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ORIGINAL ARTICLE

Hyaluronan-Irinotecan improves progression-free survival in 5-fluorouracil refractory patients with metastatic colorectal cancer: a randomized phase II trial

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Abstract
Purpose The objective of this study was to conduct a randomised phase II study in second-line metastatic colorectal cancer with the purpose of confirming preliminary clinical data indicating that the formulation of irinotecan with the drug carrier, hyaluronan (HA) reduced toxicity of the drug.
Methods Irinotecan-naïve patients were randomized to receive either irinotecan (350 mg/m^2) or HA-Irinotecan (HA 1,000 mg/m^2 and irinotecan at 350 mg/m^2) every 3 weeks for a maximum of eight cycles.
Results Seventy-six patients (41 HA-Irinotecan and 35 irinotecan-alone) were enrolled. There was no significant difference in any individual, or overall, grade 3 or 4 toxicity. There was a trend for increased diarrhea in the HA-Irinotecan-treated patients (20 versus 9%; $P = 21$), potentially explained by a disproportionate number of baseline toxicity-associated risk factors in this treatment group. The median number of cycles completed was six for HA-Irinotecan patients and two for irinotecan-alone patients ($P = 0.005$). When compared to the control arm, HA-Irinotecan patients had a significantly longer median progression-free survival of 5.2 versus 2.4 months ($P = 0.017$) and time to treatment failure (4 vs. 1.8 months; $P = 0.007$). Median overall survival was 10.1 months for HA-Irinotecan compared to 8.0 months for irinotecan patients ($P = 0.196$).
Conclusion Further studies are required to define the safety of the formulation of irinotecan with HA. While this study was not adequately powered to demonstrate survival differences, these phase II data indicated HA-Irinotecan to be a promising therapy demonstrating improved efficacy compared to irinotecan-alone.

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Introduction

Colorectal cancer (CRC) is one of the most prevalent cancers worldwide and remains a leading cause of mortality. Irinotecan, a topoisomerase-1 inhibitor, is a standard therapy for patients with metastatic disease who have failed 5-fluorouracil (5-FU)-based therapy. Single-agent irinotecan can be given according to a variety of schedules, including 350 mg/m^2 every 3 weeks, which demonstrates similar efficacy to other alternatives [1].

In an attempt to increase the benefit associated with irinotecan-based treatment and/or to reduce the dose-limiting toxicity often associated with this therapy, irinotecan has been formulated with the naturally ubiquitous polysaccharide, hyaluronan (HA), resulting in a proprietary product (HA-Irinotecan). This product utilizes the unique physiochemical and biologic properties of HA as a macromolecular carrier of drugs to solid tumors. Several intrinsic characteristics of HA highlighted its potential as a drug delivery vehicle: (1) the amphiphilic nature of HA enables it to form a large coiled meshwork at low concentrations [2, 3], making it an ideal vehicle for the solvation and entrainment of smaller molecules; (2) up-regulation and activation of the HA receptor CD44 on malignant tissue [4–7] where activation of the CD44 within the tumoral environment mediates HA internalization [8] and (3) HA is non-immunogenic and considered by regulatory bodies as a biologically inert compound. After intravenous administration, the HA-derivatized drug rapidly enters the tumor and aggregates thereby forming a vascular microembolism within the tumor where the intra-tumoral drug depot persists, increasing drug accumulation and retention [9]. The proposed mechanism of action follows that the increased intra-tumoral drug concentration enables the increased internalization of the anti-cancer agent via a CD44-mediated mechanism, ultimately enhancing efficacy. A secondary effect is the diversion of the drug from healthy tissue leading to a reduction in some commonly observed treatment toxicities [9].

Phase I studies formulating HA with irinotecan [10], 5-FU or doxorubicin [11] demonstrated the safety of these combinations. Based on promising HA-Irinotecan phase I data that demonstrated a trend toward reduced gastrointestinal and hematological toxicity, a randomized phase II study was initiated to compare this formulation with irinotecan alone. To enable a direct comparison between HA-Irinotecan and irinotecan, a standard irinotecan monotherapy regimen was used in second-line metastatic CRC patients, which permitted a single parameter comparison of toxicity and outcome data.

Patients and methods

This study was a randomized, open-label, multicenter phase II study conducted from March 2004 to November 2006. Local ethics committee approval was obtained before enrollment of any patient into the study, which was carried out in accordance with the Declaration of Helsinki and its subsequent amendments, as well as Good Clinical Practice guidelines. Signed informed consent was obtained from all patients before study entry.

The primary endpoint was the incidence of grade 3/4 diarrhea where sample size power calculations were based on an incidence of 30% grade 3/4 diarrhea in the control arm (based on literature estimates [1, 12–17]) versus 5% in the HA-Irinotecan arm (based on an incidence of 8% in the phase I study), which resulted in a sample size of 86 patients to achieve 80% power for a type 1 error of 5%. Secondary endpoints included rates of other severe toxicity, number of cycles delivered, progression-free survival (PFS), overall survival (OS) and time to treatment failure (TTF).

Inclusion criteria

All patients entered into this study had advanced or metastatic CRC with histological documentation of colorectal adenocarcinoma. Patients were required to have disease that was either refractory to or had progressed within 6 months of first line 5-FU (or capecitabine)-containing treatment. Previous oxaliplatin was permitted. Other eligibility criteria were as follows: age 18–75, $\geq$1 measurable lesion ($\geq$1 cm on spiral CT or MRI), ECOG performance status of 0 or 1, estimated survival of $\geq$12 weeks, adequate bone marrow function (neutrophil count $\geq 1.5 \times 10^9$/l, platelets $\geq 100 \times 10^9$/l, hemoglobin >8 g/dl), adequate liver function (bilirubin $\leq 1.25 \times$ upper limit of normal (ULN), ALT $\leq 5 \times$ ULN) and adequate renal function (creatinine 0.2 mmol/l). Major exclusion criteria were as follows: previous exposure to irinotecan, active inflammatory bowel disease, $\geq$grade 2 chronic diarrhea, bulky disease (>50% hepatic involvement, >25% lung involvement or abdominal mass $\geq$10 cm), cerebral metastases and previous exposure to irinotecan, any prior radiotherapy to the pelvis or to >30% of bone marrow or currently active second malignancy.

Patients were excluded from consideration based on the following factors: presence of pleural effusion or ascites requiring therapeutic thoracocentesis or paracentesis; patients with Gilbert's syndrome; patients receiving treatment with phenobarbitone, St. John's Wort, phenytoin or valproate, partial or complete bowel obstruction; concomitant active infection; other severe acute or chronic medical or psychiatric condition or laboratory abnormality that may

increase the risk associated with study participation or study drug administration or may interfere with interpretation of study results; pregnant or lactating women or women of child-bearing potential not using adequate contraception.

Treatment schedule

Patients were randomized to receive irinotecan 350 mg/m^2 formulated with hyaluronan 1,000 mg/m^2 (HA-Irinotecan) or to receive irinotecan alone 350 mg/m^2 every 3 weeks. Patients between 71 and 75 years commenced treatment at a reduced dose (irinotecan 300 mg/m^2). Irinotecan alone or HA-Irinotecan was administered intravenously over approximately 90 min every 3 weeks for a maximum of 8 cycles. All patients received pre-medication using a 5-HT3 inhibitor and dexamethasone.

Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (NCICTC), Version 3. Patients who experienced any treatment-related grade 3 or 4 toxicities (other than alopecia or thrombo-embolic events) received a 25% dose reduction of irinotecan for their subsequent treatment cycles, while the HA dose was maintained at 1,000 mg/m^2. A dose delay of up to 21 days until resolution of the toxicities to grade 1 or less was permitted. All patients received education regarding the potential for treatment-related diarrhea and the appropriate use of loperamide, which was routinely dispensed at the time of the first cycle of treatment. Prophylactic use of granulocyte-colony stimulating factors or erythropoietin was not permitted.

Treatment

Bulk raw HA was produced by a fermentation process using *Streptococcus zooepidemicus* cultures under cGMP conditions (CPN spol.s.r.o, Czech Republic). A 1% (W/V) sterile solution of HA (modal molecular weight 825 kDa) was prepared by sterile filtration (Biological Therapies, Australia), which was used for the manufacture of HA-Irinotecan where the formulation was diluted in 5% glucose (Baxter, Melbourne, Australia) to a final volume of 500 ml.

Toxicity and response evaluations during the study

Before treatment, the clinical status of each patient was assessed by medical history, physical examination, complete blood count, serum CEA and chemistry panel. Following every two cycles, radiological imaging was repeated to determine disease status. RECIST 1.0 criteria were used for assessment of response [18]. An external review of imaging was not conducted.

Pharmacokinetics of irinotecan, SN-38 and SN-38G

In the first cycle, on the day of drug administration, blood samples for quantitation of total (lactone plus carboxylate) irinotecan and its metabolites SN-38 and SN-38G were drawn at 0, 45, 90, and 105 min, 2, 2.5, 3, 4, 6, 24, and 48 h post commencement of infusion. Blood was collected in heparinized tubes and plasma was separated from blood cells by centrifugation (1,200*g* for 10 min) and frozen at minus 20°C then stored at −80°C until analyzed. The concentrations of irinotecan, SN-28 and SN-38G in thawed plasma were determined as previously described [10] using reversed phase HPLC. Concentrations were calculated by comparison to standard curves created using 1/x weighted linear regression fitted to plots of concentration versus response, where response equalled the ratio of peak area of analyte to an internal standard.

Non-compartmental pharmacokinetic parameters for irinotecan, SN-38 and SN-38G were estimated using a Microsoft Office Excel-based software package PK solutions 2™. Patients for whom less than six samples were collected could not be analyzed for all of the PK parameters. The terminal elimination constant (k) was derived from the log transformed concentration versus time plots, with the elimination half life calculated by the equation: $t1/2 = 0.693/K$. AUC (0-infinity) total was calculated by the linear trapezoidal rule from time zero up to the last measurable data point; the terminal contribution was derived by dividing the concentration at the last time point by 'k'. Clearance (CL) was calculated by dividing the dose received by the AUC. The volume of distribution was calculated by the term of CL/k. The metabolic conversion parameters were calculated by: REC of irinotecan = $AUC_{SN\text{-}38}/AUC_{CPT\text{-}11}$; the metabolic ratio (MR) was defined as $(AUC_{SN\text{-}38} + AUC_{SN\text{-}38G}) / AUC_{CPT\text{-}11}$; the glucuronidation ratio (GR) was evaluated by $AUC_{SN\text{-}38G} / AUC_{SN\text{-}38}$, and the biliary index (BI) was calculated as the equation $AUC_{CPT\text{-}11}$ X $AUC_{SN\text{-}38} / AUC_{SN\text{-}38G}$ as previously described [19].

Pharmacokinetics of hyaluronan

Serum samples collected for irinotecan pharmacokinetics were also used for the quantitation of HA. The concentration of HA was quantified by using an enzyme-linked hyaluronan-binding protein assay (Echeleon Scientific, Utah, USA) as previously described [10].

Pharmacogenomic typing of UDP-glucuronosyltransferase 1A1 (UGT1A1) polymorphisms

The UGT1A polymorphisms of UGT1A1 *28, UGT1A1 *27, UGT1A1 *6, UGT1A1 *7, UGT1A7 and UGT1A9 *22 were characterized with the specific purpose of identifying

any correlation with treatment toxicities of grade 3/4 neutropenia and/or diarrhea. Blood samples were collected for isolation of genomic DNA during venipuncture for other diagnostic laboratories at least 1 week prior to starting treatment. Genomic DNA was isolated from white cell pellets using the Qiagen QIAamp DNA Mini Kit. DNA was isolated from 39 HA-Irinotecan and 34 irinotecan patients and was used to identify UGT1A1 *28, UGT1A6, UGT1A7, UGT1A9 and UGT1A9 *22. For each batch of assays, appropriate positive and negative controls of established genotype were assayed.

The UGT1A1 variable length (TA)*n* repeat polymorphism ($n = 5–8$) was evaluated using Genescan technology (Applied Biosystems, Foster City, CA) as previously described [20–22].

Statistical considerations

Population and end-point definitions

The safety patient population (SPP) was defined as all patients who received at least one dose of study drug. Intent to treat population (ITT) was the same as the safety population since the primary endpoint was a safety endpoint, and the per protocol population (PPP) was defined as ITT who received at least two cycles of chemotherapy at the recommended dose. The primary endpoint was any late-form grade 3 or grade 4 diarrhea at any cycle during therapy. Late-form diarrhea was considered to be >24 h post drug infusion.

Progression-free survival was considered as progressive disease on radiological assessment or death from any cause. PFS was determined for each patient as the number of days from Day 1, Cycle 1 (D1C1) to the date of progression. For a patient who did not experience progression during the trial, their time was the number of days from D1C1 date to the date of the follow-up assessment. Time to treatment failure was defined as the time from Day 1, Cycle 1 to the date of discontinuation of treatment for any reason (including progression of disease, treatment toxicity and death). Survival for each patient was defined as the number of days from the date of D1C1 to the date of death. For patients alive at study follow-up, the time was from the date of D1C1 to the date of the last follow-up assessment, and the patient was censored for this analysis.

Data analysis

Tables, listings, graphs and other statistical output were produced with the SAS system, Version 9.1. Demographic and baseline characteristics were summarized as the number of observations used (*n*), mean, standard deviation, minimum, median and maximum for continuous data and as counts and percentages for categorical data. Kaplan–Meier curves were used to compare the time to first occurrence of late-form grade 3 diarrhea and the difference between the control and HA-Irinotecan group was tested using the log rank test. There were a range of secondary and additional statistical analyses. Kaplan–Meier curves, log rank tests and Cox proportional hazards regression models were used for time to event data. Fisher's exact test and logistic regression were used for analysis of data where the outcome of interest was dichotomous.

Results

From March 2004 to November 2006, a total of 80 patients from ten Australian centers were enrolled onto the study where four patients were not randomized due to withdrawal of consent ($n = 1$) or ineligibility ($n = 3$). Table 1 presents the characteristics of the patients; these were well balanced between the treatment groups. In addition, Table 1 includes known prognostic factors such as ECOG status, number of previous chemotherapy regimes, age, sex, smoking status, prior treatment with oxaliplatin, sites of disease, year since diagnosis of primary disease, lactate diagnosis, bilirubin, leukocyte number, alkaline phosphatase and prognostic factors that were used as the adjustment parameters in a Cox PH model.

Treatment compliance

Refer to Fig. 1 for the Consort flowchart following participants at each stage of the study. In the safety and intention-to-treat population, 76 patients were randomized, 41 patients received HA-Irinotecan and 35 received irinotecan alone. HA-Irinotecan patients were able to be treated for significantly more cycles, the median number of completed cycles was 6 versus 2 for irinotecan-only patients ($P = 0.005$). The total number of cycles administered was 213 for HA-Irinotecan-treated patients compared with 123 for irinotecan alone patients. Fourteen (34%) HA-Irinotecan-treated patients and 5 (14%) irinotecan-treated patients completed eight cycles ($P = 0.06$). Dose reduction due to unresolved toxicity was performed in 34% of HA-Irinotecan, compared to 20% of irinotecan administrations. The mean irinotecan dose per cycle was 590 mg for patients on the investigational arm compared to 591 mg on the control arm.

Toxicity

There were no significant differences in any individual grade 3 or 4 toxicities or in total grade 3 or 4 toxicity (Table 2). When considering the incidence of adverse events per cycle and per patient, there was no statistically

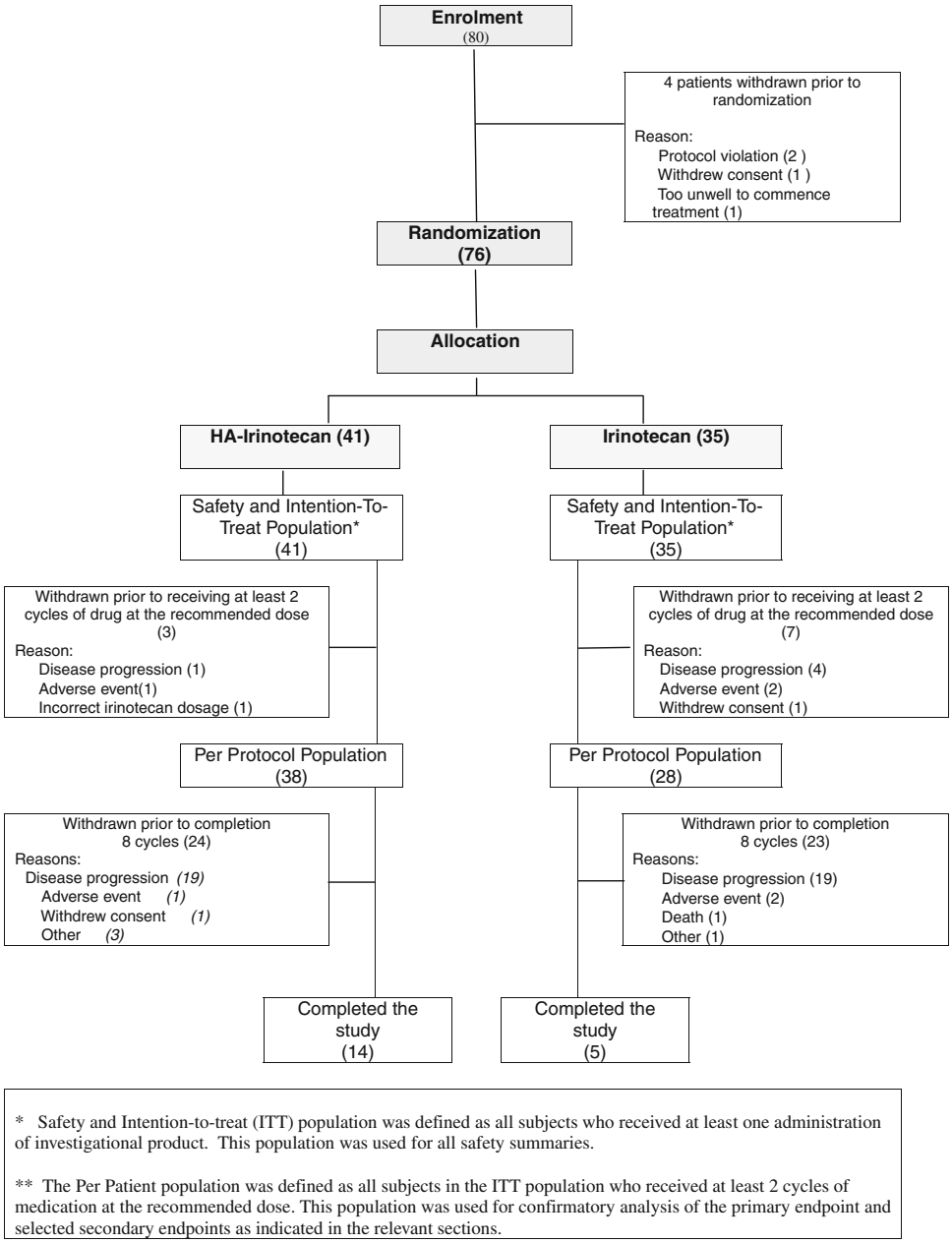
Table 1 Summary of characteristics for patients entered on the study, including known prognostic factors

Parameter	Irinotecan only (35)		HA-Irinotecan (41)	
	Number of patients	%	Number of patients	%
Age (range)				
Mean	63 (37–78)		62 (45–75)	
Median	64		63	
Sex				
Male	21		24	
Female	14		17	
Ethnicity				
Caucasian	34	97	41	100
Asian	1	3		
ECOG				
ECOG 0	16	46	19	46
ECOG 1	19	54	22	54
Time since original diagnosis (years)				
Mean (range)	2.3 (0–11)		1.6 (0–6)	
Median	1.3		1.1	
Previous therapy				
Surgery	34	97	41	100
Prior pelvic radiation	3	9	7	17
Chemotherapy				
5-Fluorouracil	32	91	38	83
Capecitabine	11	31	9	22
Folinic acid	23	66	22	54
Oxaliplatin	30	86	34	83
Bevacizumab	3	9	1	2
Number of sites with metastatic lesions				
1	9	26	14	34
2	14	40	16	39
>2	12	34	11	27
Location of metastatic lesions				
Liver	27	77	33	81
Lung	20	57	21	51
Lymph nodes	10	29	12	29
Other	19	54	16	39
Baseline CEA (μg/l)				
Mean (range)	1,642 (0.5–32,004)		306 (0.5–2,878)	
Median	29		53	
Smoking history				
Never	19	54	20	49
Past	12	34	16	39
Current	4	11	5	12
Baseline LDH (IU/l)				
Mean (range)	522 (142–1,433)		463 (128–1,301)	
Median	467		392	
Alkaline phosphatase (IU/l)				
Mean (range)	171 (45–787)		193 (51–677)	
Median	142		156	
Bilirubin (μmol/l)				
Mean (range)	12 (4–25)		12 (5–24)	
Median	10		11	

significant difference between treatment groups. In the control group, 14% of patients withdrew from the study due to adverse events compared 7% in the HA-Irinotecan arm. There was no significant difference in the rate of severe diarrhea in the investigational arm with 20% (8 patients) compared to 9% (3 patients) in the control arm, where after adjusting for the number of previous chemotherapy regimes, age, gender and prior pelvic radiation, there was no statistically significant difference in the odds of having late-form grade 3 diarrhea between the two treatment groups (odds ratio for HA-Irinotecan vs. control group = 2.53; $P = 0.21$). With the exception of one patient in the control arm, all episodes of late-form grade 3 diarrhea occurred in cycle 1. Comparison of subsequent treatment of the patients who developed late-stage grade 3 diarrhea demonstrated a differentiation between the treatment arms. On the control arm, three patients experienced grade 3 diarrhea after which two withdrew from the study due to the severity of the diarrhea and the third withdrew due to disease progression. For the HA-Irinotecan arm, the eight patients who experienced grade 3 diarrhea in cycle 1 underwent a dose reduction which enabled them to receive a total of 2 cycles ($n = 2$), 4 cycles ($n = 1$), 5 cycles ($n = 1$) or 8 cycles ($n = 4$). It was noted that 63% of HA-Irinotecan patients who experienced a grade 3 diarrhea had 1–3 of the baseline risk factors (≥65 years, increased bilirubin and/or UGT1A1 *28/*28 allele expression) listed in the irinotecan label and therefore should have ideally had a reduction in the starting dose by one dose level.

The incidence of severe neutropenia was similar in each arm, with 3 (7%) HA-Irinotecan patients experiencing febrile neutropenia compared to 4 (11%) irinotecan-only-treated patients. The incidence of other major toxicities, including anemia, thrombocytopenia and nausea/vomiting was very similar in both arms. There were three deaths while on study treatment, all in the control arm. One death was due to neutropenic sepsis, the second death was due to respiratory failure 9 days after the patient was withdrawn

Fig. 1 Consort flowchart



from study due to progressive disease, the other patient was found dead at home possibly due to coronary thrombosis.

Response to treatment

Five patients (14%) in the irinotecan-only arm did not complete the first cycle of treatment due to toxicity, consent withdrawal or non-cancer-related death were included in the efficacy analysis as non-evaluable. When the best overall response was evaluated, partial responses were seen in 3 (7%) HA-Irinotecan patients and 1 (3%) irinotecan-alone patients. The rate of stable disease in HA-Irinotecan patients was 68 versus 43% in the irinotecan-only arm. The disease control rate (PR plus SD) was 31 (76%) in the HA-Irinotecan arm versus 16 (46%) in the irinotecan arm, with this difference almost reaching statistical significance ($P = 0.053$). When a confirmatory analysis was conducted on the per protocol population (those patients who completed two cycles of therapy), the overall results were similar to those mentioned earlier with patients in the control group (41%) more likely to have PD compared to patients in the HA-Irinotecan group (21%; odds ratio for HA-Irinotecan vs. control group = 0.39; $P = 0.09$).

Survival analysis

As shown in the Kaplan–Meier curve in Fig. 2a, the median progression-free survival was 5.2 months for HA-Irinotecan

Table 2 Number and percentage of patients experiencing a study treatment-related adverse event

Organ system Number of patients (% of safety population)	Grade 1		Grade 2		Grade 3		Grade 4	
	Irinotecan	HA-Irinotecan	Irinotecan	HA-Irinotecan	Irinotecan	HA-Irinotecan	Irinotecan	HA-Irinotecan
Gastrointestinal								
Late on-set diarrhea	6 (17)	6 (15)	2 (6)	9 (22)	3 (9)	8 (20)	0	0
Nausea	15 (43)	21 (51)	6 (17)	14 (34)	3 (9)	4 (10)	0	0
Vomiting	5 (14)	12 (29)	6 (17)	10 (24)	3 (9)	2 (5)	0	0
Mucosal inflammation	1 (3)	2 (5)	0	3 (7)	0	0	0	1 (2)
Constipation	1 (3)	3 (7)	0	0	0	0	0	0
Abdominal pain	7 (20)	3 (7)	1 (3)	0	1 (3)	0	0	0
Hematological								
Neutropenia	0	1 (2)	5 (14)	4 (10)	5 (14)	8 (20)	6 (17)	4 (10)
Febrile neutropenia	0	0	0	0	2 (6)	3 (7)	2 (6)	0
Leukopenia	1 (3)	1 (2)	0	0	3 (9)	2 (5)	3 (9)	3 (7)
Anemia	0	1 (2)	4 (11)	2 (5)	1 (3)	1 (2)	0	0
Thrombocytopenia	1 (3)	1 (2)	0	0	0	1 (2)	0	0
Pulmonary								
Hypertension	0	0	0	0	0	0	0	0
Dyspnoea	1 (3)	0	0	0	0	0	0	0

patients compared with 2.4 months for those receiving irinotecan alone ($P = 0.02$). When adjusted for 12 baseline prognostic factors, the difference remained significant ($P = 0.01$) (Cox PH model, P value from Wald Statistic). The median time to treatment failure (Fig. 2b) was 4.0 versus 1.8 months ($P = 0.01$) months, again favouring HA-Irinotecan-treated patients. There was no statistically significant difference in median overall survival of HA-Irinotecan (10.1 months) versus irinotecan-treated (8.0 months) patients ($P = 0.2$) as shown in Fig. 2c.

Pharmacogenomic typing of UGT1A polymorphisms

With the exception of the UGT1A1 *28/*28 and UGT1A1 *1/*6 polymorphisms (data not shown), which had a higher incidence in the HA-Irinotecan treatment group, the UGTA1 polymorphism expression profiles were evenly matched and within the published ranges for the two treatment arms (Table 3).

Pharmacokinetics of irinotecan, SN-38 and SN-38G

The formulation of hyaluronic acid with irinotecan (HA-Irinotecan) did not alter the pharmacokinetics or metabolism of irinotecan as demonstrated by the collective pharmacokinetic data for irinotecan, SN-38 or SN-38G (Table 4). The pharmacokinetic parameters for both the irinotecan and HA-Irinotecan treatment groups were comparable to previously published ranges, which were obtained using identical treatment and dosage regimens.

Hyaluronic acid pharmacokinetics

The median baseline level of HA was 56 ng/ml (range 10–334), which were within the published range [23]. After administration of HA-Irinotecan, elimination of the exogenous HA exhibited substantial inter-patient variability. Maximum circulating levels of HA were achieved at the median time of 2.2 h (range 1.5–5.5) after the commencement of the 90-min infusion reaching a median level of 625 μg/ml (range 409–1,050), after which the majority of patients maintained constant levels of HA for 23 ± 9 h after administration.

Discussion

This randomized phase II study evaluated the efficacy and safety of a new proprietary formulation of irinotecan known as HA-Irinotecan, using irinotecan as the control treatment. As the primary endpoint of this study was the incidence of any late-form grade 3 or 4 diarrhea occurring at any cycle (an objective resulting from strong supportive phase I clinical evidence [10]), this study failed to meet its primary end-point due to the lack of a significant difference between the study treatment groups. Compared to the grade 3 or 4 diarrhea levels reported in the literature (16–41% [1, 12, 24]), this study presented a low overall incidence of late-form diarrhea. Due to improved methods of treating gastrointestinal toxicities, the best comparator study is the recently completed EPIC trial where 16% of patients who

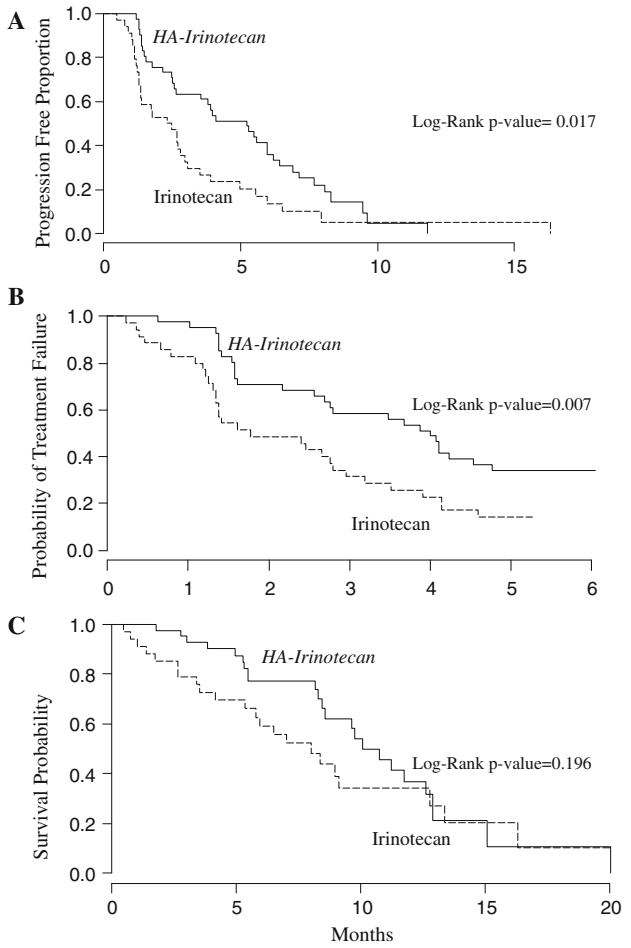


Fig. 2 HA-Irinotecan provided an efficacy advantage over irinotecan as indicated by a significant increase in median (**a**) progression-free survival with an increase of 2.8 months ($P = 0.017$) and a 2.2 month increase in time to treatment failure (**b**). Patients treated with HA-Irinotecan displayed an overall survival of 10.1 versus 8 months when treated with irinotecan only (**c**)

received irinotecan according to the same schedule (350 mg/m^2 q3w) experienced grade 3 or 4 diarrhea [24]. This percentage is very similar to the incidence observed in the HA-Irinotecan arm. The exceptionally low incidence of diarrhea in the irinotecan alone control arm of our study cannot be explained but these data should be considered in the context of the limited patient numbers.

Any direct comparison of toxicity rates was complicated by the markedly different duration of treatment in the two arms, with patients on the investigational arm receiving 3 times (median 6 vs. 2 cycles) as many treatments. Due to the greater treatment duration, an increased potential for treatment-related toxicity, and also for adverse events unrelated to study treatment, could have potentially biased against the toxicity profile of HA-Irinotecan-treated patients. Despite the difference in cycles delivered, the dose intensity was almost identical for each arm, with patients treated with HA-Irinotecan receiving a median of 590 mg per treatment cycle versus 591 mg per treatment cycle for irinotecan-treated patients. This suggests there was no cumulative toxicity in the HA-Irinotecan-treated patients. The most common non-hematological toxicities reported were nausea, fatigue and alopecia where the incidence was similar to previously cited figures. Interpretation of the "all cycles data" is difficult since there was a disproportionate number of dropouts from the control arm early in the study, thus exposure to treatment across the two arms was not balanced over all cycles. In general, there was little difference in adverse events and hence no notable safety issues between the arms. Most patients withdrew from the study due to disease progression with relatively few withdrawals due to adverse events (14% in control, 7% in HA-Irinotecan). Twelve events in the control group and 25 events in the HA-Irinotecan group required study drug dose reduction. In general, the overall toxicities were similar to those previously reported for irinotecan with no new toxicities emerging that were attributable to the HA-Irinotecan formulation.

Response rates were low in both arms, consistent with recent studies of irinotecan containing regimens in the second-line setting. In both the EPIC study [24] in the patients that received irinotecan alone, and in the study by Tourigand [25] where patients received 5-FU and irinotecan, a 4% response rate was reported. In this study, there were no statistically significant differences in response rates or stable disease rates between the two arms, but there was a trend in the DCR favouring HA-Irinotecan-treated patients 76 versus 46% ($P = 0.053$). When comparing these data to similar published studies, the responses in the irinotecan arm were consistent with the expected mean disease control rate of 50% (range 33–60% [13–16]), while the responses obtained in the HA-Irinotecan arm were higher than previously cited response rates. The increased tumor control in the HA-Irinotecan arm potentially supports the preclinical findings that demonstrated hyaluronic acid to be an effective novel excipient for anti-cancer drugs; where it targeted the drug to the tumor resulting in higher intra-tumoral drug concentrations and ultimately enhanced efficacy [9]. An analysis conducted on the patients who completed two cycles of therapy indicated a trend that patients in the control group (41%) were more likely to have progressive disease compared to patients in the HA-Irinotecan arm (21%). The improved tumor responses in the HA-Irinotecan arm enabled the patients to continue on study, finally resulting in 34% of HA-Irinotecan patients completing all eight cycles of therapy compared to 14% in the control arm.

There was a significant benefit for HA-Irinotecan-treated patients in terms of progression-free survival, 5.2 versus 2.4 months (Fig. 2a), and time to treatment failure, 4.0

Table 3 Correlation between the incidence of severe toxicity and the UGT1A1 *28 polymorphism

Polymorphism	Incidence of alleles in the treatment group[a]			Grade 3 diarrhea[b]		Grade 3/4 neutropenia[b]	
	HA-Irinotecan[c]	Irinotecan[d]	Expected incidence [14–16]	HA-Irinotecan	Irinotecan	HA-Irinotecan	Irinotecan
UGT1A1 *28							
*1/*1	19 (0.5)	8 (0.2)	0.4–0.8	5 (26%)	0	3 (16%)	2 (25%)
*1/*28	16 (0.4)	25 (0.7)	0.2–0.5	2 (13%)	3 (12%)	4 (25%)	7 (28%)
*28/*28	4 (0.1)	1 (0.05)	0.05–0.4	2 (50%)	0	2 (50%)	1 (100%)

[a] Figures in the table are (number of patients with allele combination and in brackets the incidence within the total patient population with the alleles)

[b] Figures in the table are (number of patients with toxicity and in brackets the percentage within the number of patients with the specific allele combination)

[c] HA-Irinotecan sample size = 39

[d] HA-Irinotecan sample size = 34

Table 4 Overall pharmacokinetic parameters of irinotecan, SN-38 and SN-38-glucuronide, for each treatment group

	Irinotecan Median (range)	HA-Irinotecan Median (range)	Published ranges	References
No. of patients	26 (20)[a]	26 (23)[a]		
Irinotecan				
C_{max} (ng/ml)	3,086 (1,686–6,082)	3,065 (2,000–7,676)	2,518–8,800	[17, 29–31]
$AUC_{(0\text{-infinity})}$ total (mg h/l)	24.1 (12.0–52.9)	23.0 (10.2–43.9)	14.3–40.9	[17, 29–38]
$t½_{(0\text{-infinity})}$ (h)	7.3 (4.8–13.7)	7.7 (5.2–9.5)	9.1–13.8	[32, 35, 36, 39]
Clearance (l/h/m^2)	13.5 (6.6–27.8)	14.2 (6.9–33.2)	7.2–20.55	[29, 32, 36, 39]
Vol distribution (l)	291 (154–643)	273 (126–708)		[29, 32, 36, 39]
SN-38				
C_{max} (ng/ml)	31.6 (13.1–117.1)	36.0 (15.0–65.0)	21.4–84.0	[17, 29, 33, 36]
$AUC_{(0\text{-infinity})}$ (mg h/l)	0.57 (0.10–1.67)	0.40 (0.13–1.1)	0.19–1.13	[17, 29–36, 38]
$t½_{(0\text{-infinity})}$ (h)	12.0 (7.5–81.5)	13.2 (8.2–25.6)	39.1–54.9	[35, 39]
REC	0.019 (0.009–0.051)	0.019 (0.006–0.036)	0.003–0.078	[29, 30, 32, 33, 36, 39]
SN-38G				
C_{max} (ng/ml)	107.2 (18.3–282.7)	106.7 (39.0–346.3)	99–257	[32, 39]
$AUC_{(0\text{-infinity})}$ (mg h/l)	1.9 (0.7–15.7)	2.0 (0.7–6.8)	0.75–3.53	[34, 40]
$t½_{(0\text{-infinity})}$ (h)	13.7 (7.6–83.1)	13.8 (8.2–31.6)	14.9–63.5	[35, 37]
MR	0.11 (0.05–0.53)	0.11 (0.04–0.24)	0.06–0.18	[39]
GR	4.44 (1.26–9.38)	4.81 (1.73–11.24)	0.85–28	[33, 37, 38]
BI	5,246 (1,775–22,521)	4,507 (2,220–12,781)	439–5,750	[35, 37]

MR metabolic ratio

GR glucuronidation ratio

BI biliary index

[a] Not all patients in this group had a full sample set for PK analysis—number in squared parentheses represents the number of patients in this group for whom a full set of PK samples were analyzed and upon which the PK parameters were calculated

versus 1.8 months (Fig. 2b). When comparing the median PFS of the treatment groups in this study to the control arm of the EPIC study [24], where 350 mg/m^2 of irinotecan was administered 3-weekly to 650 second-line patients, the PFS of the irinotecan arm in this study was very similar at 2.4 versus 2.6 months observed in the EPIC study. Often, the EGFR inhibitors are combined with irinotecan in the second-line setting, however, several recent studies have shown a lack of efficacy benefit (PFS of 8 vs. 3 months) in using EGFR inhibitors in patients who have tumors carrying the KRAS mutation in codon 12 or 13 [26, 27]. The KRAS mutation occurs in approximately 40% of mCRC

patients, which means that there are limited treatment options for this patient population [28]. The PFS advantage provided by HA-Irinotecan in this study, if translated in a larger patient population, could fulfill the currently unmet need in this patient population providing an urgently required clinical benefit.

The increase in overall survival of 2.1 months was not statistically significant, but this is not unexpected given the confounding effect of subsequent therapy. The study was also not powered to demonstrate an overall survival benefit. However, if a statistically significance difference of this magnitude were reproduced in a larger study, this improvement in overall survival would be considered clinically significant.

In conclusion, this study has provided preliminary clinical proof that HA can act as an effective novel excipient for irinotecan, where, when compared to an identical dose of irinotecan, HA-Irinotecan significantly enhanced the clinical benefit derived from irinotecan. An adequately powered, blinded and randomised phase III study incorporating HA-Irinotecan is being planned to provide further validation of these early clinical data.

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