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Clinical Cancer Research

A Dose-Escalation and Signal-Generating Study of the Immunocytokine L19-IL2 in Combination with Dacarbazine for the Therapy of Patients with Metastatic Melanoma

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Abstract

Purpose: L19-IL2 is an immunocytokine composed of an antibody fragment specific to the EDB domain of fibronectin, a tumor angiogenesis marker, and of human interleukin-2 (IL2). L19-IL2 delivers IL2 to the tumor site exploiting the selective expression of EDB on newly formed blood vessels. Previously, the recommended dose of L19-IL2 monotherapy was defined as 22.5 million international units (Mio IU) IL2 equivalents. In this study, safety and clinical activity of L19-IL2 in combination with dacarbazine were assessed in patients with metastatic melanoma.

Experimental Design: The first 10 studied patients received escalating doses of L19-IL2 on days 1, 3, and 5 in combination with 1 g/m^2 of dacarbazine on day 1 of a 3-weekly therapy cycle. Subsequently, 22 patients received L19-IL2 at recommended dose plus dacarbazine. Up to six treatment cycles were given, followed by a maintenance regimen with biweekly L19-IL2.

Results: The recommended dose of L19-IL2 in combination with dacarbazine was defined as 22.5 Mio IU. Toxicity was manageable and reversible, with no treatment-related deaths. Twenty-nine patients were evaluable for efficacy according to Response Evaluation Criteria in Solid Tumors (RECIST). In a centralized radiology analysis, eight of 29 (28%) patients achieved a RECIST-confirmed objective response, including a complete response still ongoing 21 months after treatment beginning. The 12-month survival rate and median overall survival of the recommended dose–treated patients ($n = 26$) were 61.5% and 14.1 months, respectively.

Conclusions: The repeated administration of L19-IL2 in combination with dacarbazine is safe and shows encouraging signs of clinical activity in patients with metastatic melanoma. This combination therapy is currently evaluated in a randomized phase II trial with patients with metastatic melanoma. *Clin Cancer Res; 17(24); 7732–42. ©2011 AACR.*

Introduction

Melanoma is the most aggressive type of skin cancer, causing over 8,700 estimated deaths per year in the United States alone (1, 2), and having a very poor prognosis, well reflected in a 5-year survival rate of less than 5% (3).

Current therapy approaches for treating metastatic melanoma include cytotoxic chemotherapy, immunotherapy, and the combination of the two. Until the recent approval of ipilimumab and vemurafenib, the melanoma therapy landscape was dominated by two agents, dacarbazine and interleukin-2 (4, 5). Dacarbazine is the only chemotherapeutic

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

T.K. Eigentler and B. Weide contributed equally to this work.

Clinical Trial registration: Clinicaltrials.gov. Identifier: NCT01055522.

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Translational Relevance

Ever since its approval in 1976, dacarbazine represents the standard of care for the treatment of patients with metastatic melanoma; however, only a minority of patients with melanoma respond to dacarbazine treatment. This article presents clinical evidence that the combination of dacarbazine with the tumor-targeting immunocytokine L19-IL2 (a biopharmaceutical consisting of the human recombinant antibody L19, specific to the alternatively spliced EDB domain of fibronectin, fused to human interleukin-2) is well tolerated in patients with metastatic melanoma and can induce long-lasting objective responses in a subset of patients.

agent approved by U.S. Food and Drug Administration (FDA); when given as single agent, it shows response rates between 5% and 15% (6). However, responses are rarely durable and most patients experience disease relapse after a few weeks or months (7). Despite such modest efficacy, dacarbazine still represents the reference treatment of metastatic melanoma, chosen as comparative arm in many clinical trials (8). Some immunotherapeutic approaches have led to more durable responses in a small number of patients. In 1998, FDA approved high-dose bolus interleukin-2 (IL2) for the treatment of metastatic melanoma, based on its ability to mediate durable and complete responses in a small subset of patients (9–11). However, major toxicities associated with high-dose IL2 limit its administration to patients with excellent performance status (8).

In consideration of the significant toxicities associated with IL2 treatment, monoclonal antibodies have been proposed as "vehicles" for the selective delivery of this immune-stimulatory cytokine to the tumor environment, thus sparing normal tissues (12–15). Reisfeld and collaborators have pioneered the use of full immunoglobulins, featuring a C-terminal fusion of IL2 with the heavy chain of the IgG molecule (16). This strategy has been moved to clinical trials in patients with neuroendocrine tumors (17) and melanoma (18, 19), using antibodies specific to EpCAM and GD2-Ganglioside, respectively. Such approaches have shown good safety profile and favorable pharmacokinetics but limited signs of antitumor activity (18, 19). To avoid the generation of multifunctional therapeutic proteins featuring the simultaneous presence of an antigen-binding moiety, IL2, and of the Fc antibody portion which could cross-link the cytokine onto cells carrying Fcγ receptors, we constructed smaller fusion proteins between a human antibody in the single-chain variable fragment (scFv) format and recombinant human IL2 (20–23). In particular, the monoclonal antibody L19, specific to the alternatively spliced extra-domain B (EDB) of fibronectin, appeared to be an ideal candidate for the pharmacodelivery of IL2, in view of its very limited binding to normal mature tissues and its ability to selectively localize to newly formed blood vessels in almost all cancers, irrespective of histopathology (22). L19-IL2 had been administered as monotherapy to patients with renal cell carcinoma and other solid tumors in a phase I/II study (23), and the recommended dose (RD) for monotherapy was determined to be 22.5 million international units (Mio IU) IL2 equivalents.

Here, we report safety and activity results of the L19-IL2/dacarbazine combination therapy in 32 patients with metastatic melanoma.

The clinical development of L19-IL2 in metastatic melanoma was justified not only by the previous reports of therapeutic activity of recombinant human IL2 in this indication (9, 10, 24) but also by the preclinical observation that L19 stains tumor tissues in biopsy sections of human melanomas (25) is capable of selective accumulation in neoplastic lesions in patients with metastatic melanoma and that it does not bind to the vast majority of normal adult human tissues (23). Furthermore, a fluorescent *ex vivo* immunostaining analysis conducted on biopsies taken 24 hours after injection of L19-IL2 confirmed that the immunocytokine selectively localizes to vascular structures within melanoma metastases (Supplementary Fig. S1).

Materials and Methods

Patient characteristics

In the dose-escalation part of the study, 3 to 4 patients were recruited into each of 3 sequential dosing cohorts. Doses were escalated using the following scheme: 10, 15, and 22.5 Mio IU IL2 equivalent (IL2e; refs. 22, 26), each in combination with 1 g/m^2 of dacarbazine. In the following expansion of the last cohort, named phase IIb step 1, 22 enrolled patients received the RD of 22.5 Mio IU of L19-IL2 in combination with 1 g/m^2 of dacarbazine.

Adult patients with histologically or cytologically confirmed unresectable metastatic (stage IV) non-uveal melanoma were enrolled into the study if following features applied: measurable disease defined as at least one lesion that could be accurately and serially measured per Response Evaluation Criteria in Solid Tumors (RECIST), cutaneous lesions measuring at least 1 cm were considered measurable; prior chemotherapy including dacarbazine for metastatic melanoma were allowed if treatment had been completed >6 months prior to study entry; an Eastern Cooperative Oncology Group (ECOG) performance status of $\leq$2; a life expectancy of at least 12 weeks; lactate dehydrogenase (LDH) < 2 $\times$ upper limits of normal; for phase IIb only, fewer than 3 organs involved or cutaneous and/or subcutaneous metastases only. The following conditions were considered exclusion criteria: primary ocular melanoma; evidence of brain metastasis by computed tomographic (CT) scan in the 2 months prior to study entry; active autoimmune disease; and long-term basis use of corticosteroids or other immunosuppressant drugs.

The complete list of inclusion and exclusion criteria is reported in the Supplementary Material.

Both competent authorities and ethical committees approved the study protocol; all patients signed an informed consent form before being admitted into the study. The trial

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was conducted according to the principles of the latest version of the Declaration of Helsinki and the guidelines for Good Clinical Practice. The trial is registered in http://clinicaltrials.gov/ with the number NCT01055522.

Study design and treatment

This was an open-label, nonrandomized phase II study involving 3 centers in Germany and Italy. During the dose-escalation part, patients were enrolled in 3 sequential cohorts (3–4 subjects per cohort) corresponding to a fixed dose of dacarbazine and increasing doses of L19-IL2; subsequently, 22 patients were enrolled and treated at the confirmed recommended dose. The phase IIb step 1 part of the study is the first step of an optimal Simon 2-step design, considering a type-one error rate of 5%, a power of 80%, a null hypothesis $p_0 = 0.10$, and a target response rate $p_1 = 0.25$. Assumption for continuation of enrollment in the following phase IIb step 2 part of the study was that at least 3 of the 22 patients enrolled in the step 1 respond to the combination treatment.

L19-IL2 immunocytokine was provided by Philogen SpA (22, 23). L19-IL2 was administered as a 1-hour intravenous infusion on days 1, 3, and 5 of each 21-day cycle (Fig. 1).

The primary objective of the phase IIa was to confirm the recommended dose of L19-IL2 (previously defined as 22.5 Mio IU; ref. 23) when administered in combination with a fixed dose of dacarbazine in patients with metastatic melanoma. The primary objective of the phase IIb step 1 was the evaluation of the objective response rate (ORR) after induction. The phase IIa secondary objectives included the investigation of the pharmacokinetic profile of L19-IL2, the analysis of the induction of human anti-fusion protein antibodies (HAFA), the study of the antitumor activity of L19-IL2 with dacarbazine in patients with metastatic melanoma, and the evaluation of the immunologic activity of study treatment. The secondary objectives of phase IIb step 1 included the evaluation of progression-free survival (PFS), overall survival (OS), as well as safety and tolerability.

During the dose-escalation, the highest investigated dose level for which a dose-limiting toxicity (DLT) incidence in not more than 1 of 3 patients is observed was designated as recommended dose. DLTs were defined using the CTCAE v3.0 considering each of the following events as DLT, if believed related to the study treatment: grade IV thrombocytopenia (platelet count < 25,000/mm^3) or grade III thrombocytopenia with hemorrhage; grade >I renal toxicity (creatinine, blood urea nitrogen); grade ≥II hypoproteinemia, hypoalbuminemia, edema, proteinuria, dyspnea, hypotension, hypoxia (decreased O_2 saturation at rest), or any other signs of acute capillary leak syndrome; grade ≥III nonhematologic toxicity despite supportive therapy; failure to recover to grade ≤I toxicity (excluding alopecia) after delaying the initiation of the next cycle by a maximum of 2 weeks; failure to deliver any of the doses due to toxicity; failure to re-treat the patient on the second cycle due to toxicity. In patients who experienced a DLT, treatment with L19-IL2 was stopped and subsequently resumed only if toxicity resolved to the pretreatment level. Patients experiencing a DLT were removed from the study if the toxicity did not recover to grade II within 2 weeks or grade <II after 4 weeks.

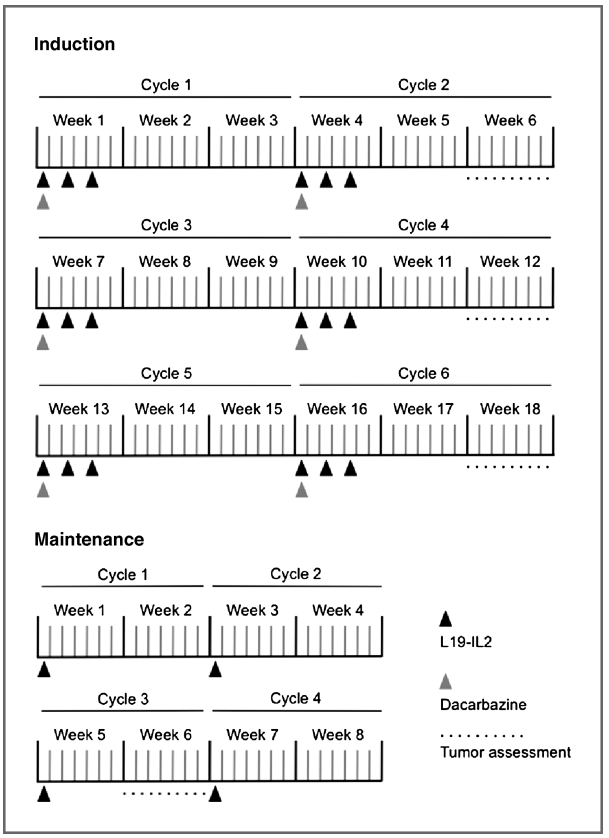

Figure 1. Treatment schedule. Patients were screened up to 14 days before beginning of the study. Each cycle of treatment comprises L19-IL2 administration on days 1, 3, and 5 (indicated by black arrowheads) and dacarbazine administration on day 1 (indicated as gray arrowheads), followed by 16-day rest (total duration of one cycle is 21 days). Patients could receive up to 6 cycles of treatment. The tumor assessments (indicated by dotted lines) were conducted according to RECIST: the lesions were measured at screening and at the end of cycles 2, 4, and 6 (between days 14 and 21). Patients with stable or responding disease after induction could receive additional L19-IL2 administration every 2 weeks, without dacarbazine, as maintenance therapy.

Safety and efficacy assessments

After obtaining patients informed consent, screening evaluations and procedures, which are fully described in the Supplementary Material, were conducted within 14 days before initiating study drug treatment. Patients returned for the end of treatment visit 30 to 37 days after the last dose of study drug, unless the objective tumor response was assessed as "progressive disease" in a previous treatment visit or the patient at some point discontinued the treatment. Adverse events and toxicities were graded as per CTCAE version 3.0 (27). Disease status was assessed at baseline, after every 2 cycles (i.e., 6 weeks) and at study discontinuation using RECIST 1.0 (28). Chest, abdomen, pelvis, and brain scans, either CT (preferred) or MRI (at discretion of the investigator), were conducted at baseline. At the following tumor

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assessments, scans of chest, abdomen, and pelvis were taken; brain scans were conducted only if patients were symptomatic. Patients remained on study until the occurrence of unacceptable toxicity, disease progression, withdrawal of consent, or until a L19-IL2 or dacarbazine infusion in the first 2 cycles was missed, except in the case of a missed dose because of DLT. Best overall responses were defined as the largest shrinkage in the sum of diameters of target lesions at any moment of time, compared with baseline.

Pharmacokinetics

Determination of L19-IL2, dacarbazine, and AICA (5-aminoimidazole-4-carboxamide) in human serum was conducted as described in Supplementary Material.

L19-IL2 production and characterization

Production, characterization, and toxicology testing of L19-IL2 were described previously (23).

Table 1. Patients' demography and characteristics

	No. of patients[a]		
Total number of patients receiving L19-IL2 + dacarbazine[b]	32		
Patients receiving L19-IL2 (10 Mio IU) + dacarbazine (1 g/m^2)	3		
Patients receiving L19-IL2 (15 Mio IU) + dacarbazine (1 g/m^2)	3		
Patients receiving L19-IL2 (22.5 Mio IU) + dacarbazine (1 g/m^2)	26		
Gender			
Male	23		
Female	9		
Patients with baseline LDH, IU/l			
<100	1		
>100 to <250	19		
>250	10		
N.A.	2		
ECOG performance status			
0	28		
1	4		
	10 and 15 Mio IU ($n = 6$)	22.5 Mio IU ($n = 26$)	Total ($n = 32$)
Stage of metastatic disease at baseline			
Stage IV M1a	1	4	5
Stage IV M1b	1	6	7
Stage IV M1c	4	16	20
Sites of metastatic lesions at baseline[c]			
Lymph nodes	5	20	25
Lung	4	18	22
Soft tissue	6	12	18
Adrenal gland	1	5	6
Spleen	1	5	6
Bone	1	4	5
Liver	-	5	5
Intestine	-	3	3
Kidney	-	1	1
Prior systemic antineoplastic therapies received			
Chemotherapy	6	6	12
Of which dacarbazine	4	2	6
Radiotherapy	6	3	9

Abbreviations: ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; N.A., not applicable.

[a]Median age, 55 years; range, 30 to 83 years.

[b]Median number of induction cycles received, 5; range. 1 to 6 cycles.

[c]Median number of months from diagnosis of metastatic disease to start of treatment, 2.9; range, 0.2 to 61.5 months

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Results

Study design

Patients could receive up to 6 cycles of treatment (induction) with L19-IL2 and dacarbazine. Patients with stable or responding disease after induction could receive additional biweekly L19-IL2 administration, without dacarbazine, as maintenance therapy.

Dose finding, pharmacokinetics, safety, and tolerability

All enrolled patients had progressing disease at the time of entering the study. The median age at the start of treatment was 55 years, 23 patients were males and 9 females (Table 1). Patients enrolled in the dose-escalation part of the study were heavily pretreated with 9 of 10 patients having received previous chemotherapy and 8 of 10 received previous radiotherapy. Further details about baseline characteristics of the single patients (i.e., site of disease, prior treatments, mutational status) are given in the Supplementary Table S1.

The maximum tolerated dose (MTD), determined as 22.5 Mio IU in a previous monotherapy study (23), was found to be safe also in combination with standard dacarbazine (1 g/m^2), and none of the 10 patients enrolled in the dose-escalation part of the study experienced a drug-related DLT.

Pharmacokinetic evaluation was conducted on all the phase IIa patients. Figure 2 shows the mean concentrations (±SD) of L19-IL2, dacarbazine, and AICA by dose group, during the first cycle of treatment. The maximum concentration (C_{max}) of L19-IL2 increased dose-proportionally within the tested L19-IL2 dose range and occurred within 1 hour after the end of the intravenous infusion. After reaching C_{max}, the L19-IL2 concentration decreased with a terminal half-life of 2 to 3 hours. Analysis of both dacarbazine and AICA revealed essentially identical pharmacokinetic profiles in patients who received different L19-IL2 doses (Fig. 2). Similar profiles were recorded for all the 3 compounds during the second cycle of treatment (data not shown). L19-IL2 was found to be nonimmunogenic even after repeated administrations, as documented by the absence of changes in the L19-IL2 pharmacokinetic profiles at different cycles and by the inability of patients' serum (1:100 dilution) to compete with a polyclonal rabbit anti-serum (1:10,000 dilution) raised against the immunocytokine (Supplementary Fig. S2).

Overall, 32 of 32 patients were evaluable for safety. Toxicity was manageable and reversible, with no serious unexpected suspected adverse reactions (SUSAR) and no treatment-related deaths. The incidence of drug-related adverse events by grade and System Organ Class (SOC) is listed in Table 2. The most frequent adverse events included chills, fatigue, and fever, which however were generally dose related and mild or moderate in severity. In general, only few and manageable grade III and IV adverse events related to the combination of L19-IL2 and dacarbazine were reported. Laboratory analyses revealed asymptomatic grade III and IV leucopenia, neutropenia, or anemia in 1 and 5 patients who received 10 Mio IU and 22.5 Mio IU of L19-IL2, respectively. Four patients experienced grade III cardiac and vascular complications, mainly hypotension; one of these patients required prolonged hospitalization but recovered without need of corrective therapy.

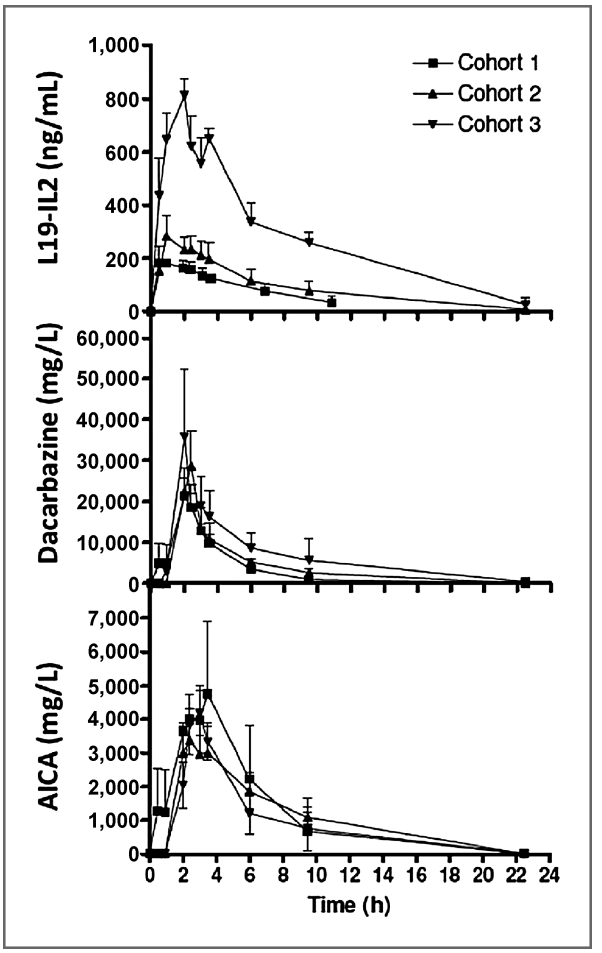

Figure 2. Pharmacokinetic analysis. Mean concentrations (±SD) of L19-IL2, dacarbazine, and its metabolite AICA during and after a 1-hour intravenous infusion by dose group are plotted. For L19-IL2, the dose is expressed as IL2 equivalent. Data were collected on day 1 of the first cycle of treatment.

L19-IL2 dosage had to be adjusted for single administrations (postponed, canceled, or reduced) in 12 patients and dacarbazine dose was reduced in 2 patients.

Effects on lymphocyte subsets, following administration of L19-IL2 and dacarbazine, were similar in the majority of the phase IIa patients. A striking but transient decrease (>90%) in the absolute number of total lymphocytes occurred during each treatment cycle after L19-IL2 and dacarbazine administration (samples were collected each cycle at day 1, 1 hour after dacarbazine infusion). Lymphopenia, which is a known transient consequence of both IL2 (29) and dacarbazine administration, was mainly observed in T-lymphocyte and natural killer (NK) cell populations (Supplementary Fig. S3A).

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Table 2. Incidence of related adverse events per grade

Drug-related adverse events	Incidence of related adverse events					
	Grade I	Grade II	Grade III	Grade IV	Unknown	Total
General disorders and administration site conditions	27/32	15/32	4/32		2/32	31/32
Pyrexia	16/32	11/32	1/32			20/32
Chills	15/32	6/32				18/32
Fatigue	12/32	4/32	1/32			14/32
Edema, peripheral	5/32				1/32	5/32
Asthenia	4/32	1/32	1/32			4/32
Hyperhidrosis	3/32					3/32
Chest pain	1/32	1/32				1/32
Drug intolerance					1/32	1/32
Extravasation		1/32				1/32
Influenza-like illness	1/32					1/32
Edema	1/32					1/32
Pain			1/32			1/32
Gastrointestinal disorders	19/32	12/32	1/32			24/32
Vomiting	10/32	7/32				14/32
Nausea	8/32	8/32	1/32			14/32
Diarrhea	5/32	2/32				6/32
Constipation	3/32					3/32
Abdominal pain	1/32	1/32				2/32
Dry mouth	2/32					2/32
Gingivitis	1/32					1/32
Oral dysesthesia	1/32					1/32
Stomatitis	1/32					1/32
Skin and subcutaneous tissue disorders	14/32	6/32			1/32	19/32
Rash	6/32	3/32				8/32
Pruritus	6/32	3/32				7/32
Erythema	3/32	1/32				4/32
Itching scar	2/32					2/32
Xeroderma	2/32	1/32				2/32
Alopecia	1/32					1/32
Dry skin	1/32				1/32	1/32
Urticaria		1/32				1/32
Musculoskeletal and connective tissue disorders	8/32	8/32				14/32
Arthralgia	4/32	7/32				9/32
Pain in extremities	4/32					4/32
Myalgia		1/32				1/32
Hypertonia	1/32					1/32
Joint range of motion decreased	1/32					1/32
Musculoskeletal pain		1/32				1/32
Trismus	1/32					1/32
Vascular disorders	7/32	5/32	4/32	1/32		11/32
Hypotension	7/32	5/32	3/32			11/32
Embolism				1/32		1/32
Presyncope	1/32					1/32
Thrombosis			1/32			1/32

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Table 2. Incidence of related adverse events per grade (Cont'd)

Drug-related adverse events	Incidence of related adverse events					
	Grade I	Grade II	Grade III	Grade IV	Unknown	Total
Nervous system disorders	9/32	2/32				10/32
Headache	4/32	1/32				4/32
Vertigo	3/32					3/32
Dizziness	2/32					2/32
Dysesthesia	1/32					1/32
Paresthesia		1/32				1/32
Tremor	1/32					1/32
Blood and lymphatic system disorders	3/32	5/32	4/32	2/32		8/32
Leukopenia	1/32	1/32	1/32	1/32		4/32
Neutropenia		3/32	3/32			4/32
Anemia	1/32	1/32	1/32	1/32		3/32
Thrombocytopenia	1/32					1/32
Metabolism and nutrition disorders	4/32	3/32				7/32
Anorexia	4/32					4/32
Decreased appetite		1/32				1/32
Dehydration		1/32				1/32
Hyperthyroidism		1/32				1/32
Metabolic and laboratory disorders	4/32	2/32		2/32		6/32
Increased alanine aminotransferase		2/32				2/32
Increased blood bilirubin	2/32					2/32
Increased platelet count	1/32			1/32		2/32
Increased blood creatine	1/32					1/32
Increased body weight	1/32					1/32
Increased γ-glutamyltransferase				1/32		1/32
Renal and urinary disorders	5/32					6/32
Oliguria	2/32					2/32
Proteinuria	2/32					2/32
Nocturia	1/32					1/32
Respiratory, thoracic, and mediastinal disorders	2/32	2/32				4/32
Cough	1/32	1/32				2/32
Laryngitis		1/32				1/32
Rhinorrhea	1/32					1/32
Infections and infestations	1/32	1/32				2/32
Candidiasis	1/32					1/32
Dental pulp disorder		1/32				1/32
Cardiac disorders	1/32	1/32	1/32		1/32	1/32
Arrhythmia					1/32	1/32
Extrasystoles		1/32				1/32
Palpitations	1/32					1/32
Tachycardia			1/32			1/32
Ear and labyrinth disorders (i.e., tinnitus)	1/32					1/32
Eye disorders (i.e., vision blurred)	1/32					1/32
Psychiatric disorders (i.e., depressed mood)	1/32					1/32
Reproductive system and breast disorders (i.e., penile swelling)	1/32					1/32

NOTE: The number of patients who experienced a certain side effect of a certain grade, over the total number of treated patients is reported in the table. Events which changed grade over time are listed as single events of the highest grade assumed.

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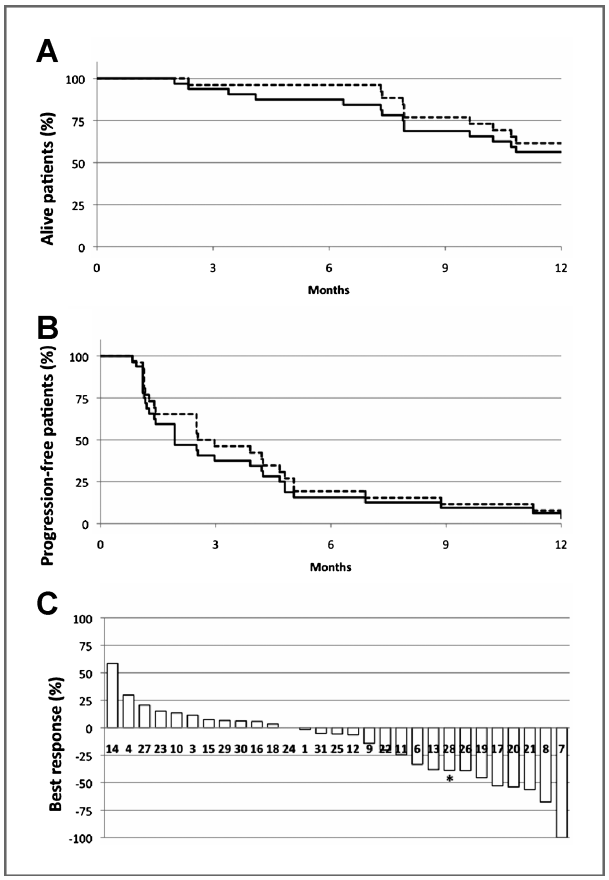

Figure 3. Waterfall plot and survival curves. A, one-year survival plot. One-year survival of all the 32 treated patients (black line) and of the 26 recommended dose treated patients (dashed line) is reported in the plot. Survival rates at 12 months were 61.5% and 56.3% for the recommended dose–treated patients and for all the 32 patients, respectively. B, PFS plot. One-year PFS of all the 32 treated patients (black line) and of the 26 recommended dose–treated patients (dashed line) is reported in the plot. Best overall response according to the central assessment. C, best responses (defined as the largest shrinkage in the sum of diameters of target lesions at any moment in time, compared with baseline) of evaluable patients are reported in the plot. Patients 1 to 3 received 10 Mio IU IL2 equivalent of L19-IL2 plus 1,000 mg/m^2 of dacarbazine; patients 4 to 6 received 15 Mio IU IL2 equivalent of L19-IL2 plus 1,000 mg/m^2 of dacarbazine. Patients 7 to 32 received 22.5 Mio IU IL2 equivalent of L19-IL2 plus 1,000 mg/m^2 of dacarbazine. Patients No. 2 and 5 are not represented as they experienced a rapid progression of the disease during the first 2 cycles of treatment, which was only assessed by clinical examination. Patient No. 32 is not reported as he was considered not evaluable according to RECIST. Patient No. 7 experienced a rapid disappearance of all lesions, as well as a resolution of pleural effusions, whereas a subcarinal lymph node, with 34 mm diameter at presentation, reduced to 10 mm (size of a normal lymph node) within 10 months. Asterisks mark patients for which the indicated percentage of target lesions shrinkage was accompanied by appearance of new lesions.

Moreover, at day 8 of every treatment cycle, a significant increase of CD71 expression on CD4$^+$ T-helper (Th) lymphocytes and CD8$^+$ cytotoxic T lymphocytes was recorded, indicating lymphocyte activation and proliferation (Supplementary Fig. S3B). At day 8 of each treatment cycle, a considerable increase of CD25 (IL2 receptor α chain) and CD122 (IL2 receptor β chain) expression on CD4$^+$ Th lymphocytes was also observed (Supplementary Fig. S3C); such expansion of CD4$^+$ T cells expressing the IL2 receptor is also a well-known effect of IL2 administration (29). No loss of immune stimulation was observed for patients treated with up to 9 cycles, (i.e., repeated therapy cycles still led to transient increase of immune effector cells; data not shown).

Antitumor activity of L19-IL2 plus dacarbazine

A waterfall plot indicating the best responses compared with baseline experienced by the evaluable patients according to a central assessment is reported in Fig. 3C. The plot reports the maximum percentage of tumor reduction for target lesions according to RECIST (28). According to the central assessment, 9 of 29 evaluable patients [31%; 95% confidence interval (CI), 14–48] achieved shrinkage greater than 30% which was not accompanied by appearance of new lesions; 8 of these patients (28%; 95% CI, 11–44) had a RECIST-confirmed objective response. These data are comparable with the efficacy analysis results given by the investigators, for which 8 of 30 evaluable patients (27%; 95% CI, 11–42) showed a shrinkage of the sum of the longest diameters of the tumor lesions greater than 30%, of which 5 (17%; 95% CI, 3–30) turned out to be RECIST-confirmed objective responses (data not shown). The two efficacy analyses (central and investigators' assessments) resulted in similar profiles with few discrepancies caused by differently selected target lesions, which do not affect substantially the overall picture. One-year survival and PFS data are reported in Fig. 3A and B, respectively. Eighteen of 32 patients were still alive 12 months after beginning of treatment, giving a 1-year survival rate of 56.3%. This rate increases to 61.5% when considering only patients treated at recommended dose (16 alive after 1 year of 26 recommended dose–treated patients; Fig. 3A). Median OS was 14.1 months (95% CI, 10.1–19.9) for patients treated at recommended dose and 13.9 months (95% CI, 7.8–17.0) for all patients. The median PFS was 2.5 months (95% CI, 1.5–4.2) for all patients and 3.8 months (95% CI, 2.5–5.0) for the recommended dose–treated patients (Fig. 3B). The median duration of responses was 86 days (95% CI, 7–325; range, 7–604 days); most of the responding patients experienced significant reduction of the lesions' diameters which lasted from 1 month to more than 1 year; one single patient (No. 8), who had a marked reduction of the target lesions at the first tumour assessment, after 7 days, discontinued treatment due to the appearance of a new cutaneous lesion, whereas the neoplastic masses progressed at later time points; 604 days correspond to the last contact with patient No. 7, who at that time was still responding to treatment. Selected examples of observed objective responses are shown in Supplementary Fig. S4.

No correlation was found between the *BRAF*, *KIT*, or *NRAS* mutational status of the treated patients and the antitumor activity of the L19-IL2/dacarbazine regimen. In

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particular, tumor responses after L19-IL2 plus dacarbazine were achieved in patients with melanoma irrespective of the *BRAF* or *NRAS* mutational status of their disease (Supplementary Table S1).

Discussion

We have reported the results of a dose-escalation and signal-generating trial conducted with L19-IL2 in combination with dacarbazine in patients with metastatic melanoma. Overall, approximately 30% of the patients exhibited an objective response, including a complete resolution of all neoplastic lesions in one patient which is still ongoing more than 21 months from study entry.

Patients who achieved an objective response or disease stabilization after the combined induction therapy were offered additional biweekly L19-IL2 administrations as maintenance a therapy. A similar treatment strategy seemed to positively affect PFS as well as OS for patients with metastatic melanoma in a recent phase II study, when compared with historical controls. In this study, treatment consisted of a biochemotherapy induction regimen [including cisplatin, vinblastine, dacarbazine, decrescendo IL2, and IFNα-2b with granulocyte macrophage colony-stimulating factor (GM-CSF) cytokine support] and a maintenance biotherapy (with low-dose IL2 and GM-CSF followed by intermittent pulses of decrescendo IL2 over 12 months; ref. 30).

Given the limited number of patients, it is of course premature to judge about the clinical significance of the therapeutic potential of L19-IL2 in melanoma. However, the observation that more than 60% of the study patients were still alive 12 months from start of treatment is highly encouraging, considering that the median survival for patients with pretreated melanoma is typically 6 to 9 months (31), and that in similar trials it was shown to range between 10 and 14 months for patients treated with recently FDA-approved ipilimumab, with or without dacarbazine (32). Moreover, our results are in line with observations in high-dose IL2–treated patients revealing response rates between 5% and 27%. In contrast to chemotherapy-induced responses, IL2-based schedules seem to be able to produce durable remissions (33, 34). Similar effects could be observed in some patients responding to L19-IL2; results from a larger ongoing trial with L19-IL2 will confirm if this trend is real.

The combined treatment with L19-IL2 plus dacarbazine was reasonably well tolerated, and all side effects encountered in the study were manageable and reversible. In comparison to high-dose IL2 therapy, which is recommended to be carried out only in hospitals with easy access to an intensive care facility, the infusion of L19-IL2 never resulted in severe adverse events necessitating intensive care support. Rather, the application of L19-IL2 plus dacarbazine could be conducted on a regular hospital ward or even in an outpatient setting (day hospital).

The immunocytokine L19-IL2 has been studied extensively in animal models of cancer, revealing an inhibitory activity on tumor growth when used as single agent (22, 35–39), which could be potentiated when the agent was used in combination with chemotherapy (36) or with antibody-based therapeutics, leading to complete tumor eradications (38, 39). Following intravenous administration, L19-IL2 preferentially localizes at the tumor site, as evidenced by quantitative biodistribution studies and by microautoradiographic investigations (22). Furthermore, the tumor-targeting ability of the L19 antibody in scFv format (40) and in small immunoprotein (SIP) format (25) has extensively been studied in animal models (20, 25, 41–45) and in more than 100 patients with cancer (46–48), using nuclear medicine techniques.

The pharmacokinetic studies conducted in this trial have confirmed a rapid clearance of L19-IL2 at the end of the infusion, in full analogy with the data observed in preclinical models (22, 23) and in patients treated with L19-IL2 as single agent (23). This rapid clearance is a favorable property of L19-IL2, which helps minimizing systemic adverse events, whereas the immunocytokine exhibits long residence times on the neoplastic lesions (15, 22, 25). While the value of area under the curve of L19-IL2 in serum increased as expected as a result of the administered dose in the different cohorts, the serum levels of dacarbazine and of its metabolite AICA did not change substantially.

On the basis of the supportive clinical results of the L19-IL2/dacarbazine combination therapy, a controlled phase IIb study with 90 patients with metastatic melanoma was started recently to prospectively analyze the antitumor activity of L19-IL2 plus dacarbazine versus dacarbazine alone in patients with metastatic melanoma.

Disclosure of Potential Conflicts of Interest

D. Neri is a co-founder and shareholder of Philogen, the biotech company which has licensed the L19 antibody from the ETH Zurich D. Neri has employment and ownership interest. T.K. Eigentler and B. Weide have commercial research support. C. Garbe and F. de Braud are consultants/advisory board members. No potential conflicts of interest were disclosed by other authors.

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A Dose-Escalation and Signal-Generating Study of the Immunocytokine L19-IL2 in Combination with Dacarbazine for the Therapy of Patients with Metastatic Melanoma

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