Original Article

Imatinib Plus Low-Dose Doxorubicin in Patients With Advanced Gastrointestinal Stromal Tumors Refractory to High-Dose Imatinib

A Phase I-II Study by the Spanish Group for Research on Sarcomas

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BACKGROUND: In KIT-expressing Ewing sarcoma cell lines, the addition of doxorubicin to imatinib increases apoptosis, compared with imatinib or doxorubicin alone. On the basis of these in vitro data, the authors conducted a phase 1-2 trial of doxorubicin with imatinib in patients with gastrointestinal sarcoma tumors refractory to high-dose imatinib therapy. **METHODS:** Patients with metastatic gastrointestinal sarcoma tumor resistant to imatinib at 400 mg by mouth (p.o.) twice a day were eligible for this multicenter study, and received imatinib (400 mg p.o. every day [q.d.]) concomitantly with doxorubicin 15-20 mg/m^2/weekly for 4 cycles (monthly cycles), followed by imatinib (400 mg p.o. q.d.) maintenance in nonprogressive patients. Spiral computed tomography and positron emission tomography with F18-fluorodeoxyglucose were done basally and after 2 months of therapy to evaluate response. An in vitro study assessed the effect of combining imatinib and doxorubicin. **RESULTS:** Twenty-six patients with progressive gastrointestinal sarcoma tumor were entered in the study. Treatment was well tolerated. Three (14%) of 22 evaluable patients had partial responses per Response Evaluation Criteria in Solid Tumors, and 8 (36%) had clinical benefit (partial response or stable disease for $\geq$6 months). Median progression-free survival (PFS) was 100 days (95% confidence interval [CI], 62-138), and median survival was 390 days (95% CI, 264-516). Interestingly, PFS was 211 days (95% CI, 52-370) in patients with wild type (WT) *KIT* and 82 days (95% CI, 53-111) in non-WT patients (10 mutant, 6 not assessed). A synergistic effect on cell line proliferation and apoptosis was found with imatinib and doxorubicin combination. **CONCLUSIONS:** Low-dose chemobiotherapy combination showed promising activity in heavily pretreated gastrointestinal sarcoma tumor patients, especially in those with WT-*KIT* genotype. ***Cancer*** **2010;116;3692–701.** © *2010 American Cancer Society.*

KEYWORDS: gastrointestinal sarcoma tumor, imatinib, doxorubicin, wild type *KIT*.

Gastrointestinal stromal tumors are characterized by gain-of-function of the KIT receptor and occasionally the platelet-derived growth factor A receptor (PDGFRA). Imatinib, a small-molecule tyrosine kinase inhibitor, has shown activity in metastatic gastrointestinal sarcoma tumor, but 50% of patients progress during the first 2 years of therapy.[1,2]

Of patients treated with imatinib, 20% show primary resistance (progressive disease during the first 6 months of therapy).[3] These patients usually present a pattern of generalized disease progression and are usually refractory to

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subsequent therapies. The majority of these patients show *KIT* exon 9 mutations, wild type (WT) genotype, or *PDGFRA* D842V mutations.[4]

Secondary resistance typically occurs in patients initially harboring *KIT* exon 11 mutations. These patients usually present a pattern of limited disease progression or the so-called "nodule in mass," suggesting that imatinib remains active, at least in nonprogressive lesions. Three major potential mechanisms of imatinib resistance are described: overexpression of KIT receptor[5]; acquisition of secondary mutations in *KIT* exons 13, 14, and 17; or *PDGFRA*[6-9] and activation of alternative tyrosine kinases, with KIT down-regulation, because of a kinase switch mechanism.[10] Regardless of KIT status, the PI3K-AKT pathway remains crucial for cell survival.[11]

In Ewing sarcoma cell lines overexpressing KIT, the addition of doxorubicin to imatinib showed improved activity compared with imatinib or doxorubicin alone.[12,13] In these cases, imatinib synergistically sensitized Ewing sarcoma cells to doxorubicin treatment by arresting cell cycle and impairing intracellular signaling, mainly through MAPK pathway inhibition. On the basis of these in vitro published data, we hypothesized that the combination of doxorubicin with imatinib could result in clinical activity in patients with gastrointestinal sarcoma tumors refractory to imatinib therapy. The aim of the study is to evaluate a metronomic strategy with doxorubicin, to improve potential imatinib interactions, minimize toxicities, and reverse resistance.

MATERIALS AND METHODS

Inclusion and Exclusion Criteria

Patients were required to be $\geq$18 years of age, with histologically proven locally advanced and/or metastatic gastrointestinal sarcoma tumor, progressive disease per Response Evaluation Criteria in Solid Tumors (RECIST) or intolerance to 800 mg/d of imatinib, no previous treatment with doxorubicin therapy, no previous tumor other than basal skin cancer within 5 years of entry into the study, an Eastern Cooperative Oncology Group performance status of 0-2, measurable disease, left ventricular ejection fraction $\geq$50%, and adequate bone marrow, liver, and renal function. Exclusion criteria were severe associated diseases or active infection, central nervous system metastases, and psychological or sociological problems that could preclude awareness of the study's implications and requirements. No concurrent investigational therapy was allowed, and written informed consent was required.

Treatment

In phase 1, patients were treated in 2 cohorts with 2 levels of doxorubicin, 15 mg/m^2/wk and 20 mg/m^2/wk. All patients received a fixed dose of imatinib: 400 mg/d. At a minimum, 3 evaluable patients were treated at each level. Dose-limiting toxicity (DLT) was defined as grade 4 hematological or grade 3 or 4 nonhematological toxicity (by National Cancer Institute Common Toxicity Criteria). Safety of the combination was assessed in the phase 1 trial, with further evaluation in the phase 2 trial. If 1 of 3 patients at a given dose level experienced DLT, 3 more patients were accrued at that dose level. If 3 or more of 6 patients experienced DLT, then the maximum tolerated dose (MTD) was exceeded. The phase 1 trial MTD was selected for development in phase 2. Concomitant treatment was given during 4 months (4 cycles), and patients without progressive disease were allowed to continue receiving imatinib 400 mg/d until disease progression.

Criteria for Evaluation

Spiral computed tomography (CT) and positron emission tomography (PET) or PET-CT was performed before treatment and at 2 months of therapy. CT scans were repeated at 4 and 6 months and every 3 months thereafter, until disease progression. Two independent radiologists centrally reviewed all cases following RECIST[14]; a specialist in Nuclear Medicine performed all PET evaluation. Response to PET was defined according to the recommendations of the European Organization for Research and Treatment of Cancer PET Study Group[15] and compared with objective CT response. Progression-free survival (PFS) was established from the date of inclusion to the first documented date of progression or death for any cause; patients were censored at the date of last follow-up (February 28, 2009) if alive and free from progression at that time. Survival time was established as the time between the date of study inclusion and date of death or final follow-up.

Statistical Analysis

Fleming's single-stage phase 2 design was used along with Gehan's criteria to proceed to the phase 2 trial. Sample size was computed under the following assumptions: α error of .05, β error of .20, and an expected response rate of 10% of the presumptive active combination. Under these assumptions, if $<$2 or $\geq$5 partial responses were found in the 15 first evaluable patients, the study would be stopped because of lack of efficacy or evidence of efficacy, respectively. If 2 to 4 responses were found among

the first 15 evaluable patients, recruitment would be extended to 25 evaluable patients. Under these conditions, 5 partial responses in 25 patients would be needed to consider the experimental treatment eligible for an extended phase 2 trial. Time-to-event distributions were estimated by Kaplan-Meier analysis and compared by log-rank hypothesis test. SPSS 15.0.1.1 software was used (SPSS Inc., Chicago, Ill).

DNA Extraction and Mutation Analysis

DNA was isolated from 3 to 5 fixed and paraffin-embedded 5-mm sections of tumor tissue. Genetic analysis of exons 9, 11, 13, and 17 of *KIT* and exons 12 and 18 of PDGFR was performed by polymerase chain reaction. Negative controls were included in every set of amplifications. A bidirectional sequencing analysis was performed on an ABI 3130xl sequencer using the BigDye terminator v3.1 kit (Applied Biosystems, Foster City, Calif) with the specific primers.

Cell Lines

GIST882, a cell line with *KIT* mutation K642E in exon 13 (a gift from Dr. Jonathan Fletcher) was cultured in RPMI medium (Gibco, Grand Island, NY; Invitrogen, Carlsbad, Calif; Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (GIBCO, Invitrogen, Life Technologies).

Drugs

AEW541, a specific inhibitor of the kinase activity of insulinlike growth factor-I receptor (IGF1R), and imatinib were kindly provided by Novartis Pharma AG, Basel, Switzerland. These compounds were resuspended in dimethylsulfoxide (10 mM) and aliquoted in the desired working concentrations. Doxorubicin, purchased from Sigma Chemicals Co. (St. Louis, Mo), was resuspended in distilled water.

Dose-response proliferation of the cell lines under the influence of imatinib or doxorubicin was analyzed to determine the concentration that inhibits 50% (IC_{50}) of proliferation. The percentage of proliferation inhibition was then evaluated in cells treated with imatinib combined with doxorubicin. To determine the rate of viability, we used the 3-(4,5-demethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.

Isobolographic Analysis

The effects of the combination of imatinib with doxorubicin were analyzed by Loewe's isobolographic analysis[16] as revised by Steel and Peckman,[17,18] which distinguishes 3 types of interactions: pure additivity, synergy, and antagonism. For isoeffective dosages of a 2-drug combination (dA + dB) and the individual drugs alone (DA, DB), combinations with combination index >1 are considered as antagonistic, those with combination index = 1 as additive, and those with combination index <1 as synergistic (combination index = dA/DA + dB/DB). If synergy exists, then a lower concentration of dA and/or dB would be required to achieve the same effects of the theoretical dosages for additivity.[19]

In our studies, combination index values for each condition were calculated using the IC_{50} of proliferation, determined by plotting the MTT assay results in a Hill curve (using Origin 6.0), as the isoeffective point. Isobolograms were done by plotting the IC_{50} of imatinib on the x axis and the IC_{50} of doxorubicin on the y axis, with the line of additivity being the line that connects these 2 points.

Western Blot

Western blot (WB) studies were performed to analyze KIT pathway inhibition after treatment with imatinib or doxorubicin, evaluating the expression and activation of AKT and MAPK42/44. This study also included a pretreatment with AEW541 to test whether acquired resistance to imatinib could be because of a possible KIT reactivation through cross-phosphorylation by increased IGF1R activity.

Preliminary studies were performed to determine optimal doses and timing of all drugs and ligands (data not shown). On the basis of our results (Martins et al.[20,21]), we pretreated all cell lines with AEW541 and imatinib for 20 minutes, doxorubicin for 1 hour, and finally with insulinlike growth factor-1 for 15 minutes. The optimal concentrations of imatinib, AEW541, and doxorubicin were, respectively, 10 μM, 150 nM, and 80 ng/mL.

Initially, cell lysates were obtained by scraping the treated cells on ice with 500 μL of lysis buffer (NP-40 1%, NaCl 150 mM, ethylenediaminetetraacetic acid 50mM, glycerol 10%, Tris-HCl pH 7 20 mM, protease inhibitor cocktail tablet [Roche Molecular Biochemicals, Nutley, NJ], NaF 50 mM, and Na_3VO_4 2 mM), sheared through a 25-gauge needle and then centrifuged at 13,000 g for 15 minutes at 4°C. Protein concentration was determined using the BCA Protein Assay Reagent (Pierce Biotechnology, Rockford, Ill). Proteins (50 μg/lane) were resolved on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene

difluoride membranes. Blots were blocked with 5% bovine serum albumin in Tris-buffered saline with Tween, probed with the specific antibodies, and observed by enhanced chemiluminescence detection reagents (Amersham, Arlington Heights, Ill). To quantitatively assess the changes of phosphorylation, the intensity of each phosphoband was analyzed by densitometry with Quantity 1 4.3.1 software, GelDoc 2000 (Biorad, Hercules, Calif), and normalized against the total protein band.

The antibodies used were: antiphospho-p44/p42 MAPK (Thr202/Tyr204), anti-p44 of 42 MAPK, antiphospho-AKT (Ser473), anti-AKT (all from Cell Signaling Technologies, Beverly, Mass), and anti-GAPDH (Abcam, Cambridge, UK).

Apoptosis

Apoptosis was measured after treatment with imatinib and/or doxorubicin for 72 hours as described elsewhere.[21]

RESULTS

Patients

Twenty-six patients with histologically proven, locally advanced and/or metastatic gastrointestinal sarcoma tumor with progressive disease were entered into the study between June 2004 and November 2007 from 13 hospitals in Spain; 2 patients did not receive imatinib at 800 mg/day (major protocol violation) after progression to 400 mg/day, and response could not be evaluated by CT scan in 2 patients. Thus, 26 patients were evaluable for toxicity and 22 for activity. Three (10%) patients were intolerant to imatinib at 800 mg/day.

Twenty patients were centrally evaluable by PET. The study did not reach the targeted accrual (n = 25) because of slow recruitment. Clinical characteristics of these patients are depicted in Table 1.

Mutational Analysis

Tissue for gene mutation analysis was available from 18 (75%) of the 24 evaluable patients (Table 2). Median duration with imatinib at 400 mg was 31 months in *KIT*-mutant patients and 10.3 months in *KIT*-WT patients. Median duration of imatinib therapy to 800 mg was similar in *KIT*-mutant or *KIT*–WT patients: 3.5 months versus 3 months, respectively.

Treatment and Toxicity

All patients received at least 1 doxorubicin dose plus imatinib treatment, and 12 (46%) received the full 4 courses of treatment. A total of 79 courses of doxorubicin with

Table 1. Patient Characteristics

Characteristic	No. of Patients	%
Patients entered	26	
Patients eligible	24	92.3
Age, median y	57	
Range	26-80	
Sex, male	24	92.3
Primary location		
Gastric	11	42.3
Small bowel	10	38.5
Other	5	19.2
Metastatic sites		
Primary	2	7.7
Liver	20	76.9
Peritoneum	19	73.1
Other	8	30.8
ECOG performance Status		
0	8	20.8
1	16	61.5
2	2	7.7
LDH levels		
<ULN	13	50
>ULN	10	38.5
Unknown	3	11.5
Albumin		
≤3.5 mg/dL	4	15.4
>3.5 mg/dL	16	61.5
Unknown	6	23.1
Neutrophils		
<4500	16	61.5
>4500	9	34.6
Unknown	1	3.8
Previous therapy		
Chemotherapy	1	3.8
Imatinib 800	24	92.3
Sunitinib	4	15.4
RAD0001	1	3.8
Imatinib 400		
Sensitive[a]	22	84.6
Refractory[b]	2	7.7
Intolerant	2	7.7
Imatinib 800		
Sensitive[a]	7	26.9
Refractory[b]	14	53.8
Intolerant	3	11.5
Mutational phenotype		
KIT	11	42.3
WT	8	20.8
Not done	7	26.9

ECOG indicates Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; ULN, upper limit of normal; WT, wild type.
[a] Nonprogressive disease >3 months.
[b] Progressive disease <3 months.

Table 2. Efficacy and Tumor Genotype

Patient No.	Age	Sex	TTF IM 400, d	TTF IM 800, d	Metastatic location	Mutation analysis	PFS, d	Status, d
1	76	F	122	31	Peritoneal, bone, soft tissue	WT[a]	81	437 (DOD)
2	42	M	255	333	Unresectable primary tumor	WT	339	440 (DOD)
3	40	M	174	607	Liver, peritoneal	WT	231	505 (DOD)
4	71	M	752	97	Peritoneal	WT	57	411 (DOD)
5	26	M	308	929	Liver	WT	84	140 (LFU)
6	57	M	275	71	Liver, peritoneal	WT[a]	1108+	1108 (NED)
7	42	M	496	52	Liver	WT	211	305 (DOD)
8	65	M	608	46	Liver	WT	264	478 (DOD)
9	73	M	415	95	Liver, peritoneal, spleen, and primary	W557C+Del 558-559	342	821 (DOD)
10	54	M	406	154	Liver, peritoneal	Dup CD568-588	55	75 (DOD)
11	53	M	1032	232	Liver, peritoneal	Dup CD573-591	122	183 (DOD)
12	71	M	1123	102	Liver	Del CD557-558	51	60 (DOD)
13	55	M	986	237	Liver, lymph node	V560D	100	390 (DOD)
14	62	M	443	186	Peritoneal	K642E	64	369 (DOD)
15	34	M	41	98	Liver, peritoneal	Dup CD573-591	204	512 (DOD)
16	40	M	781	41	Peritoneal	W557C+Del 558-559	62	88 (DOD)
17	64	M	1188	35	Liver, peritoneal	V559A	65	170 (DOD)
18	51	M	867	89	Peritoneal	Del CD557-558	84	523 (DOD)
19	57	M	462	83	Liver, spleen, lymph node, peritoneal	ND	69	325 (DOD)
20	71	M	227	80	Liver, peritoneal	ND	63	244 (AWD)
21	63	M	1879	434	Liver, peritoneal, lymph node	ND	434	508 (AWD)
22	49	M	765	190	Primary, liver	ND	154	167 (DOD)
23	44	M	1037	31	Liver, peritoneal	ND	122	154 (DOD)
24	72	F	899	62	Liver, peritoneal	ND	82	162 (DOD)

TTF indicates time to treatment failure; IM, imatinib; PFS, progression-free survival; F, female; WT, wild type; DOD, death of disease; M, male; LFU, lost to follow-up; NED, no evidence of disease; ND, not done; AWD, alive with disease.
[a] Biopsy at study entry.

Table 3. Toxicity Profile

Toxicity	Phase I Patients (n=6), No.								Phase II Patients (n=20), No.			
	DX Dose Level 15 mg/m^2 (n=3)				DX Dose Level 20 mg/m^2 (n=3)							
	1	2	3	4	1	2	3	4	1	2	3	4
Hematological toxicity												
Leukocytes	0	2	0	0	1	1	0	0	5	2	0	0
Neutrophils	0	0	0	0	0	0	0	0	2	0	0	0
Platelets	0	0	0	0	0	0	0	0	3	0	0	0
Hemoglobin	2	0	1	0	2	1	0	0	11	6	2	0
Nonhematological toxicity												
Creatinine	1	0	0	0	0	0	0	0	4	1	0	0
Bilirubin	0	1	0	0	1	0	0	0	3	3	0	0
ASAT	2	0	0	0	1	0	0	0	3	1	1	0
ALAT	1	0	0	0	0	1	0	0	4	0	1	0
Febrile neutropenia	0	0	0	0	0	0	0	0	0	0	0	0
Anorexia	1	0	0	0	1	0	0	0	4	2	1	0
Diarrhea	1	0	0	0	0	0	0	0	2	1	0	0
Nausea	2	0	0	0	1	0	0	0	8	1	0	0
Vomiting	1	1	0	0	1	1	0	0	4	5	0	0
Stomatitis	1	0	0	0	1	1	0	0	3	2	0	0
Alopecia	1	0	0	0	1	1	0	0	8	5	0	0
Edema	0	2	0	0	0	0	0	0	5	0	0	0
Periorbital edema	0	1	0	0	1	0	0	0	1	0	0	0
Asthenia	0	2	0	0	1	1	0	0	4	4	3	0

DX indicates doxorubicin; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase.

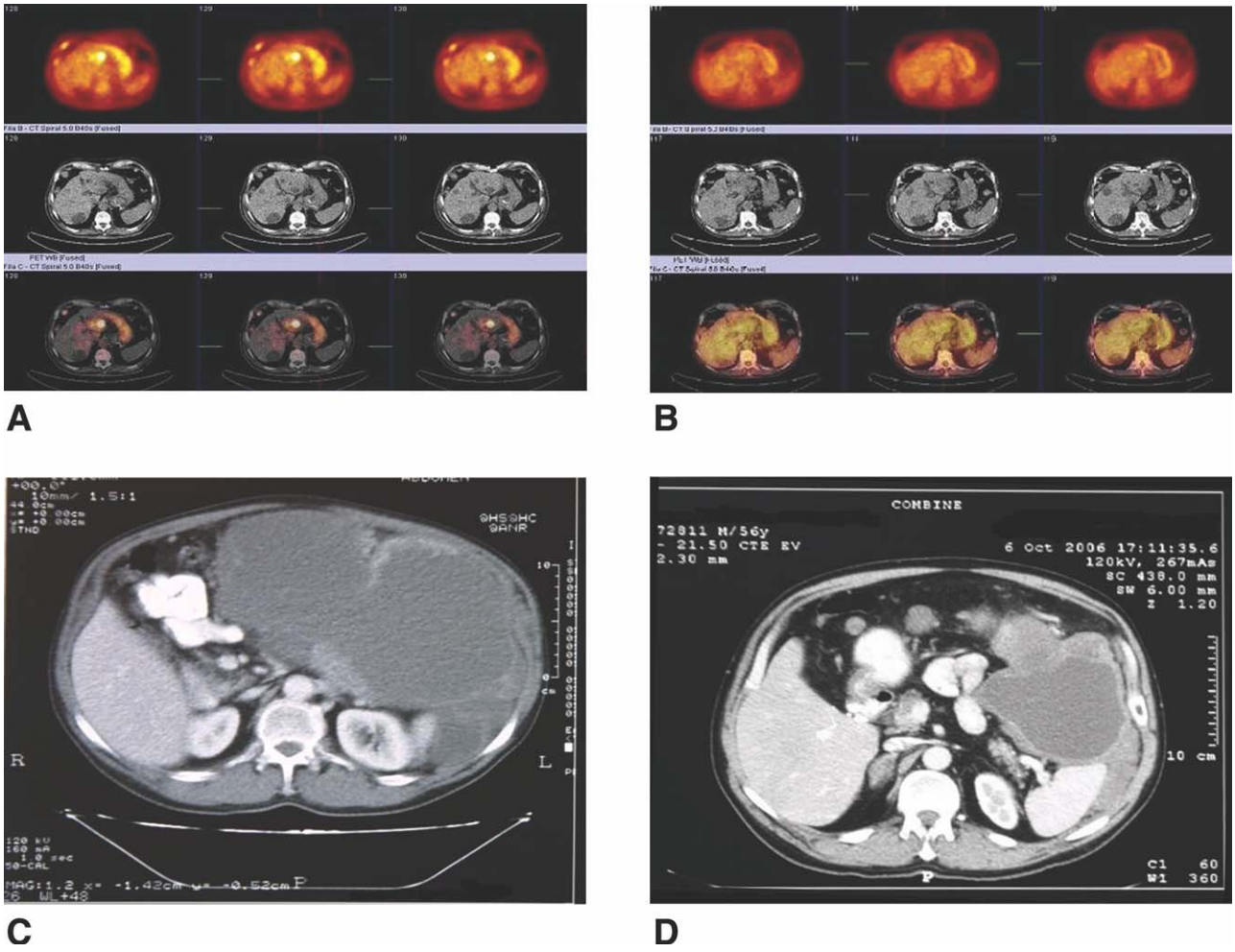

Figure 1. Patient 9 had positron emission tomography (PET)-computed tomography (CT) with primary gastric tumor and liver, peritoneal, and spleen metastases. Only 1 peritoneal implant showed PET-CT activity at study inclusion (A) and only this lesion progressed by RECIST criteria to imatinib 800 mg/d. PET, CT, and fused PET-CT transversal images show pathological uptake in the baseline study (maximum standard uptake value [SUV_{max}] = 9.40) (upper row). There was a significant decrease in the intensity of the lesion in the follow-up study (SUV_{max} = 3.71), considered a partial response by European Organization for Research and Treatment of Cancer PET criteria (lower row) (B). Initially, Patient 9 had a KIT mutation (W557C) and a critical deletion (558-559) on exon 11. At study entry, Patient 6 had a wild-type genotype and a bulky peritoneal implant on baseline CT scan (C). After 8 months of therapy (October 2006), there was a partial response per Response Evaluation Criteria in Solid Tumors criteria (D). The patient underwent surgical resection on February 2007 and in February 2009 was still alive without disease.

imatinib were given, with a median number of 3 cycles of doxorubicin given to each patient (range, 1-4 cycles). The dose was reduced at least once in 8% of the patients and in 3% of the cycles, mostly because of asthenia. Treatment was well tolerated. Most frequent adverse events included: alopecia, asthenia, edema, and mucositis (see Table 3). Cardiotoxic events were not seen in the study.

Response, PFS, and Overall Survival

Two of the 24 eligible patients could not be evaluated for activity because CT evaluation at 2 months was not done (investigator decision and death because of pneumonia). Three (14%) of 22 patients had partial responses (PRs) per RECIST, and 8 (36%) patients had clinical benefit. Significantly fewer lesions were identified by the principal investigator (n = 73; median, 2; range, 1-6) than by the radiologist (n = 122; median, 5; range, 1-10), but concordance regarding response assessment was good (quadratic-weighted kappa, 0.68; 95% confidence interval [CI], 0.47-0.90). Of 122 lesions identified by CT, 28 did not demonstrate significant appreciable glucose uptake at baseline (on 800 mg/day of imatinib treatment) on fluorodeoxyglucose (FDG)-PET. Mean baseline tumor size on CT was 5.9 cm (range, 0.9-28 cm), and mean baseline

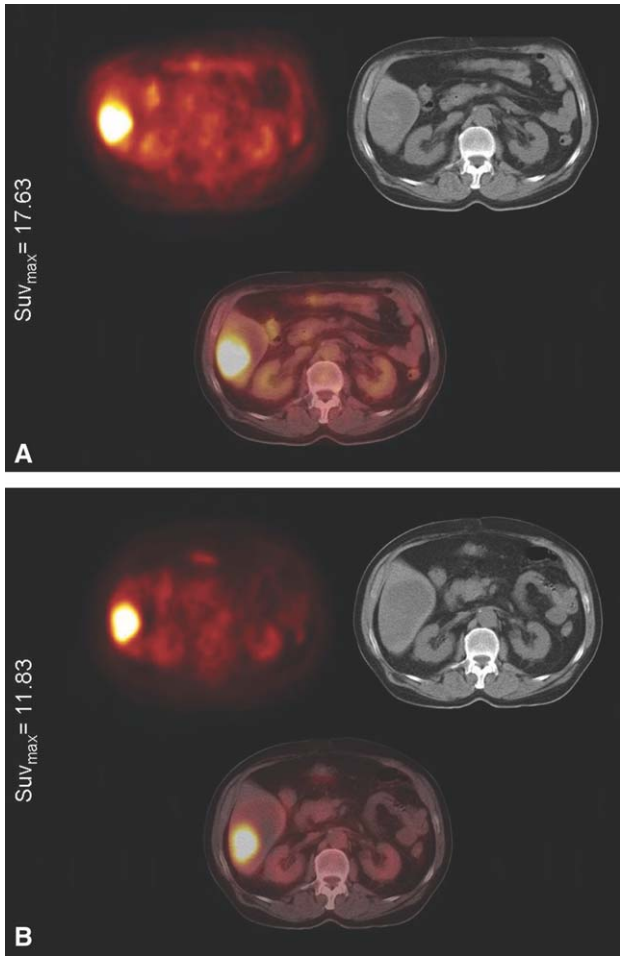

Figure 2. Patient 21 had positron emission tomography (PET)-computed tomography (CT) with an active metabolic deposit in the liver at study entry (*Top*). After 2 months of therapy, there was a partial response by PET-CT but a stable response per Response Evaluation Criteria in Solid Tumors (RECIST) (*Bottom*). At 12 months after study entry, liver metastases showed a partial response by RECIST criteria. SUV$_{max}$ indicates maximum standard uptake value.

maximum standard uptake value on FDG-PET was 7.2 (range, 1.9-26.4). Eight patients had a partial response by FDG-PET based on European Organization for Research and Treatment of Cancer criteria, compared with 2 responses identified by radiologist review per RECIST (Figs. 1 and 2). Concordance between FDG-PET and CT was poor (quadratic-weighted kappa, 0.35; 95% CI, 0.05-0.66). Patients having disease control (PR or stable disease, n = 14), assessed by PET, had longer overall survival (OS) (14.6 months; 95% CI, 12.3-17) than those patients who were classified as progressive disease (n = 6; 4.1 months; 95% CI, 0.8-7.3; $P = .008$).Median PFS was 3.3 months (95% CI, 2.1-4.6), and median survival was 13 months (95% CI, 8.2-17.2). Interestingly, median PFS and OS in patients with *KIT*-WT (n = 8) were 7 months (95% CI, 1.7-12.3) and 14.6 months (95% CI, 14.4-14.9), respectively, which was rather better than in non-WT patients (10 mutant, 6 not assessed): 2.7 months of PFS (95% CI, 1.7-3.7; $P = .134$) and 5.6 months of OS (95% CI, 0-1.8; $P = .249$).

In Vitro Studies

In vitro studies showed that imatinib treatment alone had almost no effect on proliferation or apoptosis of gastrointestinal sarcoma tumor cell line GIST882. We were only able to detect mild effects at very high concentrations; IC_{50} of proliferation was 42.88 μM, much above physiological concentrations. However, this drug was able to sensitize GIST882 cells to the effects of doxorubicin action, resulting in an additional decrease of 20% to 50% in the proliferative rate when imatinib and doxorubicin were combined. Combination was highly synergistic (with combination indexes between 0.68 and 0.57, Fig. 3A), both at the level of proliferation inhibition and at apoptosis induction (Fig. 3B). In the latter, the higher the concentration of both drugs, the greater the decrease of alive cells and the increase of apoptotic/necrotic cells. Imatinib + doxorubicin resulted in an additional increase of 20% to 0% in apoptosis.

The effectiveness of imatinib combination with doxorubicin was driven, at least partially, by the reduction of AKT and MAPK42/44 phosphorylation. As depicted in Figure 3C, the WB studies demonstrated that although the KIT signaling pathway was being activated in the control situations (showing AKT and MAPK42/44 phosphorylation), KIT signaling was almost unaffected when cells were pretreated with imatinib. However, AEW541 or doxorubicin pretreatment, combined with imatinib, dramatically decreased AKT and MAPK42/44 phosphorylation, showing additive effects. It is remarkable that imatinib combination with AEW541 was able to block AKT and MAPK42/44 phosphorylation in about 80%, suggesting that acquired resistance to imatinib could be because of a possible KIT reactivation through cross-phosphorylation by increased IGF1R activity (Fig. 3C).

DISCUSSION

Advanced nonresectable gastrointestinal sarcoma tumors are refractory to cytotoxic chemotherapy, including doxorubicin.[22] Nonetheless, we have observed that imatinib 400 mg given once a day concomitantly with metronomic

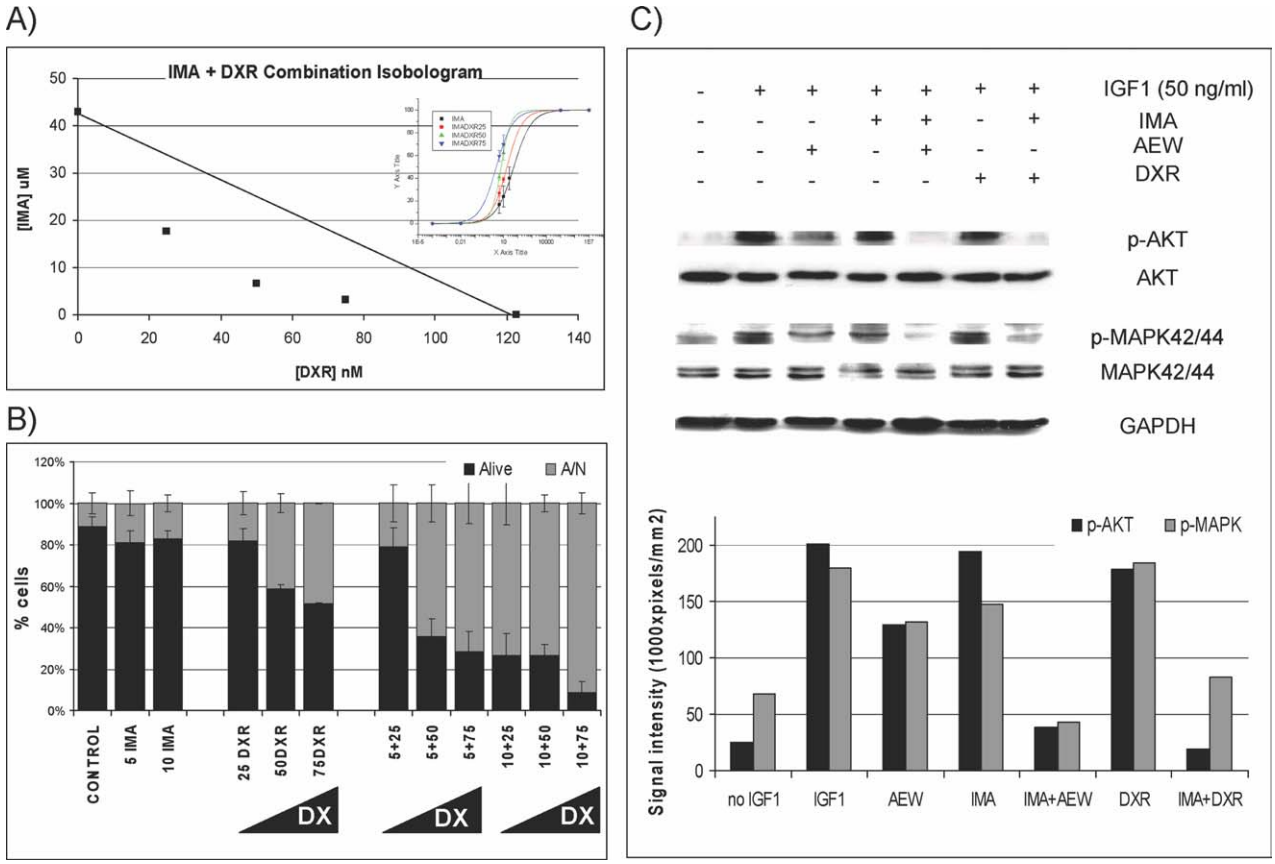

Figure 3. In vitro studies were conducted with the GIST882 cell line refractory to imatinib treatment. Subconfluent cells were treated with different combinations of 5 or 10 μM imatinib and 25 to 75 ng/mL of doxorubicin for 72 hours. (A) Antiproliferative effects of imatinib are shown, alone or combined with doxorubicin. The concentration inhibiting 50% of proliferation for imatinib was 42.88 μM; for doxorubicin, it was 122.65 ng/mL. Combined treatment resulted in an additional decrease of 20% to 50% in the proliferative rate (95% confidence interval, 0.57-0.68). Drug combination is synergistic in all cases (combination index <1), as shown in the isobologram. (B) Proapoptotic effects of imatinib and/or doxorubicin are shown. The higher the concentration of both drugs, the greater the decrease of alive cells and the increase of apoptotic/necrotic cells. The effect of combining imatinib + doxorubicin resulted in an additional increase of 20% to 40% in apoptosis. (C) Effects of imatinib, alone or combined with AEW541 or doxorubicin, on the activation of AKT and MAPK42/44 (Western blot, upper panel; densitometric analysis, lower panel). The signaling pathway was activated after treatment with insulinlike growth factor-I for 15 minutes (lanes 2-7), showing AKT and MAPK42/44 phosphorylation. Nevertheless, when pretreated with imatinib for 20 minutes, phosphorylation decreased (lanes 4, 5, and 7). AEW541 or doxorubicin pretreatment, combined with imatinib, dramatically decreased AKT and MAPK42/44 phosphorylation (60%-80% reduction). Doxorubicin alone did not have significant effects on AKT or MAPK activation. Combination of imatinib + AEW541 or doxorubicin showed additive effects. In all cases, filters were stripped and incubated with antibodies against total AKT, MAPK, and GAPDH to control for lane load. Similar bands confirmed the correct load of equal amounts of protein.

doxorubicin therapy shows activity in patients refractory to imatinib 800 mg/d. We could not rule out that metronomic doxorubicin therapy alone, a schedule never tested previously in gastrointestinal sarcoma tumors, would be active per se.[23,24] Because we were afraid of the toxic effect of the combination of high-dose imatinib plus doxorubicin, we reduced the dose of imatinib to 400 mg/d.

The reasons for the activity of the combination are not clear. Recent in vivo preclinical data show that the association of cytotoxic metronomic therapies with dual vascular endothelial growth factor receptor and PDGFR inhibitor (imatinib or sunitinib) further enhanced efficacy. In this model, the pericyte detachment induced by PDGFR inhibition sensitized the endothelial cells to metronomic chemotherapy.[25] Therefore, we could not rule out the possibility that our combined therapy could also have an antiangiogenic effect.

WT-*KIT/PDGFRA* constitutes 15% of adult patients with gastrointestinal sarcoma tumors. Despite absence of *KIT* mutation, KIT plays an important role in

oncogenesis, and the level of KIT activation is similar to that in patients with mutant *KIT*.[26] Amplification of the *IGF1R* gene has been observed recently in this subset of patients,[27] but acquired mutations were not seen at progressive disease, suggesting different mechanisms of acquired resistance than in patients with *KIT* exon 11 mutations.[28] We observed, in accordance with the literature[4,29] that first-line therapy with 400 mg imatinib resulted in shorter PFS in WT-*KIT* patients than in patients with *KIT* exon 11 mutations. Surprisingly, however, the WT-*KIT* patients experienced a major benefit from the second-line therapy, similar to findings recently published by Heinrich et al.[30] We have shown in vitro that imatinib resistance can be reversed with the addition of doxorubicin. This combination is highly synergistic and induces apoptosis through pAKT down-regulation. Our work suggests that our combination has limited activity in patients with primary *KIT* exon 11 mutations. Most of these patients acquire secondary mutations in *KIT* exon 13, 14, and 17 at progressive disease, and therefore the lack of activity seems comprehensive. Despite in vitro activity of new tyrosine kinase inhibitors in patients with acquired mutations such as sorafenib or HSP90 inhibitor (IPI-504),[31] the efficacy of these drugs in clinical settings is extremely poor, with <5% objective response per RECIST,[32,33] suggesting that alternative tyrosine kinase receptors could also be implicated in imatinib resistance in patients with acquired mutations. Conversely, KIT inhibitors could be combined with other cytotoxic agents in a more prolonged metronomic therapy without dose-limiting toxicities. This strategy has been successfully tested with docetaxel and MP470 in gastrointestinal sarcoma tumor cell lines with secondary acquired mutations.[34]

PET and CT correlate poorly in our study. In our sample, evaluation of response by PET seems to translate the efficacy better than RECIST by investigator or by radiologists. PET criteria correlate well with PFS in pretreated patients,[35] but further studies should be performed in imatinib-refractory disease, with independent data, before routinely incorporating it in future studies. Cardiotoxicity is a matter of concern in patients treated with imatinib,[36] and its administration along with an anthracycline may have synergistic effects. Nevertheless, recently published prospective data suggest that imatinib alone produces an insignificant deterioration of cardiac function[37,38] and exerts its cardiotoxic effects mostly on elderly people with pre-existing cardiac morbidities, a population excluded from this trial, and doxorubicin was administered far from its threshold toxic dose.

In conclusion, the reported low-dose chemobiotherapy combination shows promising activity in heavily pretreated gastrointestinal sarcoma tumor patients, specially in those with WT *KIT* phenotype, and appears as a reasonable and safe option for patients not responding to high-dose imatinib therapy. However, we cannot rule out that a more inherent indolent biologic behavior could also contribute to the favorable outcome of WT patients. In our opinion, the strategy of combining chemotherapy, IGF1R inhibitors, and new tyrosine kinase inhibitors such as nilotinib, sorafenib, or HSP-90 inhibitors merits attention in future clinical trials in patients resistant to imatinib and sunitinib.

CONFLICT OF INTEREST DISCLOSURES

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