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A phase I dose-escalation study of the immunocytokine EMD 521873 (Selectikine) in patients with advanced solid tumours

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Abstract ***Background:*** EMD 521873 (Selectikine), an immunocytokine comprising a DNA-targeting antibody, aimed at tumour necrosis, fused with a genetically modified interleukin-2 (IL-2) moiety, was investigated in this first-in-human phase I study.
Methods: Patients had metastatic or locally advanced solid tumours failing previous standard therapy. Selectikine was administered as a 1-hour intravenous infusion on 3 consecutive days, every 3 weeks. A subgroup of patients also received 300 mg/m^2 cyclophosphamide on day 1 of each cycle. Escalating doses of Selectikine were investigated with the primary objective of determining the maximum tolerated dose (MTD).
Results: Thirty-nine patients were treated with Selectikine alone at dose levels from 0.075 to 0.9 mg/kg, and nine were treated at doses of 0.45 and 0.6 mg/kg in combination with cyclophosphamide. A dose-dependent linear increase of peak serum concentrations and area under curve was found. The dose-limiting toxicity was grade 3 skin rash at the 0.9 mg/kg dose-level; the MTD was 0.6 mg/kg. Rash and flu-like symptoms were the most frequent side-effects. No

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severe cardiovascular side-effects (hypotension or vascular leak) were observed. At all dose-levels, transient increases in total lymphocyte, eosinophil and monocyte counts were recorded. No objective tumour responses, but long periods of disease stabilisation were observed. Transient and non-neutralising Selectikine antibodies were detected in 69% of patients.

Conclusions: The MTD of Selectikine with or without cyclophosphamide administered under this schedule was 0.6 mg/kg. The recommended phase II dose was 0.45–0.6 mg/kg. Selectikine had a favourable safety profile and induced biological effects typical for IL-2.

1. Introduction

Immunocytokines comprise a monoclonal antibody fused to a cytokine. Targeting tumour antigens via the antibody moiety facilitates cytokine enrichment in the local tumour microenvironment, achieving the concentrations required to induce an effective immune response.[1] In murine cancer models, interleukin-2 (IL-2)-containing immunocytokines demonstrated superior dose-dependent antitumour activity compared with equimolar doses of the free cytokine and antibody.[2–4]

IL-2 demonstrates antitumour activity,[5–7] and was the first cytokine to be fused to tumour-targeting antibodies, but similar to free recombinant IL-2, severe hypotension and vascular leak syndrome remained dose-limiting when immunocytokines were tested in patients with advanced solid tumours.[8–11] EMD 521873 or NHS-IL2LT (Selectikine, Merck KGaA, Darmstadt, Germany) is a novel immunocytokine comprising genetically modified IL-2 with decreased vascular toxicity fused with a DNA-targeting antibody moiety, NHS76.

NHS76, a fully human de-immunised monoclonal antibody,[12] binds free DNA fragments without requirements for specific nucleotide sequences or recognised secondary structures (Merck KGaA data on file). DNA is often released from dying tumour cells, either spontaneously, or during their exposure to radiation or chemotherapy.[13–16] Systemic or intra-tumoural administration of TNT1, (iodine131 radio-labelled precursor of NHS76) successfully targeted different tumour types.[17–20]

Selectikine acts predominantly on activated T-cells via the high-affinity IL-2 receptor, and shows minimal activity for the intermediate-affinity receptor, a suspected mediator of vascular toxicity mainly expressed by NK cells.[21] Genetic modification of the fusion junction (M1) of Selectikine has increased its serum half-life.[22]

In preclinical studies, CD8-dependent Selectikine antitumour activity was shown in murine tumour models.[21] Intermittent dosing every three weeks in cynomolgus monkeys was well tolerated, without the induction of cardiovascular side-effects.[18] Mild to moderate side-effects were observed including typical IL-2-target organ toxicity and a transient increase in lymphocyte subpopulations.[21] Selectikine generally demonstrated a better safety profile than other immunocytokines containing unmodified IL-2 (Merck KGaA, data on file).

This first-in-man phase I clinical trial investigated the safety and tolerability of Selectikine in patients with refractory, advanced solid tumours. IL-2 treatment induces an increase in regulatory T-cells, which express the high-affinity IL-2 receptor and can also be activated by Selectikine. Low-dose cyclophosphamide might potentiate the antitumour activity of immunotherapies via depletion of regulatory T-cells,[23–26] and was associated with increased lymphokine-activated killer cells in IL-2 treated melanoma patients.[27] Combining low-dose cyclophosphamide with Selectikine was explored in this trial to investigate if the anticipated effect of Selectikine on regulatory T-cells could be limited.

2. Materials and methods

2.1. Study design

This open-label, multicentre phase I study aimed to determine the Selectikine maximally tolerated dose (MTD) administered alone (group 1) or with low-dose cyclophosphamide (group 2). Secondary objectives were to evaluate pharmacokinetics (PK), immunogenicity, biological and clinical response, progression-free survival (PFS) and overall survival (OS). The regimen was selected based on preclinical data and prior clinical experience with other immunocytokines containing unmodified IL-2.[8–10,21,28]

The study was performed with local ethics committee and regulatory authority approval and in accordance with the declaration of Helsinki and the International Conference on Harmonization.

2.2. Main patient eligibility criteria

All patients gave written informed consent. Eligible patients were $\geqslant$18 years, with Eastern Cooperative Oncology Group performance status of $\leqslant$1, adequate organ function, histologically/cytologically proven solid tumours or B-cell non-Hodgkin's lymphoma after failure of standard therapy. Patients requiring treatment with immunosuppressive agents, or having autoimmune disease, inflammatory bowel disease, chronic viral infec-

tions or significant cardiovascular or pulmonary disease, were ineligible.

2.3. Treatment

Selectikine, (intravenous [iv] infusion over 1 h) was administered on days 1–3 of a 21-day cycle. Group 2 patients received 300 mg/m^2 cyclophosphamide (iv infusion over 1 h), 1 day before the first Selectikine dose in each cycle. Treatment continued until tumour progression or the occurrence of intolerable side-effects. Pre- and co-medication included a non-steroidal anti-inflammatory drug to alleviate flu-like symptoms.

2.4. Dose escalation

The starting dose of 0.075 mg/kg Selectikine was chosen after preclinical evaluation (Merck KGaA data on file). Rapid dose-escalation was planned if no relevant $\geqslant$grade 2 toxicity was observed with two single-patient cohorts. Otherwise, three-patient cohorts were treated per dose level, with expansion to six-patient cohorts in the presence of dose limiting toxicity (DLT). No intra-patient dose-escalation was allowed. Dose-escalation occurred if 0/3 or $\leqslant$1/6 patients experienced DLT, defined as any grade $\geqslant$3 treatment-related adverse events (AEs) occurring in cycle 1. Grade 3 fever and chills lasting <6 h, allergic reactions, grade 3 rash improving to grade 2 in <24 h, grade 3 nausea and vomiting improving with antiemetic treatment, and specified laboratory abnormalities were not considered DLTs. Patients receiving at least one Selectikine dose were evaluable for DLTs. Patients not experiencing DLTs should have received the full scheduled doses in cycle 1 to be included in the DLT analysis, patients discontinuing early for reasons other than toxicity were replaced.

The MTD was the highest dose in cycle 1 in which there were no DLTs/3 patients or $\leqslant$1 DLT/6 patients. Following MTD determination, additional patients were enrolled (group 1a) at dose levels below the MTD to obtain further information on changes in biological parameters.

2.5. Clinical assessments

Assessments were performed on days 1, 3, 4 and 8, and at the end of study visit 28 days after the last dose Selectikine. Vital signs were recorded at the start of infusion, every 15 min until the end of the infusion (EOI), then every 30 min until 2 h after EOI and then hourly until 6 h after EOI. AEs were graded by the National Cancer Institute (NCI) common criteria for AEs (version 3.0). Tumour response was assessed every 6 weeks according to RECIST[29] and patients were followed until death or for $\geqslant$1 year after study closure.

2.6. Pharmacokinetic and pharmacodynamic analysis

Serum Selectikine was analysed by an enzyme-linked immunosorbent assay (ELISA, Supplemental Material). PK analysis was performed using Kinetica™ (version 4.4.1 2002, Innaphase PA, USA). Patient Selectikine PK parameters were determined on day 1 of cycles 1 and 3 by non-compartmental analysis. Single-dose parameters included: maximum serum concentration (C_{max}), time to reach maximum serum concentration (t_{max}), area under the curve (AUC) from time zero to time of last concentration above lower limit of quantification ($AUC_{0\text{-}t}$), AUC from time zero to infinity ($AUC_{0\text{-}\infty}$), and terminal half life ($t_{1/2}$). Accumulation was assessed by comparing the day 1 values for $AUC_{0\text{–}t}$ and C_{max} between cycles 1 and 3 and the EOI and pre-infusion concentrations on days 1 and 2 of cycles 1–3. Selectikine dose proportionality was assessed by plotting the dose-dependent parameters C_{max} and $AUC_{0\text{-}t}$ as a function of various absolute dose levels administered on day 1 of cycle 1.

2.7. Immunogenicity

Serum Selectikine antibodies were detected using a bridging ELISA (Supplemental Material).

2.8. Statistical methods

Descriptive statistics were used for baseline characteristics, safety assessments, PK and immunogenicity variables. Survival analyses were performed by the Kaplan–Meier method. Counts of leukocyte subsets were compared between days 1 and 8 of the first two cycles using repeated measurements mixed model analysis of variance (ANOVA). Full statistical analyses are described in the Supplemental Material.

3. Results

3.1. Patient characteristics and MTD determination

Between December 2006 and May 2009, 48 patients were enrolled: 39 were treated with Selectikine alone (group 1) and nine with Selectikine plus cyclophosphamide (group 2) (Table 1). Thirty-seven patients were evaluable for DLT and 42 for efficacy.

Selectikine dose levels and DLTs are summarised in Table 2. In group 1, two potential DLTs were observed at 0.3 mg/kg, the study was continued at the previous dose level (0.15 mg/kg) with no further DLTs. The protocol was subsequently amended to exclude asymptomatic intermittent laboratory changes as DLTs, and doses were escalated by 50% instead of 100% dose-increments up to 0.9 mg/kg. Four DLTs were reported: one grade 3 dyspnoea (0.3 mg/kg) and three grade 3 skin rash (one

Table 1
Patient and disease characteristics at baseline.

Characteristic	Group 1 ($n = 39$)	Group 2 ($n = 9$)
Median age, years (range)	60 (24–76)	52 (31–78)
Sex, *n* (%)		
Male	22 (56)	3 (33)
Female	17 (44)	6 (67)
ECOG PS, *n* (%)		
0	29 (74)	7 (78)
1	10 (26)	2 (22)
Tumour type, *n* (%)		
Colorectal carcinoma	15 (39)	1 (11.1)
Ovarian carcinoma	6 (15)	1 (11.1)
Prostate carcinoma	3 (8)	0
Renal cell carcinoma	4 (10)	0
Skin carcinoma	2 (5)	2 (22.2)
Other	9[a] (18)	5[b] (55.5)
Time from metastatic/recurrence diagnosis to study entry, Median, (range in years)	2.39 (0.1–6.2)	2.05 (1.3–8.7)
Previous therapy[c]		
Surgery	35 (90)	7 (78)
Chemotherapy	36 (92)	9 (100)
Radiotherapy	16 (41)	6 (67)
Monoclonal antibody	15 (39)	2 (22)
Hormonal	6 (15)	1 (11)
Immunotherapy	7 (18)	3 (33)
Other	14 (36)	1 (11)

ECOG PS, Eastern Cooperative Oncology Group performance status.
[a] One of each: adrenal gland carcinoma; breast carcinoma; hypopharyngeal carcinoma; non small cell lung carcinoma; small cell lung carcinoma; osteosarcoma; pleural mesothelioma; urothelial carcinoma and endometrial carcinoma.
[b] One of each: parotid gland carcinoma; synovial sarcoma; tongue carcinoma; thymic carcinoma; nasopharyngeal carcinoma.
[c] Excluding palliative radiotherapy and surgery.

Table 2
Selectikine dosing and DLTs.

Dose level (mg/kg)	Number of patients			DLT and grade 3 toxicity[a]
	Group 1 ($n = 28$)	Group 2 ($n = 9$)	Group 1a ($n = 11$)	
0.075	1	0	5	None
0.15	6	0	0	One patient with grade 3 lymphopenia and PTT increase, not clinically relevant (no DLT recorded as per amended protocol)
0.225[b]	3	0	3	None
0.3	6	0	0	1 DLT; grade 3 dyspnoea without evidence of vascular leak (no oedema or infiltrates on high resolution CT scan), finally assessed as related to tumour progression One patient with grade 3 increase in lipase, not clinically relevant (no DLT recorded as per amended protocol)
0.45[b]	3	3	3	None
0.6	6	6	0	1 DLT; grade 3 rash in group 1 and 1 DLT; grade 3 rash in group 2
0.9	3	0	0	2 DLTs; grade 3 rash

DLT, dose limiting toxicity; PTT, partial thromboplastin time; CT computed tomography.
[a] Grade 3 toxicities after cycle 1 in group 1a only.
[b] These doses were included following suspected DLTs at the 0.3 mg/kg dose. Following a protocol amendment to exclude asymptomatic intermittent laboratory changes from DLT, Selectikine dosing was returned to the previous dose level and dose escalation restarted by 50% instead of 100% dose increments.

at 0.6 mg/kg and two at 0.9 mg/kg). The Selectikine MTD was determined as 0.6 mg/kg.

Eleven patients (group 1a) were subsequently enrolled and treated with Selectikine at 0.075, 0.225 and 0.45 mg/kg in a sequential order.

Nine patients were treated in group 2 (Selectikine 0.45 mg/kg and 0.6 mg/kg), one experienced a DLT (grade 3 skin rash at 0.6 mg/kg).

The median number of cycles per patient was 2 (range 1–10). The majority of patients ($n = 20$) received 2

treatment cycles. Treatment was discontinued in 37 patients (77.1%) due to progressive disease (PD), and in 11 (22.9%) due to toxicity.

3.2. Safety

Dose-dependent skin rash was the most frequently reported AE (48%) and generally improved rapidly with the use of topical steroids and antihistamine agents. Fever (⩽40 °C) occurred in 40% of patients at doses ⩾0.225 mg/kg (Table 3). Other AEs appeared to be independent of dose and included lymphopenia (44%), dry skin (40%) and mild (grade 1) hypotension (31%).

Fourteen patients discontinued treatment due to AEs: grade 2 leukocytoclastic vasculitis ($n = 1$), grade 2 autoimmune thyroiditis ($n = 1$), dyspnoea ($n = 1$), transaminase increase ($n = 1$), grade 3 skin rash ($n = 7$), exacerbation of psoriasis ($n = 1$) and grade 2 and 3 angioedema ($n = 2$). The main reason for study withdrawal was PD reported in three of these patients.

In both patient groups transient changes in lymphocyte counts were found, which decreased on day 3, returning to normal and increasing significantly above baseline on day 8 (Supplemental Material).

3.3. Pharmacokinetics

PK analysis was performed on all 48 patients during cycle 1 and on nine patients during cycle 3. Post-dose concentrations were above the lower limit of quantification (LLOQ) for all dose levels. Peak serum Selectikine concentrations were reached at the EOI, thereafter declining continuously without a noticeable distribution phase.

At the end of day 1, quantifiable concentrations were detectable in all 24-hour samples (except the 0.075 mg/kg dose; Fig. 1). Descriptive statistics are shown in Table 4. Individual C_{max} and AUC values (group 1, day 1, of cycle 1) analysed as a function of absolute administered dose (mg) indicated a proportional increase in C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ up to a threshold of approximately 40 mg, which corresponded to a dose-range between 0.45 and 0.6 mg/kg (Figs 2A–C).

Analysis of within-cycle accumulation showed that pre-dose concentrations did not increase from days

Table 3
Treatment-related adverse events (AEs) occurring in more than 20% of patients by different dose levels in groups 1 and 2.

Adverse event	Dose levels (mg/kg)									
	Group 1, *n*							Group 2, *n*		
	0.075 (*n* = 6)	0.15 (*n* = 6)	0.225 (*n* = 6)	0.3 (*n* = 6)	0.45 (*n* = 6)	0.6 (*n* = 6)	0.9 (*n* = 3)	0.45 (*n* = 3)	0.6 (*n* = 6)	Total, *n* (%) all doses (*n* = 48)
Rash										
Any grade	1	3	3	3	3	3	2	2	3	23 (47.9)
Grade 1/2	1	3	3	3	2	2	0	2	2	18 (37.5)
Grade 3/4	0	0	0	0	1	1	2	0	1	5 (10.4)
Erythema										
Any grade	2	0	2	1	1	4	0	0	3	13 (27)
Grade 1/2	2	0	2	1	1	4	0	0	3	13 (27)
Grade 3/4	0	0	0	0	0	0	0	0	0	0
Dry skin										
Any grade	4	2	1	2	1	3	1	2	3	19 (39.6)
Grade 1/2	4	2	1	2	1	3	1	2	3	19 (39.6)
Grade 3/4	0	0	0	0	0	0	0	0	0	0
Fever										
Any grade	0	0	2	2	4	3	3	2	3	19 (39.6)
Grade 1/2	0	0	2	2	4	3	3	2	3	19 (39.6)
Grade 3/4	0	0	0	0	0	0	0	0	0	0
Lymphopenia										
Any grade	1	3	4	3	1	2	0	2	5	21 (43.8)
Grade 1/2	1	0	3	1	1	1	0	1	2	10 (20.8)
Grade 3/4	0	3	1	2	0	1	0	1	3	11 (22.9)
Hypotension										
Any grade	2	2	2	2	2	2	0	1	2	15 (31.2)
Grade 1	2	2	2	2	2	2	0	1	2	15 (31.2)
Grade 2/3/4	0	0	0	0	0	0	0	0	0	0
Asthenia										
Any grade	0	2	2	1	1	1	2	1	1	11 (22.9)
Grade 1/2	0	2	2	1	1	1	2	1	1	11 (22.9)
Grade 3/4	0	0	0	0	0	0	0	0	0	0

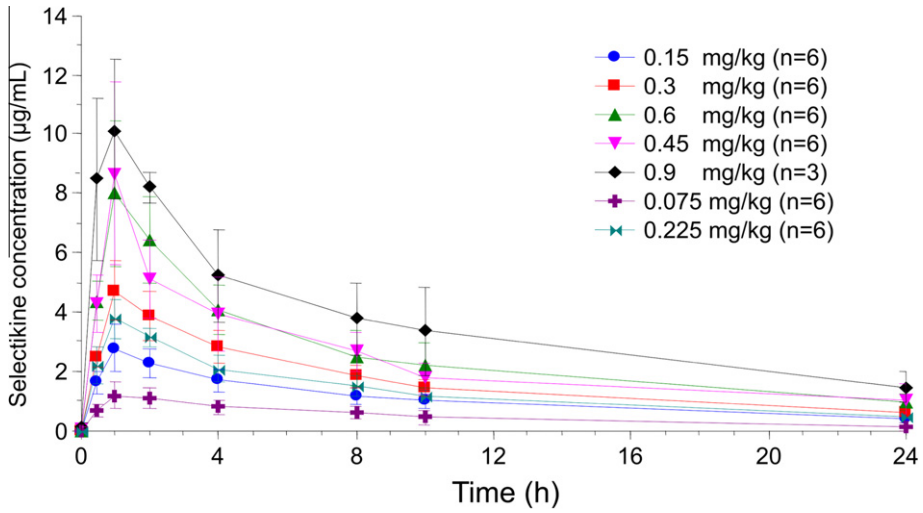

Fig. 1. Mean (standard deviation) concentration–time profile for Selectikine after the first infusion (day 1) of cycle 1.

2–3 as indicated by mean trough$_{(day3)}$/trough$_{(day2)}$ ratios across all dose-levels (1.15, 1.14 and 1.04 for cycles 1, 2 and 3, respectively). The corresponding $C_{max(day3)}/C_{max(day2)}$ ratios did not reveal significant day-to-day accumulation (0.97, 1.14 and 1.03 for cycles 1, 2 and 3, respectively). Predose concentrations on day 1 of cycle 3 were mainly below the LLOQ, suggesting no measurable accumulation from the previous cycle. Nevertheless a 1.3–1.9 fold increase for C_{max} and a 1.8–2.6 increase for $AUC_{0\text{-}t}$ were observed in cycle 3, when compared to cycle 1. The $t_{1/2}$ ranged between 7.8 and 11 h in cycle 1 and between 9.5 and 18 h in cycle 3 in both groups. Pre-treatment with cyclophosphamide may result in a slight decrease in exposure to Selectikine, but this was not statistically significant.

3.4. Immunogenicity

Serum Selectikine antibodies were detected in 33/48 patients (69%), with peaks occurring on day 8 of cycle 1 in the majority. A decline in antibody detection with subsequent administrations suggested a transient immune response against Selectikine. Antibodies did not appear to be neutralising as they influenced neither the PK nor the biological activity of Selectikine. Increases in total lymphocytes, CD4+, CD8+ and NK cells in cycles 1 and 2 were not reduced by the presence of Selectikine antibodies (data not shown).

3.5. Antitumour activity

No tumour responses were recorded, however SD for >6 weeks was noted in 12/48 (25%) patients. Median survival time was 9.6 months (95% CI 5.6–16.4) in group 1 and 7.0 months (95% CI 4.3–23.2) in group 2.

Selectikine treatment was associated with a potential clinical benefit in four patients, one patient with refractory recurrent small cell lung cancer (stable for >10 months), two patients with colorectal cancer and one patient with ovarian cancer (Supplemental Material).

4. Discussion

In this first-in-human phase I study, Selectikine treatment was associated with typical IL-2-like biological effects including lymphopenia followed by lymphocytosis and eosinophilia at all dose levels, while IL-2 related clinical AEs were mainly mild to moderate. DLT was skin rash, which responded well to topical corticosteroids.

In previous studies with intermediate to high IL-2 doses, or with other IL-2-based immmunocytokines, vascular leak syndrome and hypotension were common side-effects, thought to be due to activation of the intermediate-affinity IL-2 receptor and direct activation of endothelial cells.[8–10] In contrast, Selectikine induced only mild hypotension and no vascular leak syndrome demonstrating improved cardiovascular tolerability of the modified and more selective IL-2 moiety.

Following administration, Selectikine PK appeared to be linear. The increased exposure and prolonged Selectikine half-life in cycle 3 was surprising; predose levels measured at day 1 were below LLOQ and no identifiable changes in physiological factors, which might have impacted on clearance rate or volume of distribution were found. While the reported dose–exposure relationship for Selectikine was similar to that observed for other structurally related fusion proteins, the Selectikine half-life was notably longer than that reported for other IL-2 immunocytokines or unmodified recombinant IL-2.[8,9,28] This comparatively longer half-life can be attributed to the lysine to alanine amino acid substitution in the junction sequence between antibody and IL-2 component. This effect is assumed to be independent of binding to either Fcγ receptors or the Fc protection receptor, but was associated with changes in susceptibility to intracellular proteases.[22]

Table 4
Main pharmacokinetic (PK) parameters of serum Selectikine obtained on day1 following first infusion cycle 1 and cycle 3.[a]

Parameter[a]	Treatment groups/cycle/dose (mg/kg)													
	Group 1/cycle 1							Group 1/ cycle 3				Group 2/cycle 1		Group 2/cycle 3
	0.075 ($n = 6$)	0.15 ($n = 6$)	0.225 ($n = 6$)	0.3 ($n = 6$)	0.45 ($n = 6$)	0.6 ($n = 6$)	0.9 ($n = 6$)	0.075 ($n = 3$)	0.15 ($n = 3$)	0.3 ($n = 1$)	0.6 ($n = 1$)	0.45 ($n = 3$)	0.6 ($n = 6$)	0.6 ($n = 1$)
Dose, mg														
Mean	5.2	12.4	16.4	23.4	30.9	38.2	73.2	5.6	10.3	30	27.6	28.4	43.3	35.0
Co-efficient of variation (CV) %	33.7	14.3	20.9	15.5	24.2	21.1	3.9	34.9	19.3			9.3	15.6	
Range	2.9–7.6	9.8–14.3	13.0–21.4	19.7–30.0	20.0–39.6	27.6–49.0	70.0–75.6	3.6–7.5	8.6–12.5			26.1–31.3	33.0–50.0	
$C_{max,}$ μg/mL														
Mean	1.1	2.7	3.7	4.7	8.2	7.8	10.5	1.8	3.1	8.6	11.4	6.2	7.4	7.6
CV%	46.0	26.2	18.0	21.7	34.7	30.5	18.5	32.5	17.8			21.3	42.1	
Range	0.5–1.8	2.0–4.2	2.9–4.6	3.5–6.3	6.0–12.7	5.4–10.7	8.5–11.8	1.5–2.6	2.6–3.6			5.2–7.8	4.3–12.1	
$t_{max,}$ hrs														
Median	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.0	1.0	1.0	1.0
Range	1.0–2.0	1.0–1.1	1.0–1.4	1.0–2.0	1.0–1.1	1.0–2.0	0.5–1.0	1.0–1.1	1.0–1.1			1.0–1.0	1.0–1.0	
$AUC_{0\text{-}t}$ μg/mL*h														
Mean	7.9	24.0	30.1	39.0	55.0	57.8	83.3	15.5	40.8	110.9	153.4	44.8	46.6	84.1
CV%	67.9	22.9	9.2	17.1	28.5	24.8	29.1	92.0	28.9			20.4	31.8	
Range	2.9–5.9	16.9–32.2	26.6–33.6	30.1–47.1	33.5–73.9	39.0–69.9	63.8–112.5	6.5–29.9	29.5–50.0			37.0–55.3	33.6–78.4	
$AUC_{0\text{-}\infty}$ μg/mL*h														
Mean	12.0	29.0	35.2	47.7	68.6	70.0	106.3	21.6	60.3	171.4	249.5	52.5	56.2	115.9
CV%	52.3	25.1	11.5	16.2	33.1	21.2	27.2	104	44.4			20.3	35.2	
Range	5.0–19.8	18.9–39.7	29.8–39.5	36.1–55.7	40.3–105.1	47.7–81.7	89.4–144.5	8.4–44.5	37.2–82.0			43.8–65.2	39.4–94.4	
$t_{1/2}$, hrs														
Mean	7.8	9.3	8.3	9.9	10.0	9.5	11.1	9.5	14.0	15.5	17.7	8.7	10.1	12.5
CV%	31.2	19.0	12.6	12.7	29.2	17.3	26.5	72.7	27.9			4.8	19.8	
Range	5.2–11.9	7.2–12.3	6.6–9.7	8.6–11.6	7.5–15.5	8.0–12.7	8.6–14.4	4.5–14.5	10.2–16.9			8.4–9.2	8.4–14.5	

$AUC_{0\text{-}t}$, area under the serum concentration–time curve from time zero to the time of the last concentration above the lower limit of quantification; $AUC_{0\text{-}\infty}$, area under the serum concentration–time curve from time zero to infinity; C_{max}, maximum observed serum concentration; CV, co-efficient of variation; $t_{1/2}$, terminal half life; t_{max}, time to reach maximum serum concentration.

[a] Means are geometric means, apart from means for dose.

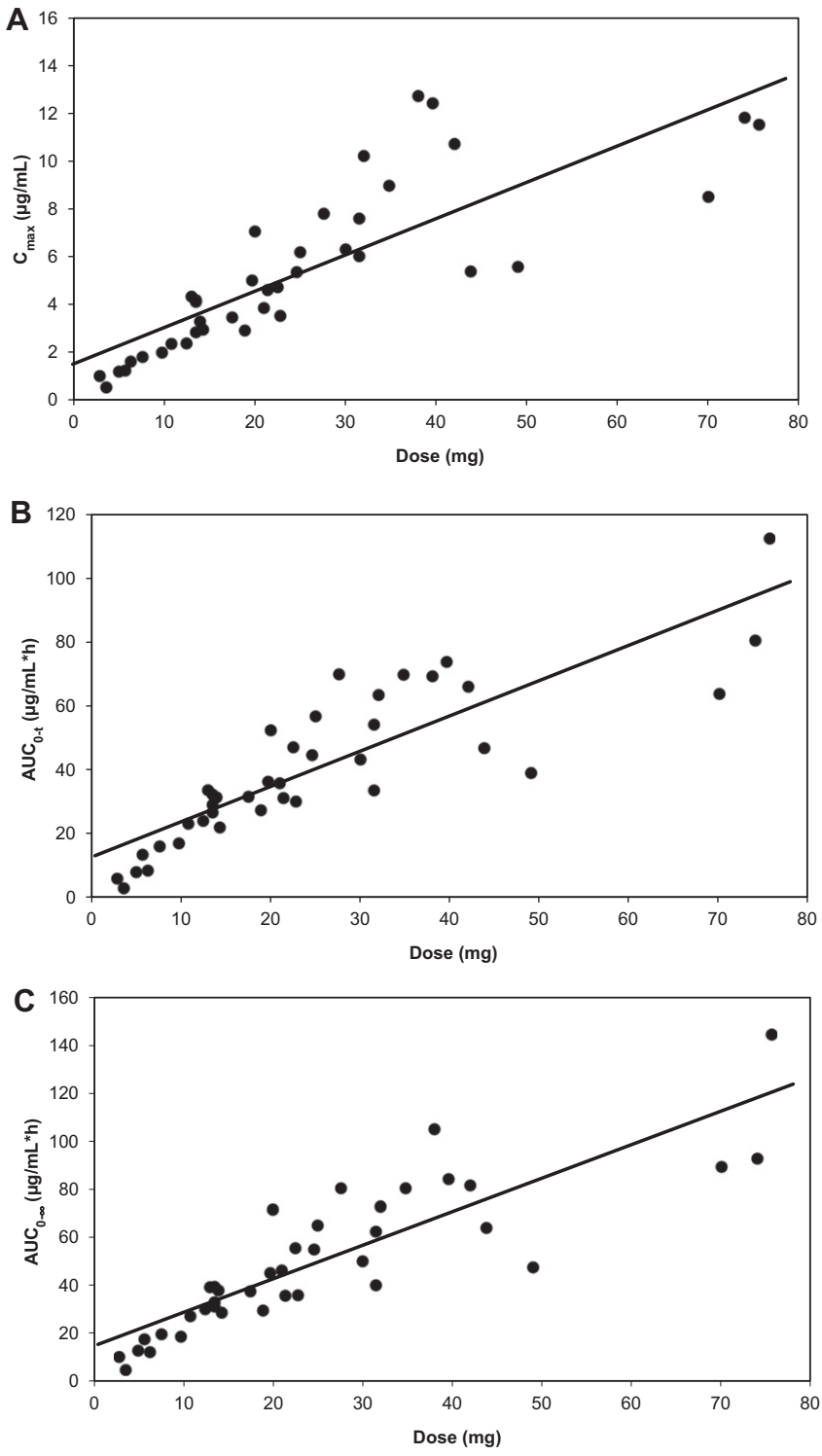

Fig. 2. Dose proportionality of PK parameters for group 1 during cycle 1. (A) C_{max}, (B) $AUC_{0\text{-}t}$ and (C) $AUC_{0\text{-}\infty}$. C_{max}, maximum observed serum concentration; $AUC_{0\text{-}t}$, area under the serum concentration–time curve from time zero to the time of the last concentration above the lower limit of quantification; $AUC_{0\text{-}\infty}$, area under the serum concentration–time curve from time zero to infinity.

A transient clinically irrelevant immunogenic effect was observed in the majority of patients (69%). Antibody responses were also observed after treatment with other IL-2-immunocytokines.[8–10] Interestingly, preliminary immunogenicity data from a phase I trial with L19-IL-2, consisting of the recombinant human antibody fragment L19 (highly specific for the extra domain B of fibronectin), fused with unmodified human IL-2 indicate a very low immunogenic potential.[9]

Antitumour activity was reported in this heterogeneous and heavily pretreated patient population as prolonged disease stabilisation and a transient drop in

tumour markers. Combination or intercalation of immunocytokine treatment with chemotherapy or radiation may facilitate the generation of an effective antitumour immune response by increased tumour antigen release, and activation of dendritic cells and tumour antigen specific T-cells.[30] Also IL-2 containing immunocytokines may be clinically more effective if administered in patients with less tumour burden or non-bulky tumour disease. In a phase II study in children with refractory neuroblastoma, whereas no responses were observed in children with bulky metastatic disease, complete responses occurred in 5/23 (21.7%) children with low disease burden.[31]

The data indicate that low-dose cyclophosphamide can be safely combined with Selectikine without the need for dose reductions of the immunocytokine. While the PK was not appreciably modified, the number of patients treated with cyclophosphamide is too low to draw reliable conclusions. Detailed analyses of a potential effect on immune responses are ongoing.

In conclusion, compared with historical data reporting IL-2 treatment, improved cardiovascular tolerance and an overall favourable safety profile were demonstrated for Selectikine, a novel immunocytokine with selective IL-2 activity targeting free DNA. DLT was skin rash and a dose of 0.45–0.6 mg/kg was recommended for further phase II evaluation. Further trials of this compound in combination with standard chemotherapy and radiotherapy or in patients with limited tumour burden are warranted.

Conflict of interest statement

Julien Laurent and Bernd Liedert are employees of Merck KGaA, Darmstadt, Germany. Maria Mattiaci was an employee of Merck Serono S.A. – Geneva, Geneva, Switzerland, a branch of Merck Serono SA, Coinsins, Switzerland, an affiliate of Merck KGaA, Darmstadt, Germany, until June 2011. Daniel Kramer an employee of Merck-Serono, Grafing, Germany. Ulrike Gnad-Vogt was an employee of Merck KGaA, Darmstadt, Germany from 2005 to 2008 and has received consultancy fees from Merck Serono from 2008 to 2011.

Role of the funding source

The legal sponsor of the study was Merck KGaA, Darmstadt, Germany. The sponsor was responsible for data management and statistical analysis. The clinical study was designed in collaboration with the study coordinating investigator RS (EudraCT number 2006-002083-26). Editing of this manuscript was commissioned by the sponsor. The corresponding author (and all authors) had full access to all study data, and all authors made the final decision to submit the manuscript for publication.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejca.2012.07.015.

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