RESEARCH PAPER

OncoImmunology 2:2, e23079; February 2013; © 2013 Landes Bioscience

Phase I clinical trial combining imatinib mesylate and IL-2 in refractory cancer patients

IL-2 interferes with the pharmacokinetics of imatinib mesylate

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Keywords: imatinib mesylate, interleukin-2, pharmacokinetics, maximum tolerate dose, cancer

Abbreviations: AUC, area under the curve; Cmax, maximum concentration; DC, dendritic cell; DLT, dose-limiting toxicity; GIST, gastrointestinal stromal tumors; IL-2, interleukin-2; IM, imatinib mesylate; MTD, maximum tolerated dose; NK, natural killer; OS, overall survival; PDGFR, platelet-derived growth factor receptor; PFS, progression-free survival; *t*1/2, half-life; T_{max}, time to reach the maximum concentration

Imatinib mesylate (IM) is a small molecule inhibitor of protein tyrosine kinases. In addition to its direct effect on malignant cells, it has been suggested IM may activate of natural killer (NK) cells, hence exerting immunomodulatory functions. In preclinical settings, improved antitumor responses have been observed when IM and interleukin-2 (IL-2), a cytokine that enhances NK cells functions, were combined. The goals of this study were to determine the maximum tolerated dose (MTD) of IL-2 combined with IM at a constant dose of 400 mg, the pharmacokinetics of IM and IL-2, as well as toxicity and clinical efficacy of this immunotherapeutic regimen in patients affected by advanced tumors. The treatment consisted in 50 mg/day cyclophosphamide from 21 d before the initiation of IM throughout the first IM cycle (from D-21 to D14), 400 mg/day IM for 14 d (D1 to D14) combined with escalating doses of IL-2 (3, 6, 9 and 12 MIU/day) from days 10 to 14. This treatment was administered at three week intervals to 17 patients. Common side effects of the combination were mild to moderate, including fever, chills, fatigue, nausea and hepatic enzyme elevation. IL-2 dose level II, 6 MIU/day, was determined as the MTD with the following dose-limiting toxicities: systemic capillary leak syndrome, fatigue and anorexia. Pharmacokinetic studies revealed that the area under the curve and the maximum concentration of IM and its main metabolite CGP74588 increased significantly when IM was concomitantly administered with IL-2. In contrast, IM did not modulate IL-2 pharmacokinetics. No objective responses were observed. The best response obtained was stable disease in 8/17 (median duration: 12 weeks). Finally, IL-2 augmented the impregnation of IM and its metabolite. The combination of IM (400 mg/day) and IL-2 (6 MIU/day) in tumors that express IM targets warrants further investigation.

Introduction

Imatinib mesylate (IM) is a 2-phenylaminopyrimidine derivative designed as a specific inhibitor of the inactive conformation of ABL protein tyrosine kinases.[1] Its activity against cells bearing the BCR-ABL translocation has yielded remarkable clinical results in the treatments of chronic myeloid leukemia (CML) with minimal side effects.[2] IM has been found to inhibit other

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Submitted: 10/12/12; Revised: 11/28/12; Accepted: 12/01/12
http://dx.doi.org/10.4161/onci.23079
Citation: Pautier P, Locher C, Robert C, Deroussent A, Flament C, Le Cesne A, et al. Phase I clinical trial combining imatinib mesylate and IL-2 in refractory cancer patients: IL-2 interferes with the pharmacokinetics of imatinib mesylate. OncoImmunology 2013; 2:e23079

tyrosine kinases including platelet-derived growth factor receptor (PDGFR), KIT (CD117), colony stimulating factor 1 receptor (CSF1R) and possibly leukocyte-specific protein tyrosine kinase (LCK).[3-6] The inhibitory activity of IM against KIT and PDGFR has enabled the development of effective treatments for patients affected by gastrointestinal stromal tumors (GIST),[7] eosinophilic disorders and systemic mast cell disease.[8,9]

In addition to its direct effects on malignant cells, IM appears to exert immunomodulatory functions by inhibiting and/or activating specific subsets of immune cells. Our group has demonstrated that IM activates natural killer (NK) cells in GIST patients, resulting in improved progression-free survival (PFS).[10,11] These results indicate that the innate immune response is a major and independent PFS predictive factor in patients with advanced GIST receiving IM.[12] To improve the functions of NK cells in the course of IM treatment, Taieb et al. investigated in preclinical studies a combinatorial regimen involving IM plus interleukin-2 (IL-2), showing that IL-2 is able to increase the antitumor effects of IM.[13-15] These observations provided a robust rationale to launch the Phase I clinical trial *IMAIL-2* at the Gustave-Roussy Institute (Paris), aimed at assessing of the maximum tolerated dose (MTD) of IL-2 administered in combination with daily IM in patients with refractory solid tumors. We also determined the pharmacokinetics of IM when combined with IL-2 and their clinical activity in this cohort of patients.

Results

Patient characteristics. Between October 2007 and October 2009, 21 patients with advanced solid tumors were enrolled in the *IMAIL-2* study at the Gustave-Roussy Institute. Of 21 patients initially enrolled, one was not treated due to a personal choice, two patients did not complete a full cycle of treatment due to disease progression and one patient was not evaluated for dose-limiting toxicity (DLT) due to incorrect medication intake. Seventeen patients hence completed the study, with a median age of 51 y (range 25–74). Patients were affected by the following malignancies: metastatic melanoma (n = 10); ovarian carcinoma (n = 3); Merkel-cell carcinoma (n = 1), GIST (n = 1), rectal adenocarcinoma (n = 1) and cervical adenocarcinoma (n = 1). The characteristics of the 17 patients that completed the study are detailed in **Table 1**.

Clinical efficacy of IM and IL-2 combination therapy. Seventeen patients were evaluated for response, three patients at IL-2 dose level I (3 MIU/day), 11 at IL-2 dose level II (6 MIU/day) and three at IL-2 dose level III (9 MIU/day). The progression-free survival (PFS) rate at six months was 18% (CI 95%: 6–41%) and the median PFS for all 17 patients was two months (**Fig. 1A**). There was no objective tumor response according to RECIST criteria but 8/17 (47%) patients experienced disease stabilization. The median duration of stable disease (SD) was 84 d (range: 61–210 d). The median overall survival (OS) for all patients was

Table 1. Patient characteristics at baseline

Dose level	I	II	III	TOTAL
IM (mg/day)	400	400	400	
IL-2 (MIU/day)	3	6	9	
No. of patients	3	11	3	17
Sex				
Male	0	7	0	7
Female	3	4	3	10
Age (years)				
Median [Range]	58 [30–61]	50 [25–74]	51 [42–57]	51 [25–74]
Height (cm)				
Median [Range]	165 [161–165]	163 [158–188]	168 [163–173]	165 [158–188]
Weight (kg)				
Median [Range]	65 [47–68]	60 [43–86]	59 [47–81]	61 [43–86]
E.C.O.G.				
ND	0	1	1	2
0	2	6	2	10
1	1	4	0	5
Primary tumor site				
Melanoma	0	8	2	10
Ovarian adenocarcinoma	2	0	1	3
Other*	1	3	0	4

*Other tumor sites (one patient each) include Merkel-cell carcinoma, gastrointestinal stromal tumor, rectal adenocarcinoma and cervical adenocarcinoma. IL-2, interleukin-2; IM, imatinib mesylate; MIU, million international unit; ND, not determined.

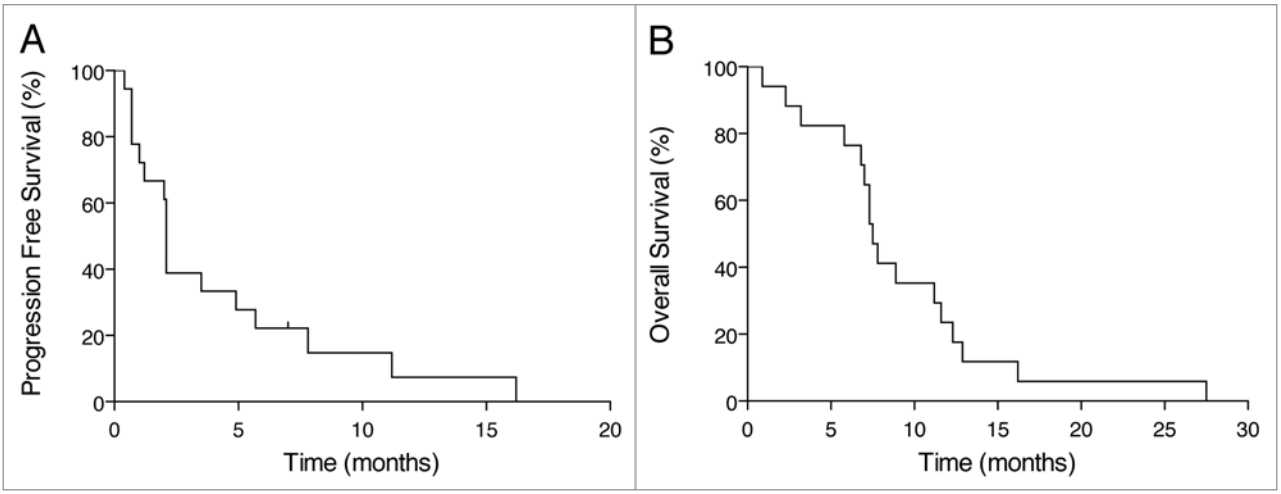

Figure 1. Clinical effects of treatment with imatinib mesylate and interleukin-2. (**A**) Progression-free survival (PFS) from the initiation of treatment with imatinib mesylate (IM) and interleukin-2 (IL-2) of n = 17 patients enrolled in the trial. (**B**) Overall survival (OS) from the initiation of treatment with IM and IL-2 of n = 17 patients enrolled in the trial.

7.7 months (**Fig. 1B**) with a survival rate at six months of 82% (IC 95%: 64–100%). Among 17 patients, nine were treated with three or more cycles (53%). The median PFS and OS of advanced melanoma patients in this trial (10/17) were two and 7.2 mo, respectively. There were no treatment-related deaths; all of them were due to disease.

Safety, tolerability and MTD. Fifty-two courses of IM + IL-2 were administered to 17 patients. All patients experienced at least one treatment-related adverse event (**Table 2**). The most frequent drug-related adverse events were fevers and chills in 16/17 (94%) patients, transaminase elevation in 11/17 (65%) patients, fatigue in 13/17 (76%) patients and nausea in 10/17 (59%) patients. In the three patients on IL-2 dose level I (3 MIU/day) and the first three patients on IL-2 dose level II (6 MIU/day), no dose-limiting toxicities were observed. Two of the three patients on IL-2 dose level III (9 MIU/day) developed a DLT consisting of an unacceptable grade 3 systemic capillary leak syndrome, fatigue and anorexia. The cohort of patients on IL-2 dose level II was then expanded to 11 patients, with no further DLT. Therefore, the MTD was determined to be 6 MIU of IL-2 combined with 400 mg IM. At this dose level, all patients (n = 11) experienced at least one treatment-related adverse event (grade 1 or grade 2 toxicities) but no grade 3 or grade 4 toxicities were observed (**Table 2**). Thus, 400 mg/day IM combined with 6 MIU/day IL-2 appears to be relatively well tolerated.

IM and CGP74588 steady-state accumulation. The pharmacokinetic parameters of IM and its main metabolite CGP74588, as determined by non-compartmental model analyses, were available for patients on IL-2 dose level II. The means and standard deviations for each parameter are given for days D1, D10 and D14 of the first cycle of treatment (**Table 3 and Table 4**).

IM was detectable in the plasma of patients one hour after the first oral administration, and T_{max} was determined to be of two hours. The maximum mean plasma concentration (C_{max}) was 2.2 ± 0.6 μg/mL after once-daily administration while the area under the curve (AUC, 0–24 h) reached 29.1 ± 7.4 μg.h/mL.

The same parameters were evaluated at D10, by which time plasma concentrations had reached the steady-state.[17,18] As anticipated, the AUC (0–24 h) and C_{max} for both IM and its metabolite were significantly higher at D10 than after the first administration (**Fig. 2**). Based on IM levels measured at D1 and D10, the means (± SD) accumulation ratios across different doses (defined as the D10 to D1 trough concentration ratio) were 1.34 ± 0.35 and 1.93 ± 0.69 for IM and CGP74588, respectively.

Effect of IL-2 on IM pharmacokinetics. IL-2 increased the exposure to IM. Indeed, at D14, that is, following five consecutive days of IL-2 co-administration, the AUC (0–24 h) of IM significantly increased from 39.6 ± 13.4 μg.h/mL at D10 to 64.0 ± 16.5 μg.h/mL at D14 (p = 0.002) (**Fig. 2A**). The AUC (0–24 h) at D14 was 61% higher than the AUC (0–24 h) at D10. Along similar lines the C_{max} of IM significantly increased from 3.3 ± 1.6 μg/mL at D10 to 4.9 ± 1.4 μg/mL at D14 (p = 0.028) (**Fig. 2A**). The same modifications were observed for CGP74588 (**Fig. 2B**). Finally, the AUC (0–24 h) of IM and CGP74588 at D14 were positively correlated with the dose of IL-2 upon normalization on the weight of each patient (IL-2/Kg) (**Fig. 2C**). The best correlation was observed for CGP74588 (R = 0.6879, p = 0.0046).

Effect of IM on IL-2 pharmacokinetics. We investigated if IM would influence IL-2 pharmacokinetics. IL-2 pharmacokinetics is well documented: the T_{max} and half-life ($t1/2$) after a subcutaneous injection of IL-2 range from 1.5 to 4.5 and from 3 to 5 h, respectively.[19,20] In patients treated from D10 to D14 with IL-2 together with 400 mg IM, IL-2 C_{max} was reached at 2 h and mean $t1/2$ was 3.1 h. Therefore, the pharmacokinetic parameters of IL-2 in patients co-treated with IM are compatible with those observed in previous pharmacokinetic studies. Altogether, these data indicate that IM does not influence IL-2 pharmacokinetics. Finally, we determined the levels of the soluble form of the α

Table 2. Toxicities according to the National Cancer Institute common toxicity criteria v. 3.0*

Dose level	I		II		III		Total	
No. of patients	3		11		3		17	
No. of courses per patients	3, 3, 3		10, 1, 1, 2, 3, 2, 1, 8, 5, 1, 3		5, 1, 1		52	
Grade	1/2	3/4	1/2	3/4	1/2	3/4	N	%
Gastrointestinal								
Nausea	2	0	5	0	3	0	10	59%
Vomiting	2	0	3	0	0	0	5	29%
Diarrhea	2	0	2	0	3	0	7	41%
Anorexia	2	0	2	0	0	1**	5	29%
Hematologic								
Anemia	0	0	2	0	1	0	3	18%
Leucopenia	0	0	1	1	0	0	2	12%
Lymphopenia	1	0	0	3	0	0	4	24%
Neutropenia	0	0	3	1	0	0	4	24%
Hepatic								
SGOT/AST elevation	3	0	7	0	1	0	11	65%
SGPT/ALT elevation	1	0	7	0	2	0	10	59%
Others								
Skin tissue disorders	1	0	6	0	0	0	7	41%
Systemic capillary leak syndrome	0	0	1	0	0	1**	2	12%
Fatigue	3	0	8	0	1	1**	13	76%
Fever/chills	3	0	10	0	3	0	16	94%
Edema/fluid retention	1	0	6	0	1	0	8	47%
Cough	0	0	3	0	1	0	4	24%
Dyspnea	0	0	2	0	0	0	2	12%

*Data for events with suspected relation to the study drugs that occurred in > 10% of patients; **Dose-limiting toxicity. ALT, alanine transaminase; AST, aspartate transaminase; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase.

subunit of the IL-2 receptor (sCD25), which represents a marker of IL-2 impregnation.[21-23] We observed a significant increase of sCD25 at the end of the first treatment cycle in our cohort ($p < 0.001$) (**Fig. 2D**).

Pharmacokinetic-pharmacodynamic relationships. The AUC (0–24 h) of IM and CGP74588 at D1, D10 and D14 were studied in relationship with the absolute count of lymphocytes, neutrophils and platelets. As shown in **Figure 3**, while the AUC (0–24 h) of IM increased between D1 and D10, no significant modifications of lymphocyte, neutrophil or platelet counts could be documented. Lymphocyte and platelet counts were significantly reduced in response to the combination therapy at D14 (**Fig. 3A and B**). Of note, neutrophil, monocyte and eosinophil counts were affected neither by IM alone nor by the combinatorial regimen (**Fig. 3C** and data not shown). Among patients on IL-2 dose level III that experienced limiting toxicities (patient #10, exhibiting a grade 3 systemic capillary leak syndrome and patient #11, exhibiting grade 3 asthenia and anorexia), only patient #10 was monitored for the pharmacokinetics of IM and IL-2. This patient had the highest AUC (0–24 h) for IM and CGP74588 (106.5 and 25.1 μg.h/mL, respectively). Patient #8, also on IL-2 dose level III but experiencing no toxicity, exhibited AUC (0–24 h) values of IM and CGP74588 (43.3 and 13.4 μg.h/mL, respectively) that were comparable to patients on IL-2 dose level II.

Discussion

We investigated the safety of IM combined with increasing doses of IL-2 in patients affected by refractory advanced solid tumors. To our knowledge, this is the first report on the combination of IM with IL-2 in late stage cancer patients. In the present study, the MTD of IL-2 in combination with 400 mg/day IM was estimated at 6 MIU/day.

This study shows that the co-administration of IL-2 increases the systemic exposure of patients to IM and its main metabolite, CGP74588. The C_{max} and AUC of IM and CGP74588 were indeed significantly increased by 49 and 61%, respectively. This could be explained by a interaction between the two drugs. IL-2 is known for its ability to limit the enzymatic activity of cytochrome P450 and monooxygenase,[24] especially at doses > 6 MIU for the former > 9 MIU for the latter. Since in our study the pharmacokinetics of CGP74588 and that of IM were altered by IM in the same manner, these effects cannot stem from the IL-2 mediated inhibition of cytochrome P450 and monooxygenase activity. In addition, IL-2 is able to affect plasma membrane transporters,

Table 3. Pharmacokinetic parameters of IM

		T_{max} (h)		C_{max} (μg/mL)		*t*1/2 (h)		AUC (0–24) (μg.h/mL)		AUC (0-inf) (μg.h/mL)		Vd (L)		Cl (L/h)	
	No. of patients	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Day 1	9	2.0	1.2	2.2	0.6	10.1	1.6	29.1	7.4	39.7	17.8	164.3	53.8	11.2	3.2
Day 10	10	2.1	1.1	3.3	1.6	13.1	8.9	39.6	13.4	64.1	28.2	126.5	64.2	7.8	4.9
Day 14	10	2.5	1.4	4.9	1.4	13.0	3.9	64.0	16.5	94.6	30.6	82.9	24.5	4.7	1.7

AUC, area under the curve; Cl, total body clearance; *t*1/2, half-life; T_{max}, time to reach the maximum concentration; Vd, volume of distribution at steady-state.

Table 4. Pharmacokinetic parameters of CGP74588

		T_{max} (h)		C_{max} (ng/ml)		*t*1/2 (h)		AUC (0–24) (μg.h/mL)		AUC (0-inf) (μg.h/mL)		Vd (L)		Cl (L/h)	
	No. of Patients	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Day 1	9	2.3	1.6	294.0	47.5	14.4	2.8	4.1	1.0	6.7	2.7	1364.5	383.6	66.6	20.9
Day 10	10	2.2	1.6	520.8	215.8	36.2	32.5	7.7	2.7	23.8	16.0	827.8	471.2	22.8	11.6
Day 14	10	3.8	2.3	727.0	200.2	32.5	20.1	13.2	3.8	37.0	28.2	531.5	170.2	14.9	7.4

AUC, area under the curve; Cl, total body clearance; *t*1/2, half-life; T_{max}, time to reach the maximum concentration; Vd, volume of distribution at steady-state.

hence leading to increased efflux of some drugs, as demonstrated in peripheral blood mononuclear cells.[25] These pharmacokinetic modifications may partly be explained by decreased P-glycoprotein expression, as described in mice by Hosten et al.[26] This hypothesis is currently under investigation in vitro, to better understand the impact IL-2 on the expression and functionality of P-glycoprotein and breast cancer resistance protein (BCRP) in human lymphocytes, as well as how this may influence the transport of IM.

Regarding the pharmacodynamic aspects of the interaction between IL-2 and IM, the significant decrease in lymphocytes and platelets observed between D10 and D14 of treatment could be ascribed to the adjunction of IL-2, since no decrease was detectable between D1 and D10 (when IM was administered alone). The impact of IL-2 on lymphocyte counts has previously been described by others[27] and has hypothetically been ascribed to an effect of lymphocyte redistribution[28] and/or to activation-induced cell death.[29,30] At high doses (up to 0.3 MIU/kg i.v. every eight hours over 3–4 d), IL-2 infusions have previously been shown to result in moderate to severe thrombocytopenia.[27] With 6 MIU IL-2, we observed a significant drop in platelet counts, but this decrease never reached levels associated with an increased hemorrhagic risk. In addition, we never observed a greater reduction in subsequent cycles compared with cycle 1 (not shown). The analysis of sCD25 levels, IL-2 *t*1/2 and AUC did not reveal any modifications of the pharmacokinetics of IL-2 when given in combination with IM.

As previously shown, IM may act as an immunomodulatory agent, in particular as it promotes the dendritic cell (DC)-NK cell crosstalk, leading to increased secretion of tumor necrosis factor α (TNFα) and interferon γ (IFNγ) by NK cells and IL-12 by DCs.[30,32] This immunomodulatory activity of IM was found to constitute an independent prognostic factor and was associated with longer PFS in GIST patients.[31-33] Moreover, a preclinical study has shown that IM may synergize with IL-2 at reducing lung metastases after an i.v. injection of B16F10 melanoma cells, which are not sensitive to the direct cytotoxic effects of IM.[34] Such a synergistic antitumor effect was mainly due to an increased cytotoxicity of TNF-related apoptosis-inducing ligand (TRAIL)-dependent HLA-DR$^+$CD11c$^+$B220$^+$NK1.1$^+$ cells, the so-called interferon-producing killer DCs (IKDCs).[34] Thus, IL-2 has been envisaged to boost IM-elicited antitumor immunity in cancer patients, in particular by inducing IKDCs in vivo. A comprehensive immunomonitoring of this patient cohort was undertaken (see companion paper in *OncoImmunology*, 2:e23080).

In our cohort, 8/17 patients (47%) experienced disease stabilization after a median treatment duration of 12 weeks. Unfortunately, we were only able to include one patient affected by GIST in this study, and, among the other patients, ten were affected by metastatic melanoma. It would be interesting to evaluate this combinatorial regimen in tumors bearing *KIT* mutations and see whether the adjunction of IL-2 can boost the efficacy of IM. Many teams have tried to combine IM with other immunotherapeutic agents in order to enhance its clinical efficacy against solid tumors, and many of them have investigated the combination of IM with conventional chemotherapy. However, only modest signs of clinical benefits were documented, often in association with a relatively poor tolerance. In one study, IM was combined with IFNα in patients affected by metastatic renal cell carcinoma (RCC). The response rate was 6% with a median time to progression of two months, de facto constituting no improvement as compared with the administration of IFNα alone.[35] As significant toxicities were observed, the investigators concluded that further studies of IM combined with IFNα would not be recommended in patients with metastatic RCC.[36] The clinical efficacy of this combinatorial regimen in GIST patients, which

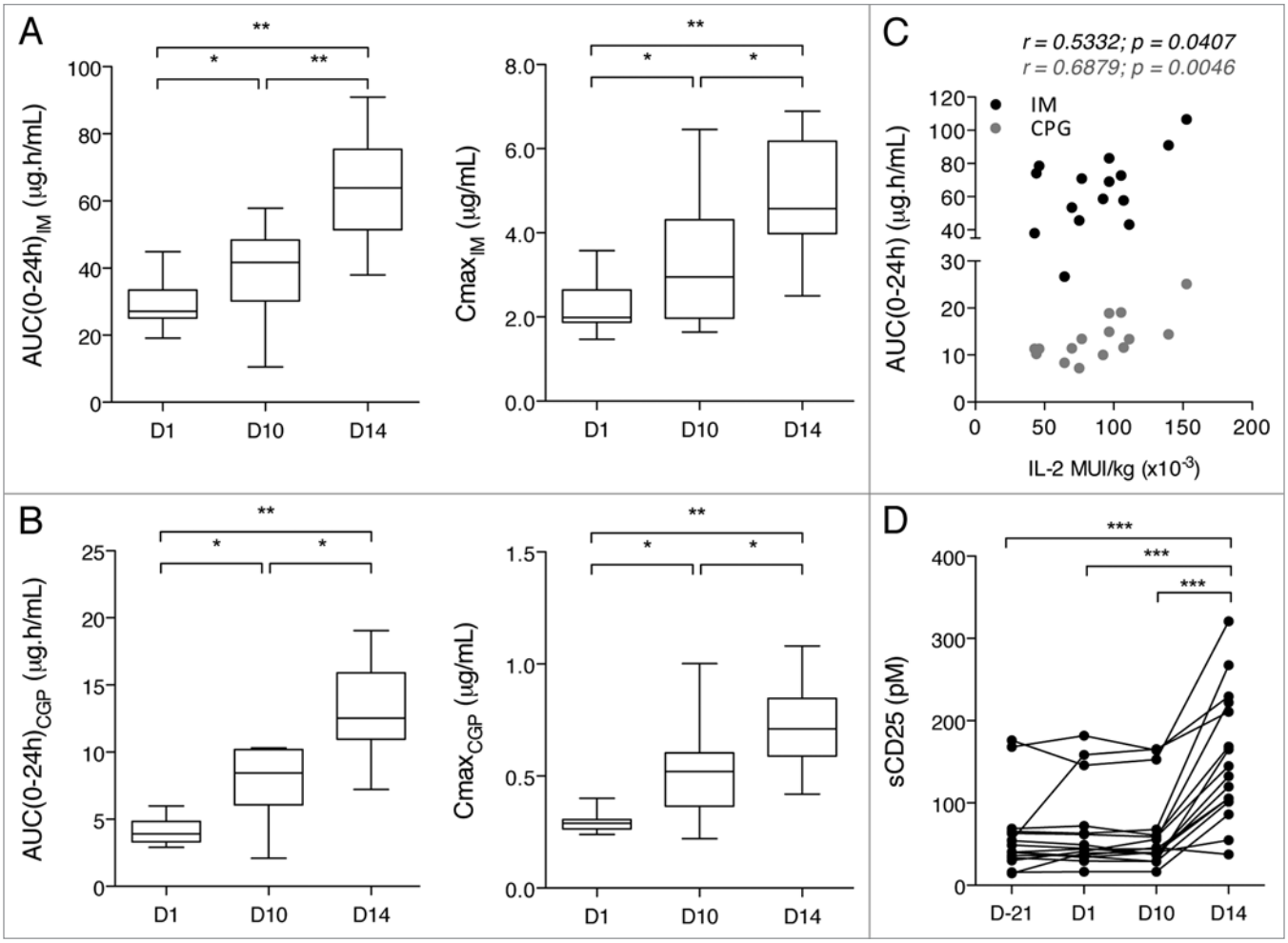

Figure 2. Interleukin-2 impacts on imatinib mesylate pharmacokinetic. (**A and B**) Area under the curve (AUC, left panel) and C_{max} (right panel) of imatinib mesylate (IM, (**A**) and CGP74588 CGP, (**B**) immediately after the initiation of the treatment (D1), after 10 d of treatment with IM alone (D10) and after 14 d of treatment with IM plus interleukin-2 (IL-2) at dose level II (6 MIU/day) on the last 4 d (D14). Wilcoxon signed rank test was used and results from statistical analyses are depicted on each graph. (**C**) Correlation between IL-2 MIU/kg body weight and AUC (0–24h) of IM (dark circles) or CGP74588 (gray circles) (**D**) Dynamic pattern of the soluble form of CD25 (sCD25).

has a comparatively stronger rationale because IM specifically targets GIST-associated KIT or PDGFR mutants,[37] appears to be far superior to that observed in individuals affected by RCC.

Approximately 71% of GIST patients exhibit mutations in exon 11 of *KIT*, followed by mutations in *KIT* exon 9 (8.2%), *KIT* exon 13 (1.2%), *PDGFRA* exon 18 (1.2%) and *KIT* exon 17 (1.0%). These mutations are important as they often dictate the patient sensitivity to IM therapy. In particular, *KIT* exon 11 mutations are associated with comparatively longer time to progression (median 27.2 mo) and OS (median 60 mo).[37] IM and IFNα have recently been combined in patients bearing stage III/IV GIST in a study designed to promote IM antitumor immunity with IFNα.[38] IM is thought to undermine tumor-induced immune tolerance[39] and, owing to its cytotoxic activity, may promote the shedding of tumor-associated antigens for DC uptake, presentation and priming of T_H1 responses.[38] In 8 patients evaluated in this respect, the combination of IM and IFNα was associated with increased T_H1 and NK cell activity. Clinical responses were encouraging, since an objective response rate of 100% (including both complete and partial responses) and an OS of 100% with a median follow-up of 3.6 y were observed. These clinical findings appear superior than those obtained with IM monotherapy, yet, so far, remain preliminary.[38]

Our study did not demonstrate any major therapeutic effects stemming from the combination of IM and IL-2. However, we confirmed that IM can be associated with 6 MIU IL-2 with an acceptable tolerance profile. Improving IM exposure may bring about clinical benefits, as suggested by pharmacokinetic studies in individuals affected by CML and GIST. Among CML patients, a significant correlation has been detected between IM trough plasma levels and cytogenetic and molecular responses.[40-42] In GIST patients, a relationship between IM pharmacokinetics and clinical responses is emerging.[43] Finally, as shown in the companion paper (*OncoImmunology* 2:e23080), the combination of IM and IL-2 was able to induce the accumulation of HLA-DR^+ NK cells, which are linked to clinical outcome. As IM has been shown to decrease intratumoral regulatory T cells (Tregs) in an indoleamine 2,3-dioxygenase (IDO)-dependant manner by

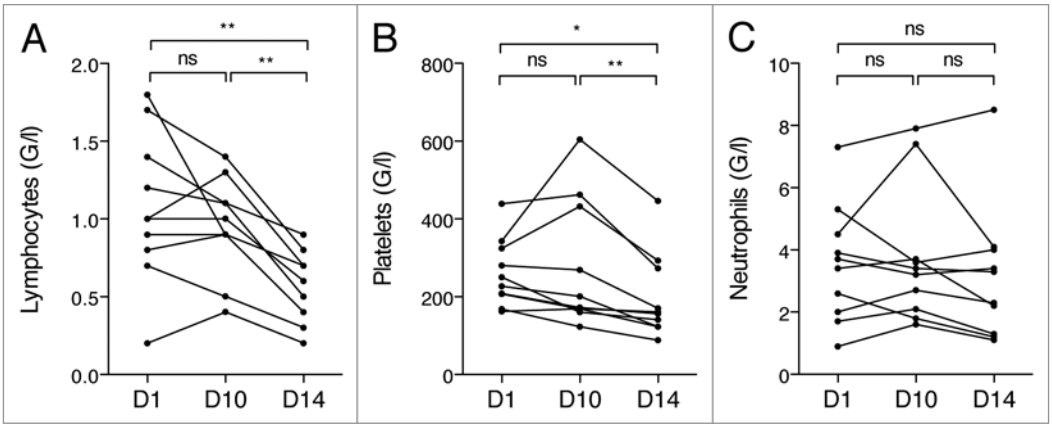

Figure 3. Impact of imatinib mesylate alone or combined with interleukin-2 on the hematopoietic compartment. (**A–C**) Lymphocyte (**A**), platelet (**B**) and neutrophil (**C**) counts at day one after starting treatment (D1), immediately after the initiation of the treatment (D1), after 10 d of treatment with imatinib mesylate (IM) alone (D10) and after 14 d of treatment with IM plus interleukin-2 (IL-2) at dose level II (6 MIU/day) on the last 4 d (D14). Wilcoxon signed rank test was used and results from statistical analyses are depicted on the graph.

inhibiting oncogenic KIT signaling,[39] and as Tregs are capable of inhibiting NK cell functions,[44,45] further studies on the synergistic clinical activity of IL-2 and IM should involve patients affected by tumors bearing *KIT* mutations such as GIST.

Patients and Methods

Patient eligibility. Adult patients with metastatic or locally advanced solid malignancy, measurable or evaluable disease who were refractory to standard therapy were eligible for the study (Phase I *IMAIL-2* trial approved by the Kremlin Bicêtre Hospital Ethics Committee [no 07–019] and the Agence Française de Sécurité Sanitaire des Produits de Santé [no A70385–27; EudraCT N°:2007–001699–35 in 2007]. Further requirements included: chronological age > 18 y and physiological age < 70 y; more than four weeks since the last disease-specific treatment, adequate bone marrow function defined as an absolute white blood count ≥ 4 × 10^9/L, neutrophil count ≥ 1.5 × 10^9/L, platelets ≥ 100 × 10^9/L and hemoglobin ≥ 9 g/dL; SGOT, SGPT and alkaline phosphatases ≤ 2-fold the upper limit of normal (ULN) (≤ 3 ULN in case of liver metastases); bilirubin < 1.25 ULN; creatinine < 1.25 ULN, creatinine clearance ≥ 60 mL/min; good cardiac function (left ventricular ejection fraction > 50%), an Eastern Cooperative Oncology Groups (ECOG) performance status ≤ 2; no concurrent uncontrolled medical illness; no active viral hepatitis or HIV infection; no other current or previous malignancy in the past five years (except for adequately treated cone-biopsied in situ carcinoma of the cervix and basal or squamous cell carcinoma of the skin); a life expectancy of 12 or more weeks and a signed informed consent. Patients recruited had not received IM and IL-2 previously, except for GIST patients who could have received IM. No concomitant antitumor treatment was allowed. Patients with active brain metastases, severe concomitant cardiac disease (cardiac insufficiency, myocardial infarction during the previous six months, severe/unstable angina or stroke, non-controlled arterial hypertension), peripheral neuropathy ≥ grade 2 and pregnant or breast-feeding women were not eligible.

Dose escalation and toxicity assessment and clinical efficacy. The study was conducted as a single-center, Phase I, dose-escalation trial. A minimum of three patients were monitored for at least one complete cycle before dose escalation. If none of the first three patients in any cohort experienced a DLT, the next three patients were enrolled at the next higher dose level. If one instance of DLT was observed, three additional patients were treated at that dose level. When one of these additional patients had a DLT, the dose level of the previous cohort was considered as the MTD. No intra-patient dose escalation was allowed. Safety was evaluated at day one (D1), D10 and D14. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 3.0. DLT was defined as any unacceptable grade three or greater non-hematologic toxicity, grade 4 hematologic toxicity or treatment-related death. Response to therapy was assessed using the Response Evaluation Criteria in Solid Tumors (RECIST),[16] with scans evaluation at baseline, after one cycle of treatment (between D14 and D21) and every two cycles (every six weeks) until progression of the disease.

Treatment schedule. A low dose (50 mg/day) of the alkylating agent cyclophosphamide (CTX) was given orally from D-21 until D14, exclusively during the first cycle. IM (Gleevec®) was administered orally once daily at 400 mg from D1 to D14. IL-2 was administered subcutaneously from D10 to D14. Cycle 2 was started at day 21 (day 1 = day 21). Four dose levels of IL-2 (Proleukine®) were explored according to toxicity: 3 million international units (MIU) per day (level I), 6 MIU/day (level II), 9 MIU/day (level III) and 12 MIU/day (level IV). In case of DLT at the first level, a level 0 with 1 MIU/day had to be evaluated. If two patients presented a DLT at level 0, the association would be considered too toxic to be explored in a Phase II trial. IM and IL-2 cycles were repeated every 3 weeks until patients experienced disease progression or DLT. A dose reduction of no more than two dose levels of IL-2 was permitted after the first cycle and decided by the investigators. No dose escalation was permitted. A reduction of the IM dose was permitted in case of specific toxicity. Treatment could be stopped prematurely in case

of inacceptable toxicity, disease progression or patient refusal to continue the study. Data from patients who withdrew prematurely from the study were not included in the final analyses.

Chemicals. IM, [d8]-imatinib and *N*-desmethyl-imatinib (CGP74588) were kindly provided by Novartis, with purities of 99.6, 97.0 and 99.7%, respectively. Stock solutions of each compound were prepared at 1 mg/mL in methanol and stored at -20°C.

Blood sampling. In order to determine IM and CGP74588 concentrations, venous blood samples were collected during cycle 1 on D1, D10 and D14 at 0, 1, 2, 5, 8 and 24 h after drug administration. For each plasma sample, blood was collected in a tube containing heparin, inverted several times, centrifuged at 5,000 × g for 10 min and stored at -20°C.

Quantification of IM and CGP74588 by LC-MS/MS. Plasma samples (0.25 mL) were mixed with 50 μL of internal standard [d8]-imatinib (500 ng/mL in methanol), deproteinized with 200 μL of cold acetonitrile and centrifuged at 4,000 × g for 10 min at 4°C. The resulting supernatant was drawn into a glass vial and stored at 4°C until LC-MS/MS analysis. IM and CGP74588 were quantified in plasma samples by liquid chromatography/electrospray ionization tandem mass spectrometry (LC-ESI/MS/MS) using the Quattro-Ultima LCZ® triple-quadrupole mass spectrometer (Waters, Micromass) coupled with the 1100 series HPLC system (Agilent Technologies) and fitted with a Uptiphere® C18 column (5 μm, 100 mm × 2 mm i.d.) supplied by Interchim. The isocratic mobile phase composed of methanol/ammonium acetate 6 mM (72/28, v:v) was run at a flow-rate of 0.25 mL/min.

IM and CGP74588 were detected with the mass spectrometer operating in positive electrospray ionisation mode, using a capillary voltage, a cone voltage and a collision energy set at 3.5 kV, 40 V and 25 eV, respectively. IM, CGP74588 and the [d8]-imatinib internal standard were detected by multiple reaction monitoring (MRM) using the transitions 494→394, 480→394 and 502→394, respectively. The concentrations of both compounds were quantified using Masslynx® and Quanlynx® software with a weighted ($1/x^2$) least-square linear calibration over the range set from 10 to 10,000 ng/mL. Nine plasma standards (10, 20, 50, 100, 200, 1000, 2000, 5000 and 10000 ng/mL) were prepared in duplicate each day. Quality control plasma samples (10, 200, 2000 and 4000 ng/mL) were prepared over three days. Within-run and between-run accuracies and precision were evaluated to be lower than 15% according to FDA and ICH recommendations. The lower limit of quantification for both compounds was 10 ng/mL. The retention time of IM and CGP74588 were found at 2.1 and 2.6 min, respectively.

Pharmacokinetic analysis. Pharmacokinetic parameters were calculated from plasma IM and CGP74588 concentration-time using non-compartmental methods of WinNonlin, version 3.2 (Pharsight). The mean maximal plasma concentration of IM and CGP74588 (C_{max}) and the time to reach C_{max} (T_{max}) were recorded from the individual subject concentration-time curve. The terminal phase elimination rate constant (*K*e) was determined from the slope of the terminal portion of the log-concentration vs. time curve by linear least-squares regression analysis of the plasma concentration-time profile. The terminal phase elimination half-life (*t*1/2) was calculated as ln2/*K*e. The area under the plasma concentration-time curve from time 0 to the last measurable concentration at time t [AUC (0-t)] was calculated using the linear trapezoidal method. For IM and CGP74588, the AUC (0-inf) was computed as AUC (0-t) plus the extrapolation from the last time point to infinity using Ct/*K*e, where Ct is the last quantifiable concentration. The extrapolated AUC before the first time point and after the last time point did not exceed 20% of the total AUC (0–inf). For IM, total body clearance (Cl) was calculated from the dose/AUC (0–inf). The volume of distribution (Vd) at steady-state was calculated as Dose/Cl. Plasma concentrations below the assay lower limit of detection were treated as 0.0 ng/mL in order to calculate pharmacokinetic parameters. Actual times after the start of infusion were used in the calculation of pharmacokinetic parameters.

Serum concentration of the soluble form of sCD25 and IL-2. sCD25 levels of serum were determined in accordance with the manufacturer's procedure using the Soluble IL-2 Receptor EIA Kit obtained from IMMUNOTECH (IM10559; Beckman Coulter, Inc.). Patient's sera were collected at cycle 1 before treatment (D21) after metronomic cyclophosphamide (D1) and after IM treatment (D10) and at the end of IM+IL-2 treatment (D14). Serum IL-2 concentration was measured with the EIA Kit obtained from BD Bioscience (BD OptEIA). As in the pharmacokinetic study of IM, serum IL-2 concentrations were determined at D14 at 0, 1, 2, 5, 8 and 24 h after IL-2 administration.

Statistical analyses. For the efficacy analysis, the response rate was estimated on patients who had already received one cycle of treatment and for whom response had been evaluated. Descriptive data were used to summarize the characteristics of patients, the administration of and compliance to treatment, tolerance and efficacy and pharmacokinetics parameters. No interim analysis was planned. Descriptive data were compared, using the χ^2 test or Fisher's exact test for proportions and the Student's t-test or the Wilcoxon rank-sum test for continuous measures. Correlation analyses were performed between two parameters using the Pearson's test. OS and PFS were estimated according to the Kaplan-Meier method. OS was defined as the time from the diagnosis to death from any cause or to the last follow-up if no death had occurred. PFS was defined as the time from the diagnosis to progression or to the last follow-up if no progression has occurred. Patients who had not experienced an event at the time of the analysis were censored at the date of the last follow up.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by Novartis SA, Institut National du Cancer (INCa), la Ligue contre le cancer (LIGUE labelisée, L.Z.), l'Association pour la Recherche sur le Cancer (A.R.C.), Fondation pour la Recherche Médicale and Fondation de France. We acknowledge Novartis for providing chemicals used for pharmacokinetic analyses, i.e., [d8]-imatinib and *N*-desmethyl-imatinib (CGP74588). The authors thank Lorna Saint Ange (Institut Gustave Roussy) for editing.

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