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ORIGINAL ARTICLE

Phase I study of liposomal irinotecan (LY01610) in patients with advanced esophageal squamous cell carcinoma

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Abstract

Purpose This phase I trial was performed to determine the maximum-tolerated dose (MTD), dose-limiting toxicities (DLTs), preliminary efficacy, and pharmacokinetics (PK) of LY01610, a novel liposome-encapsulated irinotecan, in patients with advanced esophageal squamous cell carcinoma (ESCC).

Methods This trial was conducted in two stages. In the dose-escalation stage, patients with advanced ESCC refractory or intolerant to previous chemotherapy received escalating doses of LY01610. A recommended dose based on patient tolerance was then expanded in the second stage. LY01610 was administered intravenously every 2 weeks, except that the first cycle in dose escalation was 3 weeks to allow observation of DLTs.

Results Twenty-four patients were enrolled across 4 dose levels (30, 60, 90 and 120 mg/m^2). The DLTs included vomiting and febrile neutropenia, and the MTD was 90 mg/m^2. The most common grade 3/4 adverse events were leukopenia in six patients (25.0%), anemia in six patients (25.0%) and neutropenia in five patients (20.8%). One patient achieved complete response, and four had partial response, including one patient receiving LY01610 at the starting dose of 30 mg/m^2. Compared with conventional irinotecan, the PK profile of LY01610 was characterized by increased and prolonged exposure of total irinotecan and the active metabolite SN-38 in plasma.

Conclusion LY01610 demonstrated manageable toxicity and promising anti-tumor activity in patients with advanced ESCC. Future clinical development of LY01610 as single agent or in combination with other anti-cancer agents in treating ESCC patients is warranted.

Trial registration NCT04088604 at ClinicalTrials.gov.

Introduction

Esophageal cancer is the sixth driving cause of cancer-related death around the world [1, 2]. In Eastern Europe and Asia, esophageal squamous cell carcinoma (ESCC) remains the most prevalent histological subtype, accounting for roughly 90% of all esophageal cancer cases [3, 4]. About two-thirds of patients with esophageal cancer are unresectable at the time of initial diagnosis, and 43.3–54.5% of patients undergoing radical esophagectomy have local recurrence or distant metastasis within 5 years [5, 6]. Treatment options for patients with advanced ESCC are limited. Cisplatin combined with 5-fluorouracil is commonly used in the first-line setting. For ESCC patients whose disease has progressed with first-line therapy, irinotecan is among the preferred cytotoxic agents in the National Comprehensive Cancer Network (NCCN) Guidelines [7]. The response rate reported in a phase 2 trial with single-agent irinotecan in pretreated esophageal cancer patients was 15.4% [8].

Irinotecan is an inhibitor of DNA topoisomerase I. To be clinically effective, irinotecan must be converted to its active metabolite SN-38 by carboxylesterase primarily in the liver. The mechanism of anti-tumor activity is through the binding of SN-38 to topoisomerase I-DNA complex, causing double-strand DNA damage during DNA synthesis [9]. SN-38 can subsequently be converted via glucuronosyl transferase

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1A1 (UGT1A1) conjugation to its inactive metabolite SN-38 glucuronide (SN-38G). Upon irinotecan administration, biotransformation of SN-38 to SN-38G protects against gastrointestinal toxicity.

Liposomal irinotecan is an encapsulated nanoliposomal formulation of irinotecan hydrochloride. The new formulation protects the drug from premature conversion and activation in the liver, therefore the plasma circulation of liposomal irinotecan is prolonged [10]. It also enables the slow release of encapsulated drug to lower the maximum plasma concentration (C_{max}) and to alleviate drug-associated side effects. Moreover, the leaky vasculature in tumor facilitates the extravasation of liposomal nanoparticles and the defective lymphatic drainage helps increase the retention of the drug within tumor, thereby enhancing its local accumulation in the tumor tissue [10–13]. Irinotecan liposome injection (Onivyde, Merrimack Pharmaceuticals; formerly PEP02 or MM-398) in combination with leucovorin (LV) and 5-fluorouracil (5-FU) has been approved by the FDA for the treatment of patients with metastatic pancreatic adenocarcinoma that relapsed after gemcitabine-based chemotherapy. However, no reports ever addressed treatment outcomes of liposomal irinotecan in patients with esophageal cancer.

LY01610 (Irinotecan hydrochloride liposome injection, Nanjing Luye Pharmaceutical Co., Ltd., Nanjing, China) is a novel nanoparticle formulation of irinotecan hydrochloride encapsulated with polyethylene glycolated liposome. Based on the efficacy of irinotecan in esophageal cancer patients in previous reports, and the presumed advantage of improved bioavailability and anti-tumor activity with LY01610 over conventional irinotecan, we performed a phase I study to evaluate the safety, pharmacokinetics and anti-tumor activity of LY01610 in treatment-refractory advanced ESCC patients.

Patients and methods

Patient selection

This is a phase I, open-label, non-randomized, dose escalation study of LY01610. Key inclusion criteria included: (i) having histologically confirmed solid tumors and have experienced disease progression or intolerance to standard systemic treatment; (ii) age ≥ 18 and ≤ 70 years; (iii) Eastern Cooperative Oncology Group performance status score < 2; (iv) life expectancy ≥ 3 months; (v) adequate bone marrow, liver and renal functions: absolute neutrophil count $\geq 1.5 \times 10^9$/L, platelet count $\geq 100 \times 10^9$/L and hemoglobin ≥ 90 g/L; total bilirubin $\leq 1.5 \times$ upper limit of normal (ULN), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) $\leq 2.5 \times$ ULN (ALT and AST $\leq 5 \times$ ULN for patients with liver metastasis); serum creatinine $\leq 1.5 \times$ ULN or creatinine clearance rate ≥ 50 mL/min (Cockcroft-Gault formula); (vi) no chemotherapy or radiotherapy within 4 weeks before treatment initiation.

The exclusion criteria included: (i) active systemic infections requiring intravenous antibiotics; (ii) significant cardiovascular diseases, such as myocardial infarction, unstable angina, congestive heart failure (New York Heart Association $\geq$ class II), or unstable arrhythmia within 6 months before screening; (iii) uncontrolled ascites or pleural effusions; (iv) pregnancy, lactating or refusal to use effective contraception; (v) a second malignancy within 5 years prior to screening; (vi) symptomatic brain metastasis; (vii) previous exposure to irinotecan; (viii) UGT1A1 *28 homozygous 7/7 variant.

The independent ethics committee approved all versions of the protocol and informed consent form (ICF). All participants voluntarily signed the written ICF. The study was conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. This trial was registered on ClinicalTrials.gov (Identifier: NCT04088604).

Study design and treatments

This study comprised of two stages: dose escalation and dose expansion. The starting dose of LY01610 was 30 mg/m^2, and the planned subsequent dose levels were 60, 90, 120, 150 and 180 mg/m^2. To effectively reduce the number of patients who are under-treated, dose escalation followed a modified patient cohort accelerated titration design [14], in which a single-patient cohort for 30 mg/m^2 and three-patient cohorts for 60 mg/m^2 or above would be recruited until any DLT was observed in the first circle. Once a patient experienced DLT, then additional patients would be enrolled at the same dose. Further dose escalation would be terminated if two or more of the patients experienced any DLT at a particular dose level, and the prior lower dose would be defined as the maximum-tolerated dose (MTD). Intra-patient dose escalation was not permitted. In the dose-expansion stage, 12–15 patients were enrolled to further evaluate the safety, PK characteristics and efficacy of LY01610. The dose for expansion was chosen based on the patient tolerance in dose escalation.

LY01610 was diluted in 250 mL of 5% dextrose and delivered as a 90-min intravenous infusion without premedication. In the dose-escalation stage, the first 3-week was defined as the observation period for DLTs. The treatment was then repeated every 2 weeks starting from the second dose until disease progression, unacceptable toxicity, treatment delay for ≥ 2 weeks, or patient's refusal or death. In the dose-expansion stage, LY01610 was administered every 2 weeks.

Safety and response evaluation

Adverse events (AEs) and DLTs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5.0. DLT was defined as any of the following events occurring during the first 3-week period: (i) grade 4 neutropenia lasting for at least 3 days or grade 3 febrile neutropenia; (ii) grade 3 thrombocytopenia with bleeding; (iii) any other grade 4 hematologic or grade 3 non-hematologic toxicities. Grade 3 diarrhea, nausea, vomiting, or electrolyte disorders were considered DLT only when these disorders persistent despite appropriate medical management.

Radiographical studies were conducted every 8 weeks, and responses were assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [15].

Pharmacokinetics

Pharmacokinetic sampling was done during the first cycle of LY01610 administration. Blood samples were collected from an arm vein contralateral to the site of injection into K_2-ethylenediaminetetraacetic acid (EDTA) Vacutainer tubes before treatment, during the infusion at 45 min, at the end of infusion, after infusion at 1, 2, 3, 6, 9, 12, 24, 48, 72, 96, 168, 240 and 336 h, and before the second dose. Blood samples were kept at 4 °C while being processed for laboratory analyses. Plasma was separated by centrifugation within 1 h of blood collection. The original plasma samples were stored at − 80 °C until batched analyses. Plasma levels of total irinotecan (encapsulated + free), free irinotecan, SN-38 and SN-38G were measured by validated liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS) analytical methods. Irinotecan-d10 used as an internal standard for the quantification of free irinotecan was purchased from Toronto Research Chemicals (Toronto, ON, Canada). Separation of free irinotecan from liposomal was performed using Centrifree® Ultrafiltration Devices (1 mL, Merck Millipore Ltd. Carrigtwohill, IRL). An aliquot of 50-µL plasma sample marked by irinotecan-d10 was diluted with 1 mL 0.9% saline and transferred to the ultrafiltration device and centrifuged at 4 °C for 20 min at 2000*g* in a centrifuge. A 200-µL aliquot of the ultrafiltrate was processed for LC–MS/MS analysis. The average recovery rate reached 82.4% using quality control plasma samples at three different concentration level, and the coefficient of variation was 1.8%, indicating that this method could effectively extract free irinotecan from plasma samples. In this trial, the assay used for sample detection had completed methodological verification and was consistent with FDA and European Medicines Agency (EMA) guidance. Study samples were reanalyzed and matched incurred sample reanalysis (ISR) criteria with a 100% pass rate. The lower limits of quantitation for total irinotecan, free irinotecan, SN-38 and SN-38G were 100 ng/mL, 1 ng/mL, 0.2 ng/mL and 1 ng/mL, respectively.

PK parameters were calculated by a noncompartmental method using the Phoenix WinNonlin software (Version 8.1.0; Certara USA, Inc.; Princeton, NJ). Maximum concentration (C_{max}) and time to reach C_{max} (T_{max}) were taken directly from the observed data. Area under the plasma concentration–time curve from time zero to the time of the last observable concentration ($AUC_{0\rightarrow t}$) was determined by the linear-up/log-down trapezoidal rule. The AUC_{inf} was extrapolated from $AUC_{0\rightarrow t}$ upon the observed last concentration using the Phoenix WinNonlin software. Phoenix first attempted to estimate the rate constant, Lambda Z (λ_Z), associated with the terminal elimination phase for concentration data. If λ_Z was estimable, parameters for concentration data would be extrapolated to infinity. To estimate the best fit for λ_Z, Phoenix repeats regressions of the natural logarithm of the concentration values using the last three points with non-zero concentrations, then the last four points, last five points, etc. The point at C_{max} for intravenous infusion model was not used in the Best Fit method. For each regression, R^2 was computed in Supplementary Table 1. The proportion of AUC from observed last concentration to infinity was shown in Supplementary Table 2.

Endpoints and statistical analysis

The primary endpoints were the MTD, DLT and safety profile of LY01610. Secondary endpoints were PK, objective response rate (ORR, defined as the percentage of patients whose best overall response was complete or partial response), disease control rate (DCR, defined as the percentage of patients whose best overall response was complete response, partial response, or stable disease), progression-free survival (PFS, defined as the time from treatment initiation to the first disease progression or death from any cause), and overall survival (OS, defined as the time from treatment initiation to death from any cause).

The analyses were descriptive. Categorical variables were summarized as frequency and percentage, and continuous variables were summarized as mean with standard deviation (SD) or median with range. The Kaplan–Meier method was used to estimate time-to-event variables. All analyses were carried out on SPSS statistics 26 (International Business Machines (IBM) Corp., USA) and Phoenix WinNonlin software.

Results

Patient characteristics

Between February 2019 and August 2020, 34 patients were screened for this trial and 24 patients with advanced ESCC

were eligible, with 13 in the dose-escalation stage and 11 in the dose-expansion stage. The median age was 59.0 years (range: 44–70), and 22 of the patients (91.7%) were men. All patients had received at least one systemic therapy before treatment with LY01610. Apart from chemotherapy, other prior treatments included surgical resection ($n = 10$, 41.7%) and radiotherapy ($n = 14$, 58.3%). The demographics and baseline characteristics of all patients are listed in Table 1. Of note, this study was suspended from January 31, 2020 to March 6, 2020 due to the COVID-19 pandemic in China. As a result, two patients did not receive the planed treatment during the study suspension. One patient had disease progression after the treatment interruption, and another patient missed two cycles of the study treatment before resuming LY01610. The median follow-up duration was 13.1 months (95% CI 11.3–14.8) as of the data cut-off date (October 7, 2020), and all patients had discontinued the study treatment. The reasons for treatment discontinuation were progressive disease ($n = 19$; 79.2%), adverse event ($n = 1$; 4.2%), patient refusal ($n = 1$; 4.2%), and death ($n = 3$; 12.5%).

Table 1 Demographic characteristics

	N (%)
Patients enrolled	24
Age [median (range)]	59 (40–77)
Gender	
Male	22 (91.7)
Female	2 (8.3)
ECOG PS score	
0	20 (83.3)
1	4 (16.7)
Histologic grade	
G1	1 (4.2)
G2	11 (45.8)
G3	8 (33.3)
Unknown	4 (16.7)
Number of organs with metastasis	
≤ 1	13 (54.2)
≥ 2	11 (45.8)
Site of metastases	
Lymph node	19 (79.2)
Lung	7 (29.2)
Liver	7 (29.2)
Bone	2 (8.3)
Previous treatment	
Surgery	10 (41.7)
Radiotherapy	14 (58.3)
Number of previous chemotherapy regimens	
1	21 (87.5)
2	3 (12.5)

ECOG Eastern Cooperative Oncology Group, *PS* performance status

DLT, MTD and safety

Four dose levels were evaluated in the dose-escalation stage, with 2, 3, 6 and 2 patients enrolled in the 30, 60, 90 and 120 mg/m^2 groups, respectively. One patient in the 30 mg/m^2 group died of upper gastrointestinal hemorrhage due to primary tumor invasion during the DLT observation period, and a second patient was enrolled at the same dose. No DLTs were observed at the 30 and 60 mg/m^2 dose groups. One patient in the 90 mg/m^2 group developed grade 3 febrile neutropenia, and a total of six patients were enrolled at this dose level, with no additional DLTs observed. Two patients in the 120 mg/m^2 group developed grade 3 vomiting that did not improve after appropriate supportive therapy. Therefore, 90 mg/m^2 was determined as the MTD of LY01610. In the dose-expansion stage, we enrolled 11 additional patients at 60 mg/m^2 based on the safety findings in dose escalation.

Among the 24 patients who had received at least 1 dose of LY01610, the total cycles of LY01610 administered were 114, with a median of 5 cycles per patient (range: 1–17). Treatment delay and dose modification in each dose level are summarized in Table 2. The most frequent treatment-related AEs (TRAEs) were fatigue (100.0%), anorexia (91.7%), and nausea (87.5%), and the majority of these TRAEs were of grade 1–2. Without preventive atropine administration, no patients experienced cholinergic syndrome throughout the study. Treatment-related serious AEs were reported in four patients (16.7%), including one case of grade 4 febrile neutropenia along with grade 4 leukopenia and grade 4 neutropenia, one case of grade 3 febrile neutropenia, and two cases of grade 3 vomiting. All TRAEs were managed with appropriate medical care. There were no treatment-related deaths. TRAEs observed in patients treated with LY01610 are listed in Table 3.

The frequency and grade of TRAEs were dose related. As dose escalation proceeded, hematologic toxicity became the most common grade ≥ 3 AEs, among which leucopenia and neutropenia were more frequent at higher dose levels, whereas thrombocytopenia was uncommon across all dose levels. LY01610 was infused without premedication in this phase I study. Four patients experienced transient flushing, palpitation, and chest tightness at the beginning of first infusion, then the symptoms were relieved by reducing the infusion rate immediately. No infusion reactions recurred in these patients in the subsequent cycles.

Antitumor activity

Twenty-one of the 24 patients treated with LY01610 were evaluable for response. The ORR was 20.8%, and the DCR was 33.3%. One patient had a best response of complete response (CR) (4.2%), four patients had partial response (PR) (16.6%), and three (12.5%) had stable disease (SD) (Fig. 1).

Table 2 Dosing details and anti-tumor activity of LY01610

Dose level (mg/m^2)	Total No. of cycles completed	No. of patients with dose reduction	No. of cycles of dose reduction	No. of patients with dose delay	DLT (*N*)	CR (*N*)	PR (*N*)	SD (*N*)	PD (*N*)	Not evaluable(*N*)
Stage 1—dose escalation										
30 (*N* = 2)	9	0	0	0	0	0	1	0	0	1
60 (*N* = 3)	16	0	0	0	0	0	0	1	2	0
90 (*N* = 6)	32	4	12	3	1[a]	1	2	1	2	0
120 (*N* = 2)	4	1	2	0	2[b]	0	0	0	2	0
Stage 2—dose expansion										
60 (*N* = 11)	53	0	0	2	NA	0	1	1	7	2

DLT dose-limiting toxicities, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

[a]Grade 3 febrile neutropenia

[b]Grade 3 vomiting

Among the five patients achieving objective responses, one received LY01610 at 30 mg/m^2, one at 60 mg/m^2, and three at 90 mg/m^2. The response to LY01610 of each patient is summarized in Table 2. Median PFS (95.8% maturity, 23 events from 24 patients) was 1.9 months (95% CI: 1.7–2.1), and median OS (62.5% maturity, 15 deaths from 24 patients) was 6.8 months (95% CI: 3.9–9.7). Three patients were not assessed for response due to deterioration of cancer-related symptoms before the planned first imaging evaluation. They discontinued the study treatment afterwards and received supportive care.

Pharmacokinetics

The C_{max}, $AUC_{0\rightarrow t}$, AUC_{inf}, $T_{1/2}$ and T_{max} of total irinotecan, free irinotecan, SN-38 and SN-38G at different dose levels are listed in Table 4. We used the power function model to analyze the dose linearity of total irinotecan, free irinotecan, SN-38 and SN-38G. The C_{max}, $AUC_{0\rightarrow t}$ and AUC_{inf} of total irinotecan showed good linearity within the dose range of 30–120 mg/m^2, with all R^2 of the linear regression curves greater than 0.97 (shown in Supplementary Fig. 1). On the other hand, due to the small number of patients and inter-individual variation, the PK linearity across the entire range for free irinotecan, SN-38 and SN-38G were unable to be determined. But higher AUC was observed at higher dose levels.

The mean plasma concentration–time curve with positive SDs of total irinotecan, free irinotecan, SN-38 and SN-38G at different dose levels are graphed in Fig. 2. LY01610 mainly presented in the plasma in an encapsulated form after infusion, with extremely low levels of free irinotecan (accounts for about 0.5–0.8% of total irinotecan) detected at different timepoints in the circulation. These results suggest the slow release of irinotecan from LY01610 over time.

Discussion

Irinotecan obtained approval for the treatment of cancer over 20 years ago. Up till now, successful development of a liposomal form of irinotecan with reduced toxicity and improved efficacy have been few. Onivyde (formerly PEP02 or MM-398) was the only marketed liposomal formulation, approved in combination with fluorouracil and folinic acid in patients with metastatic pancreatic adenocarcinoma who previously received gemcitabine-based therapy. IHL-305, another pegylated liposomal irinotecan, showed relatively low SN-38 AUC in its MTD on a 2-week dosing schedule [16]. The anti-tumor activity was limited, with only 1 case of PR observed in 60 enrolled patients, and further clinical development was terminated. In our present first-in-human study, we evaluated the safety, PK and preliminary efficacy

Table 3 Treatment-related adverse event

Dose level, mg/m^2	30 ($N=2$)			60 ($N=14$)			90 ($N=6$)			120 ($N=2$)		
Adverse events/Grade	1/2 *N* (%)	3 *N* (%)	4 *N* (%)	1/2 *N* (%)	3 *N* (%)	4 *N* (%)	1/2 *N* (%)	3 *N* (%)	4 *N* (%)	1/2 *N* (%)	3 *N* (%)	4 *N* (%)
Hematologic toxicities												
Neutropenia	1 (50)	0 (0)	0 (0)	4 (28.6)	2 (14.3)	0 (0)	1 (16.7)	1 (16.7)	1 (16.7)	0 (0)	1 (50)	0 (0)
Febrile neutropenia	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	1 (16.7)	0 (0)	0 (0)	0 (0)
Leukopenia	1 (50)	0 (0)	0 (0)	3 (21.4)	3 (21.4)	0 (0)	2 (33.3)	1 (16.7)	1 (16.7)	0 (0)	1 (50)	0 (0)
Anemia	1 (50)	0 (0)	0 (0)	7 (50)	3 (21.4)	0 (0)	3 (50)	2 (33.3)	0 (0)	1 (50)	0 (0)	0 (0)
Thrombocytopenia	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Non-hematologic toxicities												
Nausea	2 (100)	0 (0)	0 (0)	13 (92.9)	0 (0)	0 (0)	4 (66.7)	0 (0)	0 (0)	1 (50)	1 (50)	0 (0)
Diarrhea	0 (0)	0 (0)	0 (0)	5 (35.7)	0 (0)	0 (0)	2 (33.3)	2 (33.3)	0 (0)	1 (50)	0 (0)	0 (0)
Vomiting	2 (100)	0 (0)	0 (0)	6 (42.9)	0 (0)	0 (0)	3 (50)	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)
Fatigue	1 (50)	0 (0)	0 (0)	14 (100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)
Anorexia	1 (50)	0 (0)	0 (0)	13 (92.9)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)	1 (50)	1 (50)	0 (0)
Alopecia	0 (0)	0 (0)	0 (0)	5 (35.7)	0 (0)	0 (0)	2 (33.3)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)
Rash	0 (0)	0 (0)	0 (0)	3 (21.4)	0 (0)	0 (0)	2 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Infusion related reaction	2 (100)	0 (0)	0 (0)	1 (7.1)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Dizziness	0 (0)	0 (0)	0 (0)	5 (35.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
ALT increased	0 (0)	0 (0)	0 (0)	3 (21.4)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
AST increased	0 (0)	0 (0)	0 (0)	3 (21.4)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Blood bilirubin increased	0 (0)	0 (0)	0 (0)	4 (28.6)	0 (0)	0 (0)	3 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Hypokalemia	0 (0)	0 (0)	0 (0)	2 (14.3)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Hyponatremia	0 (0)	0 (0)	0 (0)	7 (50)	1 (7.1)	0 (0)	3 (50)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)
Hypoalbuminemia	0 (0)	0 (0)	0 (0)	8 (57.1)	0 (0)	0 (0)	3 (50)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)

TRAEs observed in ≥ 10% of the patients and all the TRAEs of grade 3 or higher are listed

ALT alanine aminotransferase, *AST* aspartate aminotransferase

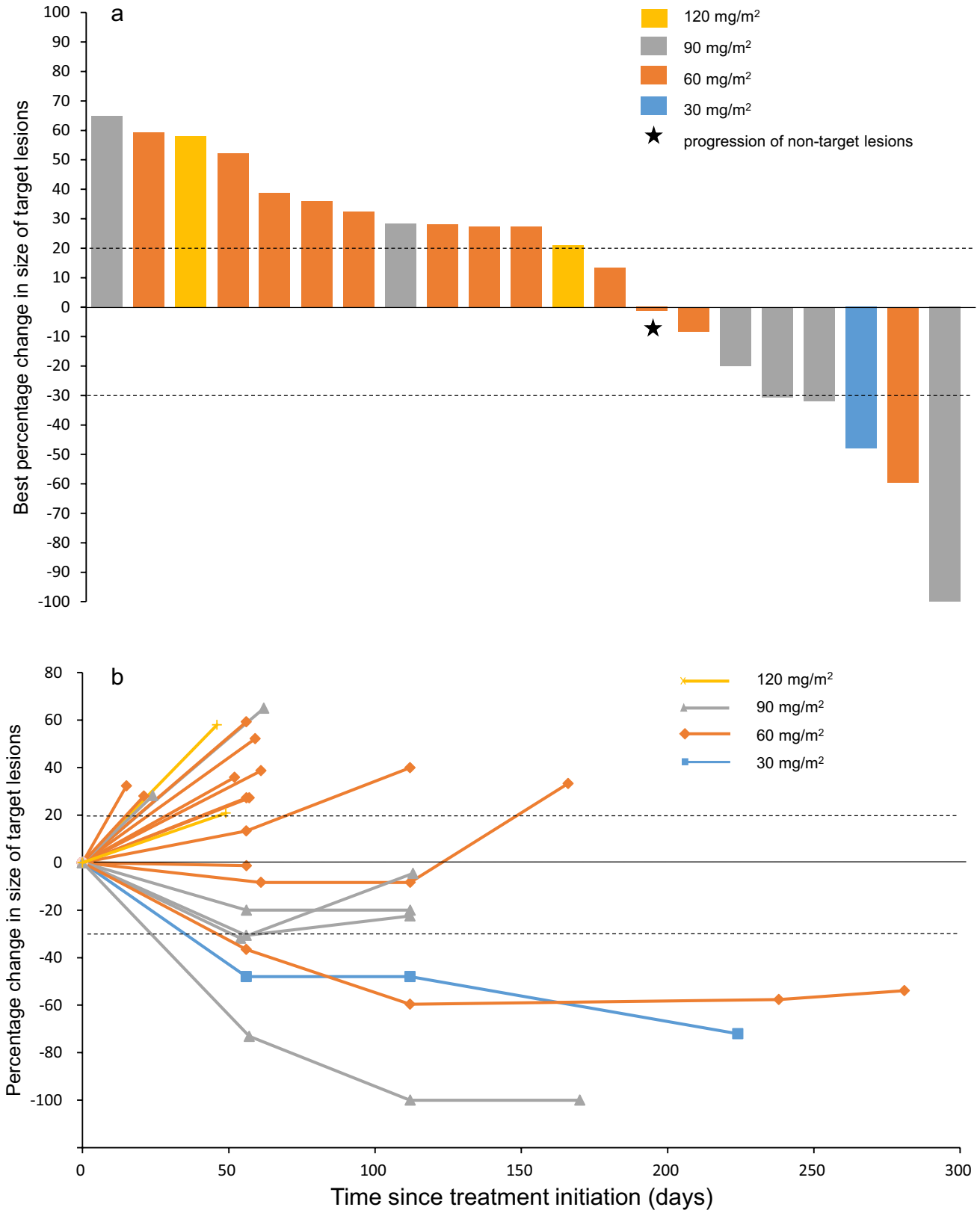

Fig. 1 Response to the study treatment. **a** Waterfall plot of best percent change in target lesions from baseline. The star indicates a patient with a new lesion despite stable target lesions. **b** Percentage change of target lesions from baseline

Table 4 Pharmacokinetic parameters of LY01610 at each dose level

Dose level (mg/m^2)	Mean (SD)				
	C_{max} (ng/mL)	AUC_{0-t} (ng/mL × h)	AUC_{inf} (ng/mL × h)	$t_{1/2}$ (h)	T_{max}[a] (h)
Total irinotecan					
30 mg/m^2 (n = 2)	14,451.58 (2162.03)	316,593.99 (45,555.62)	319,173.23 (44,619.65)	11.70 (1.48)	3.5
60 mg/m^2 (n = 14)	28,461.74 (7405.29)	611,697.63 (214,012.85)	615,722.68 (215,128.93)	11.75 (2.47)	1.5
90 mg/m^2 (n = 6)	48,806.27 (6167.02)	1,298,712.05 (204,104.06)	1,314,436.08 (210,911.72)	14.25 (1.24)	2.5
120 mg/m^2 (n = 2)	71,663.25 (8156.77)	1,497,515.80 (516,081.32)	1,507,701.25 (524,511.66)	13.32 (1.35)	3
Free irinotecan					
30 mg/m^2 (n = 2)	33.86 (4.59)	1639.52 (13.64)	1723.52 (16.15)	19.35 (1.88)	15
60 mg/m^2 (n = 14)	97.62 (41.00)	4916.26 (1940.08)	5034.00(2034.16)	33.18 (18.42)	25.5
90 mg/m^2 (n = 6)	105.41 (43.95)	6250.23 (1762.89)	6322.10 (2087.52)	25.44 (1.42)	25.4
120 mg/m^2 (n = 2)	211.47 (72.19)	8673.69 (5262.09)	8825.92 (5403.62)	27.10 (5.18)	7.5
SN-38					
30 mg/m^2 (n = 2)	2.71 (0.91)	147.97 (57.80)	171.35 (76.68)	25.86 (4.45)	10.5
60 mg/m^2 (n = 14)	6.44 (3.29)	426.54 (281.81)	455.42 (286.11)	42.93 (11.72)	13.5
90 mg/m^2 (n = 6)	6.45 (4.09)	458.96 (239.81)	475.81 (238.99)	39.45 (4.05)	25.5
120 mg/m^2 (n = 2)	11.05 (6.48)	529.33 (457.73)	553.01 (452.53)	53.57 (8.60)	19.5
SN-38G					
30 mg/m^2 (n = 2)	7.35 (1.28)	479.19 (102.87)	458.19 (NA)	24.82 (NA)	37.5
60 mg/m^2 (n = 14)	28.55 (16.72)	1993.81(1397.08)	2109.76(1439.28)	36.15 (8.77)	25.5
90 mg/m^2 (n = 6)	18.5 (5.78)	1332.62 (445.13)	1467.02 (443.59)	35.19 (3.34)	25.5
120 mg/m^2 (n = 2)	38.71 (8.44)	2415.88 (186.16)	2601.83 (26.06)	48.19 (0.42)	16.5

NA: one of the patients had less than three measurable plasma samples after T_{max}, which could not fit the elimination parameters of AUC_{inf} and $t_{1/2}$

[a]T_{max} is represented by the median

of LY01610, a novel nanoparticle formulation of irinotecan, in patients with advanced ESCC. The safety profile of LY01610 administered every 2 weeks was favorable. Myelosuppression and gastrointestinal events were the major DLTs, and 90 mg/m^2 was defined as the MTD. The PK results suggested a slow but durable release of total irinotecan and SN-38. In addition, LY01610 was active in patients with pretreated advanced ESCC, with 1 case of CR and 4 cases of PR among the 24 enrolled patients. Although LY01610 was developed as an analog of Onivyde, the category and proportion of phospholipid vesicle (liposome) encapsulating irinotecan hydrochloride were different. Besides, the distinctive preparation technology and quality control standards may contribute to the different PK characteristics. To our knowledge, this is the first report on the treatment outcomes and PK parameters of a liposomal irinotecan in patients with advanced ESCC, the differences of clinical efficacy between LY01610 and Onivyde were equivocal and warranted further clinical investigations.

No new safety signals were identified with this novel liposomal formulation, and the AEs observed in the present trial were comparable to those with Onivyde [17]. Treatment-related adverse events were generally manageable with appropriate supportive care or dose interruptions or reductions, indicating that LY01610 was well tolerated in patients with ESCC. Additionally, we did not administer prophylactic atropine before LY01610 dosing, and no cholinergic reactions were observed throughout the study. This was consistent with the findings in the phase 1 study of Onivyde [17]. In contrast, the reported incidence of overall irinotecan-related cholinergic syndrome ranged from 31.3 to 83.0% in patients receiving irinotecan-based chemotherapies [18–20]. The patients in the present trial might have benefited from the lower C_{max} of free irinotecan, since the frequency and severity of cholinergic syndrome are likely irinotecan concentration dependent [21, 22].

Irinotecan was commonly used at 180 mg/m^2 every 2 weeks as monotherapy or in combination with other cytotoxic drugs in treating gastrointestinal cancers. Comparing the PK of SN-38 in our present study with that from a phase 1 study evaluating conventional irinotecan at 180 mg/m^2, C_{max} was significantly lowered with LY01610 at the MTD (90 mg/m^2) (Mean: 6.45 vs 26.2 ng/mL), meanwhile, longer $T_{1/2}$ (39.45 vs 19.7 h) and higher AUC_{inf} (475.81 vs 367.6 ng/mL*h) were observed [23]. Notably, the AUC_{inf} of SN-38 with LY01610 at 60 mg/m^2 (455.42 ng/mL*h, listed in Table 4) was already higher than that achieved with 180 mg/m^2 conventional irinotecan, suggesting equivalent or

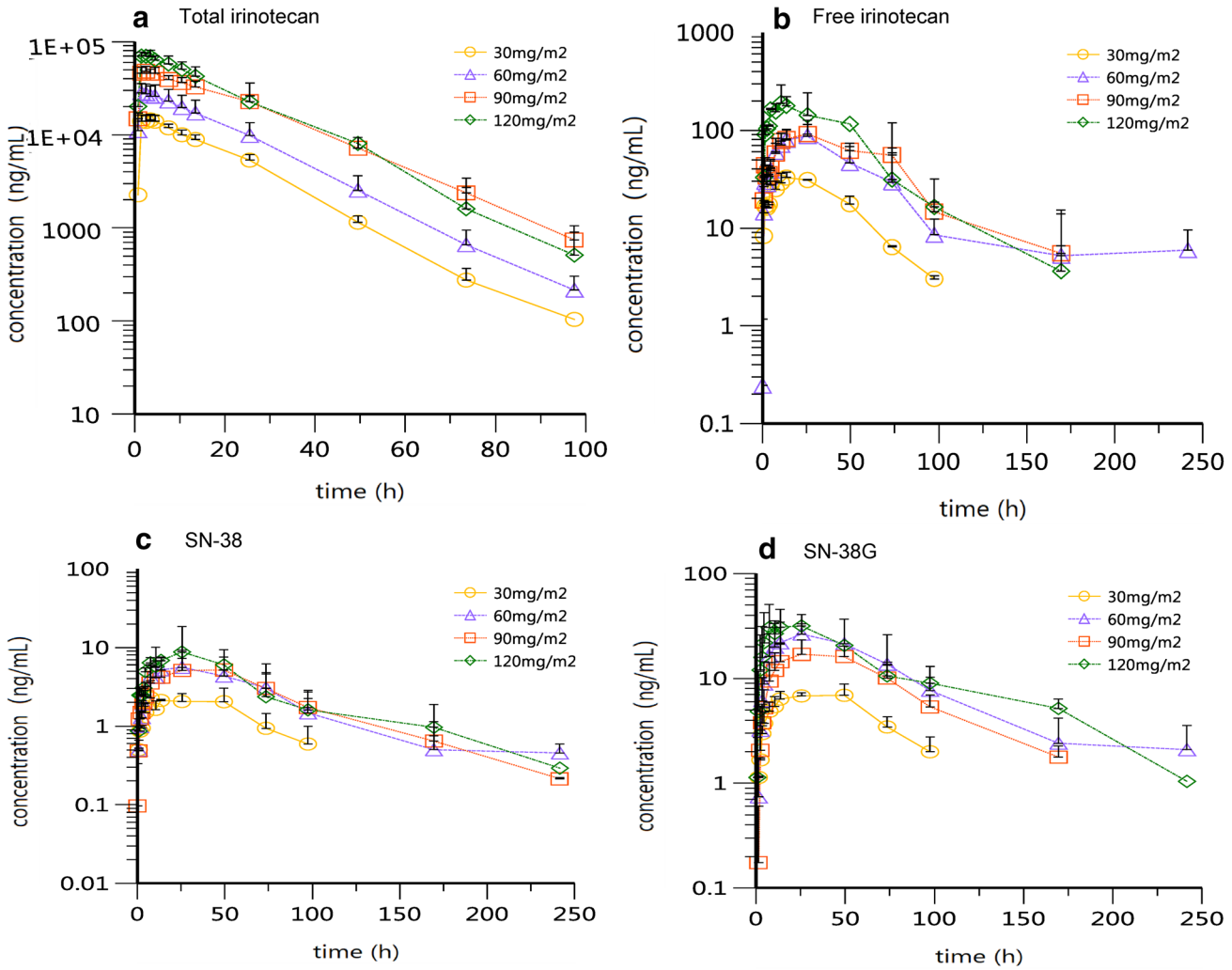

Fig. 2 Mean plasma concentration–time curve with positive standard deviations (SDs). **a** Total irinotecan, **b** free irinotecan, **c** SN-38 and **d** SN-38G at 30, 60, 90 and 120 mg/m2 dose levels of LY01610

improved efficacy with lower toxicities. Indeed, comparing the safety profile with single-agent irinotecan at 180 mg/m^2 administered every 14 days in a phase 2 clinical trial, LY01610 at 60 mg/m^2 was better tolerated with a numerically lower incidence of grade 3 or 4 neutropenia (14.3% vs 23.3%) and thrombocytopenia (0% vs 3.3%), although the differences were not statistically tested [24].

Currently, evidence of the anti-tumor activity of single-agent irinotecan in patients with advanced ESCC was limited. In a phase 2 study including both patients with pretreated ESCC and esophageal adenocarcinoma, the response rate of weekly irinotecan monotherapy was 15%, and the median PFS and OS were 2 months and 5 months, respectively [8]. Recently, the ESCORT study compared camrelizumab (a PD-1 inhibitor) with investigator's choice of chemotherapy in patients with advanced ESCC, in which the investigator's choice of chemotherapy included irinotecan and docetaxel. In the chemotherapy arm, 80.5% (177/220) patients received irinotecan monotherapy, and the reported ORR was 6.4% and the median OS was 6.2 months (95% CI: 5.7–6.9). Taking as reference the response rates in these two prior trials, the efficacy of LY01610 in the present study was encouraging, with an ORR of 20.8%. The median PFS and OS observed in our present study (1.9 months and 6.8 months, respectively) were similar to the survival data in previous trials. However, it is noteworthy that the suspension of the current study during the COVID-19 pandemic in early 2020 might have caused an underestimation of the PFS, since two patients who responded to LY01610 missed the scheduled study treatment during the suspension. Besides, one patient having a best response of PR received LY01610 at the starting dose of 30 mg/m^2. The potential of LY01610 to achieve robust anti-tumor activity at a dose level significantly lower than the MTD suggested a wide therapeutic

index. This is clinically meaningful in that a lower dose level might be used in the setting of maintenance therapy until disease progression, or unacceptable toxicity in the future.

In the era of immunotherapy, three randomized phase 3 trials with immune checkpoint inhibitors have established anti-PD-1 antibodies as the standard second-line regimen for advanced ESCC, and thus changed the management of this devastating malignancy [25–27]. However, the benefit in response and survival with single-agent anti-PD-1 antibodies in an unselected patient population was limited, and chemotherapy was therefore still of important value. In the KEYNOTE-590 trial, an improvement in OS was observed in ESCC patients treated with pembrolizumab plus chemotherapy versus chemotherapy alone as first-line therapy, validating the promise of this combination strategy in ESCC [28]. To achieve better treatment outcomes in the future, the optimal chemotherapy backbone in combination with immunotherapy remained to be investigated. Since irinotecan is among the effective cytotoxic agents against ESCC, the antitumor activity observed with LY01610 in our present trial and the improved tolerance support future clinical development this novel agent in combination with other cytotoxic drugs and/or PD-1 inhibitors in ESCC patients.

In conclusion, LY01610 was safe and effective in patients with advanced ESCC refractory or intolerant to previous chemotherapy in our phase 1 study, with slow but prolonged release of total irinotecan and SN-38 as revealed by PK studies. LY01610 warrants further validation in randomized trials as single agent or in combination with other anti-cancer agents in treating ESCC patients.

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Availability of data and materials Data supporting the results reported in this article are not publicly available but are accessible from the corresponding author on reasonable request and approval from study sponsor according to available guidelines at time of request.

Declarations

Conflict of interest The author(s) declare that they have no conflict of interest.

Ethics approval and consent to participate This study was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. The study protocol was approved by the ethics committee. Informed consent was obtained from all individual participants included in the study.

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