n* = 1)	1.00	1,941.13	0.84	2,902.93	2,918.71
100 mg (*n* = 3)	0.75 (0.5, 4.0)	1,719.31 ± 647.13	1.85 ± 0.67	5,013.59 ± 1,080.16	5,068.96 ± 1,126.93
200 mg (*n* = 1)	0.50	5,992.01	2.39	7,802.51	7,846.41
400 mg (*n* = 1)	0.50	12,184.50	2.34	18,202.83	18,329.96
600 mg (*n* = 6)	1.00 (0.5, 2.0)	16,107.12 ± 6,081.68	3.56 ± 0.70	37,637.51 ± 6,739.48	37,719.57 ± 6,769.90
800 mg (*n* = 3)	1.50 (0.5, 4.0)	12,724.92 ± 4,301.27	4.04 ± 1.21	38,728.71 ± 10,986.15	38,935.84 ± 11,156.53
1,100 mg (*n* = 1)	0.50	17,275.90	3.73	115,684.15	116,619.12

All parameters were expressed as mean ± standard deviation except for T_{max}, which was expressed as median (range).
Abbreviations: AUC_{0-24}, area under the plasma concentration-time curve from time 0 to 24 h; $AUC_{0-\infty}$, area under the plasma concentration-time curve from time 0 to infinity; C_{max}, maximum plasma concentration; T_{max}, time to reach maximum (peak) plasma concentration following drug administration; $t_{1/2}$, terminal elimination half-life.



Examining Lesion	Parameters	C1D0	C1D3	C1D7	C1D14	C1D28	C3D28
Right pleura	Lesion size (mm)	37×25	37×25	37×25	37×27	32×26	34×28
	Active area (cm^2)	3.12	3.12	3.12	0.53	0.67	0
	Peak intensity (dB)	-37	-41	-45	-50	-55	No signal
	Observation	/	/	Small cavities	Massive necrosis	Massive necrosis	No blood supply

Figure 3. Changes of blood supply of a typical tumor lesion after DX1002 medication observed by CEUS

We used CEUS to observe the pleura metastasis lesion in patient 01 with advanced colon cancer, which was outlined by the white circle. An obvious decrease in the blood supply to the tumor lesion was observed after DX1002 medication.

anlotinib, and a combination of albumin-bound paclitaxel and carboplatin (despite previous failure to this regimen), respectively. This observation suggests the intriguing possibility that DX1002 administration, aimed at disrupting the existing tumor vasculature, may resensitize tumors to chemotherapy. Consequently, the combination of DX1002 and chemotherapy warrants further investigation in clinical trials.

In conclusion, the present study provides the preclinical and first-in-human clinical evidence for DX1002 and shows that this drug is generally well tolerated with early anti-tumor signals in several types of advanced solid tumors. The MTD and RP2D for DX1002 were established as a once-daily dose of 600 mg for consecutive 21 days in a 28-day treatment cycle. Based on these findings, further large-scale investigations of DX1002 in phase 2 setting were launched (CTR20200996), and combination of DX1002 with chemotherapy is warranted.

Limitations of the study

Several limitations of this study should be acknowledged. Firstly, based on the mechanism of action of DX1002, the method to select patients with tumors of sufficient blood supply may be employed to get better efficacy. Secondly, as observed in this

study, patients with potential risk of bleeding should be ruled out at study entry, and the pre-clinical long-term repeat-dose toxicity study may be necessary to identify the characteristics of bleeding adverse effects of DX1002. Thirdly, although we incorporated a pharmacodynamic marker in the study design, baseline tumor lesions with abundant blood supply suitable for CEUS observation were not consistently present, making it impractical to detect and analyze the pharmacodynamicprofile of DX1002. Better pharmacodynamic markers and detecting methods should be considered in further studies. Lastly, the sample size of this phase 1 study was limited; further phase 2 study of single-agent DX1002 or combinational therapy with chemotherapy is warranted, and the chemotherapy regimens used in combination should be carefully selected.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to corresponding author, Hong-Yun Zhao (zhaohy@sysucc.org.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The clinical data of the participants in this study have been recorded at Research Data Deposit: http://www.researchdata.org.cn with ID of RDDA2024895067. The data are available from Research Data Deposit, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Research Data Deposit public platform.
- This paper does not report any original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, H.-Y.Z. and R.-H.X.; methodology, H.-Y.Z. and R.-H.X.; data curation, X.-L.W., H.-X.W., D.-Y.R., F.W., L.X., Y.-H.L., Y.-X.M., Z.-Q.W., and Y.-P.Y.; supervision, H.-Y.Z. and R.-H.X.; formal analysis, X.-L.W., H.-X.W., D.-Y.R., L.-W.T., B.-L.C., and Z.-Q.Y.; drafting, X.-L.W., H.-X.W., D.-Y.R., H.-Y.Z., and R.-H.X. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

L.-W.T., B.-L.C., and Z.-Q.Y. are employees of the Guangzhou Anhao Pharmaceuticals Co., Ltd.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

No generative AI and AI-assisted technologies were used in the writing process.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-human PCNA	Abcam	Cat# ab81289; RRID: AB_303394
Anti-human CD34	Abcam	Cat# ab29; RRID: AB_1640331
Biological samples		
Blood	Patients in this study	N/A
Chemicals, peptides, and recombinant proteins		
DX1002	Guangzhou Anhao Pharmaceuticals Co., Ltd.	N/A
CA4P	Guangzhou Anhao Pharmaceuticals Co., Ltd.	N/A
5-FU	Shanghai Xudong Haipu Pharmaceutical Co., Ltd	N/A
Carboplatin	Qilu pharmaceutical Co., Ltd.	N/A
Critical commercial assays		
HTS-Tubulin Polymerization Assay Kit	Cytoskeleton, Inc	BK004P
Deposited data		
The data of patients	This manuscript	https://www.researchdata.org.cn (ID: RDDA2024895067)
Experimental models: Cell lines		
BEL-7404	NCACC	N/A
QGY-7703	NCACC	N/A
SMMC-7721	NCACC	N/A
A-549	NCACC	19375.09.3101HUMSCSP503
NCI-H23	NSTI-BMCR	3101HUMSCSP581
95-D	NCACC	CSTR:19375.09.3101HUMTCHu 61
SNU-5-luc	Nanjing Cobioer Gene Technology Co. Ltd.	NA
Experimental models: Organisms/strains		
BALB/c nude mouse	Shanghai SLAC Laboratory Animal Co. Ltd.	N/A
B-NDG mouse	Biocytogen JiangSu Co. Ltd.	N/A
ICR mouse	Shanghai Sippe-Bk Lab Animal Co., Ltd.	N/A
S-D rat	Shanghai Sippe-Bk Lab Animal Co., Ltd.; Beijing Vital River Laboratory Animal Technology Co., Ltd.	N/A
Beagle dog	Shanghai Xingang Experimental Animal Yard; Beijing Marshall Biotechnology Co., Ltd	N/A
Software and algorithms		
GraphPad Prism	GraphPad Software	9.00
SAS	SAS	9.4
Phoenix WinNonlin	Certara	7.0

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Cell culture and cell lines

Human liver cancer cell lines (BEL-7404, QGY-7703, SMMC-7721) and human lung cancer cell lines (A-549, 95-D) were obtained from the National Collection of Authenticated Cell Cultures (NCACC). The human lung cancer cell line (NCI-H23) was sourced from the National Science and Technology Infrastructure-National Biomedical Cell-Line Resource (NSTI-BMCR). The human gastric cancer cell line (SNU-5-luc) was sourced from the Nanjing Cobioer Gene Technology Co., Ltd. All cell lines were cultured in specialized growth media according to their product specifications and were incubated in humidified incubators maintained at 37°C with 5%

CO_2. All cells were authenticated on the strength of short tandem repeat (STR) fingerprinting, and tested negatively for mycoplasma contamination before use.

Animals

Our experimental design did not involve gender-related factors. *In vivo* anti-tumor effect of DX1002 were conducted using 6 to 7-week-old male BALB/c nude mice and 4 to 5-week-old female B-NDG mice, sourced from Shanghai SLAC Laboratory Animal Co. Ltd and Biocytogen JiangSu Co. Ltd, respectively. The mice were maintained under specific pathogen-free (SPF) conditions in a laminar flow rack at a constant temperature of 20°C–25°C and humidity levels of 40–70%. All mice were transplanted with specific cell lines subcutaneously or orthotopically. Prior to grouping and treatment, the weight of each animal was recorded. Tumor volumes were measured using calipers for subcutaneous tumor transplantation in nude mice. Given that tumor volume can influence the determination of treatment efficacy, it served as a numerical parameter for randomizing animals into designated groups ($n = 8$ per group).

The study protocol involving animal models was approved by the Sun Yat-sen University Cancer Center Ethics Committee (L102012015088A, L102012024228T).

Patients

This study enrolled Chinese patients (aged between 18 and 75) with histopathologically- or cytologically-confirmed, locally advanced or metastatic solid tumors, who had failed, or could not tolerate standard treatment or had no standard treatment. Other key eligibility criteria included at least one measurable lesion according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 and sufficient organ function. Key exclusion criteria were prolonged QT interval, as well as clinically significant abnormalities that may increase the risk of gastrointestinal bleeding or perforation and intrabronchial lesions or lesions infiltrating the main pulmonary blood vessels.

This was a non-randomized phase I study with no formal hypothesis testing. As a dose-escalation study, the number of patients was dependent on the occurrence of dose-limiting toxicities and the nature of the "3 + 3" design, with all patients allocated to the experimental group as a non-randomized manner.

The clinical study protocol was approved by the he Sun Yat-sen University Cancer Center Ethics Committee (A2018-024-05), and complied with the Declaration of Helsinki and guidelines for Good Clinical Practice, as defined by the International Conference on Harmonization. All patients provided written informed consent.

METHOD DETAILS

Preclinical methods

Assessment of anti-tumor effect of DX1002 in animal models

In the xenograft study, six cell lines, including (BEL-7404, QGY-7703, SMMC-7721, A-549, 95-D, and NCI-H23) were inoculated in nude mice. As the average tumor size reached 100 mm^3, DX1002 was given intragastrically once a day for consecutive 14 days. Combinational therapy of DX1002 and carboplatin in 95-D cell line, and combination of DX1002 and 5-fluorouracil in QGY-7703 cell line were also assessed. The tumor size was measured twice a week. For pathological analysis, the nude mice were euthanized to dissect the tumor tissue 24h after first administration of DX1002. Hematoxylin-eosin (HE) and immunohistochemistry (IHC) staining with anti-CD34 and anti-PCNA antibodies were performed to observed necrosis inside the tumor, together with cell proliferation and tumor vascular destruction.

To further evaluate the anti-tumor effect of DX1002 *in vivo*, an orthotopic model using human gastric cancer SNU-5-luc cells was established. Under sterile conditions, a 10μL cell suspension was intraperitoneally inoculated into the gastric wall of each female B-NDG mouse at a concentration of 8×10^5 cells/10μL/mouse. After 14 days post-inoculation, mice were randomly grouped based on total bioluminescence flux and body weight ($n = 8$ per group). DX1002 was administered intragastrically once daily for a consecutive 21 days, and relative total bioluminescence flux of was SNU-5-luc cells recorded on days 0, 3, 7, 10, 14, 17, and 21.

Assessment of inhibitory effect of DX1002 on tumor vasculature

The inhibitory effect of DX1002 on tubulin polymerization was assessed using the HTS-Tubulin Polymerization Assay Kit according to the manufacturer's instructions. Briefly, 96-well plates were preheated at 37°C for 30 min. G-PEM buffer (80 mM PIPES[pH 6.9], 2 mM MgCl2, 0.5 mM EGTA, 1 mM GTP) was pre-cooled to 4°C. Tubulin (4 mg) was added to 900μL of the pre-cooled G-PEM buffer, and the solution was placed on ice for 3 min until the tubulin was fully dissolved. Subsequently, 10μL of the sample solution was added to a 96-well plate and incubated at 37°C for 2 min. Blank control samples (G-PEM buffer), 1μM DX1002 solution, 10μM DX1002 solution, and 1μM CA4P solution were then added. The optical density (OD) value was measured every 30 s at a wavelength of 340 nm for a total of 121 readings (60 min). A tubulin polymerization curve was graphed, with time on the x axis and OD value on the y axis.

To further assess the effect of DX1002 on human umbilical vein endothelial (HUVECs) mobility, a wound-healing assay was performed. HUVECs (2×10^5/mL) were seeded in 24-well plates and cultured in α-MEM medium until confluent monolayers were obtained. A sterile 200μL pipette tip was used to create a liner wound. After washed with phosphate-buffered saline (PBS), DX1002, CA4P or PBS were added to serum-free α-MEM medium. The scratch distance was measured at 0 h and 10 h.

Furthermore, to evaluate the inhibitory effects of DX1002 on non-confluent HUVECs (mimicking tumor blood vessels) and confluent HUVECs (mimicking normal blood vessels), non-confluent HUVECs (2000 cells/well) and confluent HUVECs (8000 cells/well) were seeded in 96-well plates. Subsequently, various concentrations of DX1002 and CA4P were added to the wells. The cells were then incubated for 24 h, followed by the addition of 10μL of CCK-8 solution and a further incubation for 2.5 h. The optical density (OD) value was measured at 450 nm using an MK-2 microplate reader to calculate the half-maximal inhibitory concentration (IC50) of DX1002 and CA4P on non-confluent HUVECs (mimicking tumor blood vessels) and confluent HUVECs (mimicking normal blood vessels), respectively.

Phase I clinical trial

Trial design

This study was a first-in-human, single-center, single-arm, open-label phase I dose-escalation study investigating DX1002 tablets in adult patients with advanced solid tumors (CTR20180958, Supplementary file 1). The primary objectives were to assess the safety, tolerability, PK profile, and define the maximum tolerated dose (MTD) and recommended phase II dose (RP2D). The secondary objectives were preliminary anti-tumor activity (for detail, see trial protocol in Supplementary file 2).

The starting dose of DX1002 was set as 50mg based on preclinical toxicology studies. Followed by exploratory study of the following dose groups with 1–3 patients per group; after which a standard 3 + 3 design was employed to determine the MTD and RP2D. Totally, 8 dose groups were set in this study as follows, 50mg, 100mg, 200mg, 400mg, 600mg, 800mg, 1100mg, and 1500mg.

The MTD was defined as the highest dose of DX1002 not causing a dose-limiting toxicity (DLT) in >33% patients during the first 28 days. After that, the RP2D would be determined by the investigators based on the safety and tolerance profile as well as the PK parameters obtained in the study.

According to the trial design, we anticipated enrolling eight dose groups ranging from 50mg to 1500mg, with 2–12 subjects initially enrolled in the starting dose group (comprising two dosing regimens, 50mg dose A and 50mg dose B). Subsequent exploratory studies for effective dosing would enroll 1–3 patients per group. The timing and initial dose for the "3 + 3" dose escalation study was to be determined collaboratively by the investigators and sponsors.

After enrolling one patient each in the 50mg dose A and 50mg dose B groups, and three patients in the 100mg group, a conference was convened with investigators, sponsors, and pharmacologists to discuss the trial's progression. Based on the pharmacokinetic profile (well below the estimated minimum effective dose) and the efficacy and safety data (indicating no signal of anti-tumor response but good tolerance), it was proposed and subsequently approved that the 200mg and 400mg dose groups each would enroll one patient, aiming to minimize participant exposure in low-dose, non-effective groups.

After three patients were enrolled in the 600mg dose group, another conference was convened to deliberate the trial's continuation. Considering the favorable safety profile observed in patients receiving the 600mg dose, it was recommended to escalate to higher dose groups. Consequently, three patients were enrolled in the 800mg dose group. However, following the enrollment of one patient in the 1100mg group, two patients in the 800mg group experienced major bleeding events successively. Although these events occurred beyond the DLT observation period, and acknowledging the inherent tumor-related bleeding risks, a potential association with bleeding could not be disregarded given DX1002's nature as a vascular-disrupting agent. Following extensive discussion between the sponsors and investigators, it was determined that 600mg should be considered as the MTD. Accordingly, three additional patients were enrolled in the 600mg group, and no DLT was observed thereafter. Therefore, it was determined that the 600mg should be designated as the RP2D.

This process resulted in the patient allocation as detailed in the results Section.

Study treatment

Based on preclinical toxicology studies in accordance with section "Reference for Clinical Dose" guidelines, the starting dose of DX1002 was set as 50mg once daily (QD) for the first 2 or 3 weeks in a 28-day treatment cycle, and was administered orally at the same time each day ±1h in a fasted state. Based on results of the 50mg dose group, the DX1002 was given once daily for the first 3 weeks in a 28-day treatment cycle in the following 100mg, 200mg, 400mg, 600mg and 800mg dose groups; Based on the PK and safety data of the 50-600mg dose group, as well as the mechanism of action of DX1002, the investigators and sponsor estimated that the maximum single dose should be set as 1500 mg, and the dose of DX1002 was split into twice a day and given every day in a 28-day treatment cycle in the 1100 mg and 1500 mg groups.

Safety assessment

Safety was evaluated by incidence, nature, severity, and relatedness of adverse events (AEs), and graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v4.0.3. DLTs were defined as any ≥ Grade 2 neurotoxicity, ≥ Grade 3 hematologic or non-hematologic treatment-related AEs (TRAEs) that occurred within the first 28-day cycle. All AEs regardless of attribution were collected for 28 days following the last administration of treatment or study discontinuation/termination.

Pharmacokinetic analysis

The PK of DX1002 was evaluated using 3mL of plasma samples by liquid chromatography–mass spectrometry (LC-MS). When DX1002 was given for the first 2 weeks in a 28-day treatment cycle, plasma samples are collected at multiple time points on C1D1/14, and on D7/10 pre-dose. When DX1002 was given for the first 3 weeks in a 28-day treatment cycle, plasma samples are

collected at multiple time points on C1D1/21, and on D7/14 pre-dose. When DX1002 was given every day in a 28-day treatment cycle, plasma samples are collected at multiple time points on C1D1/28, and on D7/14/21 pre-dose.

PK parameters including area under the curve (AUC), maximum serum concentration (C_{max}), plasma half-life ($t_{1/2}$) and time taken to reach C_{max} (T_{max}) were calculated using non-compartmental methods with WinNonlin8.4. Descriptive statistical analysis was performed on pharmacokinetic parameters of different dose groups, and the arithmetic mean, standard deviation, coefficient of variation, median, maximum, minimum and geometric mean of pharmacokinetic parameters of each dose group were calculated.

Efficacy assessment

The objective response rate (ORR), disease control rate (DCR) and time to progression (TTP) of DX1002 was evaluated according to RECIST v1.1. ORR was defined as the proportion of patients that achieved complete response and partial response, while TTP was defined as the date from the first administration until disease progression. DCR were defined as the proportion of patients without disease progression at first assessment.

QUANTIFICATION AND STATISTICAL ANALYSIS

For anti-tumor effect of DX1002 in animal models, a minimum of eight mice were included per experimental group, the results are presented as the mean values ± S.D and the *p* values were calculated by two-way ANOVA. For cellular experiments, each group contains a minimum of three independent biological replicates. All patients enrolled with at least one medication record were included in the full analysis set (FAS), all patients who received at least one study medication were included in the safety analysis set (SS), while all patients who were enrolled and received DX1002 at least once and had post-medication blood samples were included in the pharmacokinetic dataset (PKS). Statistical analysis was performed using SAS software v9.4 (Cary, North Carolina, USA).

ADDITIONAL RESOURCES

This study has been registered on chictr.org.cn with the register number ChiCTR2400080298 (available at https://www.chictr.org.cn/hvshowproject.html?id=245401&v=1.0).