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SHORT COMMUNICATION

A phase I dose escalation study of the pharmacokinetics and tolerability of ZK 304709, an oral multi-targeted growth inhibitor (MTGI™), in patients with advanced solid tumours

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Abstract
Purpose The toxicities, pharmacokinetics and recommended dose of oral once daily ZK 304709, a novel multi-targeted growth inhibitor (MTGI™) with activity against cell-cycle progression and angiogenesis, was investigated in patients by administration for 14 consecutive days followed by 14 days recovery.
Methods Patients with solid tumours resistant to standard treatments were enrolled in an accelerated titration design.
Results Thirty-seven patients received ZK 304709 from 15 to 285 mg daily. The most common drug-related adverse events were vomiting, diarrhoea and fatigue. Systemic exposure to ZK 304709 increased with dose up to 90 mg daily but plateaued thereafter, with high inter-individual variability at all doses. Thirteen patients had stable disease as best response as per RECIST criteria.
Conclusions There was no increase in exposure to ZK 304709 with dose escalation above 90 mg, and the MTD was not determined. This study illustrates the importance of phase I pharmacokinetic data to guide dose escalation and drug development.

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Introduction

Angiogenesis and escape from regulatory cell-cycle check points have both been recognised as essential pathophysiological processes involved in carcinogenesis. Cyclin-dependent kinases (CDKs) are a family of Ser/Thr kinases, which control cell-cycle progression and RNA transcription [1]. CDKs inhibitors have been investigated in phase I and II clinical trials [2, 3]. Vascular endothelial growth factor (VEGF) receptors are a family of tyrosine kinases involved in both neoangiogenesis and lymphangiogenesis [4], with the licensing of bevacizumab [5] being the proof of concept that blockade of this pathway results in clinical efficacy.

ZK 304709 is a first in class oral multi-target growth inhibitor (MTGI™) that blocks tumour cell proliferation and induces apoptosis via inhibition of cell-cycle progression and tumour-induced angiogenesis. It inhibits CDKs 1, 2, 4, 7 and 9; VEGF receptor-1, -2, and -3 tyrosine kinases (VEGF-R1TK, -R2TK, -R3TK); and platelet-derived growth factor receptor beta tyrosine kinases (PDGF-R beta TK). In vitro, ZK 304709 was active at low levels with an inhibitory concentration 50% (IC_{50}) of 4 nM for CDK2/cyclinE and 30 nM for VEGFR-2 and PDGF-Rβ. In vivo, ZK 304709 was evaluated in more than 20 tumour xenograft models in nude mice with significant activity especially for breast, pancreatic, prostate, colorectal and renal cancer [6].

In animals, ZK 304709 is rapidly and extensively distributed throughout the body with no permeation through the blood–brain barrier. It undergoes little metabolism and is excreted mainly unchanged via renal and hepatic routes.

Preclinical studies demonstrated reversible gastrointestinal, renal and haematologic toxicities. Given the unique mode of action of ZK 304709, we evaluated its safety and pharmacokinetics in patients with advanced cancer in this phase I study.

Patients and methods

Patients

Patients eligible for study were ≥18 years of age, WHO performance status (PS) 0–2, with histologically or cytologically confirmed solid tumours resistant to standard treatments. Patients had to have recovered from the effects of previous surgery or chemotherapy and have a life expectancy of ≥3 months. All patients required measurable disease (by Response Evaluation Criteria in Solid Tumours, RECIST) [7] and laboratory parameters within the following ranges: absolute neutrophil count > 1.5×10^9/l, haemoglobin > 9 g/dl, platelets ≥ 100×10^9/l, aspartate aminotransferase and alanine aminotransferase ≤ 2.5 × upper limit of normal (ULN) (≤ 5.0 × ULN in presence of liver metastases), serum bilirubin ≤ 1.5 × ULN, and serum creatinine ≤ 1.5 × ULN. Concurrent severe or uncontrolled other disease, pregnancy or breast-feeding and the presence of brain metastases were all exclusion criteria. The study protocol was approved by the Leicestershire Rutland & Northamptonshire Research Ethics Committee (ref no. 7261) and all patients gave their written informed consent.

Study design

This was a phase I, open-label, non-randomized, inter-individual dose escalation study of once daily oral ZK 304709 dosed with 14 days of treatment and 14 days recovery conducted in two centres.

The primary objective was determination of the maximum tolerated dose (MTD) and identification of dose limiting toxicities (DLTs) of ZK 304709. Secondary objectives included evaluation of safety and tolerability, and determination of the pharmacokinetic profile. Adverse events were graded according to National Cancer Institute Common Toxicity Criteria (CTC) version 2.0. A DLT was defined as any of the following occurring in cycle 1: grade 4 neutropenia for >5 days, febrile neutropenia, grade 4 thrombocytopenia, any grade 3 or 4 non-haematological toxicity (except nausea, vomiting, hypertension or gamma-glutamyltransferase elevations, which were DLTs under circumstances described below); any toxicity (except anaemia, alopecia, nausea and vomiting) not resolved to ≤grade 1 within 5 weeks from the first day of study drug administration; anaemia not resolved to ≤grade 2 within 5 weeks from the first day of study drug administration. Nausea or vomiting was considered DLTs only when occurring despite the use of standard antiemetics. Hypertension was considered DLT if not manageable with ≤ 2 oral antihypertensive drugs, or associated with evidence of end-organ damage, or grade 4 in severity. Gamma-glutamyltransferase elevations were considered DLTs if grade 4. The MTD was defined as a dose producing DLTs in ≤1 of six assessable patients when ≥2 patients experienced DLTs at the next higher dose.

Patients were treated in consecutive cohorts of three to seven patients and received sequentially increasing doses of ZK 304709 (15–285 mg). Dose doubling occurred between cohorts until possible drug-related grade 2 toxicities occurred (excluding alopecia, nausea and vomiting unless standard anti-emetics were used). After the occurrence of possible drug-related grade 2 toxicities, the dose level was escalated by 50% and then by 33%. No intra-patient dose escalation was permitted. Dose escalation was permitted following investigator and Sponsor review of the results of the adverse events in cycle 1 for the previous dose level.

Treatment

ZK 304709 was provided by Bayer Schering Pharma AG and was formulated as uncoated tablets containing the active ingredient at 5, 25 or 100 mg. There were no restrictions regarding concomitant food or fasting at dosing times. The starting dose was determined as 1/20th of the MTD of rats as the more sensitive species from preclinical data, based on presumed 2 m^2 patient surface area. Dosing was daily for 14 consecutive days followed by 14 days recovery. Doses could be held or delayed according to protocol-defined criteria. Dose reduction was not allowed. Dosing continued until disease progression or unacceptable toxicity.

Evaluation criteria and procedures

Baseline evaluations within 14 days prior to study entry were as follows: history, physical examination including PS assessment, full blood count, clotting screen and serum biochemistry, urinalysis and electrocardiogram (ECG). Tumour assessment was carried out within 28 days prior to study entry. Physical examination with PS, laboratory tests, urinalysis, urine pregnancy tests in female patients of childbearing potential, ECG, adverse events (AEs) and toxicity were carried out within 24 h prior to day 1 of each treatment cycle.

Pharmacokinetics

Blood was taken for pharmacokinetics within 0.5 h prior to first administration of ZK 304709 in cycle 1, and at 2, 4, 6, 10, 12 and 24 h post dose. Additional pharmacokinetic

sampling was carried out on days 14, 15, 21 and 28 of cycle 1. Concentrations of ZK 304709 in whole blood and plasma were determined by a validated liquid chromatography/mass spectrometry (LC-MS/MS) method at Function Pharmacokinetics, Bayer Schering Pharma AG, Berlin, Germany. The hydrochloride salt ZK 304709 dissolves in aqueous solution to its free base ZK 250765 and hydrochloride. The free base ZK 250765 is the smallest active moiety of the drug and was therefore quantified.

Efficacy

Tumour responses were evaluated using RECIST by contrast-enhanced computerized tomography (CT scans) imaging at baseline, and then after the even numbered cycles (i.e. every 8 weeks). Tumour assessment was carried out during the follow up visit if no evaluation was performed during the previous 4 weeks.

Statistical methods

Statistical methodology was descriptive and focused on AEs per dose cohort, particularly those that were serious, of CTC grades 3/4, dose limiting or associated with discontinuation of study drug. Safety, pharmacokinetic and pharmacodynamic data analyses included all patients who received ZK 304709 (intent to treat population). The qualitative data were summarised by frequency and percentages, while the quantitative data were summarised by descriptive statistics.

Results

Patients

Thirty-seven patients were enrolled in the study and were included in the intent-to-treat analyses. Twenty-six patients (70%) were male, the median age was 57 years old range (32–74 years), and 31 patients (84%) were Caucasian. The majority of patients had colorectal (8), urological (7) or pancreatic (5) tumours and sarcomas (5). PS was 0 in 18 patients, 1 in 16 patients and 2 in 3 patients. Overall four patients completed the planned six courses and were allowed to continue on ZK 304709 until disease progression, receiving 9–15 courses in total. A further two patients completed the 14 days of study drug treatment in cycle 6 but withdrew due to progressive disease (PD) during the 14-day recovery period. Of the remaining patients, the majority (24 patients) withdrew due to progressive disease, 5 withdrew due to adverse events (discussed below) and 2 withdrew consent to study participation.

Safety

Due to the low patient numbers and variable exposure, it was not possible to formally evaluate the relationship between adverse event frequency or severity and dose. The most frequent drug-related adverse events were nausea (76%), vomiting (73%), fatigue (62%) and diarrhoea (38%). The majority of these were grade 1/2. There were 36 non-fatal SAEs documented, with only one (grade 2 vomiting) assessed as being possibly related to study drug which recovered/resolved at the end of study. Six deaths were recorded in this study, four of which were documented as fatal SAEs which were all unrelated to the study drug but consistent clinically with disease progression.

Four drug-related laboratory abnormalities were reported, namely grade 2 elevation in urea, grade 2 and 3 anaemia and grade 1 increase in liver enzymes. All other abnormalities were assessed as being consistent with advanced underlying malignancy or disease progression.

The dose escalation schedule as per protocol was followed: three patients were treated at 15 and 30 mg. Six patients were treated at 60 mg as DLT of grade 3 lightheadedness was documented in one patient. Seven patients were treated at 90 mg as grade 3 hypertension was seen in one patient. Six patients received 120 mg with DLT of grade 3 fatigue documented in one patient. Three patients were treated at 160, 215 mg and four patients at 285 mg prior to the study stoppage.

ZK 304709 was prematurely discontinued in five patients due to adverse events: three patients experienced nausea and vomiting, one patient experienced light-headedness and one patient had fatigue, nausea, vomiting and tumour pain. The final DLT pattern could not be identified as MTD was not reached.

Pharmacokinetics

Pharmacokinetic analyses from whole blood are summarised in Table 1. ZK 250765, the smallest molecular entity of ZK 304709, was rapidly absorbed with a t_{max} of 2–4 h but there were high inter-individual differences with a t_{max} range of 0.5–12 h. The C_{max} and AUC (0–24 h) for ZK 250765 achieved after oral administration of ZK 307620 increased less than dose proportionally especially at doses of >90 mg, and showed high inter-individual variability. The most likely underlying reason is due to the poor solubility and physiochemical properties of the compound, leading to dose limited absorption. There was no significant drug accumulation with multiple dosing, as evidenced by similar systemic exposure parameters (C_{max} and AUC) from Day 1 and Day 14 samples in Cycle 1 at all doses (see Fig. 1).

Table 1 Day 1 and Day 14 of Cycle 1 pharmacokinetic parameters of ZK250765 in whole blood

Dose level	Day	C_{max} (ng/ml)	t_{max} (h)	C_{min} (ng/ml)	AUC (0–24 h) (h/ng/ml)
15 mg ($N = 5$)	1	513 (28.3%)	2 (2–4)	25.4 (69.1%) ($N = 4$ only)	2,940 (32.2%) ($N = 4$ only)
	14	690 (13.3%)	2.25 (0.5–3.9)	47.9 (51.4%)	5,108 (24.1%)
30 mg ($N = 3$)	1	1,519 (8.04%)	2 (2–4)	106 (59.2%)	10,865 (35.9%)
	14	1,446 (7.63%)	1.5 (1.4–2)	105 (49.5%)	9,876 (44.6%)
60 mg ($N = 6$)	1	724 (73.2%)	2 (1.97–2)	53.5 (107%)	4,293 (80.8%)
	14	1,219 (32.5%)	2.01 (1.95–3.75)	168 (30.5%)	8,648 (30.1%) ($N = 5$ only)
90 mg ($N = 7$)	1	2,059 (67.7%)	4 (0.48–11.9)	153 (65.8%)	15,799 (59.9%)
	14	2,341 (53.5%)	2.25 (1.98–6)	222 (129%)	15,037 (70.8%)
120 mg ($N = 6$)	1	1,549 (34.9%)	2.14 (2–10.1)	93.2 (28.7%)	11,140 (28.9%)
	14	1,925 (65.9%)	3.92 (2-4.1)	196 (58.4%)	16,009 (69.2%)
160 mg ($N = 3$)	1	2,204 (105%)	4.08 (4–4.1)	203 (5.12%) ($N = 2$ only)	20,735 (87.8%)($N = 2$ only)
	14	2,683 (86.1%)	4.08 (2–4.1)	448 (79%)	24,855 (50.5%)
215 mg ($N = 3$)	1	2,846 (61.3%)	4.08 (2–6.17)	195 (73.5%)	17,838 (40.0%)
	14	1,940 (28.1%)	5.2 (2–6)	220 (25.4%)	14,873 (30.6%)
285 mg ($N = 4$)	1	1,976 (32.2%)	6.09 (0.58–10)	329 (169%)	20,941 (74.4%)
	14	2,080 (85.3%)	4 (0.55–6)	535 (545%)	16,918 (123%)

C_{max} = mean maximum serum concentration (geometric mean, geometric coefficient of variation shown in brackets)

t_{max} = median time to reach maximum concentration (median, range shown in brackets)

C_{min} = minimal concentration 24 h following oral administration (geometric mean, geometric coefficient of variation shown in brackets)

AUC = area under the concentration-time curve from 0 h data point up to 24 h post administration (geometric mean, geometric coefficient of variation shown in bracket)

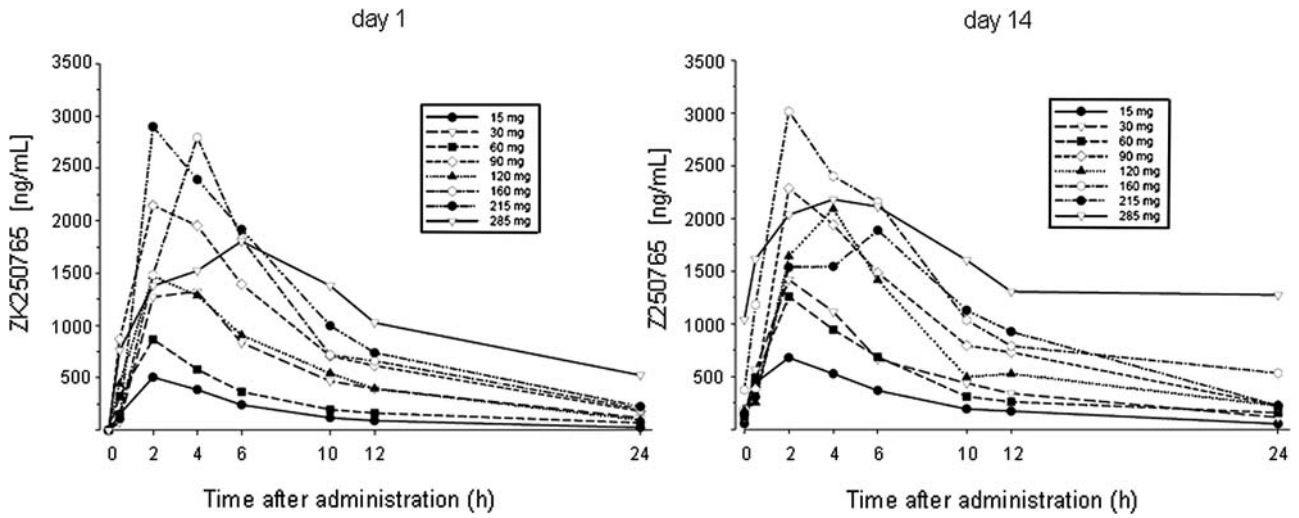

Fig. 1 Mean concentration, time profiles of ZK250765, the measured free base of ZK 304709, in whole blood for all dose groups on Day 1 and Day 14 in Cycle 1

Efficacy

Twenty-seven patients were evaluable for response according to RECIST. No partial or complete responses were documented although 13 patients (48% of evaluable cohort) had stable disease as the best overall response. The mean duration of stable disease according to RECIST was 75.5 days (SD ± 109.9 days).

Discussion

This study investigated the first in class oral Multi-Target Growth Inhibitor (MTGI™) with activity against both VEGF receptor tyrosine kinases and CDKs. It failed in its primary aim to establish the MTD and DLTs of the study drug when administered for 14 days in a 28-day cycle since systemic exposure plateaued at doses beyond 90 mg. On

this schedule, however, the administration of ZK 304709 was associated with a manageable adverse event profile and lengthy treatment durations in some patients. Although efficacy was not a primary endpoint, the duration of stable disease for 6–15 months in six patients was encouraging but speculative because preclinical data suggested that the exposures achieved in this study were insufficient for anti-tumour activity. In a parallel phase I study, using drug administration for seven consecutive days in a 21-day cycle, similar pharmacokinetic results were seen [8]. The poor solubility/physiochemical properties of ZK 304709 probably were responsible for the non-linear dose exposure. On this basis, future development of the ZK 304709 formulation was terminated.

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