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Phase II trial with ISIS 5132 in patients with small-cell (SCLC) and non-small cell (NSCLC) lung cancer. A European Organization for Research and Treatment of Cancer (EORTC) Early Clinical Studies Group report

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Abstract

Two multicentre phase II trials were designed to determine if tumour responses can be achieved in progressive small-cell lung cancer (SCLC) or non-small cell lung cancer (NSCLC) patients treated with ISIS 5132, an inhibitor of C-*raf* kinase mRNA expression (CGP 69846A; ISIS Pharmaceuticals Inc, Carlsbad, CA), and to further characterise the safety of the compound. Between August 1998 and November 1999, 26 patients (18 NSCLC, 8 SCLC) were entered. Out of these, 23 were eligible, 22 (18 NSCLC, 4 SCLC) were treated with ISIS 5132 (2 mg/kg/day, 21 days continuous intravenous (i.v.) infusion every 4 weeks) and were evaluable for toxicity and 18 (15 NSCLC, 3 SCLC) were evaluable for efficacy. For the whole group haematological toxicity did not exceed grade 2. One patient experienced a grade 4 increased prothrombin time. Non-haematological toxicity was mild to moderate, with the observation of asthenia and nausea and vomiting. Progressive disease (PD) was diagnosed in 10 patients (8 NSCLC and 2 SCLC). 8 more patients (7 NSCLC, 1 SCLC) were considered as treatment failures. In conclusion, this study using ISIS 5132 with this dose and schedule of administration excludes a 20% response rate with 95% confidence intervals for NSCLC and cannot draw any conclusions for SCLC patients as only a few were involved in the study.

Keywords: ISIS 5132; Raf kinase; Antisense; Non-small cell lung cancer; Small-cell lung cancer

1. Introduction

Lung cancer is the leading cause of cancer mortality in men and women in industrialised countries. For the treatment of non-small cell lung cancer (NSCLC), cis-platin-based chemotherapy, when compared with best supportive care alone, significantly improves survival [1]. However the differences are fairly small with an increased median survival of 6 weeks and an improvement in the 1-year survival rate of only 10% in metastatic disease. For small-cell lung cancer (SCLC), chemotherapy is rarely curative, except in limited-stage patients, and those patients are candidates for radiotherapy and chemotherapy combinations. However, multiple strategies designed to improve systemic treatments have not yielded significant breakthroughs [2]. These modest results in lung cancers have made the development of new agents imperative.

Recent advances in cancer biology have led to the identification of signalling proteins, like the RAF-kina-

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ses, that may be rational therapeutic targets. RAF kinases serve as central regulators of mitogenic signalling pathways by connecting upstream growth factor-mediated tyrosine kinase stimulation with downstream activation of serine threonine kinases [3]. Deletion of the normal amino-terminal coding sequence is able to activate *raf* genes [4] and H-*ras* is capable of transforming semi-malignant cells [5]. There are two general mechanisms for aberrant *ras* gene activation (H-, K- and N-*ras*). Mutational activation of H-*ras* and N-*ras* genes are rare events in human lung cancer, but K-*ras* gene activation can occur in 30% of adenocarcinomas [6] and has an important prognostic role [7]. The second model of *ras*-mediated oncogenesis involves protein overexpression, but is still controversial in lung cancer. *Raf* genes (A-, B- and C-*raf*) code for serine-threonine specific protein kinases that play a pivotal role in signalling processes by being downstream effectors for RAS. Activated RAS interacts directly with the NH2-terminal regulatory domain of the RAF kinase resulting in a cascade of reactions by direct activation of mitogen-activated protein kinase (MAPK) [8]. The MAPK signalling pathway has been shown to be essential for cellular proliferation and mediation of cellular transformation by most oncogenes [3]. Mutated *ras* and *raf-1* are constitutively active and have transforming potential *in vitro* [4,7,9]. RAF-1 may play a broader role in tumorigenesis: it is activated independently of RAS by the anti-apoptotic protein Bcl-2 [10] and protein kinase C-alpha [11], and promotes the expression of the multidrug resistance gene *mdr1* [12].

The elucidation of the gene sequences for the A549 lung carcinoma cell line has revealed the presence of K- and H-*ras* mutations. Studies in culture have demonstrated that ISIS 5132 (CGP 69846A; ISIS Pharmaceuticals Inc, Carlsbad, CA, USA) inhibits C-*raf* kinase mRNA expression in the A549 lung carcinoma cell line, in a nucleotide sequence- and concentration-dependent manner relative to controls, while having no effect on A-*raf* or H-*ras* mRNA expression. Also, reduction of c-*raf*-1 mRNA expression *in vivo* occurred in mice bearing A549 human tumour xenografts within hours of ISIS 5132 therapy at a dose of 6 mg/kg/day [13].

Two phase I studies were performed. In the first one, 31 patients with refractory malignancies received ISIS 5132 as a 2-h intravenous (i.v.) infusion three times weekly for 3 consecutive weeks. ISIS 5132 was well tolerated at doses up to 6.0 mg/kg body weight and clinical toxicities included fever and fatigue, transient prolongation of aPTT (prothrombin time) recovering to baseline within 2 h of the end of infusion, but these were not dose-limiting. 2 patients experienced prolonged stable disease (SD) lasting more than 7 months [14]. Significant reduction in C-*raf*-1 expression in peripheral-blood mononuclear cells was observed in 13 out of 14 patients and this paralleled the clinical benefit in 2 patients [15]. In the second phase I study, a continuous i.v. infusion of ISIS 5132 was administered for 21 days every 4 weeks to 34 patients with a variety of solid tumours refractory to standard therapy. The initial dose of ISIS 5132 was 0.5 mg/kg body weight and was successfully increased incrementally to 5.0 mg/kg body weight, while toxicity was minimal. 2 patients had prolonged stabilisation of their disease, and 1 patient with ovarian carcinoma had a significant response [16]. Although the maximum tolerated dose (MTD) has not been reached on either schedule, the recommended dose and schedule for phase II single agent trials was 2.0 mg/kg/day by 3-week continuous infusion.

This report, concerning the results of two multicentre phase II trials, describes the experience of the European Organization for Research and Treatment of Cancer (EORTC) Early Clinical Studies Group in order to determine if partial or complete responses (PR or CR) can be achieved with ISIS 5132 in patients with SCLC and NSCLC, and to further characterise the safety of this compound in these groups of patients.

2. Patients and methods

Between August 1998 and November 1999, a total of 26 patients with NSCLC (18 patients) or SCLC (8 patients) were entered into two simultaneous, but independent, multicentre, open-label, non randomised phase II trials.

Eligibility criteria included: histologically- or cytologically-verified locally advanced, unresectable or metastatic NSCLC not pre-treated with chemotherapy (prior platinum radiosensitiser was permitted) or progressive recurrent SCLC pre-treated with only one prior chemotherapy regimen with, for both histological subtypes, documented progression before the start of the study; age $\geqslant 18$ years; presence of at least one bidimensionally measurable lesion; performance status (PS) (World Health Organization (WHO) scale) $\leqslant 2$ and life expectancy $\geqslant 3$ months; adequate bone marrow (neutrophils $\geqslant 1.5\times10^9$ cells/l, platelets $\geqslant 100\times10^9$ cells/l), renal (creatinine $\leqslant 140$ μmol/l or a creatinine clearance $\geqslant 1$ ml/s, urine protein <3 g/l), liver (serum bilirubin $\leqslant 35$ μmol/l, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) $\leqslant 2\times$the upper limit of normal (ULN) or $\leqslant 5$ the ULN if related to liver metastases) functions. Exclusion criteria were: concomitant therapeutic use of anticoagulants; underlying disease state associated with active bleeding; abnormal clotting tests at screening; superior Vena Cava obstruction, unless this had been successfully treated at least 2 months before entry into the study; more than one prior chemotherapy regimen in the case of SCLC or prior chemotherapy in the case of NSCLC; radiotherapy within the last 4 weeks; previous or current malig-

nancies at other sites. All patients gave written informed consent.

Patient baseline evaluation consisted of: complete medical history; physical examination; PS assessment; clinical assessment of tumour parameters; chest and abdominal computed tomography (CT) scan. Laboratory studies at presentation included: determination of complete blood cell and platelet counts; biochemical profile; clotting screen; urine analysis. A weekly full blood count was scheduled, clotting parameters every 2 weeks, while the other laboratory tests were done every 4 weeks. Evaluation of the disease was organised every two cycles using the WHO criteria [17]. Toxicity was determined according to the National Cancer Institute (NCI) Common Toxicity Criteria [18].

2.1. Treatment

ISIS 5132, 2 mg/kg/day was administered through a central venous line, as a continuous i.v. infusion for 21 consecutive days followed by a treatment-free interval of 7 days. Missing a longer period of dosing of more than 5 days in any treatment course or delaying the next treatment course more than 2 weeks after the last day of dosing meant the patient was taken off the study. The following complications could lead to a 50% dose modification after recovery to $\leqslant$grade 1 toxicity: grade 4 haematological toxicity; infection associated with grade 4 neutropenia or bleeding associated with grade 3 or 4 thrombocytopenia; grade 3 or 4 organ toxicity.

The treatment duration depended on the response to therapy. Patients with progressive disease (PD) were to be removed from the study. Patients with SD, PR or CR could remain on the study until disease progression or excessive toxicity occurred.

3. Results

Patients' characteristics are shown in Table 1. A total of 26 patients (18 NSCLC, 8 SCLC) were entered in the study and among these, 23 were considered eligible (3 SCLC patients were ineligible and did not receive treatment; the first patient for rapid progression and Vena Cava obstruction after registration, the second patient did not have SCLC, the third patient did not have a non-irradiated measurable lesion). Thus, only 22 patients (18 NSCLC, 4 SCLC) received the study treatment because one patient with SCLC died just after registration. 3 more patients (2 with NSCLC and 1 with SCLC) died during the study. 2 patients died because of progressive disease. One patient died from dyspnoea and pulmonary insufficiency (relationship to ISIS 5132 unknown). 2 other patients died within 28 days after they went off the study because of disease progression. One patient with NSCLC went off the study because the Hickman line had fallen out and the patient was too weak to have it re-inserted due to a deterioration in his malignant condition. One patient with SCLC went off the study due to utilisation of an anticoagulant treatment.

3.1. Efficacy

A total of 18 out of the 23 eligible patients (15 NSCLC, 3 SCLC) were considered evaluable for efficacy. PD was diagnosed in 10 patients (8 NSCLC and 2 SCLC). 2 patients with NSCLC formally experienced SD, but are considered as treatment failures as they had the sum of lesions increased by 18 and 23% and did not receive any more ISIS 5132. 5 more NSCLC patients were formally not evaluable according to protocol, but could also be considered as treatment failures: 1 patient had clinical progression after the first cycle without the formal tumour evaluation being performed; 3 patients did not fully receive the complete first cycle necessary for evaluability, but had either formal PD or 'clinical' disease progression. The last patient died most probably due to disease progression (from dyspnoea and pulmonary insufficiency). One SCLC patient was also taken off study with clinical disease progression. Therefore, a 20% response rate with 95% confidence intervals can be excluded for NSCLC. However, this is difficult to evaluate for the SCLC patients as only a few were assessed (Table 2).

3.2. Toxicity

3.2.1. Haematological toxicity

Haematological toxicity related to ISIS 5132 was scarce and did not exceed grade 2 (Table 3). A patient

Table 1
Patient characteristics

	NCSLC	SCLC
Number of registered patients	18	8
Number of eligible patients	18	5
Number of treated patients	18	4
Number evaluable for toxicity	18	4
Number evaluable for efficacy	15	3
Sex		
Female	4	1
Male	14	7
Age (years)		
Median (range)	60 (40–76)	54 (43–74)
Performance status		
0	5	1
1	10	4
2	3	3
Previous therapy		
Surgery	10	5
Radiotherapy	5	6
Chemotherapy	0	8

NSCLC, non-small cell lung cancer; SCLC, small-cell lung cancer.

with SCLC experienced a grade 4 increased prothrombin time. The patient had a normal baseline value. During cycle one of ISIS 5132 treatment the prothrombin time increased to a grade 4 toxicity. After 2 weeks of treatment, ISIS 5132 was discontinued and the grade 4 toxicity was then reduced to a grade 1 level. Although the patient was on low dose warfarin treatment (to prevent occlusion of the central line) a relationship with the study drug was not completely ruled out.

3.2.2. Non-haematological toxicity

For the entire group of patients (NSCLC and SCLC), non-haematological toxicity was generally mild to moderate with asthenia and nausea and vomiting being the most frequently observed toxicities (Table 4). Grade 1–3 nausea/vomiting as observed in 10 out 22 (45%) patients. Grade 1-3 asthenia affected 7 out 22 (32%) patients. Grade 1-2 increased liver enzyme levels were noted in 3 out of 22 (14%) patients.

Table 2
Drug administration and efficacy data

	NCSLC	SCLC
Number of administered cycles	30	5
Cycle number		
1	18	4
2	10	1
3	1	–
4	1	–
Patients receiving treatment	18	4
Efficacy		
Complete response	–	–
Partial response	–	–
Progressive disease	8	2
Treatment failure	7	1
Non evaluable	3	1

NSCLC, non-small cell lung cancer; SCLC, small-cell lung cancer.

Table 3
Reported haematological and abnormal clotting adverse events (possibly/probably/definitively related) worst grade per patient

	NSCLC ($n=18$)		SCLC ($n=4$)			
	Maximum CTC grade		Maximum CTC grade			
	Grade 1	Total	Grade 1	Grade 2	Grade 4	Total
Leucopenia	1	1	2	1	–	3
Neutropenia	–	–	–	1	–	1
Thrombocytopenia	4	4	2	–	–	2
Anaemia	2	2	1	–	–	1
Increased aPTT	2	2				
Increased prothrombin time			–	–	1	1

CTC, common toxicity criteria; NSCLC, non-small cell lung cancer; SCLC, small-cell lung cancer.

4. Discussion

Since 1996, preclinical data in culture cells and in mice models, has demonstrated antitumour activity with ISIS 5132, a drug that inhibits C-*raf* kinase mRNA expression. Ras/raf targeting approaches are highly attractive to the clinical investigator because the *ras* oncogene is dysregulated or mutated more frequently in human cancers (including in lung cancers) [19] than any other oncogene studied. Two phase I studies in 52 patients demonstrated the safety of the compound with encouraging tumour responses and prolonged stabilisation. The two multicentre phase II trials which are reported here were designed in order to determine if PR or CR could be achieved with ISIS 5132 in patients with SCLC and NSCLC, and to further characterise the safety of this compound in these groups of patients. The haematological and non-haematological toxicity was mild, but no response was seen in the 18 patients evaluable for efficacy.

However, the mild toxicity encountered suggests that the dosage and schedule of administration could have been sub-optimal. Thus, it may still be possible that activity would be observed using a different dose and schedule.

Also, no determination of the ras or raf status was performed in the tumours. Would this determination have been meaningful? Perhaps a better specificity in the choice of the patients could have yielded better results as ISIS 5132 is a highly specific drug.

Table 4
Non-haematological/clotting adverse events (possibly/probably/definitively related) worst grade per patient ($n=22$) (NSCLC and SCLC analysed together)

Type of adverse event	Maximum CTC grade				
	Grade 1	Grade 2	Grade 3	Grade 4	Total
Nausea	7	1	2	–	10
Vomiting	2	3	–	–	5
Constipation	1	–	–	–	1
Asthenia, malaise/fatigue	1	5	1	–	7
Pain	1	–	–	–	1
Skin	1	–	–	–	1
Liver enzymes (ASAT, ALAT)	2	1	–	–	3
Headache	1	1	–	–	2
Allergy	1	–	–	–	1
Haemoptysis	–	1	–	–	1
Fever in absence of infection	1	1	–	–	2
Other[a]	1	4	1	–	6

NSCLC, non-small cell lung cancer; SCLC, small-cell lung cancer; CTC, common toxicity criteria; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase.

[a] Maximal other toxicity per patient: grade 1 flu-like symptoms; grade 2 hyponatremia, asymptomatic thrombosis at catheter, heartburn, dry mouth; grade 3 oedema in the legs.

In conclusion, this study using ISIS 5132 with this dose and schedule of administration excludes a 20% response rate with 95% confidence intervals for the NSCLC patients, but cannot draw any conclusions for the SCLC patients as only a few were involved in this study.

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