available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Target-driven exploratory study of imatinib mesylate in children with solid malignancies by the Innovative Therapies for Children with Cancer (ITCC) European Consortium ☆

Birgit Geoerger[a,], Bruce Morland[b], Anna Ndiaye[c], Francois Doz[d], Gabriel Kalifa[e], Anne Geoffray[f], Fabienne Pichon[g], Didier Frappaz[h], Etienne Chatelut[i], Paule Opolon[j], Sharon Hain[k], Francoise Boderet[l], Jacques Bosq[m], Jean-Francois Emile[n], Marie-Cecile Le Deley[c], Renaud Capdeville[o], Gilles Vassal[a], on behalf of the Innovative Therapies for Children with Cancer (ITCC) European Consortium*

[a]*Department of Paediatrics, Institut Gustave Roussy, 39 Rue Camille Desmoulins, 94805 Villejuif; University Paris-XI, France*
[b]*Children's Hospital, Steelhouse Lane, B4 6NH Birmingham, United Kingdom*
[c]*Biostatistics and Epidemiology, Institut Gustave Roussy, 39 Rue Camille Desmoulins, Villejuif; University Paris-XI, France*
[d]*Paediatrics, Institut Curie, 26 Rue d'Ulm, 75248 Paris cedex 05, France*
[e]*CHU Cochin Port Royal, 27 Rue du Faubourg Saint-Jacques, 75014 Paris, France*
[f]*CHU Fondation Lenval, 57 Avenue de la Californie, 06000 Nice, France*
[g]*Centre Oscar Lambret, 3 Rue Frédéric Combemale, BP 307 – 59020 Lille, France*
[h]*Centre Léon Bérard, 28 Rue Laennec, 69373 Lyon, France*
[i]*EA3035 Institut Claudius-Regaud, 31052 Toulouse, France*
[j]*UMR8121 Vectorology and Gene Transfer, Institut Gustave Roussy, 39 Rue Camille Desmoulins, Villejuif, France*
[k]*University College Hospital, 235 Euston Road, NW1 2BU London, United Kingdom*
[l]*CHU Nantes, 50 Route St Sébastien sur Loire, 44093 Nantes, France*
[m]*Pathology Departments, Institut Gustave Roussy, 39 Rue Camille Desmoulins, Villejuif, France*
[n]*Department of Pathology, Centre Ambroise Pare, 9 Avenue Charles de Gaulle, 92104 Boulogne, France*
[o]*Novartis Pharma AG, Lichtstrasse 35, Basel CH-4002, Switzerland*

ARTICLE INFO

Article history:
Received 16 January 2009
Received in revised form 18 February 2009
Accepted 9 March 2009
Available online 9 April 2009

Keywords:
Tyrosine kinase inhibitors

ABSTRACT

Aim: To explore imatinib efficacy and pharmacokinetics in children and adolescents with refractory/relapsing solid tumours, expressing imatinib-sensitive receptor tyrosine kinases.

Methods: Exploratory study on imatinib in tumours expressing, at least, one of the receptors KIT or platelet-derived growth factor receptor (PDGFR). Standard radiological response evaluation, pharmacokinetics, gene mutations and positron emission tomography imaging were assessed.

Results: Thirty-six patients (median age: 13.7 years) with brain (12), mesenchymal/bone (14) or other solid tumours, received imatinib 340 mg/m^2/d over a total of 255 months. Fifteen

☆ The study was funded by a grant from the Ligue Nationale Contre le Cancer within the framework of the project entitled Early Therapeutics Development in Pediatric Oncology and Novartis and has been presented in part at the Annual Meetings of the ASCO, SIOP and EORTC-NCI-AACR in 2006.

* *Corresponding author:* Tel.: +33 1 42 11 46 61; fax: +33 1 42 11 53 08.
E-mail address: geoerger@igr.fr (B. Geoerger).

0959-8049/$ - see front matter
doi:10.1016/j.ejca.2009.03.007

Childhood solid tumours
Exploratory study

tumours expressed KIT in ⩾30% cells, 19 expressed PDGFRA and 25 expressed PDGFRB. Twenty patients experienced grades 1–2 treatment-related toxicities. Ten patients achieved stable disease; one chordoma had metabolic response. Pharmacokinetic data showed high inter-patient variability (variation coefficient: 44% and 53% for plasma imatinib and CGP 74588 AUCs, respectively).

Conclusions: Imatinib was tolerated well, but failed to show efficacy according to standard criteria in paediatric malignancies expressing KIT or PDGFR.

1. Introduction

Imatinib mesylate (STI571, Glivec®, Gleevec®) selectively inhibits the ATP-binding site of platelet-derived growth factor (PDGFR), ABL (p210 BCR-ABL, p185 BCR-ABL, v-ABL and c-ABL) and c-KIT tyrosine kinases. It has significantly changed the treatment of patients with chronic myeloid leukaemia (CML) and gastrointestinal stromal tumours (GISTs), both diseases driven by constitutive activation of BCR/ABL, c-KIT or PDGFRA through gene translocation or activating mutation, respectively. Clinical activity of imatinib has subsequently been reported in dermatofibrosarcoma protuberans and giant-cell fibroblastoma exhibiting a *COL1A1/PDGFB* gene translocation,[1,2] CMML carrying the *ETV6-PDGFRB* or the *RAB5-PDGFRB* fusion genes,[3] and *del(4)(q12)-FIP1L1/PDGFRA* in hypereosinophilic syndrome.[4–6] However, the potential for imatinib treatment in other solid malignancies including those in children still needs to be determined. Various types of cancers have been reported to co-express growth factors and their receptors such as KIT and PDGFR. While growth factor receptor pathways activated by translocations (as in CML), mutational events (as in GIST), or gene amplification (as found in GBM), have been proven relevant for oncogenesis and successful targeted treatment, the role of receptor and ligand co-expression for the underlying disease and its relevance for targeted agents is unknown.

Since it is difficult to identify patients whose tumours are driven by the targeted pathway, this exploratory study evaluated the efficacy of imatinib when used as a single agent in paediatric solid malignancies expressing at least one imatinib-sensitive receptor tyrosine kinase, using standard radiological assessments as well as investigational metabolic activity, and extended the knowledge on safety and pharmacokinetics of imatinib in children and adolescents. This is the first clinical trial run by the European Consortium for Innovative Therapies for Children with Cancer.

2. Patients and methods

2.1. Patient eligibility

Eligibility criteria included: age at inclusion between 6 months and 21 years; progressive, refractory or relapsing solid malignancy; measurable disease; immunohistochemical (IHC) positivity of KIT, PDGFRA or PDGFRB in ⩾30% of tumour cells on archive tumour material (after amendment PDGFRs in ⩾80%); centrally re-analysed IHC positivity; life expectancy >6 weeks; no concomitant anticancer or investigational drug; Karnofsky performance status or Lansky play score >50%; completion of anticancer therapy ⩾4 weeks prior to study entry; adequate bone marrow reserve (neutrophils $> 1.0 \times 10^9$/L; platelets $> 75 \times 10^9$/L); AST/ALT ⩽ 2.5 times the upper limit of normal (ULN) (<5 × ULN if hepatic disease involvement); bilirubin ⩽1.5 × ULN; creatinine < 1.5 × ULN for age; no organ toxicity ⩾ 2; no other serious concomitant illness; negative pregnancy test and use of contraception if adequate and written informed consent by patient or parents/guardian. The protocol was approved by the institutional review board/independent ethics committee.

2.2. Study design

!This multi-centre, non-comparative, exploratory study aimed to support the proof-of-concept that inhibition of deregulated phospho-tyrosine kinase activity might be an important anticancer intervention for the targeted diseases. Since little data on KIT and PDGFR expression or gene alterations in paediatric cancer existed prior to the study, a screening of different paediatric solid malignancies and of patients who are potentially eligible was performed by immunohistochemistry for the three targets. In the study protocol, up to five patients per indication were initially accepted. Where evaluations according to conventional clinical response and/or pharmacodynamic criteria (i.e. reduced metabolic activity) suggested a potential role of imatinib, additional patients with the same type of disease were enrolled to enable adequate evaluation of imatinib effects.

2.3. Study treatment

Imatinib mesylate (Glivec®; supplied by Novartis as 100-mg hard capsules) was administered orally at 340 mg/m^2/day during 4-week cycles. Dose escalation to 440 mg/m^2/day was permitted if no improvement occurred after 4–8 weeks. Transfer of the capsule content to mineral water was allowed for patients unable to swallow the capsules. It was recommended to take imatinib with the breakfast or main meal (avoiding xanthines or grapefruit).

If grade ⩾2 non-haematologic toxicity occurred, imatinib was to be stopped until the toxicity resolved to grade ⩽1. For grade 2 toxicity, the treatment was restarted at the same daily dose; for recurrent grade 2 or grades 3–4 toxicities, the daily dose was reduced by 100 mg/m^2/day. No dose interruption or reduction was recommended for grade ⩽3 haematological toxicity. In case of grade 4 haematological toxicity, imatinib was to be withheld until toxicity resolved to grade ⩽2 and then restarted at the same dose if toxicity had

resolved to grade 2 within 2 weeks or reduced by 100 mg/m^2 if toxicity recurred or persisted for longer than 2 weeks.

Concomitant treatment with drugs known to interact with the CYP450 isoenzymes 2D6 and 3A4 was recommended to be used only after careful consideration.

2.4. *Efficacy and toxicity assessments*

Tumour response was assessed according to World Health Organisation (WHO) criteria[7] for every two cycles. Objective responses defined as complete (CR) or partial (PR) were to be confirmed at 4–6 weeks. When applicable, tumour biological markers were assessed to evaluate tumour response for non-radiological measurable diseases. On an exploratory basis, ^{18}FDG or methionine tumour avidity was assessed by PET scan after 4 weeks (if initially positive) to detect metabolic response. All imagings were reviewed by an independent committee of two radiologists and two PET reviewers. Efficacy end-points included progression-free survival (PFS) and overall survival (OS) also. PFS and OS were estimated on the whole series (intention to treat analysis). Study-cut-off was December 2007.

Adverse events (AEs) and laboratory tests were assessed throughout the study using NCI-CTCAE version 3.0. Adverse reactions are reported for the first 24 months of treatment.

2.5. *Immunohistochemistry for KIT, PDGFRA and PDGFRB expression*

Patients to be included in the study had IHC centrally performed. Formalin-fixed paraffin-embedded tissues were cut into 4-μm sections. KIT expression was determined using the polyclonal rabbit antibody anti-CD117 (A4520 DAKO, Denmark) diluted at 1:50, following microwave treatment in citrate buffer (10 mM, pH 7) and detection using the ABC Detection Kit DAB and Ventana automate (model NEXES) (Ventana, Illkirch, France). PDGFRA expression and PDGFRB expression were determined using the goat anti-human PDGFRA antibody (AF-307-NA, RD Systems), 1:50 for 45 min, and the rabbit anti-human PDGFRB (P-20, sc-339, Santa Cruz), 1:300 for 60 min, respectively, following microwave antigen retrieval in citrate buffer (pH 7.3). Staining was revealed using Universal LSAB® and Envision+ (DAKO), respectively, and the chromogen diaminobenzidine in the DAKO Autostainer system. Nuclei were counterstained with haematoxylin. Percentage of positive staining in tumour cells was estimated; cytoplasmic and membranous staining was obliged for KIT according to the criteria for GIST. All IHCs were reviewed by an independent pathologist (P.O.).

2.6. *Pharmacokinetic methods*

Heparinised blood samples were collected before and during 24 h after the first imatinib administration, before, 2–4 h after drug intake on day 30 and day 60 and were centrifuged and plasma was stored at –20 °C. Quantitative analyses of imatinib and CGP 74588 were performed using reverse-phase HPLC with fluorescence detection and coupled with tandem mass spectrometry (HPLC–MS–MS) as described before.[8] Plasma imatinib and CGP 74588 concentrations were analysed according to a non-linear mixed effects ('population') approach using the NONMEM program (version V, level 1.1) running on a PC (Pentium 200 pro) using the first-order method.[9] A proportional error model was used for both inter-patient variability and residual variability. Systemic exposures (i.e. area under the curve (AUC)) to imatinib and to CGP 74588 were calculated using individual post hoc clearance: AUC = -Dose/CL for imatinib, and AUCm = Dose/(CLm/fm) for CGP 74588 on days 1, 30 and 60.[8,10]

2.7. *Mutation analysis*

Mutations within exons 9, 11, 13 and 17 of *KIT* and within exons 12 and 18 of *PDGFRA* were detected as described previously.[11,12] Briefly, DNA was extracted from formalin-fixed paraffin-embedded tumour samples. In all cases, histology

Table 1 – Patients' characteristics (total number of patients: #36).

Characteristics	#	%
Age (years)		
Median	13.7	
Range [min–max]	2.2–22.5	
Sex		
Male	21	58
Female	15	42
Karnofsky performance status or Lansky play score		
100–90%	19	54
80–70%	12	34
60–50%	2	6
<50%[a]	2	6
Months since initial diagnosis		
Median	22	
Range [min–max]	1.6–175	
Tumour staging at study entry		
Metastatic	21	58
Localised	15	42
Disease at study entry		
Relapse or progression	32	89
Refractory disease	3	8
No standard therapy[b]	1	3
Prior anticancer treatment		
Chemotherapy	30	
Median lines of chemotherapy [range]	3 [1–13]	
High-dose chemotherapy regimens	11	
Radiation therapy	22	
Surgical resection	27	
Immunotherapy	1	
Hormone therapy	1	
Immunohistological expression in ⩾30% tumour cells		
KIT	15	
PDGFRA	19	
PDGFRB	25	

a Low performance status was due to neurological deficits in pontine glioma.
b Patient with GIST.

Table 2 – Target expression and tumour response to imatinib in 36 patients.

Disease	Patients (%)	IHC positivity in ⩾ 30% tumour cells	Gene Mutation[a]	Clinical Response, TTP	PET after one cycle
Mesenchymental or bone tumour	15 (42%)				
Fibromatosis					
Popliteal		70% KIT, 80% PDGFRB	None	SD, 7 mo	Decreased
Dorsal		60% KIT	None	PD	Increased
Mandible		80% PDGFRB	ND	SD, (–8%), 5 mo	Unchanged weak
Abdomino-pelvic		80% PDGFRB	ND	SD (–44%), >42 mo[f]	ND
Popliteal		80% PDGFRB	None	PD	Unchanged strong
Deltoid		80% KIT, 100% PDGFRB	ND	PD	Increased
Synovialosarcoma		80% PDGFRA	None	SD, 4 mo	Unchanged strong
Synovialosarcoma		100% PDGFRB	None	PD	Increased
Osteosarcoma		80% PDGFRA	ND	PD	ND
Osteosarcoma		40% KIT, 90% PDGFRB	ND	NE[b]	ND
Ewing tumour		40% KIT, 90% PDGFRA, 100% PDGFRB	ND	PD	ND
Ewing tumour		80% KIT, 80% PDGFRB	ND	PD	ND
Gastrointestinal stromal tumour		100% KIT, 80% PDGFRB	None	NE[d,f]	Unchanged strong
Malignant juvenile xanthogranuloma		100% PDGFRA, 80% PDGFRB	ND	PD	ND
Pseudo-inflammatory tumour		100% PDGFRA, 100% PDGFRB	None	SD[e], >41 mo[f]	Unchanged strong
Cerebral tumour	12 (33%)				
Brain stem glioma**		50% PDGFRA, 50% PDGFRB	ND	SD (–31%)[e], 10 mo	ND
Brain stem glioma		70% PDGFRA	ND	PD	ND
Brain stem glioma		80% KIT, 90% PDGFRA, 80% PDGFRB	ND	NE	ND
Brain stem glioma		100% PDGFRA	ND	PD	ND
Medulloblastoma		80% KIT	None	SD, 38 mo	Decreased
Medulloblastoma		60% KIT, 80% PDGFRB	ND	PD	ND
Atypical teratoid rhabdoid tumour		40% PDGFRA, 80% PDGFRB	NE	PD	ND
Atypical teratoid rhabdoid tumour		100% PDGFRB	NE	PD	ND
Oligodendroglioma		80% PDGFRA	ND	PD	ND
Choroid plexus carcinoma		50% PDGFRA, 90% PDGFRB	None	PD	ND
Low-grade glioma		60% KIT, 80% PDGFRA, 80% PDGFRB	NE	SD, 5 mo	ND
Cerebral germ cell tumour		100% PDGFRB	NE	PD	ND
Other tumours	9 (25%)				
Chordoma					
Clivus		80% KIT, 90% PDGFRA, 90% PDGFRB	None	SD, 2.5 mo	PR
Clivus		70% KIT, 70% PDGFRA, 90% PDGFRB	ND	PD	Unchanged strong
Vertebral		90% PDGFRA, 100% PDGFRB	None	PD	Unchanged strong
Neuroblastoma		100% PDGFRB	ND	NE[c]	ND
Renal cell carcinoma		90% PDGFRA	ND	SD, 7 mo	ND
Hepatoblastoma		40% PDGFRA, 80% PDGFRB	ND	PD	ND
Hepatocellular carcinoma		90% KIT	ND	PD	New lesion
Undifferentiated carcinoma of nasopharyngeal tract		90% KIT	ND	PD	New lesion
Germ cell tumour		80% PDGFRA, 50% PDGFRB	None	PD	ND

TTP: time to progression, PD: progressive disease, SD: stable disease, PR: partial response, ND: not done, NE: not evaluable. ** Objective effect with clinical improvement.

a Mutations in exons 9, 11, 13 and 17 of *KIT*, exons 12 and 18 of *PDGFRA*; NE: not evaluable; ND: not done.

b Early stop due to toxicity.

c Palliative irradiation of the target, but clinical progression.

d Non-measurable lesion (hepatic nodules < 10 mm) at baseline. The patient is free of progression and on treatment at >40 months.

e Documented progressive disease at study entry.

f Patient still with ongoing treatment at >42 months.

confirmed that the analysed sample contained more than 80% of tumour cells. Screening of insertions and deletion within exons 9 and 11 of *KIT* and within exons 12 and 18 of *PDGFRA* was performed by LAPP.[13] Mutations were identified by direct sequencing of PCR products.

3. Results

3.1. Study patient characteristics

Thirty-six patients were included in the study between November 2003 and October 2005 (Table 1). Median age at inclusion was 13.7 years (range 2.2–22.5). The majority of patients (88%) had good performance status. Most patients had a mesenchymal or bone tumour (42%) or brain tumour (33%). Thirty patients had received prior chemotherapy, five patients received radiotherapy only and one patient (GIST) was enrolled after surgical resection.

All patients had positive tumour expression for KIT, PDGFRA and/or PDGFRB in at least 30% of tumour cells (Table 2). Tumour material for mutation analysis was available in 16 patients. In 12 samples analysed, no gene mutation was found in exon 9, 11, 13 or 17 of the *KIT* tyrosine kinase gene and exon 12 or 18 of *PDGFRA*; DNA was insufficient in four samples.

3.2. Study treatment

Due to Glivec® capsule size, daily dose ranged from 294 to 425 mg/m^2 (median 343). Eighty percent of patients received a dose of 340 mg/m^2 ±10%. In eight patients with stable disease and three patients with progressive disease on treatment, the dose was escalated 1 to 12 months after treatment initiation (median 2.3) to 440 mg/m^2 (590 mg/m^2 in one GIST). Total treatment duration was 255 months (median 1.9/patient). Eight patients had more than 6 months of treatment; three of them were still on treatment at study cut-off.

Study dose was reduced in two patients due to toxicity (aggressive behaviour and thrombocytopaenia). Temporary treatment interruptions (median 2 days) were reported for 14 patients at 17 occurrences. Five patients had interruptions lasting for $\geqslant$10 days due to non-compliance (one), thrombocytopaenia (one), surgery (two), progressive disease and restart at 440 mg/m^2 (one). Most patients stopped study treatment due to inefficacy (25 progressive disease and 4 stable disease), two patients for toxicity in cycle 1 (transaminase elevation and thrombocytopaenia) and two following their decision.

3.3. Efficacy

Thirty-two patients were evaluable for tumour response; four were non-evaluable due to early treatment stop for toxicity (two cases), irradiation on the target site (one), and non-measurable disease at study entry (one). No objective response was observed. Overall 10 of 32 patients (31%, 95% confidence interval (CI) 16–50%) had stable disease (Table 2). In two of these patients, imaging prior to study demonstrated progressive disease: a relapsing pontine glioma experienced disease stabilisation (–31%) and clinical improvement for 10 months; a pseudo-inflammatory tumour was stable for 41 months at cut-off date. Prolonged stable disease was also noted for 5 months in a low-grade glioma, 4 months in a synovial sarcoma, 7 months in a renal cell carcinoma, 37 months in a medulloblastoma and for 5, 7 and >42 months in fibromatoses.

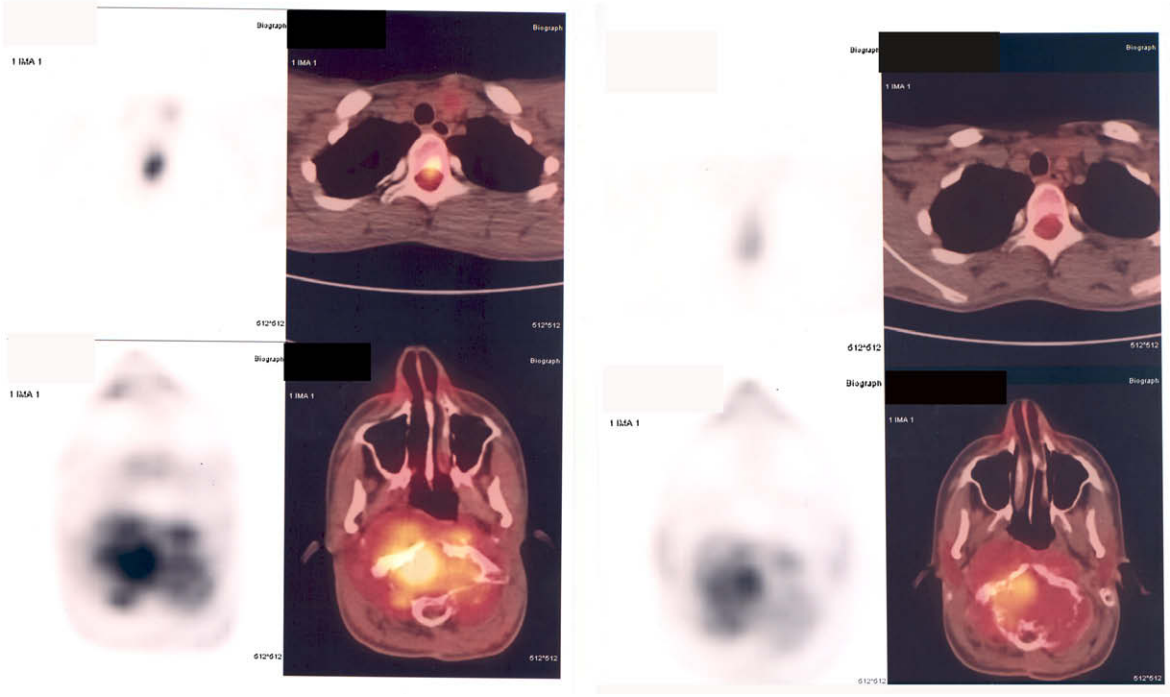

Fig. 1 – Glucose uptake in PET scan in a clivus and spinal chordoma at baseline (left panel) and after 1 month (right panel).

In total, six patients with fibromatosis were included: three experienced prolonged tumour stabilisation, while three progressed. One patient with a large abdominal mass had been considered progressive at 2 and 4 months, but experienced subsequent tumour regression (–44%) and is still on treatment. Two patients experienced tumour mass induration and inflammatory signs during treatment, one patient in particular after an increase of imatinib dose to 440 mg/m^2, which was reversible after dose reduction.

Considering the whole series followed up for a median of 41 months (range 20–45), four patients are alive with no disease progression (one GIST, pseudo-inflammatory tumour, medulloblastoma and fibromatosis, respectively) and seven patients are alive with progressive disease.

3.4. *Investigational PET scan*

PET scan was performed at study entry and at least after 1 month in 15 patients. A weaker uptake, qualified as partial metabolic response, was observed in a clivus chordoma after 1 month of treatment (Fig. 1). The disease was stable by radiological criteria; however the patient died due to tumour progression and the particular tumour localisation at 2.5 months. Decreased glucose uptake intensity has further been found in a fibromatosis after 1 month of treatment, although a metabolic increase was noted at 3 months. One medulloblastoma showed reduced methionine uptake at 2 months. Seven patients had metabolic activity comparable to that before treatment. The five other patients had increased glucose uptake (three) or new lesions (two), confirmed by standard radiology after 2 months of treatment.

3.5. *Safety*

Over a cumulative treatment period of 203 months analysed (truncated at 24 months in case of prolonged treatment), a total of 113 clinical AEs that were considered related to the study treatment were reported in 20 of 36 patients (Table 3).

Table 3 – Treatment-related adverse events in 36 patients during a total treatment duration of 203 months.

Adverse event	Grade 1	Grade 2	Total (patients)
Gastrointestinal disorders			45 (18)
Nausea/vomiting	25	13	38 (17)
Diarrhoea	1	0	1 (1)
Abdominal pain	1	0	1 (1)
Anorexia	1	0	1 (1)
Fungal infection mouth	1	1	2 (1)
Teeth discolouration	2	0	2 (1)
Constitutional symptoms			18 (10)
Asthenia	7	5	12 (10)
Cold extremities	4	0	4 (1)
Weight loss	1	0	1 (1)
Yellow sclera	1	0	1 (1)
Lymphatics			21 (9)
Orbital/face oedema	20	1	21 (9)
Dermatology			9 (5)
Cutaneous eruption	1	0	1 (1)
Loss of eyebrows	0	1	1 (1)
Pruritus	0	1	1 (1)
Skin rash	0	4	4 (1)
Depigmentation	1	0	1 (1)
Exanthema	1	0	1 (1)
Musculoskeletal/soft tissue			10 (3)
Cramps	2	0	2 (2)
Induration and inflammatory signs of tumour mass	5	0	5 (1)
Reduced leg mobility	2	0	2 (2)
Myalgia	1	0	1 (1)
Neurology			5 (2)
Dysaesthesia	1	0	1 (1)
Headache	1	3	4 (1)
Behaviour disorders			1 (1)
Aggressivity	1	0	1 (1)
Cardiac			3 (1)
Systolic murmur	2	1	3 (1)
Sexual/reproductive function			1 (1)
Gynaecomastia	0	1	1 (1)
Total	82	31	113 (20)

Table 4 – Post hoc pharmacokinetic parameters – mean values in 33 patients analysed.

Parameter	Mean	Coefficient of variation (%)
Imatinib		
AUC (mg/L h)	76.6	44
Clearance (L/h)	6.27	51
Clearance (L/h/kg)	0.17	36
Clearance (L/h/m^2)	5.14	34
Volume of distribution (L)	165	93
Volume of distribution (L/kg)	4.13	68
Half-life (h)	16.6	61
CGP 74588		
AUC (mg/L h)	10.4	53
Apparent clearance (L/h)	53.9	62
Apparent volume of distribution (L)	26.3	105

All AEs were of grade 1 or 2: gastrointestinal symptoms, mainly nausea/vomiting (18 patients), orbital or facial oedema (nine) and constitutional symptoms such as asthenia (10). Additionally, transient grades 1–2 AST, ALT or bilirubin elevation occurred in 14 (39%), 7 (19%) and 11 patients (30%), respectively. Five patients experienced grade 3 neutropaenia and two experienced grade 4 thrombocytopaenia; in two of them biological abnormality was already present at study entry.

3.6. *Pharmacokinetic parameters*

Plasma samples in 33 patients were available and were included in the population pharmacokinetics analysis. The daily area under the curve of plasma concentrations versus time (corresponding to clearance and dose on day 1) ranged between 38 and 210 mg/L h for imatinib, and between 3.1 and 26.9 for CGP 74588. There was substantial inter-patient variability in PK parameters. Mean clearance of imatinib on day 1 was 6.27 L/h with a coefficient of variation (CV) of 51%, and 5.14 L/h/m^2 (CV 34%). Mean half-life time of imatinib was 16.6 h (CV 61%). Apparent clearance of CGP 74588 was 53.91 L/h (CV 62%) (Table 4). Fig. 2 shows imatinib and CGP 74588 concentrations for each patient within time and model predicted concentrations (corresponding to mean PK parameters).

4. Discussion

The original design of this study aimed at exploring a strategy for accrual enrichment in patients whose tumours were more likely to be sensitive to imatinib. To this purpose, tumours' molecular characteristics are thought to be relevant for deciding which targeted therapy to propose for an individual patient. Whilst it is difficult to determine in a patient the dependency on an oncogenic pathway in an underlying tumour, we had hypothesised that malignancies expressing one or several of the imatinib-sensitive targets may exhibit some level of sensitivity to inhibition by imatinib as determined by radiological or metabolic tumour response. We therefore included patients if at least 30% tumour cells were expressing KIT, cytoplasmic and membranous, a criterion that had been used for GIST. However, KIT expression pattern was, with few exceptions, less intense than that observed in GIST. In the absence of data on PDGFR expression prior to our study, this inclusion criterion had been modified with the advances of our findings in IHC (see Table 5 for our screening programme on the three imatinib targets in 230 patients) and based on the hypothesis that a receptor may play a more relevant role if expressed in a high percentage of tumour cells. Although the three growth factor receptors were found in various paediatric tumours, none of the tumour entities showed a consistent expression pattern or positivity in all the samples analysed. Furthermore, none of the 12 tumours evaluated for known *KIT* or *PDGFRA* gene mutations associated with sensitivity to imatinib in GIST,[14] exhibited an activating mutation in these kinase domains. This is consistent with the limited published data on paediatric malignancies (including those on GIST) demonstrating that despite receptor expression,[15–18] gene mutations are rare.[19–23]

Using WHO radiological criteria for response, we observed no objective responses in the diseases treated. Nevertheless, minor tumour regression, prolonged stabilisation in progressive tumours, as well as observations in metabolic imaging suggest a role of PDGFR or KIT pathway in some of these tumours. In this regard, a chordoma overexpressing all three receptor kinases on most tumour cells experienced metabolic response at 1 and 2.5 months as reported by Casali and colleagues.[24] However, imatinib may participate in changes in vascular permeability, intra-tumour pressure and tumour cell metabolism through inhibition of PDGFRB in pericytes, which could be responsible for metabolic changes in the tumours

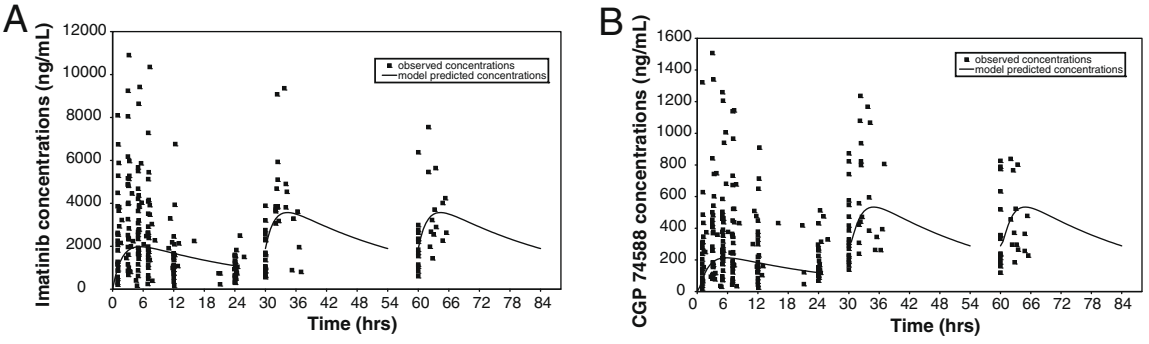

Fig. 2 – Observed imatinib (A) and CGP 74588 (B) model predicted concentrations versus time.

Table 5 – Immunohistochemical expression for KIT, PDGFRA and PDGFRB in 230 patients.

Disease	Patients	KIT	PDGFRA	PDGFRB
% Positive tumour cells		0–29%/⩾30%	0–29%/30–79%/⩾80%	0–29%/30–79%/⩾80%
Cerebral tumours	110			
High-grade glioma	14	14/0	9/1/4	4/4/6
Oligodendroglioma	8[b]	8/0	5/1/2	4/1/3
Pontine glioma	15	13/2	6/6/3	3/4/7[a]
Low-grade glioma	7	7/0	6/0/1	3/2/2
Primitive neuroectodermal tumour (PNET)	9	9/0	7/1/1	6/1/2
Medulloblastoma	32	18/13[a]	30/1/0[a]	17/3/10[a]
Atypical teratoid/rhabdoid tumour	6	6/0	5/1/0	3/0/2[a]
Ependymoma	11	10/1	10/0/1	5/1/5
Choroid plexus carcinoma	2	2/0	0/1/1	0/0/2
Cerebral germ cell tumour	1	1/0	1/0/0	0/0/1
Other brain tumours	5	5/0	5/0/0	5/0/0
Mesenchymal and bone tumours	68			
Fibromatosis	13	9/4	13/0/0	1/2/10
Fibrosarcoma	2	2/0	2/0/0	2/0/0
Osteosarcoma	15[b]	13/1[a]	11/1/2[a]	4/2/7[a]
Ewing tumours	12	4/8	11/0/1	8/1/3
Rhabdomyosarcoma	11	11/0	10/0/1	8/2/1
Synovialosarcoma	2[b]	2/0	1/0/1	1/0/1
Other mesenchymal tumours	6	6/0	5/0/1	3/0/3
Malignant schwannoma	2	2/0	1/0/1	0/0/2
Desmoplastic small round cell tumour	2	2/0	2/0/0	0/1/1
GIST	1	0/1	1/0/0	0/0/1
Pseudo-inflammatory tumour	1	1/0	0/0/1	0/0/1
Malignant juvenile xanthogranuloma	1	1/0	0/0/1	0/0/1
Other tumours	52			
Chordoma	7	4/3	3/1/3	1/2/4
Neuroblastoma	20	16/1[a]	18/2/0	12/3/4[a]
Nephroblastoma	6	5/0[a]	4/1/0[a]	1/1/3[a]
Renal cell carcinoma	4	4/0	1/1/2	1/1/2
Hepatoblastoma	2[b]	2/0	1/1/0	0/0/2
Hepatocellular carcinoma	1[b]	0/1	1/0/0	0/1/0
Undifferentiated carcinoma of nasopharyngeal tract	1	0/1	1/0/0	1/0/0
Germ cell tumour	2	2/0	0/1/1	0/1/1
Other solid tumours	9	8/1	6/1/2	4/2/3

In total, 23% brain tumours expressed PDGFRA in ⩾30% tumour cells, 12% tumours being positive in ⩾80% cells. PDGFRB was found in 53% tumours in ⩾30% tumour cells and 38% tumours expressed PDGFRB in ⩾80% cells. KIT expression was rare (15%) with the exception of medulloblastoma (42%). In extra-cranial tumours, 18% tumours expressed KIT in ⩾30% cells, 22% tumours expressed PDGFRA in ⩾30% cells, 14% being positive in ⩾80% cells. PDGFRB was found in 60% tumours in ⩾30% tumour cells and 43% tumours expressed PDGFRB in ⩾80% cells.

a Discrepancy between total numbers and reported figures is due to lack of available slides or non-evaluable tissue section.

b For some patients, multiple samples were available: in two patients with oligodendroglioma, samples at diagnosis and after chemotherapy showed identical expression of PDGFRA, both KIT and PDGFRB were negative. In a patient with synovialosarcoma, IHC showed PDGFRA expression in 60% tumour cells at diagnosis and 80% post chemotherapy, while both KIT and PDGFRB were negative. In a patient with osteosarcoma, PDGFRB expression was not found at diagnosis but in 100% tumour cells at relapse, both KIT and PDGFRA were negative. In a patient with hepatoblastoma, PDGFRA expression was found in 40% tumour cells in the liver sample at diagnosis and at relapse in 20% cells in the liver tissue and in 10% of the pulmonary metastasis; PDGFRB was expressed in 50% tumour cells in the pulmonary metastasis while in 80% cells of the liver. In a patient with hepatocellular carcinoma, KIT was not found at diagnosis while in 90% tumour cells at relapse.

that are captured by PET-scanning but does not reflect direct antitumour effects. Half of our patients with fibromatosis showed stable disease (one with 44% tumour reduction) and, in some cases, reduced contrast enhancement in the tumour centre which extends findings recently reported in adult clinical studies.[25] In addition, KIT and PDGFRB were expressed in respectively 31% and 92% of the samples analysed which contradicts Leithner and colleagues.[26] One patient with a progressive relapsing pontine glioma had 31% tumour regression and clinical benefit from imatinib treatment during 10 months. The study of Pollack and colleagues reported on imatinib with irradiation in children with newly diagnosed pontine gliomas and those with recurrent malignant intracranial gliomas.[27] The median OS of patients with pontine glioma treated with irradiation and imatinib was around 11 months which is insignificantly different to historical controls and one relapsed glioma patient had transient partial response. PDGFR pathway has been reported to be implicated in the development of malignant glioma,[28] and preclinical models have been shown to be sensitive to imatinib.[29] However, to date only limited activity has been reported in patients when imatinib was used as a single agent[30,31] or in

combination with hydroxyurea.[32,33] None of these studies evaluated PDGFR expression, gene mutations or amplification. Our own preclinical evaluations showed limited effects of imatinib when used as a single agent in *PDGFR* gene-amplified malignant brain tumours, but showed synergy with irradiation or chemotherapy (B. Geoerger et al., manuscript in preparation). Biological and genetic evaluations need to be included in clinical studies to evaluate the real potential of imatinib in these diseases.

As reported previously, imatinib was tolerated well in children with only mild treatment-related symptoms. The two selected dose levels for our study have been previously explored in the paediatric phase I study in CML.[34] The toxicity profile was similar to these and those reported in adults with a lower incidence of orbital or face oedema.

Our pharmacokinetic analysis showed AUC values at 340 mg/m^2 which were similar to those reported in children with leukaemia by Champagne and colleagues and by Menon-Andersen D and colleagues and that in adults at 600 mg/day (350 mg/m^2), as well as an already described marked high interpatient variability.[27,34–36] None of our patients had enzyme-inducing antiepileptic concomitant medication.

The observed limited antitumour activity, as recently reported for sarcomas by Bond and colleagues,[37] does not suggest a further use of imatinib as a single agent in these paediatric tumours even if KIT/PDGFRs are expressed in tumour biopsy. However, minor tumour responses (<50% according to WHO criteria) with clinical improvement and reduced metabolic activity observed in some patients affected with fibromatosis, chordoma and pontine glioma, suggest a potential role of this kinase inhibitor in some paediatric malignancies. Preclinical data showed increased activity of imatinib when used in combination with other targeted agents, chemotherapy or radiotherapy, and it needs to be awaited if this translates into effects in clinics for these combination regimens. This trial clearly highlights the inherent challenges in patients' selection or 'stratification' when the knowledge on the relevance of the markers used is still not fully understood, as this was the case for KIT and PDGFR in this trial. More upfront work is needed on tumour specimens or preclinical models before actually selecting patients. This could help to clarify the most appropriate screening evaluation and to choose the right design for these new targeted therapies.

Authors' disclosure

Renaud Capdeville is an employee of Novartis Pharma AG, Basel, Switzerland.

Conflict of interest statement

None declared.

Acknowledgements

We thank all the participating patients, their parents and the ITCC centres (Institut Gustave Roussy, Villejuif; Institut Curie, Paris; Centre Oscar Lambret, Lille; Centre Leon Berard, Lyon; Hôpital Trousseau, Paris; CHU La Timone, Marseille, CHU Toulouse, France; Royal Marsden, Sutton; Royal Infirmary, Newcastle upon Tyne, United Kingdom; AMC, Amsterdam, The Netherlands), Ms Sylviane Iacobelli for the data management, Dr Marie-Jose Terrier-Lacombe for immunohistochemistry analysis and Ms Micheline Re for the analysis of the PK samples. The authors also thank Sara Calmanti for editorial assistance.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2009.03.007.

REFERENCES

1. Maki RG. Gastrointestinal stromal tumors respond to tyrosine kinase-targeted therapy. *Curr Treat Options Gastroenterol* 2004;**7**:13–7.
2. Rubin BP, Schuetze SM, Eary JF, et al. Molecular targeting of platelet-derived growth factor B by imatinib mesylate in a patient with metastatic dermatofibrosarcoma protuberans. *J Clin Oncol* 2002;**20**:3586–91.
3. Magnusson MK, Meade KE, Nakamura R, Barrett J, Dunbar CE. Activity of STI571 in chronic myelomonocytic leukemia with a platelet-derived growth factor beta receptor fusion oncogene. *Blood* 2002;**100**:1088–91.
4. Gleich GJ, Leiferman KM, Pardanani A, Tefferi A, Butterfield JH. Treatment of hypereosinophilic syndrome with imatinib mesilate. *Lancet* 2002;**359**:1577–8.
5. Salem Z, Zalloua PA, Chehal A, et al. Effective treatment of hypereosinophilic syndrome with imatinib mesylate. *Hematol J* 2003;**4**:410–2.
6. La Starza R, Specchia G, Cuneo A, et al. The hypereosinophilic syndrome: fluorescence in situ hybridization detects the del(4)(q12)-FIP1L1/PDGFRA but not genomic rearrangements of other tyrosine kinases. *Haematologica* 2005;**90**:596–601.
7. World Health Organization. *WHO handbook for reporting results of cancer treatment*. WHO offset publication no. 48. Geneva, Switzerland: World Health Organization; 1979.
8. Delbaldo C, Chatelut E, Ré M, et al. Pharmacokinetic–pharmacodynamic relationships of imatinib and its main metabolite in patients with advanced gastrointestinal stromal tumors. *Clin Cancer Res* 2006;**12**:6073–8.
9. Beal SL. Commentary on significance levels for covariate effects in NONMEM. *J Pharmacokinet Pharmacodyn* 2002;**29**:403–10.
10. Pétain A, Kattygnarath D, Azard J, et alOn behalf of the Innovative Therapies with Children with Cancer European consortium. Population pharmacokinetics and pharmacogenetics of imatinib in children and adults. *Clin Cancer Res* 2008;**14**:7102–9.
11. Emile JF, Théou N, Tabone S, et al. Clinicopathologic, phenotypic, and genotypic characteristics of gastrointestinal mesenchymal tumors. *Clin Gastroenterol Hepatol* 2004;**2**:597–605.
12. Théou N, Tabone S, Saffroy R, et al. High expression of both mutant and wild-type alleles of c-kit in gastrointestinal stromal tumors. *Biochim Biophys Acta* 2004;**1688**:250–6.
13. Emile JF, Lemoine A, Bienfait N, Terrier P, Azoulay D, Debuire B. Length analysis of polymerase chain reaction products: a sensitive and reliable technique for the detection of

mutations in KIT exon 11 in gastrointestinal stromal tumors. *Diagn Mol Pathol* 2002;**11**:107–12.
14. Antonescu CR, Besmer P, Guo T, et al. Acquired resistance to imatinib in gastrointestinal stromal tumor occurs through secondary gene mutation. *Clin Cancer Res* 2005;**11**:4182–90.
15. Ahmed A, Gilbert-Barness E, Lacson A. Expression of c-kit in Ewing family of tumors: a comparison of different immunohistochemical protocols. *Pediatr Dev Pathol* 2004;**7**:342–7.
16. Scotlandi K, Manara MC, Strammiello R, et al. C-kit receptor expression in Ewing's sarcoma: lack of prognostic value but therapeutic targeting opportunities in appropriate conditions. *J Clin Oncol* 2003;**21**:1952–60.
17. Bar-Sela G, Kuten A, Ben-Eliezer S, Gov-Ari E, Ben-Izhak O. Expression of HER2 and C-KIT in nasopharyngeal carcinoma: implications for a new therapeutic approach. *Mod Pathol* 2003;**16**:1035–40.
18. Vitali R, Cesi V, Nicotra MR, et al. C-Kit is preferentially expressed in MYCN-amplified neuroblastoma and its effect on cell proliferation is inhibited in vitro by STI-571. *Int J Cancer* 2003;**106**:147–52.
19. Chilton-Macneill S, Ho M, Hawkins C, Gassas A, Zielenska M, Baruchel S. C-kit expression and mutational analysis in medulloblastoma. *Pediatr Dev Pathol* 2004;**7**:493–8.
20. Prakash S, Sarran L, Socci N, et al. Gastrointestinal stromal tumors in children and young adults: a clinicopathologic, molecular, and genomic study of 15 cases and review of the literature. *J Pediatr Hematol Oncol* 2005;**27**:179–87.
21. Sakuma Y, Sakurai S, Oguni S, Satoh M, Hironaka M, Saito K. C-Kit gene mutations in intracranial germinomas. *Cancer Sci* 2004;**95**:716–20.
22. Tzankov A, Bourgau C, Kaiser A, et al. Rare expression of KIT (CD117) in lymphomas: a tissue microarray study of 1166 cases. *Histopathology* 2004;**45**:398–404.
23. Tamborini E, Miselli F, Negri T, et al. Molecular and biochemical analyses of platelet-derived growth factor receptor (PDGFR) B, PDGFRA, and KIT receptors in chordomas. *Clin Cancer Res* 2006;**12**:6920–8.
24. Casali PG, Messina A, Stacchiotti S, et al. Imatinib mesylate in chordoma. *Cancer* 2004;**101**:2086–97.
25. Heinrich MC, Joensuu H, Demetri GD, et al. Phase II, Open-label study evaluating the activity of imatinib in treating life-threatening malignancies known to be associated with imatinib-sensitive tyrosine kinases. *Clin Cancer Res* 2008;**14**:2717–25.
26. Leithner A, Gapp M, Radl R, et al. Immunohistochemical analysis of desmoid tumours. *J Clin Pathol* 2005;**58**:1152–6.
27. Pollack IF, Jakacki RI, Blaney SM, et al. Phase I trial of imatinib in children with newly diagnosed brainstem and recurrent malignant gliomas: a Paediatric Brain Tumor Consortium report. *Neuro Oncol* 2007;**9**:145–60.
28. Mauro A, Bulfone A, Turco E, Schiffer D. Coexpression of platelet-derived growth factor (PDGF) B chain and PDGF B-type receptor in human gliomas. *Childs Nerv Syst* 1991;**7**: 432–6.
29. Kilic T, Alberta JA, Zdunek PR, et al. Intracranial inhibition of platelet-derived growth factor-mediated glioblastoma cell growth by an orally active kinase inhibitor of the 2-phenylaminopyrimidine class. *Cancer Res* 2000;**60**:5143–50.
30. Wen PY, Yung WK, Lamborn KR, et al. Phase I/II study of imatinib mesylate for recurrent malignant gliomas: North American Brain Tumor Consortium Study 99-08. *Clin Cancer Res* 2006;**12**:4899–907.
31. Raymond E, Brandes AA, Dittrich C, et al. Phase II study of imatinib in patients with recurrent gliomas of various histologies: a European Organisation for Research and Treatment of Cancer Brain Tumor Group Study. *J Clin Oncol* 2008;**26**:4659–65.
32. Desjardins A, Quinn JA, Vredenburgh JJ, et al. Phase II study of imatinib mesylate and hydroxyurea for recurrent grade III malignant gliomas. *J Neurooncol* 2007;**13**:1253–9.
33. Reardon DA, Egorin MJ, Quinn JA, et al. Phase II study of imatinib mesylate plus hydroxyurea in adults with recurrent glioblastoma multiforme. *J Clin Oncol* 2005;**23**:9359–68.
34. Champagne MA, Capdeville R, Krailo M, et al. Imatinib mesylate (STI571) for treatment of children with Philadelphia chromosome-positive leukemia: results from a Children's Oncology Group phase 1 study. *Blood* 2004;**104**:2655–60.
35. Menon-Andersen D, Mondick JT, Jayaraman B, et al. Population pharmacokinetics of imatinib mesylate and its metabolite in children and young adults. *Cancer Chemother Pharmacol* 2009;**63**:229–38.
36. Peng B, Hayes M, Resta D, et al. Pharmacokinetics and pharmacodynamics of imatinib in a phase I trial with chronic myeloid leukemia patients. *J Clin Oncol* 2004;**22**:935–42.
37. Bond M, Bernstein ML, Pappo A, et al. A phase II study of imatinib mesylate in children with refractory or relapsed solid tumors: a Children's Oncology Group study. *Pediatr Blood Cancer* 2008;**50**:254–8.