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PHASE I STUDIES

A phase I, dose escalation, pharmacodynamic, pharmacokinetic, and food-effect study of α_2 integrin inhibitor E7820 in patients with advanced solid tumors

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Summary *Introduction E7820* is an orally administered sulfonamide that inhibits alfa-2-integrin mRNA expression. Pre-clinically E7820 showed tumor anti-angiogenic effects in various tumor cell lines and xenograft mouse models. Human daily dosing of 100 mg QD had previously been shown to be safe and tolerable. *Methods* The study consisted of two parts: Part A (food effect) and Part B (determination of maximum tolerated dose (MTD) for bi-daily (BID) dosing). E7820 dosing started at 50 mg BID with planned escalation to 60, 80 and 100 mg BID every 28 days. *Results* Fifteen patients were enrolled in Part A and 26 in Part B. The most frequent adverse events of all grades were constipation, diarrhea, nausea, and fatigue while anemia, neutropenia, and fatigue were most frequent grade ≥3 toxicities. At dose-level 60 mg BID, two patients experienced dose-limiting toxicities (grade 3 neutropenic sepsis and grade 4 neutropenia). Therefore the recommended dose (RD) was 50 mg BID. Food had no effect on E7820 exposure. E7820 exposure following twice daily administration was dose-proportional. Expression of platelet integrin-α_2 measured as a response biomarker in Part B, generally decreased by a median 7.7 % from baseline following treatment with 50 mg BID E7820. Reduction was most pronounced within 1-week post treatment. The median duration of treatment was median 54, range 20–111 days. The best overall response in any treatment group was stable disease (SD): 23.1 % in Part A (100 mg QD); at the RD 66.7 % (12 of 18 patients) and 40 % in the 60 mg BID group in Part B. *Conclusions*:Food had no effect on E7820 exposure. A dose of 50 mg BID was considered the MTD. Treatment with E7820 is safe and tolerable with 2/3 of patients (66.7 %) at MTD having SD as their best response.

Key Message E7820 inhibitor of alfa-2 integrin showed preclinically an antitumor effect. We confirmed that oral E7820 is safe and tolerable at 50 mg BID in patients with solid tumors. The most common adverse events are constipation, diarrhea, nausea and fatigue. Food does not have effect on E7820 pharmacokinetics and 2/3 of patients at 50 mg BID showed stable disease as their best response.

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Keywords *E7820* · Integrin-α2 · Aromatic sulfonamide · Maximum tolerated dose · Food effect · Pharmacokinetics

Introduction

Integrins are transmembrane receptors that are implicated in migration, proliferation and differentiation of human

endothelial precursor cells and therefore may be important in tumor angiogenesis. Integrins may also inhibit apoptosis and prolong survival of endothelial cells [1, 2]. It has been demonstrated that inhibition of integrins results in tumor regression [3]. *E7820* is an orally administered, aromatic sulfonamide that inhibits integrin alfa-2(α_2) mRNA expression. It has anti-antiangiogenic activity by inhibition of endothelial cell proliferation and tube formation [4]. In vivo, twice-daily (BID) oral treatment with E7820 inhibited tumor growth and inhibited tumor induced angiogenesis in mouse xenograft models derived from human colon, breast, pancreas, kidney cancers with complete suppression of growth of human pancreatic and colon cell lines [4, 5].

At IC50 concentration, E7820 inhibited integrin-α_2 expression on epithelial cells and on platelets [5] suggesting that platelet integrin-α_2 expression level may be a potential biomarker for E7820 anti-tumor activity [5].

In the first-in-man, dose-escalation study of E7820, 37 patients with advanced or refractory malignancies were enrolled. The treatment included once daily (QD) dosing of E7820 for 28 days in cycle 1, followed by a 7-day no-treatment rest period and thereafter continuous daily dosing [6]. The maximal tolerated dose (MTD) and the recommended dose (RD) of E7820 was set at 100 mg QD based on a fasting schedule. It showed that E7820 is rapidly absorbed and eliminated. The relatively short elimination half-life suggested that BID, rather than daily administration, would result in plasma exposure that better sustains efficacious levels of E7820 over 24 h. Additionally, lower C_{max} levels achieved following BID dosing might ameliorate toxicity that is associated with higher C_{max} levels following daily dosing at the MTD, thus raising the possibility of achieving a higher total daily dose a higher MTD with BID dosing and possibly higher biologically active. Further, a pharmacokinetic / pharmacodynamic (PK/PD) modeling and simulation analysis showed that daily 200 mg dosing would result in a reduction in integrin-α_2 expression accompanied by 90 % tumor stasis in more than 95 % of subjects, while BID 50 mg dosing was predicted to result in greater and more sustained inhibition than was predicted for 200 mg QD dosing [7]. E7820 administration with food resulted in delayed absorption (median time to reach maximum concentration t_{max} - 6 h (h) fed vs. 2.5 h fasted) and increased exposure by 58 % at the 100 mg dose-level with 1/3 of patients having stable disease as their best response. However, those results were considered inconclusive due to the small number of patients tested ($n = 7$) and the parallel study design.

Therefore this second phase I dose-escalation study was planned to define the MTD for BID dosing of E7820 and further investigate its safety, pharmacokinetics, the effect of a high-fat meal on E7820 pharmacokinetics and preliminary anti-tumor activity in patients with advanced solid tumors.

Patients and methods

Study design

This open-label, multi-center study was conducted at 6 sites in Europe (4 sites in the UK and 2 sites in The Netherlands) between 30 Jun 2011 and 30 Apr 2014 (data cut-off) and consisted of 2 parts: Part A (food effect study) and Part B (determination of the MTD for BID Dosing). The study was performed according to ICH-GCP guidelines. All hospital ethics committees and national regulatory bodies approved the study prior to study start. All patients gave written, informed consent prior to undergoing any study-related procedures.

Part A was a 2-way cross-over study with the primary objective of determining the effect of a high fat meal (containing approximately 50 % fat) [8] on the oral bioavailability of E7820 in comparison with fasting conditions. In the treatment phase each subject received a single 50 mg dose of E7820 on Day 1, either after fasting for 10 h, or immediately after consuming a high fat breakfast. Following a 7-day washout period, the subjects crossed-over and a second 50 mg dose of E7820 was administered on Day 8. After the second washout period patients transitioned into the extension phase and received E7820 100 mg once daily (QD) in the fasted state until disease progression or unacceptable toxicity.

Part B was a multiple dose study with the primary objective to establish the MTD of E7820 given by BID dosing, starting with 50 mg BID and with planned escalation of the dose to 60, 80, and 100 mg BID. The results from Part A were evaluated to determine if E7820 was to be administered with or without food in Part B.

Patients were enrolled into Part B using a conventional algorithm (3 + 3 subjects per dose-level) to identify the maximum tolerated dose (MTD) defined as the highest dose-level at which no more than 1 of 6 subjects experienced a dose-limiting toxicity (DLT). DLTs were determined based on the toxicities observed during the first treatment cycle (28 days) and E7820 was administered in subsequent 28-day cycles until disease progression or unacceptable toxicity. In Part B, treatment phase ended when the last enrolled subject per dose-level completed six cycles of treatment or discontinued early. At this time, all subjects could transition into the Extension Phase.

In the Extension Phase, subjects were to continue to receive the same treatment they received during the Treatment Phase.

Dose-limiting toxicities (DLTs)

DLTs were defined as neutropenia $<0.5 \times 10^9$/L for >5 days or neutropenia $<1 \times 10^9$/L with fever; thrombocytopenia $<25 \times 10^9$/L accompanied by bleeding or thrombocytopenia $<10 \times 10^9$/L; any grade 3 or 4 non-hematological toxicity for

which the study drug could not be excluded as a cause (other than nausea, vomiting or diarrhea in the absence of appropriate prophylaxis); treatment delay of greater than 14 days required to recover from E7820-related toxicities.

Inclusion/exclusion criteria

Patients ≥18 years with histological or cytological evidence of an advanced or refractory solid tumor, ECOG performance status ≤2 and stable or asymptomatic brain metastases were eligible. Eligible patients needed to have adequate liver, bone marrow and renal function, as evidenced by bilirubin ≤1.5 times the upper limits of normal (ULN) and alkaline phosphatase, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) ≤3 × ULN (in the case of liver metastases ≤5 × ULN); serum creatinine ≤2.0 mg/dL (177 μmol/L) or calculated creatinine clearance ≥40 mL/min per the Cockcroft and Gault formula and absolute neutrophil count $\geq 1.5 \times 10^9$/L, hemoglobin ≥9 g/dL and platelet count $\geq 100 \times 10^9$/L.

Pregnant or lactating females were ineligible. Other exclusion criteria included presence of leptomeningeal metastases and unstable brain metastases, active hemoptysis within 3 weeks prior to the first dose of study drug, hypersensitivity to sulfonamide derivatives, patients who had radiation to ≥30 % of their bone marrow, and patients who required therapeutic anti-coagulant therapy with warfarin or related vitamin K antagonists. Prophylactic doses of heparin or low molecular weight heparin or thrombin inhibitors could be used instead of warfarin.

Other important exclusion criteria included: left ventricular ejection fraction (LVEF) <50 % on echocardiography or multiple-gated acquisition (MUGA) scanning; anticancer therapies that had not been completed/discontinued at least 28 days (7 days in case of protein kinase inhibitor and 42 days in the case of mitomycin C or nitrosoureas) prior to treatment with E7820.

Dose modifications

If patient experiences E7820-related toxicity > grade 1 (other than alopecia, anemia, lymphocytopenia, asymptomatic neutropenia, and nausea, vomiting and/or diarrhea despite optimal medical management), E7820 was interrupted and/or dose reduced. The maximum permissible dose interruption of E7820 was 14 days. No intrapatient dose escalation was permitted.

Pharmacokinetics

Blood samples for PK analyses were collected at the following time points in cycle 1: for Part 1: day 1 and 8 at pre-dose, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 24, 30 and 48 h post-dose; for Part B: Day 1 and 8 at pre-dose, 0.5, 1, 1.5, 2, 3, 4, 5, 8 and 12 h post-dose.

E7820 concentration in plasma samples was measured using a validated liquid chromatography/mass spectrometry/mass spectrometry (LC-MS/MS) method as previously described [6]. Part A was a comparative bioavailability study to estimate the effect of food on the primary E7820 PK parameters ($AUC_{(0\text{-inf})}$, $AUC_{(0\text{-t})}$, C_{max}). The effect of food was estimated using a mixed linear model of logarithmically transformed values of the primary PK parameters with fixed effects for treatment, period and sequence and a random effect of subject. Ratios of geometric means and associated two-sided 90 % confidence intervals were presented. If the 90 % confidence interval (CI) of the model-based geometric mean ratio of fed to fasted were to fall within 70–143 % for clinical significance, then the fasted state could be declared to have similar bioavailability to the fed state.

Pharmacodynamic studies

Blood for platelet integrin-α_2 expression was collected predose on day 1 of cycle 1, then weekly during cycle 1 and on day 1 of every subsequent cycle in Part B and directly stained with fluorescein-conjugated anti-integrin-α_2 antibodies (Ab) or anti-CD49b Ab, as previously reported [5]. Platelet integrin-α_2 expression levels were analysed by flow cytometry.

Safety and anti-tumor activity

Weekly safety assessments consisted of clinical laboratory parameters, vital signs, 12-lead ECG results, physical examinations, and MUGA scans or echocardiograms were performed every 12 weeks. Safety variables were shown as adverse events (AEs) graded by Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

Tumor assessments were performed and assessed by each site investigator using RECIST 1.1 (with the modification that chest disease could not be followed using chest x-ray alone) at baseline and every 8 weeks thereafter during treatment using the same imaging techniques as at baseline.

Results

In Part A, 15 patients were randomized into the study, completed the treatment phase and entered the extension phase after day 8. In Part B, 26 patients were treated (Tables 1 and 2). At the time of data cutoff (30 Apr 2014), one subject was ongoing in Part B of the study. The majority of patients were male 53.3 % (Part A) and 69.2 % (Part B). Median age was 58.0 (range: 41 to 77) years in Part A and 59.0 (range: 38 to 77) in Part B. Almost all subjects had a baseline ECOG performance status ≤1. For safety and PK analyses all patients were evaluable. For efficacy, in Part A 13 (87 %) and for Part B 23 (88 %) patients were evaluable.

Table 1 Parts A and B: Demography and Baseline Characteristics (Other tumor types: thymus, uterine, renalcell and pancreas carcinoma, melanoma, mesothelioma, renalcell, parotis and oesophageal adenocarcinoma)

Category	Part A Overall ($N=15$)	Part B E7820 Total ($N=26$)
Age (year)		
Median	58.0	59.0
Min, Max	41, 77	38, 77
Sex, n (%)		
Male	8 (53.3)	18 (69.2)
Female	7 (46.7)	8 (30.8)
ECOG Status, n (%)		
0	5 (33.3)	8 (30.8)
1	9 (60.0)	17 (65.4)
2	1 (6.7)	1 (3.8)
Tumor type		
Colon/rectum	5 (33.3)	15 (57.7)
Lung	3 (20)	1 (57.7)
Sarcoma	3 (20)	2 (3.8)
Other	4 (26.7)	8 (30.7)
Metastatic disease and/or locally advanced disease, n (%)		
Both	3 (20.0)	5 (19.2)
Locally advanced	1 (6.7)	2 (7.7)
Metastatic	11 (73.3)	19 (73.1)
Time from original histological/cytological diagnosis to first dose (months)		
Mean (SD)	35.0 (20.34)	44.3 (28.41)
Median	30.5	35.0
Min, Max	9.8, 69.4	14.6, 126.4
Time from last progression to first dose (months)		
Mean (SD)	3.6 (1.90)	9.6 (18.97)
Median	3.4	3.0
Min, Max	1.3, 8.3	1.4, 92.4

Safety (worst toxicity - all treatment courses)

The most frequent AE (>50 % in any treatment group, all grades) were fatigue, constipation, diarrhea, infection and nausea (Table 2). Grade ≥3 AE were experienced by 7/15 (46.7 %) of patients in Part A and 19/26 (73.1 %) in Part B. The most frequent grade ≥3 AE (>15 % in any treatment group) were anemia, neutropenia, and fatigue. There was one grade 5 (fatal) event of bronchitis not related to treatment (100 mg QD). Adverse events of special interest were present in the following percentages of patients: anemia (6.7 % in the extension phase of Part A and 30.8 % in Part B), leukopenia (6.7 and 3.8 %), neutropenia (6.7 and 7.7 %, 2 DLTs at 60 mg BID), thrombocytopenia (6.7 and 11.6 %), ALT increased (26.7 and 19.2 %), AST increased (13.3 and 11.6 %), dry skin (20.0 and 11.6 %), pruritus (20.0 and 13.8 %) and rash (0 and 15.4 %). Thirty-three percent of patients in the extension phase of Part A (100 mg QD) withdrew from the study because of AEs: 36.8 % in the 50 mg BID group in Part B, and 42.9 in the 60 mg BID group. Neutropenia resulted in the withdrawal of 3 subjects (1 in Part A Extension and 2 in Part B 60 mg BID); 2 of these 3 subjects were also withdrawn due to leukopenia and thrombocytopenia. All other events resulted in withdrawal of only 1 subject per event. Dose was interrupted in 13.3 % subjects in the Extension Phase of Part A (100 mg QD), 36.8 % in the 50 mg BID group in Part B, and 28.6 % in the 60 mg BID group.

Determination of MTD

Sequential cohorts of subjects were enrolled to determine the MTD during the treatment phase of Part B (dose escalation portion of the study). In cohort 1 (50 mg BID), 3 subjects were treated without experiencing DLTs. In cohort 2 (60 mg BID), 2 out of 7 treated patients (including 1 replacement) experienced DLT. One patient experienced grade 3 neutropenic sepsis considered related to treatment with E7820, starting on cycle 1, day 23 and lasting 5 days. The second patient experienced grade 4 neutropenia considered related to treatment with E7820, starting on cycle 1, day 19 and lasting more than 5 days (27 days). Following de-escalation to 50 mg BID, 4 additional subjects (including 1 replacement) were enrolled at the 50 mg BID dose-level to a total of 7 patients without reporting DLT (Table 2, Part B). The study investigators and sponsor agreed that 50 mg BID should be considered the MTD for this treatment. Twelve additional subjects were treated at the 50 mg BID dose-level to confirm the MTD and further establish the safety of this dose.

Dose modifications

Patients experiencing drug interruption due to adverse events were: two patients (13.3 %) in the extension Phase of Part A (100 mg QD) due to increased bilirubin and liver enzymes; 7 (36.8 %) in the 50 mg BID group in Part B due to anemia, thrombocytopenia, pancreatitis, fatigue, pyrexia, increased blood alkaline phosphatase, haemoptysis, rash and pruritus; and 2 (28.6 %) in the 60 mg BID group due to nausea, small intestinal obstruction and increased lipase. There were no dose reductions in this study. Two subjects (both in the 50 mg BID group) required dose interruptions due to fatigue. All other events resulted in dose interruption in only 1 patient each. Most events leading to study drug interruption were grade 2 or 3.

Table 2 Part A and B - overview of treatment-emergent adverse events during the treatment and extension phases

MedDRA system organ class preferred term	Part A			Part B	
	Treatment phase E7820 50 mg Fed (*N* = 15) *n* (%)	Extension phase E7820 50 mg Fasted (*N* = 15) *n* (%)	Treatment and extension phases E7820 100 mg QD (*N* = 15) *n* (%)	E7820 50 mg BID (*N* = 19 = 3 + 4 + 12) *n* (%)	E7820 60 mg BID (*N* = 7 = 3 + 4) *n* (%)
Diarrhoea	0	0	2 (13.3)	10 (52.6)	1 (14.3)
Fatigue	0	1 (6.7)	5 (33.3)	10 (52.6)	4 (57.1)
Anaemia	1 (6.7)	0	1 (6.7)	8 (42.1)	0
Infections and infestations	0	1 (6.7)	7 (46.7)	8 (42.1)	4 (57.1)
Constipation	0	1 (6.7)	4 (26.7)	8 (42.1)	4 (57.1)
Nausea	2 (13.3)	1 (6.7)	2 (13.3)	5 (26.3)	4 (57.1)
Vomiting	0	0	3 (20.0)	5 (26.3)	3 (42.9)
Decreased appetite	0	0	4 (26.7)	7 (36.8)	1 (14.3)
Lethargy	1 (6.7)	0	2 (13.3)	7 (36.8)	1 (14.3)
Abdominal pain	0	1 (6.7)	3 (20.0)	6 (31.6)	2 (28.6)
Blood bilirubin increased	0	1 (6.7)	5 (33.3)	1 (5.3)	0
Neutropenia	0	0	1 (6.7)	0	2 (28.6), 2 DLTs
Thrombocytopenia	0	0	1 (6.7)	1 (5.3)	2 (28.6)
Dyspepsia	1 (6.7)	0	3 (20.0)	2 (10.5)	2 (28.6)
Oedema peripheral	0	0	2 (13.3)	3 (15.8)	1 (14.3)
Alanine aminotransferase increased	0	0	4 (26.7)	3 (15.8)	2 (28.6)
Aspartate aminotransferase increased	0	0	2 (13.3)	2 (10.5)	1 (14.3)
Blood alkaline phosphatase increased	0	0	2 (13.3)	3 (15.8)	2 (28.6)
Hyperglycaemia	1 (6.7)	0	2 (13.3)	3 (15.8)	1 (14.3)
Back pain	1 (6.7)	0	2 (13.3)	4 (21.1)	1 (14.3)
Hypertension	1 (6.7)	0	0	2 (10.5)	2 (28.6)
Leukopenia	0	0	1 (6.7)	0	1 (14.3)

Pharmacokinetics (PK) and food effect

Administration of E7820 shows a dose-dependent exposure with no significant effect of food on PK. Immediately after the high fat breakfast a slight delay in absorption could be seen (time to reach maximum concentration [T_{max}]: fed 4 h vs. fasted 3 h) with a similar maximum concentration (mean C_{max}: fed 1030 ng/mL vs. fasted 901 ng/mL) and exposure (mean $AUC_{(0\text{-inf})}$: fed 12500 ng•h/mL vs. fasted 11500 ng•h/mL). Mean elimination half-life ($t_{1/2}$) was comparable in both treatment arms ($t_{1/2}$: fed 10.2 h vs. fasted 11.7 h) (Table 3).

The ratio of geometric means (fed/fasted) for $AUC_{(0\text{-inf})}$, $AUC_{(0\text{-t})}$, and C_{max} were 1.06, 1.11, and 1.13, respectively, and the corresponding 90 % confidence intervals (CI) all fell within 70 to 143 %, the pre-specified criteria for concluding no clinically significant effect of food on E7820 exposure. Thus, subjects receiving E7820 can be dosed with or without food (Fig. 1).

E7820 exposure following BID administration was dose-related. E7820 accumulation (~2.5-fold) following BID administration is consistent with ($t_{1/2}$) (Table 3).

Pharmacodynamics: Platelet Integrin Alpha2 Expression

The reduction of expression of platelet integrin-α_2 was most pronounced in the first week after treatment. Measured only in Part B as a pharmacodynamic biomarker, it decreased by a median of 7.7 % (range 72 % reduction to 17.2 % increase) from baseline until cycle 1 day 8 following treatment with 50 mg BID E7820 (Fig. 2).

Anti-tumor activity

The best overall response in any treatment group was stable disease (SD): 23.1 % of all patients in Part A (100 mg

Table 3 Part A (food effect, QD), Part B (BID) and historic PK data (previous phase I study, QD) - Mean (SD) pharmacokinetic parameters of E7820. Last sampling time point was 12 h after administration

Pharmacokinetic Parameter	Part A E7820 50 mg			
	Fed ($N=14$)[a]		Fasted ($N=15$)	
C_{max} (ng/mL)	1030 (311)		901 (242)	
t_{max} (h)[b]	4.05 (1.02, 10.05)		3.07 (0.50, 6.03)	
$AUC_{(0\text{-}t)}$ (ng•h/mL)	11200 (3680)		10500 (3460)	
$AUC_{(0\text{-}inf)}$ (ng•h/mL)	12500 (3510)[c]		11500 (3490)[d]	
$t_{½}$ (h)	10.2 (2.77)[c]		11.7 (4.81)[d]	
CL/F (L/h)	4.29 (1.21)[c]		4.89 (2.19)[d]	
Vz/F (L)	63.6 (27.9)[c]		76.8 (31.5)[d]	
Pharmacokinetic parameter	Part B E7820 50 mg BID		Part B E7820 60 mg BID	
	Cycle 1 Day 1 $n=19$	Cycle 1 Day 8 $n=18$[a]	Cycle 1 Day 1 $n=7$	Cycle 1 Day 8 $n=7$
C_{max} (ng/mL)	1150 (338)	1880 (591)	1310 (424)	2950 (1530)
DNC_{max} (ng/mL)	23.0 (6.76)	37.6 (11.8)	21.8 (7.05)	49.1 (25.5)
t_{max} (h)[b]Median	2.00 (0.50, 5.00)	1.75 (1.00, 8.08)	3.05 (1.55, 5.08)	2.02 (0.50, 4.08)
$AUC_{(0\text{-}t)}$ (ng•h/mL)	6780 (2070)	14500 (4850)	9620 (3500)	26500 (14500)
$DNAUC_{(0\text{-}t)}$ (ng•h/mL)	136 (41.2)	289 (96.8)	160 (58.3)	442 (242)
$RacC_{max}$	NA	1.70 (0.445)	NA	2.19 (0.643)
$RacAUC_{(0\text{-}t)}$	NA	2.19 (0.597)	NA	2.73 (0.805)
Pharmacokinetic parameter	Historic data (first in man study) – E7820 100 mg QD			
	After single oral dose, Cycle 1, Day 1 ($N=17$)		After multiple oral doses Cycle 1, Day 28 ($N=15$)	
C_{max} (ng/mL)	1486.9 (682.6)		2068.3 (806.56)	
t_{max} (h)[e]	2.5		2.25	
$AUC_{(0\text{-}t)}$ (ng•h/mL)	14785 (7665.8)		20346.4 (8942.57)	
$AUC_{(0\text{–}24)}$ (ng•h/mL)	14760.3 (7657.9)		20331.2 (8923.75)	
CL/F (L/h)	8.44 (5.23)[f]		6.43 (4.29)	

$AUC_{(0\text{-}t)}$ area under the concentration × time curve from time zero to time of last measurable concentration, *C_{max}* maximum drug concentration, *$DNAUC_{(0\text{-}tau)}$* dose-normalized $AUC_{(0\text{-}tau)}$, *DNC_{max}* dose-normalized C_{max}, *NA* not applicable, *t_{max}* time to reach maximum (peak) concentration after drug administration, *$RacAUC_{(0\text{-}tau)}$* accumulation index based on $AUC_{(0\text{-}tau)}$, *$RacC_{max}$* accumulation index based on C_{max}, *CL/F* apparent clearance

a: No samples were collected from one subject on Day 8; b: Median (range); c: $N=12$; d: $N=14$; e: Median

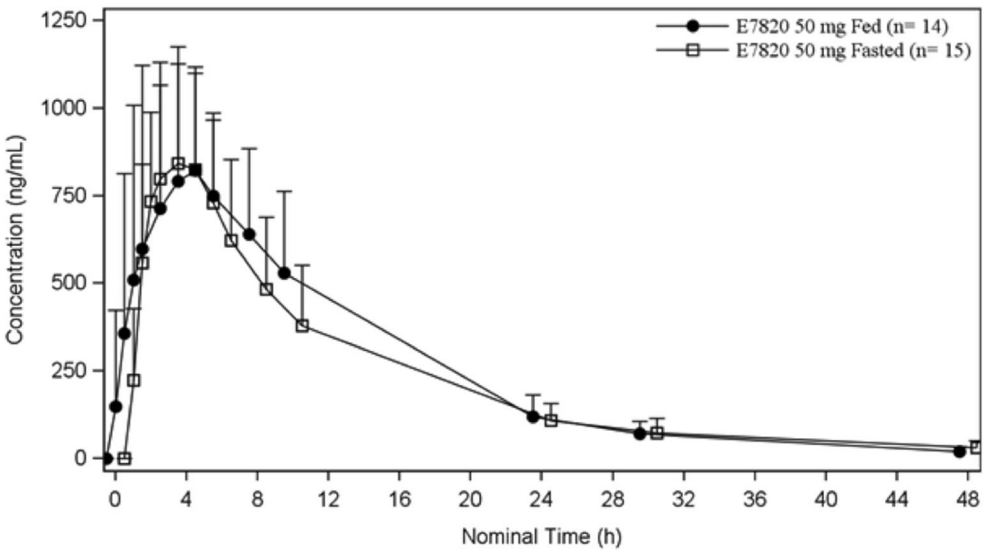

Fig. 1 Part A - Mean plasma concentration-time curve of E7820 following administration of a single dose of 50 mg E7820 to subjects with solid tumors on linear scale

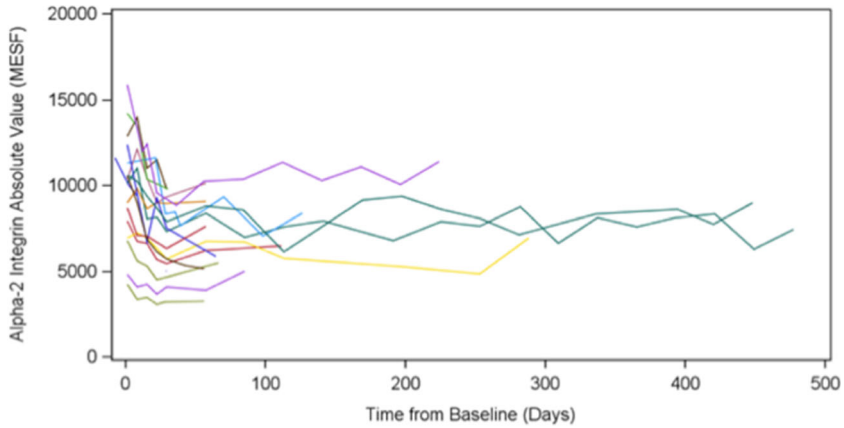

Fig. 2 Part B: Individual absolute values of platelet alpha-2 integrin in the 50 mg BID group

QD); 66.7 % in the 50 mg BID group in Part B, and 40.0 % in the 60 mg BID group in Part B (Fig. 3).

The median duration of treatment in Part A (100 mg QD) was 55 days and in Part B 54 days (60 mg BID) and 60 days (50 mg BID). The median duration of SD (in 18 patients) was 141 days (range 50–440 days). Progression free survival longer than six cycles was recorded in six patients: in two patients with synovial sarcoma and colorectal cancer treated at 100 mg QD E7820 and four patients with malignant mesothelioma, adenoid cystic, non-small cell lung and colorectal cancer, treated with 50 mg BID E7820. The patient in 50 mg BID cohort with adenoid cystic carcinoma completed 26 cycles of E7820 (Fig. 3).

Discussion

This phase I study in patients with unresectable solid tumors treated with twice-daily E7820 showed that treatment with E7820 is safe and well tolerated. The study met its primary objective of establishing the maximum tolerated dose (MTD) as 50 mg BID.

The effect of food on E7820 exposure that was seen as a trend in the Study 102 could not be confirmed in this study [6]. The effect of food in 15 patients dosed both in a fed and fasted state with 1 week in between showed no statistically significant differences in exposure to E7820. The elimination half-life ($t_{1/2}$) was found to be around the time for the second

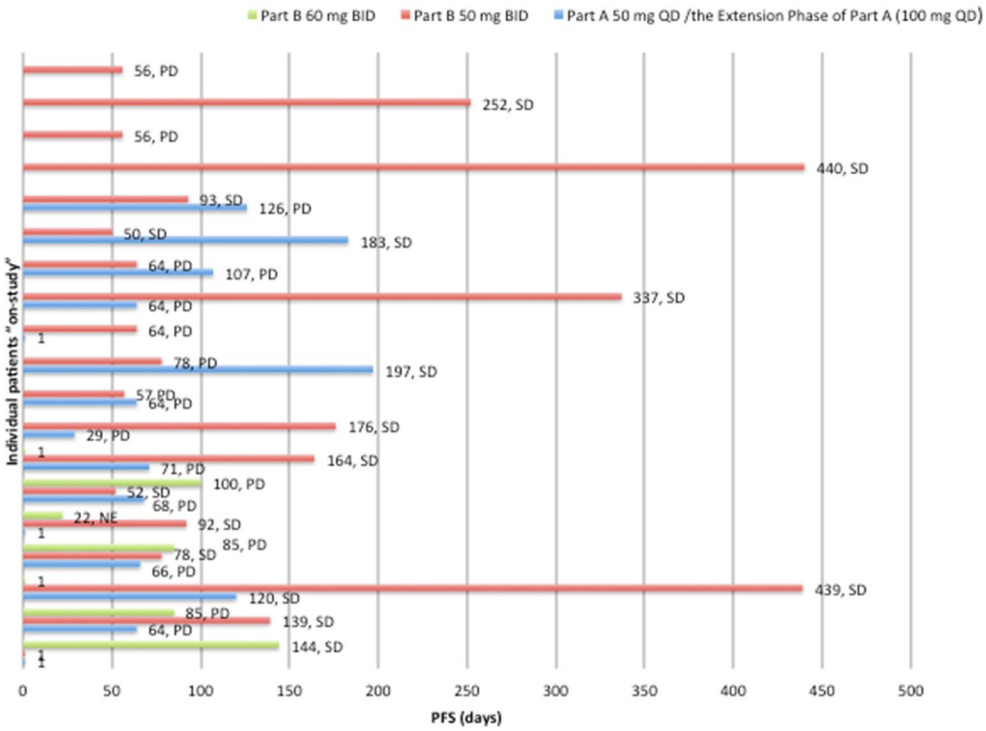

Fig. 3 Parts A and B – Progression Free Survival (PFS) and Best Overall Response of individual patients during extension phase

E7820 dose (between 10.2 and 11.7 h) and accumulation of approximately 2.5-fold was found to be consistent with the twice-daily dosing and $t_{1/2}$.

The most frequent adverse events of all grades were constipation, diarrhea, nausea, and fatigue, while anemia, neutropenia, and fatigue were adverse events with grade ≥3. No treatment related adverse events leading to death occurred. Thrombocytopenia grade 4, reported in the previous phase I study with E7820 (Study 102) was not observed [6]. Two DLTs were observed at 60 mg BID E7820: grade 3 neutropenic sepsis lasting 5 days and grade 4 neutropenia lasting 27 days. Both DLTs were considered related to E7820 treatment. Therefore, 60 mg BID was judged to have exceeded the MTD and one dose-level below, 50 mg BID, was defined as the MTD. This dose is comparable with the MTD previously defined for QD regimen (100 mg E7820 QD) [6].

No partial responses were observed but two thirds (66.7 %) of patients treated with 50 mg BID showed stable disease as their best response. In total 10 % of the patients had clinical benefit for more than 6 months and one patient at the time of data cut off is completing his 27th cycle at 50 mg E7820 BID.

This study confirmed the findings from the previous phase I clinical study and PK/PD modelling and simulation analysis that E7820 down-regulates integrin α-2 expression in surrogate tissues platelets. A predicted >50 % decrease of platelet integrin-α_2 expression in 3 of 4 patients at 200 mg and moderate (<30 %) decreases at 70 - and 100-mg dose levels could not be confirmed in our study. We showed the reduction of platelet integrin-α_2 expression in patients treated with 50 mg BID was most pronounced at cycle 1, day 8 after treatment with a median reduction of 7.7 %. Unfortunately, due to hematological toxicity, 200 mg/day, the dose predicted to target and inhibit adequate mRNA integrin expression could not be achieved in our study.

Integrin inhibitors with diverse molecular structures are safe and potentially active. However best tumor response has been reported as prolonged stable disease, similar to what was observed for E7820 in our study.

A humanized monoclonal immunoglobulin G2 antibody against the αv-subunit of human integrins was investigated as a single agent in patients with progressive castration-resistant prostate cancer with bone metastases. Drug-related toxicities included septicemia and an increase in $^{\gamma}$-glutamyltransferase (GGT) grade ≥3. Two out of 26 enrolled patients showed clinically significant PSA reductions and pain relief with one confirmed partial response (PR) [9]. Another β integrin inhibitor, the antiangiogenic peptide, ATN-161 (Ac-PHSCN-NH(2)), administered in patients with solid tumors, showed few side effects ≤ grade 2 and manifested prolonged SD in one third of enrolled patients [10]. In combination with dacarbazine in stage IV melanoma, intetumumab (CNTO 95), a fully human anti-αv integrin monoclonal antibody showed trend towards improved overall survival, although it was not significant [11]. Volociximab, an anti-α5β1 integrin antibody, in combination with carboplatin and paclitaxel showed preliminary anti-tumor activity in advanced non-small-cell lung cancer (NSCLC). Eight (24 %) out of 33 patients achieved PR and 17 (52 %) had SD. Neutropenia was reported in 24 % of patients. The median progression-free survival was 6.3 and levels of potential biomarkers of angiogenesis or metastasis were reduced following six cycles of treatment [12]. However, the most extensively investigated integrin inhibitor, cilengitide, did not demonstrate better overall survival compared to temozolomide alone in a phase III study in patients with glioblastoma [13, 14] and in recurrent/metastatic squamous cell carcinoma of the head and neck in combination with cisplatin, 5-fluorouracil, and cetuximab. Its further development was discontinued [15]. However, a new phase II study (CORE) in patients with newly diagnosed glioblastoma and unmethylated MGMT gene promoter showed some more promising data [16].

Therefore, a careful choice of the chemotherapeutic drug combination(s) and trial design are of importance in the development of anti-integrin cancer treatment. Based on the preclinical knowledge and trends shown in the clinical trials this treatment combination could prevent drug resistance and tumor relapse [2]. Based on the results of this study further development of E7820 is warranted. Phase 1b/2 Study of Irinotecan Plus E7820 Versus FOLFIRI in Second-Line Therapy in Patients with Locally Advanced or Metastatic Colon or Rectal Cancer is currently in progress.

Conclusions

A maximal tolerated dose of E7820 was confirmed as 50 mg E7820 BID. The tolerability of E7820 was acceptable and no significant safety concerns were identified. The best overall response in any treatment group was stable disease, with two third of patients showing stable disease (66.7 %) at RD. Food had no effect on E7820 exposure.

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Compliance with ethical standards

Conflict of interest This work was supported by Eisai Inc.

L.Reyderman and L.Ottesen are employed by Eisai. Other authors have declared no conflicts of interest.

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