




# A phase IIa study of HA-irinotecan, formulation of hyaluronic acid and irinotecan targeting CD44 in extensive-stage small cell lung cancer

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Received: 20 November 2017 / Accepted: 15 December 2017 / Published online: 26 December 2017  
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## Summary

Preclinical studies in small cell lung cancer (SCLC) have shown that hyaluronic acid (HA) can be effectively used to deliver chemotherapy and selectively decrease CD44 expressing (stem cell-like) tumour cells. The current study aimed to replicate these findings and obtain data on safety and activity of HA-irinotecan (HA-IR). Eligible patients with extensive stage SCLC were consented. A safety cohort ( $n = 5$ ) was treated with HA-IR and Carboplatin (C). Subsequently, the patients were randomised 1:1 to receive experimental (HA-IR + C) or standard (IR + C) treatment, to a maximum of 6 cycles. The second line patients were added to the study and treated with open label HA-IR + C. Tumour response was measured after every 2 cycles. Baseline tumour specimens were stained for CD44s and CD44v6 expression. Circulating tumour cells (CTCs) were enumerated before each treatment cycle. Out of 39 patients screened, 34 were evaluable for the study. The median age was 66 (range 39–83). The overall response rates were 69% and 75% for experimental and standard arms respectively. Median progression free survival was 42 and 28 weeks, respectively ( $p = 0.892$ ). The treatments were well tolerated. The incidence of grade III/IV diarrhea was more common in the standard arm, while anaemia was more common in the experimental arm. IHC analysis suggested that the patients with CD44s positive tumours may gain survival benefit from HA-IR. HA-IR is well tolerated and active in ES-SCLC. The effect of HA-IR on CD44s + cancer stem-like cells provide an early hint towards a potential novel target.

**Keywords** Small cell lung cancer · CD44 · Hyaluronic acid · Cancer stem cells

## Introduction

Small cell lung cancer (SCLC) is often sensitive to platinum based chemotherapy, but early relapses limit the effectiveness

of this form of therapy [11, 23, 43, 46, 48]. The current standard of care to treat extensive stage small cell lung cancer (ES-SCLC) is a combination of platinum analogues (either cisplatin or carboplatin) [43, 46] and etoposide [14, 39]. The addition of a third agent, such as paclitaxel to a platinum-etoposide doublet has been associated with increased toxicity without improvement in survival [36], whereas alternatives to etoposide have been combined with platinum agents in various studies. Several studies have shown high response rates with irinotecan-based regimens [4, 26, 27, 41]. Noda et al. in [31] reported a clinical trial comparing the use of cisplatin with either irinotecan or etoposide in patients with ES-SCLC showing significant improvement in median survival and toxicity profile in favour of irinotecan [31]. Subsequently, another phase III also showed improved survival with irinotecan [20]; however, several other trials failed to confirm positive results due to reasons unexplained [19, 24, 33, 42, 45, 51]. A recent meta-analysis of 8 clinical trials did confirm the superiority of irinotecan over etoposide [18]. A related compound topotecan has also shown to have activity in this disease in a

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first-line and relapse setting, but so far there are no randomised clinical trials suggesting an improved outcome with this agent [5, 8].

Currently there is no well-accepted standard second line therapy for advanced SCLC. Anthracyclines, taxanes and alkylating agents have all been trialed with very little benefit and at the cost of significant toxicity. A clinical trial evaluated the activity of irinotecan in combination with carboplatin in patients with ES-SCLC who were chemotherapy-naïve or previously treated [10]. The data showed that this combination of chemotherapy drugs was promising for the treatment of relapsed ES-SCLC. A recent meta-analysis confirmed these findings [53]. Therefore, irinotecan in combination with a platinum agent serves as a reasonable alternative to platinum-etoposide to treat patients with ES-SCLC.

The existence of cancer stem cells (CSC) is thought to explain the failure of chemotherapy and other treatments to eradicate metastatic disease. CD44, in combination with other biomarkers, is often used as a marker of CSCs in solid tumours [21, 49, 55]. The expression of CD44 in lung cancer is well described [40, 47], though the frequency of expression may vary according to the histological subtypes [29, 50]. The human CD44 gene is alternatively spliced into many different isoforms with tissue and differentiation-specific expression [34]. The standard isoform of CD44 (CD44s) is the smallest isoform and lacks all of the 9 variable exons, whereas the variant isoforms (CD44v) include variable number of exon insertions (v2-v10) and are expressed mostly by cancer cells (exon v1 is not expressed in humans) [34]. The CD44v6 isoform appears to be the most commonly reported marker of stem cell phenotype in lung cancer [25, 54]. The CD44-hyaluronic interaction plays an important role in tumorigenesis as well as invasion and migration of malignant cells [32, 34]. Recent data from preclinical studies suggest that CD44-Hyaluronon interaction could be an attractive therapeutic target in lung cancer [28, 37, 44].

Hyaluronic Acid ChemoTransport (HyACT®) is a novel anti-cancer technology (Hyaluronan Laboratory, Monash University) specifically designed to target and transport chemotherapeutic agents to the activated intra-tumoral CD44. This technology involves the unique biologic and physiochemical properties of hyaluronic acid as a macromolecular carrier of the chemotherapy agent [7]. Preclinical studies have strongly correlated the efficacy of the HyACT platform with enhanced therapeutic responsiveness in CD44 positive tumour cells [7]. Safety of this combination has been demonstrated in early phase clinical trials [16, 38]. A randomised phase II study of HA-Irinotecan (HA-IR) showed improvement in progression free survival compared to irinotecan in metastatic colorectal cancer [17]. A subsequent phase III trial however, did not reach the primary endpoint of statistically significant improvement in progression free survival. Our aim was to obtain clinical evidence to determine whether HA-IR, is

capable of targeting CD44-expressing lung cancer stem cells by demonstrating that HA-IR/carboplatin (HA-IR + C) provides a clinically significant benefit (safety and efficacy) over IR + C, in ES-SCLC patients.

## Methods

### Study design

#### Study population

Patients eligible for enrolment were  $\geq 18$  years old with ES-SCLC (confirmed on histology or cytology) with measurable disease on computed tomography (CT) scans. Additional inclusion criteria were as follows: Eastern Co-operative Oncology Group (ECOG) performance status (PS) of 0 or 1; life expectancy  $\geq 3$  months; asymptomatic/controlled brain metastasis; adequate haematological, renal and hepatic function. Patients with concomitant medical illness, poor performance status (ECOG  $\geq 2$ ), and inflammatory bowel disease or concurrent active malignancy other than non-melanoma skin cancers were excluded from the study. Prior radiotherapy was allowed, provided it was delivered more than 4 weeks prior to study treatment. The study was approved by the human research and ethics committee at Monash Medical Centre, Melbourne, Australia and Monash University, Melbourne, Australia. Informed consent was obtained from all individual participants included in the study.

#### Study design and evaluation

All first line patients were assigned to treatment using a central 1:1 randomisation system prior to the first dose of study treatment. Patients randomised to the experimental arm received HA-IR + C, once every 3 weeks for up to six treatment cycles. Patients randomised to the standard arm received six cycles of IR + C at the same dose and schedule. The first five patients were treated on the experimental arm (HA-IR + C), to establish the safety of the combination. Once the safety was established, the remaining patients were randomised to one of the two treatment arms. Due to slow initial recruitment, a third arm of second line patients (patients previously treated with only one line of chemotherapy for ES-SCLC) was subsequently added. All second line patients received HA-IR + C at the similar doses (Fig. 1).

#### Chemotherapy schedule

With a 3-weekly schedule, patients on the experimental arm received IR at the dose of  $150 \text{ mg/m}^2$  in combination with HA at the dose of  $1000 \text{ mg/m}^2$ , over a 90-min intravenous infusion. This was followed by carboplatin dosed to an area under

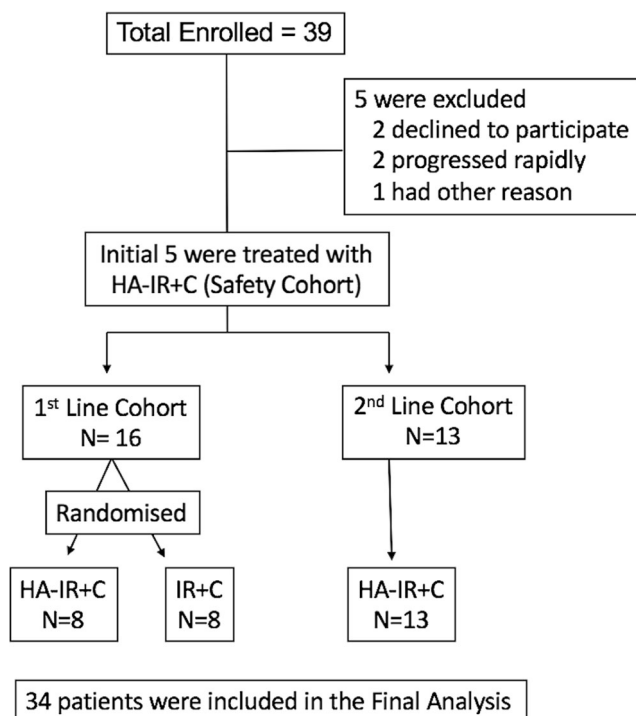


Fig. 1 Study Design

the curve (AUC) of 5 mg/mL per minute, intravenously over 60 min. Carboplatin was calculated using the formula of Calvert et al., with the creatinine clearance estimated by the Cockcroft and Gault method [9, 12]. Patients randomised to the standard arm, received similar treatment without HA.

### Assessment of toxicities and response

Patients continued to receive treatment according to the protocol specifications if there was no evidence of excessive toxicity or progression of the disease, or a patient request to discontinue therapy. Toxicities (based on the National Cancer Institute Common Terminology Criteria for Adverse Events [version 3.0] grading criteria) were recorded. All toxicities were recorded before each treatment cycle. Any grade 3 or above toxicity prompted dose reduction by 25%. A maximum of 2 dose reductions was allowed for excessive toxicity. Patients requiring more than 2 dose reductions were discontinued from the study. Treatment was to be withheld on day 1 of each treatment cycle if the absolute neutrophil count was  $<1.2 \times 10^9/L$  or the platelet count  $<85 \times 10^9/L$ , until the counts rose above these levels. For non-haematological toxicities the treatment was to be withheld on day 1 of each treatment cycle until the severity returned to  $<$  grade 2.

All patients underwent a clinical examination, tumour evaluation with a CT scan of chest, abdomen and pelvis, a complete blood count (white blood cell count, platelet count and haemoglobin) and a serum chemistry (creatinine, sodium,

potassium, bilirubin, AST and ALT). Physical examination, blood counts, chemistry and toxicity evaluation were performed before each cycle of treatment until treatment was discontinued. Tumour response was evaluated using RECIST criteria (version 1.1) every 6 weeks during treatment.

### Immunohistochemistry

Tumour specimens obtained before treatment were stained for CD44s and CD44v6 expression. The primary antibodies used for CD44s were, clone DB105, Miltenyl Biotech (dilution 1:100) and for CD44v6 it was clone ab78960 (Abcam, dilution 1:200). Tissue sections were deparaffinised in xylene and rehydrated in ethanol. Antigen retrieval was performed by microwaving the slides in citrate buffer (pH 6.0) for 10 min. Endogenous peroxidase activity was blocked by 15 min incubation in 1% hydrogen peroxide. A protein block with a 10% normal serum was performed for 30 min. Incubation with primary antibody was carried out at 4 °C overnight. The secondary antibody was applied for 30 min, after washing with tris-buffered saline (TBS). Diaminobezadine solution was used for colour detection, followed by counterstaining with haematoxylin. All staining runs were accompanied by appropriate control slides.

### Scoring for CD44 expression

The stained slides were scanned into a digital slide scanner (APERIO Scan ScopeXT, San Diego®) and *e-slides* were created as described before [1, 2]. Two pathologists (BK & DNW) independently evaluated all the scanned sections in a blinded manner. Tumour specific expression of markers was evaluated and scored according to the method described earlier [2]. A cutoff point of 5% was selected to divide the specimens into positive and negative categories.

### Circulating tumour cells (CTCs)

Blood samples for circulating tumour cells were collected prior to each treatment cycle and subsequently at each follow up visit. All samples were collected and stored at room temperature and were processed within 48 h for CTC counting. The CellSearch system was used for the CTC counting, the technical details of which have been previously described by Allard et al. [3]. CTCs were defined as EpCAM-isolated intact cells showing positive staining for cytokeratin and negative staining for CD45. The results were reported quantitatively as the number of CTCs per 8 ml of blood. The selected threshold to define patients into favourable and unfavorable groups was 8/8 ml of blood. Those with  $<8/8$  ml of CTCs were categorized into the favourable group and vice versa. This threshold was selected based on a previous publication [30].

## Statistical analysis

The primary endpoints of this study were to determine the objective response rate (ORR) and to estimate the expression of CD44s and CD44v6 in tumour samples. ORR was defined as the proportion of eligible patients whose disease met the RECIST 1.1 criteria for complete response (CR) or partial response (PR) on two consecutive evaluations at least 6 weeks apart. Secondary endpoints were the estimation of disease free survival, defined as the duration (in months) between the date of surgery and the date of first recurrence or death due to any cause and overall survival, defined as the duration (in months) between the date of surgery and the date of death due to any cause and to determine all the toxicities. An exploratory analysis to correlate CTC numbers (at baseline and during treatment) with clinical outcomes was also performed.

All analyses were performed using SPSS for windows version 20 (SPSS Inc., Chicago, IL). The survival curves were plotted using the Kaplan-Meier method and log-rank test was used to assess the statistical difference between the groups. Variables with *p* value 0.1 or less were entered into multivariable analysis and the Cox proportional hazard model was used to carryout group comparisons. The expression of CD44s and CD44v6 were dichotomised into either ‘low’ or ‘high’ scores according to the criteria described above. The correlation between CD44 expression and clinical-pathological characteristics and OTRR were then analyzed using a Chi-Square test. Statistical significance was set at a probability value of <0.05, with two-tailed *p* values.

## Results

### Patients characteristics

From September 2011 to September 2014, 39 patients were enrolled in the study, out of which 5 were excluded while 34 were analysed for efficacy analysis (Fig. 1). Patient characteristics of all 34 patients are shown in Table 1. Twenty-one (21) were male, 13 were female. The median age was 66 (range 39–83). The ECOG performance status was normal (0) in 11 out of 34 patients (32%), while it was impaired (1) in the rest (68%) of the population. Three patients had asymptomatic brain metastasis at the time of entry into the study. Twenty-one (21) patients were treated as first line, not having received any prior chemotherapy, while 13 patients were treated as second line, having progressed following first line chemotherapy. The first five patients (first line) were treated with HA-IR + C to establish the safety. Of the remaining 16 first line patients, 8 were treated on the experimental arm (HA-IR + C), while 8 were treated on the standard arm (IR + C). All of the second line patients (*n* = 13) received HA-IR + C. Patient characteristics and baseline demographics were similar in the

**Table 1** Patients characteristics (*N* = 34)

	Whole Cohort	HA-IR + C 1st line	IR + C 1st line	HA-IR + C 2nd line
Total	34	13	8	13
Median Age	66 (39–83)	66 (40–83)	67 (57–78)	67 (39–77)
Male	21 (61)	8 (61)	4 (50)	9 (69)
Female	13 (39)	5 (39)	4 (50)	4 (31)
ECOG 0	11 (32)	5 (38)	3 (37)	3 (24)
ECOG 1	23 (68)	8 (61)	5 (63)	10 (76)
Brain Metastasis	3 (8)	2 (15)	0	1 (8)
Median chemo cycles received	6	6	6	4
Median Time since 1st Line	NA	NA	NA	4.5 Months

treatment arms. In total, 22 patients (65%) received all six cycles of chemotherapy, of which 13/13 (100%) were in the experimental arm, 7/8 (87%) in the standard arm while 2/13 (15%) were second line. Reasons for stopping treatment earlier than 6 cycles were, disease progression (7), toxicity (3), concomitant illness (1) patient’s request in 1 patient.

### Efficacy

All 34 patients received at least one cycle of chemotherapy and were therefore evaluated for efficacy. The overall response rate was 53% (18/34), with 13 (38%) partial responses and 5 (15%) complete responses. There was no statistically significant difference in ORRs according to the treatment arm (69% and 75% in the experimental and standard arms respectively, *p* = 1.000). Amongst the 13 s-line patients who progressed after previous one line of chemotherapy, 2 partial responses and one complete response were seen (ORR of 22%). The difference in response rates amongst first and second line patients was statistically significant (70% vs. 22%, *p* = 0.013). Three patients (two in experimental arm and 1 in second line cohort) experienced disease stabilisation. Response rates according to treatment type and line of therapy are shown in Table 2.

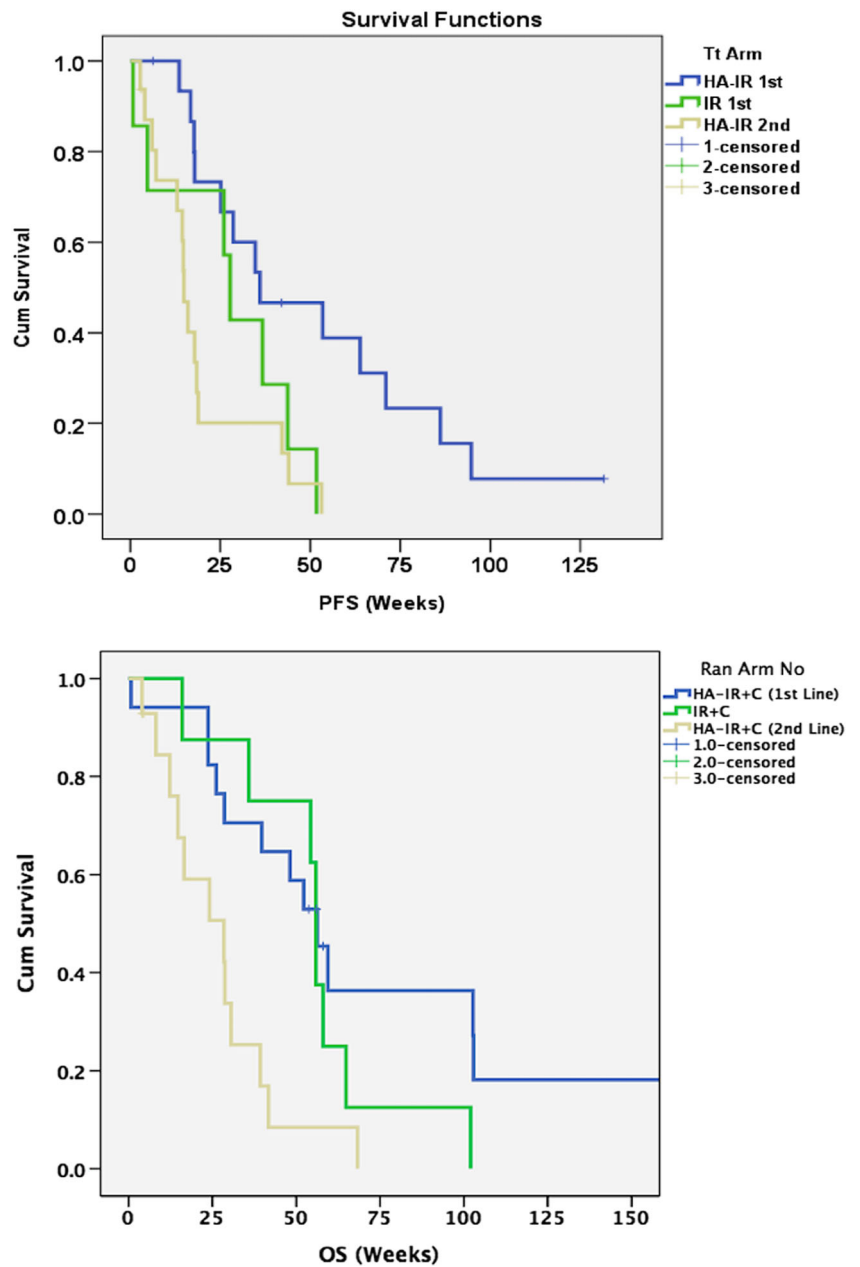
The cutoff date of overall survival update was 12 Dec 2016. At the time of last follow up 3 out of 34 patients were alive. There was no significant difference in PFS or OS among patients treated on the experimental or the standards arms (Fig. 2). The median progression free survival was 42 weeks for the experimental arm compared to 28 weeks for standard arm (Hazard ration [HR] 0.93, 95% CI 0.36–20.42, log rank *P* = 0.892). The median OS was 56.1 weeks for the experimental arm vs. 55.2 weeks for the standard arm (HR 0.99 95% CI 0.44–21.43, *P* = 0.943) (Fig. 2).

When compared according to the treatment line, patients with refractory disease (second line) had significantly worse

**Table 2** Efficacy summary (N = 34)

Response	1st Line HA-IR + C N = 13	1st Line IR + C N = 8	2nd Line HA-IR + C N = 13
Complete Response (CR)	2 (15)	2 (25)	1 (7)
Partial Response (PR)	7 (54)	4 (50)	2 (15)
Stable Disease (SD)	2 (15)	0 (0)	1 (7)
Progressive Disease (PD)	1 (7)	1 (12)	5 (38)
Not evaluable	1 (7)	1 (12)	4 (30)
Objective Response Rates (ORR)	69%	75%	22%
Disease Control Rates (DCR)	85%	75%	31%
Duration of Response (weeks) Median (Range)	53.43 (17.86–113.00)	42.14 (28.5–63.86)	18.86 (18.43–44.0)
No. of Events	11	8	12

**Fig. 2** Kaplan-Meier survival analysis according to the treatment arm. First line patients had significantly better survival than those with refractory disease





survival compared to those treated as first line. Median PFS was 36.4 weeks in the first line cohort vs. 14.2 weeks in the second line cohort ( $P = 0.001$ ). Median OS was 55.3 weeks in first line cohort compared to 28.5 weeks in second line ( $P = 0.003$ ).

### Safety and toxicities

Overall, the most common adverse events (AEs) were haematological (anaemia, neutropenia and thrombocytopenia) or gastrointestinal (diarrhea and vomiting). The majority of the toxicities were grade 1 or 2 (Table 3). In general, no major differences were observed in the incidences of common AEs across the three treatment arms, although the incidences of grade III/IV diarrhea were higher in the standard arm, while anaemia in the experimental arm. One patient died during treatment in the experimental arm, due to condition unrelated to chemotherapy.

### Expression of CD44s and CD44v6 in SCLC

Of the entire population, tumour samples from 24 patients (70%) were evaluable for the expression of CD44s and for CD44v6 expression. CD44s staining were observed predominantly in the cytoplasmic membrane, while CD44v6 was expressed both on the membrane as well as in the cytoplasm. Six (6/24 = 25%) tumour samples were considered positive for CD44s, while 16/24 (66%) were positive for CD44v6 expression. Of the 6 CD44s positive cases, 5 were treated with HA-IR + C while only one was treated with IR + C. In the CD44s negative cohort ( $n = 18$ ), 14 were treated with HA-IR + C while 4 were treated with IR + C. Similarly, in the

CD44v6 positive cohort ( $n = 16$ ), 13 were treated with HA-IR + C, in CD44v6 negative cohort ( $n = 8$ ), 6 patients received HA-IR + C.

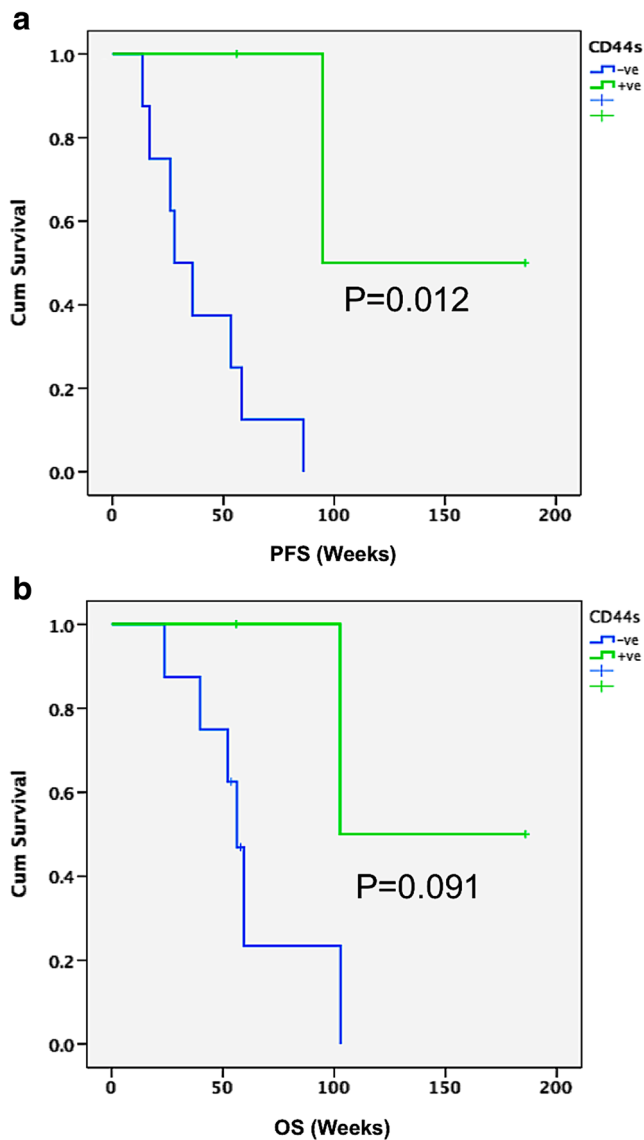
### CD44 expression and the efficacy of HA-IR

There was no significant difference in the overall response rates according to the expression of CD44s and CD44v6 in the entire cohort. Response rates were 66% and 58% respectively for CD44s positive and negative populations respectively ( $P = 0.999$ ), while 50% and 62% for CD44v6 positive and negative populations respectively ( $P = 0.679$ ). When analysed according to the treatment arms, in the HA-IR + C group, the overall response rates were 50% in CD44s positive, while 36% in the CD44s negative group ( $P = 0.602$ ). Similarly, the response rates were 54% and 50% in the CD44v6 positive and negative groups respectively ( $P = 0.999$ ). There were only 5 patients in the IR + C cohort, with suitable tumour samples for CD44 analysis. Of those 5, only one was positive for CD44s and 3 were positive for CD44v6. All 5 patients had a confirmed response to the treatment, regardless of the CD44 status.

Further survival analysis was performed according to CD44 expression. In the entire cohort, there was no difference in median PFS and OS in the CD44s or D44v6 positive and negative groups. However, when analysed according to the treatment arms, first line patients with CD44s positive tumours had significantly longer median PFS and a trend towards improved OS when treated with HA-IR + C, compared to CD44s negative patients treated with the same treatment (PFS 94.7 vs 28 weeks,  $P = 0.013$ , HR (Fig. 3).

**Table 3** Summary of toxicities

	HA-IR + C		IR + C		HA-IR + C	
	1st line		1st line		2nd line	
	N (%)		N (%)		N (%)	
	All Grades	Grade 3/4	All Grades	Grade 3/4	All Grades	Grade 3/4
Anemia	5 (38)	3 (23)	3 (37)	1(14)	2 (15)	1(7)
Thrombocytopenia	4 (30)	0	4 (50)	1(14)	1 (7)	1(7)
Neutropenia	6 (46)	4 (30)	4 (50)	2(28)	3 (39)	2(14)
Febrile Neutropenia	–	2 (15)	–	1(14)	–	0
Diarrhoea	4 (30)	2 (13)	4 (50)	2(28)	3 (39)	2(14)
Nausea	5 (38)	2 (15)	3 (37)	2(28)	2 (15)	1(7)
Vomiting	5 (38)	3 (23)	3 (37)	2(28)	2 (15)	2(14)
Anorexia	1 (7)	0	1 (12)	0	1 (7)	1(7)
Dyspnoea	1 (7)	1(7)	1 (12)	0	0	0
Fatigue	5 38)	2(15)	2 (25)	1(8)	4 (30)	2(14)
Hypocalcaemia	0	0	0	0	1 (7)	1(7)
Hypomagnesaemia	1 (7)	1(7)	1 (12)	0	1 (7)	1(7)



**Fig. 3** Survival analysis according to CD44s status. First line patients treated on experimental arm and positive for CD44s had significantly improved PFS ( $P=0.012$ ) (a) and a trend towards improved OS ( $P=0.091$ ) (b), compared to CD44s negative patients treated with the same regimen

In the same treatment group, there was no difference in survival according to CD44v6 status. Similarly, there was no difference in PFS or OS according to CD44s or CD44v6 expression in the second line patients treated with HA-IR + C. In the standard (IR + C) first line treatment arm, though there was numerically longer PFS in the CD44s positive group, but the number of patients were too small to get any significant results (data not shown).

A scatterplot of CD44 expression versus PFS and OS in the entire first line patients is shown in Fig. 4. There appears a positive correlation between CD44s expression and both PFS and OS ( $R^2$  0.65 and 0.61, respectively), but not with CD44v6.

## CTCs and efficacy

Blood samples from 28 patients were analysed for CTCs. At baseline, two or more CTCs were detected in 86.6% of the patients (95% confidence interval [CI], 55.0–95.7). Nine (9) patients (32%) had a baseline CTC count of 1000/8 mls of blood or above. The median CTC count at baseline was 361 (range 0–19,000). There was no difference between first line and refractory patients in terms of median CTC count (338 and 359 respectively).

## Baseline CTC levels and survival

To estimate the predictive value of baseline CTC level, a threshold of 8 cells per 8 mls of blood was used. The data showed that the favourable group (patients with CTC < 8/8 ml) had a trend towards better PFS compared to the unfavourable group ( $P=0.186$ , HR 2.1 95% CI 0.69–6.13) (Fig. 5). Survival analysis according to the treatment arms was not performed, as the numbers in the standard arm were too small to get any meaningful result.

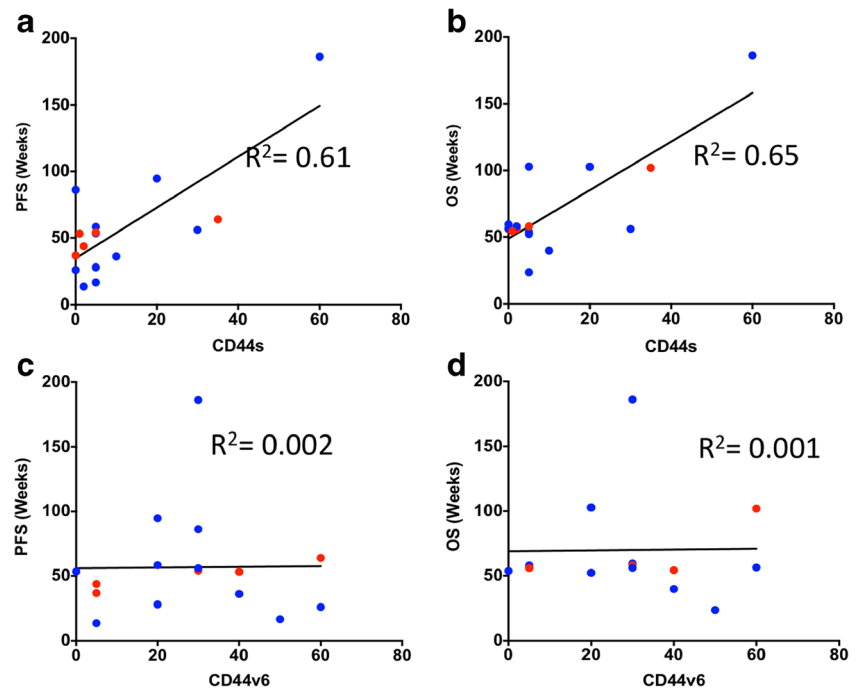
## Changes in CTC count with treatment

To estimate the effect of treatment on CTC levels, the cohort was divided into three groups, responders (CR/PR), patients with stable disease (SD) and progressive disease (PD). Since the data for CTC after 6 cycles were available in only 35% of the patients, analysis only up to cycle 4 was performed. In the responders group ( $N=18$ ), patients had a low (below median) baseline CTC count, the SD group had almost similar while PD group had much higher (above median) CTC level at baseline. Serial monitoring of CTCs also revealed that patients in the PD group had the highest (2.1 folds) rise in median CTC numbers after 4 cycles of chemotherapy compared to baseline. In PR/CR and SD groups, same rise was 1.3 and 1.6 folds respectively (Table 4).

## Discussion

This randomised study evaluated the safety and activity of CD44 targeting therapy (HA-IR) in small cell lung cancer, using irinotecan as a control arm. The regimen has been designed to deliver chemotherapy in the tumour microenvironment as well as to target CD44+ subpopulation within the tumour. Our results show that HA-IR is an active and tolerable option for patients with ES-SCLC. Although the previous phase III study in colorectal cancer did not show improvement in progression free survival despite positive phase II results [17], there was no bio-marker data reported in those studies. The current study on the other hand, was not adequately

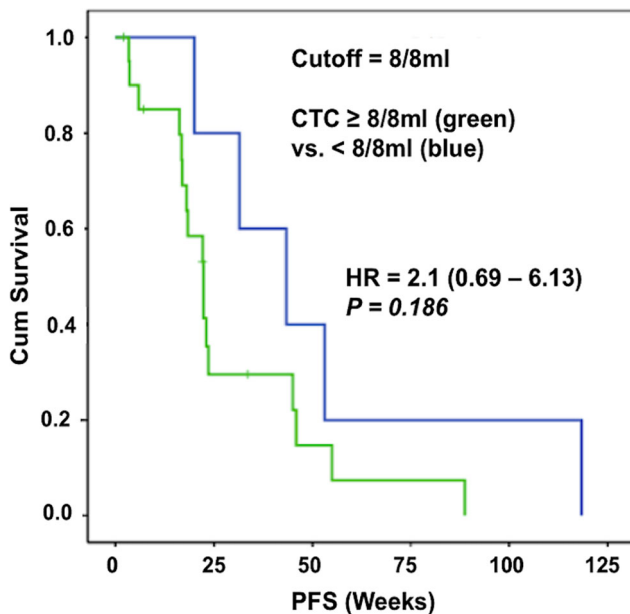
**Fig. 4** Scatterplot showing correlation between CD44 expression and survival in the first line patients. Blue dots represent patients on experimental while red dots represent standard therapy. There is a significant correlation between CD44s expression and PFS and OS (A&B) but not between CD44v6 and survival (C&D)



powered but the preliminary data suggest that CD44 may predict survival.

Various therapeutic strategies to target CD44 in solid cancers are being tested in preclinical models [6, 13, 15]. Formulation of irinotecan with HA was previously tested in a phase II study in colorectal cancer [17]. The study by Gibbs et al. showed that HA-IR was safe and resulted in the

improvement in progression free survival in patients with colorectal cancer, compared to irinotecan [17], but the role of CD44 as a bio-marker was not reported. The data on expression and hence the prognostic role of CD44 molecule and its variants in lung cancer is conflicting. A large body of evidence suggests that the CD44 variant 6 may be a more specific marker of tumour progression and poor prognosis in lung cancer [22, 25, 54], but no predictive role of CD44 or any of its variants has been described. Moreover, CD44 is expressed in a large variety of normal tissues such as immune system and epithelia [35, 52]; the risk of toxicity with unselective CD44 targeting therapies could be a concern. In the current study, CD44v6 expression was observed in over 50% of tumour samples, compared to on 35% expression in case of CD44s. Since our regimen (HA-IR) appears to show improved



**Fig. 5** Survival analysis according to baseline number of circulating tumour cells in blood samples from patients with small cell lung cancer. Patients with low (<8/8 ml) CTCs had a trend towards better survival

**Table 4** Trend in CTC levels according to tumour response post chemotherapy. Patients with progressive disease (PD) had definite progression in CTC levels after chemotherapy

	Baseline	Post C2	Post C4
PR/CR	343 (0–3000) n = 18	531 (9–22,173) n = 15	449 (6–25,600) n = 17
SD	384 (17–19,000) n = 3	30 (0–665) n = 3	651 (7–8000) n = 3
PD	657 (1–2600) n = 7	1600 (19–6834) n = 6	1381 (88–15,705) n = 4
Total	361 (0–19,000) n = 28	668 (0–22,173) n = 24	399 (6–25,600) n = 24



survival in CD44s positive tumours, with a lack of excessive toxicities to normal tissues, it can be hypothesized that CD44s is the potential therapeutic target. Certainly these findings need to be validated in a large phase III clinical trial.

We also explored the prognostic value of circulating tumour cells (CTCs). Our results were compatible with previous reports, suggesting a worse survival in patients with high baseline levels of CTCs [30]. Moreover, serial measurements of CTCs showed some correlation with clinical response to the treatment; however, the number of patients was too small to make it a meaningful conclusion. If confirmed in a larger study, serial CTC measurement could serve as a useful surrogate to assess response to treatment. Moreover, the methodology used to isolate serial CTCs in our study appears to be efficient and reproducible. Isolation and propagation of CTCs from patients with ES-SCLC also allows for downstream analysis of potentially important molecular mechanisms of chemotherapy resistance and biomarkers of response to subsequent/novel therapies such as checkpoint inhibitors. It is a non-invasive technique and, therefore, has potential to replace the invasive biopsy of tissues from humans, which may be associated with complications.

In conclusion, this proof of concept study has provided preliminary evidence that HA can act as an effective novel excipient for irinotecan. HA-IR could potentially enhance the clinical benefit derived from irinotecan. Moreover, CD44s could be a potential predictive biomarker of response to HA-chemotherapy delivery system. An adequately powered, blinded and randomised phase III study incorporating HA with irinotecan or chemotherapy drugs would be needed for further validation of these early clinical data.

**Acknowledgements** The authors thank Peter Midolo and Zdenka Prodaniv for their technical support.

**Funding** The study was supported by a grant from Alchemia Oncology (Project ACO-003).

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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