Rovalpituzumab tesirine, a DLL3-targeted antibody-drug conjugate, in recurrent small-cell lung cancer: a first-in-human, first-in-class, open-label, phase 1 study

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Summary

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See Online for appendix

Background—Rovalpituzumab tesirine is a first-in-class antibody-drug conjugate directed against delta-like protein 3 (DLL3), a novel target identified in tumour-initiating cells and expressed in more than 80% of patients with small-cell lung cancer. We aimed to assess the safety and activity of rovalpituzumab tesirine in patients who progressed after one or more previous regimen.

Methods—We conducted a phase 1 open-label study at ten cancer centres in the USA. Eligible patients were aged 18 years or older and had histologically or cytologically confirmed small-cell lung cancer or large-cell neuroendocrine tumours with progressive measurable disease (according to Response Evaluation Criteria in Solid Tumors [RECIST], version 1.1) previously treated with one or two chemotherapeutic regimens, including a platinum-based regimen. We assigned patients to dose-escalation or expansion cohorts, ranging from 0·05 mg/kg to 0·8 mg/kg rovalpituzumab tesirine intravenously every 3 weeks or every 6 weeks, followed by investigation of the dose schedules 0·3 mg/kg and 0·4 mg/kg every 6 weeks and 0·2 mg/kg every 3 weeks. Primary objectives were to assess the safety of rovalpituzumab tesirine, including the maximum tolerated dose and dose-limiting toxic effects. The primary activity endpoint was objective response by intention-to-treat analysis. This study is registered with ClinicalTrials.gov, number NCT01901653. The study is closed to enrolment; this report focuses on the cohort with small-cell lung cancer.

Findings—Between July 22, 2013, and Aug 10, 2015, 82 patients were enrolled, including 74 patients with small-cell lung cancer and eight with large-cell neuroendocrine carcinoma, all of whom received at least one dose of rovalpituzumab tesirine. Dose-limiting toxic effects of rovalpituzumab tesirine occurred at a dose of 0·8 mg/kg every 3 weeks, including grade 4 thrombocytopenia (in two of two patients at that dose level) and grade 4 liver function test abnormalities (in one patient). The most frequent grade 3 or worse treatment-related adverse events in 74 patients with small-cell lung cancer were thrombocytopenia (eight [11%]), pleural effusion (six [8%]), and increased lipase (five [7%]). Drug-related serious adverse events occurred in 28 (38%) of 74 patients. The maximum tolerated dose of rovalpituzumab tesirine was 0·4 mg/kg every 3 weeks; the recommended phase 2 dose and schedule is 0·3 mg/kg every 6 weeks. At active doses of rovalpituzumab tesirine (0·2 mg/kg or 0·4 mg/kg every 3 weeks or 0·3 mg/kg or 0·4 mg/kg every 6 weeks), 11 (18%) of 60 assessable patients had a confirmed objective response. 11 (18%) of 60 assessable patients had a confirmed objective response, including ten (38%) of 26 patients confirmed to have high DLL3 expression (expression in 50% or more of tumour cells).

Interpretation—Rovalpituzumab tesirine shows encouraging single-agent antitumour activity with a manageable safety profile. Further development of rovalpituzumab tesirine in DLL3-expressing malignant diseases is warranted.

Introduction

Small-cell lung cancer is a tumour with neuroendocrine features that comprises about 13–15% of all lung cancers, accounting for more than 275 000 new cases worldwide every year.[1] It is characterised by aggressive growth and early metastasis to distant sites, resulting in most patients being diagnosed with extensive-stage disease.

Treatment and survival of patients with small-cell lung cancer has not changed substantially in more than 40 years. The disease is rarely cured with local therapy alone (surgery,

radiotherapy, or both), and systemic chemotherapy remains a cornerstone of treatment. Standard, initial, systemic chemotherapy for all patients with adequate performance status consists of a platinum salt (eg, carboplatin or cisplatin) in combination with a second agent (eg, etoposide).[2,3] Responses to first-line treatment are high, but recurrence is frequent, and is universal in patients with extensive-stage disease: median survival is 14–20 months for limited-stage disease and 9–11 months for extensive-stage disease.[2,3] When small-cell lung cancer recurs, prognosis is especially poor, and few therapeutic options are available. Topotecan is the only treatment approved by the US Food and Drug Administration (FDA) for second-line treatment of recurrent small-cell lung cancer.[3] However, this drug is challenging to use because of haematological toxic effects and modest antitumour activity, with reported responses in 5–24% of patients and median survival of roughly 25 weeks. In the third-line setting, no approved treatments for small-cell lung cancer exist, and current guidelines recommend best supportive care, consideration of investigational drugs, or systemic treatment in patients who maintain a good performance status.[2,3] Development of novel and more durably effective treatments for small-cell lung cancer is needed desperately. However, by contrast with non-small-cell lung cancer, progress in small-cell lung cancer has been hampered by the scarcity of specific molecular targets.[1,2,4,5]

Research in context

Evidence before this study

We searched PubMed between July 22, 2003, and July 22, 2013, with the terms "SCLC", "second-line", "third-line", "phase 1", "phase 2", "phase 3", "relapsed", "refractory", "recurrent", "DLL3", and "rovalpituzumab". We focused on reports and meta-analyses for treatment options and outcomes after failure of first-line treatment in patients with small-cell lung cancer that were published during the 10-year period before the start of our study. Recurrent, refractory, and relapsed small-cell lung cancer shows very poor survival outcomes, with no approved drugs beyond topotecan as second-line treatment, and no identified molecular biomarkers to guide targeted treatments.

Added value of this study

Our study shows activity of rovalpituzumab tesirine in small-cell lung cancer. Patients with relapsed or refractory disease, a population with few treatment options, achieved objective responses and had manage able toxic effects. The novel therapeutic target DLL3 is a potential predictive biomarker for small-cell lung cancer.

Implications of all the available evidence

These data have prompted the initiation of several trials in small-cell lung cancer, including in relapsed and refractory disease (NCT02674568) and as part of a first-line chemo therapeutic regimen (NCT02819999). Moreover, a trial in other DLL3-expressing neuroendocrine cancers has begun (NCT02709889).

Growing evidence supports a tumour-suppressor role for Notch-1 signalling in neuroendocrine tumours. Delta-like protein 3 (DLL3) is an atypical member of the Notch receptor ligand family that, unlike related family members, seems to inhibit Notch receptor activation.[6,7] DLL3 has been identified as a novel putative therapeutic target in high-grade

neuroendocrine carcinomas including small-cell lung cancer, based initially on whole transcriptome sequencing of tumour-initiating cells isolated from small-cell lung cancer and large-cell neuroendocrine cancer patient-derived xenografts.[8] DLL3 was expressed in most small-cell lung cancers and large-cell neuroendocrine tumours, by contrast with non-malignant adult tissues and non-neuroendocrine tumour types in which membrane protein expression is scant. DLL3 has been implicated in the regulation of cell-fate decisions during development and might function as an oncogenic driver in high-grade neuroendocrine tumours, including small-cell lung cancers; in these tumours, DLL3 appears to be a downstream transcriptional target of the ASCL1 transcription factor. By inhibiting the Notch receptor pathway, DLL3 might promote neuroendocrine tumorigenesis.[6–8]

Rovalpituzumab tesirine (SC16LD6.5) is a DLL3-targeted antibody-drug conjugate consisting of the humanised DLL3-specific IgG1 monoclonal antibody SC16, the DNA cross-linking agent SC-DR002 (D6.5), and a protease-cleavable linker that covalently links SC-DR002 to SC16. We aimed to do a first-in-human, open-label, phase 1 study to investigate the safety, tolerability, pharmacokinetics, and antitumour activity of rovalpituzumab tesirine in patients with small-cell lung cancer or large-cell neuroendocrine tumours. Most patients had relapsed metastatic small-cell lung cancer and represent the focus of this report.

Methods

Study design and participants

We did a first-in-human, open-label, phase 1 study of single-agent rovalpituzumab tesirine at ten cancer centres in the USA (appendix p 4). Eligible patients were aged 18 years or older and had histologically or cytologically confirmed small-cell lung cancer or large-cell neuroendocrine tumours with progressive measurable disease (according to Response Evaluation Criteria in Solid Tumors [RECIST], version 1.1) previously treated with one or two chemotherapeutic regimens, including a platinum-based regimen. Further inclusion criteria were: Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; minimum life expectancy of 12 weeks; and adequate haematological counts and hepatic and renal function. Key exclusion criteria included: presence of active CNS metastases; administration of previous chemotherapy within 21 days; any concurrent anticancer treatment; and uncontrolled infection or systemic disease or clinically significant cardiac disease.

The protocol and its amendments were approved by the relevant institutional review board or ethics committee at every study centre. We did the study in accordance with Good Clinical Practice guidelines. All patients provided written informed consent before any study-related procedures were done.

We did not do a preplanned phase 2 portion of the study. Instead, a separate clinical trial was initiated in patients with relapsed or refractory small-cell lung cancer (NCT02674568).

Procedures

We initially planned an accelerated dose-escalation scheme followed by expansion cohorts, to estimate the tolerability of potential recommended phase 2 doses of rovalpituzumab tesirine. Full details are provided in the protocol (appendix pp 28–110). We enrolled patients to dose-escalation or expansion cohorts, ranging from 0·05 mg/kg to 0·8 mg/kg rovalpituzumab tesirine administered intravenously every 3 weeks or every 6 weeks (appendix p 5). For dose escalation, we planned to enrol between one and six patients to every dose level: 0·05 mg/kg, 0·1 mg/kg, 0·2 mg/kg, 0·4 mg/kg, 0·8 mg/kg, and 1·6 mg/kg, every 3 weeks. At the first dose level, we enrolled three patients; if we noted no treatment-related adverse events of grade 2 or higher, we enrolled between one and three patients to subsequent dose cohorts. We aimed to continue with these dose cohorts until we recorded a grade 2 or worse treatment-related adverse event. At that point, we adopted a standard 3 + 3 design: if a dose-limiting toxic effect was seen in any of the three patients during the first cycle of a cohort, three additional patients were to complete one cycle at that dose. If a second patient developed a dose-limiting toxic effect, dose escalation was ceased. A final dose level, midway between the last dose level assessed and the previously tolerated dose level, could be tested before declaring a maximum tolerated dose. Moreover, if a dose level was not tolerated with a dosing schedule every 3 weeks, but the dose level immediately lower was tolerable on the 3-week schedule, the option to increase the dosing interval to every 6 weeks could be investigated in addition to or in place of testing an intermediate dose level on a 3-week schedule. If the 6-week dosing interval improved tolerability, dose escalation could resume with this schedule. Based on pharmacokinetic data, we introduced dose-escalation regimens of 0·3 mg/kg and 0·4 mg/kg, administered every 6 weeks.

We initially intended patients to receive rovalpituzumab tesirine until disease progression. We did not permit intrapatient dose escalation. We allowed patients who had a grade 3 or 4 adverse event or a clinically intolerable grade 2 adverse event at any time to continue on study drug at one dose level below their enrolled dose, as long as the adverse event had resolved to lower than grade 1 or to baseline within 2 weeks of the scheduled subsequent dose (a dose delay of >2 weeks was needed for discontinuation), their ECOG performance status was 2 or lower, and the adverse event was not an increase in alanine aminotransferase or aspartate aminotransferase greater than three times the upper limit of normal (ULN) and concomitant bilirubin greater than twice the ULN. We allowed only one dose reduction per patient.

We assessed tumour burden by CT or MRI every cycle (6 weeks), until three cycles (18 weeks) of treatment had been completed, at which time we did assessments every two cycles (12 weeks). We did safety laboratory testing (blood count, blood chemistry) at baseline or day 1, and weeks 2 and 3 of every treatment cycle. We monitored adverse events every week throughout the study; investigators graded toxic effects with the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4.03. We coded adverse events with the Medical Dictionary for Regulatory Activities (MedDRA), version 19.0. We assessed best responses in individual patients at every participating site by dedicated radiology review using RECIST version 1.1, and by independent radiographic review when available. We confirmed objective responses with at least one sequential

tumour assessment at least 4 weeks later; we only report confirmed objective responses by RECIST version 1.1. Patients were judged not assessable by independent central review or by the investigator who discontinued treatment before obtaining a post-baseline scan for lesion assessment. Patients were regarded as not evaluable if they were judged not assessable by independent central review or by the investigator who discontinued treatment before obtaining a post-baseline scan for lesion assessment.

For pharmacokinetic analyses, we did intensive sampling every week or more frequently during cycles 1 and 4 for patients on a 3-week dosing schedule, and during cycles 1 and 3 for those on a 6-week schedule. We took samples for immunogenicity analysis before dosing in every cycle for both 3-week and 6-week dosing schedules.

When biopsy samples were available, we did immunohistochemistry for DLL3 expression retro spectively (CDx CAP/CLIA laboratory, Ventana Medical Systems, Tucson, AZ, USA). Briefly, we sectioned formalin-fixed, paraffin-embedded, tumour biopsy samples at 4 μm and placed them on glass slides. We stained slides with an anti-DLL3 mouse monoclonal antibody (Stemcentrx, South San Francisco, CA, USA) and analysed them by light microscopy. We defined positive DLL3 staining as any cytoplasmic or membranous staining at any intensity in tumour cells. We scored patients' biopsy samples by the percentage of tumour cells staining positively for DLL3. In exploratory population analyses, we included the subset of patients whose tumours expressed DLL3 in at least 50% of cells by immunohistochemistry (referred to here as DLL3-high), an exploratory threshold identified during retrospective analyses as encompassing all investigator-assessed responders, and which might be pursued for a DLL3 companion diagnostic. We referred to the subset of patients whose tumours expressed DLL3 in fewer than 50% of cells (by immunohistochemistry) as DLL3-low.

Outcomes

The primary objectives of our study were to assess the safety of rovalpituzumab tesirine, including the maximum tolerated dose and dose-limiting toxic effects. Secondary objectives were to characterise the pharma cokinetics and immunogenicity of rovalpituzumab tesirine, to estimate its antitumour activity, and to establish the recommended phase 2 dose and schedule. The safety endpoints for the study were treatment-emergent adverse events, treatment-emergent serious adverse events, clinical laboratory tests (haematology, blood chemistry), vital signs (weight, pulse, systolic blood pressure, diastolic blood pressure, temperature), and QTc interval. We defined the maximum tolerated dose as the dose level immediately below that at which at least two of the first three patients per cohort (or at least two of six patients during the first cycle) had a dose-limiting toxic effect (related to study drug). At least six patients must have been treated at the designated maximum tolerated dose, with no more than one dose-limiting toxic effect recorded among the six patients. We defined the maximum tolerated dose in terms of toxic effects (graded with CTCAE version 4.03) during the patients' first treatment cycle. Antitumour activity endpoints were objective response according to RECIST version 1.1, defined as a confirmed partial or complete response, duration of response (defined as the time from the initial confirmed objective response to the time of disease progression or death, whichever occurs first), progression-

free survival (defined as the time from the first day of treatment to disease recurrence or progression, or death), and overall survival (defined as the time from the the first day of treatment to death).

This study is registered with ClinicalTrials.gov, number NCT01901653.

Statistical analysis

We calculated the sample size during dose escalation with the dose-escalation rules for determining the maximum tolerated dose described above. For dose expansion, we judged a sample size of roughly 20 people per cohort would provide a reasonable degree of confidence around the point estimates of tolerability.

The full analysis set comprised all patients who received at least one cycle of treatment. The population for activity analyses included all patients treated with any dose of rovalpituzumab tesirine, corresponding to the dose levels at which investigator-assessed objective responses were observed. The measurement of activity in patients not treated at the recommended phase 2 dose was post hoc. The safety analysis set included all patients who received at least one dose of treatment. The pharmacokinetic analysis set consisted of all patients who satisfied inclusion criteria for the study, who received any amount of study drug, and who had at least one post-baseline pharmacokinetic assessment.

The date of data analysis was May 11, 2016. We estimated duration of response, progression-free survival, and overall survival using the Kaplan-Meier method. We used the Greenwood formula to ascertain 95% CIs and calculated the Pearson coefficient for pharmacokinetic analysis. Patients without a respective event as of the date of analysis were censored at the last known assessment. All activity endpoints were preplanned but analysed as exploratory endpoints. We did post-hoc analyses of chemotherapy-sensitive and resistant or refractory subpopulations and of patients treated in the second-line or third-line setting. We did exploratory analyses to investigate the association between DLL3 expression and response and time-to-event outcomes. Analyses were done with SAS version 9.4.

Role of the funding source

Representatives of the funder designed the study, with assistance from academic advisers, including authors. Data were collected by the investigators and their site personnel. The authors and representatives of the funder did data analyses and data interpretation. The report was prepared by the corresponding author, with input from all coauthors, including those employed by the funder. All authors had full access to data in the study and the corresponding author had final responsibility for the decision to submit for publication.

Results

Between July 22, 2013, and Aug 10, 2015, 82 patients were enrolled in our study (74 patients with small-cell lung cancer and eight with large-cell neuroendocrine carcinoma), all of whom received at least one dose of rovalpituzumab tesirine. At the time of data cutoff (May 11, 2016), the median duration of follow-up was 3·9 months (IQR 2·2–7·4; range 0·4–22·0). No patients remained on active treatment, and seven (9%) remained in follow-up. The

entire study cohort received a median of two doses (IQR 1–3; range 1–14) of rovalpituzumab tesirine. Because patients with large-cell neuroendocrine tumours comprised a small proportion of the study population (10%), and outcomes can differ from those of patients with small-cell lung cancer,[9] they were excluded from endpoint analyses. Clinical characteristics of the 74 participants (age range 38–81 years) with small-cell lung cancer were typical of patients with advanced recurrent disease (table 1).

During dose escalation in patients with small-cell lung cancer, dose-limiting toxic effects arose in two (100%) of two patients given 0·8 mg/kg rovalpituzumab tesirine every 3 weeks (both had small-cell lung cancer), consisting of grade 4 thrombocytopenia (n=2) and grade 4 liver function test abnor malities (n=1), all of which improved by the end of the first treatment cycle. No dose-limiting toxic effects occurred at other dose levels; the maximum tolerated dose was declared as 0·4 mg/kg every 3 weeks. Because rovalpituzumab tesirine was found to have a half-life of around 10–14 days (appendix pp 6–8), dosing regimens of 0·3 mg/kg and 0·4 mg/kg every 6 weeks were also studied. However, the 0·4 mg/kg dose was associated with an unacceptable level of delayed toxic effects with regimens every 3 weeks and every 6 weeks, including grade 3 treatment-related serosal effusion adverse events in three (50%) of six patients (one of three treated every 3 weeks and two of three treated every 6 weeks). As a result, expansion cohorts included 0·2 mg/kg every 3 weeks and 0·3 mg/kg every 6 weeks, which were later capped at a total initial dose of 0·6 mg/kg (three cycles of 0·2 mg/kg every 3 weeks or two cycles of 0·3 mg/kg every 6 weeks). In total, three patients received 0·05 mg/kg every 3 weeks, one was given 0·1 mg/kg every 3 weeks, 25 were treated with 0·2 mg/kg every 3 weeks, three received 0·4 mg/kg every 3 weeks, two were given 0·8 mg/kg every 3 weeks, 45 were treated with 0·3 mg/kg every 6 weeks, and three received 0·4 mg/kg every 6 weeks (appendix p 5). The recom mended dose and schedule for future studies with rovalpituzumab tesirine is two cycles of 0·3 mg/kg every 6 weeks. 68 patients with small-cell lung cancer were treated with an active dose of rovalpituzumab tesirine (0·2 mg/kg or 0·4 mg/kg every 3 weeks or 0·3 mg/kg or 0·4 mg/kg every 6 weeks).

Rovalpituzumab tesirine (antibody-drug conjugate with an average drug-to-antibody ratio of 2) showed roughly linear pharmacokinetics, with dose-proportional increases in exposure and a half-life of around 10–14 days, based on an analysis of 41 patients with small-cell lung cancer (appendix pp 6–8). For the dosing schedule every 3 weeks, steady-state was achieved by cycle 3 or 4, and for the schedule every 6 weeks it was achieved by cycle 2 or 3 (ie, between 50 and 70 days). For the dosing schedule every 3 weeks, modest accumulation of 30% was noted by steady-state (cycle 4), and for the schedule every 6 weeks, no accumulation was seen (cycle 3), in line with theoretical calculations. The pharmacokinetics of total antibody (conjugated, partly deconjugated, and fully deconjugated antibody-drug conjugate) and antibody-drug conjugate were correlated (Pearson correlation coefficient 0·94), with total antibody exposures roughly 5–25% higher than those associated with antibody-drug conjugates across cohorts with no dose dependence. Circulating amounts of the DNA cross-linking agent SC-DR002 were generally not measurable (only seven of 427 samples from 26 patients had levels above the lower limit of detection of 40 pg/mL). No anti-therapeutic antibodies against rovalpituzumab tesirine were detected.

Rovalpituzumab tesirine was generally well tolerated in the 74 patients with small-cell lung cancer. Treatment-related adverse events of any grade arose in 65 (88%) patients, and those of grade 3 or worse were noted in 28 (38%) patients (table 2). The most frequent grade 3 or worse treatment-related adverse events were thrombocytopenia (eight [11%] patients), pleural effusion (six [8%]), and increased lipase (five [7%]). The most frequent groups of treatment-related adverse events of grade 3 or worse were thrombo cytopenia (nine [12%] of 74; including thrombocytopenia and decreased platelet count), serosal effusions (eight [11%] of 74; including pleural and pericardial effusions, ascites, and capillary leak syndrome [comprising serosal effusions, peripheral oedema, and hypoalbuminaemia; re-coding was done after patients were not adjudicated as having capillary leak syndrome by a data monitoring committee of experts in that disorder]), and skin reactions (six [8%] of 74; consisting of maculopapular rash, erythema, photosensitivity, dermatitis acneiform, erythema multiforme, and palmar-plantar erythrodysaesthesia syndrome; appendix pp 19–22). Median onset of thrombocytopenia was 15 days (IQR 10–16) and median duration was 22 days (8–42); median onset of serosal effusions was 74 days (43–97) and median duration was 15 days (7–28); and median onset of skin reactions was 30 days (16–42) and median duration was 21 days (13–42). All-causality adverse events and serious adverse events are shown in the appendix (pp 11–25). Compared with individuals with DLL3-low expression, patients with DLL3-high expression had a greater frequency of treatment-related adverse events (nine [69%] of 13 *vs* 28 [97%] of 29) and adverse events of grade 3 or worse (three [23%] of 13 *vs* 12 [41%] of 29). However, DLL3-high patients also had a longer treatment duration and follow-up (mean 7·6 months [SD 6·0]) than did those with DLL3-low expression (2·9 months [2·4]).

Dose reductions due to adverse events occurred for six (7%) of 82 patients. Rovalpituzumab tesirine was withdrawn because of adverse events in 18 (22%) patients (appendix p 11), most frequently for pleural effusion (n=4), pericardial effusion (n=2), and maculopapular rash (n=2; appendix p 26). Drug-related serious adverse events occurred in 35 (43%) of 82 patients, the most frequent including pleural effusion in 14 (19%) and pericardial effusion in five (7%; appendix p 25).

65 (79%) of 82 patients died during the study, 56 (86%) because of disease, four (6%) because of an adverse event, four (6%) for unknown reasons, and one (2%) because of a bleeding ulcer (appendix p 27; data corresponding to the end-of-study forms are missing for two patients who discontinued because of an adverse event and have died). Of deaths attributable to an adverse event, two were judged treatment-related. In one patient, endobronchial tumour haemorrhage developed on study day 10 in association with local disease progression, which subsequently prompted withdrawal of care. However, a platelet count of 16×10^9 platelets per L was also noted and a contribution of drug-related thrombocytopenia could not be ruled out. In the second patient, acute kidney injury developed on study day 128 in the context of disease progression and poor oral intake, in addition to antecedent use of diuretics, corticosteroids, and non-steroidal anti-inflammatory drugs administered for peripheral oedema attributed to rovalpituzumab tesirine. Care was also withdrawn for this patient; because rovalpituzumab tesirine was judged likely to be the cause of the peripheral oedema, which prompted administration of potentially nephrotoxic

agents, contribution of rovalpituzumab tesirine to the fatal adverse event could not be ruled out.

Figure 1 shows the best change in tumour burden from baseline, as assessed by the investigator. Nine (12%) of 74 patients were not assessable for activity analyses: five died before the post-baseline scan, one had a serious adverse event, two withdrew consent, and one did not complete the scan and died at day 71. Of 65 patients assessable for activity analyses who received any dose of rovalpituzumab tesirine, 11 (17%) achieved a confirmed objective response and 35 (54%) had stable disease; therefore, 46 (71%) patients achieved disease control. For the exploratory analysis of DLL3 expression in tumour tissue, 39 patients provided samples for analysis. Of 29 assessable patients who were defined as DLL3-high, ten (35%) had a confirmed objective response and 26 (90%) achieved disease control. Of ten assessable patients defined as DLL3-low, none had a confirmed objective response and six (60%) achieved disease control (appendix p 9). Of 60 assessable patients receiving an active dose of rovalpituzumab tesirine (0·2 mg/kg or 0·4 mg/kg every 3 weeks or 0·3 mg/kg or 0·4 mg/kg every 6 weeks), 11 (18%) achieved a confirmed objective response and 30 (50%) had stable disease; therefore, 41 (68%) patients achieved disease control (table 3). In an exploratory analysis of available tumour tissue samples (n=34), the proportion of patients with a response was higher among assessable DLL3-high patients (ten [38%] of 26 had a confirmed objective response and 23 [88%] achieved disease control) than among assessable DLL3-low patients (no confirmed objective responses and four [50%] of eight patients achieved disease control).

Median duration of response among all 65 assessable patients was 5·6 months (95% CI 2·5–8·3), based on nine of 11 responders with uncensored progression. Figure 2 shows the duration of treatment and responses among patients treated at active doses of rovalpituzumab tesirine (0·2 mg/kg or 0·4 mg/kg every 3 weeks or 0·3 mg/kg or 0·4 mg/kg every 6 weeks; n=60). Of 65 assessable patients, 59 had disease progression or died and median progression-free survival was 3·1 months (95% CI 2·7–4·1). In an exploratory analysis, median progression-free survival was 4·5 months (95% CI 3·0–5·4) for DLL3-high patients (based on 26 of 29 patients who had disease progression or died) and 2·3 months (1·3–3·3) for DLL3-low patients (based on nine of ten patients who had disease progression or died). Of the nine patients who could not be assessed, eight were dosed at active doses (0·2 mg/kg or 0·4 mg/kg every 3 weeks or 0·3 mg/kg or 0·4 mg/kg every 6 weeks) and one was dosed outside the active range. Progression-free survival for the 60 assessable patients who were treated at active doses is shown in table 3. Of note, several patients derived long-term clinical benefit after receiving only two or three doses of rovalpituzumab tesirine in the absence of an objective response or subsequent systemic treatment (eight with overall survival >6 months, two with overall survival >12 months), in some cases, associated with an initial increase in tumour burden not meeting criteria for progressive disease.

A retrospective, independent, radiographic review was done in a subset of 56 patients treated with an active dose of rovalpituzumab tesirine in whom scans could be accessed and anonymised. This central review validated the primary findings based on investigator site assessments (table 3). In a post-hoc analysis, the proportion of patients achieving an

objective response did not differ between those treated in the second-line or third-line setting (appendix p 10).

In 68 patients treated at the active dose levels of rovalpituzumab tesirine, overall survival was 4·6 months (95% CI 3·9–7·1; based on 54 deaths). In an exploratory analysis, in 29 patients in the DLL3-high subset, median overall survival was 5·8 months (95% CI 4·4–11·6; based on 22 deaths); and in ten patients in the DLL3-low subset, median overall survival was 2·7 months (1·2–10; based on nine deaths). 1-year overall survival was 18% (95% CI 9–29) in patients treated at the active dose level, 32% (15–49) in DLL3-high patients, and 0% in DLL3-low patients (appendix pp 2, 3). In a post-hoc analysis of chemotherapy-sensitive versus refractory or resistant patients, 1-year overall survival was 21% in patients with resistant or refractory disease; it was 29% in the DLL3-high patients and 0% in the DLL3-low patients. 1-year overall survival was 17% in patients with chemotherapy-sensitive disease; it was 33% in the DLL3-high patients and 23% in the DLL3-low patients.

Discussion

This first-in-human, phase 1 study of rovalpituzumab tesirine, a novel DLL3-targeted antibody-drug conjugate, defined a dose range in which the drug is well tolerated and shows encouraging single-agent antitumour activity in recurrent small-cell lung cancer. Expression of DLL3 in tumours can identify patients who are more likely to achieve a response and better long-term outcomes during treatment with rovalpituzumab tesirine, suggesting DLL3 as a potential biomarker and tractable therapeutic target in small-cell lung cancer.

These findings with rovalpituzumab tesirine are especially encouraging in third-line small-cell lung cancer, for which no currently approved treatment exists. In a retrospective analysis of 120 patients at five centres in Canada, the UK, and Australia—the largest published experience in this setting—1-year survival for patients with third-line small-cell lung cancer was 12% with conventional therapeutic options.[10] In the DLL3-high population in our study, 1-year survival with rovalpituzumab tesirine in the third-line setting was 36% (appendix p 10).

In this study, all investigator-assessed responses were recorded in patients with expression of DLL3 in at least 50% of tumour cells, defined here as DLL3-high. However, disease control, including stable disease, often extended in duration, was noted among several patients with DLL3 expression below 50%. Subsequent clinical development of rovalpituzumab tesirine will include assessment of activity both in patients with tumours expressing any detectable level of DLL3 and within the cohort defined here as DLL3-high. Including these patients in subsequent studies might further validate a DLL3-high threshold while also permitting investigation of the use of a lower companion diagnostic cut off that could better define the population having meaningful disease control.

Preclinical data showing that rovalpituzumab tesirine can target effectively tumour-initiating cells within small-cell lung cancer suggest potential application in other lines of treatment. The response to first-line chemotherapy is high with platinum-based regimens, but disease

invariably recurs in extensive-stage disease, attributable to the persistence of tumour-initiating cells through conventional chemotherapy. By targeting this residual cell population that leads to disease recurrence, rovalpituzumab tesirine has a unique mechanistic rationale for assessment in the first-line setting, either as monotherapy, in combination with conventional therapeutic approaches, or possibly in combination with immune-checkpoint inhibitors, which have shown activity in small-cell lung cancer.[11]

Observations of long-term clinical benefit after limited dosing of rovalpituzumab tesirine in the absence of objective responses accord with the hypothesis that rovalpituzumab tesirine can target effectively tumour-initiating cells as underlying drivers of tumour progression and metastasis and suggest that traditional RECIST-based objective responses can underestimate disease control of cancer stem cell-directed agents. Alternative response criteria might be appropriate to consider with such therapeutic agents, analogous to use of immune-related response criteria to account for pseudoprogression after treatment with immunotherapies. [12] At the same time, the perhaps modest median progression-free survival and overall survival reported in this study could reflect a still incompletely understood therapeutic approach to these target cells: for instance, rovalpituzumab tesirine might be especially effective at reducing tumour volume, but a pharmacological sink created by high burden, high-DLL3 tumours could compromise effective targeting of the rare tumour-initiating cell population.

Rovalpituzumab tesirine has a unique toxicity profile, notable for thrombocytopenia, serosal effusions, and skin reactions. The mechanism of these toxic effects is not clear, but probably relates to the pyrrolobenzodiazepine dimer component of the antibody-drug conjugate, SC-DR002, since DLL3 protein is not expressed on the vasculature, platelets, megakaryocytes, or skin tissues, and no clear independent relation is noted between treatment response or DLL3 expression in the tumour and the incidence of these adverse events (data not shown). Other pyrrolobenzodiazepine-based compounds, such as SJG-136, have shown analogous toxic effects in human beings.[13] The observed serosal effusions were clinically significant and dose-limiting, prompting dose modification, delay or discontinuation in five (8%) patients in that study.[13] In our study, no serosal effusion-related dose modifications were necessary within the first two cycles of the 0·3 mg/kg every 6 weeks regimen, or within the first three cycles of the 0·2 mg/kg every 3 weeks regimen, and variably these effusions and associated symptoms were ameliorated by systemic corticosteroids. The apparently higher incidence of toxic effects in patients with higher DLL3 expression is confounded by total drug exposure, duration of treatment, and follow-up. Future clinical development of rovalpituzumab tesirine will incorporate and analyse strategies to manage these toxic effects, such as limited repeated rovalpituzumab tesirine dose exposure and prophylactic administration of systemic corticosteroids or use prompted by toxic effects. Molecular and cellular characterisation of treatment-emergent serosal fluids might further define the mechanism and guide appropriate management of these toxic effects.

The PD-1 antagonist nivolumab, alone or in combination with the CTLA4 antagonist ipilimumab, has shown encouraging activity in recurrent small-cell lung cancer.[11] Although direct comparisons between these studies might be misleading, it is tempting to note the relatively low (10–23%) but durable responses with these immunotherapy regimens, by contrast with the higher responses recorded with rovalpituzumab tesirine. Since the

mechanisms of action and toxic effects of these different therapeutic approaches do not seem to overlap, future therapeutic regimens entailing combinations of rovalpituzumab tesirine with antagonists of PD-1 and CTLA4 pathways could be relevant and of clinical interest.

In conclusion, the findings of this study suggest DLL3 is a clinically relevant, novel target in small-cell lung cancer and that rovalpituzumab tesirine is a novel antibody-drug conjugate agent for DLL3-positive small-cell lung cancer. Limitations of the study include its exploratory trial design, including the absence of an active comparator, and the somewhat limited numbers of patients at each active dose level in every dose cohort. Nonetheless, testing for DLL3 expression seems to be feasible and could identify patients with an enhanced likelihood of clinical benefit from treatment with the antibody-drug conjugate rovalpituzumab tesirine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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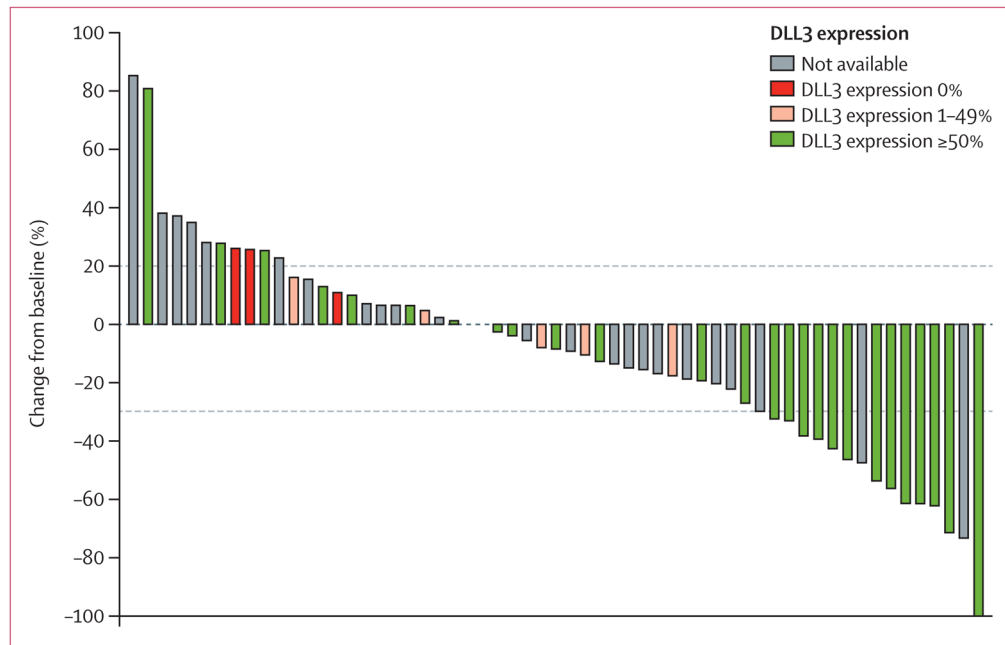
Figure 1. Waterfall plot showing best change in tumour burden from baseline at active treatment doses (n=60)

Investigator-assessed best change from baseline was the change in the sum of longest diameters of target lesions for patients treated with rovalpituzumab tesirine 0·2 mg/kg or 0·4 mg/kg every 3 weeks or 0·3 mg/kg or 0·4 mg/kg every 6 weeks. Grey dotted line at 20% indicates the threshold for progressive disease and the line at –30% the threshold for partial response. One patient did not have a measurable target lesion.
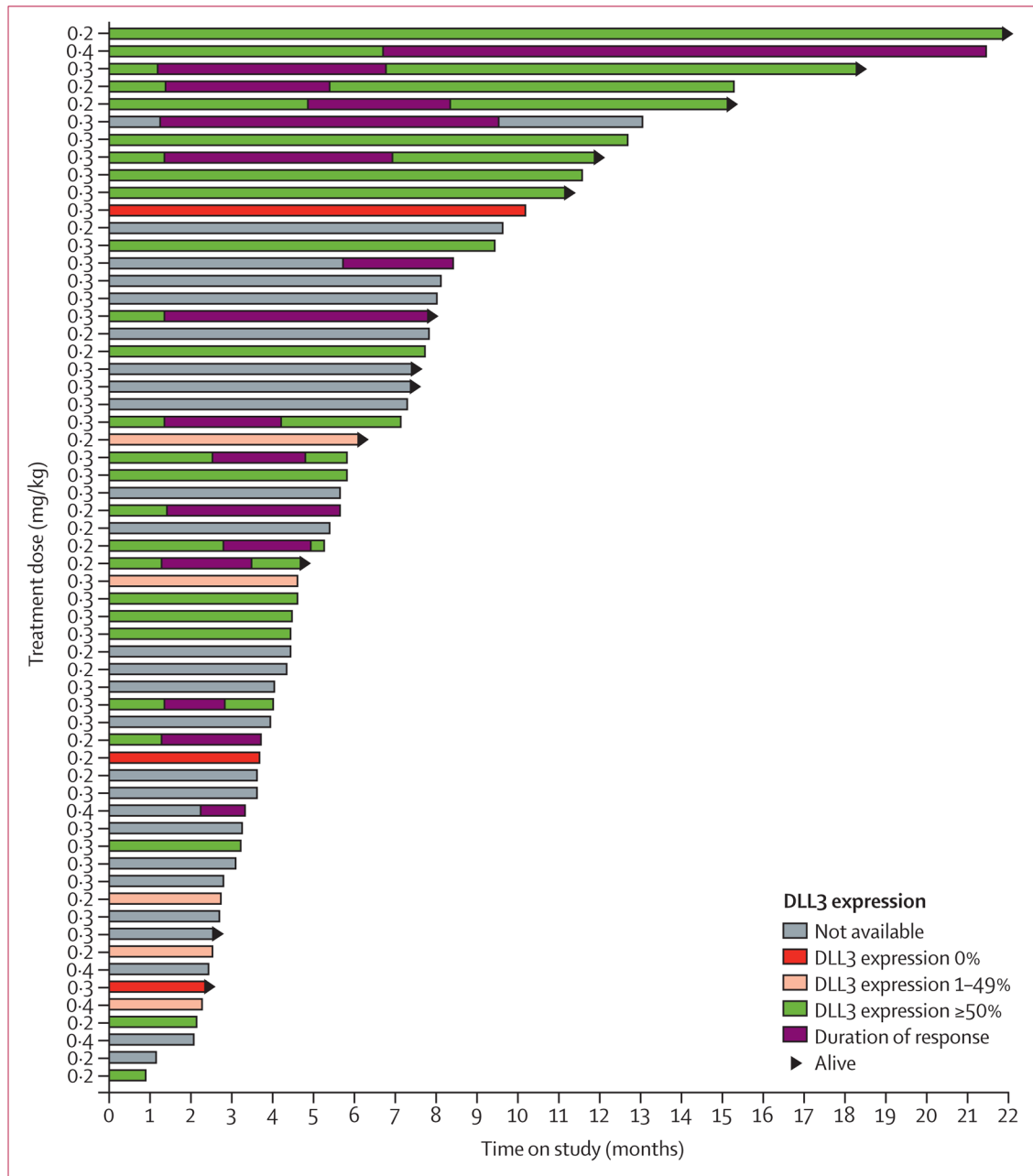
Figure 2. Swimmer's plot showing time on study and duration of response for patients treated with active treatment doses (n=60)

Outcomes are shown for patients treated with rovalpituzumab tesirine 0·2 mg/kg or 0·4 mg/kg every 3 weeks or 0·3 mg/kg or 0·4 mg/kg every 6 weeks. Lines without an arrow indicate deaths (patients who withdrew were still followed up). Duration of response includes confirmed and unconfirmed responses.

Table 1

Baseline characteristics in patients with small-cell lung cancer

	Patients (n=74)
Age (years)	61 (55–69)
Sex	
Female	32 (43%)
Male	42 (57%)
ECOG performance score	
0	21 (28%)
1	50 (68%)
2	3 (4%)
Response to first-line treatment	
Sensitive [*]	39 (53%)
Resistant [†]	23 (31%)
Refractory [‡]	7 (9%)
Not assessable	5 (7%)
Treatment-free interval before second-line treatment (months)	4·1 (1·8–7·9)
Previous lines of treatment	
One	39 (53%)
Two	35 (47%)
History of CNS metastases	21 (28%)
Previous treatments	
Platinum and etoposide	71 (96%)
Platinum and other drug	5 (7%)
Platinum, etoposide, and other drug	7 (9%)
Topotecan	8 (11%)
Temozolomide	10 (14%)
ABT-888	8 (11%)
Radiation	61 (82%)
Other	16 (22%)
Tumour DLL3 expression [§]	
1%	42/48 (88%)
50%	32/48 (67%)

Data are median (IQR) or number of patients (%). ECOG=Eastern Cooperative Group.

[*] Defined as a best response of stable disease or better to first-line treatment, and a treatment-free interval between first-line and second-line treatment of 90 days or longer.

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Table 2

Treatment-related adverse events in 74 patients with small-cell lung cancer

	Grade 1–2	Grade 3	Grade 4	Grade 5
Acute kidney injury	0 (0%)	0 (0%)	0 (0%)	1 (1%)
Alanine aminotransferase increased	2 (3%)	1 (1%)	1 (1%)	0 (0%)
Amylase increased	2 (3%)	1 (1%)	0 (0%)	0 (0%)
Anaemia	7 (9%)	2 (3%)	0 (0%)	0 (0%)
Arthralgia	8 (11%)	1 (1%)	0 (0%)	0 (0%)
Aspartate aminotransferase increased	5 (7%)	1 (1%)	1 (1%)	0 (0%)
Asthenia	1 (1%)	1 (1%)	0 (0%)	0 (0%)
Blood alkaline phosphatase increased	3 (4%)	1 (1%)	0 (0%)	0 (0%)
Cardiac tamponade	0 (0%)	0 (0%)	1 (1%)	0 (0%)
Conjunctivitis	0 (0%)	1 (1%)	0 (0%)	0 (0%)
Decreased appetite	12 (16%)	0 (0%)	0 (0%)	0 (0%)
Dermatitis acneiform	3 (4%)	1 (1%)	0 (0%)	0 (0%)
Dyspnoea	6 (8%)	2 (3%)	0 (0%)	0 (0%)
Electrocardiogram ST segment elevation	0 (0%)	1 (1%)	0 (0%)	0 (0%)
Erythema	10 (14%)	0 (0%)	0 (0%)	0 (0%)
Erythema multiforme	5 (7%)	1 (1%)	0 (0%)	0 (0%)
Fatigue	23 (31%)	3 (4%)	0 (0%)	0 (0%)
Hypoalbuminaemia	13 (18%)	0 (0%)	0 (0%)	0 (0%)
Hypotension	1 (1%)	2 (3%)	1 (1%)	0 (0%)
Hypoxia	0 (0%)	1 (1%)	0 (0%)	0 (0%)
Infection	0 (0%)	0 (0%)	1 (1%)	0 (0%)
Lipase increased	1 (1%)	2 (3%)	3 (4%)	0 (0%)
Liver function test abnormal	0 (0%)	0 (0%)	1 (1%)	0 (0%)
Maculopapular rash	10 (14%)	2 (3%)	0 (0%)	0 (0%)
Nausea	14 (19%)	0 (0%)	0 (0%)	0 (0%)
Neutrophil count decreased	0 (0%)	1 (1%)	0 (0%)	0 (0%)
Palmar-plantar erythrodysaesthesia syndrome	1 (1%)	1 (1%)	0 (0%)	0 (0%)
Pancytopenia	0 (0%)	0 (0%)	1 (1%)	0 (0%)
Pericardial effusion	7 (9%)	0 (0%)	2 (3%)	0 (0%)
Peripheral oedema	18 (24%)	2 (3%)	0 (0%)	0 (0%)
Photophobia	0 (0%)	1 (1%)	0 (0%)	0 (0%)
Photosensitivity reaction	7 (9%)	1 (1%)	0 (0%)	0 (0%)
Platelet count decreased	2 (3%)	1 (1%)	0 (0%)	0 (0%)
Pleural effusion	17 (23%)	6 (8%)	0 (0%)	0 (0%)
Pulmonary oedema	0 (0%)	1 (1%)	0 (0%)	0 (0%)
Thrombocytopenia	4 (5%)	3 (4%)	5 (7%)	0 (0%)
Troponin increased	0 (0%)	1 (1%)	0 (0%)	0 (0%)

	Grade 1–2	Grade 3	Grade 4	Grade 5
Tumour haemorrhage	0 (0%)	0 (0%)	0 (0%)	1 (1%)
Vomiting	10 (14%)	0 (0%)	0 (0%)	0 (0%)
White blood cell count decreased	0 (0%)	1 (1%)	0 (0%)	0 (0%)

Data are number of patients (%). The table shows grade 1–2 treatment-related adverse events in 10% of patients in any treatment cohort and all grade 3–5 adverse events. All-cause adverse events and serious adverse events are shown in the appendix (pp 11–25).

Table 3

Activity outcomes in response-assessable patients treated at active doses, assessed by the investigator and by central review

	Investigator-assessed			Central review		
	All patients (n=60)	DLL3 expression 0–49% (n=8)	DLL3 expression ≥50% (n=26)	All patients (n=56)	DLL3 expression 0–49% (n=6)	DLL3 expression ≥50% (n=26)
Confirmed objective response (complete response and partial response)	11 (18%)	0 (0%)	10 (38%)	9 (16%)	0 (0%)	8 (31%)
Confirmed disease control (complete response, partial response, and stable disease)	41 (68%)	4 (50%)	23 (88%)	36 (64%)	2 (33%)	22 (85%)
Duration of response (months)	5·6 (2·5–8·3)	0	4·3 (2·2–15)	4·4 (2·2–6·5)	0	4·6 (2·2–6·9)
Progression-free survival (months)	2·8 (2·5–4·0)	2·2 (1·3–2·5)	4·3 (2·8–5·6)	4·0 (2·6–4·8)	2·2 (1·1–3·7)	4·6 (4·0–5·7)

Data are number of patients (%) or median (95% CI). Responses reflect confirmed responses according to RECIST version 1.1, based on two consecutive assessments at least 4 weeks apart, in patients treated with 0·2 mg/kg or 0·4 mg/kg every 3 weeks or 0·3 mg/kg or 0·4 mg/kg every 6 weeks. RECIST=Response Evaluation Criteria in Solid Tumors.