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The tumour-targeting human L19-IL2 immunocytokine: Preclinical safety studies, phase I clinical trial in patients with solid tumours and expansion into patients with advanced renal cell carcinoma ☆

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ABSTRACT

Background: L19-IL2, a tumour-targeting immunocytokine composed of the recombinant human antibody fragment L19 (specific to the alternatively-spliced EDB domain of fibronectin, a well characterised marker of tumour neo-vasculature) and of human IL2, has demonstrated strong therapeutic activity in animal cancer models. This phase I/II trial was performed to evaluate safety, tolerability, recommended phase II dose (RD) and early signs of activity of L19-IL2.

Patients and methods: Five cohorts of patients with progressive solid tumours ($n = 21$) received an intravenous infusion of L19-IL2 (from 5 to 30 Mio IU IL2 equivalent dose) on days 1, 3 and 5 every 3 weeks. This treatment cycle was repeated up to six times. In the following expansion phase, patients with metastatic renal cell carcinoma (RCC) ($n = 12$) were treated at the RD of L19-IL2. Clinical data and laboratory findings were analysed for safety, tolerability and activity.

Results: Preclinical studies in rats and monkeys did not raise any safety concerns. The RD was defined to be 22.5 Mio IU IL2 equivalent. Pharmacokinetics of L19-IL2 were dose propor-

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tional over the tested range, with a terminal half-life of 2–3 h. Toxicities were manageable and reversible with no treatment-related deaths. We observed stable disease in 17/33 patients (51%) and 15/18 with mRCC (83%) after two cycles. Median progression-free survival of RCC patients in the expansion phase of the study was 8 months (1.5–30.5).
Conclusions: L19-IL2 can be safely and repeatedly administered at the RD of 22.5 Mio IU IL2 equivalent in advanced solid tumours. Preliminary evaluation suggests clinical activity of L19-IL2 in patients with mRCC.

1. Introduction

One of the most promising new avenues for the development of more selective and efficacious cancer therapies relies on antibody-mediated targeted delivery of bioactive agents to the tumour environment.[1,2] The antibody-based targeted delivery of therapeutics such as cytokines to antigens located within tumour stroma appears to be particularly attractive. The alternatively-spliced extra-domain B (EDB) of fibronectin is an excellent target for such a strategy because it is virtually undetectable in normal healthy adults (exception made for the endometrium in the proliferative phase), but is strongly expressed in stromal and neo-vascular structures during cancer progression.[3]

L19-IL2 is a recombinant fusion protein consisting of human interleukin-2 (IL2) fused to single chain fragment variable [scFv(L19), an antibody fragment specific to EDB.[4–6] ScFv(L19) selectively localises to tumour tissues in animal models of cancer[5,7–11] and its ability to target solid tumours and lymphomas in humans has been demonstrated in two immunoscintigraphic clinical studies in patients with cancer.[12,13] Preclinical biodistribution studies with human and murine tumour-bearing animals showed that L19-IL2 is selectively delivered to the tumour tissue and deposited at high local concentrations over an extended time period (beyond 72 h), resulting in a dramatic enhancement of the therapeutic properties of IL2.[7,14,15] In these models, the inhibitory effects of L19-IL2 on tumour growth were much stronger and more sustained as compared with a control IL2 fusion protein not containing L19 and a free IL2 plus the L19 antibody in an equimolar mixture.[7,14,15] In tumour-bearing mice, the anticancer activity was shown to be mainly mediated by natural killer (NK) cells.[7,14]

Preclinical toxicology studies performed in primates and rats with L19-IL2 are in complete agreement with the results described for human recombinant IL2, and overall they did not raise any safety concerns.

This is the first clinical study of L19-IL2 conducted in cancer patients. Because of known activity of recombinant human IL2 in advanced renal cell carcinoma (RCC) patients, and as EDB is strongly expressed in this tumour type, RCC was considered a relevant target for a treatment with L19-IL2.[16]

2. Patients and methods

2.1. Study design and treatment

This was an open-label, non-randomised, multicentre, phase I/II study. In the dose escalation part of the study, 3–6 patients with advanced solid tumours were recruited into five dosing cohorts according to the modified Fibonacci scheme. The cohorts were sequentially enrolled and doses were escalated according to the following scheme: 5, 10, 15, 22.5, and 30 Mio IU IL2 equivalent (IL2e), corresponding to L19-IL2 doses of 0.835, 1.67, 2.51, 2.76 and 3.01 mg, respectively.[7] In the second part of the study, patients with advanced RCC were to be given a fixed dose of L19-IL2 at the recommended phase II dose (RD). It was anticipated that a maximum of 18 patients would be recruited in the dose escalation part of the study and a total of 12 patients in the expansion phase of the study. No formal sample size calculation was performed.

Clinical-grade L19-IL2 immunocytokine was provided by Philogen S.p.A (Siena, Italy; see also Supplementary Fig. 2).[7]

The primary objective of the study was to determine the maximum administered dose (MAD), maximum tolerated dose (MTD) and RD of L19-IL2. The secondary objectives were to determine the toxicity and pharmacokinetic profile, to characterise the pharmacodynamic activity of L19-IL2 on blood mononuclear cells, to assess the presence of anti-fusion protein antibodies in treated patients, to evaluate the safety profile of repeated administrations of L19-IL2 in patients treated at the RD, and to identify early signs of antitumour activity. The MAD was defined as the dose at which 2 out of 3–6 patients experience dose-limiting toxicity (DLT) during the first cycle. The definition of DLTs is provided in Supplementary material.

2.2. Patient characteristics

Adult patients were included if they had histologically or cytologically confirmed advanced solid cancer (phase I) or renal cell carcinoma (phase II); an Eastern Cooperative Oncology Group (ECOG) performance status of $\leqslant 2$; a life expectancy of at least 12 weeks; no other available standard treatment; at least one measurable lesion as detected by computed tomography (CT); the complete list of inclusion/exclusion criteria is provided in Supplementary material. The local ethical committees approved the study protocol and all patients signed an informed consent form before being admitted into the study. The trial was conducted according to the principles of the latest version of the Declaration of Helsinki and the guidelines for Good Clinical Practice.

2.3. Safety and efficacy assessments

At screening, a medical history and complete physical examination (including ECG, functional spirometry), ECOG performance status, haematology and clinical chemistry analyses, urinalysis, signs and symptoms, vital signs, and disease assessment were performed. Each patient returned for an

end-of-treatment visit within 30 d after the last dose of L19-IL2. The follow-up lasted until the occurrence of subsequent treatment onset, death or latest follow-up, whichever occurred first. Adverse events and toxicities were graded as per NCI-CTCAE version 3.0.[17] Disease status was assessed after every two cycles (corresponding to six infusions) and at study discontinuation using the Response Evaluation Criteria in Solid Tumours (RECIST).[18] Patients remained on study until the occurrence of unacceptable toxicity, disease progression, withdrawal of consent, treatment delay (>2 weeks), or until the maximum of six treatment cycles was reached.

2.4. *Pharmacokinetics*

Blood samples for pharmacokinetic analysis were collected on days 1, 3 and 5 of cycle 1 at the following time-points: prior to infusion, then 30, 55, 80 and 100 min and 2, 4, 7, 11 and 24 h after start of infusion. Analytical methods for pharmacokinetics analysis are described in the legend to Supplementary Table 2.

2.5. *Safety testing in rats and primates*

Preclinical toxicology studies were performed by Inveresk (a Charles River Company, Edinburgh, UK) in order to find a safe first dose for dose escalation studies in man, to identify potential target organs for toxicity, and to evaluate L19-IL2 immunogenicity. The studies were performed in two species considered relevant: rats and monkeys. Performed studies included: L19-IL2 acute intravenous toxicity study in rats; L19-IL2 intravenous 1 h infusion dose range finding study in Sprague–Dawley rats; L19-IL2 intravenous 1 h dose range finding study in cynomolgus monkeys; L19-IL2 four cycles intravenous 1 h infusion toxicity study in cynomolgus monkeys.

Moreover, the following safety pharmacology investigations, aimed at studying potential undesirable pharmacodynamic effects on physiological functions, were performed in cynomolgus monkeys: electrocardiography; ophthalmoscopy; central nervous system; immunotoxicology assessments.

3. Results

3.1. *EDB expression in RCC*

Prior to study initiation, the L19 antibody[11,19] was used to investigate the expression of the alternatively-spliced EDB domain of fibronectin in frozen specimens of human RCC. Clear cell renal cell carcinomas consistently showed strong EDB expression, both in the immunohistochemical and in the immunofluorescence analysis. L19 staining was particularly intense around tumour blood vessels (Supplementary Fig. 1). L19 exhibits no detectable staining in a panel of normal human tissues, exception made for placenta and for the endometrium in the proliferative phase (Supplementary Fig. 2).

3.2. *L19-IL2*

The immunocytokine L19-IL2 used in the clinical trial was produced in mammalian cells[7] and purified to homogeneity as a non-covalent homodimer, consisting of two identical subunits of 42 kDa each. Supplementary Fig. 3 illustrates the schematic structure of L19-IL2 and provides analytical data (SDS–PAGE, gel filtration on a Superdex 200 column and MALDI-TOF analysis), as well as information on product production and formulation. The *in vitro* lymphocyte stimulatory activity of L19-IL2 is indistinguishable from the one of recombinant IL2 on an equimolar basis (Supplementary Fig. 3).

Table 1 – Patient demography and characteristics.

Solid tumours patients from phase I and phase II – demography and characteristics		
Characteristics	*n*	
Screened	38	
Eligible	33	
Male/females (%)	27/6 (82/18)	
Median age (range years)	58 (35–74)	
ECOG PS[a] 0/1 (%)	18/15 (55/45)	
Histology	*Phase I (21) + phase II (12)*	
Renal cell carcinoma (RCC)	18 [Phase I (6) + phase II (12)]	
Colon adenocarcinoma	4	
Melanoma	3	
Rectal adenocarcinoma	2	
Biliary tract cell adenocarcinoma	1	
Breast carcinoma	1	
Thymic carcinoma	1	
Neuroendocrine cervix carcinoma	1	
Peritoneal mesothelioma	1	
Parotid gland carcinoma	1	
RCC patients from phase I ($n = 6$) and phase II ($n = 12$) – baseline characteristics		
Median Age	62	
Range (years)	52–74	
Number of cycles	70	
Median (range)	4 (2–6)	
Characteristics patients ($n = 18$)	*n*	*%*
Females	3	16.7
Males	15	83.3
Histology		
Clear cell RCC	18	100
Non-clear cell RCC	0	0
MSKCC criteria		
Good risk	8	44.4
Intermediate risk	9	50
Poor risk	1	5.6
Line of treatment[b]		
First-line	8	44.4
Second-line	7	38.9
Third-line	3	16.7

[a] ECOG PS = Eastern Cooperative Oncology Group Performance status.

[b] Among the 15 RCC patients who received L19-IL2 at the RD: 10 had previous surgery, two received previous IL2 monotherapy, one received INFα monotherapy, two received previous IL2 + INFα + 5-fluoruracil combination therapy, two were previously treated with TK inhibitors, and two received previous radiotherapy.

3.3. *Safety pharmacology in rats and monkeys*

In studies performed in monkeys and rats, L19-IL2 did not raise any safety concerns and had no effects on the cardiovascular system, central nervous system, nor on the eye at the tested doses. Rats received 1 h intravenous infusions at days 1, 3, and 5 of 0, 1.32, 3.96, or 10.57 mg/kg/d of L19-IL2, which corresponded to 0, 2.5, 7.5 and 20 times the maximum anticipated human dose. Cynomolgus monkeys received 1 h intravenous infusions at days 1, 3, 5, and 8, followed by a post-dose observation period of 16 d, of 0, 0.57, 1.42, or 2.85 mg/kg/d of L19-IL2, which corresponded to 0, 2, 5 and 10 times the maximum anticipated human dose. A description of the main safety pharmacology findings can be found as Supplementary material. The L19-IL2 toxicity effects were in complete agreement with the reported toxicity profile of human recombinant IL2 in the same species.[20,21]

3.4. *Patient characteristics*

Twenty-one patients with progressive cancer were enrolled in the first part of the study (Table 1 and Supplementary Table 1) and treated with L19-IL2 (schedule see Fig. 1). Four patients were recruited at the first dose level, as one experienced two non-drug-related serious adverse events (SAEs; a pleural effusion and a superior vena cava compression due to progressive disease). Five patients were recruited into the second dose level (in order to compensate for a patient who omitted an L19-IL2 dose for a non-drug related thrombocytopenia, and one patient who withdrew from the study for progressive disease). At the first dose level, one patient omitted one dose of L19-IL2 for non-drug related grade 1 thrombocytopenia which starting day 1 cycle 1 prior to treatment, lasting 5 d. The patient also omitted the second dose of cycle 1. Another patient withdrew from study for progressive disease. In the expansion part, 12 progressive RCC patients were enrolled. Baseline characteristics of all the metastatic RCC patients (phase I and expansion phase) are depicted in Table 1. Overall, 102 cycles of L19-IL2 therapy were administered (median three cycles per patient, range 1–6, for RCC patients only, see Table 1). All patients were evaluable for safety and 31 for anticancer activity.

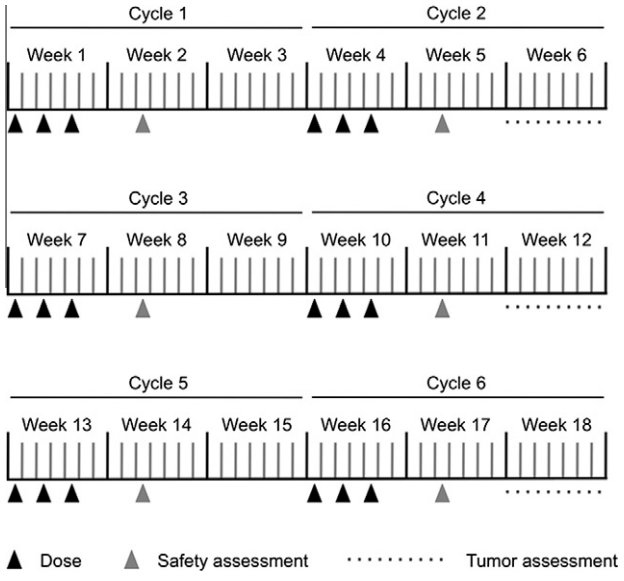

Fig. 1 – Treatment schedule. Patients were screened up to 14 d before the start of the study and then received a minimum of two cycles of treatment. Each cycle comprises treatment on days 1, 3 and 5 (indicated by black arrowheads) followed by 16 d rest (total duration of one cycle is 21 d). At the investigator's discretion, patients may have received up to four further cycles of treatment (i.e. a maximum of six cycles in total). Safety assessments (indicated as grey arrowheads) were performed 5 d after the administration of the third dose of the cycle. The tumour assessments (indicated by dotted lines) were performed according to RECIST: the lesions were measured at screening and at the end of cycles 2, 4 and 6 (between days 14 and 21).

3.5. *Toxicity*

The incidence of treatment-related adverse events is depicted in Table 2. The most frequent adverse events included: fever with chills, nausea, vomiting, asthenia, oedema, skin rash, pruritus, elevated serum creatinine levels and pain at tumour site. Pain at the tumour site is an uncommon effect of human recombinant IL2 that could be related to L19-mediated targeting of this pro-inflammatory cytokine. However, this event occurred only in phase I patients irrespective of the L19-IL2 dose and was adequately controlled with intravenous morphine injections not requiring dose modifications. Signs of mild capillary leak syndrome appeared in two patients at the 15 Mio IU IL2e after repeated administration (3 and 4 doses, respectively), with no clinical intervention necessary. At the 22.5 Mio IU IL2e, a 50% dose reduction was necessary in 4 of 9 patients after repeated doses (12, 12, 5, and 4 doses, respectively) to avoid worsening of capillary leakage. At the 30 Mio IU IL2e, however, L19-IL2 had to be stopped in all patients (after dose 4, 3, and 2, respectively) due to severe capillary leak syndrome or associated complications. Two DLTs occurred at 30 Mio IU: a grade 3 syncope during the first dose of cycle 2 requiring vasopressor support and a grade 2 increase of serum creatinine after the second dose of cycle 1. One DLT occurred at 22.5 Mio IU during cycle 1 (after the second dose) with signs of transient capillary leak. Although the MTD was stated as $\leqslant$1 DLTs during cycle 1 of each dose level, we considered the study closed at 30 Mio IU because two of three patients at this dose level were not able to complete two cycles due to L19-IL2-associated toxicity. In fact, one patient at 30 Mio IU omitted the third dose of cycle 1 because of grade 2 renal failure (DLT) and received cycle 2 at a 50% dose reduction. Additionally, one patient at 22.5 Mio IU required a 25% dose reduction for cycle 2 and a 50% dose reduction was planned for cycle 3 because of grade 2 oedema. Another patient at 22.5 Mio IU received 50% of the planned dose during the fifth cycle and omitted the last day for grade 2 oedema. All other patients received L19-IL2 treatment as planned. During the expansion phase of the study, grade 3 hypertension and hypoxia were recorded in two patients and grade 3 dyspnea in one of these two patients (Table 2). There were no treatment-related deaths.

Table 2 – Incidence of adverse events.

Category of adverse events	Phase I																					Phase II			
	5 Mio IU				10 Mio IU				15 Mio IU				22.5 Mio IU				30 Mio IU				Total	22.5 Mio IU			Total
	G1	G2	G3	Total	G1	G2	G3	Total	G1	G2	G3	Total	G1	G2	G3	Total	G1	G2	G3	Total		G1	G2	G3	
Constitutional symptoms	4	2		4/4	4	2		4/5	3	1		3/3	6			6/6	3	1		3/3	20/21	12	9	1	12/12
Gastrointestinal	3	1		3/4	3			3/5	2			2/3	4	2		5/6	2			2/3	15/21	10	4	1	10/12
Pain	3			3/4	1			1/5	3	1		3/3	4			4/6	2	1		3/3	14/21	8	7		10/12
Dermatology	2			2/4	4			4/5	1			1/3	4			4/6	1			1/3	12/21	12	7	1	12/12
Metabolic/laboratory	2	1		2/4	2			2/5	1			1/3	3			3/6	2	1		2/3	10/21	5	7		9/12
Cardiac general	1			1/4	3			3/5					2			2/6	2	3		3/3	9/21	5	2	2	6/12
Allergy	1			1/4					1			1/3	4			4/6					6/21	1	1		2/12
Blood/bone marrow	1		1	1/4	2			2/5					2	1		2/6					5/21	3	7	5	8/12
Cardiac arrhythmia													2			2/6	3	1		3/3	5/21				
Pulmonary/upper respiratory	1			1/4	1			1/5	2			2/3	1			1/6					5/21	5	3	2	6/12
Renal/genitourinary	1			1/4	1			1/5					1	1		2/6	1			1/3	5/21	3	2		4/12
Lymphatics					1			1/5					1			1/6	1			1/3	3/21	3	2	1	5/12
Infection													2			2/6					2/21		1		1/12
Vascular													1	1		2/6					2/21	1	2	1	3/12
Auditory													1			1/6					1/21				
Neurology																			1	1/3	1/21	6	2		7/12
Ocular/visual																						2	1		3/12
Muscoskeletal/soft tissue																						1	1		2/12

The number of patients who experience a certain side effect over the number of total patients enrolled in each cohort and in each phase of the study is reported. G1 stands for mild, G2 for moderate and G3 for severe adverse events.

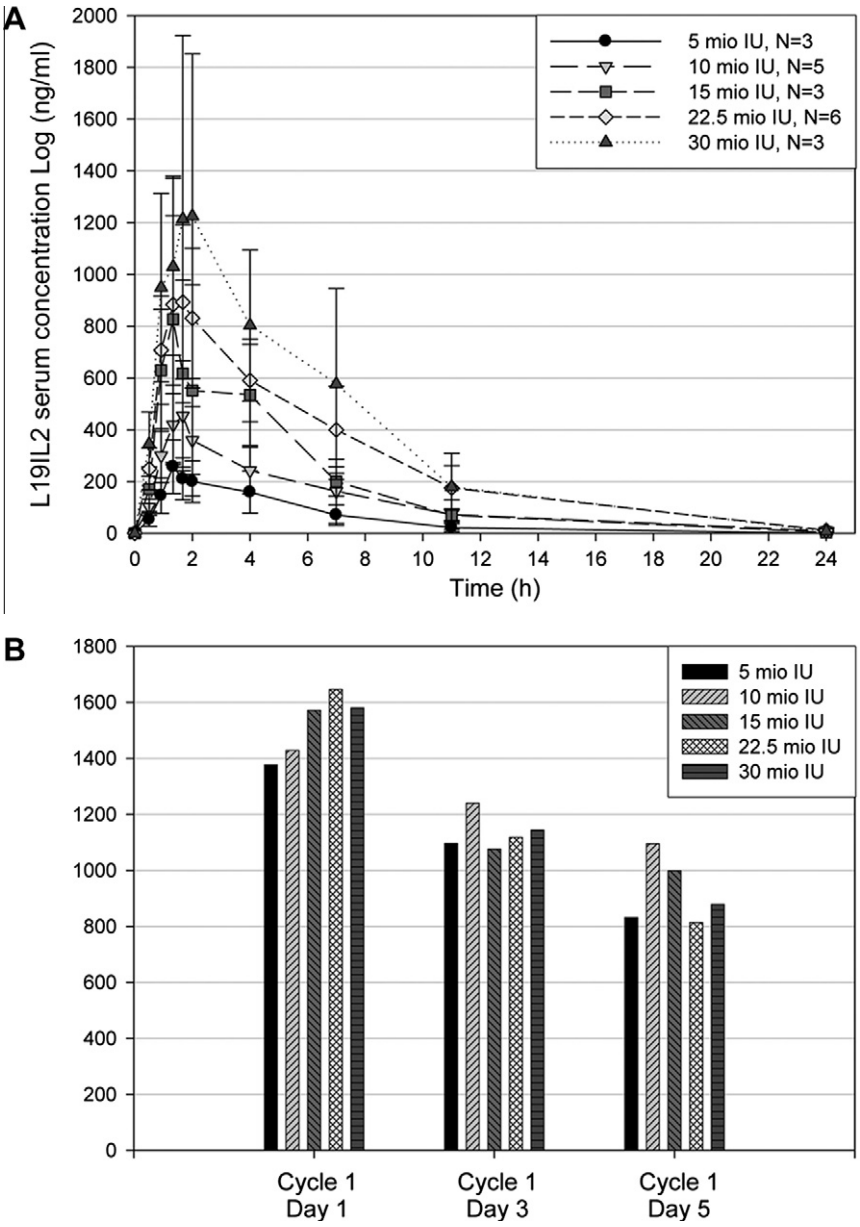

Fig. 2 – (A) Mean concentrations (±standard deviation) of L19-IL2 during and after a 1-h intravenous infusion by dose group. The dose is expressed as IL2 equivalent, N = number of patients. (B) Dose normalised mean maximum L19-IL2 serum exposure (AUC/D) by dose group and treatment day during cycle 1 (dose in IL2 equivalent; AUC = area under the curve; D = dose).

3.6. *Pharmacokinetic analysis*

Pharmacokinetic evaluation was performed on 20 patients enrolled in the phase I. Fig. 2A shows the mean concentrations (±standard deviation) of L19-IL2 by dose group during cycle 1. The Cmax increased dose proportionally within the tested range (Supplementary Table 2) and occurred within 1 h after the end of the 1-h IV infusion. Post-maximum L19-IL2 concentrations decreased with a terminal half-life of 2–3 h and most patients had serum levels below the lower limit of quantification 24 h after the end of each L19-IL2 infusion. No accumulation of L19-IL2 was observed upon subsequent dosing on days 3 and 5. AUC increased in a roughly dose proportional manner (Fig. 2B, Supplementary Table 3). An increase in clearance as indicated by a shortened terminal half-life and an about 40% reduction in AUC was observed after repeated administrations within cycle 1, possibly due to the known vasoactive properties of IL2.

3.7. *Human anti-fusion protein antibodies (HAFA)*

Supplementary Fig. 4 shows that the serum of patients at baseline, day 10 of cycle 1 and day 10 of cycle 2 (i.e. 30 d after the first administration of L19-IL2), in spite of the molar excess used in the assay, never competed the rabbit antiserum, indicating a low immunogenic potential of L19-IL2 even after repeated administrations. The data of these HAFA studies and the observation of IL2 activity (e.g. comparable blood pressure

drop values after infusion) even after 18 administration of L19-IL2 indicate a negligibly low immunogenic potential of L19-IL2 at the doses used.

3.8. *Pharmacodynamic analysis*

Changes in peripheral blood lymphocyte counts have been long noted as a dose-dependent immune modification observed during IL2 therapy. As expected, a peripheral lymphopenia was found on days 2–5 of each therapy cycle, followed by a rebound lymphocytosis on day 10 (Supplementary Fig. 5A). The strongest pharmacodynamic effects were observed for activated NK cells and T8 cells, where substantial increases could be observed during each treatment cycle, and no loss of immune stimulation was seen even after the sixth cycle of L19-IL2 therapy.

In addition, we investigated the levels of circulating Treg cells (defined as CD4+ CD25high Foxp3+) by FACS analysis in patients during the expansion phase of the study. Examples of two representative patients are shown in Supplementary Fig. 5B. An expansion of CD4+/CD25high, CD4+/CD25high/Foxp3+ and CD4+/CD25int/Foxp3+ cells was observed between the first day and the end of the cycle, with a subsequent reduction in cell numbers at later time-points.

3.9. *Response*

The median follow-up was 40 months (range 22–48). Thirty-one patients were evaluable for activity (the remaining two patients progressed after cycle 1). We observed stable disease in 17/33 patients (51%, 15 RCC, 1 biliary tract adenocarcinoma and 1 peritoneal mesothelioma) and in 15/18 with advanced RCC (83%) after two cycles of L19-IL2. In the 18 RCC patients, median progression-free survival (PFS) was 5 months [range 1.5–30.5 (on-going); 95% confidence interval (CI) 3.9–11.5. Median PFS was 6.7 months (range 1.5–30.5; 95% CI 4.3–13.2) in the 15 patients with RCC who received the RD of 22.5 Mio IU (3 in phase I and 12 in the expansion phase of the study) and 8 months (range 1.5–30.5; 95% CI 4.5–15.4) in the 12 patients from the expansion phase. Two patients from the expansion phase of the study, who both received four cycles of L19-IL2, are still free of progression beyond 24 months. A waterfall plot of best responses (defined as the largest shrinkage in the sum of diameters of target lesions at any moment of time, compared to baseline) to L19-IL2 treatment in RCC patients who received the RD is depicted in Fig. 3. Moreover, selected computed tomography scans are reported in Fig. 4, showing minor responses in two patients, one of whom (Fig. 4B) still experiencing long-lasting disease stabilisation.

4. Discussion

This is the 'first-in-man' study of L19-IL2. The results of this study demonstrate that L19-IL2 can be safely administered at 22.5 Mio IU IL2 equivalent dose corresponding to 3.75 mg of fusion protein. All reported toxicities were temporary and resolved completely within a few hours or days of L19-IL2 administration. There was no evidence of motor neuropathy or allergic reactions associated with L19-IL2. All frequent toxicities observed with L19-IL2, except for pain at the tumour site, are commonly associated with IL2 treatment and have also been observed in clinical trials with an immunocytokine consisting of IL2 combined with an anti-GD2 mAb in IgG format.[22] L19-IL2 toxicity worsened after repeated administrations as was expected from preclinical studies.[7] In determining the MTD for the study, we used a more cautious approach compared to the MTD definition as per protocol. In order to protect patients from cumulative toxicities that may develop in subsequent treatment courses, we took into consideration severe L19-IL2-associated toxicities that developed during or after all subsequent treatment cycles. None of three patients enrolled into the 30 Mio IU dose cohort was able to complete the planned treatment (stated as at least two courses) due to adverse events. Thus, the MTD was determined to be 22.5 Mio IU, which was chosen as the RD.

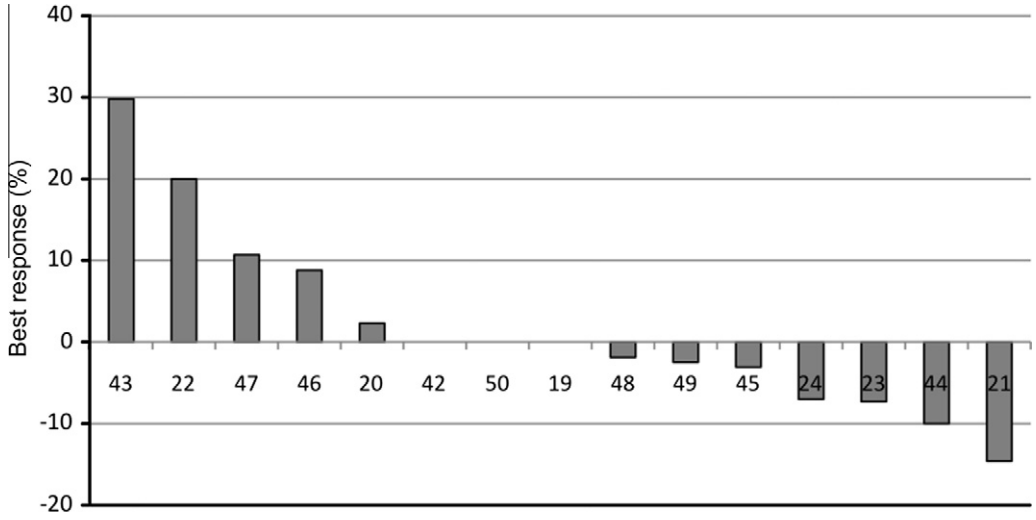

Fig. 3 – Waterfall plot. Best responses, defined as the largest shrinkage in the sum of diameters of target lesions at any moment of time, compared to baseline are reported in the graph. Bars represent individual patients; 15 patients with renal cell carcinoma, who were treated at the RD of 22.5 Mio IU, are shown.

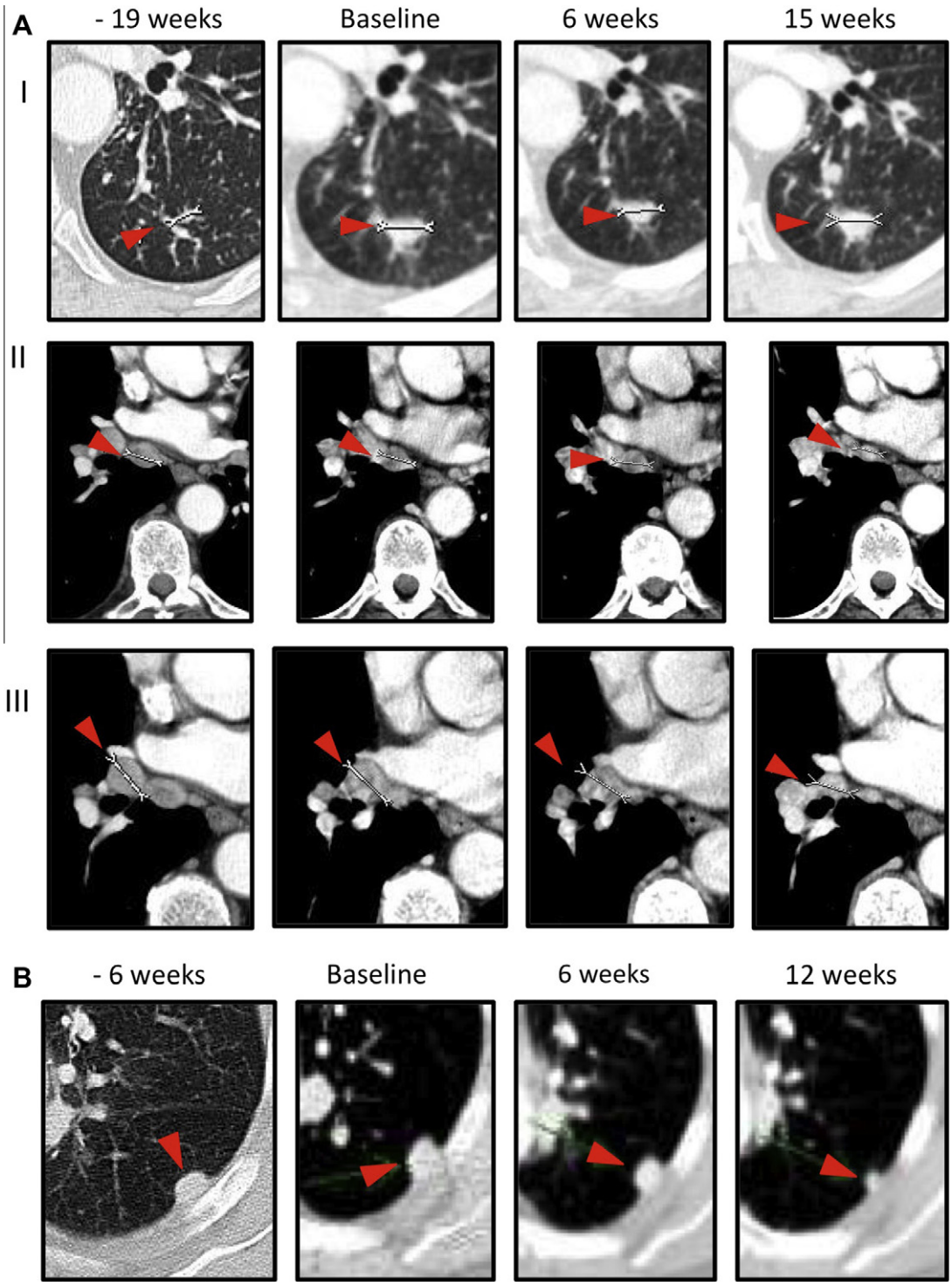

Fig. 4 – Computed tomography documentation of responses observed in two RCC patients. (A) CT images of stabilisation of a previously progressing lung lesion (I: pre-baseline 7 mm, baseline 13 mm, first TA 13 mm, second TA 13 mm) and of minor responses regarding a right hilar lymph node (II: pre-baseline 16 mm, baseline 18 mm, first TA 18 mm, second TA 12 mm) and a subcarenal lymph node (III: pre-baseline 18 mm, baseline 21 mm, first TA 19 mm, second TA 15 mm) of patient no. 21 (best response –14.6%; previous treatment with IL2) are indicated by red arrowheads. CT images were taken 19 weeks before baseline, at baseline and at later time-points indicated in figure. (B) CT images of minor responses of a lung lesion, indicated by the red arrowheads, of patient no. 45 (best response –3%; previous treatment with interferon monotherapy) are shown. CT images were taken at baseline and at later time-points indicated in figure.

The toxicological profile could be correlated with L19-IL2 exposure, as high doses (30 Mio IU) and/or more frequent application of the 22.5 Mio IU dose appeared to coincide with more serious adverse events. No accumulation in L19-IL2 was observed over the three dosing days. Rather, an increase in clearance as indicated by a shortened terminal half-life and a reduction in AUC were observed after repeated administrations within cycle 1, in line with the well-known action of IL2, mediating an increase in vascular permeability.[23]

Pharmacodynamic evaluation revealed that NK cells and T8 cells appear to be the subset of leucocytes which are most upregulated as a result of L19-IL2 treatment. This observation is encouraging, in light of the fact that studies performed with L19-IL2 in tumour-bearing mice revealed a dominant role of NK cells for the therapeutic activity of L19-IL2.[7] A reversible expansion of Treg cells was observed in peripheral blood as a consequence of L19-IL2 treatment. To further support dose selection, data from these studies will be used to help refine the dose–pharmacodynamic response relationship.

Although no objective tumour responses were seen in this phase I/II study, we observed stable disease in 51% of all patients and in 83% of patients with advanced RCC after two cycles. While responses were not durable in patients treated in the phase I part of the study, the median PFS of 8 months in patients with RCC in the expansion phase of the study, who were also mostly pretreated, suggests activity of L19-IL2. Two out of 12 patients from this cohort, who both received four cycles of L19-IL2, are still progression-free after 27.5 and 30.5 months (on-going), respectively, without having received any anticancer treatment after the last L19-IL2 administration. Although conclusions cannot be drawn from such a small study, these results compare well with those published for some of the novel anti-angiogenetic targeted drugs in first- and second-line treatment of advanced RCC.[24–26]

While objective responses have been reported for sunitinib in certain patient sub-populations,[27] objective responses for other drugs and/or RCC patients types (e.g. poor prognosis patients) are infrequent.[24–26] Thus, given the predominant role of disease stabilisation as best response in the systemic treatment of this disease, which has remained essentially unchanged in the era of molecularly targeted therapy, more emphasis may be laid on this clinically relevant type of outcome when evaluating novel anticancer therapies.

Moreover, in all patients in our study who achieved disease stabilisation, L19-IL2 was discontinued during the follow-up, resulting in a progression-free period without toxicity. This is in contrast to the necessity of long-term continuous targeted therapy with anti-angiogenetic drugs, where discontinuation results in rapid re-growth of metastases, except possibly in rare and selected cases of complete responses.[28]

In view of these considerations and of the fact that a considerable portion of patients does not tolerate long-term treatment with tyrosine kinase inhibitors well, there is still a need for safe and efficient agents in advanced RCC and other treatments including IL2 based therapies should be considered.[29]

In conclusion, L19-IL2 can be safely administered to patients with solid tumours at up to 22.5 Mio IU IL2 equivalents. At the tested doses, toxicities were mild and reversible. Preliminary evidence of L19-IL2 efficacy was demonstrated with the induction of stable disease in patients with progressive advanced RCC and by the selective expansion of NK cells in treated patients. The data presented here lay the foundations for future clinical trials with L19-IL2 alone or in combination with approved anti-tumour drugs for advanced solid cancers such as RCC, metastatic melanoma, and others.

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Conflict of interest statement

Manfred Johannsen has received honoraria from Pfizer, Germany, Roche Pharma, Wyeth, Bayer AG and Novartis, Germany; Jan Roigas has received honoraria from Pfizer, Germany, Bayer AG, Wyeth and Roche Pharma; Steffen Weikert has received honoraria from Pfizer, Germany, Wyeth and Novartis, Germany; Kurt Miller is a consultant for Sanofi-Aventis, Novartis, Germany and AstraZeneca, Germany; Valeria Lovato, Eveline Trachsel, Manuela Kaspar are employees of Philochem AG, Zurich, Switzerland; Reinerio Gonzalez is a QA Manager Philogen, Siena, Italy; Leonardo Giovannoni is the Head of CMC Production and Clinical Development Philogen, Siena, Italy; Hans D. Menssen is a Senior Director Global Medical Development Oncology, Bayer HealthCare Pharmaceuticals, Berlin, Germany; Dario Neri is Co-Founder and Shareholder of Philogen, Siena, Italy; the remaining authors declare no potential conflict of interest .

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2010.07.033.

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