Clinical Cancer Research

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Phase I Pharmacokinetic and Pharmacodynamic Study of the First-in-Class Spliceosome Inhibitor E7107 in Patients with Advanced Solid Tumors

Ferry A.L.M. Eskens[1], Francisco J. Ramos[2], Herman Burger[1], James P. O'Brien[3], Adelaida Piera[2], Maja J.A. de Jonge[1], Yoshiharu Mizui[4], Erik A.C. Wiemer[1], Maria Josepa Carreras[2], José Baselga[5], and Josep Tabernero[2]

Abstract

Purpose: To assess the safety, tolerability, pharmacokinetics, pharmacodynamics, and clinical activity of E7107 administered as 5-minute bolus infusions on days 1, 8, and 15 in a 28-day schedule.

Experimental Design: Patients with solid tumors refractory to standard therapies or with no standard treatment available were enrolled. Dose levels of 0.6 to 4.5 mg/m^2 were explored.

Results: Forty patients [24M/16F, median age 61 years (45–79)] were enrolled. At 4.5 mg/m^2, dose-limiting toxicity (DLT) consisted of grade 3 diarrhea, nausea, and vomiting and grade 4 diarrhea, respectively, in two patients. At 4.0 mg/m^2, DLT (grade 3 nausea, vomiting, and abdominal cramps) was observed in one patient. Frequently occurring side effects were mainly gastrointestinal. After drug discontinuation at 4.0 mg/m^2, one patient experienced reversible grade 4 blurred vision. The maximum tolerated dose (MTD) is 4.0 mg/m^2. No complete or partial responses during treatment were observed; one patient at 4.0 mg/m^2 had a confirmed partial response after drug discontinuation. Pharmacokinetic analysis revealed a large volume of distribution, high systemic clearance, and a plasma elimination half-life of 5.3 to 15.1 hours. Overall drug exposure increased in a dose-dependent manner. At the MTD, mRNA levels of selected target genes monitored in peripheral blood mononuclear cells showed a reversible 15- to 25-fold decrease, whereas unspliced pre-mRNA levels of DNAJB1 and EIF4A1 showed a reversible 10- to 25-fold increase.

Conclusion: The MTD for E7107 using this schedule is 4.0 mg/m^2. Pharmacokinetics is dose-dependent and reproducible within patients. Pharmacodynamic analysis revealed dose-dependent reversible inhibition of pre-mRNA processing of target genes, confirming proof-of-principle activity of E7107. *Clin Cancer Res; 19(22); 6296–304. ©2013 AACR.*

Introduction

Protein-encoding genes of the human genome are composed of multiple exons interrupted by introns. Exons consist of 50 to 250 base pairs, whereas introns are usually larger and can comprise up to several thousand base pairs. In general, exon sequences encode for proteins, whereas introns are generally noncoding. During gene transcription, exon and intron sequences are converted into single precursor messenger ribonucleic acid (pre-mRNA). In a process called "splicing," intron sequences are removed from pre-mRNA and exons are fused together, resulting in the formation of mature mRNA, which is processed further and subsequently transported out of the nucleus into the cellular cytoplasm to be translated into proteins. Most genes give rise to multiple spliced transcripts by a process called alternative splicing. These various transcripts contain different combinations of exons, leading to different mRNA variants and the synthesis of different proteins. Alternative splicing can, among other things, induce the formation of a large number of different oncoproteins. The spliceosome, an intracellular complex of multiple proteins and ribonucleoproteins, is the main cellular machinery guiding splicing. Recently, two natural compounds interfering with the spliceosome were found to display antitumor activity *in vitro* and *in vivo* (1–3). Therefore, it is conceivable that inhibiting the spliceosome could

Authors' Affiliations: [1]Erasmus University Medical Center, Daniel den Hoed Cancer Center, Rotterdam, the Netherlands; [2]Vall d'Hebron University Hospital and Vall d'Hebron Institute of Oncology (VHIO), Universitat Autònoma de Barcelona, Barcelona, Spain; [3]Eisai Inc., Woodcliff Lake, New Jersey; [4]Eisai Inc., Tsukuba, Japan; and [5]Memorial Sloan-Kettering Cancer Center, New York, New York

Note: The data in this article were previously presented in part at the 2009 AACR-NCI-EORTC Molecular Targets and Cancer Therapeutics Meeting, Boston, MA (abstract #C32).

Corresponding Author: Ferry A.L.M. Eskens, Department of Medical Oncology, Room HE120, Erasmus University Medical Center, Daniel den Hoed Cancer Center, P.O. Box 2040, 3000 CA, Rotterdam, the Netherlands. Phone: 311-0703-4897; Fax: 311-0703-4627; E-mail: f.eskens@erasmusmc.nl

doi: 10.1158/1078-0432.CCR-13-0485

Translational Relevance

Alternative splicing can induce formation of many different oncoproteins. The spliceosome, an intracellular complex of multiple proteins and ribonucleoproteins, is the main cellular machinery guiding splicing. E7107, a first-in-class spliceosome inhibitor, is a semisynthetic derivative of the natural product pladienolide B and induces cell-cycle arrest at G_1–G_2–M and apoptosis. In this first-in-human study of E7107, administered as a bolus infusion on days 1, 8, and 15 of a 28-day schedule, E7107 was generally well tolerated with manageable side effects and a maximum tolerable dose (MTD) in this schedule of 4.0 mg/m^2. The pharmacokinetic profile of E7107 revealed dose-dependent drug exposure. At MTD, mRNA levels of selected target genes monitored in peripheral blood mononuclear cells showed a 15- to 25-fold decrease, whereas unspliced pre-mRNA levels of DNAJB1 and EIF4A1 showed a 10- to 25-fold increase, confirming proof-of-concept biologic activity. No other specific spliceosome inhibitors are currently being tested in clinical studies.

serve as a novel target for the development of anticancer drugs (4–6).

E7107, a first-in-class spliceosome inhibitor, is a semisynthetic derivative of the natural product pladienolide B, originally isolated from *Streptomyces platensis*, which strongly inhibits hypoxia-induced vascular endothelial growth factor expression and cell proliferation (1). In preclinical studies, E7107 induced cell-cycle arrest at G_1–G_2–M, and eventually apoptosis (1). It is administered by intravenous injection and is stable in aqueous solution at around pH 6. Recent studies indicate that E7107 inhibits activity of the spliceosome most likely by interacting with the spliceosome-associated protein-130 (7–10). The result of this interaction is that E7107 modulates "maturation" processing of pre-mRNA to mRNA, with consequent changes in protein expression profiles. E7107 has shown the most activity against tumors that express a functional loss of retinoblastoma gene, an increased p16 expression, and upregulated cyclin E (10). In cell-based assays, E7107 inhibited proliferation of a panel of eight human tumor cell lines with a half maximal inhibitory concentration of 1.0 to 20 nmol/L. Most human xenograft tumors tested, including lung, breast, and colon carcinomas, responded to treatment with E7107 with tumor regression (mT/C <42%; ref. 8). Pharmacokinetic studies in mice, rats, and dogs indicated a large distribution volume, high plasma clearance, and linear increase in overall drug exposure (area under the curve) when the dose was increased. CYP3A4 appears to metabolize E7107, but other cytochromes as yet uncharacterized may be involved as well (Eisai; data on file).

We conducted this first-in-human phase I pharmacokinetic and pharmacodynamic study of E7107 administered intravenously as a 5-minute bolus infusion on days 1, 8, and 15 in a 28-day schedule. The objectives of this study were to identify dose-limiting toxicities (DLT) of E7107, explore the safety and tolerability of E7107, determine the pharmacokinetic profile of E7107, determine biomarkers of the pharmacodynamic effect and potential efficacy by identifying changes in pre-MRNA and mRNA expression in peripheral blood cells, and explore the antitumor activity of E7107.

Patients and Methods

Eligibility criteria

The main inclusion criteria (full description in the Supplementary text) were histologically confirmed advanced tumors unresponsive to standard therapy, age >18 years, life expectancy >3 months, Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and adequate bone marrow, hepatic, and renal function. All patients gave written informed consent before any study-related procedure, and approval was obtained from the ethics committees at the participating institutions and regulatory authorities. The study followed the Declaration of Helsinki and good clinical practice guidelines.

Pretreatment and follow-up examinations

Before therapy, a complete medical history was taken and a physical examination was conducted. A complete blood cell count (CBC), including white blood cell (WBC) differential and serum biochemistry, was conducted, as were urinalysis and chest X-ray. A 12-lead electrocardiogram (ECG) was made at screening, preinfusion, within 1 hour postinfusion at days 1, 8, and 15 (cycles 1 and 2 only), and at study termination. Weekly evaluations included physical examination and vital signs, adverse events (AE), ECOG performance status assessment, and CBC, including WBC differential and serum chemistry. Patients were evaluated for AEs and toxicity according to the National Cancer Institute Common Toxicity Criteria, Version 3.0. Tumor measurements were assessed before enrollment and every other cycle. Response was assessed using Response Evaluation Criteria in Solid Tumors (RECIST; ref. 11). Patients were allowed to continue treatment in the absence of progressive disease or unacceptable toxicity.

Drug administration and dose-escalation procedure

E7107 was administered intravenously as 5-minute bolus infusions for 3 consecutive weeks in a 28-day schedule. On the basis of animal toxicology data, the starting dose was 0.6 mg/m^2, which corresponds with one tenth of the dose inducing severe toxicity or death in rats, the most sensitive species in toxicology studies. Patients were enrolled sequentially into escalating dosing cohorts using a standard 3+3 Fibonacci-modified design. Dose increases in subsequent cohorts were by approximately 100% increments until a patient at a given dose level experienced toxicity of grade 2 or higher. From then on, dose increases were by 50% or less, depending upon the seriousness of the toxicity. Intrapatient dose escalation was not allowed.

The dose-limiting toxicity was defined as any grade 3 to 4 hematologic toxicity with the exception of neutropenia

lasting less than 7 days and lymphopenia; any grade 3 or higher nonhematologic toxicity considered to be treatment related, with the exceptions of nausea and vomiting in the absence of appropriate antiemetics and diarrhea abating within 12 hours. In addition, any repeated grade 2 hematologic or nonhematologic toxicity considered to be directly related to E7107 and requiring dose reduction was considered a DLT, as was delayed recovery from toxicity related to treatment delaying scheduled retreatment for more than 14 days and toxicities requiring dose reductions during cycle 1. Finally, an ECG showing QTc interval >500 msec during therapy or 60 msec increase over baseline was considered a DLT.

The maximum tolerable dose (MTD) was defined as the highest dose level that could be given to 6 patients with no more than 1 patient experiencing DLT. If, during dose escalation, 1 in 3 patients experienced DLT during cycle 1, an additional 3 patients were to be treated at that dose level. If no additional DLT was observed, dose escalation could be continued until MTD was reached. Once the MTD was established, this cohort was to be expanded with 10 more patients, to have a minimum of 15 fully evaluable patients and to achieve a more complete description of the safety and pharmacokinetic profile.

Pharmacokinetic evaluation

Complete pharmacokinetic evaluation was conducted by collecting blood samples via an indwelling intravenous catheter in the opposite arm of the infusion. In cycle 1, on days 1 and 15, 4 mL samples were collected before dosing; at the end of infusion; at 5, 10, 15, and 30 minutes; and at 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, and 48 hours after the end of infusion. Urine samples were collected on days 1 and 15 of cycle 1 for 48 hours in the following intervals: 0 to 8 hours, 8 to 24 hours, and 24 to 48 hours. A preinfusion sample was taken.

Pharmacokinetic parameters included the peak plasma concentration (C_{max}), area under the plasma concentration–time curve extrapolated to infinity ($AUC_{0-\infty}$), total body clearance from plasma (CL), volume of distribution at steady state (V_{ss}), and terminal phase elimination half-life ($t_{1/2}$). Plasma concentrations were measured using a validated liquid chromatography–tandem mass spectrometry technique. The lower limit of quantification of the analytic method of each compound was 0.01 ng/mL for plasma.

Pharmacodynamic analysis

To investigate the effect of E7107 on levels of mature mRNA and unspliced pre-mRNA, the expression of certain specific candidate genes was examined in peripheral blood mononuclear cells (PBMC). The candidate genes were determined using exon array and quantitative real-time PCR analysis of human and animal tissues and cell lines. Genes were selected for inclusion in this analysis based on the presence of detectable changes at early time points following exposure, durability of the changes in expression, and detectability in multiple tissues and cell lines. Blood samples (3 mL) were taken preinfusion and 2, 6, 24, and 48 hours relative to end of bolus infusion in cycle 1 at days 1 and 15, and an additional preinfusion sample was taken at day 1 of cycle 2. Blood samples were collected at these 11 time points, and total RNA was isolated and reverse transcribed, after which the pre-mRNA and mRNA expression levels of selected target genes were determined by real-time PCR (12). A more detailed description of this analysis and the candidate gene selection criteria are presented in Supplementary Data.

Statistical analysis

Descriptive statistics were used for baseline characteristics, safety assessments, pharmacokinetic variables (including C_{max}, $AUC_{0-\infty}$, $t_{1/2}$, CL, and V_{ss}), and exploratory assessments, including time to progressive disease and tumor response. Comparison between values before and on treatment was conducted with the paired sample t test or Wilcoxon signed rank test as appropriate. Assessments of the correlation between dose and fold change in mRNA or pre-mRNA transcript levels were conducted using the Spearman rank test. All analyses were conducted using SPSS version 12.01 (SPSS).

Results

Forty patients [24 male/16 female, median age 61 years (range 45–79)] were screened and enrolled. The patients' characteristics are summarized in Table 1. Dose levels studied were 0.6 mg/m^2 ($n = 4$), 0.9 mg/m^2 ($n = 3$), 1.3 mg/m^2 ($n = 3$), 2.0 mg/m^2 ($n = 3$), 3.0 mg/m^2 ($n = 7$), 4.0 mg/m^2 ($n = 17$), and 4.5 mg/m^2 ($n = 3$). The median number of cycles per patient was 2 (range, 1–7). with no obvious relation between dose and duration of exposure. Dose reductions were made in 12% of patients. Reasons for study drug discontinuation were primarily lack of efficacy (29 patients, 72%) or the occurrence of AEs (6 patients, 15%).

Table 1. Patient characteristics

Total	40
Male/female	24/16
Median age, y	61
Range, y	45–79
ECOG performance status	
0	13
1	27
Prior chemotherapy	
1–3 prior regimens	23
≥4 prior regimens	13
Prior radiotherapy	17
Tumor type	
Colorectal carcinoma	16
Esophageal carcinoma	8
Pancreatic carcinoma	3
Gastric carcinoma	2
Renal cell cancer	2
Uterine cancer	2
Miscellaneous	7

Table 2. Drug-related toxicities irrespective of grade

	E7107 initial dose group (mg/m^2)							
	0.6	0.9	1.3	2.0	3.0	4.0	4.5	Overall
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
Total number of patients	4	3	3	3	7	17	3	40
Nausea	1 (25.0)	1 (33.3)	1 (33.3)	3 (100.0)	3 (42.9)	12 (70.6)	3 (100.0)	24 (60.0)
Vomiting	1 (25.0)	1 (33.3)	0	2 (66.7)	2 (28.6)	8 (47.1)	2 (66.7)	16 (40.0)
Diarrhea	1 (25.0)	2 (66.7)	0	1 (33.3)	1 (14.3)	7 (41.2)	3 (100.0)	15 (37.5)
Anorexia	0	1 (33.3)	1 (33.3)	2 (66.7)	2 (28.6)	4 (23.5)	1 (33.3)	11 (27.5)
Fatigue	1 (25.0)	1 (33.3)	1 (33.3)	2 (66.7)	1 (14.3)	3 (17.6)	0	9 (22.5)
Abdominal pain	0	1 (33.3)	0	2 (66.7)	1 (14.3)	4 (23.5)	0	8 (20.0)
Headache	0	3 (100.0)	0	0	1 (14.3)	3 (17.6)	0	7 (17.5)
Constipation	0	3 (100.0)	0	0	2 (28.6)	1 (5.9)	1 (33.3)	7 (17.5)
Asthenia	2 (50.0)	0	0	0	0	4 (23.5)	0	6 (15.0)
Pain	0	0	1 (33.3)	2 (66.7)	1 (14.3)	2 (11.8)	0	6 (15.0)
Cough	0	1 (33.3)	1 (33.3)	0	0	3 (17.6)	0	5 (12.5)
Dyspnea	0	0	0	1 (33.3)	1 (14.3)	3 (17.6)	0	5 (12.5)
Back pain	0	0	1 (33.3)	2 (66.7)	0	1 (5.9)	0	4 (10.0)
Dehydration	1 (25.0)	0	0	1 (33.3)	1 (14.3)	1 (5.9)	0	4 (10.0)
Peripheral edema	1 (25.0)	1 (33.3)	0	0	1 (14.3)	1 (5.9)	0	4 (10.0)
Hypertension	0	0	0	1 (33.3)	0	2 (11.8)	1 (33.3)	4 (10.0)
Pyrexia	0	0	0	1 (33.3)	1 (14.3)	1 (5.9)	1 (33.3)	4 (10.0)

Treatment-related toxicities irrespective of grade are summarized in Table 2; the most frequently occurring side effects were gastrointestinal, while fatigue of any grade occurred in approximately one third of patients. Nausea, vomiting, and diarrhea could be treated and/or prevented in the large majority of patients with routinely administered antiemetic and antidiarrheal drugs, respectively. Hematologic toxicity throughout all dose levels was mild (not exceeding grade 2) and, apart from 1 patient at 4.0 mg/m^2 who did not receive day-15 administration in cycle 1 due to grade 2 thrombocytopenia, did not cause dose reduction or dose delay.

Dose-limiting toxicity and cohort expansion

DLT was observed in 2 patients at 4.5 mg/m^2: 1 patient developed grade 4 diarrhea leading to collapse at day 15 in cycle 1, hampering further drug administration, and another patient developed a combination of grade 3 diarrhea, nausea, and vomiting after the first administration in cycle 1, resulting in a dose delay of more than 14 days. In the next-lower dose level of 3.0 mg/m^2, no DLT was observed in 7 consecutive patients, and therefore an intermediate dose level of 4.0 mg/m^2 was explored in 6 patients. In this cohort, 1 patient required dose reduction due to the onset of a combination of grade 3 nausea, vomiting, and anorexia during cycle 1. The dose of 4.0 mg/m^2 was set to be the recommended dose for further testing, and per protocol 10 additional patients (1 patient was not evaluable for safety due to rapid disease progression) were enrolled to obtain additional safety and pharmacologic data. In this expanded cohort, 1 patient required dose reduction in cycle 2 due to the onset of treatment-related hypertension, but no further DLTs were observed at this dose.

In a parallel phase I study with E7107 that explored a day 1 and 8 administration in a 21-day schedule, 2 patients developed visual problems, leading to a decision made in January 2009 that all patients were to be taken off medication immediately. In the current study, 1 patient who was at 4.0 mg/m^2 in his seventh cycle of treatment when taken off the study had started to complain of mild visual alterations, consisting of difficulty in reading and watching television; after treatment discontinuation he subsequently developed grade 4 blurred vision and central scotomas. Extensive and repeated ophthalmologic investigations, including visual evoked potential measurements, electroretinography, HFA-24-2 visual field analysis, and Goldman perimetry, revealed signs of bilateral optic neuritis. High-dose corticosteroids were prescribed that ultimately led to improvement of the presenting findings with only mild residual symptoms.

Pharmacokinetics

Pharmacokinetic data per dose level are summarized in Table 3. The pharmacokinetic parameters of E7107 were well characterized at all dose levels on days 1 and 15 given the limit of quantification of E7107 in plasma (0.01 ng/mL). Mean C_{max} and AUC showed some variation between patients, but these findings generally were comparable on days 1 and 15 for each patient. Mean C_{max} and AUC increased in a dose-related manner on day 1 (C_{max} ranged from 31.61 ng/mL with 0.6 mg/m^2 to 273.72 ng/mL with 4.5 mg/m^2, AUC_{0-t} (AUC from time zero to last measurable concentration) ranged from 6.17 ng·h/mL with

Table 3. Summary of E7107 pharmacokinetic parameters on days 1 and 15

	E7107 initial dose group (mg/m^2)						
	0.6	**0.9**	**1.3**	**2.0**	**3.0**	**4.0**	**4.5**
Day 1							
C_{max} (ng/mL)	31.6 (7.9)	50.2 (28.0)	197.6 (122.8)	121.6 (40.2)	230.7 (116.1)	228.5 (92.3)	273.7 (13.5)
	$n = 4$	$n = 3$	$n = 3$	$n = 3$	$n = 7$	$n = 17$	$n = 3$
AUC_{0-t} (ng·h/mL)	6.2 (2.7)	8.0 (1.3)	20.0 (7.8)	25.4 (8.3)	30.5 (8.1)	34.8 (10.2)	34.0 (0.9)
	$n = 4$	$n = 3$	$n = 3$	$n = 3$	$n = 7$	$n = 17$	$n = 3$
$AUC_{0-\infty}$ (ng·h/mL)	6.5 (2.7)	8.4 (1.2)	20.3 (7.9)	25.6 (8.3)	29.0 (7.4)	34.6 (9.4)	34.4 (1.4)
	$n = 4$	$n = 3$	$n = 3$	$n = 3$	$n = 6$	$n = 15$	$n = 2$
t_{max} (h)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	$n = 4$	$n = 3$	$n = 3$	$n = 3$	$n = 7$	$n = 17$	$n = 3$
$t_{1/2}$ (h)	12.9 (2.8)	12.7 (2.0)	10.5 (0.8)	7.9 (1.8)	8.8 (2.2)	8.0 (2.3)	6.3 (2.6)
	$n = 4$	$n = 3$	$n = 3$	$n = 3$	$n = 6$	$n = 15$	$n = 2$
CL (L/h)	215.9 (134.7)	176.8 (26.7)	136.7 (45.6)	160.3 (67.4)	213.8 (51.4)	227.0 (61.4)	239.7 (28.1)
	$n = 4$	$n = 3$	$n = 3$	$n = 3$	$n = 6$	$n = 15$	$n = 2$
V_{ss} (L)	1262.9 (668.7)	1100.4 (217.7)	556.3 (185.6)	418.9 (81.7)	676.3 (294.2)	612.2 (269.6)	428.4 (58.5)
	$n = 4$	$n = 3$	$n = 3$	$n = 3$	$n = 6$	$n = 15$	$n = 2$
$\%F_{e0-48}$ (%)	5.6 (2.4)	5.7 (1.3)	5.1 (-)	8.1 (1.4)	6.2 (2.6)	6.5 (2.2)	5.4 (1.9)
	$n = 2$	$n = 2$	$n = 1$	$n = 3$	$n = 6$	$n = 15$	$n = 2$
Day 15							
C_{max} (ng/mL)	70.4 (94.2)	70.4 (57.5)	260.7 (145.0)	162.8 (33.8)	185.5 (88.8)	260.0 (108.8)	258.1 (151.1)
	$n = 4$	$n = 3$	$n = 3$	$n = 3$	$n = 6$	$n = 15$	$n = 2$
AUC_{0-t} (ng·h/mL)	6.9 (5.4)	9.2 (1.7)	22.4 (3.9)	21.1 (2.5)	34.5 (8.9)	35.9 (11.4)	34.5 (7.7)
	$n = 4$	$n = 3$	$n = 3$	$n = 3$	$n = 6$	$n = 15$	$n = 2$
$AUC_{0-\infty}$ (ng·h/mL)	8.6 (5.5)	9.5 (2.4)	22.6 (3.9)	22.8 (0.9)	34.8 (8.8)	37.1 (11.3)	29.2 (-)
	$n = 3$	$n = 2$	$n = 3$	$n = 2$	$n = 6$	$n = 14$	$n = 1$
t_{max} (h)	0.0 (0.0)	0.03 (0.05)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	$n = 4$	$n = 3$	$n = 3$	$n = 3$	$n = 6$	$n = 15$	$n = 2$
$t_{1/2}$ (h)	15.7 (4.8)	13.1 (1.1)	9.9 (1.0)	9.0 (2.6)	9.8 (1.4)	9.2 (1.9)	9.5 (-)
	$n = 3$	$n = 2$	$n = 3$	$n = 2$	$n = 6$	$n = 14$	$n = 1$
CL (L/h)	181.1 (109.4)	156.5 (37.1)	116.1 (35.6)	162.8 (8.8)	173.8 (44.4)	219.5 (70.2)	282.1 (-)
	$n = 3$	$n = 2$	$n = 3$	$n = 2$	$n = 6$	$n = 14$	$n = 1$
V_{ss}(L)	1494.1 (1196.0)	885.1 (459.4)	561.2 (435.4)	542.3 (327.2)	582.4 (289.3)	679.8 (353.5)	787.1 (-)
	$n = 3$	$n = 2$	$n = 3$	$n = 2$	$n = 6$	$n = 14$	$n = 1$
$\%F_{e0-48}$ (%)	2.0 (1.2)	7.2 (4.1)	10.3 (-)	4.5 (3.2)	5.0 (2.3)	6.0 (2.8)	9.7 (-)
	$n = 4$	$n = 3$	$n = 1$	$n = 3$	$n = 5$	$n = 12$	$n = 1$

NOTE: Mean (SD) values are shown. AUC_{0-t} noted as AUC_{last} in source table.

Abbreviations: *n*, number; %Fe, percent fraction of dose excreted in urine; t_{max}, time to peak concentration.

0.6 mg/m^2 to 33.95 ng·h/mL with 4.5 mg/m^2, and $AUC_{0-\infty}$ ranged from 6.46 ng·h/mL with 0.6 mg/m^2 to 34.36 ng·h/mL with 4.5 mg/m^2).

Pharmacodynamics

We were able to show the biologic activity of E7107 with modulation of mRNA and unspliced pre-mRNA of candidate genes in the vast majority of patients (38/40) in PBMC samples. These pharmacodynamic analyses revealed that mature mRNA levels of particular candidate genes, *TRAPPC4*, *SLC25A19*, and *GTF2H1*, evidently decreased (Fig. 1). Furthermore, the unspliced precursor pre-mRNA levels of DNAJB1 and EIF4A1 clearly showed a marked increase, whereas the mature mRNA levels of these particular genes remained virtually unchanged. These observed changes in relative mRNA and unspliced pre-mRNA levels were clearly dose dependent and resulted in an almost linear dose–effect correlation (Fig. 1A). At the MTD, the mRNA levels of TRAPPC4, SLC25A19, and GTF2H1 showed a 15- to 25-fold decrease, whereas the unspliced pre-mRNA levels of both DNAJB1 and EIF4A1 showed an opposite 10- to 25-fold increase (Fig. 1B). Notably, comparable

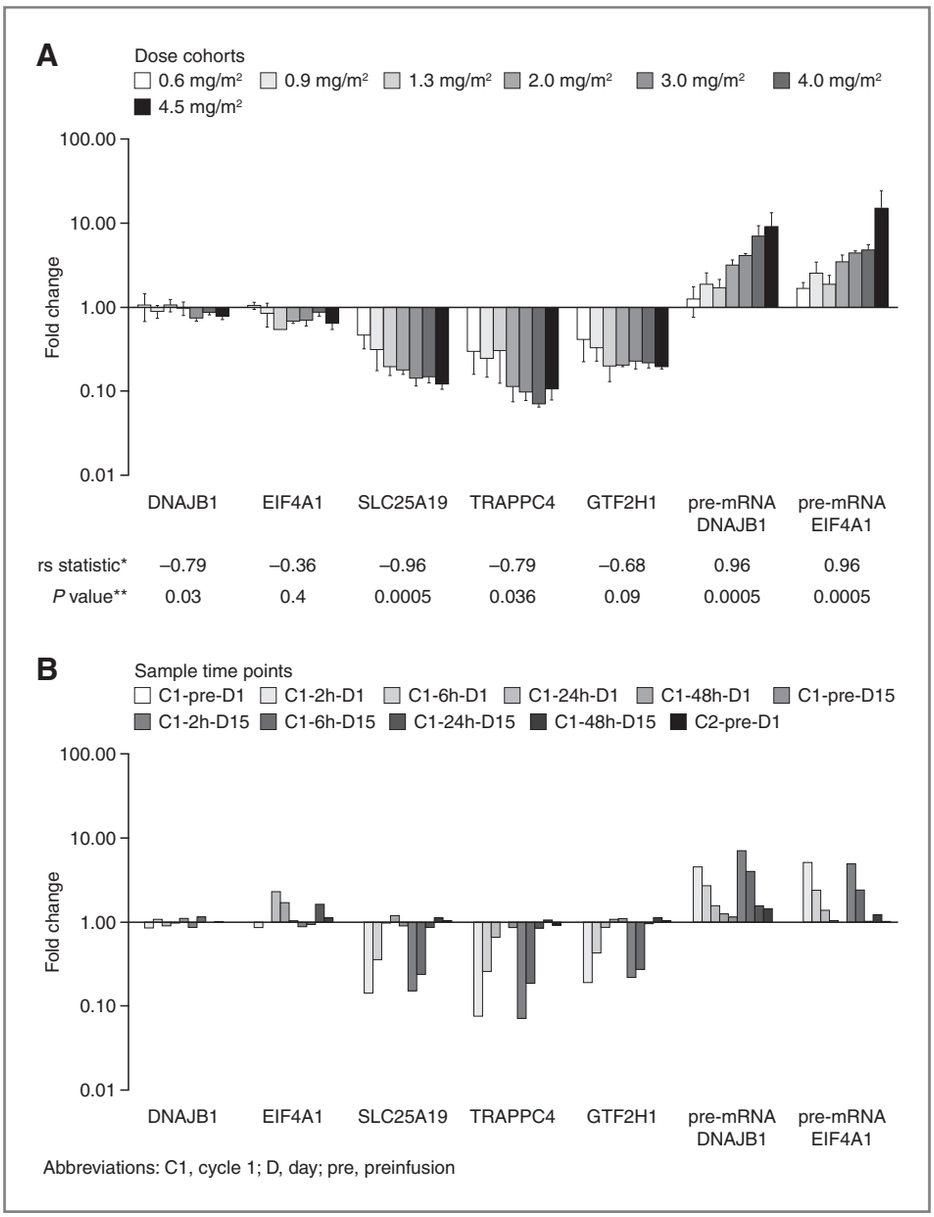

Figure 1. E7107-induced modulation of mRNA and unspliced pre-mRNA candidate genes. A, dose-dependent E7107-induced modulation of mRNA and unspliced pre-mRNA candidate genes monitored 2 hours after start of the last infusion of cycle 1 at day 15 (C1-2h-D15). Different dose cohorts are indicated and the observed fold changes were calculated per dose cohort and presented relative to the predose day-1 sample time point of cycle 1 (baseline level before treatment). *, Spearman rank correlation coefficient; **, two-tailed *P* value. B, observed changes of all treated patients ($n = 17$) at the MTD (4.0 mg/m^2) were averaged and concomitantly the fold change was calculated and presented relative to the predose day-1 sample time point of cycle 1. The presented sample time points are cycle 1 (C1) preinfusion day 1 (D1, #1), C1-2h-D1 (#2), C1-6h-D1 (#3), C1-24h-D1 (#4), C1-48h-D1 (#5), C1-pre-D15 (#6), C1-2h-D15 (#7), C1-6h-D15 (#8), C1-24h-D15 (#9), C1-48h-D15 (#10), and C2-pre-D1 (#11), respectively.

results were seen at days 1 and 15 and treatment-induced changes generally peaked at 2 to 6 hours after the end of the bolus infusion and almost completely recovered to baseline levels 24 to 48 hours after the end of infusion. These observed pharmacodynamic effects provide proof-of-principle with respect to the expected biologic activity of E7107 and further show that this drug administration schedule resulted in a temporary dose-dependent and reversible inhibition of pre-mRNA processing of particular target genes monitored in PBMCs of patients treated with E7107.

Anticancer activity

One patient with acinar pancreatic carcinoma and hepatic metastases had a confirmed partial response lasting 8 months that occurred after he had been taken off the study drug due to the onset of visual disturbances in the parallel study with E7107 (Fig. 2). During participation in this study, which lasted three cycles, hepatic metastases had regressed after two cycles but did not constitute a partial response. The first follow-up computed tomography following drug discontinuation showed a decrease in size and number of hepatic metastases constituting a partial response, which was maintained without additional treatment for the next 11 months when asymptomatic RECIST-defined progressive disease was noted. No other partial responses were noted, but stable disease lasting up to seven cycles was observed with no clear correlation between dose administered and duration of

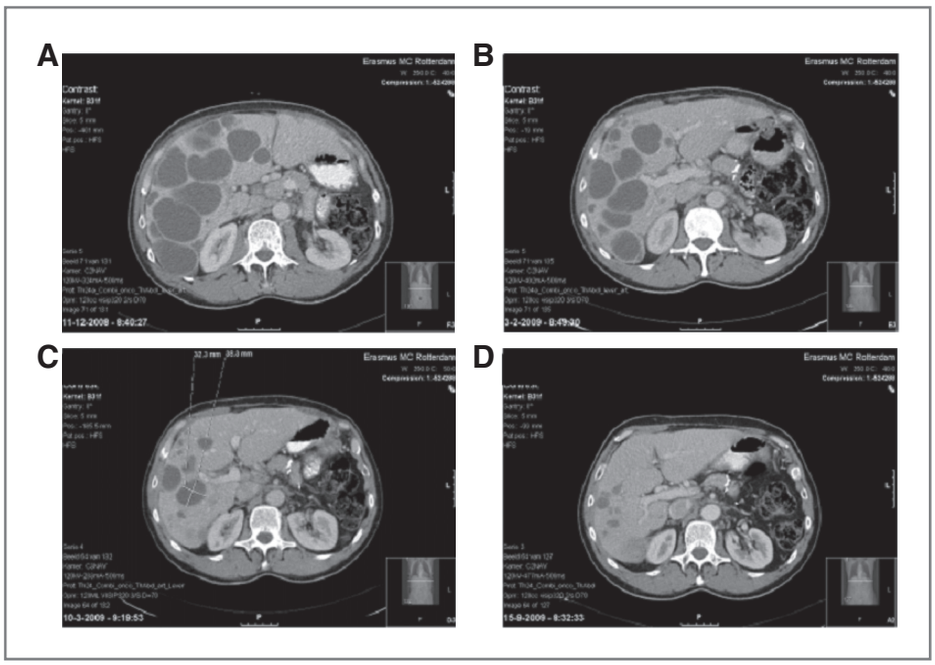

Figure 2. Confirmed partial response in a patient with acinar pancreatic carcinoma. A, before enrollment, after premature discontinuation (B), at follow-up and to confirm response (C), and at long-term follow-up (D).

disease stabilization. Eight patients presented disease control for more than 3 months.

Discussion

This is the first clinical study that has been conducted with the spliceosome inhibitor E7107. Because it was a phase I study, the most emphasis was put on the analysis of safety and tolerability, whereas extensive pharmacologic research was done aiming at an optimal assessment of the pharmacokinetic and pharmacodynamic behavior.

In this study, using a 5-minute bolus intravenous administration on days 1, 8, and 15 in a 28-day schedule, DLT was observed at 4.5 mg/m^2, mainly consisting of gastrointestinal toxicity. Side effects were rapidly reversible in all cases, whereas in subsequent patients treated at next-lower dose levels, routinely prescribed antiemetics were largely able to prevent these side effects. Strikingly, hematologic toxicity was only very mild and, apart from 1 patient, did not lead to either dose reduction or dose delays. A toxicity that was not initially seen in our study was visual disturbance, and the first patient that presented with it was treated in a companion U.S.-based study. Although recruitment in the present study had been completed, the appearance of this severe toxicity precluded continuation of the two studies. Hence, 2 patients who were on treatment in our study at that time were immediately discontinued until more knowledge and mechanistic information could be gained on this infrequent side effect. One of these patients, who was in his seventh cycle of treatment when taken off the study drug, just mentioned the onset of mild visual alterations. Shortly thereafter, this patient developed grade 4 blurred vision. A full ophthalmologic evaluation led to a diagnosis of optic neuritis that improved following treatment with steroids. The nature of this AE and its relationship with the mechanism of action of this spliceosome inhibitor are not completely understood. Although none of the other patients complained of any visual disturbance and all the surviving patients who had participated in the study underwent complete ophthalmologic evaluations without any notable findings, we cannot rule out that this AE is attributable to the study drug. Notwithstanding the fact that this side effect has been shown to be almost completely reversible upon discontinuation of the drug and treatment with steroids, the unpredictable nature of this side effect has prevented further clinical development of E7107.

We have shown that stabilized blood is an easy and excellent source to monitor mRNA and unspliced pre-mRNA levels in surrogate tissue like normal PBMCs upon treatment with spliceosome inhibitors. Evidently, the clear decrease in mRNA levels of particular target genes and the accompanying increase in unspliced pre-mRNA levels of some other candidate genes support the proposition that E7107 functions as a potent *in vivo* spliceosome inhibitor in drug-treated patients. Furthermore, the observed modulation of these efficacy biomarkers as measured in PBMCs of E7107-treated patients clearly provides proof-of-concept that can readily be used in clinical settings. The observed pharmacodynamic effects also provide proof-of-principle with respect to the expected biologic activity of E7107 and further show that this drug administration schedule resulted in a temporary dose-dependent and reversible inhibition of pre-mRNA processing of particular target genes monitored in PBMCs of E7107-treated patients. The finding that the pre-mRNA levels of DNAJB1 and EIF4A1 apparently accumulated

upon treatment with E7107 without affecting the level of mature mRNA was quite unexpected and is not yet fully understood. A likely explanation for this could be that the mature mRNA molecules of these genes have a relatively long half-life.

The potential clinical importance of the spliceosome as a target for therapeutic development has been recently highlighted by the identification of mutation of the *SF3B1* protein, the target of E7107, in myelodysplastic syndrome with ringed sideroblasts (13–16) and chronic lymphocytic leukemia (CLL; 17). In CLL, the *SF3B1* mutation was observed in 15% of cases studied and was associated with 11q deletions and alterations in pre-mRNA splicing.

Apart from this compound, we are not aware of any other clinical studies with spliceosome inhibitors conducted to date. Some spliceosome inhibitors that were developed and analyzed in preclinical studies, such as isoginkgetin, have not yet been developed for clinical purposes (18). The observed biologic effects of E7107 in this study provide proof-of-principle of spliceosome inhibitors and will boost future research into this class of potential new anticancer agents.

In summary, this phase I study with weekly bolus infusion of E7107 has shown that this agent can be clinically administered up to doses that produce the desired target effect with dose-dependent pharmacokinetic behavior. We have shown dose-dependent reversible inhibition of pre-mRNA processing of target genes, confirming proof-of-principle activity of E7107 by splicesome machinery inhibition. This pharmacodynamic evaluation can therefore be included in other trials evaluating splicesome inhibitors, as it may help to define the most feasible and active recommended dose for further development. Specifically regarding E7107, the development of infrequent, but severe ophthalmologic AEs, at least some of which were reversible, currently hampers further clinical development of this agent.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: F.A.L.M. Eskens, Y. Mizui, J. Baselga, J. Tabernero
Development of methodology: F.A.L.M. Eskens, H. Burger, Y. Mizui, E.A.C. Wiemer, J. Tabernero
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): F.A.L.M. Eskens, F.J. Ramos, H. Burger, J.P. O'Brien, A. Piera, M.J.A. de Jonge, E.A.C. Wiemer, M.J. Carreras, J. Tabernero
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): F.A.L.M. Eskens, H. Burger, J.P. O'Brien, M.J.A. de Jonge, E.A.C. Wiemer, J. Tabernero
Writing, review, and/or revision of the manuscript: F.A.L.M. Eskens, F.J. Ramos, H. Burger, J.P. O'Brien, A. Piera, M.J.A. de Jonge, E.A.C. Wiemer, M.J. Carreras, J. Tabernero
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): F.J. Ramos
Study supervision: F.A.L.M. Eskens, J.P. O'Brien, M.J. Carreras

Acknowledgments

The authors thank A.T. den Dekker and N. Azaouagh for technical assistance with the TaqMan analyses. Editorial support was provided by Annette Smith of Complete Medical Communications and was funded by Eisai Inc.

Grant Support

This study was funded by Eisai Inc.

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Received February 20, 2013; revised August 14, 2013; accepted August 19, 2013; published OnlineFirst August 27, 2013.

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