Pharmacogenetics and cost-effectiveness of systemic treatment in soft tissue sarcoma

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Voor Caroline

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1

General introduction

This thesis on systemic treatment options in soft tissue sarcoma (STS) focusses on two topics. In the first part single nucleotide polymorphisms (SNPs) are explored that potentially influence drug effects in the treatment of gastrointestinal stromal tumors (GIST). In the second part the introduction of trabectedin chemotherapy for the treatment of STS is examined with a cost-effectiveness analysis (CEA) and description of venous access related adverse events.

Part I: Pharmacogenetics of systemic GIST-treatment

Gastrointestinal stromal tumors

Gastrointestinal stromal tumor (GIST) is a tumor arising from mesenchymal cells of the gastro-intestinal tract and has a unique biology and clinical course. Most GISTs are the result of a gain-of-function mutation in the *KIT* gene, encoding for the KIT/CD117 transmembranous receptor.[1,2] This receptor can be blocked by intracellular active tyrosine kinase inhibitors (TKIs), such as imatinib.[3] Imatinib is an oral drug that was first used in the treatment of chronic myeloid leukemia due to its binding to the oncogenic BCR-ABL protein.[4] This potent drug can be employed in the neo-adjuvant, adjuvant and palliative stages of GIST therapy.[5] In case the GIST is resectable, surgery can cure patients. In case the tumor has metastasized, TKIs are used to suppress tumor activity for as long as possible.

Imatinib is firmly positioned as the first-line option for advanced GIST.[6] Its position has been challenged by nilotinib, a drug that has even higher affinity for the wild-type BCR-ABL kinase, while retaining its activity against KIT and PDGFR.[7] A head-to-head phase III trial in advanced GIST patients showed, however, that imatinib treatment resulted in longer progression free survival and overall survival compared to nilotinib.[8] A recent trial investigated the activity of dasatinib, another TKI, as first-line agent for GIST, but the progression free survival was far shorter than obtained in previous imatinib trials.[9] The fact that this study failed to meet the envisioned enrollment of 52 patients over a period of almost four years is a clear sign of imatinib's paramount position. Masitinib might have been a useful alternative, as the results phase II trial with GIST patients during first line therapy who received this drug are comparable to imatinib, but the future of masitinib is uncertain.[10]

Sunitinib is the second-line treatment option following imatinib resistance or intolerability.[6] The majority of trials with sunitinib in GIST patients were performed in a setting following imatinib treatment. Long term safety and efficacy have been shown in large international patient cohorts.[11,12] Thus far only one randomized clinical trial directly compared two TKIs in a setting of advanced GIST after imatinib failure, the TKIs being

sunitinib and masitinib.[13] Masitinib yielded somewhat better progression free survival, but in patients receiving sunitinib survival was far shorter than what has been reported in previous studies with sunitinib.

Regorafenib is the third-line option for GIST patients following imatinib and sunitinib resistance or intolerance.[6] It was one of many agents tested in this setting and its activity has been demonstrated in clinical trials.[14,15] As patients receive more lines of therapy, each line offers less survival gains than the previous line of therapy, as a result of ongoing development of TKI-resistance in the heterogeneous GIST metastases.[16]

Currently, there is no established fourth-line treatment option. Many targeted agents have been explored in patients with advanced GIST. Several clinical trials are currently investigating the activity of new drugs compared to imatinib, sunitinib and regorafenib in first, second and third line setting. Among these are the new TKIs DCC-2618 and BLU-285. DCC-2618 has anti-tumor potential against GISTs with *KIT* mutations exon 13, 14, 17 or 18. In a dose escalation trial partial responses have been observed. Clinical trials with DCC-2618 in the second line versus sunitinib (NCT03673501) and in the fourth line versus placebo (NCT03353753) have subsequently been initiated. Due to the clinical success of the phase I study with DCC-2618 (NCT02571036), this study was expanded to include patients in the second and third line of therapy. Of the 46 patients treated with 150mg once daily in the second or third line 10 had a response and the median progression free survival was 36 weeks with 61% of patients censored.[17] BLU-285, now called avapritinib, has activity against GISTs with specific mutation that other TKIs do not inhibit. It is being investigated in clinical trials as third line therapy versus regorafenib (NCT03465722) and in a fourth line phase II setting (NCT02508532).

Pharmacogenetics

Whereas treatment for illnesses such as malignancies are based on evidence derived from clinical trials involving large numbers of patients, the response of individual patients to a certain drug is dependent on patient specific characteristics.[18] These characteristics include age, sex, body size, kidney function, co-medication, as well as a patient's specific germline genetic traits.[19] The most prevalent genetic variations are single nucleotide polymorphisms and the focus of the research in this part of the thesis.

SNPs may or may not affect gene function and patient phenotype. Some SNPs will not alter which amino acid is built into the protein, termed synonymous SNPs. Non-synonymous SNPs, on the other hand, do have an effect. These SNPs will either change an amino acid at particular location, being a missense SNPs, or in case of a nonsense SNPs will result in the premature insertion of a stop codon and ending further amino acids being added to the protein. SNPs do also occur in non-coding regions and these

can affect the splicing, binding and alteration of the pre-mRNA molecule. SNPs studied in this thesis were all selected to have a functional effect, as found in the National Institute of Environmental Health Sciences SNP database.[20]

SNPs in genes related to the pharmacokinetics or pharmacodynamics of drugs may influence the response to these drugs. In case of GIST, imatinib or sunitinib efficacy may be enhanced or reduced in terms of longer or shorter survival. Equally, the adverse effects of these drugs may vary according to a patient's genetic profile. One such example that has found its way into clinical practice is the determination of *DPYD* polymorphisms in patients receiving 5-fluorouracil.[21]

Pharmacogenetic research has shown associations of SNPs in genes related to TKI pharmacokinetics and pharmacodynamics with clinical outcome in TKI treated malignancies. Imatinib is primarily metabolized by CYP3A4 and CYP3A5, while other CYP-enzymes have a limited role.[19] The drug is a substrate for the influx transporters hOCT1 (*SLC22A1*), OCTN1 (*SLC22A4*), OCTN2 (*SLC22A5*) and OATP1B3 (*SLCO1B3*).[22-24] Active efflux transporters are the ATP-binding cassette (*ABCB1*) and the breast cancer resistance protein (*ABCG2)*.[19] Time to progression in advanced GIST patients who were treated with imatinib has been associated to SNPs in *SLC22A4* (rs1050152) and *SLC22A5* (rs2631367, rs2631372).[25] Response to imatinib in the treatment of chronic myeloid leukemia has been associated to SNPs in *ABCB1* (rs868755, rs1045642, rs28656907), *ABCG2* (rs2231137), *CYP3A5* (rs776746), *SLC22A1* (rs683369) and in *SLCO1B3* (rs4149117).[26-28] Imatinib trough levels have been associated in multiple studies to SNPs in *ABCB1*, *ABCG2* and *CYP3A4*.[24,29,30]

Even more pharmacogenetic studies have been performed with sunitinib, many of them in patients with metastatic renal cell carcinoma.[31] Sunitinib is metabolized into the SU12662 metabolite by CYP3A4 and both are active compounds.[19] CYP3A4 expression or activity is in turn influenced by *NR1I2*, *NR1I3* and *POR* effects.[32,33] CYP3A5 and CYP1A1 may also metabolize sunitinib, as these CYPs are active in other TKIs.[19] Sunitinib is a substrate for the drug efflux transporters ATP-binding cassette (*ABCB1*) and breast cancer resistance protein (*ABCG2)*.[19] Survival during sunitinib treatment in metastatic renal cell carcinoma has been associated with SNPs in *ABCB1* (rs1045642, rs1128503, rs2032582) and *CYP3A5* (rs776746) and these associations have been confirmed in a separate patient cohort.[34,35] Individual sunitinib adverse events were associated with several SNPs in *ABCB1*, *ABCG2* , *CYP1A1*, *NR1I3*, *IL8* and *IL13*.[36,37] Sunitinib clearance has been associated with a SNP in *CYP3A4* (rs35599367).[38] In sunitinib treated GIST, associations have been found with SNPs in *VEGFR3* (rs6877011, rs7709359) and time to progression, and with a SNP in *VEGFA* (rs7709359) and toxicity.[39] Until the work described in this thesis was started, pharmacogenetic studies with a large cohort of advanced GIST patients had not yet been performed.

Part II: Use of trabectedin in STS

Soft tissue sarcoma

STS comprise 50 to 60 distinct types of histology and constitute one percent of all solid malignancies. Due to its rarity STS have long been grouped together in treatment and research.[40] As knowledge on the tumor biology of histologic subtypes expands, it has become evident that specific subtypes should be treated as specific as possible. Due to its unique pathophysiology, treatment and clinical course, GIST already has its separate guideline.[6]

Systemic agents tested in STS trials may have anti-tumor activity in only some STS subtypes. The first line therapy in advanced STS is doxorubicin for almost all subtypes.[40] For second line options, the specific STS histology is to be taken into account when selecting treatments. Most patients will be offered the oral TKI pazopanib, but patients with adipocytic tumors will not respond. Adipocytic tumors such as liposarcomas, as well as the otherwise unrelated leiomyosarcomas have shown favorable response to trabectedin.[41] Trabectedin is a marine derived compound with a unique mechanism of action involving DNA binding, influencing transcription factors and modulating the tumor micro-environment.[42]

In the past decade, the number of available systemic agents and combinations thereof in advanced STS has increased. First line doxorubicin can be augmented by adding ifosfamide to increase the chance of a response.[43] Alternately, adding the PDGFR-inhibitor olaratumab to doxorubicin might prolong survival. This was seen in a phase II trial, but not in the subsequent phase III trial.[44] Apart from trabectedin and pazopanib, some patient may benefit from ifosfamide monotherapy, or from eribulin in case of a liposarcoma.[45,46] In patients with undifferentiated pleomorphic sarcoma, gemcitabine-docetaxel cycles may be considered. Angiosarcomas can respond to taxanes.[47] In all, while the number of treatment options has grown and survival may be prolonged, advanced STS still is disease with very slim chances of survival and almost all affected patients will die due to it.

Cost-effectiveness analysis

Cost-effectiveness analysis requires data on the efficacy and toxicity of a certain drug in a certain clinical setting and a comparator that can be seen as a valid option for that disease. Together with data on health care usage and costs, an analysis can be performed. In the analysis an incremental cost effectiveness ratio is calculated, denoting the costs per QALY of the new treatment compared to the other treatment. In the Netherlands, health care authorities have published a report entitled 'Sensible and sustainable care'

in which an ICER of a maximum of €80,000 is considered acceptable.[48] This number is now frequently used as the acceptable cost per QALY in the Netherlands.

As the number of systemic anti-cancer drugs grows, choices have to be made concerning treatment allocation on a single patient basis, as well as on a group level. Apart from data on efficacy and toxicity, the societal costs of drug will need to be taken in consideration.[48] Treatment with new drugs, with their patent still active, will usually have substantial costs and health care regulators are keen to learn whether a new drug is worth its price tag. When trabectedin was introduced into the Dutch market, Dutch health care regulators also wished to see its cost-effectiveness investigated in STS.

Outline of this thesis

The subject of **Part I** of this thesis is further introduced in an updated review article (**chapter 2**) on the systemic treatment in GIST. The development of imatinib, sunitinib and regorafenib are described, the results of the most relevant trials, as well as mechanisms of drug resistance. Additionally, other drugs tested in phase II or phase III trials are summarized. In the subsequent chapters, SNPs involved in the pharmacokinetics and pharmacodynamics of imatinib are investigated for an association with treatment effect in GIST. The efficacy of imatinib is studied in advanced GIST patients (**chapter 3**), seeking SNPs that are predictive for survival duration during first-line imatinib treatment. A similar study was performed with sunitinib (**chapter 4**), exploring associations with sunitinib efficacy during second-line of therapy. These two studies aim to identify SNPs that may serve to predict the duration of survival and the associated risk of progressive disease. SNPs are selected using a pharmacologically informed pathway approach. In case SNPs are associated with reduced survival, patients with these SNPs may benefit from intensified follow-up. In case SNPs are associated with prolonged survival, these SNPs could potentially influence future treatment decisions in favor of the specific drug if more active agents become available. In regard to GIST, the specific mutation causing the disease also is an important factor influencing therapy. Therefore, in these two chapters with pharmacogenetic studies with imatinib and sunitinib, the associations of SNPs with survival is corrected for the mutation found in the primary tumor.

The relation of imatinib adverse events was studied next (**chapter 5**), aiming to find SNPs that will predict the clinical impact of severe toxicity requiring therapy restriction. Although imatinib has a relatively mild toxicity profile, clinical trials have shown a need for dose reduction in around 15% of patients.[49-51] If patients in need of a dose reduction can be identified through their genetic polymorphisms before therapy is initiated, adverse events necessitating the dose reduction may be prevented. Averting toxicity in this way, pharmacogenetics may contribute to improving patients' safety and quality

of life. The last study in this section (**chapter 6**) aims to associate SNPs in *CYP2C8* with imatinib steady-state trough levels after prolonged period of use. CYP3A4 and CYP3A5 are the primary metabolizers of imatinib, but chronic use of imatinib leads to auto-inhibition of these CYP enzymes and then CYP2C8 becomes an important metabolizer.[52] CYP2C8 activity in regard to imatinib has been shown *in vitro* to vary according to polymorphism is present, but an *in vivo* study has not yet been performed.[53]

The subject of **Part II** of this thesis is further introduced in a review article (**chapter 7**) on the development of trabectedin and the first clinical studies with this drug in STS. The cost-effectiveness of trabectedin was tested in patients with advanced STS after treatment with first line doxorubicin. This study (**chapter 8**) originally was meant to compare trabectedin with best supportive care in this regard, but as is explained in further detail later, the study eventually went to compare the cost-effectiveness of trabectedin versus ifosfamide chemotherapy in a second line setting. Data from EORTC trials with ifosfamide in STS patients was used. This study was started on the request of Dutch health care authorities as part of the registration process of trabectedin. The adverse events relating to the venous access devices for trabectedin (**chapter 9**) are reported as last study in this thesis. In some patients sterile inflammation along the catheter trajectory of the Port-a-Cath developed and this had not yet been reported as a possible adverse event when administering trabectedin. Placing the catheter deeper in the skin resolved this issue.

This thesis is concluded with a general discussion (**chapter 10**) on the studies performed. It highlights the key results and delivers comments on how to interpret these results and the studies in general.

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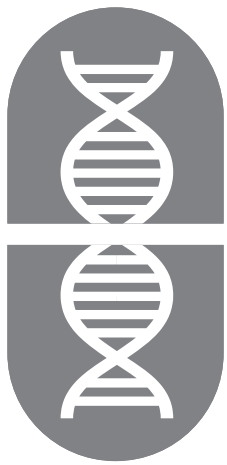
Part I: Pharmacogenetics of systemic GIST-treatment

2

Systemic treatment of advanced gastro-intestinal stromal tumors

Michiel Verboom, Hans Gelderblom



Summary

The treatment of advanced gastrointestinal stromal cell tumors (GIST) includes three lines of tyrosine kinase inhibitors: imatinib, sunitinib and regorafenib. Each of these agents bind intracellular to KIT and PDGFRA receptors, that may cause unlimited cell proliferation due to a somatic mutation in the tumor. Sunitinib and regorafenib also inhibit angiogenesis. Imatinib and sunitinib have been registered for some years; in July 2014 regorafenib was registered as well. The development of nilotinib has been terminated after a negative phase III trial. In this chapter the development of these drugs in GIST are reviewed, as well as their respective mechanisms of resistance. Furthermore, new developments in systemic therapy are evaluated, and current and future clinical trials with GIST patients in the Netherlands are highlighted.

Introduction

Gastrointestinal stromal tumors (GIST) is a rare mesenchymal tumor, that can arise in the entire digestive tract.[1] It is estimated that up to 35% of the population have microscopic small GISTs, but only 250 patients are diagnosed in a clinically relevant stage in the Netherlands each year.[2,3] In case of advanced disease multiple options for systemic therapy can be considered. This chapter aims to review the developments in the systemic treatment of GIST, as well as current clinical studies in the Netherlands.

GIST is characterized by immunohistochemical staining of CD117 (KIT) and the even more specific DOG1 (Discovered On GIST 1).[4] Malignant transformation from the interstitial cells of Cajal, that function as a pacemaker in intestinal peristalsis, occurs due to mutations in the tyrosine kinase receptor KIT in the majority of cases.[5,6] In physiologic conditions, this receptor can be activated by the stem cell factor, for instance in melanocytes, gametogenesis, mast cells and in hematopoiesis. In GIST, a somatic mutation in the KIT receptor or in the platelet-derived growth factor receptor (PDGFRA) causes permanent activation of the downstream pathway through receptor autophosphorylation, leading to unbridled growth.[7] In a subset of GISTs a mutation in either of these receptors is not found. In this 'wild type' group more new mutations are found, for instance in NF1 and SDH*x*, making the term wild type possibly obsolete in the future.[8,9] For an overview of the prevalent KIT-, PDGFR- and so-called 'wild type mutations', see Table 1.

Imatinib (Glivec®, Novartis) has a clear position in the treatment of advanced GIST and the agent can also be used in the neo-adjuvant or adjuvant stage in locally advanced or high risk GIST, respectively.[10] Imatinib is an oral tyrosine kinase inhibitor (TKI) of KIT and PDGFR, among others. The drug is well tolerated, with gastro-intestinal adverse events, peri-orbital edema and muscle spasms as most frequent side effects.[11] Mutations in KIT exon 11 are sensitive to imatinib in the standard dose of 400 mg. For tumors with a mutation in KIT exon 9 an double dose of 800 mg is advised.[12]

The majority of patients have an objective response to imatinib.[11] Patients with stable disease as best response have an equal as good chance of long term efficacy. Very long term results have been published from a large randomized trial investigating the optimal imatinib dose. In the EORTC-Italian-Australasian trial, patients receiving imatinib 400 mg once daily had a median PFS of 20.4 months and median OS of 46.8 months at median 10.9 years of follow-up.[13] Sunitinib is indicated as second-line therapy after progression on imatinib.[10] Sunitinib (Sutent®, Pfizer) is a TKI and an inhibitor of KIT, PDGFR and vascular endothelial growth factor receptor (VEGFR) 1, 2, and 3 and so also has an anti-angiogenic effect. The most frequent adverse events are hypertension, hand-foot syndrome and gastro-intestinal symptoms.[14] Tumors with mutations in KIT exon 9 are relatively sensitive to sunitinib, which has two standard starting regimens; either 50

mg every four out of six weeks, or 37.5 mg continuously.[15] The median progression free survival is only 5.3 months, despite long term clinical benefit in some patients.[14]

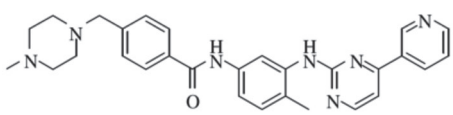
For an overview of published phase III studies with imatinib and sunitinib, see Table 2.

For the structure of imatinib, sunitinib, regorafenib, sorafenib and nilotinib, see Figure 1.[37]

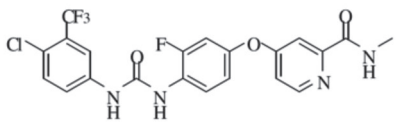
Table 1: overview of oncogenic mutation in GIST

KIT		
	exon 8	± 0.2 %
	exon 9	9 - 10 %
	exon 11	60 - 70 %
	exon 13	1 - 2 %
	exon 17	1 - 2 %
KIT total		70 - 80 %
PDGFRa		
	exon 12	1 - 2 %
	exon 14	± 0.6 %
	exon 18	10 - 14 %
PDGFRa total		11 - 15 %
'wild-type'		
	NF1 associated	± 1.1 %
	SDHx associated	1 - 4 %
	BRAF associated	1 - 2 %
	unknown	3 - 12 %
'wild-type' total		10 - 15 %

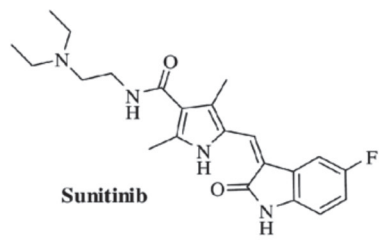
Figure 1: structure of imatinib, sunitinib, regorafenib, sorafenib and nilotinib



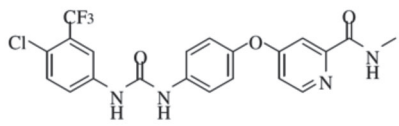
Imatinib



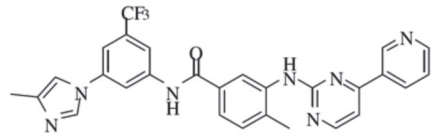
Regorafenib



Sunitinib



Sorafenib



Nilotinib

Mechanisms of resistance

Tumor growth continues in around 15% of patients, despite start of imatinib treatment, which is referred to as primary resistance.[11] In a similar proportion of patients, the (remaining) GIST remains sensitive to imatinib for a very long time, often more than 10 years, and it poses the question if those are cured by then. In the remaining 70% of patients secondary resistance develops over time.
Primary resistance occurs more often in wild-type GIST, in which a mutation in KIT or PDGFR is not found.[16] Possibly, mutations in other pathways play a part in this. Primary resistance also occurs frequently in case of a specific PDGFRa D842V mutation.[16]

Imatinib blood levels are reduced by 30% during the first three months of treatment, which could lead to so called pharmacokinetic resistance.[17] In a subset of patients, the blood level drops 1.100 mg/ml, which is the retrospectively defined target value.[18,19] These cases indicate a possible role for therapeutic drug monitoring and dosage adjustment.[19] Patients with extensive gastric surgery also have lower imatinib and sunitinib blood levels.[20,21] Furthermore, intracellular levels of imatinib can in theory decrease due to an increase of efflux transporters in GIST cells.

Secondary resistance most commonly happens due to growth of tumor clones with a second mutation in KIT or PPDGFR, after which imatinib is unable to bind to the receptor. Possible locations of the secondary mutations are the ATP-binding part (KIT exon 13 or 14), or the kinase activation loop (KIT exon 17 or 18).[22] Secondary mutations can lead to KIT hyperactivation and strong activation of the PI3-K/AKT pathway.[23] Separate tumor clones can have different secondary mutations and this heterogeneity can also occur within a single metastasis. A biopsy taken from a progressive lesion may very well not be representative for the tumor as a whole.[24] Other possible mechanisms of resistance include KIT gene amplification, increasing the quantity of this kinase, and the loss of wild-type GIST, losing the healthy allele.[25] Loss of KIT expression is another possibility, after which the tumor keeps proliferating due to overexpression of other kinases.[26]

In sunitinib treatment resistance also occurs. In around 40% of the patients, the agent does not have effect on tumor growth in the second line after imatinib.[14] Sunitinib is more frequently active if the secondary KIT mutation is located in the ATP-binding part (KIT exon 13 of 14), but much less active if the extra mutation has arisen in the KIT activation loop (KIT exon 17 or 18).[22]

Table 2: overview of clinical studies with imatinib and sunitinib in advanced GIST patients

Trial	Study type	Previous TKI treatment	Drug	No. of patients	Dose	Median PFS (months)	Median OS (months)	Median follow-up (months)	CR (%)	PR (%)	SD (%)	ORR (%)	CBR (%)
Verweij et al. 2003 [55]	phase II	none	Imatinib	27	800 mg	not reached	not reached	> 13	3.7	66.7	18.5	70.4	88.9
Demetri et al. 2002 [56], Blanke et al. 2008 [57]	phase II	none	imatinib	73	400 mg	20.0	57.0	63.0	0.0	68.5	13.7	68.5	82.2
				74	600 mg	26.0			2.7	64.9	17.6	67.6	85.1
Verweij et al. 2004 [11], Casali et al. 2017 [13]	phase III	none	imatinib	473	400 mg	20.4	46.8	40.0	5.1	45.0	31.7	50.3	81.8
				473	800 mg	24.0			5.9	48.4	31.7	54.3	86.0
Blanke et al. 2008 [58]	phase III	none	imatinib	345	400 mg	18.0	55.0	54.0	4.9	39.7	24.6	44.6	69.3
				349	800 mg	20.0	51.0		3.4	42.4	21.8	45.8	67.6
Nishida et al 2008 [59]	phase II	none	imatinib	28	400 mg	17.0	40.1	Not specified	0.0	60.7	39.3	60.7	100.
				46	600 mg	24.7	not reached		0.0	73.9	17.4	73.9	91.3
Ryu et al. 2009 [60]	phase II	none	imatinib	47	400 mg	40.0	65.0	62.0	0.0	68.1	19.1	68.1	87.2
Yeh et al. 2011 [61]	phase II	none	imatinib	171	400 mg	37.6	71.0	33.6	2.3	55.0	29.8	57.3	87.1
Schlemmer et al. 2011 [62]	compas-sionate use trial	none	imatinib	95	400 mg	not reached	not reached		4.6	29.9	47.1	34.5	81.6
Demetri et al. 2009 [63]	phase I-II	imatinib	sunitinib	97	25-75 mg every 4 out of 6 weeks	7.8	19.0	not specified	0.0	7.2	46.4	7.2	53.6

Shirao et al. 2010 [64]	phase I-II	imatinib	sunitinib	36	50 mg every 4 out of 6 weeks	6.5	not specified	not specified	0.0	11.1	27.8	11.1	38.9
George et al. 2009 [15]	phase II	imatinib	sunitinib	60	37.5 mg continuously	7.9	24.7	not specified	0.0	13.3	66.7	13.3	80.0
Demetri et al. 2006 [14], Demetri et al. 2012 [65]	phase III	imatinib	sunitinib	243	50 mg every 4 out of 6 weeks	5.3	16.8	41.7	0.0	6.6	52.7	6.6	59.3
			placebo	118	placebo	1.4	15.0		0.0	0.0	42.4	0.0	42.4
Reichardt et al 2015 [66]	compas-sionate use trial	imatinib	sunitinib	1124	50 mg every 4 out of 6 weeks	8.3	16.6	34.6	0.0	8.0	60.0	8.0	68.0

PFS = progression free survival, OS = overall survival, CR = complete response, PR = partial response, SD = stable disease
CBR = clinical benefit rate (at least stable disease), ORR = objective response percentage

Third line agents

Regorafenib

Regorafenib (Stivarga®, Bayer) is an oral multiple TKI and derived from sorafenib. In this 'fluoro-sorafenib' an extra fluorine-atom protrudes halfway the molecule from the carbon ring, expanding the list of target receptors. Next to VEGFR 1, 2 and 3 the agents inhibits tyrosine kinase with immunoglobin and epidermal growth factor domain 2 (TIE2), the fibroblast growth factor receptor (FGFR) and PDGFR. The oncogenic kinases KIT, RET and RAF are also inhibited.[27] The standard dose regimen is 160 mg each day during 3 weeks in cycles of 4 weeks.[28]

The efficacy in GIST was demonstrated in a phase II study with 34 GIST patients, who had progressive disease on imatinib and sunitinib, of whom 27 patients (79%) had stable disease for at least 3.7 months. The median progression free survival was 10 months in the original publication.[29] Efficacy data have also been updated and the median PFS went to 13.9 months with the longer follow-up, and median OS was 25.0 months instead of not being reached.[30] In a subsequent randomized placebo controlled phase III GRID study with 199 GIST patients, who were progressive after imatinib and sunitinib, regorafenib gave a median progressive free survival of 4.8 months versus 0.9 for placebo (P= 0,0001).[31] After progression on placebo patients switched to regorafenib. In part due to this, the overall survival was not significantly different (hazard ratio 0,77, P= 0.199). The drug has a considerable toxicity profile and in the majority of patients (72%) the dose had to be reduced, but in only 6% of patients was it stopped. The most frequent grade 3 adverse event was hypertension (23%), which is a class effect. Hand-foot syndrome is also prevalent (20%), but could be treated adequately.[31]

In July 2014, regorafenib was approved by the EMA for the treatment for imatinib and sunitinib resistant GIST, following FDA approval in February 2013. The CieBOM has published a positive advice in February 2014 and called the drug an effective third line therapy for GIST with manageable toxicity.[32]

Nilotinib

Nilotinib (Tasigna®, Novartis) is an oral inhibitor of Bcr-Abl, KIT and PDGFR. The recommended dose is 400 mg twice daily, as was found in a phase I study, which also demonstrated efficacy in imatinib-resistant CML.[33] The intracellular concentration of nilotinib in GIST cell lines is higher than of imatinib, and as such pharmacologic resistance would pose a smaller risk.[34]

A phase III study in which nilotinib and imatinib were evaluated in the *first* line was terminated prematurely after 397 patients, because the risk of progressive disease was twice as large for the nilotinib treatment versus imatinib treatment (Hazard ratio 2.032).[35]

To test the clinical value of nilotinib in GIST patients in the *third* line a phase III study was performed with 248 patients.[36] Nilotinib was compared to best supportive care, with the option to prescribe imatinib and sunitinib in the latter arm. To be eligible for inclusion patients had to either have progressive disease on imatinib and sunitinib, or to be intolerant for both of these agents. Due to this study design nilotinib was not consistently assessed as third line agent.

The median progression free survival at central radiologic review, the primary end point, was not different in either treatment group (3.6 months, p= 0.56); at local evaluation of progression nilotinib was superior to the best supportive care group with 3.9 months versus 2.3 months, respectively (p=0.0007). In a subgroup analysis, in which only 197 imatinib and sunitinib *resistant* patients were compared, nilotinib had a 4 months longer overall survival (13.2 months versus 9.2 months).[36] Unfortunately, this was not the primary end point, meaning further development of nilotinib for the indication GIST was ceased.

For an overview of clinical studies with nilotinib and regorafenib as third line treatment, see Table 3.

Other agents

A large number of other agents have been tested in phase II studies in GIST, most of which are TKI's. For an overview of clinical studies with drugs that have tested in advanced GIST patients, see Table 4.

Combination therapies

Despite the success of TKI monotherapy, new treatment options are needed for patients with progressive disease after treatment with registered agents. As previously mentioned, GIST metastases are often heterogeneous at progressive disease and a treatment is desired that interferes at a lower point in the downstream pathway of KIT, such as the PI3-K/AKT pathway. This concept is investigated in studies that combine simultaneous PI3-K inhibitors and imatinib.

Phosphatidylinositol 3-kinases (PI3-K) comprises a group lipase kinases in the PI3-K/AKT pathway, which in physiologically conditions are involved in protein synthesis, glucose metabolism, angiogenesis and cell proliferation and migration.[38] PI3-K activity can be inhibited by PTEN, a tumor suppressor enzyme. Activation of the PI3-K/AKT pathway is an important step in tumor genesis and cell growth in a large number of tumors. This can lead to inhibition of PTEN and overexpression of AKT. In GIST, it can be activated dependent or independent of KIT.[39] There are three different classes of PI3-kinases, and generic and specific inhibitors of PI3-kinas are being explored. The new agents are tested as monotherapy and in combination with other drugs in different tumors, including GIST.

Table 3: overview of clinical studies with regorafenib and nilotinib in advanced GIST patients

Trial	Study type	Previous TKI treatment	Drug	No. of patients	Dose	Median PFS (months)	Median OS (months)	Median follow-up (months)	CR (%)	PR (%)	SD (%)	ORR (%)	CBR (%)
George et al. 2012 [29], Ben-Ami 2016 [30]	phase II	imatinib and sunitinib	regorafenib	33	160 mg every 3 of 4 weeks	13.2	25.0	41.0	0.0	18.1	57.6	18.1	75.6
Demetri et al. 2013 [31]	phase III	imatinib and sunitinib	regorafenib	133	160 mg every 3 of 4 weeks	4.8	not reached	not specified	0.0	4.5	71.4	4.5	75.9
			placebo	66	placebo	0.9	not reached		0.0	1.5	33.3	1.5	34.6
Montemurro et al. 2009 [67]	compassionate use trial	imatinib and sunitinib	nilotinib	52	400 mg twice daily	2.8	7.8	6.5	1.9	7.7	36.5	9.6	46.2
Kim et al. 2011 [68]	compassionate use trial	imatinib and sunitinib	nilotinib	17	400 mg twice daily	5.4	17.1	not specified	0.0	11.8	58.8	11.8	70.6
Sawaki et al. 2011 [69]	phase II	imatinib and sunitinib	nilotinib	35	400 mg twice daily	3.7	10.2	not specified	0.0	2.9	65.7	2.9	68.6
Cauchi et al. 2012 [70]	phase II	imatinib and sunitinib	nilotinib	13	400 mg twice daily	2.0	not specified	not specified	0.0	0.0	30.8	0.0	30.8
Reichardt et al. 2013 [36]	phase III	imatinib and sunitinib	nilotinib	165	400 mg twice daily	3.6	10.9	not specified	0.0	0.6	52.1	0.6	52.7
			Best supportive care, TKI's allowed	83	BSC, including TKI treatment	3.6	9.2		0.0	0.0	44.6	0.0	44.6
Blay et al. 2015 [35]	phase III	none (first line)	nilotinib	324	400 mg twice daily	25.9	not reached	not specified	-	-	-	-	-
			imatinib	320	400 mg	29.7	not reached		-	-	-	-	-

PFS = progression free survival, OS = overall survival, CR = complete response, PR = partial response, SD = stable disease
CBR = clinical benefit rate (at least stable disease), ORR = objective response percentage, BSC = best supportive care

Table 4: overview of clinical studies with (combinations of) drugs that have been tested in advanced GIST patients (continued on next pages)

Trial	Study type	Previous TKI treatment	Drug	No. of patients	Dose	Median PFS (months)	Median OS (months)	CR (%)	PR (%)	SD (%)	ORR (%)	CBR (%)
Bendell et al. 2016 [71]	phase II	imatinib and sunitinib	AUY922	25	70 mg/mg^2	3.9	8.5	0.0	4.0	60.0	4.0	60.0
Leahy et al. 2007 [72]	phase II	imatinib	brostallicin	21	10 mg/m^2 every three weeks	2.2	9.9	0.0	0.0	42.1	0.0	42.1
Edmonson et al. 2002 [73]	phase II	none	combination chemotherapy	21	a cycle every three weeks	7.3	16.7	0.0	4.8	-	4.8	-
Dickson et al. 2013 [74]	phase II	imatinib and sunitinib	BIIB021	25	400 or 600 mg thrice per week	1.2	not specified	0.0	0.0	43.5	0.0	43.5
Judson et al. 2014 [75]	phase II	imatinib and sunitinib	cediranib	24	45 mg once daily	2.0	not specified	0.0	0.0	45.8	0.0	45.8
Trent et al. 2011 [76]	phase II	imatinib	dasatinib	50	70 mg twice daily	2.0	19.0	0.0	31.9	-	31.9	-
Montemurro et al. 2018 [77]	phase II	none	dasatinib	42	70 mg twice daily	13.6	not reached	33.3	40.4	14.3	73.8	88.1
Kang et al. 2013 [78]	phase II	imatinib and sunitinib	dovitinib	30	500 mg every 5 of 7 days	3.6	9.7	0.0	3.3	70.0	3.3	70.0
Joensuu et al. 2017 [79]	phase II	imatinib and sunitinib	dovitinib	38	500 mg every 5 of 7 days	4.8	not specified	0.0	2.6	50.0	2.6	52.6
Kang et al. 2013 [80]	phase II	imatinib and sunitinib	imatinib	41	400 mg once daily	1.8	8.2	0.0	0.0	41.4	0.0	41.4
Schöffksi et al. 2010 [81]	phase II	imatinib	imatinib and everolimus	28	600 mg/ 2.5 mg once daily	1.9	14.9	0.0	0.0	35.7	0.0	35.7

Table 4: (continued)

Trial	Study type	Previous TKI treatment	Drug	No. of patients	Dose	Median PFS (months)	Median OS (months)	CR (%)	PR (%)	SD (%)	ORR (%)	CBR (%)
Schöffksi et al. 2010 [81]	phase II	imatinib and sunitinib	imatinib and everolimus	47	600 mg/ 2.5 mg once daily	3.5	10.7	0.0	2.1	42.6	2.1	44.7
Le Cesne et al. 2010 [51]	phase II	none	masitinib	30	7.5 mg/kg once daily	41.3	not reached	3.3	50.0	43.3	53.3	96.7
Adenis et al. 2014 [52]	phase II	imatinib	masitinib	23	12 mg/kg/day	3.7	29.8	-	-	-	-	-
			sunitinib	21	50 mg every 4 out of 6 weeks	1.9	17.4	-	-	-	-	-
Sawaki et al. 2010 [82]	phase II	imatinib	motesanib	35	125 mg once daily	3.7	not specified	0.0	2.9	54.3	2.9	57.1
Benjamin et al. 2011 [83]	phase II	imatinib	motesanib	102	125 mg once daily	3.7	14.8	0.0	2.9	58.8	2.9	61.8
Chugh et al. 2005[84]	phase II	none	9-Nitro-Camptothecin	13	1.25 mg/m^2 every 5 of 7 days	1.8	not specified	-	-	-	-	-
Trial	Study type	Previous TKI treatment	Drug	No. of patients	Dose	Median PFS (months)	Median OS (months)	CR (%)	PR (%)	SD (%)	ORR (%)	CBR (%)
Wagner et al. 2017 [85]	phase II	imatinib and sunitinib	olaratumab	21	20 mg/kg every 14 days	not specified	not specified	0.0	0.0	23.8	0.0	23.8
Ganjoo et al. 2014[86]	phase II	imatinib and sunitinib	pazopanib	25	800 mg once daily	1.9	10.7	0.0	0.0	48.0	0.0	48.0

Mir et al. 2016 [87]	phase II	imatinib and sunitinib	pazopanib	40	800 mg once daily	3.4	17.8	0.0	0.0	80.0	0.0	80.0
Heinrich et al. 2015 [88]	phase II	imatinib, sunitinib and regorafenib	ponatinib	45	45 mg once daily	2.0	13.5	0.0	4.4	48.9	4.4	53.3
Demetri et al. 2010 [89]	phase III	imatinib and sunitinib	retaspimycin (IPI-504)	32	400 mg/m^2 twice weekly	1.2	not specified	0.0	0.0	68.8	0.0	68.8
Kindler et al. 2011 [90]	phase II	imatinib and sunitinib	sorafenib	38	400 mg twice daily	5.2	11.6	0.0	13.2	55.3	13.2	68.4
Park et al. 2012 [91]	phase II	imatinib and sunitinib	sorafenib	31	400 mg twice daily	4.9	9.7	0.0	12.9	51.6	12.9	64.5
Trent et al. 2003 [92]	phase II	none	temozolomide	19	75 mg/m^2 once daily	2.3	26.4	0.0	0.0	22.2	0.0	22.2
Garcia del Muro et al. 2005 [93]	phase II	none	temozolomide	18	75 mg/m^2 once daily	2.4	19.4	0.0	0.0	-	0.0	-
Blay et al. 2004 [94]	phase II	none	trabectedin	28	1.5 mg/m^2 every three weeks	1.7	19.6	0.0	0.0	33.3	0.0	33.3
Ryan et al. 2012 [95]	phase II	none	trabectedin	20	1.5 mg/m^2 every three weeks	1.3	8.6	0.0	0.0	10.5	0.0	10.5
Joensuu et al. 2008 [96]	phase II	imatinib and sunitinib	vatalanib	15	1250 mg once daily	8.5	not specified	0.0	13.3	53.3	0.0	66.7
Joensuu et al. 2011 [97]	phase II	imatinib and sunitinib	vatalanib	45	1250 mg once daily	4.5	not specified	0.0	4.4	35.6	4.4	40.0

PFS = progression free survival, OS = overall survival, CR = complete response, PR = partial response, SD = stable disease
CBR = clinical benefit rate (at least stable disease), ORR = objective response percentage

BKM120 (buparlisib, Novartis) is an oral PI3-K inhibitor with high specificity for all classes of I PI3-kinases.[40] In GIST cell lines, synergy of imatinib and BKM120 has been established. Recently, an international phase I study was performed, wherein imatinib and sunitinib resistant patients were treated with imatinib and an escalating dose of BKM120. This study has been completed but the results have yet to be published (NCT01468688).

BYL719 (Novartis) is another PI3-K inhibitor which specifically inhibits class I α PI3-kinases, and the β, γ and δ isoforms much less so.[41] Just as BKM120, it is an oral agent and it should in theory have less central nervous system toxicity. BYL719 has also recently been tested in a phase I study in combination with imatinib. This study has an estimated completion date at the end of 2018 (NCT01735968).

New tyrosine kinase inhibitors

The treatment of GIST has developed beyond histology driven therapy to mutation driven therapy. An early example of this, is the recommendation to treat patients with a KIT exon 9 mutation with imatinib 800 mg instead of the usual 400 mg.[12] The *PDGFR* D842V mutation is insensitive to imatinib and patients with this mutation should not be treated with imatinib.[42] In cell line studies, the TKI crenolanib was found to inhibit the kinase activity and cells with this mutation.[43] Based on these findings, a phase II trial was performed for patients with this specific mutation (NCT01243346) which has been completed, but results have not been published. Also, a phase III trial has been initiated for this population in which crenolanib is tested versus placebo (NCT02847429). GIST clones may also revert to different tyrosine kinases to promote proliferation, and GIST growth was found to be inhibited in several xenograft models by the TKI cabozantinib, which is also an inhibitor for MET, AXL and VEGF-receptors.[44] An EORTC coordinated phase II trial investigating the efficacy of cabozantinib has completed patient accrual and follow-up data is being collected (NCT02216578).

DCC-2618

Overcoming drug resistance due to secondary mutations is a challenge in GIST research. TKI's currently approved are only active against a number of possible secondary mutations. A new agent named DCC-2618 has been reported to confer activity against a broad set of mutations, including mutations in KIT exon 13 and 14, as well exon 17 and 18. In advanced pretreated GIST patients a dose-escalation study was performed and a dose of 150 mg per day of DCC-2618 tablets was selected for further studies (NCT02571036).[45] Partial responses were seen in a number of patients. This has prompted the initiation of a randomized, placebo-controlled, double-blind multi-center study in which DCC-2618

is compared to placebo in GIST-patients who already received imatinib, sunitinib, and regorafenib (NCT03353753). In a different study with DCC-2618, the drug is compared to sunitinib in an randomized open-label multicenter study in patients who had imatinib and now need second line systemic therapy (NCT03673501).

BLU-285

Another new agent with potency against the activity of KIT harboring a broad spectrum of exon mutations is BLU-285. This oral drug has been named avapritinib. This drug has shown activity against *KIT* D816V and *PDGFRA* D842V mutations that other TKI do not inhibit. The safety of BLU-285 has been studied in a phase I study, in which no dose limiting toxicities were seen while the drug did show anti-tumor activity (NCT02508532).[46] A dose of 300 mg per day was selected for further studies. Preliminary results showed that despite pretreatment, 9 of the 40 patients had an partial remission. These results lead to study expansion, aiming to enroll more patients in a phase II setting. An randomized open-label study has been started to investigate BLU-285 in a third line setting comparing it to regorafenib and is currently recruiting (NCT03465722).

Immunotherapy

As has been the case in other types of cancer, the successes of checkpoint inhibitors has prompted the use of immunotherapy in clinical trials with advanced GIST patients. A phase I trial sought to combine ipilimumab with imatinib in patients with various tumors including GIST.[47] The recommended phase II dose was determined at ipilimumab 3 mg/kg every 3 weeks with imatinib 400 mg twice daily. No dose limiting toxicities were observed among 35 GIST patients, one of whom with a wild-type GIST had a partial response.[47] A clinical trial investigating pembrolizumab in combination with metronomic cyclophosphamide showed limited activity in 10 GIST patients.[48] Based on post-treatment tumor samples the investigators concluded that macrophage infiltration led to an immunosuppressive tumor microenvironment. In a randomized phase II nivolumab is currently tested against the combination of nivolumab and ipilimumab.[49] After accrual of the first 14 of a projected 40 advanced GIST patients, the clinical benefit rate for both treatment arms is around 40% with a median PFS of 1.9 months (NCT02880020). Another study recruiting GIST patients is a phase II trial investigating epacadostat and pembrolizumab to assess the efficacy of combined IDO and PD-1 inhibition (NCT03291054).

Clinical studies in the Netherlands

In the five Dutch soft tissue sarcoma centers a number of trials are performed or prepared for the first, second and third line of treatment. Furthermore, studies are set up for the adjuvant setting and for long term responders, and work is being done into biomarkers like germ line DNA polymorphisms, circulating tumor DNA (the KWF sponsored GALLOP study) and blood level monitoring. Some studies are briefly highlighted below.

ALT GIST

In this randomized phase II trial patients with advanced GIST are treated with either standard imatinib treatment, or with imatinib alternated with regorafenib and a brief interval without medication. The idea is that cells re-enter the proliferation cycle during the treatment-free interval and then will be more sensitive to imatinib. Regorafenib should suppress imatinib resistant cells before these can grow to clinically relevant clones. The EORTC coordinates this study in the Netherlands. This study has been completed and results are to be reported shortly (NCT02365441).

Masitinib

Masitinib (AB1010, AB Science) is an inhibitor of KIT, PDGFRa and Lyn and preclinical research suggests that it has a stronger and more specific binding to KIT than imatinib does.[50] In a first line phase II study almost all of 30 patients (97%) had at least stable disease and a median survival of 41.3 months.[51] Recently, a randomized phase II study was published in which 44 imatinib resistant patients were treated with masitinib or sunitinib; the group of 23 patients who received masitinib had a longer progression free survival compared to the group of 21 patients who received sunitinib; 3.7 versus 1.9 months, respectively.[52] The median PFS of sunitinib is far shorter than the original trials designed to asses sunitinib efficacy. Two phase III trials were started; one study which compares masitinib with imatinib in the first line (NCT00812240), and a study in which masitinib is compared to sunitinib in the second line (NCT01694277). Both these studies have been closed for inclusion for some time and results have not yet been reported.

LOP628

A recent development in targeted therapy is the antibody drug conjugate (ADC). These conjugates use an antibody to guide a cytotoxic drug to malignant cells. This should result in less toxicity of non-sensitive cells and the delivery of a cytotoxic agent at or in the targeted cells. An ADC has been developed called LOP628, which consists of an anti-KIT antibody that is linked to a DM1 maytansinoid toxin. This toxin interferes in

microtubule assembly and thus prevents cell proliferation. A preclinical study showed anti-proliferative activity on c-KIT-positive cell lines, including some imatinib-resistant cell lines.[53] A phase I trial aiming to establish a maximum tolerated dose in patients with a KIT positive tumor has been performed (NCT02221505).[54] All three included patients suffered a hypersensitivity reaction requiring rescue medication in the form of steroids and antihistaminic drugs. Mast cell degranulation was determined as the cause for the reaction and the trial was subsequently terminated.

GALLOP study

On a different note, one noteworthy study currently performed in the Netherlands is the GALLOP study (NCT02331914). Collaborating in the Dutch GIST consortium, all five Dutch sarcoma referral centers participate in this study. This study aims to asses GIST mutation during treatment, as well as measure TKI serum. In a bio-database, clinical data, tumor and blood samples are collected. Blood samples are analyzed during treatment for TKI serum levels in order to adjust dosing and thus optimize anti-tumor treatment. Next to mutation analysis of the primary GIST , blood samples during treatment are used to routinely perform mutation analysis on circulating tumor DNA. In case of disease progression, patients are asked to have a biopsy of a progressive lesion taken in order to test for secondary mutations. Using circulating tumor DNA, disease progression may be discovered before CT scans show tumor growth or spread. Receiving optimal TKI treatment may influence whether secondary mutations in circulating tumor DNA emerge at all. The DNA collected in these blood samples may also serve as a validation set for the pharmacogenetic studies presented in the subsequent chapters.

Conclusion

In the past 18 years, the median survival of advanced GIST has risen from less than 12 months to more than 60 months. Factors that contribute to this include improved understanding of GIST pathogenesis, mechanisms of resistance to available TKI's and the opportunities that new (combination) therapies offer. The clinical introduction of imatinib, sunitinib and regorafenib facilitates long term treatment. This chapter highlights current developments in systemic treatment as well as current trials. Sadly, most patients with metastasized disease will eventually die of their disease. Therefore, patient participation in clinical trials is vital to discover new effective treatment strategies. These trials are performed in specialized centers, so patients will have to be treated at those hospitals. As shown, numerous trials have been and are currently performed to improve the systemic treatment of advanced GIST patients.

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3

Genetic polymorphisms in angiogenesis related genes are associated with worse progression free survival of patients with advanced gastro-intestinal stromal tumors treated with imatinib

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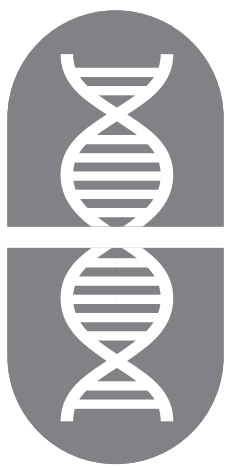

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Abstract

Background

Imatinib 400mg per day is first line therapy for patients with gastrointestinal stromal tumors (GIST). Although clinical benefit is high, progression free survival (PFS) is variable. This study explores the relationship of single nucleotide polymorphisms (SNPs) in genes related to imatinib pharmacokinetics and pharmacodynamics and PFS in imatinib-treated patients with advanced GIST.

Methods

In 227 patients a pharmacogenetic pathway analysis was performed. Genotype data from 36 SNPs in 18 genes were tested in univariate analyses to investigate their relationship with PFS. Genetic variables which showed a trend ($p<0.1$) were tested in a multivariate model, in which each singular SNP was added to clinicopathological factors.

Results

In univariate analyses, PFS was associated with synchronous metastases ($p=0.0008$) and the mutational status ($p=0.004$). Associations with rs1870377 in *KDR* (additive model, $p=0.0009$), rs1570360 in *VEGFA* (additive model, $p=0.053$), and rs4149117 in *SLCO1B3* (mutant dominant model, 0.027) were also found. In the multivariate model, significant associations and trends with shorter PFS were found for synchronous metastases (HR 1.94, $p=0.002$), *KIT* exon 9 mutation (HR 2.45, $p=0.002$), and the SNPs rs1870377 (AA genotype, HR 2.61, $p=0.015$), rs1570360 (AA genotype, HR 2.02, $p=0.037$), and rs4149117 (T allele, HR 0.62, $p=0.083$).

Conclusion

In addition to *KIT* exon 9 mutation and synchronous metastases, SNPs in *KDR*, *VEGFA* and *SLCO1B3* appear to be associated with PFS in patients with advanced GIST receiving 400mg imatinib. If validated, specific SNPs may serve as predictive biomarkers to identify patients with an increased risk for progressive disease during imatinib therapy.

Introduction

Imatinib mesylate (Gleevec®, Glivec®) is first line therapy for chronic myeloid leukemia (CML) and gastrointestinal stromal tumors (GIST).[1,2] It has revolutionized the treatment of both malignancies by achieving significant survival benefit with limited toxicity.[3] Clinical response to this oral tyrosine kinase inhibitor (TKI) is determined by somatic mutations, as well as by germline genetic variations.[4,5] Single Nucleotide Polymorphisms (SNPs) are the most common germline genetic variations. SNPs can have various functional effects, ranging from silent mutations to affecting gene expression and enzyme function. The pharmacokinetics and pharmacodynamics of imatinib may be changed in patients carrying SNPs in genes encoding for enzymes and target proteins involved in imatinib pharmacology.

GIST is a mesenchymal tumor of the digestive tract, often caused by gain-of-function mutations in the genes encoding for KIT or PDGFR-α.[6-8] *KIT* mutations are routinely screened in GIST to predict imatinib efficacy which is dependent on the location of the *KIT* mutation.[4] Disease progression has also been associated with clinical factors, such as the location of the primary tumor.[9,10]

In CML treatment, complete cytogenetic response to imatinib has been associated with SNPs in genes encoding for enzymes which have a role in imatinib metabolism. Also, polymorphisms in the genes encoding for the efflux transporter ABCG2 (rs2231137) and for the influx transporter SLC22A1 (rs683369) have been associated with poor response and progression to advanced disease, respectively.[5] In 54 patients with advanced GIST who were treated with imatinib, associations have been reported for SNPs in *SLC22A4* (rs1050152) and *SLC22A5* (rs2631367 and rs2631372) and time to progression.[11] Since this report, no similar studies have been published. A review highlighting SNPs found in relation to imatinib in CML and GIST has been published elsewhere.[12]

This study aims to investigate the relationship of genetic variants in genes encoding proteins involved in the pharmacokinetics and pharmacodynamics of imatinib and efficacy in patients with locally advanced and metastatic GIST.

Methods

Patients

For this exploratory retrospective study GIST patients were included who had been treated in four Dutch referral centers. All patients had a histologically proven GIST and documented non-curative disease, being either non-resectable locally advanced or metastatic disease at the time of start of imatinib. Patients started imatinib therapy in a dose of 400mg once daily between January 2001 and May 2013 and follow-up lasted

until July 2014. All patients had to be treated until the first treatment evaluation, with the exception of patients with clinical progression before this moment. Patients with *KIT* exon 9 mutation were retained in the analysis despite having received imatinib in a 400mg daily dose, as the objective of the study was to test the pharmacogenetic effects of 400mg daily and 800mg daily induce more toxicity. Furthermore, it is common practice in the Netherlands to start with imatinib 400mg daily in case of a *KIT* exon 9 mutation if the tumor load is low and a patient is asymptomatic, and only escalate to 800mg in case of progressive disease.

DNA was obtained from residual blood samples or, in the Erasmus MC Cancer Institute, after specific informed consent was obtained. Samples were stored at -20°C until genotyping. In one location serum of these samples was stored. If a residual blood or serum sample was not available, DNA was obtained from residual formalin fixated paraffin embedded (FFPE) specimen. All samples were anonymized by a third party and the Code for Proper Secondary Use of Human Tissue was adhered to (www.federa.org/codes-conduct).[13]

SNP selection

SNPs in genes related to imatinib pharmacokinetics and pharmacodynamics were selected using a pathway approach.[14] The literature was screened for SNPs in relevant genes. Using Haploview and HapMap data (release 28), SNPs in linkage disequilibrium (>95%) were identified to select candidate SNPs. SNPs were included if the minor allele frequency was at least 0.1. Additionally, the NIEHS database was used to select the SNPs with an expected functional change. A total of 36 SNPs in 18 genes were included, as shown in Table 1.

Table 1: selected SNPs in pharmacokinetics and pharmacodynamics of imatinib

Gene	Rs number	Chromosome	Allele change	Change type
In pharmacokinetics				
ABCG2	rs2231137	4	G/A	Splicing
ABCG2	rs2231142	4	C/A	Splicing
SLC22A5	rs2631367	5	C/G	TFBS
SLC22A5	rs2631370	5	T/C	TFBS
SLC22A5	rs2631372	5	C/G	TFBS
SLC22A1	rs628031	6	G/A	Splicing
SLC22A1	rs683369	6	C/G	Splicing
SLC22A1	rs6935207	6	G/A	TFBS

ABCB1	rs1045642	7	C/T	Splicing
ABCB1	rs868755	7	G/T	Splicing
ABCB1	rs28656907	7	C/T	TFBS
SLC22A4	rs1050152	5	C/T	Splicing
CYP3A4	rs2740574	7	A/G	TFBS
POR	rs1057868	7	C/T	nsSNP
ABCC2	rs717620	10	C/T	TFBS
CYP1A1	rs1048943	15	A/G	nsSNP
CYP1A2	rs762551	15	A/C	TFBS
SLCO1B3	rs4149117	12	G/T	Splicing
In pharmacodynamics				
PDGFRA	rs1800810	4	C/G	TFBS
PDGFRA	rs1800812	4	G/T	TFBS
PDGFRA	rs1800813	4	A/G	TFBS
PDGFRA	rs2228230	4	C/T	Splicing
PDGFRA	rs35597368	4	C/T	Splicing
KDR	rs1870377	4	A/T	nsSNP
KDR	rs2071559	4	C/T	TFBS
KDR	rs2305948	4	C/T	nsSNP
VEGFA	rs1570360	6	G/A	TFBS
VEGFA	rs2010963	6	G/C	TFBS
VEGFA	rs25648	6	C/T	Splicing
VEGFA	rs3025039	6	C/T	miRNA
VEGFA	rs699947	6	A/C	TFBS
VEGFA	rs833061	6	C/T	TFBS
FLT4	rs6877011	5	C/G	miRNA
RET	rs1799939	10	G/A	Splicing
FLT3	rs1933437	13	T/C	Splicing
FLT1	rs7993418	13	A/G	Splicing

Selected SNPs in pharmacokinetics and pharmacodynamics of imatinib: Splicing= Splicing modifying, TFBS= Transcription Factor Binding Site, nsSNP= Non-Synonymous SNP, miRNA= Micro RNA alteration

Genotyping

DNA was isolated from blood (197 patients), serum (20 patients) using the MagnaPure Compact (Roche Diagnostics, Almere, the Netherlands) or from FFPE samples (10 patients) using the Tissue Preparation System (Siemens Diagnostics, The Hague, The Netherlands) and stored at -20°C. For optimal genotyping results, DNA isolated from serum and FFPE samples was pre-amplified using real-time PCR genotyping assays as described before.[15] A custom made array was developed for the QuantStudio 12K Flex Real-time PCR system (Life Technologies, Bleiswijk, the Netherlands) and DNA was genotyped according to the manufacturer's protocol. To achieve a satisfactory call rate for all SNPs (>90%), a number of SNPs were subsequently genotyped using commercially available realtime PCR genotyping assays (Life Technologies, Bleiswijk, the Netherlands) according to the manufacturer's protocol or in-house developed Pyrosequencing assays (Qiagen, Venlo, the Netherlands).

The average call rates did not differ significantly between blood, serum or FFPE samples (99.4%, 96.5% and 95.4%, respectively). All 36 SNPs had a call rate of >90%, 32 of which had >95%. Out of 36 SNPs, 31 were in the Hardy-Weinberg Equilibrium (HWE) and the remaining 5 SNPs were so if just 2 patients (0.9%) had another genotype, meaning that allele frequency is not different from expected. In this patient cohort the minor allele frequencies were in accordance to those reported in the NCBI database. To explore haplotypes in the study population Haploview 4.2[16] and Plink 1.7[17] were used. SNPs in the same gene were considered to be in a haplotype in case D' was at least 95%.

Statistics

Clinical variables were collected from patient files. Progression free survival (PFS) was the primary endpoint and defined as the time between the date of start of imatinib treatment and the date of progressive disease, according to clinical progression or to RECIST 1.1 definition of progressive disease. If patients were still on treatment at the last date of follow-up, PFS was censored at that date. The secondary endpoint overall survival (OS) was defined as the time between the date of start of imatinib treatment and death due to GIST. OS was censored at the last date of follow-up if a patient was alive at that time, or a day before death if a patient had died due to an unrelated illness.

The clinical variables age, sex, synchronous metastases and mutational status (either *KIT* exon 11, *KIT* exon 9 or an 'other' group consisting of other mutations in *KIT, PDGFRA* or 'wild-type') were tested univariately with Cox regression or Kaplan Meier analysis. These factors were included in the multivariate analysis, as they were deemed to affect imatinib efficacy. SNPs and haplotypes were univariately tested with Kaplan Meier analysis for an association with PFS and OS. If univariate analyses showed a trend for a

difference in survival (p< 0.1), these genetic factors were selected for inclusion into the multivariate Cox regression model. In the multivariate model, the effect of combined clinical factors was calculated without inclusion of SNPs. To determine the impact of SNPs, singular SNPs were added to combined clinical factors. SNPs were tested in the additive model, unless frequency of mutant homozygote patients did not allow for this. Variables with p<0.05 in the multivariate analyses were considered statistically significant. Due to the explorative nature of this study no correction for multiple testing was performed. SPSS version 20 (IBM Corp., Armonk, NY, United States) was used.

Results

Study population

A total of 365 patients were screened for study selection, but 68 patients had imatinib only as neo-adjuvant treatment, 41 patients had imatinib only as adjuvant treatment, in 1 patient the indication was unclear. Of the remaining 255 patients who received imatinib for locally advanced and metastatic GIST 28 had imatinib in another dose than 400mg once daily. Therefore 227 patients were included in the study. The baseline characteristics of the study population are shown in Table 2. In 69 patients (39.2%) metastases were found at diagnosis, and in 137 patients (60.4%) either metachronous metastases or a locally advanced relapse developed in time. The median PFS for the study population was 39.0 months (95% confidence interval (CI): 27.4-50.6 months) and the median OS 86.5 months (95% CI: 70.8-102.2 months). At the time of analysis, 116 patients (51.1%) had progressive disease and 80 patients (35.2%) had died due to GIST. The median time of follow-up was 71 months, as calculated by the reversed Kaplan Meier estimator.

PFS was significantly longer in patients without synchronous metastases (p= 0.0008) and in patients who had a *KIT* exon 11 mutation as compared to *KIT* exon 9 (p= 0.004), while age and sex did not show an association, as shown in Table 3. Overall survival was longer in females (p= 0.042) and if metastases were absent at diagnosis (p= 0.0002), but not with other selected clinical variables, as shown in Table 4.

Table 2: baseline characteristics of study population

		Number	%
Age at diagnosis	median, in years	59.1	
Sex	male	139	61.2
	female	88	38.8
WHO performance	0-1	189	83.3
score at start of imatinib	2-3	8	3.5
	unknown	30	13.2
Previous operation for GIST	yes	158	30.4
	no	69	69.6
Mutation found	KIT exon 11	110	48.5
	KIT exon 9	22	9.7
	other	54	23.8
	unknown	41	18.1
Metastases or relapse with	synchronous metastases	89	39.2
locally advanced disease	metachronous or relapse	137	60.4
	unknown	1	0.4

Baseline characteristics of 227 advanced GIST patients; other mutation: KIT exon 13 (3), KIT exon 14 (1), KIT exon 17 (2), PDGFR exon 12 (4), PDGFR exon 18 (4), 'wild type' (40)

Pharmacogenetic factors associated with PFS

In the univariate analysis of PFS, three SNPs related to the pharmacodynamics of imatinib showed (a trend for) an association with survival. These were for rs1870377 in *KDR* (TT *vs* AT *vs* AA, p= 0.0009), rs1570360 in *VEGFA* (GG *vs* GA *vs* AA, p= 0.035) and rs4149117 in *SLCO1B3* (GG *vs* GT+TT, p= 0.027), see Table 3.

In the multivariate analysis, the combined clinical factors were associated with shorter PFS in the case of synchronous metastases and a *KIT* exon 9 mutation (HR 1.94, p= 0.002 and HR 2.45, p= 0.002, respectively). When one of the selected SNPs was added to this model, the AA genotype in rs1870377 and the AA genotype in rs1570360 were associated with shorter PFS (HR 2.61, p= 0.037 and HR 2.02, p= 0.015, respectively), whereas GT or TT genotype in rs4149117 showed a trend for longer PFS (HR 0.62, p= 0.083).

Table 3: univariate and multivariate analyses of progression free survival of advanced GIST with 400 mg imatinib

			Univariate Kaplan Meier analyses			Multivariate Cox regression analyses		
		N patients	**median PFS**	**95% CI**	***p* value**	**HR**	**95% CI**	***p* value**
Clinical factors								
Age		(HR per year increase = 0.998)		0.984 - 1.013	0.812	0.9993	0.98 - 1.02	0.936
Sex	male	139	35.8	24.8 - 46.9	0.201	1		0.348
	female	88	47.9	2.9 - 92.8		0.82	0.53 - 1.25	
Metastasis at diagnoses	absent	137	60.9	18.2 -103.7	0.0008	1		0.002
	present	89	24.9	18.5 - 33.8		1.94	1.28 - 2.94	
Mutation	KIT exon 9	22	19.1	5.2 - 32.9	0.004	2.45	1.40 - 4.30	0.002
	KIT exon 11	110	44.3	32.6 - 57.9		1		
	* other group	54	26.2	17.3 - 35.9		1.34	0.86 - 2.11	0.201
Genetic factors								
rs1870377 *(KDR)*	TT vs	132	37.7	29.8 - 45.6	0.0009	1		
	AT	78	50.6	28.5 - 72.6		0.76	0.48 - 1.19	0.225
	vs AA	11	8.7	3.0 - 14.4		2.61	1.06 - 6.43	0.037
rs1570360 *(VEGFA)*	GG vs	109	39.4	23.3 - 55.5	0.053	1		
	GA	81	60.9	1.7 -120.2		0.67	0.42 - 1.08	0.102
	vs AA	29	28.0	14.7 - 41.4		2.02	1.15 - 3.56	0.015
rs4149117 *(SLCO1B3)*	GG vs	161	28.5	19.1 - 37.9	0.027	1		
	vs GT + TT	** 48	50.2	45.6 - 53.8		0.62	0.36 - 1.06	0.083

Univariate and multivariate analyses of progression free survival of GIST with 400mg imatinib: only univariate analyses with $p < 0.1$ shown; HR > 1.0 indicates association with worse survival and vice versa; in the multivariate Cox regression the effect of combined clinical factors are reported without inclusion of SNPs and SNP results are presented for the singular SNP added to the model of combined clinical factors. PFS = progression free survival; survival in months; 95% CI = 95% confidence interval; HR = Hazard ratio; * other group consists of other mutations in KIT, PDGFR or 'wild-type'; ** only 2 patients were homozygote mutant for rs4149117

Table 4: univariate and multivariate analyses of overall survival of advanced GIST with 400 mg imatinib

			Univariate Kaplan Meier analyses			Multivariate Cox regression analyses		
		N patients	median OS	95% CI	*p* value	HR	95% CI	*p* value
Clinical factors								
Age	(HR per year increase = 0.9999991)			0.999 -1.000	0.971	1.0005	0.98 - 1.02	0.960
Sex	male	138	80.1	62.2 - 97.8	0.042	1		0.327
	female	86	- #	- ##		0.77	0.46 - 1.29	
Metastasis at diagnoses	absent	135	119.1	- ##	0.0002	1		0.0001
	present	88	66.6	50.7 - 82.4		2.71	1.63 - 4.50	
Mutation	KIT exon 9	22	71.8	57.5 - 86.0	0.419	1.94	0.96 - 3.93	0.065
	KIT exon 11	125	89.8	73.0 -106.7		1		
	* other group	56	80.9	- ##		1.11	0.65 - 1.91	0.702
Genetic factors								
rs1870377 *(KDR)*	TT vs	132	86.4	64.0 -108.8	0.057	1		
	AT	76	100.6	67.9 -133.3		0.87	0.51 - 1.51	0.632
	vs AA	10	67.3	24.4 -110.1		2.69	0.98 - 7.38	0.054
rs4149117 *(SLCO1B3)*	GG vs	158	75.7	60.5 - 90.9	0.030	1		0.081
	GT + TT	** 48	- #	- ##		0.54	0.27 - 1.08	

Univariate and multivariate analyses of overall of GIST with 400mg imatinib: only univariate analyses with $p < 0.1$ shown; HR > 1.0 indicates association with worse survival and vice versa; in the multivariate Cox regression the effect of combined clinical factors are reported without inclusion of SNPs and SNP results are presented for the singular SNP added to the model of combined clinical factors. OS = overall survival; survival in months; 95% CI = 95% confidence interval; HR = Hazard ratio; # = median not reached; ## = 95% CI not computed; * other group consists of other mutations in KIT, PDGFR or 'wild-type'; ** only 2 patients were homozygote mutant for rs4149117

Pharmacogenetic factors associated with OS

In the univariate analysis of OS, a trend for association was seen in rs1870377 in *KDR* (TT *vs* AT *vs* AA, p= 0.057) and a statistically significant association for rs4149117 in *SLCO1B3* (GG *vs* GT+TT, p= 0.030), see Table 4. In the multivariate model only synchronous metastases was associated with OS (HR 2.71, p= 0.0001), while a *KIT* exon 9 mutation showed a trend for worse survival (HR 1.94, p= 0.065). Addition of a SNP to the combined clinical factors showed trends for shorter survival in case of the *AA* genotype in rs1870377 (HR 2.69, p= 0.054) and longer survival for the GT or TT genotype in rs4149117 (HR 0.54, p= 0.081).

Discussion

This exploratory pharmacogenetic study shows that SNPs in the genes encoding for VEGFA, KDR (also known as VEGFR2) and SLCO1B3 (also known as OATP1B3) are associated with PFS in patients with advanced GIST treated with 400mg imatinib once daily. To the best of our knowledge, this cohort of 227 GIST patients is the largest patient group in which the pharmacogenetics of imatinib was explored. The SNP selection for this study was performed using a candidate gene approach based on imatinib pharmacology and expected functionality. This, however, does not exclude the possibility that the SNPs which show an association with PFS, are in fact independent prognostic biomarkers.

So far, only one study exploring the effects of SNPs in genes related to imatinib pharmacokinetics on its efficacy was performed in patients with advanced GIST. This study investigated 31 SNPs in a population of 54 patients.[11] SNPs in *SLC22A4* (rs1050152) and *SLC22A5* (rs2631367 and rs2631372) were associated with time to progression, independent of mutational status, tumor size, age and sex. These SNPs were also tested in the present study, but univariate tests with the additive model did not show a trend for an association with survival. Possibly, the small sample size can account for this discrepancy.

Several SNPs in vascular endothelial growth factor A (*VEGFA*) were included in this study. VEGFA plays a crucial role in inducing angiogenesis. Compared to weak or non-expressers, high VEGF expression in GIST has been associated to inferior PFS during imatinib therapy.[18] Also, imatinib may lead to decreased VEGF expression in a subset of patients.[18] In this study, rs1570360 in *VEGFA* was associated with PFS. Other SNPs in *VEGFA* such as rs699947 have been associated with a reduced effect of imatinib in CML patients, but none other of the tested SNPs showed a significant association in this study population.[19] In this study, rs7993418 in *FLT1* (encoding for vascular endothelial growth factor receptor 1) and rs6877011 in FLT4 (encoding for the receptor of vascular endothelial growth factor C and D) did not show an association with survival. [20]

The rs1870377 SNP in kinase insert domain receptor (KDR, also known as VEGFR2) was associated with shorter PFS (and less so with OS) in the present study population. This may be due to increased micro-vessel density seen in tumors with this SNP mutation.[21] The effect of enhanced tumor angiogenesis may be stronger in terms of increased nutrient supply as compared to improved accessibility for imatinib. Having a variant in this SNP has also been shown to increase GIST susceptibility, pointing to a role of VEGF in GIST biology.[22] A study investigating SNPs in *KDR* for an effect on GIST relapse rate did not show a similar effect, in contrast to a study with CML patients, which reported better clinical outcome for patients with the wildtype genotype in rs1870377.[19,20]

Patients with at least one T allele in rs4149117 in *SLCO1B3* had a trend for longer OS. The solute carrier organic anion transporter family member (SLCO) 1B3 is an influx transporter with imatinib as a substrate.[23] A study performed in CML patients reported that the frequency of patients with the *TT* genotype was higher in the responder group than in the non-responder group.[24] These results are in line with a study from Japan, which found enhanced transporter function in patients with the *TT* genotype, as measured by higher intracellular imatinib levels.[25]

As previously reported, the effect of the oncogenic somatic mutation on imatinib efficacy were also found in this study. Tumors with a *KIT* exon 11 mutation were more sensitive to imatinib compared to tumors with a *KIT* exon 9 mutation.[4] Patients with a *KIT* exon 9 mutation received imatinib at a dosage currently considered too low, but this was corrected for in the multivariate analysis. Presence of synchronous metastases was clearly associated with reduced survival. These metastases may be considered heterogeneous and some clones will progress despite imatinib activity in the majority of GIST lesions.[26] Other clinical factors were not associated with survival, even though factors such as the primary tumor site have been reported in other studies.[9]

Remarkably, SNPs in the pharmacokinetic genes encoding for ABCB1, ABCG2, SLC22A1, SL22A5 or CYP3A4 were not associated with a difference in survival, despite previous, sometimes conflicting, reports.[5,10,11,19,20,27-29] A hypothetical explanation may be, that most patients had an imatinib serum level higher than the threshold needed for clinical activity, negating any effects that these SNPs may have on the actual serum level above this threshold.

This study has limitations, mainly due to the retrospective nature of the data. In addition, DNA derived from blood was not available for all patients. FFPE samples were used instead, as it has been demonstrated to be a valid proxy for DNA from peripheral blood.[30] Out of the 36 SNPs tested, 5 were not in HWE. These SNPs were retained in the analyses, as an allele change in only 2 patients would mean these SNPs are in HWE, and patient selection due to the retrospective nature of the study was considered the most plausible reason.

This study investigated the associations of polymorphisms in genes related to the pharmacokinetics and pharmacodynamics of imatinib in the treatment of advanced GIST. One SNP in the pharmacokinetic pathway (rs4149117 in *SLCO1B3*) and two SNPs related to pharmacodynamics (rs1870377 in *KDR*, and rs1570360 in *VEGFA*) were significantly associated with PFS. When replicated, these polymorphisms, together with tumor mutation and metastases, may identify patients who are most at risk of developing progressive disease and it may select patients whom may benefit from more frequent treatment evaluation or alternative first line treatments that are currently being developed (e.g. NCT02365441).

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Genetic polymorphisms as predictive biomarker of survival in patients with gastro-intestinal stromal tumors treated with sunitinib

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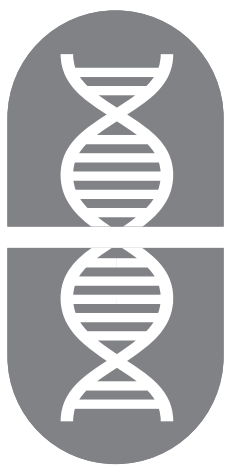

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Abstract

This study aimed to identify single-nucleotide polymorphisms (SNPs) that are associated with outcome to treatment with sunitinib in patients with advanced gastrointestinal stromal tumors (GIST). Forty-nine SNPS involved in the pharmacokinetic and pharmacodynamic pathway of sunitinib were associated with progression-free survival (PFS) and overall survival (OS) in 127 patients with advanced GIST who have been treated with sunitinib. PFS was significantly longer in carriers of the TT genotype in POR rs1056878 (hazards ratio (HR) 4.310, 95% confidence interval (CI):1.457-12.746, p= 0.008). The presence of the T-allele in SLCO1B3 rs4149117 (HR 2.024, 95% CI:1.013-4.044, p= 0.046), the CCC-CCC alleles in SLC22A5 haplotype (HR 2.603, 95% CI: 1.216-5.573, p= 0.014), and the GC-GC alleles in the IL4 R haplotype (HR 7.131, 95% CI:1.518-33.496, p= 0.013) were predictive for OS. This shows that polymorphisms in the pharmacokinetic and pharmacodynamic pathways of sunitinib are associated with survival in GIST. This may help to identify patients that benefit more from treatment with sunitinib.

Introduction

As the introduction of imatinib as first line treatment for advanced gastrointestinal stromal tumors (GIST), progression-free survival (PFS) and overall survival (OS) of patients with this malignancy has markedly improved. Unfortunately, eventually the vast majority of patients develop resistance to imatinib, mainly due to secondary mutations, while in others severe toxicity occurs, both resulting in the need to switch to second line treatment with sunitinib (Sutent; Pfizer Pharmaceuticals Group, New York, USA).[1] Sunitinib is a multi-targeted tyrosine kinase inhibitor.[2,3] Its clinical value in the treatment of patients with metastatic GIST has been shown in a randomized trial showing a median time to tumor progression of 27.3 weeks for patients treated with sunitinib, versus 6.4 weeks for patients treated with placebo.[1] However, there is a large interindividual difference in the efficacy of sunitinib in patients with GIST. This may in part be explained by the presence of specific mutations within the tumor but another factor that may contribute to the variability in efficacy may be germline genetic variation.[4] In patients treated with sunitinib for metastatic renal cell cancer, single-nucleotide polymorphisms (SNPs) in genes related to the pharmacokinetic and pharmacodynamic pathways of sunitinib have been associated with outcome in terms of PFS and OS.[5]

In patients with GIST, the role of germline genetic polymorphisms as biomarkers predicting outcome has never been investigated. To further personalize treatment in this group of patients, it is meaningful to get better insight into the factors predicting the efficacy of a drug before starting, especially when alternative treatment options exist such as in the case of advanced GIST. Therefore, a multicenter association analysis was performed to explore whether polymorphisms in candidate genes within the pharmacokinetic or pharmacodynamic pathway of sunitinib are associated with PFS and OS in patients with GIST.

Materials and methods

Study population and design

From a large multicenter Dutch cohort of 365 patients with GIST, those patients who have been treated with second line sunitinib were selected. Patients had started sunitinib treatment between March 2004 and June 2014 in the Erasmus MC Cancer Institute, Leiden University Medical Center, Netherlands Cancer Institute-Antoni van Leeuwenhoek, or University Medical Center Groningen. Sunitinib could be administered in a 4 weeks on/2 weeks off treatment scheme, or in a continuous dosing regimen (or both), with any dose of sunitinib. Patients who have had dose reductions or dose escalations were allowed to be included in this study.

Demographic data of patients was retrospectively collected in an electronic case record form, designed for this study. Collected patient characteristics were age, gender, self-declared ethnicity, Eastern Cooperative Oncology Group (ECOG) WHO performance score, weight, length, tumor characteristics (*i.e.* histology, mutation status, mitotic index (per 50 HPF), site of origin tumor, previous surgery), prior therapy and therapy after sunitinib, and survival estimates. For PFS and OS, data collection took place until August 2014.

From each patient one sample of whole blood, serum or tumor surrounding tissue containing germline DNA was collected for DNA isolation. Samples could be either residuals or prospectively obtained samples in a study approved by the local medical ethical board. Samples were stored at –20 °C or colder at the local hospital laboratory until further process. All samples were anonymized, according to the Codes for Proper use and Proper Conduct in the Self-Regulatory Codes of Conduct (www.federa.org).

Genetic polymorphisms and haplotype estimation

Forty-nine SNP in 23 genes involved in the pharmacokinetics and pharmacodynamics of sunitinib were selected for genotyping, based on literature (see Table 1). SNPs were selected from the genes *ABCB1, ABCC2, ABCG2, CYP1A1, CYP1A2, CYP3A4, NR1I2, NR1I3, POR* (Cytochrome P450 oxidoreductase), *SLCO1B3, SLC22A1, SLC22A4* and *SLC22A5* within the pharmacokinetic pathway and the genes *FLT1, FLT3, IL-4R, IL-8, KDR* (Kinase Insert Domain Receptor), *PDGFRA, RET* and *VEGFA* within the pharmacodynamic pathway.

Table 1: Selected polymorphisms within the pharmacodynamic and pharmacokinetic pathway of sunitinib

Gene	Protein	SNP	Allele change
Pharmacodynamic genes			
IL4	IL4	rs224350 (Chu *et al.*[9])	C/T
IL4R	IL4R	rs1801275 (Chu *et al.*[9]) rs1805010 (Chu *et al.*[9]) rs1805015 (Chu *et al.*[9])	A/G A/G T/C
IL8	IL8	rs4073 (Xu *et al.*[12]) rs1126647 (Xu *et al.*[12])	A/T A/T
IL13	IL13	rs1800925 (Chu *et al.*[9]) rs20541 (Chu *et al.*[9])	C/T G/A
FLT1	FLT1	rs7993418 (Beuselinck *et al.*[13])	A/G
FLT3	FLT3	rs1933437 (van Erp *et al.*[14])	T/C
FLT4	VEGFR3	rs6877011 (Scartozzi *et al.*[15])	C/G

KDR	VEGFR2	rs1870377 (Garcia-Donas *et al.*[16]) rs2071559 (van Erp *et al.*[14]) rs2305948 (Garcia-Donas *et al.*[16])	A/T C/T C/T
PDGFRA1	PDGFRA1	rs1800810 (van Erp *et al.*[14]) rs1800812 (van Erp *et al.*[14]) rs1800813 (van Erp *et al.*[14])	C/G G/T A/G
PDGFRA2	PDGFRA2	rs2228230 (Bruck *et al.*[17]) rs35597368 (Garcia-Donas *et al.*[16]; van Erp *et al.*[14])	C/T C/T
RET	RET	rs1799939 (van Erp *et al.*[14])	G/A
VEGFA	VEGFA	rs1570360 (Garcia-Donas *et al.*[16]) rs2010963 (Eechoute *et al.*[18]; Garcia-Donas *et al.*[16]) rs25648 (Scartozzi *et al.*[15]) rs3025039 (Kim *et al.*[19]) rs699947 (Eechoute *et al.*[18]; Garcia-Donas *et al.*[16]; Kim *et al.*[19]) rs833061 (Eechoute *et al.*[18]; Kim *et al.*[19])	G/A G/C C/T C/T A/C C/T
Pharmacokinetic genes			
ABCB1	ABCB1	rs1045642 (Maffioli *et al.*[20]; Takahashi *et al.*[21]) rs868755 (Angelini *et al.*[8]; Takahashi *et al.*[21]) rs28656907 (Loeuillet *et al.*[22])	C/T G/T C/T
ABCC2	ABCC2	rs717620 (Takahashi *et al.*[21])	C/T
ABCG2	ABCG2	rs2231137 (Angelini *et al.*[8]) rs2231142 (Angelini *et al.*[8]; Takahashi *et al.*[21])	G/A C/A
CYP1A1	CYP1A1	rs1048943 (van Erp *et al.*[14])	A/G
CYP1A2	CYP1A2	rs762551 (van Erp *et al.*[14])	A/C
CYP3A4	CYP3A4	rs2740574 (Angelini *et al.*[8])	A/G
NR1I2	NR1I2	rs3814055 (van Erp *et al.*[14]) rs1054191 (van Erp *et al.*[14])	C/T G/A
NR1I3	NR1I3	rs2307424 (van der Veldt *et al.*[5]; van Erp *et al.*[14]) rs2307418 (van der Veldt *et al.*[5]; van Erp *et al.*[14]) rs4073054 (van der Veldt *et al.*[5]; van Erp *et al.*[14])	C/T A/C G/T
POR	POR	rs1057868 (de Jonge *et al.*[23])	C/T
SLC1B3	OATP1B3	rs4149117 (Angelini *et al.*[8])	G/T
SLC22A1	hOCT1	rs628031 (Maffioli *et al.*[20];Takahashi *et al.*[21]) rs683369 (Angelini *et al.*[8]; Takahashi *et al.*[21]) rs6935207 (Maffioli *et al.*[20])	G/A C/G G/A
SLC22A4	OCTN1	rs1050152 (Angelini *et al.*[8])	C/T
SLC22A5	OCTN2	rs2631367 (Angelini *et al.*[8]) rs2631370 (Angelini *et al.*[8]) rs2631372 (Angelini *et al.*[8])	C/G T/C C/G

Selected polymorphisms within the pharmacodynamic and pharmacokinetic pathway of sunitinib

DNA isolation and genotyping were performed at the department of Clinical Pharmacy and Toxicology, Leiden University Medical Center. DNA was isolated from serum or whole blood using Magna Pure compact (Roche, Almere, The Netherlands), or from tumor surrounding tissue using Maxwell (Promega, Leiden, The Netherlands). DNA isolated from serum or tissue was pre-amplified as described before.[6]

SNPs were determined using the QuantStudio 12K Real-Time PCR System (Life Technologies, Bleiswijk, the Netherlands), with custom designed arrays. Custom designed pyrosequencing assays were used to enhance the call-rate above 90%. The mean genotype call-rate was 98.6% with a lowest call-rate of 93.2% and highest call rate of 100%. The allele frequencies of seven out of 49 SNPs were not in Hardy Weinberg equilibrium, but frequencies were comparable to the frequencies reported in the National Center for Biotechnology Information (NCBI) website (www.ncbi.nlm.nih.gov) and all SNPs were therefore kept within the analysis.

SNPs within a gene were tested for linkage disequilibrium (LD) using Haploview (Broad Institute). Haplotypes were estimated for polymorphisms with an LD of more than 95%. The maximum likelihood estimates of haplotype probabilities were calculated using PLINK software, version 1.7 (http://pngu.mgh.harvard.edu/purcell/plink/). Haplotype probabilities with a likelihood ≥ 95% were included in the statistical analysis. Haplotypes were formed from SNPs in *NR1I3* (rs2307418, rs2307424, rs4073054), *PDGFRA1* (rs1800810, rs1800812, rs1800813), *PDGFRA2* (rs2228230, rs35597368), IL8 (rs1126647, rs4073), *SLC22A5* (rs2631367, rs2631370, rs2631372), *VEGFA* (rs2010963, rs699947, rs833061), *IL4R* (rs1801275, rs1805015). Separate statistical analyses were performed for the SNPs and the haplotypes. In case a haplotype contained a certain SNP that was significant, the analysis of the SNP was dropped.

Statistics

PFS was defined as the time between the first day of sunitinib treatment, and the day of progressive disease (PD), or death due to PD, whatever came first. If PD had not occurred in a patient, or in those cases where a patient was lost to follow up, the patient was censored at the day of last follow up. OS was defined as the time between the first day of sunitinib treatment and the date of death. Patients who had not died or of whom that was unknown were censored at the last day of follow up.

All SNPs and haplotypes were univariately tested against PFS and OS using the Kaplan-Meier method with the log-rank test. Patient characteristics were also univariately tested against PFS and OS, using either the Kaplan-Meier method with the log-rank test, or Cox regression analysis, based on the type of data. Variables and SNPs or haplotypes with a P-value$\leqslant$0.10 in the univariate analysis were selected for inclusion

in a multivariate Cox regression analysis, using PFS and OS as dependent variables. For SNPs, the best fitted model (multiplicative, wild-type dominant or mutant dominant based on genotype distribution) was chosen to enter into the multivariate analysis, based on the univariate analyses. Missing data from baseline characteristics that were associated with PFS or OS in the univariate analysis, were randomly imputed before entering the variable in the multivariate regression model. Depending on the variable, 1-40% of data was imputed. Multivariate analysis were performed twice, with and without replacement of missing variables. If results were similar in size and direction of effect, replacement was considered legitimate.

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 17.0 (SPSS, Chicago, IL, USA). Given the explorative nature of this study, all results from multivariate analysis with P-value $\leqslant 0.05$ were considered statistically significant and no correction for multiple testing was performed.

Results

Study population

The study population consisted of 127 patients with GIST treated with sunitinib, of whom 63% were men. The mean age at start of sunitinib was 61.2 ± 13.4 year. The stomach was the most frequent site of primary GIST location (38%). In 14 patients (11%) a *c-KIT* exon 9 mutation was found, and 58 patients (46%) had a tumor with an exon 11 mutation in *c-KIT* in the primary tumor. Other mutations were found in *c-KIT* exon 13 (*n*= 2), exon 14 (*n*= 1), exon 17 (*n*= 2) or in *PDGFR* exon 18 (*n*= 7). In 43 patients (33.8%) the mutation in the primary tumor was unknown. Most patients (76%) received sunitinib in an intermittent dosing scheme, starting sunitinib with 50 mg a day (*n*= 91, 72%) during the first 4 weeks, continued by 2 weeks off-dosing.

At the time of analysis, 110 patients had stopped sunitinib treatment. In 87 patients (85%), this was because of PD and in all other cases because of severe toxicity. In the entire population, the median PFS was 7.6 months (interquartile range 3.1-17.0 months) and the median OS was 18.3 months (interquartile range 9.7-29.3 months). The baseline characteristics of the study population are presented in Table 2.

Table 2: Baseline characteristics

Variable	N (%) or mean (sd)
Gender	
Male	80 (63)
Female	47 (37)
Age at start sunitinib (years)	61.2 (13.4)
Hospital	
LUMC	60 (47)
EMC	43 (34)
NKI	18 (14)
UMCG	6 (5)
Primary location tumor	
Stomach	48 (38)
Small bowel	36 (28)
Colon	7 (5)
Rectum	6 (5)
Unknown	30 (24)
Histology of primary tumor	
Spindle cell	70 (55)
Epitheloid	12 (9)
Mixed	21 (17)
Unknown	24 (19)
Mutation	
Exon 9	14 (11)
Exon 11	58 (46)
other mutation or wild type	32 (25)
Unknown	21 (16)
WHO PS at start sunitinib	
0-1	98 (77)
2-3	11 (9)
Unknown	18 (14)
Type of sunitinib treatment	
Intermittent	97 (76)
Continuous	28 (22)
Unknown	2 (2)
Dose of sunitinib at start treatment	
12.5 mg	1 (1)
25 mg	5 (4)
37.5 mg	28 (21)
50 mg	91 (72)
unknown	3 (2)
Reason to stop sunitinib	
Progressive disease	87 (69)
Toxicity	23 (18)
Continued treatment	17 (13)

Baseline characteristics. BSA: body surface area, LUMC: Leiden University Medical Center, EMC: Erasmus MC Cancer Institute, NKI: Netherlands Cancer Institute, UMCG: University Medical Center Groningen, WHO PS: World Health Organization Performance Score

Pharmacogenetic biomarkers for PFS

In the univariate analysis, PFS was longer for patients with the presence of the T-allele in *KDR* rs1870377 T/A (p= 0.033), the presence of the G-allele in *IL13* rs20451 G/A (p= 0.025), the presence of the C-allele in *VEGFA* rs25648 T/C (p= 0.014), and in the absence of two GCT copies in the *VEGFA* haplotype (p= 0.042) in the pharmacodynamic genes. With respect to the pharmacokinetic SNPs that were tested, the presence of the homozygous TT- allele in *POR* rs1057868 C/T (p= 0.008), and the absence of two CCC-copies in the *SLC22A5* haplotype (p= 0.007) were univariately associated with prolonged PFS. From the baseline characteristics length (per cm increase HR 1.028; 95% confidence interval (CI): 1.002-1.055, p= 0.032), mitotic index of the primary tumor (per unit increase HR 1.006, 95% CI: 1.000-1.012, p= 0.042), age at start of sunitinib (per year increase HR 0.986; 95% CI: 0.972-0.999, p= 0.037) and the reason to stop imatinib (PD 13.7 months, other than PD 29.9 months; p= 0.01) were included in the multivariate analysis.

Only the homozygous TT genotype in *POR* rs1057868 C/T (HR 0.232, 95% CI: 0.078-0.686, p= 0.008) was associated with PFS in the multivariate Cox regression analysis (Table 3). A trend toward shorter PFS was seen for the presence of 2 copies of the CCC *SLC22A5* haplotype, compared with 1 or 0 copies (HR 2.358, 95% CI: 0.978-5.684, p= 0.056).

Table 3: Univariate and multivariate analysis of progression free survival in patients with GIST treated with sunitinib (Continued on next page)

Factors		**Univariate analysis***			**Multivariate analysis****		
	No.	*Mean PFS (months)*	*95% CI*	*p value*	*HR*	*95% CI*	*p value*
Clinical factors							
Reason to stop imatinib				0.10			0.238
Progressive disease	102	13.7	11.3 - 16.1		1.565	0.744 - 3.929	
Other	23	29.9	14.9 - 45.0		1		
Length (HR 1.028)	96		1.002-1.055	0.032	1.008	0.994 - 1.007	0.582
Mitotic index (HR 1.006)	76		1.000-1.012	0.042	1.001	0.994 - 1.007	0.804
Age at start sunitinib (HR 0.986)	125		0.972-0.999	0.037	0.990	0.974 - 1.007	0.240
Genetic factors pharmacodynamic pathway							
KDR rs1870377				0.033			0.423
TT & TA	114	17.9	13.6 - 22.2		0.696	0.286 - 1.691	
vs AA	9	8.1	1.9 - 14.2		1		

Table 3: Continued

Factors		Univariate analysis*			Multivariate analysis**		
IL13 rs20541				0.025			0.756
GG & GA	113	18.0	13.7 - 22.3		0.870	0.362 - 2.090	
vs AA	11	8.0	4.8 - 11.3		1		
VEGFA rs25648				0.014			0.347
CC & CT	117	17.7	13.5 - 21.8		0.626	0.236 - 1.661	
vs TT	8	7.0	2.5 - 11.4		1		
VEGFA GCT-haplotype				0.042			0.081
GCT-GCT *vs*	1	3.0	3.0 - 3.0		6.488	0.793 - 53.06	
GCT-other & other-other	116	16.5	12.8 - 20.3		1		
Genetic factors pharmacokinetic pathway							
POR rs1057868				0.001			0.008
TT	9	46.5	17.6 - 75.4		0.232	0.087 - 0.686	
CC & CT	115	14.5	11.8 - 17.2		1		
SLC22A5 CCC-haplotype				0.007			0.056
CCC-CCC *vs*	15	7.7	4.3 - 11.1		2.358	0.987 - 5.684	
CCC-other or other-other	105	18.5	14.1 - 23.0		1		

Univariate and multivariate analysis of progression free survival in patients with GIST treated with sunitinib. 95% CI: 95% confidence interval

*Only factors with P-value < 0.10 level are presented; these were selected for multivariate analysis. PFS: progression free survival

**Hazard ratio. HR < 1 indicates that the factor is associated with improved PFS, HR > 1 indicated that the factor is associated with worse PFS.

Pharmacogenetic biomarkers for OS

In the univariate analysis two pharmacodynamic SNPs within *VEGFA* were predictive for longer OS (rs1570360 G/A, absence of the A allele; p= 0.005 and rs699947 C/A, presence of the C-allele; p= 0.036), as well as the presence of a CGG-copy in the *PDGFRA1* haplotype (p= 0.007) and the presence of the GC-other or other-other alleles in the *IL4R* haplotype (p= 0.008). Within the pharmacokinetic pathway, the presence of the C-allele in *ABCC2* rs717620 C/T (p= 0.006), as well as presence of the T-allele in *SLCO1B3* rs4149117 G/T (p= 0.054). Two haplotypes within the pharmacokinetic pathway were associated with longer OS: the absence of two CTT-copies in NR1I3 (Po0.0001) and the absence of two CCC-copies in *SLC22A5* (p= 0.001).

From the baseline characteristics that were univariately tested against OS, a better survival was seen in patients who stopped imatinib for another reason than PD (PD 25.8 months OS, other than PD 55.4 months OS, p= 0.001), the absence of liver metastasis at start of sunitinib (44.2 vs 27.4 months, p= 0.093), and the absence of metastases at the time of diagnosis (37.6 vs 25.8 months OS, p= 0.025). Multivariate Cox regression analysis

showed *SLCO1B3* rs4149117 G/T, the absence of a T-allele (HR 2.024, 95% CI: 1.013-4.044, p= 0.046), the presence of two copies of the CCC *SLC22A5* haplotype (HR 2.603, 95% CI: 1.216-5.573, p= 0.014), and the presence of two copies of the GC *IL4R* haplotype (HR 7.131, 95% CI: 1.518-33.496, p= 0.013) as predictors for OS, as well as PD as a reason to stop imatinib (HR 3.025, 95% CI: 1.358-6.742, p= 0.007) and the presence of metastases at the time of the primary diagnosis GIST (HR 1.773, 95% CI: 1.044-3.012, p= 0.034). Data are presented in Table 4.

Table 4: Univariate and multivariate analysis of overall survival in patients with GIST treated with sunitinib (continued on next page)

Factors		**Univariate analysis***			**Multivariate analysis****		
	No	Mean OS (months)	95% CI	p value	HR	95% CI	p value
Clinical factors							
Reason to stop imatinib				0.001			0.007
Progressive disease	102	25.8	21.8 - 29.8		3.025	1.358 - 6.742	
Other	24	55.4	37.5 - 73.3		1		
Metastasis at time of diagnosis				0.025			0.034
No	66	37.6	28.8 - 46.4		1		
Yes	59	25.8	19.5 - 32.2		1.773	1.044 - 3.012	
Liver metastasis at start sunitinib				0.093			0.127
No	37	44.2	28.1 - 30.3		1		
Yes	86	27.4	23.2 - 31.6		0.660	0.315 - 1.155	
Genetic factors pharmacodynamic pathway							
VEGFA rs1570360				0.005			0.128
GG *vs*	66	38.9	29.6 - 48.2		0.654	0.378 - 1.130	
GA & AA	58	22.0	18.1 - 25.9		1		
VEGFA rs699947				0.036			0.390
CC & CA	94	35.8	28.6 - 43.0		0.775	0.398 - 1.433	
vs AA	28	21.6	17.6 - 25.5		1		
PDGFRA CGG-haplotype				0.007			0.066
CGG-CGG & CGG-other	120	33.1	27.1 - 39.1		0.189	0.085 - 0.418	
vs other-other	6	13.7	6.6 - 20.7		1		
IL4R GC-haplotype				0.008			0.013
GC-GC *vs*	4	8.2	2.0 - 14.5		7.131	1.518 - 33.50	
GC-other & other-other	117	32.8	26.7 - 38.8		1		

Table 4: (continued)

Factors		Univariate analysis*			Multivariate analysis**		
Genetic factors pharmacokinetic pathway							
ABCC2 rs717620				0.006			0.168
CC & CT	121	32.7	26.8 - 38.6		0.248	0.090 - 0.682	
vs TT	5	10.2	8.5 - 11.8		1		
SLCO1B3 rs4149117				0.054			0.046
GG *vs*	97	28.1	23.3 - 32.9		2.024	1.013 - 4.044	
GT & TT	23	47.9	28.5 - 67.2		1		
NR1I3 CTT-haplotype				<0.001			0.062
CTT-CTT *vs*	4	9.1	3.1 - 15.0		4.599	0.927 - 22.81	
CTT-other & other-other	122	33.0	27.0 - 38.9		1		
SLC22A5 CCC-haplotype				0.001			0.014
CCC-CCC *vs*	14	15.6	10.5 - 20.8		2.603	1.216 - 5.573	
CCC-other & other-other	107	34.9	28.4 - 41.5		1		

Univariate and multivariate analysis of overall survival in patients with GIST treated with sunitinib. 95% CI: 95% confidence interval

*Only factors with P-value < 0.10 level are presented; these were selected for multivariate analysis. OS: overall survival

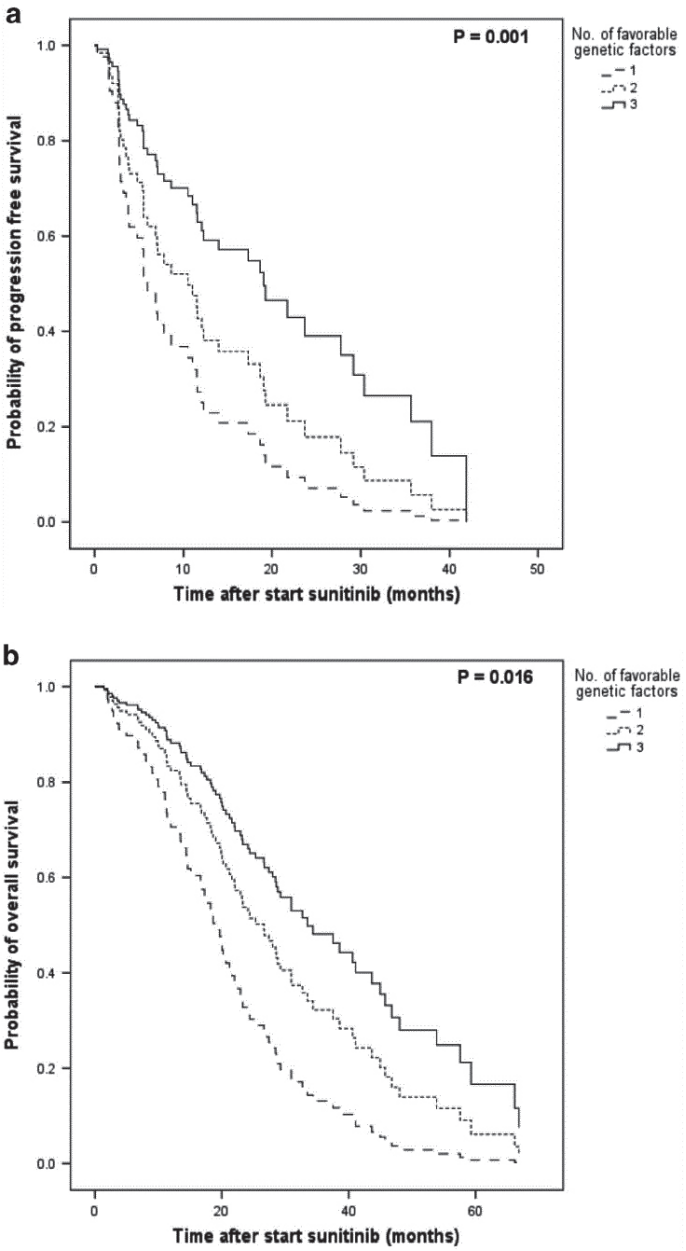
**Hazard ratio. HR < 1 indicates that the factor is associated with improved PFS, HR > 1 indicated that the factor is associated with worse PFS.

Favorable genetic profile

Polymorphisms and haplotypes that were significantly associated with OS (*SLCO1B3* rs4149117 G/T, the presence of the T-allele, the absence of a CCC-copy in the *SLC22A5* haplotype and the absence of a GC-copy in the *IL4R* haplotype) were combined in a favorable genetic profile for PFS and OS, using the number of favorable genetic factors. The number of favorable genetic factors was significantly associated with longer survival (PFS 9.2 vs 15.6 vs 28.4 months for respectively one, two or three favorable genetic factors, p= 0.005). There was only one patient with no favorable genetic factors in this population. In a multivariate regression model including the clinical factors (reason to stop imatinib, length and mitotic index of the primary tumor), this was confirmed (HR 0.654, 95% CI 0.512-0.836, p= 0.001, Figure 1a).

OS was significantly longer with an increasing number of positive predicting genetic factors (mean OS 16.0 vs 31.5 vs 49.5 months for respectively one, two or three positive predictive genetic factors, p= 0.001). This was confirmed in a multivariate regression analysis, including the amount of favorable genetic factors and the clinical factors reason to stop imatinib, metastasis at primary diagnosis and liver metastasis at the start of sunitinib (HR 0.359, 95% CI 0.156-0.826, p= 0.016, Figure 1b).

Figure 1: Progression free survival (a) and overall survival (b) in patients with GIST treated with sunitinib being carriers of one, two or three favorable genetic variations.



Discussion

Patients with GIST treated with sunitinib have a large inter-patient difference in PFS and OS. This may in part be explained by various tumor cell-related factors such as secondary mutations and by some clinical factors.[4] However, genetic polymorphisms within the pharmacokinetic and pharmacodynamic pathways may add to this, as the exposure to and the efficacy of the drug is affected, and thereby influence the outcome of treatment as well. In this explorative study in a population of 127 patients with GIST, it was shown that polymorphisms in both the pharmacokinetic (*SLCO1B3*, *SLC22A5* and *POR*) and the pharmacodynamic (*IL4R*) pathway of sunitinib are associated with PFS and OS in patients with advanced GIST treated with sunitinib.

These findings indirectly suggest that survival to sunitinib in patients with GIST is subjected to exposure to sunitinib and its active metabolite. Sunitinib is metabolized by CYP3A4 and CYP3A5 into its active metabolite SU12662. This is converted to several inactive compounds by the same enzymes. The activity of cytochrome P450-enzymes is regulated by P450 oxydoreductase (POR). In this study, rs1056878, otherwise known as *POR**28, was associated with prolonged PFS in sunitinib treated patients with GIST. Rs1056878 encodes for the amino acid variant A503V, and has been associated with lower activity of CYP1A2, CYP2D6, CYP3A5, but not of CYP3A4.[7] The finding that the polymorphic variant of rs1056878 is associated with better PFS suggests that carriers of this variant have a lower activity of metabolizing enzymes resulting in higher plasma concentrations.

Sunitinib is a substrate of the ATP-binding cassette ABCB1 and ABCG2 efflux transporters, playing a role in both uptake and efflux of sunitinib. However, none of the SNPs in these genes were associated with survival in this analysis. The precise role of members of the organic cation transporter novel (OCTN) family and the organic anion-transporting peptide (OATP) family in sunitinib absorption and elimination is unclear. However, SNPs in *SLC22A5*, which is the gene encoding for OCTN2, have been found to be associated with survival to imatinib in patients with GIST and CML.[8] Interestingly, the *SLC22A5* haplotype, consisting of rs2631367, rs2631370 and rs2631372, was found to be significantly associated with longer OS. Carriers of the two CCC-copies had significantly shorter OS than patients with other allelic combinations. This is consistent with the finding in imatinib treated patients with GIST.[8] Other members of the OCTN family that were tested in this study did not show a significant association with PFS or OS. In *SLCO1B3*, which encodes OATP1B3, rs4149117 was also associated with prolonged OS. Possibly, sunitinib is a substrate of these efflux transporters as well, but this needs to be elucidated.

The homozygous GC-copy in the *IL4R* haplotype consisting of rs1801275, rs1805015 (Ser478Pro and Gln551Arg) was significantly associated with longer OS. In a previous study, SNPs in *IL4R* have been associated with the development of renal cell carcinoma.[9] The finding that SNPs within *IL4R* are associated with OS in patients with GIST treated with sunitinib may be related to IL4R being involved in the tumor biology of GIST as well. A limitation of this study is that no pharmacokinetics of sunitinib as an intermediate endpoint were measured in this group of patients. Therefore, it can only be assumed that the effects of the SNPs on survival is caused by differences in pharmacokinetics. In a recent pharmacogenetic-pharmacokinetic study, *CYP3A4*22* was found to have an effect size of >20% on clearance.[10] However, this finding was not statistically significant.

Another limitation of this study is the sample size. Although this is the largest pharmacogenetic study in patients with GIST treated with sunitinib so far, the number of patients with specific genotypes is too small to draw conclusions from. Since this was an exploratory study, no formal correction for multiple testing was performed and results from the multivariate analyses with a p-value less than 0.05 were considered significant. Currently, the false discovery rate is frequently used to control for reporting false positives in exploratory studies. Therefore, false discovery rate values were calculated for each separate endpoint in a post hoc analysis. False discovery rate was below 10% for all SNPs with $P< 0.05$ indicating a low likelihood of false positive findings.

In this current study, SNPs that were found associated with prolonged PFS, were not associated with OS and *vice versa*. This is somewhat surprising, since PFS and OS can be expected to be related to each other. However, while PFS only includes the effects of sunitinib treatment, OS also embodies the effects of any subsequent lines of treatment. Patients in this study received sunitinib over a broad area of time. In the first years after the registration of sunitinib, no good third line of treatment was available, but patients were frequently offered other treatment in the context of clinical studies. Since recently, regorafenib has been approved for third line treatment of GIST after failure of imatinib and sunitinib.[11] This may have caused a bias in the OS in this analysis, as most patients did not receive this drug during earlier years. Still, it was shown in a large group of patients that genetic polymorphisms can serve as a biomarker for OS. In one of this previous studies; studying polymorphisms associated with survival in RCC, a favorable genetic profile was found, including mutations in *CYP3A5, NR1I3,* and *ABCB1*.[5] The only reason for the discrepancy with the current findings is the tumor type (GIST *versus* RCC). Progressive disease as the reason to stop imatinib treatment was univariately associated with both worsened PFS and worsened OS in this current study. In the multivariate analysis this was only confirmed for OS, but not for PFS. The existence of metastases at the time of the primary diagnosis was also associated with worse OS. Possibly, the tumor has a more aggressive behavior when metastasis are present at first diagnosis and when

the tumor has already progressed on imatinib, rather than the patient switched to sunitinib for other reasons, resulting in shorter OS.

Previously it has been described that primary mutations in *c-KIT* and *PDGFRA* may be predicting for the survival obtained by sunitinib in patients with GIST. This was not seen in this study. This may be explained by the fact that all patients were pre-treated with imatinib. It has been shown that during the treatment with imatinib, secondary mutations may arise, leading to imatinib-resistance.[4] Therefore, mutations that are found in the primary tumor may not be representative of the mutations within the tumor after treatment with imatinib. Moreover, not in all tumor samples mutations in *c-KIT* and *PDGFRA* were determined. A lack of correlation between *c-KIT* and *PDGFRA* in univariate analysis may be (partly) due to missing data.

Altogether it may be concluded that polymorphisms in genes encoding for proteins related to the pharmacokinetic and pharmacodynamic pathways of sunitinib may be associated with survival in patients with GIST treated with sunitinib. When validated in the future, this may be useful to predict which patient is going to respond to sunitinib therapy, and which patients may better respond to other treatment types.

Funding

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Conflict of interest

J. Swen, H. Gelderblom and H.-J. Guchelaar have an unrestricted grant from Pfizer regarding pharmacogenetic research in patients treated with sunitinib.

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5

Genetic polymorphisms in *ABCG2* and *CYP1A2* are associated with imatinib dose reduction in patients treated for gastro-intestinal stromal tumors

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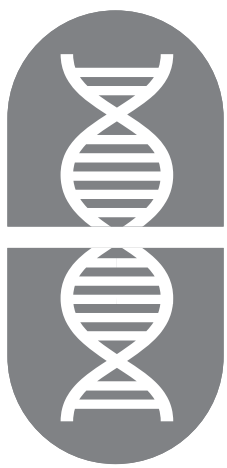

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Abstract

Imatinib has a mild toxicity profile, although severe adverse events may develop. In this pharmacogenetic pathway analysis the need for dose reduction and cessation of therapy was tested for an association with single nucleotide polymorphisms (SNPs) in genes related to imatinib pharmacology. Retrospective data from 315 patients with a gastrointestinal stromal tumor who received imatinib 400mg o.d. was associated with 36 SNPs. SNPs that showed a trend in univariate testing were tested in a multivariate model with clinical factors and correction for multiple testing was performed. Dose reduction was associated with carriership of the A-allele in rs2231137 in *ABCG2* (OR 7.35, p=0.0002) and two C-alleles in rs762551 in *CYP1A2* (OR 7.12, p=0.001). Results remained significant after correction for multiple testing. Therapy cessation did not show an association with any of the tested SNPs. These results may help identifying patients at increased risk for toxicity who could benefit from intensified follow-up.

Introduction

Imatinib mesylate (Glivec®, Novartis, Switzerland) is a tyrosine kinase inhibitor (TKI) which primarily blocks the Bcr-Abl protein and the KIT receptor in the treatment of chronic myeloid leukemia (CML) and gastrointestinal stromal tumors (GIST), respectively.[1,2] Imatinib offers a significant survival benefit in these malignancies and is considered first line therapy. Despite being a selective TKI, it confer a broad range of toxicities, albeit less than conventional cytostatic agents.[3] These adverse effects range from mild and amendable symptoms, to rare but fatal hepatitis.[4]

The incidence of imatinib adverse events has been associated with a range of clinical factors. In a large collaborative effort, Van Glabbeke et al. found associations of sex, age and performance score with the incidence of several imatinib-induced, non-hematological adverse events such as fatigue, nausea, diarrhea and edema.[5] In addition to clinical factors, germline genetic polymorphisms have also been shown to be associated with TKI-induced toxicity.[6-8] Single nucleotide polymorphisms (SNPs) are the most prevalent genetic polymorphisms and known to potentially alter protein function. Therefore, SNPs in genes involved in the pharmacokinetic and pharmacodynamic pathways of imatinib may affect its toxicity.[9] Our group identified SNPs that may predict for worse progression free survival in GIST patients who received imatinib 400 mg once daily.[10] If SNPs are also associated with imatinib related toxicity, patients at risk for toxicity may be identified at the onset of treatment and serious adverse effects may possibly be avoided by starting with a reduced dose of imatinib.

This study aims to explore genetic variants in genes involved in the pharmacokinetics and pharmacodynamics of imatinib for an association with treatment-restricting toxicity.

Methods

Patients and DNA samples

Patients from four Dutch referral centers (Erasmus MC Cancer Institute, Leiden University Medical Center, Antoni van Leeuwenhoek - Netherlands Cancer Institute and University Medical Center Groningen) were recruited. Patients were included to the same standards, being the documented use of imatinib for GIST and the availability of a DNA sample, without further selection criteria. All patients were treated with imatinib with a standard starting dose of 400 mg once daily. Therapy was given for neoadjuvant, adjuvant, or palliative indications. The observation period lasted from January 2001 to July 2014. Clinical and toxicity data were collected from patient files. The decision for dose reduction or cessation of treatment was made upon the treating physician's

discretion. The level of toxicities were scored according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0.

DNA was obtained from residual blood samples that were collected for routine patient care and stored at -20°C. In the Antoni van Leeuwenhoek - Netherlands Cancer Institute only serum samples were stored, and in the Erasmus MC Cancer Institute informed consent was signed by patients. If a residual blood or serum sample was not available, DNA was obtained from residual pathology specimens. All samples were anonymized by a third party and the Code for Proper Secondary Use of Human Tissue was applicable and was adhered to (www.federa.org/codes-conduct).[11]

The etiologic (KIT) mutation was not determined, because in contrast to the evident effect of these mutations in GIST on survival, such an effect is not probable for toxicity. Etiologic mutations will not affect imatinib pharmacokinetics, and toxicity is a result of imatinib interaction with healthy cells, which do not carry these mutations.

SNP selection

Using the candidate gene pathway approach, a review of literature was performed to identify SNPs in genes encoding for imatinib metabolizing enzymes and targets.[12] For selection, Haploview and HapMap data (release 28) was used to find SNPs in linkage disequilibrium (LD, >95%), and only one SNP was selected if multiple were in high LD. The National Institute of Environmental Health Sciences database was used to select the SNPs with an expected functional change, and SNPs were required to have a minor allele frequency of at least 0.1 for inclusion.[10] A total of 36 SNPs in 18 genes were selected (see Supplementary Table S1).

Genotyping

DNA was isolated from blood (270 patients), serum (32 patients) or FFPE samples (13 patients) using the MagnaPure Compact (Roche Diagnostics, Almere, the Netherlands). To enhance genotyping results, DNA isolated from serum and FFPE samples was pre-amplified using real-time PCR genotyping assays.[13] Using the QuantStudio™ 12K Flex Real-time PCR system (Life Technologies, Bleiswijk, the Netherlands) with a custom-made array, DNA was genotyped according to the manufacturer's protocol. A number of SNPs were additionally genotyped with real-time PCR genotyping assays (Life Technologies, Bleiswijk, the Netherlands) according to the manufacturer's protocol or in house developed Pyrosequencing assays (Qiagen, Venlo, the Netherlands) in order to achieve a satisfactory call rate for all SNPs (>90%).

All 36 SNPs had a call rate of >90%, 33 of which had a call rate of >95%. Out of the 36 SNPs, 32 were in Hardy-Weinberg Equilibrium (HWE). For the remaining 4 SNPs, the relative low minor allele frequency was deemed to be the cause for the deviation. The minor allele frequencies were in accordance to those reported in the NCBI database. Haploview 4.2[14] and Plink 1.7[15] were used to explore haplotypes in the study population. SNPs in the same gene were considered to be in a haplotype in case D' was at least 95%. Only patients with a ≥95% probability of the assigned allele were included in the analyses.

Statistics

Two co-primary endpoints were deemed clinically relevant: the need for dose reduction and the need for therapy cessation due to toxicity. Toxicity was *a-priori* considered to be related to the clinical variables age, sex, and WHO performance score, based on clinical experience and literature.[5] The endpoints were first tested for associations with clinical variables using the Students's t-test or chi-square test, depending on the variable. To univariately test for associations with SNPs and haplotypes the chi-square test was also used. The general genetic model was used for the genetic variables, unless the paucity of the homozygote variant necessitated otherwise. If this test showed a trend for an association, with $p< 0.1$, it was selected for inclusion as covariate in the multivariate analysis. The multivariate analysis used logistic regression and included the clinical variables and the selected genotypes, the latter as a categorical variable. A single SNP or haplotype was added to these variables. To account the 36 SNPSs tested, a correction for multiple testing was performed. A p-value in the multivariate analysis was considered significant when it was lower than 0.00139 (that is 0.05 divided by 36, the number of tested SNPs). SPSS version 20 (IBM Corp., Armonk, NY, USA) was used.

Results

Study population

A total of 315 patients were included in the study, see Table 1 for the baseline characteristics.

Table 1: baseline characteristics of study population

	median	**range**
Age at start imatinib		
in years	62.2	17.9 - 92.6
	number	%
Sex		
male	200	63.5
female	115	36.5
WHO performance score		
0-1	279	88.6
2-3	10	3.2
unknown	26	8.3
Metastases at diagnosis		
no	225	71.4
yes	89	28.3
unknown	1	0.3
Previous surgery for GIST		
no	123	39.0
yes	192	61.0
Indication for imatinib		
neo-adjuvant	63	20.0
adjuvant	38	12.1
palliative	213	67.6
unknown	1	0.3

Baseline characteristics of study population, in which all patients received imatinib in a starting dose of 400 mg.

In 32 patients (10.2%) a dose reduction due to toxicity was performed, and 28 patients (8.9%) ceased imatinib treatment due toxicity, see Table 2. Only 5 patients had dose reductions prior to ceasing imatinib entirely due to toxicity. The final imatinib dose was 200 mg in 12 patients, and 300 mg in 14, whereas in 6 patients the dose was later escalated to 400 and 800 mg (in between dosing not recorded). The time between start of imatinib and dose reduction was a median 3.1 months (range 0.7 to 68.8 months), and the majority of dose reductions occurred early in treatment (see Supplementary Figure S2). In case of subsequent dose escalation, it followed in median 2.9 months (range 1.2 to 22.4 months).

Table 2: Incidence of imatinib toxicity

	number	%
Dose reduction	32	10.2
Cessation of therapy due to toxicity	28	8.9
Final dose of imatinib		
200 mg	12	37.5
300 mg	14	43.8
400 mg	4	12.5
800 mg	2	6.3
Highest toxicity, any	298	94.6
grade 1	206	65.4
grade 2	68	21.6
grade 3	20	6.3
grade 4	4	1.3
Toxicity absent	17	5.4

Almost all patients (N=298, 94.6%) suffered from at least one adverse event, 92 patients (29.2%) had at least a grade 2 toxicity, and 24 patients had a grade 3 toxicity or higher (7.6%). Just 20 patients (6.3%) had a grade 3 toxicity. Only 4 patients (1.3%) had a grade 4 adverse event. None of the patients died as a direct result of imatinib toxicity. The toxicities to cause cessation of treatment were diverse in nature and in some patients it was a combination of several adverse events (see Supplementary Table S3).

Dose reduction needed

Increased age was associated with the need for dose reduction in the multivariate analysis (OR 1.05 per year, p=0.015), and sex and WHO performance score were not, as shown in Table 3. Carriers of the A allele in rs2231137 in *ABCG2* had higher chance of dose reduction (34.8%) compared to wildtype patients (8.4%) and this showed a significant difference in both univariate and multivariate analysis (OR 7.35, p=0.0002). Two C alleles in rs762551 in *CYP1A2* conferred a higher chance of dose reduction compared to CA and AA genotypes (28.6% *vs* 10.3% and 6.3%, respectively). This was also found to be a statistically significant difference in the multivariate tests (OR 7.12, p=0.0010). For homozygous carriers of the T allele in rs28656907 in *ABCB1* an association was found (OR 0.19, p=0.040) and patients with this genotype had a lower change of needing a

dose reduction. However, this result failed to match the significance level set by the correction for multiple testing.

Table 3: Multivariate logistic regression analyses of toxicity

Dose reduction needed		**N event (%)**	***p* value**	**OR**	**95% CI**	***p* value**
Age			0.004	1.05	1.01 - 1.08	0.015
Sex	male	16 (8.0)	0.094	1		
	female	16 (13.9)		1.87	0.86 - 4.04	0.114
WHO score	0-1	28 (10.0)	0.278	1		
	2-3	2 (20.0)		1.57	0.30 - 8.28	0.595
rs2231137 *(ABCG2)*	GG *vs*	23 (8.4)	<0.001	1		
	GA + AA	8 (34.8)		7.35	2.55 - 21.2	*0.0002
rs762551 *(CYP1A2)*	AA *vs*	16 (10.3)	0.002	1		
	AC	8 (6.3)		0.81	0.32 - 2.07	0.657
	vs CC	8 (28.6)		7.12	2.21 - 22.9	*0.001
rs28656907 (ABCB1)	CC *vs*	11 (13.9)	0.079	1		
	CT	14 (9.7)		0.67	0.27 - 1.66	0.385
	vs TT	2 (3.0)		0.19	0.04 - 0.93	0.040
Cessation of therapy due to toxicity		**N event (%)**	***p* value**	**OR**	**95% CI**	***p* value**
Age			0.079	1.03	0.99 - 1.06	0.163
Sex	male	14 (7.0)	0.120	1		
	female	14 (12.2)		1.88	0.81 - 4.37	0.143
WHO score	0-1	23 (8.2)	0.843	1		
	2-3	1 (10.0)		0.95	0.11 - 8.10	0.961
rs2631370 *(SLC22A5)*	TT *vs*	6 (4.9)	0.068	1		
	TC	19 (12.3)		2.07	0.76 - 5.62	0.153
	vs CC	2 (5.3)		1.01	0.19 - 5.36	0.988
rs1045642 *(ABCB1)*	CC *vs*	9 (10.5)	0.003	1		
	CT	6 (3.9)		0.40	0.12 - 1.33	0.136
	vs TT	13 (17.3)		1.98	0.72 - 5.41	0.184
rs1050152 (*SLC22A4)*	CC *vs*	11 (10.2)	0.095	1		
	CT	16 (10.9)		0.95	0.39 - 2.32	0.919
	vs TT	1 (1.7)		0.15	0.02 - 1.25	0.080

Multivariate logistic regression analyses of toxicity during imatinib treatment, OR= odds ratio, 95% CI= 95% confidence interval, * these results remained significant after statistical correction for the number of tested SNPs. Multivariate results are reported for the base model with clinical variables characteristics as covariates without inclusion of SNPs. Genetic variables results are presented for the singular SNP or haplotype added to the base model.

Cessation of therapy due to toxicity

Ceasing imatinib therapy due to toxicity was not associated with age, sex or WHO performance score. SNPs in *SLC22A5, ABCB1* and *SLC22A4* only showed an association in the univariate analysis, but these associations did not remain significant in the multivariate analysis.

Discussion

This exploratory pharmacogenetic study on the toxicity of imatinib 400 mg once daily has found an association of rs2231137 in *ABCG2* and rs762551 in *CYP1A2* with the need for a dose reduction. To the best of our knowledge, this is the largest pharmacogenetic study to explore imatinib toxicity, and the results remained statistically significant after correction for the number of tested SNPs.

This study used the need for dose reduction and cessation of therapy due to toxicity as primary endpoints as they were deemed to be clinically relevant endpoints for a drug that has a relatively mild toxicity profile. Although toxicity can be debilitating, non-hematological toxicity can be treated with other drugs and hematological toxicity is often asymptomatic and acceptable considering the need for anti-tumor therapy. A combination of several mild ailments, however, may lead to a decision to reduce the dosage, or stop treatment altogether. The treatment setting, being neo-adjuvant, adjuvant or palliative, was not associated with one of the clinical endpoints, nor with the prevalence of polymorphisms in the selected SNPs (data not shown).

A dose reduction was needed more often in older patients, in line with results of a large cohort which showed age to be associated with toxicity.[5] In large clinical trials, the need for a dose reduction has consistently been reported to be around 15% in patients receiving a dose of 400 mg daily for GIST.[16-18] The percentage of 10% found in this study is comparable and the slightly lower percentage may better reflect clinical practice, as most trial protocols dictate a mandatory dose reduction in case of certain grade of (non-)hematological toxicities. Furthermore, the percentage of patients with a poor performance score was lower than in most trials.

An association for dose reduction was found with the A-allele in rs2231137 in *ABCG2*, with 34.8% needing dose reduction *vs* 8.4% in wild type patients. The ATP-binding cassette sub-family G member 2 is encoded by the *ABCG2* gene and it functions as a cellular transmembrane transporter able to excrete xenobiotic molecules.[19] Imatinib is known to be transported through this molecule in the intestinal epithelium.[20] Associations with selected (non-)hematological adverse events and this SNP were neither found in a study with Malaysian patients, nor in Chinese patients who had a

GIST.[7,21] The A-allele has been associated with better response to imatinib in Korean patients, but a mechanism in which this SNP may lead to higher imatinib plasma levels is uncertain, as an association with imatinib steady state trough levels was not found in two cohorts of GIST patients from China and Korea.[22-24] Possibly, the minor allele frequency is too low in Asian patients to provide enough statistical power to detect a difference in serum levels. An alternative possibility may be that this SNP influences the intracellular imatinib level, instead of the serum level.

Several other studies have reported an association with rs2231142, another frequently investigated SNP in *ABCG2*, and imatinib efficacy, but it was not associated with the toxicity endpoints in that study, nor were SNPs in the genes encoding for organic cation (influx) transport proteins.[21-23] SNPs previously reported to be associated with imatinib efficacy in advanced GIST were not associated to one of the clinical endpoints.[10]

Patients with the less prevalent CC genotype in rs762551 in *CYP1A2* had a significantly higher chance of the need for a dose reduction; 28.6% had a dose reduction *vs* 10.3 and 8.3%. The CC genotype is considered to yield a slow metabolizers CYP1A2 phenotype compared to the AA genotype, and if patients with a CC genotype have a higher plasma level, it may explain for the increased need for dose reduction. Obviously, *in vivo* other enzymes in the cytochrome P450 system could compensate in case of slow acting CYP1A2, but the effect of the CC genotype is strong enough to show in the multivariate analysis. This effect has not yet been reported previously.

An association with dose reduction was found for rs28656907 in *ABCB1*, but this did not remain significant when corrected for multiple testing. This SNP has been shown to increase *ABCB1* expression.[25] This gene (also known as MDR1) encodes for the drug transporter P-glycoprotein. SNPs in this gene, such as with rs1045642, rs1128503 and rs2032582, have been studied extensively in CML patients receiving imatinib. The T-allele in rs1128503 has been shown to confer a better response in Asian patients.[26] However, association studies with imatinib toxicity have yielded mixed results.[21] One study found rs1045642 to associated with periorbital edema in a co-dominant model, but this was not tested in a multivariate analysis.[7]

Cessation of treatment due to toxicity was not associated with any of the tested SNPs. Possibly, this is due to the low frequency of events. Imatinib is a drug with a relatively mild toxicity profile. Phase I studies showed dose limiting toxicity to occur in patients taking imatinib 500 mg twice daily, whereas currently the standard dose for imatinib is much lower at 400 mg once daily, and all patients in this cohort received that dose.[27]

Specific grades of toxicity were not explored because of the retrospective character of data collection. Instead, the clinically relevant endpoints were used, that are accurately noted in patient files. By choosing these particular endpoints, any result this

study would find, was prone to be challenging in determining a molecular explanation. Therapy restricting toxicities may well be due a combination of adverse events, with each having a different molecular pathway. Although the etiologic mutations for GIST do not have a direct effect on toxicity, there could be an indirect time-related effect. Etiologic mutations influence patient survival and longer survival allows for more time for an endpoint to occur. This potential effect can be considered to be negligible as the median duration until the event was 3 months and the sheer majority of events was early in treatment.

For this study, DNA was obtained from blood samples, serum samples and FFPE samples. FFPE material contained tumor specimen and genotyping could potentially have been affected by loss of heterozygosity, as seen in GIST. However, almost all of the tested SNPs were in HWE, and deviations from HWE did not point towards loss-of-heterozygosity. DNA was obtained from FFPE samples in only 13 out of 315 patients (4%).The multivariate analysis did not yield different conclusions if performed without these patients (data not shown), which have therefore been retained in the analysis.

In conclusion, this pharmacogenetic study found SNPs in *ABCG2* and in *CYP1A2* in association with the need for a dose reduction of imatinib 400 mg in patients being treated for GIST. In 10% of patients dose reduction is needed and, these SNPs, if validated could potentially identify those patients in advance of the adverse events occurring. This may help in identifying which patients will suffer more from imatinib toxicity and could benefit from intensified follow-up. This would be a step towards personalizing and optimizing imatinib therapy in GIST patients.

Funding

A grant by Stichting Een Gift voor GIST was used for SNP genotyping.

Supplementary material

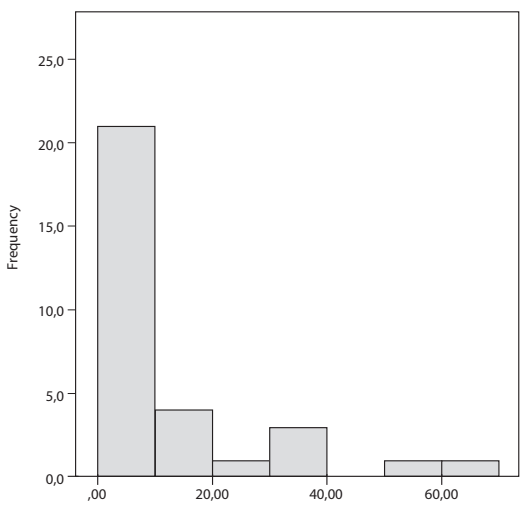
Supplementary Table S1: selected SNPs in pharmacokinetics and pharmacodynamics of imatinib

Gene	Rs number	Chromosome	Allele change	Change type	Study MAF
SNPs in pharmacokinetics					
ABCG2	rs2231137	4	G/A	Splicing	0.044
ABCG2	rs2231142	4	C/A	Splicing	0.124
SLC22A5	rs2631367	5	C/G	TFBS	0.477
SLC22A5	rs2631370	5	T/C	TFBS	0.366
SLC22A5	rs2631372	5	C/G	TFBS	0.275
SLC22A1	rs628031	6	G/A	Splicing	0.419
SLC22A1	rs683369	6	C/G	Splicing	0.276
SLC22A1	rs6935207	6	G/A	TFBS	0.240
ABCB1	rs1045642	7	C/T	Splicing	0.482
ABCB1	rs868755	7	G/T	Splicing	0.414
ABCB1	rs28656907	7	C/T	TFBS	0.522
SLC22A4	rs1050152	5	C/T	Splicing	0.422
CYP3A4	rs2740574	7	A/G	TFBS	0.065
POR	rs1057868	7	C/T	nsSNP	0.325
ABCC2	rs717620	10	C/T	TFBS	0.188
CYP1A1	rs1048943	15	A/G	nsSNP	0.043
CYP1A2	rs762551	15	A/C	TFBS	0.294
SLCO1B3	rs4149117	12	G/T	Splicing	0.135
SNPs in pharmacodynamics					
PDGFRA	rs1800810	4	C/G	TFBS	0.216
PDGFRA	rs1800812	4	G/T	TFBS	0.218
PDGFRA	rs1800813	4	A/G	TFBS	0.206
PDGFRA	rs2228230	4	C/T	Splicing	0.175
PDGFRA	rs35597368	4	C/T	Splicing	0.121
KDR	rs1870377	4	A/T	nsSNP	0.216
KDR	rs2071559	4	C/T	TFBS	0.436

KDR	rs2305948	4	C/T	nsSNP	0.089
VEGFA	rs1570360	6	G/A	TFBS	0.318
VEGFA	rs2010963	6	G/C	TFBS	0.336
VEGFA	rs25648	6	C/T	Splicing	0.177
VEGFA	rs3025039	6	C/T	miRNA	0.142
VEGFA	rs699947	6	A/C	TFBS	0.521
VEGFA	rs833061	6	C/T	TFBS	0.508
FLT4	rs6877011	5	C/G	miRNA	0.068
RET	rs1799939	10	G/A	Splicing	0.166
FLT3	rs1933437	13	T/C	Splicing	0.410
FLT1	rs7993418	13	A/G	Splicing	0.198

Selected SNPs in pharmacokinetics and pharmacodynamics of imatinib: Splicing= Splicing modifying, TFBS= Transcription Factor Binding Site, nsSNP= Non-Synonymous SNP, miRNA= Micro RNA alteration, MAF= minor allele frequency

Supplementary Figure S2: Time between imatinib start and dose reduction, in months



Supplementary Table S3: Toxicity to stop imatinib

	N
Nausea	5
Hepatic dysfunction	4
Dermatitis	4
Myalgia	3
Diarrhea	2
Edema	2
Intestinal necrosis	2
Pneumonitis	2
Agranulocytosis	1
Erectile dysfunction	1
Kidney failure	1
Combination of several	6

The total number of toxicities is larger than the number of patients ceased treatment due to toxicity, as some patients stopped due to more than one distinct toxicity that were all severe in nature (grade ≥3), whereas other stopped due a combination of several grade 1-2 toxicities.

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6

Influence of *CYP2C8* polymorphisms on imatinib steady-state trough level in chronic myeloid leukemia and gastro-intestinal stromal tumor patients

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Pharmacogenetics and Genomics. 2017 Jun;27(6):223-226

Abstract

Imatinib trough levels have been associated with its clinical effects. During chronic use of imatinib CYP2C8 becomes an important metabolizing enzyme due to cytochrome P450 3A4 (CYP3A4) auto-inhibition. Single Nucleotide Polymorphisms (SNPs) in CYP2C8 may affect imatinib trough levels. This study investigates the effect of common CYP2C8 polymorphisms (*1B (rs7909236), *1C (rs17110453), *3 (rs11572080 and rs10509681), and *4 (rs1058930)) on steady state trough levels of imatinib during chronic imatinib use in 43 patients with chronic myeloid leukemia (CML) or gastrointestinal stromal tumors (GIST). Standardized imatinib trough levels did not show a significant difference between wild type and variant groups for any tested SNPs, but an association with age was found with older patients having higher trough levels. This suggests that common CYP2C8 SNPs have no effect on the pharmacokinetics of imatinib.

Introduction

The tyrosine kinase inhibitor imatinib has dramatically improved the treatment of patients with Chronic Myeloid Leukemia (CML) or with Gastrointestinal Stromal Tumors (GIST).[1,2] However, clinical response varies significantly between patients. This short communication aims to address the influence of several common SNPs in *CYP2C8* as potential pharmacogenetic biomarkers of this inter patient variability.

Clinical response of imatinib has been shown to be influenced by both patient and tumor factors. Associations with plasma trough level have also been reported and large inter-individual differences in trough levels have been described.[3,4] Imatinib is primarily metabolized to its active metabolite, N-demethylated piperizine derivative, by cytochrome P450 (CYP) 3A4 and CYP3A5. Chronic use of imatinib results in reduced CYP3A4 and CYP3A5 activity through auto-inhibition. When this occurs, CYP2C8 becomes an important metabolizer of imatinib.[5] The *CYP2C8* gene has several so called functional single nucleotide polymorphisms (SNPs), one of which has shown in *in vitro* tests to have a gain-of-function effect on imatinib, but which results in reduced enzyme activity in *in vitro* tests with other drugs.[6,7] The influence of CYP2C8 polymorphisms on imatinib pharmacokinetics has not yet been studied *in* vivo before. Therefore, this study aims to investigate the relationship between CYP2C8 polymorphisms and pharmacokinetics of imatinib, in patients who have used this drug for at least 30 days, which is long enough for CYP2C8 to have become the primary metabolizer.

Material and methods

In a prospective study patients from three Dutch hospitals were included. Inclusion criteria were a confirmed diagnosis of CML or GIST, continuous imatinib usage of at least 30 days and written informed consent. In case of suspected non-adherence to imatinib (at the physician's discretion) or if the time of imatinib intake was not precisely known or was within 3 hours of sampling, the patient was excluded. During routine blood sampling for standard care, additional blood samples were taken for imatinib plasma concentration determination and for CYP2C8 genotyping. The study protocol was approved by the local science or ethics commission at each study site.

The plasma concentration of imatinib was determined using a validated LC-MS/MS assay in a single laboratory. These concentrations were recalculated into trough levels using the following formulas:

$$Conc_{24h} = Conc_{measured}\ x\ 0{,}5\ \left(\frac{24-interval}{t^{1/2}}\right)\ Conc_{12h} = Conc_{measured}\ x\ 0{,}5\ \left(\frac{12-interval}{t^{1/2}}\right).$$[8]

The calculated trough levels were standardized to a once-daily dose of 400 mg imatinib by dividing the calculated concentration with the daily dose and then multiplying it by 400. SNPs in *CYP2C8* were selected with a minimum minor allele frequency (MAF) of 0.02 in Caucasians. For the genotyping of SNPs *CYP2C8 *1C - rs17110453, *3 - rs10509681, *3 - rs11572080, *4 - rs1058930*, commercially available Taqman assays were used (Applied biosystems/Thermo Fisher Scientific, Waltham, MA, USA). For *CYP2C8 *1B - rs7909236* genotyping custom TaqMan assay was used (forward: GTATTGGATTGGAGCCCAGGTATTT, reverse: TGTTTCTCCATCATCACAGCACAT; Probes, VIC labeled: AAGTCCCTGGTTGTTCCA, FAM labeled: TCCCTGGTTTTTCCA). The genotyping results were tested for deviation of the Hardy Weinberg equilibrium, to exclude non-normally distributed genetic variation. As standardized trough levels were non-normally distributed, non-parametric tests were used for univariate analysis. For testing the association of standardized trough levels and patient characteristics the Spearman's rho and the Mann–Whitney U test were used, and for genotypes the latter test as well. When testing for differences between wild type and variant alleles, patients with heterozygous and homozygous variant genotypes were grouped together due to the paucity of the latter group in the patient cohort. A multivariate linear regression analysis was performed using the significantly associated patient characteristics and each SNP. A p value of less than 0.05 was considered statistically significant. SPSS version 22 was used (IBM Corp., Armonk, NY, USA).

Results

From June 2014 to February 2015, 47 consecutive patients were included, of which 4 patients were excluded due incomplete sampling or samples taken within 3 hours in imatinib intake. Table 1a shows the characteristics of 43 included patients and Table 1b the genotyping results. The only significant difference between patients with CML and GIST was sex (p=0.009), all genotype results did not differ significantly (data not shown). All SNPs were in HWE (data not shown). Table 2 shows the association of standardized trough level with patient characteristics and with SNP genotypes. None of the tested SNPs were significantly associated. Only age showed an association, with older patients having higher trough levels (r=0.359, p=0.018). Figure 1 shows the distribution of the standardized trough level per genotype for each SNP. Table 3 shows the results of multivariate regression analyses of the standardized trough level with age and each genotype. This confirms the effect of age regardless of CYP2C8 genotype.

Table 1a: patient characteristics
Table 1b: genotyping results

		All patients	CML	GIST
Patients N				
Age	median (range), in years	63 (36 - 83)	62 (36 - 83)	63 (47 - 76)
Weight	median (range), in kg	78 (51 - 108)	75 (51 - 100)	84 (62 - 108)
Standardized trough level	median (range), in µgl/L	1029 (444 - 2790)	1086 (444 - 2430)	1028 (603 – 2790)
Sex	male (%)	24 (56)	8 (36)	16 (76)
	female (%)	19 (44)	14 (64)	5 (24)
Race	Caucasian (%)	37 (86)	19 (86)	18 (86)
	other (%)	6 (14)	3 (14)	3 (14)
***CYP2C8* SNPs**				
*1B (rs7909236, G/T)	wild-type (%)	26 (60)	13 (59)	13 (62)
	variant (%)	17 (40)	9 (41)	8 (38)
*1C (rs17110453, A/C)	wild-type (%)	33 (77)	16 (73)	17 (81)
	variant (%)	10† (23)	6 (27)	4† (19)
*3 (rs11572080, C/T)	wild-type (%)	31 (72)	14 (64)	17 (81)
	variant (%)	12 (28)	8 (36)	4 (19)
*3 (rs10509681, T/C)	wild-type (%)	31 (72)	14 (64)	17 (81)
	variant (%)	12† (28)	8† (36)	4 (19)
*4 (rs1058930, G/C)	wild-type (%)	39 (91)	20 (91)	19 (90)
	variant (%)	4 (9)	2 (9)	2 (10)

Table 1a: patient characteristics, CML = chronic myeloid leukemia, GIST = gastrointestinal stromal tumor
Table 1b: genotyping results, all patients were heterozygote for the variant allele, except for †, where one patient was homozygous for the variant allele

Table 2: associations with standardized imatinib trough level

	r	p value
Age	0,359	**0.018**
Weight	-0,221	0.155
Sex	-0,224	0.142
Race	-0,059	0.700
Disease	0,022	0.884
*1B (rs7909236)	-0,152	0.320
*1C (rs17110453)	-0,162	0.287
*3 (rs11572080)	-0,116	0.448
*3 (rs10509681)	-0,116	0.448
*4 (rs1058930)	-0,115	0.452

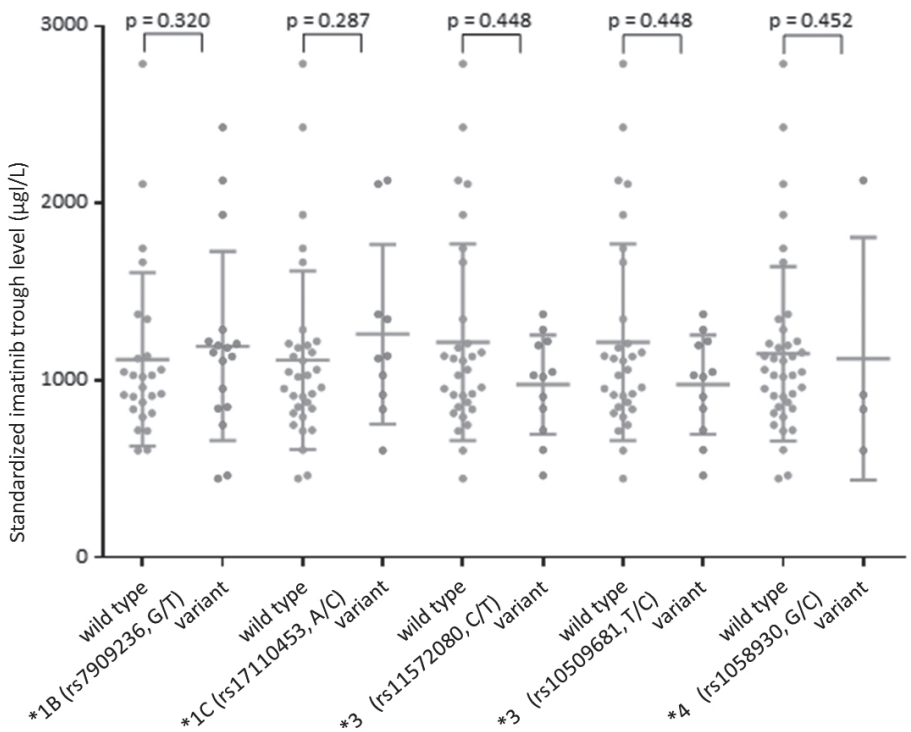
Univariate associations with imatinib trough level standardized for a daily dose of 400mg, r notes effect size, only age is significantly associated

Table 3: regression analysis of standardized imatinib trough level with age and SNP

	Beta	p value
Age	0.434	**0.004**
**1B (rs7909236)*	0.114	0.428
Age	0.418	**0.005**
**1C (rs17110453)*	0.105	0.465
Age	0.394	**0.010**
**3 (rs11572080)*	-0.117	0.428
Age	0.394	**0.010**
**3 (rs10509681)*	-0.117	0.428
Age	0.425	**0.005**
**4 (rs1058930)*	0.023	0.876

Multivariate regression analysis of imatinib trough level standardized for a daily dose of 400mg with age and SNP, Beta denotes effect size

Figure 1: Distribution of standardized imatinib trough level per SNP



Distribution of standardized imatinib trough level per SNP for wild type and variant allele groups, above each SNP the p-value of the Mann-Whitney U test is shown, the horizontal line denotes the mean and the vertical line the standard deviation.

Discussion and conclusion

This study shows no statistically significant difference in standardized imatinib trough level between wild-type CYP2C8 and variant allele groups in patients with CML or GIST, who have used imatinib for at least 30 days. While *in vitro* studies of variant allele groups *CYP2C8 *2, *3, *4* have shown a reduced activity relative to wild type CYP2C8, an effect on metabolic clearance has not been seen *in vivo*.[7]

Based on a previous study, it is assumed that after auto-inhibition of the primary CYP3A4 metabolic pathway imatinib is metabolized by CYP2C8.[5] Possibly, polymorphisms of other CYP enzymes and transporters outweigh the effects of CYP2C8 SNPs on imatinib trough level.[9,10] Furthermore, by the time a slow-acting CYP2C8 becomes an imatinib metabolizer other CYP enzymes may also come into play, such as CYP2C9, CYP2C19, CYP2D6, or CYP1A2, and diminish CYP2C8 SNPs effects.[9] The association of age and increased imatinib standardized trough level was in line with a previously reported

weak correlation, but these authors considered this effect not likely to be to be clinically relevant due to large inter patient variability.[11]

The calculation of the imatinib trough level is one of this study's limitations. The formula uses a fixed elimination constant which makes it highly dependent on the interval between the intake and sampling of imatinib. Incorrect registration may thus influence the calculated trough level. Future studies may yield more precise results if the investigated drug is taken during clinical supervision. Furthermore, this study has a relative small number of patients, so only strong associations are likely to show in the present data.

In conclusion, this study suggests common *CYP2C8 SNPs *1B, *1C, *3 (rs10509681 and rs11572080)* and **4* have no effect on the pharmacokinetics of steady state imatinib in patients with GIST or CML, but age does show an association.

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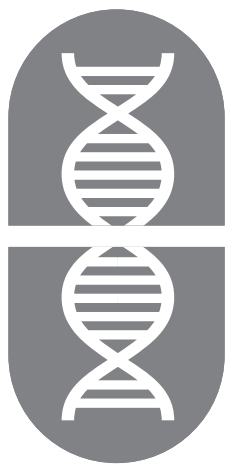
Part II: Use of trabectedin in STS

7

Trabectedin in soft tissue sarcoma

Michiel Verboom, Hans Gelderblom



Summary

Trabectedin is a relatively new chemotherapeutic agent for the treatment of soft tissue sarcoma. Doxorubicin and ifosfamide are the front-line agents used for most subtypes since decades. Trabectedin is available as orphan drug in a number of hospitals in The Netherlands for soft tissue sarcoma after previous treatment with standard chemotherapy. Trabectedin is effective in leiomyosarcoma and myxoid liposarcoma as well as in some other subtypes, such as synovial sarcoma. The most frequent adverse events are fatigue, liver and bone marrow toxicity, and in very rare cases rhabdomyolysis. A great part of the toxicity can be prevented with 20 mg dexamethasone pre-medication. Treatment with trabectedin has to be given by experienced doctors, due to mandatory dose reductions based on extensive laboratory tests before cycles. In this chapter the clinical development of trabectedin in soft tissue sarcoma is being reviewed.

Introduction

Trabectedin is a relatively new agent in the treatment of soft tissue sarcomas. Soft tissue sarcomas are a heterogenic group of tumors that have arisen from connective tissue. As each of the more than 80 different subtypes has its own etiology and biology, trabectedin is not equally effective in all subtypes of soft tissue sarcomas. Most data have been generated in the treatment of leiomyosarcoma and myxoid liposarcoma, the so-called L-sarcomas.

About a thousand patients in the Netherlands develop a non-GIST soft tissue sarcoma each year, 53 per 1.000.000 per year.[1] The most prevalent subtypes are leiomyosarcoma (19%), synovial sarcomas (13%), and liposarcoma (12%). The 5-year survival of all high-grade non-GIST sarcomas is 60%.[1] Due to the rareness of the different subtypes of soft tissue sarcomas, it is advised to either refer these patients to specialized centers for their treatment and participation in clinical trials, or to discuss these cases with those centers for case specific treatment or advice.

In half of the patients metastases occur, usually pulmonary metastasis.[2,3] If metastasectomy is not possible or meaningful, palliative treatment commences with first line doxorubicin monotherapy.[4] Combining doxorubicin with other agents such as ifosfamide has not been shown to improve overall survival (OS).[5] For some specific subtypes of soft tissue sarcoma specific drugs are being used, such as taxanes for angiosarcoma or gemcitabine for leiomyosarcoma.[4] During the past decades, many trials have aimed to find new agents active in soft tissue sarcomas.[6] Trabectedin is one of these drugs and has shown to be of use in stabilizing tumor growth.[7] Other second line options include pazopanib for non-adipogenic tumors and eribulin for adipogenic tumors.[4]

This chapter aims to review the existing knowledge of the treatment with trabectedin for soft tissue sarcomas. Apart from the adverse events and effectiveness in sarcomas in general, some of the soft tissue sarcomas subtypes will be highlighted. Furthermore, phase III and phase IV trials will be discussed.

Development

In the sixties of last century, during a National Cancer Institute screening for new agents, extracts of the sea squirt *Ecteinascidia turbinata*, a tunicate from the Caribbean Sea, were found to have anti-tumor effect (Figure 1).[8] In 1984 the structure of one of these extracts was determined; ET-743, or trabectedin.

Figure 1: The sea squirt Ecteinascidia turbinata

Though ET-743 was initially obtained through an aqua culture, it is being synthetically formed since 1996.[9] The commercial name for trabectedin is Yondelis (PharmaMar, Madrid, Spain). In vitro and xenograft studies have shown that trabectedin has anti-tumor effect in soft tissue sarcomas among others.[10,11] An in vitro study showed that trabectedin is more cytotoxic for sarcoma tissue than for other types of type tissue, and is more effective against sarcoma tissue than other established chemotherapeutics.[12]

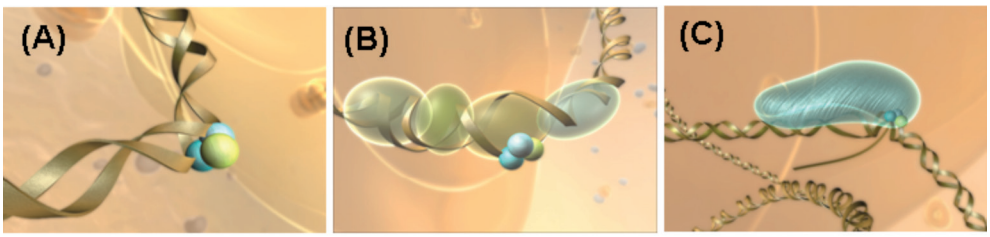
Using the data from a comparative phase II study, the European Medicines Agency has approved trabectedin in September 2007. As of 2015, trabectedin has been assigned the orphan drug status in the Netherlands for the second line treatment of all subtypes of soft tissue sarcoma.

Mechanism of action

Trabectedin has a unique mechanism of action, which is not yet fully understood. The drug consists of three interconnected rings; two of the rings bond to DNA whilst the third ring protrudes from this complex, free to interact with DNA surrounding enzymes.[13] In contrast to most alkylating agents, which bind to the major groove of the DNA molecule, trabectedin binds to the minor groove.[14] Through this bond the DNA is bent to the major groove and trabectedin inhibits transcription by preventing binding of transcription factors to DNA, and interferes with DNA-binding proteins and DNA repair mechanisms (Figure 2).[15] These interactions lead to double-strand breaks and cell cycle disruption by a delay in the S-phase and blockage of the G_2M-phase resulting in apoptosis.[16] Furthermore, trabectedin interaction with the elongating RNA polymerase

II stops transcription and leads to degradation of the polymerase, in a transcription coupled nucleotide excision repair dependent manner.[17]

Figure 2: The mechanism of action of trabectedin



(A) Trabectedin binds to the DNA's minor groove and bends the DNA-molecule to the major groove. **(B)** This makes the cell enter apoptosis caused by failure of DNA repair mechanisms, which cannot function due to binding of trabectedin to Xeroderma Pigmentosum Group D and upon which double-strand breaks appear. **(C)** Trabectedin interferes with the surrounding transcription factors.

Trabectedin also influences the tumor microenvironment and it inhibits the production of and angiogenic and pro-inflammatory cytokines. In myxoid liposarcoma cell lines, the production of CCL2, CXCL8, VEGF and PTX3 was lowered after trabectedin admission, which was subsequently confirmed in a xenograft model.[18] This type of sarcoma is associated with the FUS-DDIT3 fusion protein. Trabectedin blocks the activating ability of this protein by displacing it from its target promoters.[15] The reduced binding to the target genes was also seen in a myxoid liposarcoma xenograft, with a histologic response that showed diminishment of non-lipogenic tumor cells and vasculature.[15] The (progression free) survival of trabectedin-treated patients is influenced by expression of ERCC1 (Excision Repair Cross-Complementing group 1), ERCC5 (also known as XPG; Xeroderma Pigmentosum Group D) and BRCA1 (Breast cancer 1). Patients with a high expression of ERCC1 and XPG, and a low expression of BRCA1 had a longer survival.[19,20] This could be a potential predictor of clinical response.

Efficacy of trabectedin as monotherapy

The clinical relevance of attaining a response by RECIST criteria when treating sarcomas is considered of limited relevance.[21,22] The EORTC Soft Tissue and Bone Sarcoma Group therefore advises to use progression free survival as a surrogate end point. Second line anti-tumor agents are considered to be active when they achieve a progression free survival of 39% after 3 months and 14% after 6 months.[23] For soft tissue sarcomas in general, the objective response percentage to trabectedin as second line agent is about 10% in a series of phase II studies.[24-30] This is in line with large national retrospective studies.[31,32] The percentage of progression free patients after 6 months consistently was around 25%.[26-28]

For the different subtypes of sarcomas variation in the effectiveness of trabectedin is evident. The most sensitive tumors are the L-sarcomas.[33] Additionally, anti-tumor effect was also seen in a considerable number of patients with synovial sarcoma. Three second line phase II studies with a total of 86 patients showed the response percentage varying from 6% to 18%, and 50 to 61% of patient had clinical benefit.[26,34,35] A retrospective study in 61 patients showed that 23% synovial sarcoma patients were progression free at 6 months.[36] For treatment of the much rarer fibrosarcoma and undifferentiated pleomorphic sarcoma trabectedin may be effective, but the numbers of patients treated are too small for any definite conclusion. In case of alveolar soft part sarcoma, trabectedin has shown only limited activity, in contrast to the more active pazopanib.[37]

Based on the positive results of a combined analysis of three non-randomized phase II studies in patients with leiomyosarcoma and liposarcoma, a randomized study was started in L-sarcomas.[33] In that study 270 patients were randomized for two infusion schemes, with time to progression as primary end point. The 3-weekly 24-hour 1.5 mg/m^2 regimen was significantly better than the weekly 0.58 mg/m^2 3-hour regimen. Of 136 patients treated with the 3-weekly schedule 6% had an objective response, 53% was progression free at 3 months and 37% after 6 months.[38] Subsequently, the 3-weekly schedule has become the standard treatment regimen.

In other second line phase II studies a total of 161 L-sarcoma patients had an objective response chance of 7% to 13%, and in 27% to 58% of patients the treatment had a clinical effect (at least stable disease on the first scan in a pre-treatment progressive patient).[25-29] In an American expanded access program with 664 patients having a L-sarcoma, 7% had an objective response, while 54% had a clinical effect.[32] Trabectedin appears most effective in myxoid liposarcomas; in a retrospective study of 51 patients 51% responded and a14 months median progression free survival was reached.[39]

To investigate the efficacy of trabectedin head to head with doxorubicin-based chemotherapy as first line treatment, a randomized phase III trial was performed in patients with advanced translocation-related sarcomas, such as myxoid liposarcoma and synovial sarcoma.[40] The primary endpoint PFS did not differ significantly, meaning neither treatment was superior. Doxorubicin did appear to cause more toxicity than trabectedin did. In another fist line randomized study doxorubicin was compared to trabectedin in two treatment regimens in patients, the TRUSTS trial.[41] Patients with advanced STS were included. Doxorubicin gave a somewhat better PFS than either the 3-hour infusion (1.3 mg/m^2) or the 24-hour infusion (1.5 mg/m^2) trabectedin regimen did. However, due to severe toxicity in the trabectedin arms, the study was terminated with doxorubicin remaining the standard first line systemic agent in advanced STS.

Trabectedin in the treatment of L-sarcomas was further studied in a randomized phase III trial in patients who had received at least two previous lines of therapy.[42] The study

showed that trabectedin achieved superior disease control compared to dacarbazine, with the median PFS at 4.2 and 1.5 months, respectively. OS did not differ, being 12.4 and 12.9 months, respectively. As disease control is considered to be clinically relevant in sarcomas, this study led to FDA approval for trabectedin in L-sarcoma treatment after the use of doxorubicin.[43] A post-hoc analysis of the uterine leiomyosarcoma subgroup showed similar results, as did a subgroup analysis which focused on elderly patients.[44,45]

Trabectedin has also been tested in relation to best supportive care (BSC). A randomized phase II trial included 73 patients with translation-related sarcomas.[46] Treatment with trabectedin resulted in a median PFS of 5.6 months compared to 0.9 months for BSC, with a Hazard Ratio of 0.07 ($p< 0.0001$) for progressive disease. In another trial, 103 advanced STS patients have been randomized to receive either trabectedin or BSC as second or later line of treatment. PFS was the primary endpoint.[47] The median PFS was 3.1 months for trabectedin and 1.5 months for BSC, with a Hazard Ratio of 0.39 ($p< 0.0001$). After cross-over the difference in median OS was not significant, with 13.6 months for trabectedin and 10.8 months for BSC. Sixty percent of patients had an L-sarcoma and the response rate for trabectedin was 12% in this subgroup. The difference in median PFS between treatment arms was larger in L-sarcoma, being 5.1 months for trabectedin and 1.3 months for BSC.[47]

Efficacy of trabectedin in combination with other systemic agents

Pre-clinical research has shown a synergistic effect of trabectedin with doxorubicin, irinotecan and cisplatin.[48-50] Phase I studies have been performed to demonstrate this effect in patients.

In a phase I study in which trabectedin and doxorubicin were combined, the maximal tolerable dose trabectedin was 1.1 mg/m^2 with doxorubicin given at 60 mg/m^2. The most frequent adverse events were bone marrow suppression and transaminase elevation. Although this type of study does not allow for statements on efficacy, the response rate was 12%.[51] In a phase II trial patients with advanced leiomyosarcoma received six cycles with this combination as first line systemic therapy.[52] The response rate for 108 patients was 48%, with 82% achieving progression free survival after 24 weeks. The adverse events were in line with the phase I study. This study has prompted a phase III trial in which doxorubicin will be compared to the doxorubicin-trabectedin combination followed by further trabectedin cycles in case of clinical effect (NCT02997358). For patients with advanced STS a randomized phase II trial has compared the combination of trabectedin and doxorubicin to doxorubicin 75 mg/m^{2}monotherapy in a first line

setting.[53] In the interim analysis, the median PFS did not differ between study arms and the study was stopped for futility.

Trabectedin in combination with the poly ADP-ribose polymerase 1 (PARP1) inhibitor olaparib was tested in a phase I trial.[54] For further studies the recommend dose was trabectedin at 1.1 mg/m^2 and olaparib at 150 mg twice daily. The adverse events were manageable and consisted mainly of bone marrow depression and hypophosphatemia. A randomized phase II study will commence in which trabectedin monotherapy is compared to trabectedin plus olaparib (NCT03838744).

The combination of trabectedin and doxorubicin appears to be an improvement to the combination of gemcitabine and doxetaxel, but a direct comparison has not been performed.[52,55,56] The phase I study investigating the combination of trabectedin and gemcitabine has been terminated as too much toxicity was seen to suggest a tolerable regimen.[57] A new research foray in improving systemic STS treatment is aimed at combining trabectedin with immunotherapy and clinical trials with ipilimumab and nivolumab have started (NCT03138161, NCT03590210). As of present, trabectedin in combination with other agents is not being used in routine soft tissue sarcoma patient care.

Adverse events

Trabectedin is well tolerated by most patients, provided that they receive dexamethasone as pre-treatment. Hepatic toxicity was the most frequent adverse event during the first clinical studies with trabectedin. Animal experiments showed that pre-treatment with dexamethasone prevented liver toxicity without impairing the anti-tumor effect.[58] Therefore, 20 mg dexamethasone pre-treatment half an hour before trabectedin infusion has become standard of care. Additionally, this reduces bone marrow suppression and has an anti-emetic effect.[59] Pre-treatment with dexamethasone can also be administered 24 hours before the cycle; it is not yet clear which regimen lowers toxicity best.

The most frequently mentioned complaint is fatigue.[7] At times the fatigue is progressive during several cycles, 0% to 15% of patients has grade 3-4 fatigue. Nausea is easy to control with anti-emetics and 5% to 9% has grade 3-4 nausea and vomiting.[25-30,38] Alopecia or stomatitis are very rare (<1%), as are cardiomyopathy related adverse events.[60,61]

The incidence of grade 3-4 transaminase elevation has been reduced to 11% to 57% of patients, since the advent of dexamethasone pre-treatment.[25-30,38] This elevation usually is temporary and noncumulative, but can be a reason for dose reduction or delay of the next cycle.[62]

Bone marrow suppression is variably seen in all patients and seems to be dependent on the extent of pre-treatment, 17% to 61% of patients has grade 3-4. Some of these patients also develop fever, which necessitates hospital admission in 1% to 10% of patients. Grade 3-4 anemia happens in 0% to 22%, grade 3-4 thrombocytopenia in 10% to 29% of patients.[25-30,38] In order to reduce the chance on febrile neutropenia, primary prophylaxis with G-CSF should be considered.

A number of patients have died due to trabectedin treatment, caused by multi-organ failure, severe liver toxicity or rhabdomyolysis. To prevent this, the bilirubin should be normal before start of treatment and the other liver enzymes and creatine kinase not more elevated than 2.5 times the upper normal limit. This is different from an oncologists routine clinical practice, when normally often only hematological values are considered. In addition, in between cycles patients are checked with laboratory test (mainly creatine kinase, kidney and liver function). If test values exceed 2.5 times the upper normal limit, the dose should be reduced for the following cycle. Also, the creatine kinase may not be rising rapidly. This requires extra discipline, as not only the laboratory tests on the day of the new cycle are of importance, but also the tests in between the cycles. In case of leads for rhabdomyolysis, swift action is required to proactively treat this condition.

The standard trabectedin dose is 1.5 mg/m^2 in 24 hours, every 3 weeks. However, due to the frequent need for dose reductions to 1.2 mg/m^2, it is advised to start with this reduced dose in patients who previously have had more than one line of chemotherapies. Infusion via a port-a-cath is recommended. Thus far, no cumulative toxicity has been reported for trabectedin. Advanced age does not predispose for significantly more adverse events when trabectedin is prescribed, although neutropenia and asthenia is seen more often.[63] A subgroup analysis of a phase III trial showed that elderly patients had a comparable adverse event profile to the younger study population.[45]

Discussion

Trabectedin is active against several soft tissue sarcoma subtypes; the best results have been achieved in patients with either leiomyosarcoma or liposarcoma, however it may also be effective in the rarer subtypes. It is a relatively safe drug to prescribe to patients with a normal liver function and with dexamethasone pre-treatment.The biologic diversity of soft tissue sarcomas makes the efficacy of trabectedin in several diverse subtypes particularly noteworthy.

Furthermore, trabectedin in combination with pegylated liposomal doxorubicin (PLD) has shown to be active in relapsed ovarian cancer.[64] For this indication, trabectedin in combination with PLD has been approved by the European Medicine Agency in

2009. A phase III study showed this combination to have a longer progression free survival than PLD alone.[65] Additionally, subsequent re-challenge with platinum after a longer platinum-free interval was also effective for a longer duration of time.[66]

The current first line chemotherapy for soft tissue sarcoma is doxorubicin. Adding ifosfamide enhances the response chance, but does not lengthen survival.[4] Trabectedin counts as a third line option. One can also choose for trabectedin treatment before high dose ifosfamide is given, due to the considerable shorter time of admission for each cycle.

After decades without new drugs with a broad indication for soft tissue sarcomas, two new agents have been developed in rapid succession; besides trabectedin, pazopanib and eribulin have also shown to be effective. Pazopanib is a multi-targeted tyrosine kinase inhibitor with a toxicity profile comparable to other tyrosine kinase inhibitors. In a phase III study pazopanib has been compared as second line option to placebo. Pazopanib has a substantial gain for the primary endpoint progression free survival of 3 months (Hazard Ratio 0.31) and also a modest survival gain of 2 months (Hazard Ratio 0.86) in non-lipo-soft tissue sarcomas.[67]

Eribulin has a seaborne origin, like trabectedin. It is a synthetic copy of halichondrin B which was first derived from the marine sponge Halichondria okadai.[68] It interferes with microtubule dynamics and blocks mitosis which leads to cell apoptosis, next to effects on the tumor microenvironment.[69,70] It has been compared in a randomized phase III clinical trial to dacarbazine in 452 patients advanced L-sarcoma.[71] About half these patients had two lines of previous systemic therapy, and another half even more lines of previous therapy. While the median PFS was 2.6 months for both treatment arms, the median OS for eribulin was 13.5 months compared to 11.5 months for dacarbazine. This resulted in a Hazard Ratio of 0.77 (p= 0.0169). Patients had similar post-study treatments, regardless of treatment arms. In a subgroup analysis of this trial, the difference in survival was more profound in patients with a liposarcoma.[72] Median PFS was 2.9 months for eribulin and 1.7 months for dacarbazine and median OS 15.6 months and 8.4 months, respectively. The differences in the subgroup analysis were statistically significant, with similar adverse events. This has led to FDA approval for eribulin in liposarcoma.[73] Eribulin is less active in other types of STS.[74] Pazopanib and eribulin have yet not been directly compared to trabectedin.

A ZonMW funded phase IV observational study with trabectedin will be presented in the next chapter. This study aimed to register advanced STS patients' survival and health care needs when receiving trabectedin treatment. This observational study was intended to establish the cost-effectiveness of trabectedin per gained QALY compared to an alternative treatment (NCT01299506).

Conclusion

Trabectedin has shown its value as a new agent in the treatment of soft tissue sarcomas. It seems to have a clear position as effective second line agent for certain histological subtypes, such as leiomyosarcoma, liposarcoma and maybe also in other soft tissue sarcoma subtypes.

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8

Survival and cost-effectiveness of trabectedin compared to ifosfamide monotherapy in advanced soft tissue sarcoma patients

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Abstract

Trabectedin and ifosfamide are among the few cytostatic agents active in advanced soft tissue sarcomas (STS). Trabectedin is most potent against so-called L-sarcomas (leiomyosarcoma and liposarcoma). The survival gain and cost-effectiveness of these agents in a second line setting were analyzed in the setting of advanced STS after failure of anthracyclines. A prospective observational trial had previously been performed to assess the use of trabectedin in a Dutch real-world setting. Data on ifosfamide monotherapy was acquired from previous studies and an indirect comparison of survival was made. A state-transition economic model was constructed in which patients could be in mutually exclusive states of being pre-progression, post-progression or deceased. The costs and quality adjusted life years (QALYs) for both treatments were assessed from a Dutch health care perspective. Separate analyses for the group of L-sarcomas and non- L-sarcomas were performed. Trabectedin treatment resulted in a median progression free survival of 5.2 months for L-sarcoma patients, and 2.0 months for non-L-sarcoma patients, and a median overall survival of 11.8, and 6.0 months, respectively. For L-sarcoma patients, trabectedin offered an increase of 0.368 life years and 0.251 QALYs compared to ifosfamide and € 20,082 in additional costs, for an incremental cost-effectiveness ratio (ICER) of € 80,000 per QALY gained. In the non-L-sarcoma patients, trabectedin resulted in 0.413 less life years and 0.266 less QALYs, at the increased cost of € 4,698. The difference in survival between drugs and the acquisition costs of trabectedin were the main influence in these models. Trabectedin was shown to have anti-tumor efficacy in advanced L-sarcoma. From a health economics perspective the costs per QALY gained compared to ifosfamide monotherapy may be acceptable, considering what is currently regarded as acceptable in the Netherlands.

Introduction

Soft tissue sarcomas (STS) are a rare group of malignancies arising from mesenchymal cells comprising one percent of all adult malignancies. STS in general are relatively insensitive to chemotherapy compared to tumors of epithelial origin. Some drugs, like doxorubicin, have been found active in a range of different sarcoma subtypes, whereas others show only activity in specific subtypes, such as crizotinib in the inflammatory myofibroblastic tumor.[1] Trabectedin is a drug active in several subtypes, with most notable effect in leiomyosarcoma and liposarcoma. It has a unique mechanism of action in binding to the minor groove of DNA, and also in influencing the tumor environment.[2,3]

Trabectedin was approved for clinical use in Europe in 2007 for patients with advanced STS after failure to anthracyclines and ifosfamide or for patients unsuited to receive these agents. At this time, studies with a randomized comparison with other treatment options were not available. Therefore, before market authorization in the Netherlands could be granted, a prospective observational trial was designed, which aimed to analyze the use of trabectedin in STS in a real-world setting.

The original aim of this observational trial was to analyze the use of trabectedin compared to best supportive care (BSC) and derive an incremental cost-effectiveness ratio (ICER) for its use compared to BSC. All patients eligible for trabectedin were also given the option of BSC, but only a few patients opted for BSC, which made it impossible to draw meaningful conclusions from this small number of patients. Instead, as an alternative, a comparison with ifosfamide in retrospective data was sought, as this drug is a treatment option for patients with advanced STS after failure to anthracyclines. Ifosfamide is an alkylating agent and available since the 1980s for the treatment of STSs.

Therefore, this study aims to compare both survival and cost-effectiveness between trabectedin and ifosfamide in the setting of second line cytostatic treatment of STS in the Netherlands.

Methods

Patient selection

In order to facilitate the entry and reimbursement of trabectedin in the Dutch health care system a cost-effectiveness analysis was designed to evaluate trabectedin and BSC usage patterns and outcomes in advanced STS in a real-world setting, including data on quality of life and associated utilities. This prospective observational phase IV trial was to provide the Dutch health authority (Zorginstituut Nederland) with sufficient data on the effectiveness and optimal use of trabectedin to ensure a proper evaluation for permanent registry in the Regulation Orphan Drugs. This trial was named ET-D-

010-10, with trial registration number NCT01299506. The RECIST 1.1 criteria were used for response evaluation. Quality of life data was scored using patient-reported EQ-5D questionnaires. Patients with all subtypes of STS were recruited in this trial if they were eligible for trabectedin, after the failure of anthracyclines and/or ifosfamide, or in case these patients were unsuited to receive these drugs. The patients in this observational trial were offered treatment with trabectedin or BSC, and the latter could consist of no systemic chemotherapy or other systemic anti-tumor therapy. Some of the included patients received trabectedin in a different line of therapy than second line and those patients were not used in the current analysis. All patients were adult and signed an Institutional Review Board approved informed consent form.[4]

At the time of the ET-D-010-10 observational trial no study had yet directly compared the efficacy of trabectedin to BSC. Hence, the choice of treatment was with the patient and local physician, as long as the patient was deemed fit enough to receive chemotherapy. It was intended to include 100 patients, of whom 80 would have received trabectedin and 20 would have chosen BSC. In reality, however, a larger portion of patients wished to be treated with trabectedin (91%) than predicted, and too few patients chose the BSC-arm (total 9%; 6% only BSC, 3% received additional or other systemic anti-tumor therapy). Despite an extension of the trial duration, accrual of the BSC-arm was insufficient to be able to perform a viable comparison of the collected data.

To account for the lack of a trial-generated comparator group, it was decided to perform an indirect comparison of the data in the trabectedin arm with data obtained from previous studies. As appropriate data on patients on BSC was not available, an agent active as second line treatment was sought. These data were obtained from two EORTC clinical trials with ifosfamide in patients with advanced STS, published by Van Oosterom et al. and Nielsen et al, hereafter termed the EORTC trials.[5,6] These two trials used the 1979 WHO criteria for response evaluation. According to the 2018 ESMO guideline on STS treatment, after doxorubicin patients may be treated with ifosfamide, if they did not progress on it previously.[1] Therefore, a second line setting was chosen for comparing the phase IV ET-D-010-10 data on trabectedin with the EORTC data on ifosfamide.

The efficacy of ifosfamide differs in STS subtypes to a certain extent, but has not been shown to differ as much between subtypes as trabectedin does. Trabectedin has a markedly better efficacy in leiomyosarcoma and liposarcoma subtypes, the so-called L-sarcomas. This difference in efficacy between L-sarcomas and non-L-sarcomas has led to clinical trials which specifically included patients with one of these two subtypes.[2] Due to the prominence of the L-sarcomas in trabectedin clinical research, it was decided to split the study population into two subsets, consisting of L-sarcomas and non-L-sarcomas.

Out of all patients included in the phase IV trial, 54 patients received trabectedin as second line treatment. The remaining 39 patients received trabectedin as third or higher line of treatment and were excluded from the cost effectiveness analysis. The drug was prescribed in the accepted regimen of 1.5 mg/m2 once every 3 weeks during a 24 hour hospital admission. Due to dose reductions, the average administered trabectedin dose was 1.3 mg/m2 From the ifosfamide trials a total of 50 patients were identified to fit the criterion of second line treatment. The ifosfamide dosage was 9 g/m2 given in 3 consecutive days every 3 weeks (19 patients),[6] or 12 g/m^2 as a 3 day continuous infusion every 4 weeks (31 patients),[5] together with intravenous MESNA to prevent hemorrhagic cystitis. For the cost-effectiveness analysis the regimen of 9 g/m2 was modelled, as it is current practice in the Netherlands. Based on the EORTC trials a dose intensity of 95% was implemented.

Survival analysis

The duration of progression free survival (PFS) was taken as the time from the first dose of either study drug until disease progression. The latter could be based on radiology findings or in case of trabectedin on clinical evaluation and cessation of treatment due to it. Duration of overall survival (OS) was counted from the day of the first study drug dose until death by any cause. To perform an indirect non-parametric analysis of survival, the Kaplan Meier method and the log rank test were used. The ECOG performance score was considered to be prognostic for survival, more so than sex or age in patients who require second line chemotherapy for STS. An ECOG performance score of 0 classified as low and a score of 1 or 2 as high. A Cox regression analysis was used for multivariate tests, in which ECOG performance score and the drug received were included into the analysis. Survival probabilities at 3 and at 6 months per treatment and group of sarcomas were calculated based on observed progression free survival, and the number of treatment cycles was noted.

Cost-effectiveness analysis

A state-transition model was constructed to estimate healthcare costs and quality-adjusted life expectancy (QALYs), separately for the L-sarcoma and the non-L-sarcoma patients. In this model patients were in a mutually exclusive state of either pre-progression survival, post-progression survival (being overall survival (OS) minus pre-progression survival), or deceased. The pre- and post-progression average discounted life expectancies (DLE) were calculated for each treatment. Lifetime costs and QALYs for treatment *T*, being either trabectedin or ifosfamide, were calculated as:

$$Costs_T = C_T + C_{Pre\text{-}progression} \times DLE_{T,\,Pre\text{-}progression} + C_{Post\text{-}progression} \times DLE_{T,Post\text{-}progression}$$

$$QALY_T = -U_T + U_{Pre\text{-}progression} \times DLE_{T,Pre\text{-}progression} + U_{Post\text{-}progression} \times DLE_{T,Post\text{-}progression}$$

In these formulae, C_T are the costs for treatment, such as drug acquisition and administration, and also those due to adverse events, and U_T is the QALY loss due to adverse events. The $C_{Pre\text{-}progression}$ and $C_{Post\text{-}progression}$ denote the annual treatment-unrelated costs before and after progression. Similarly, $U_{Pre\text{-}progression}$ and $U_{Post\text{-}progression}$ denote the utilities before and after progression. Each of these model parameters is described in more detail below. Subsequently the incremental cost-effectiveness ratio (ICER) was calculated as:

$$ICER = (Costs_{trabectedin} - Costs_{ifosfamide}) / (QALY_{trabectedin} - QALY_{ifosfamide}).$$

Consistent with the Dutch guidelines[7], life years (LY), QALYs and costs were discounted at 0%, 1.5%, and 4%, respectively. A life-time horizon was used and costs are reported in Euro's at price level 2018. Other model components are described below. Additional lines of anti-tumor therapies had not been recorded in the EORTC or ET-D-010-10 trial and these were not assumed in the cost-effectiveness analysis.

-- Survival

PFS and OS data of trabectedin and ifosfamide treatments were directly taken from the ET-D-010-10 and EORTC trials, respectively. Table 1 details the number of patients from each study, as well as baseline characteristics. To estimate average survival times, parametric survival analyses were used in which all patients were pooled, regardless of treatment. This facilitated extrapolating survival beyond study follow-up, and correcting for the (non-significant) difference in ECOG performance score between the prospective trabectedin and retrospective ifosfamide patients. LogNormal distributions were used, based on the Akaike Information Criterion (data not shown, considered alternative distributions were Loglogistic, Exponential, Gamma, Gompertz and Weibull distributions).

Table 1: baseline characteristics of study population.

Baseline characteristics of study population		**L-sarcoma**		**non-L-sarcoma**	
		Trabectedin	Ifosfamide	Trabectedin	Ifosfamide
Age at first dose	Mean (SD)	55 (12)	54 (10)	57 (14)	45.3 (14)
		number (%)	number (%)	number (%)	number (%)
Sex	Female	16 (42.1)	9 (47.4)	9 (56.3)	19 (61.3)
	Male	22 (57.9)	10 (52.6)	7 (43.8)	12 (38.7)
ECOG PS	0	18 (47.4)	10 (52.6)	9 (56.3)	8 (25.8)
	1+2	20 (52.6)	9 (47.4)	7 (43.8)	23 (74.2)
Study size	ET-D-010-10	38 (100.)	-	16 (100.)	-
	Nielsen et al.	-	14 (73.7)	-	17 (54.8)
	Oosterom et al.	-	5 (26.3)	-	14 (45.2)
Drug received	Trabectedin	38 (100.)	-	16 (100.)	-
	Ifosfamide	-	19 (100.)	-	31 (100.)
Disease status	Local disease	10 (26.3)	1 (5.3)	2 (12.5)	9 (29.0)
	Metastatic disease	28 (73.7)	18 (94.7)	14 (87.5)	22 (71.0)
Tumor histology	Leiomyosarcoma	19 (50.0)	13 (68.4)	-	-
	Liposarcoma	19 (50.0)	6 (31.6)	-	-
	UPS	-		6 (37.5)	4 (19.4)
	Synovial sarcoma	-		5 (31.3)	7 (22.6)
	Neurogenic sarcoma	-		-	4 (12.9)
	Hemangiosarcoma	-		-	3 (9.7)
	Rhabdomyosarcoma	-		-	3 (9.7)
	Other	-		5 (31.3)	8 (25.8)

SD: standard deviation, ECOG-PS: ECOG performance score, UPS: undifferentiated pleomorphic sarcoma

Table 2 shows the estimated μ and σ for each treatment in each group of sarcomas for PFS and OS, as well as the associated average survival duration in months and years.

Table 2: progression free survival

Progression free survival		**L-sarcoma**		**non-L-sarcoma**	
		Trabectedin	Ifosfamide	Trabectedin	Ifosfamide
PFS probability (%)	at 3 months	59.5	47.4	37.5	51.6
	at 6 months	41.7	15.8	18.8	22.6
N treatment cycles	mean	6.1	3.8	3.8	3.4
	median	6	4	3	3
Parametric analysis of survival					
PFS	mu	1.50	1.08	1.00	1.20
	sigma	1.05	1.05	1.04	1.04
Average PFS	in months	7.75	5.07	4.64	5.71
	in years	0.65	0.42	0.39	0.48
OS	mu	2.42	2.15	1.77	2.21
	sigma	1.00	1.00	0.94	0.94
Average OS	in months	18.58	14.17	9.09	14.18
	in years	1.55	1.18	0.76	1.18

Progression free survival rate age at 3 and at 6 months, the mean and median number of treatment cycles received, the parametric description of survival with the LogNormal distribution, and average survival times. The estimated average survival time with the LogNormal distribution is calculated by: exp ($\mu + \sigma 2 / 2$). PFS: progression free survival, OS: overall survival.

-- Utilities

Utility values represent the valuation of health, on a scale anchored at 1 for perfect health and 0 for health as poor as deceased. The ET-D-010-10 trial could only provide pre-progression utility data, which was scored using the EQ-5D and on average was 0.764. Therefore, EQ-5D utility estimates for patients receiving second line chemotherapy from the SABINE trial were used. In the SABINE trial the health-related quality of life was assessed in patients with metastatic sarcoma from North-America and Europe, including patients from the Netherlands.[8] Converting the UK tariff to the Dutch tariff resulted in pre- and post-progression utility score of 0.754 and 0.614, respectively. As the pre-progression utility in the SABINE trial was very similar to the utility found in the ET-D-010-10 trial, the usage of the SABINE utilities was considered appropriate. Utilities were assumed equal for L- sarcoma and non-L-sarcoma patients and equal for both trabectedin and ifosfamide treatment groups, except for the disutility caused by adverse events.

-- Health care costs

Costs for trabectedin and ifosfamide cycles included drug acquisition costs and drug administration costs, as shown in Table 3. Drug administration costs included the costs for hospitalization and blood tests and imaging. The majority of costs for trabectedin cycles consisted of trabectedin acquisition costs; € 4,238 out of € 5,877 per cycle. For ifosfamide cycles, on the other hand, the 5 days hospitalization formed the largest part of the costs; € 2,470 out of € 4,474 per cycle. A one-time treatment cost was added to include the cost for insertion of a central venous catheter (CVC), which was mandatory for all patients receiving trabectedin and amounted to € 1,015. Non-treatment related monthly healthcare costs were estimated for patients by extracting these data from the ET-D-010-10 study. These costs were estimated separately from the pre- and post-progression period and assumed equal for L-sarcoma and non-L-sarcoma patients and equal for the trabectedin and ifosfamide treatment groups. During pre-progression survival, monthly costs were € 284, and during post-progression survival this rose to € 461, as show in Table 4. Costs were taken from Dutch publicly available sources. Prices were corrected for inflation to obtain 2018 levels.

Table 3: treatment related costs.

Treatment related costs			**Trabectedin**		**Ifosfamide**	
Unit	price	source	use	costs	use	costs
Trabectedin 1 mg vial	€ 1,956	ref [19]	2.17	€ 4,238	-	-
Trabectedin 0.25 mg vial	€ 506	ref [19]	1.85	€ 938	-	-
Ifosfamide 2 mg vial	€ 121	ref [19]	-	-	8.87	€ 1,070
Dexamethasone 20mg	€ 9	ref [19]	1	€ 9	-	-
Granisetron 1 mg vial	€ 4	ref [19]	2	€ 8	4	€ 16
Dexamethasone 8 mg vial	€ 3	ref [19]	1	€ 3	4	€ 11
MESNA 0.4 mg vial	€ 9	ref [19]	-	-	1	€ 718
Hospitalization per day	€ 494	ref [7]	1	€ 494	5	€ 2,470
Full laboratory test	€ 43	ref [20]	1	€ 43	1	€ 43
Hematological test	€ 18	ref [20]	0.25	€ 5	0.25	€ 5
CT-scan	€ 157	ref [21]	0.25	€ 71	0.25	€ 71
MRI-scan	€ 264	ref [21]	0.25	€ 13	0.25	€ 13
Blood transfusion	€ 224	ref [7]	0.25	€ 56	0.25	€ 56

Table 3: treatment related costs. (Continued)

Treatment related costs				Trabectedin		Ifosfamide
Drug costs per cycle						
Drug acquisition costs				€ 5,175		€ 1,070
Drug administration costs				€ 702		€ 3,403
Drug costs, total per cycle				€ 5,877		€ 4,474
One-time treatment costs						
CVC insertion	€ 1,015	ref [21]	1	€ 1,015	0.30	€ 305
Total treatment costs						
- L-sarcoma				€ 36,895		€ 17,081
- non-L-sarcoma				€ 23,595		€ 15,601

Treatment related costs of trabectedin and ifosfamide, for the average number of treatment cycles (see Table 2), MESNA: 2-mercaptoethane sulfonate sodium, ref: reference, with numbers

Table 4: non-treatment related costs per month during pre-progression survival and post-progression survival

Non-treatment related costs per month			Pre-progression survival		Post-progression survival	
Unit	price	source	average use	cost	average use	cost
Hospitalization per day	€ 494	ref [7]	0.21	€ 106	0.48	€ 236
Full laboratory test	€ 43	ref [20]	1.02	€ 44	1.24	€ 54
Hematological test	€ 18	ref [20]	0.16	€ 3	0.13	€ 2
CT-scan	€ 157	ref [21]	0.31	€ 49	0.37	€ 58
MRI-scan	€ 265	ref [21]	0.01	€ 2	0.00	€ 0
Blood transfusion	€ 224	ref [7]	0.09	€ 20	0.00	€ 0
General Practitioner visit	€ 34	ref [7]	0.01	€ 0	0.03	€ 1
Medical oncologist visit	€ 102	ref [7]	0.58	€ 59	1.08	€ 110
Nurse	€ 34	ref [7]	0.01	€ 0	0.02	€ 1
Psychologist	€ 82	ref [7]	0.01	€ 1	0.00	€ 0
Total costs per period				€ 284		€ 461

-- Adverse events

Adverse events were scored in the EORTC and wider ET-D-010-10 trials, and the incidence and duration of adverse events were taken directly from these trials, as shown in Table 5. Adverse events were assumed equal for L-sarcoma and non-L-sarcoma patients. Disutility and costs data per adverse event were taken from the literature and converted to Dutch tariffs and 2018 price levels. In this indirect comparison, trabectedin resulted in more frequent elevation of liver enzymes compared to ifosfamide, whereas ifosfamide gave more neutropenia, with its associated febrile neutropenia. The total QALY loss due to adverse events was 0.00153 for trabectedin and 0.00352 for ifosfamide, with costs of € 1,119 and € 1,841 respectively.

-- Sensitivity analyses

To assess the sensitivity of the model for variations of key parameters, univariate sensitivity analyses were performed and presented in a tornado diagram. The difference in PFS and OS between trabectedin and ifosfamide was varied over the 95% confidence interval (95% CI) in the parametric survival analysis. The other tested variables were increased or decreased by 20%, which included costs of trabectedin, costs of ifosfamide, costs of hospitalization per day, utility pre-progression, utility post-progression and body surface area.

Results

Patient characteristics

A total of 54 patients received trabectedin after doxorubicin in the phase IV trial from December 2010 to April 2014, and a total 50 patients were included from the EORTC trials published by Nielsen et al and Van Oosterom et al. The subsets of L-sarcoma and non-L-sarcoma consisted of 57 and 47 patients, respectively, as shown in Table 1.

Survival analysis

L-sarcoma patients had a median PFS of 5.2 months on trabectedin, and 2.6 months on ifosfamide, as shown in Table 6. The difference in PFS in this indirect comparison showed a trend favoring trabectedin, but did not reach statistical significance with a p value of 0.074. In the multivariate regression the drug received continued to show a trend with a hazard ratio (HR) of 0.60 (95% CI 0.33-1.07), p value of 0.086. The median OS for L-sarcoma patients on trabectedin was 11.8 months, and on ifosfamide 8.2 months, also a non-significant difference (p value 0.184). For OS, high ECOG performance score at baseline

Table 5: Adverse events

Adverse events	Frequency of patients (%)		Average duration (days)		Disutility value	Source *
	Trabectedin	Ifosfamide	Trabectedin	Ifosfamide		
Fatigue, asthenia	2.1	2.2	5.0	5.0	0.216	ref [22]
Nausea	1.0	7.0	10.0	10.0	0.295	ref [22]
Vomiting	2.1	5.3	7.5	7.5	0.295	ref [22]
Anemia	5.3	9.6	5.0	5.0	0.098	ref [23]
Neutropenia	14.9	39.0	7.5	5.0	0.124	ref [25]
Febrile neutropenia	4.3	19.7	12.5	13.0	0.124	ref [25]
Thrombocytopenia	13.0	6.1	8.5	1.0	0.089	ref [26]
Elevation of liver enzymes	44.7	0.0	3.0	0.0	0.089	#
Alopecia	0.0	8.3	0.0	36.0	0.094	ref [25]
Neurotoxicity	0.0	5.7	1.0	1.0	0.195	ref [22]
Acute Renal Failure	0.0	1.8	0.0	16.0	0.124	ref [28]
Catheter related infection	2.1	0.1	3.0	0.0	0.161	ref [26]
						Total QALY loss

Adverse events; frequency, duration, disutility, and costs during trabectedin and ifosfamide treatment, * translated to Dutch

showed an association with reduced survival in both univariate and multivariate tests, with a HR of 1.91 (95% CI 1.06-3.45), p value 0.032, in the multivariate analysis.

For non-L-sarcoma patients receiving trabectedin the PFS was 2.0 months and for patients who received ifosfamide PFS was 3.3 months, p value 0.819. High ECOG performance score was associated with a worse PFS in both univariate and multivariate test, with a HR of 2.43 (95% CI 1.16-5.07) and p value 0.018 in the latter test. Median OS in this group was 6.0 months for trabectedin and 8.9 months for ifosfamide treatment (p value 0.903). High ECOG performance score was associated with shorter duration of OS, and in the multivariate test a HR of 2.99 (95% CI 1.44-6.20), p value 0.003.

Patients with an L-sarcoma had a PFS probability at 3 months of 59.5%, and at 6 months of 41.7% when receiving trabectedin, as shown in Table 2. In this group, a mean of 6.1 and median of 6 treatment cycles were given. Patients who had a non-L-sarcoma or who received ifosfamide had shorter survival and received fewer cycles of chemotherapy.

QALY loss		Cost per event	Source †	Total costs	
Trabectedin	Ifosfamide			Trabectedin	Ifosfamide
0.00006	0.00007	€ 153	ref [13]	€ 3	€ 3
0.00008	0.00056	€ 1,464	ref [13]	€ 15	€ 120
0.00013	0.00032	€ 1,464	ref [13]	€ 31	€ 78
0.00007	0.00013	€ 1,864	ref [24]	€ 99	€ 179
0.00038	0.00066	€ 1,329	ref [24]	€ 198	€ 518
0.00018	0.00087	€ 2,919	ref [24]	€ 6	€ 575
0.00027	0.00001	€ 3,503	ref [24]	€ 445	€ 214
0.00033	-	€ 153	ref [13]	€ 68	-
-	0.00077	€ 512	ref [27]	-	€ 42
-	0.00003	€ 1,650	ref [13]	-	€ 94
-	0.00010	€ 1,593	ref [29]	-	€ 29
0.00003	-	€ 5,920	ref [30]	€ 124	€ 6
0.00153	0.00352	**Total costs**		€ 1,119	€ 1,841

utilities, † translated to 2018 costs in euro's, #assumed similar to thrombocytopenia, ref: reference, with number

Table 6: non-parametric analysis of survival

Non-parametric survival		L-sarcoma			non-L-sarcoma		
Progression free survival							
Univariate Kaplan-Meier		median PFS	95% CI	p value	median PFS	95% CI	p value
Age			0.96 - 1.02	0.395		0.97 - 1.01	0.460
Sex	Female	3.19	0.00 - 6.62	0.621	2.89	1.76 - 4.03	0.931
	Male	4.57	2.43 - 6.71		2.30	0.81 - 3.80	
ECOG PS	0	3.68	1.08 - 6.28	0.602	3.22	0.00 - 6.45	**0.022**
	1+2	3.29	0.00 - 7.90		1.91	0.46 - 3.35	
Drug received	Trabectedin	5.19	3.31 - 7.07	**0.074**	2.04	1.52 - 2.55	0.819
	Ifosfamide	2.63	0.43 - 4.83		3.25	2.33 - 4.18	
Disease status	Local	3.29	0.00 - 7.01	0.740	3.25	0.00 - 6.80	0.875
	Metastatic	3.94	1.40 - 6.48		2.43	1.27 - 4.30	

Table 6: (Continued)

Non-parametric survival		**L-sarcoma**			**non-L-sarcoma**		
Multivariate Cox regression		HR	95% CI	p value	HR	95% CI	p value
- Drug received		0.60	0.33 - 1.07	**0.086**	1.35	0.67 - 2.74	0.403
- ECOG PS		1.11	0.63 - 1.95	0.715	2.43	1.16 - 5.07	**0.018**
Overall survival							
Univariate Kaplan-Meier		median OS	95% CI	p value	median OS	95% CI	p value
Age			0.96 - 1.02	0.498		0.97 - 1.01	0.273
Sex	Female	8.35	2.66 - 14.0	**0.071**	9.17	5.63 - 12.7	0.796
	Male	14.85	10.2 - 19.5		5.55	3.68 - 7.42	
ECOG PS	0	13.41	7.57 - 19.2	**0.033**	13.77	7.72 - 19.8	**0.008**
	1+2	8.35	3.92 - 12.8		5.23	3.64 - 6.81	
Drug received	Trabectedin	11.80	7.78 - 15.8	0.184	5.98	0.70 - 11.3	0.903
	Ifosfamide	8.22	0.00 - 21.2		8.94	5.97 - 11.9	
Disease status	Local	7.43	2.82 - 12.0	0.666	11.80	6.30 - 17.3	0.594
	Metastatic	13.41	7.85 - 19.0		6.97	2.81 - 11.1	
Multivariate Cox regression		HR	95% CI	p value	HR	95% CI	p value
- Drug received		0.66	0.37 - 1.18	0.162	1.73	0.85 - 3.50	0.128
- ECOG PS		1.91	1.06 - 3.45	**0.032**	2.99	1.44 - 6.20	**0.003**

Table 6: non-parametric analysis of survival of for L-sarcoma and non-L-sarcoma patients, univariate Kaplan Meier analysis with median survival in months and log rank test, and multivariate Cox regression. The univariate Hazard Ratio for age per year increase was 0.99 for all tests. PFS: progression free survival, OS: overall survival, 95% CI: 95% confidence interval, ECOG PS: ECOG performance score, HR: Hazard ratio

Cost-effectiveness analysis

The results from the cost-effectiveness model are shown in Table 7. Results from the parametric survival analysis were consistent with the non-parametric survival analyses. For L-sarcoma patients trabectedin produced longer PFS and OS than ifosfamide did. For non-L-sarcoma patients, ifosfamide treatment calculated to longer PFS and OS than trabectedin.

For patients with L-sarcoma the total discounted costs were € 44,879 for trabectedin, and € 24,797 for ifosfamide. Costs for trabectedin acquisition were higher than for

ifosfamide (€ 31,597 *vs* € 4,113, respectively), but drug administration costs were lower for trabectedin than ifosfamide (€ 5,298 *vs* € 13,380, respectively). The latter difference was due to longer hospitalization needed for ifosfamide cycles. The non-treatment related monthly costs were higher for trabectedin owing to longer survival compared to ifosfamide (€ 6,866 *vs* € 5,464, respectively). The costs for adverse events were lower for trabectedin than for ifosfamide (€ 1,119 *vs* € 1,841, respectively). These treatments resulted in 1.524 and 1.169 LY gained, respectively, which gives an ICER of € 56,000 per LY gained. QALYs were 1,025 for trabectedin and 0.773 for ifosfamide, leading to an ICER of € 80,000 per QALY gained.

For patients with a non-L-sarcoma ifosfamide dominated trabectedin since the costs were higher for trabectedin than ifosfamide (€ 27,497 *vs* € 22,799, respectively), while effectiveness for trabectedin was worse in terms of LYs (0.754 *vs* 1.170, respectively) and in terms of QALYs (0.516 *vs* 0.781, respectively).

Table 7: estimated average costs and effectiveness

Cost-Effectiveness Model		**L-sarcoma**			**non-L-sarcoma**	
	Trabectedin	Ifosfamide	Difference	Trabectedin	Ifosfamide	Difference
Costs (all discounted)	€ 44,879	€ 24,797	€ 20,082	€ 27,497	€ 22,799	€ 4,698
- Drug acquisition	€ 31,597	€ 4,113	€ 27,484	€ 19,407	€ 3,660	€ 15,747
- Drug administration	€ 5,298	€ 13,380	- € 8,082	€ 3,646	€ 11,941	- € 8,295
- Non-related costs	€ 6,866	€ 5,464	€ 1,402	€ 3,325	€ 5,357	- € 2,032
- Adverse events costs	€ 1,119	€ 1,841	- € 722	€ 1,119	€ 1,841	- € 722
Effectiveness						
- QALYs, discounted	1.025	0.773	0.251	0.516	0.781	- 0.265
- Pre-progression LYs, undiscounted	0.646	0.423	0.223	0.386	0.476	- 0.090
- Post-progression LYs, undiscounted	0.902	0.758	0.144	0.371	0.694	- 0.323
Cost-Effectiveness Ratio's						
Costs per LY gained		**€ 56,000**			**Ifosfamide dominant**	
Costs per QALY gained		**€ 80,000**			**Ifosfamide dominant**	

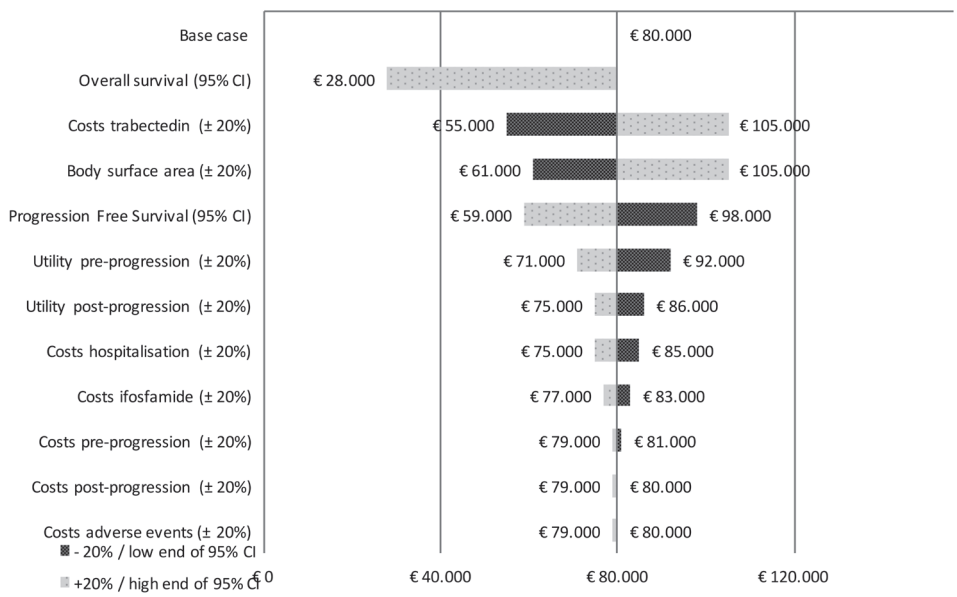
Estimated average costs and effectiveness, comparing trabectedin and ifosfamide in advanced L-sarcoma and non-L-sarcoma. QALY: quality adjusted life years, LY: life years.

Sensitivity analyses

The sensitivity analysis of L-sarcoma showed the ICER to be most affected by the difference in survival between trabectedin and ifosfamide, as shown in Figure 1. This effect was most prominent in OS. The 95% CI of the difference in OS for trabectedin and ifosfamide was -3.6 to 18.4 months and this meant an overlap of OS duration. This resulted in ICER ranging from € 28,000 per QALY gained in favor of trabectedin, to ifosfamide being dominant for OS. The ICER across the 95% CI of PFS also varied substantially, but QALYs remained in favor of trabectedin, with the ICER ranging from € 59,000 to € 98,000 per QALY gained. Another clear influence on ICER variation was the cost of trabectedin, with the ICER ranging from € 55,000 to € 105,000.

As the base-case analysis showed ifosfamide to dominate trabectedin in patients with non-L sarcoma, a sensitivity analysis for non-L-sarcoma was not performed.

Figure 1: Tornado diagram representing the univariate sensitivity analysis for L-sarcoma, numbers abbreviated to thousands.



All variables other than survival were increased (light shaded bars) or decreased (dark shaded bars) by 20%. For progression free survival and overall survival the 95% confidence interval (95% CI) of the survival difference between trabectedin and ifosfamide was used (low end: light shaded bars, high end; dark shaded bars). Note that the bar for the low end of the difference in OS does not stop and no number is given, as ifosfamide dominated trabectedin at that point. OS: overall survival, PFS: progression free survival.

Discussion

Trabectedin was shown to be an active drug in the second line treatment of L-sarcomas (either leiomyosarcoma or liposarcoma). In this non-randomized comparison, the median survival of patients with L-sarcomas was 2.5 months longer if they received trabectedin instead of ifosfamide, not meeting the criterion for statistical significance (p=0.074). In non-L-sarcoma ifosfamide resulted in longer survival, but the difference was not significant. The cost-effectiveness analysis of trabectedin compared to ifosfamide showed an ICER of € 80,000 per QALY gained in case of L-sarcoma. For non-L-sarcoma, ifosfamide dominated trabectedin as ifosfamide costs were lower but survival and QALYs gained higher compared to trabectedin treatment. Survival differences and trabectedin acquisition costs had the strongest impact on the ICERs found. Future changes in trabectedin pricing would alter the ICER. However, given the status of trabectedin as 'orphan drug' due to the low incidence of malignancies trabectedin is currently registered for, its price is not expected to change in the foreseeable future.

When this cost-effectiveness analysis was designed, a comparator group was sought that could provide for a sensible comparison to second line trabectedin. Ifosfamide was chosen as this drug was widely tested in STS and data for second line treatment was available at the EORTC. Due to the adverse events and the long hospital admission per treatment cycle, this drug has been used less extensively over last decade and alternatives are available. In terms of expected anti-tumor effect, ifosfamide was still considered to represent a realistic comparator group. Additionally, potential alternative data sets would not match the patient population of the trabectedin treated patients.

This cost-effectiveness study was not a randomized comparison, contrary to the designs of the original ifosfamide studies. To reduce bias, survival was counted from the moment of first drug infusion, not the moment of trial inclusion as in the original trials. This was done to evade a potential bias, wherein the duration of survival of either ifosfamide or trabectedin would have been longer due to effects other than drug effect. Therefore, the difference in survival now reported is accurately reflecting survival following treatment. The EORTC STBSG has used progression free rates (PFR) as an indicator whether a drug is active as an second line agent in STS.[9] Agents considered active, have an estimated PFR at 3 months of 39% and at 6 months of 14%. For L-sarcoma, trabectedin showed, by this standard, to be an active drug in this population with a PFR of 59% and 42%, respectively. For non-L-sarcoma, trabectedin was less potent with a PFR at 3 months just below the threshold at 37%, and PFR at 6 months at 19%. The PFR's for ifosfamide were above the EORTC STBSG number in both L-sarcoma as non-L-sarcoma.

Several studies have previously investigated the cost-effectiveness of trabectedin in STS compared to other treatments. In a 2011 study by Soini et al, trabectedin was

compared to ifosfamide. Trabectedin data was taken from a 2009 randomized trial comparing trabectedin treatment regimen and ifosfamide data from the same studies by Van Oosterom and Nielsen used in the current study.[2,10] All patients on trabectedin had an L-sarcoma, whereas sarcoma subtypes were not clear for patients ifosfamide. The study found an ICER per LY gained of € 31,590 and € 42,633-47,735 per QALY gained when prescribing trabectedin. These ICER's are lower than in the current study, suggesting better trabectedin cost-effectiveness. The most evident cause for this difference is the higher survival benefit due to ifosfamide in the current study compared to Soini et al. (1.17 LY *vs* 0.60 LY, respectively), whereas there were higher costs of ifosfamide treatment in Soini et al. (€ 13,053-14,286 *vs* € 7,568, respectively). The difference in survival gained due to ifosfamide, even though these are taken from the same studies, suggests a difference in patient selection between the cost-effectiveness studies.

A 2013 indirect comparison into the cost-effectiveness of doxorubicin-ifosfamide combination *vs* trabectedin also showed more QALYs gained at lower health care costs for doxorubicin-ifosfamide.[11] A pooled patient cohort from four phase II studies of patients receiving trabectedin for advanced STS was used in a 2015 study comparing the cost-effectiveness of trabectedin and the tyrosine kinase inhibitor pazopanib.[12] The HR calculated was 1.11 in favor of pazopanib (with 95% CI of 0.94 - 1.31). Pazopanib treatment costs were half the cost of trabectedin cycles. As pazopanib is oral medication which is taken without the need for hospital admissions, the majority of patients will prefer pazopanib for that fact alone, regardless of costs. A study comparing pazopanib to placebo in advanced STS patients resulted in an ICER of € 77,120 per QALY gained when taking pazopanib treatment, illustrating the high costs of therapies aimed at treating advanced STS.[13]

Compared to the 2016 randomized phase III trial by Demetri et al comparing trabectedin *vs* dacarbazine in pre-treated metastatic L-sarcoma patients, patients on trabectedin in the current study in a real life setting had a higher median PFS (4.2 *vs* 5.2 months respectively), whereas OS was slightly lower (12.4 months *vs* 11.8 months, respectively).[14] A possible explanation for the PFS difference is the blinded radiologic evaluation of imaging to asses PFS in the randomized trial. The efficacy of trabectedin *vs* dacarbazine showed better PFS for trabectedin but equal OS.[14] Unfortunately, this trial did not include QALY assessments.

A study by Le Cesne at al. presented at the 2018 ASCO meeting randomized pre-treated advanced STS patients between trabectedin and BSC, giving the comparison originally attempted for this cost-effectiveness analysis.[15] In that trial, trabectedin showed better PFS than BSC for L-sarcomas (5.3 *vs* 1.4 months, respectively), but not for non-L-sarcomas (1.8 *vs* 1.5 months, respectively). OS did not differ and this was deemed due to per-protocol crossover to trabectedin after progressive disease on BSC. This trial

demonstrates the efficacy of trabectedin for L-sarcomas compared to BSC. The efficacy of trabectedin within the group of L-sarcomas also varies and it offers the largest benefit in patients with myxoid liposarcoma.[16] The actual size of the anti-tumor effect in myxoid liposarcoma is blunted in clinical trials as other liposarcoma subtypes, in which trabectedine is less active, are included in the same trials. The number of patients in this analysis was too small to detect any differences between leiomyosarcomas *vs* liposarcomas or myxoid liposarcomas *vs* other liposarcoma subtypes.

This cost-effectiveness analysis has several limitations, especially since it was not possible to perform the study originally set out to do. The number of included patients was constrained by the number of eligible patients in the ET-D-010-10 and EORTC trials. The criteria for response evaluation were slightly different, but this was considered not to have an impact on the study's conclusion. The non-randomized nature of the comparison may have introduced bias, especially since the sensitivity analysis showed that the estimated survival difference was the most influential variable in the analysis. The correction for ECOG performance score was enacted to reduce this potential bias. Nevertheless, the p-values that are reported in the survival analysis disregard the non-randomized nature of the data.

The use of data on patients treated with ifosfamide did provide a sensible alternative, but those patients were treated some twenty years before the patients who received trabectedin. In those years, experience with safely administering ifosfamide has increased, probably leading to lower adverse events rates than those used in the current study. This may constitute a bias in favor of trabectedin in the study. Other possible explanations for the difference in survival include additional treatment options developed since the ifosfamide trials were performed and advancements in supportive and palliative care.

This study was performed for a Dutch health care setting with chemotherapy given during hospital admissions. Administrating trabectedin in an outpatient setting using ambulatory pump is also possible.[17] This method of administration would be less costly and will affect the ICER in favor of trabectedin therapy. However, this method is currently not standard in the Netherlands. Obviously, the prices of health care items will differ in other countries and the ICER may be different as a result.

Conclusions

Trabectedin was shown to offer a non-significant survival gain compared to ifosfamide for L-sarcoma and this results in an estimated ICER of € 80,000. This ICER is at the top end of what is generally considered acceptable in the Netherlands.[18] As there is a clinically unmet need for anti-tumor agents in the group of rare malignancies, this threshold may

not be the most relevant factor in the decision to continue to prescribe trabectedin to these patients. For non-L-sarcoma, ifosfamide treatment dominated trabectedin.

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9

Central venous access related adverse events after trabectedin infusions in soft tissue sarcoma patients; experience and manage-ment in a nationwide multi-center study

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Abstract

Background

Trabectedin has shown efficacy against soft tissue sarcomas (STS) and has manageable toxicity. Trabectedin is administered through central venous access devices (VAD), such as subcutaneous ports with tunneled catheters, Hickman catheters and PICC lines. Venous access related adverse events are common, but have not yet been reported in detail.

Methods

A retrospective analysis of patient files of STS patients receiving trabectedin monotherapy between 1999 and 2014 was performed in all five STS referral centers in the Netherlands. This survey focused on adverse events related to the VAD and the actions taken in response to these events.

Results

In the 127 patients included in this analysis, 102 venous access ports (VAP), 15 Hickman catheters and 10 PICC lines were used as primary means of central venous access. The most frequently reported adverse events at the VAD site were erythema (30.7%), pain (28.3%), inflammation (11.8%) and thrombosis (11.0%). Actions taken towards these adverse events include oral antibiotics (17.3%), VAD replacement (15.0%) or a wait-and-see policy (13.4%). In total, 45 patients (35.4%) with a subcutaneous port developed a varying degree of inflammation along the trajectory of the tunneled catheter. In all but three patients, this was a sterile inflammation, which was considered a unique phenomenon for trabectedin. Microscopic leakage of trabectedin along the venous access device and catheter was considered the most plausible cause for this adverse event. Placing the catheter deeper under the skin resolved the issue almost completely.

Conclusion

Trabectedin infusion commonly leads to central venous access related adverse events. Sterile inflammation along the catheter trajectory is one of the most common adverse events and can be prevented by placing the catheter deeper under the skin.

Background

Cytostatic drugs infused directly into peripheral veins can have very damaging effects on these blood vessels. To ensure safe and durable administration of such agents, several methods have been developed in the past, like the arteriovenous shunt, which is no longer used for the infusion of chemotherapy.[1] In 1982, a central venous access device was introduced, that used a subcutaneous reservoir and a tunneled catheter to provide access to the superior vena cava.[2] This type of central venous catheters (CVC) allows for easy access to a patient's circulation, incur minimal restriction in normal activities and usually at a low risk of complications.[3] Next to venous access ports (VAP), other methods have also been introduced, such as the Hickman catheter and peripherally inserted CVC (PICC) lines.[4,5] However, all devices constitute some risk of venous access related adverse events (VARAE).

As anticancer drug, trabectedin stands out as a drug with a unique mechanism of action, having effect both at the level of tumor DNA and on the tumor microenvironment.[6] It is one of the few drugs active in STS.[7] The drug has a manageable toxicity profile, but life-threatening toxicity due to uncommon adverse events has been reported.[8] Thus far several papers have mentioned VARAE, including reports on trabectedin extravasation and associated thrombi on the line tip, but no papers focusing on VARAE in detail have been published.[9-12] This article aims to systematically study VARAE observed in 127 consecutive sarcoma patients treated with trabectedin and to evaluate the measures taken to handle these problems.

Methods

A retrospective analysis of VARAE in all patients treated with trabectedin was performed in all five participating Dutch sarcoma referral centers: the Leiden University Medical Center (LUMC), the Netherlands Cancer Institute Antoni van Leeuwenhoek (NKI-AvL), the Erasmus MC Cancer Institute (EMC), the Radboud University Medical Center (RUMC) and the University Medical Center Groningen (UMCG). Patients were eligible when treated with trabectedin monotherapy for advanced STS. Data on patient characteristics were reported as well as the type of venous access device, its placement, adverse events related to its usage and the interventions to counter these events. Adverse events related to the venous access devices (VAD) placement were ignored, as these have no direct relation with trabectedin infusions. Hence, all events described occurred after at least one cycle of trabectedin had been given.

To test for a difference in the number of cycles per VAD, a Kruskal-Wallis non-parametric test was used. To assess differences between VARAE per VAD cross tables and the chi-square were computed. All statistical analyses were performed using SPSS version 20.

Results

Patients

In total, 127 advanced STS patients were treated with single agent trabectedin between November 1999 and November 2014. Almost all patients were treated as part of an observational phase IV study or of the TRUSTS trial.[13,14] Due to the inclusion criteria of these studies, trabectedin was given either as first line (15.0%), second line (59.1%), third line (16.5%), fourth line (7.1%) or as a further line of treatment (2.4%). The trabectedin treatment regimen was given at a dosage of 1.5 mg/m^2 as 24 hour infusion every three weeks in 89.8% of patients, the remaining patients received a lower dose (1.1 to 1.3 mg/m^2) and/or a 3 hour infusion. The most prevalent types of STS histology were leiomyosarcoma (41%), liposarcoma (26%) and synovial sarcoma (12.6%), as shown in Table 1.

Table 1: patient characteristics

		N (%)
Sex	Female	66 (52.0)
	Male	61 (48.0)
Age	Median (years)	54.3
	Range (years)	25.6 - 79.5
WHO performance score	0	52 (40.9)
	1	66 (52.0)
	2	9 (7.1)
Histology	Leiomyosarcoma	52 (40.9)
	Liposarcoma	33 (26.0)
	Synovial sarcoma	16 (12.6)
	Various others	26 (20.5)
Best response	Partial response	8 (6.3)
	Stable disease	64 (50.4)
	Progressive disease	45 (35.4)
	Not evaluable	10 (7.9)
Hospital	LUMC	48 (37.8)
	NKI-AvL	40 (31.5)
	EMC	15 (11.8)
	RUMC	12 (9.4)
	UMCG	12 (9.4)

VADs inserted

The VAP was used in 102 (80.3%) patients, of which 87 were identified as a Smith Medical Port-a-Cath®. Hickman catheters and PICC lines were inserted in 15 (11.8%) and 10 (7.9%) of patients, respectively. A total of 540 cycles of trabectedin were given with a median number of 4 cycles for the entire patient group. The number of cycles given did not differ significantly per VAD (data not shown).

Each hospital had a clear preference for a particular type of VAD that was initially inserted; in the LUMC VAPs (100% of patients), in the NKI-AvL VAPs (95%), in the EMC the Hickman catheter (100%), in the RUMC a PICC line (66.7%) and in the UMCG VAPs (100%). VADs were inserted by a dedicated team of health care workers to ensure low incidence of complications related to the VAD placement.

Of all patients, only three patients with a Hickman catheter requested their VAD to be replaced by another type of VAD. Two of these patients preferred a VAP, but did not have a VARAE at the time of replacement. In another patient, the catheter was chronically obstructed due to a thrombus at the catheter tip, which required catheter flushing by a radiologist, despite adequate antithrombotic treatment.

Sterile inflammation along the catheter trajectory

Out of the 127 patients, 45 patients (35.4%) with a VAP developed a varying degree of inflammation along the catheter trajectory, which could include erythema, pain or swelling, as shown in Figure 1. In between cycles these symptoms waned, but a few days after the following infusion a flare up was often noted. Fever was neither reported by patients, nor observed during physical examination at admission or at the outpatient clinic. The skin surrounding the port's reservoir was not affected and the VAD could be used for infusions normally. Bacterial cultures could not identify an etiological micro-organism for these symptoms in all, but three patients.

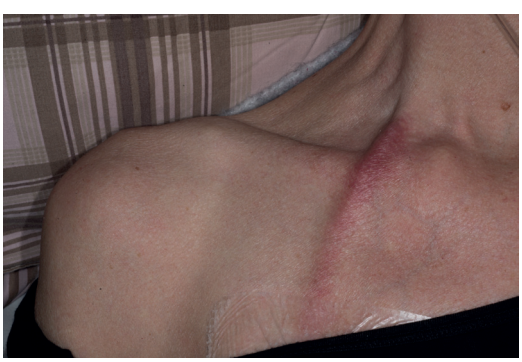

Figure 1: Typical sterile inflammation along the venous access port catheter trajectory

In the first instances these symptoms were deemed a result of cellulitis and oral antibiotics were prescribed (flucloxacillin 500mg four times daily). However, the symptoms abated only mildly and the erythema remained unchanged for weeks and existed even after the discontinuation of trabectedin therapy. Extra intravenous infusion of normal saline fluids during trabectedin infusion appeared to ease the symptoms, especially the pain.

In a single patient with port VAD, the inflammation became rampant and in the course of several weeks it lead to severe skin erosion along the catheter trajectory, as shown in Figure 2. At progression of the inflammatory aspect of the skin, the patient was treated with oral antibiotics. Due to the skin destruction, a local secondary cellulitis developed. Despite this, the patient did not feel ill. As the patient did not show symptoms of acute infection, it was decided to continue trabectedin treatment. After trabectedin was stopped due to progressive disease, the VAP remained in place and was used for dacarbazine cycles without VARAE.

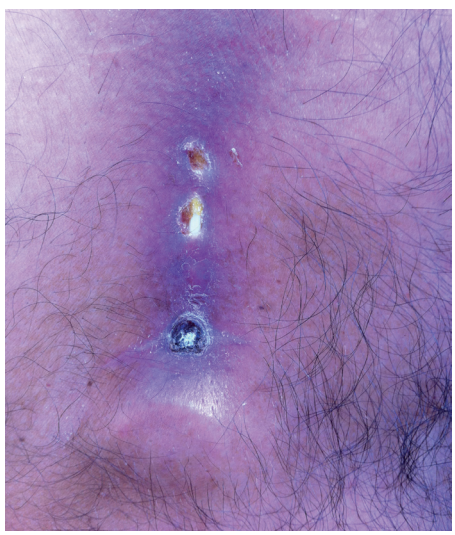

Figure 2: Skin erosion along Venous Access Port catheter trajectory due to sterile inflammation

Remarkably, this complication appeared only in patients from one hospital and only after receiving several trabectedin cycles, and did not occur with any other type of cytostatic agent. As the same brand and type of VAD was used in another hospital without this complication, the dedicated teams compared their respective methods of VAD insertion. The only apparent difference found, was in the depth of the subcutaneous insertion for tunneling the catheter. Catheter insertion can be performed

more or less deeper under the skin and the latter method was associated with the sterile inflammation along the catheter trajectory. Upon changing the local protocol to deepen the tunneling of the catheter, no further events of sterile inflammation of the catheter trajectory were observed.

Adverse events related to VAD

All types of VADs used had VARAE, as shown in Table 2. For the whole patient cohort, the most common adverse events were erythema (30.7%) and pain (28.3%) at the VAD site or along the catheter trajectory. In 11.8% of patients these symptoms were diagnosed as an inflammation and/or infection, where inflammation consisted of swelling, painfulness and erythema. Blood cultures did not grow pathogenic micro-organisms. In some of these patients the 'infection' diagnosis could retrospectively be reclassified as the previously described sterile inflammation with near certainty. Several patients (11.0%) had a thrombus at the catheter tip at one or several instances. Often, these thrombi could be flushed with urokinase solution before proceeding to administer the trabectedin infusion without further complications. However, catheter thrombosis could also lead to VAD impairment. Remarkably, all of these patients were treated in the same hospital, which was also the hospital were VAPs were inserted with tunneled catheters deep in the subcutis. Thrombosis at the catheter tip and the sterile inflammation were not significantly associated (data not shown). The skin erosion and extravasation of trabectedin were seldom seen. Dislocation or pinch-off was not seen in any patient. Due to the small number of patients with a Hickman catheter or PICC line, no statistical differences in the incidence of VARAE could be detected. Only a single patient (0,8%), who had a Hickman catheter, had an extravasation.

Interventions for VARAE

Oral antibiotics were given in 17.3% of patients, most often flucloxacillin, as shown in Table 3. Some patients received a prescription for oral antibiotics to be taken in case VAD related symptoms worsened. Although this was not sufficient to stop the erythema along the catheter trajectory, it may have helped against a secondary infection. In 5 patients (3.9%) VAD an infection necessitated IV antibiotics (2 patients with a VAP, 3 patients with a PICC line). Due to the severity of symptoms or VAD impairment VAD replacement was needed in 15.0% of patients. Patients with a VAP usually had the same type of VAP inserted at the contralateral side, patients with a Hickman catheter or PICC line most often received a VAP. As the problem of the sterile inflammation and other VARAE were better understood and recognized, in due course a wait-and-see policy was applied in a considerable number of patients (13.4%). Despite frequent complaints of pain at the VAD site, analgesics were only needed in a minority of these patients.

Table 2: adverse events per venous access device

N (%)	Inflammation	Erythema	Pain	Infection	Thrombosis	Impairment	Erosion	Extravasation	All AE *
Venous Access Port (102)	35 (34.3)	30 (29.4)	28 (27.5)	9 (8.8)	11 (10.8)	5 (4.9)	1 (1.0)	0 (0.0)	44 (43.1)
Hickman line (15)	5 (33.3)	4 (26.7)	4 (26.7)	3 (20.0)	1 (6.7)	3 (20.0)	0 (0.0)	1 (6.7)	7 (46.7)
PICC line (10)	5 (50.0)	5 (50.0)	4 (40.0)	3 (30.0)	2 (20.0)	1 (10.0)	0 (0.0)	0 (0.0)	5 (50.0)
TOTAL	45 (35.4)	39 (30.7)	36 (28.3)	15 (11.8)	14 (11.0)	9 (7.1)	1 (0.8)	1 (0.8)	56 (44.1)

Adverse events at venous access devices site/trajectory per venous access device, * summary of all types of adverse events (AE) per venous access device

Table 3: interventions per venous access device

N (%)	Antibiotics (oral)	VAD replaced	Wait-and-see	Analgesics	Urokinase (IV)	Antibiotics (IV)
Venous Access Port (102)	19 (18.6)	10 (9.8)	13 (12.7)	8 (7.8)	8 (7.8)	2 (2.0)
Hickman line (15)	1 (6.7)	6 (40.0)	3 (20.0)	2 (13.3)	1 (6.7)	0 (0.0)
PICC line (10)	2 (20.0)	3 (30.0)	1 (10.0)	0 (0.0)	0 (0.0)	3 (30.0)
Total	22 (17.3)	19 (15.0)	17 (13.4)	10 (7.9%)	9 (7.1)	5 (3.9)

Interventions for venous access devices related adverse events per venous access device, IV = intravenous treatment

Discussion

Trabectedin is one of the proven active drugs in the treatment of soft tissue sarcoma and is given through a central venous catheter to avoid peripheral vein damage. As treatment continues until progressive disease or unacceptable toxicity, is it important to evaluate catheter related complications. The sterile inflammation along the catheter trajectory found in this study was an unexpected VARAE and was initially poorly understood. Erythema or pain is usually taken as a sign of skin infection and treated as such. However, there were no other signs of infection such as positive cultures, and the severity of the skin complications appeared to be related to the administration of trabectedin. In addition, the erythema was most prominent along the catheter trajectory, which made a porous catheter likely to be the cause. A direct effect of trabectedin on the tissue surrounding the catheter could cause the inflammation, but this catheter porosity implies that only a small quantity of trabectedin permeates. This small quantity leads to fewer symptoms compared to a full trabectedin extravasation, as has been reported in literature.[11]

To investigate the hypothesis of catheter porosity, the manufacturer of trabectedin, PharmaMar, offered to test a used catheter. A VAP was available that was previously used in a patient who had received several cycles of trabectedin with symptoms of sterile inflammation alongside the catheter trajectory and from whom the VAP was removed because of disease progression. The objective of the test was to determine if trabectedin permeates from the internal surface to the outside of the catheter during a 24 hour infusion. High-performance liquid chromatography with diode array detection (HPLC-DAD) and multi-syringe flow injection system (MS-FIA) methods were used for detection of trabectedin in the dextrose 5% solution the VAP was submerged in. Neither test could detect trabectedin in samples taken from the dextrose 5% solution, which ruled out gross catheter porosity (PharmaMar communication). In our view, however, this could not rule out sub lower-limit of quantification leakage.

Non-infectious inflammation of the VAD site of various severity was also reported by Hoicyk et al, in addition of thrombi at the catheter tip. It was hypothesized that increased resistance due to small thrombi may be associated with drug backspill.[12] In the current study, neither an association of sterile inflammation and thrombosis was found, nor was reduced flow through the catheter observed. Catheter thrombosis occurred in several patients, which was treated by flushing the catheter with an urokinase solution. Thrombosis prophylaxis was not initiated at the start of trabectedin therapy in any of the participating centres.

In the patient cohort only a small number of patients had PICC lines. A larger retrospective series of STS and ovarian cancer patients was reported by Martella and

colleagues . Out of 45 patients with a PICC line receiving trabectedin a device dislocation was reported in two patients and an infection in another two. PICC line malfunction or VARAE requiring VAD removal did not occur.[10] This implies that PICC lines may have lower incidence of associated toxicity than our current cohort suggests. However, the number of VARAE in patients using a PORT was also lower. Due to the retrospective nature of this patient series, relative underreporting compared to our study may have occurred, as almost all patient in this cohort where treated as part of a clinical trial.
The usage of a disposable elastomeric pump to administer a 24-hour trabectedin infusion has been described.[9] Patients could choose for a regular VAP or a Baxter LV10 Pump which allowed patients to spend the night at home. Out of 28 patients 21 chose the ambulatory pump. This method was considered feasible and safe. However, most patients will receive trabectedin trough conventional VAPs reported on in this paper, and no data is available comparing these different techniques.

Compared to published safety data, the rate of observed trabectedin extravasation of 0.8% in our series was similar to 0.5% reported in large pooled analysis of 1132 patients who received single agent trabectedin.[8]

Conclusions

Despite the use of central venous access devices, trabectedin can cause local sterile inflammation along the catheter trajectory, in particular in venous access ports. Positioning the port's catheter deeper in the subcutis appears to be the most efficient way to prevent this complication.

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10

General discussion

This thesis on systemic treatment options in soft tissue sarcoma (STS) is divided into a part on the pharmacogenetics of systemic treatment for gastrointestinal stromal tumors (GISTs) and a part on the use of trabectedin in the treatment of STS, as noted in the general introduction (**chapter 1**).

Part I: Pharmacogenetics of systemic GIST-treatment

In this thesis single nucleotide polymorphisms (SNPs) have been investigated for associations with clinical outcome with regard to survival and toxicity in the treatment of GISTs. Additionally, in one study SNPs are associated to standardized imatinib trough levels. These were exploratory studies, using a rational candidate gene approach in a substantial numbers of patients recruited in referral centers in the Netherlands. The drugs of interest were imatinib and sunitinib; tyrosine kinase inhibitors (TKIs) which are the registered first- and second-line of systemic treatment in case of advanced GIST (**chapter 2**).

The study on the efficacy of imatinib in 227 advanced GIST patients treated with 400mg imatinib once daily (**chapter 3**) was the largest study in which the pharmacogenetics of imatinib was explored. It showed germline SNPs in *VEGFA* (rs1570360), in *KDR* (also known as VEGFR2, rs1870377) and in *SLCO1B3* (rs4149117) to be associated with worse progression free survival (PFS). Furthermore, synchronous metastases and somatic *KIT* exon 9 mutations were also associated with worse PFS, whereas somatic *KIT* exon 11 mutations led to longer PFS, in line with previous reports.[1] As an imatinib dose of 400mg once daily is considered too low for patients with a *KIT* exon 9 mutation by the standards of today, this was corrected for in the multivariate analysis. An Italian study also investigated imatinib efficacy in GIST and it explored 31 germline SNPs in 54 GIST patients.[2] In that study, independent of tumor mutational status, tumor size, age and sex, SNPs in SLC22A4 (rs1050152) and SLC22A5 (rs2631367 and rs2631372) were associated with PFS, but not with rs4149117. These SNPs were also tested in this chapter, but univariate analysis did not show associations with survival.

SNPs in *VEGFA* were included this chapter, as high VEGF (vascular endothelial growth factor) expression has been associated with a shorter PFS during imatinib therapy, and imatinib can lead to reduced VEGF expression in some GIST patients.[3] This could help to explain the role of rs1570360. VEGF plays a crucial role in inducing angiogenesis. The antiangiogenic properties of sunitinib have clearly been described, but imatinib may apparently also have comparable effects.[4] Tumor micro-vasculature and its density may also be influenced by germline SNPs, such as rs1870377 in *KDR*.[5] Micro-vasculature and its density may make GIST better accessible to serum imatinib and it also confers increased nutrient and oxygen supply. This *KDR* SNP has also been associated to increased GIST susceptibility, suggesting a role of VEGF in the pathophysiology of GIST.[6]

Only one SNP related to imatinib pharmacokinetics was associated to survival; rs4149117 in *SLCO1B3*. This gene encodes for the drug-influx transporter SLCO (Solute carrier organic anion transporter family) with imatinib as a substrate and the *T* allele has been associated with higher intracellular imatinib levels and response.[7-9] The other SNPs in the pharmacokinetic pathway (such as *ABCB1*, *ABCG2*, *SLC22A1*, *SL22A5* or *CYP3A4*) were not associated with survival. The effects of these SNPs could be absent or too small to affect imatinib serum level in a manner profound enough to influence survival.

The study on the efficacy of sunitinib in patients with advanced GIST (**chapter 4**) showed survival to be associated with SNPs in *SLCO1B3* (rs4149117), in *POR* (1056878), in *SLC22A5* (haplotype consisting of rs2631367, rs2631370 and rs2631372) and in *IL4R* (haplotype consisting of rs1801275 and rs1805015). Synchronous metastases were associated with reduced survival in these 127 patients, as it was for imatinib efficacy. The primary somatic mutation did not show an association with survival in this study, even though primary mutations have previously been found predictive for sunitinib efficacy in GIST.[10]

Several SNPs in the pharmacokinetic pathway of sunitinib were associated with survival. Sunitinib is metabolized by cytochrome P450 (CYP) 3A4 and 3A5. The activity of these CYP-enzymes are regulated by *P450 oxydoreductase* (POR). A SNP in *POR*, rs1056878, was associated with PFS, but not overall survival (OS). This SNP has been associated with reduced CYP3A5 and CYP1A2 activity.[11] Possibly, patients with this SNP have higher sunitinib trough levels due to lower enzyme activity and better survival as a result. SNPs in *ABCB1* and *ABCG2* were not associated with survival on sunitinib, despite being sunitinib efflux transporters and despite previous associations of these SNPS with imatinib efficacy.[12]

Survival on sunitinib was associated with a haplotype in *SLC22A5* (consisting of rs2631367, rs2631370 and rs2631372) which encodes for the transporter OCTN (organic cation transporter novel) type 2. The OCTN group of transporters facilitates intracellular imatinib uptake, but such a role for the uptake of sunitinib was not observed in mouse models.[13,14] SNPs found in *SLC22A5* may be more of a prognostic nature, instead of being predictive for sunitinib effect.

A suggestion of an effect on survival in case of advanced GIST is given by the univariate association of rs4149117 in *SLCO1B3* with imatinib PFS, imatinib OS and sunitinib OS. This association did not remain significant in the multivariate analyses. Organic anion-transporting polypeptide 1B3 (OATP1B3) is the drug transporter encoded for by *SLCO1B3* and has a broad set of TKIs as substrate, with the exception of sunitinib.[15] The same explanation may be given for these results as for *SLC22A5*, being either due to doubtful role of this transporter for sunitinib or the prognostic role of these SNPs.

The study on the toxicity of imatinib in patients with GIST patients treated with 400mg imatinib once daily (**chapter 5**) showed SNPs in *ABCG2* (rs2231137) and in *CYP1A2* (rs762551) to be associated with the need for dose reduction. These results remained statistically significant after correction for the 36 SNPs tested. Dose reduction and treatment cessation were used as primary endpoints as these are considered clinically relevant. In the 315 included GIST patients, dose reduction was needed in about one in ten patients. Older patients experienced more toxicity, in line with previous studies.[16,17] Patients received treatment in a neo-adjuvant, adjuvant or palliative setting. As most instances of dose reduction occurred early in treatment, an indirect effect on the somatic mutation in the GIST was considered negligible.

The rs2231137 SNP in *ABCG2* was associated with the need for dose reduction. Imatinib is known to be a substrate for the transporter ABCG2 (ATP-binding cassette sub-family G member 2) and the SNP encoding for the transporter has been associated with enhanced response in GIST patients.[18,19] The effect on toxicity could be a result of increased intracellular imatinib levels, as this SNP was not associated with imatinib trough levels in two groups of ethnic Asian patients.[19,23] The rs2231142 SNP in *ABCG2* has also been frequently studied in patients receiving imatinib, but it was not associated with the need for dose reduction in this chapter.

The rs762551 SNP in *CYP1A2* was associated with dose reduction. This effect was seen despite a possible compensatory effect of other CYP-enzymes in the metabolization of imatinib. Patients with this SNP have a slow acting CYP1A2 enzyme, conceivably resulting in higher imatinib serum levels, and thus causing increased toxicity.

None of the tested SNPs were associated to the endpoint of cessation of imatinib due to toxicity. Lack of events due to the relatively mild toxicity profile of imatinib can be one reason. Another reason could be that this event is prevented in patients with a high burden of toxicity, by reducing the dose well in advance of toxicity to become so severe that imatinib is stopped.

The study into imatinib serum levels in patients with GIST or chronic myeloid leukemia treated with imatinib (**chapter 6**) did not show SNPs in *CYP2C8* to be associated with standardized imatinib trough levels. In vitro studies of CYP2C8 have shown reduced activity of CYP2C8 variants compared to wild type CYP2C8, but this was not seen in vivo studies.[20]After the assumed auto-inhibition of the primary metabolic pathway via CYP3A4, imatinib is then to be metabolized by CYP2C8.[21] CYP2C8 is just one the CYP-enzymes, and it could be that other CYP enzymes develop a more prominent role after CYP3A4 auto-inhibition in imatinib metabolization and subsequent effect on trough levels.[22] The association of increased imatinib standardized trough level and age was in line with a previous report.[23]

Several comments can be made regarding these pharmacogenetic studies in general. At the beginning of the research leading to this thesis, it could be hypothesized that the same SNPs in the pharmacokinetic pathway would be associated with survival on both imatinib and on sunitinib. These drugs are both TKIs, and both are metabolized by CYP3A4 and CYP3A5, but not all transporters and enzymes overlap. Only a SNP in the pharmacodynamic pathway was associated with PFS when tested univariately for both drugs. For the *KDR* SNP rs1870377, the association with the mutant AA genotype and PFS remained significant in the multivariate analysis for imatinib, but not for sunitinib. OS was associated with rs4149117 in *SLCO1B3* for both imatinib and sunitinib in the univariate analyses, but not in the multivariate model. This SNP could be prognostic for survival in GIST, as sunitinib is thus far not observed to be a substrate for OATP1B3, the transporter the *SLCO1B3* gene encodes. The rs1570360 SNP in *VEGFA* could also be associated to both imatinib and sunitinib efficacy, but the mutant homozygote group in the sunitinib analysis consisted of only a single patient. That means no definite conclusions can be made regarding rs1570360 in this setting, other than the rarity of homozygous patients, despite the number of patients included in these studies.

A strength of the pharmacogenetic studies is the number of patients analyzed, considering the relative low incidence of GIST. This was made possible by the multicenter approach in which four of the five Dutch GIST referral centers cooperated. This allowed for an increased number of patients and thus more SNPs to be reliably tested. Nevertheless, the number of patients was not sufficient to adhere to the rule of thumb to have at least ten patients per SNPs analyzed. In the study concerning imatinib toxicity (chapter 5) results held even after correction for multiple testing. Still, those results need to be validated, particularly as in that study the clinical endpoint of the need for dose reduction was used.

To allow for a biologic rationale, selected SNPs needed to have expected functionality. However, it is possible that SNPs that were found to be associated with clinical outcome were actually independent prognostic biomarkers. These SNPs may results some sort of altered protein function, but that does not directly imply a causal relationship. Nonetheless, the SNP selection is a crucial element in this type of research.

The SNPs were selected using the candidate gene approach. Other methods of pharmacogenetic research include the use of a large single array of pre-selected SNPs such a DMET pack (Drug Metabolizing Enzymes and Transporters). This DMET includes 1936 SNPs in genes active in drug absorption, distribution, metabolism, and excretion. Only one study has thus far used a DMET in the analysis of imatinib pharmacogenetics.[24] It did include advanced GIST patients treated with imatinib 400mg once daily, but the number of 49 patients is too low for the 482 SNPs investigated. Furthermore, instead of the general genetic model, both the dominant and recessive model were used.

Therefore, any result this study yielded, has to be treated with substantial caution. Despite the low incidence of GIST, the pharmacogenetic studies in this thesis have shown that performing DMET studies with adequate number of patients is possible and could well yield interesting results.

In the candidate gene approach, SNPs had to have a minimum minor allele frequency of 0.1, to ensure sufficient number of patients having mutant type alleles. This was required for statistical considerations as enough events, whether disease progression or severe adverse events, are needed to determine if differences between groups are statistically significant. Furthermore, a very rare SNP, while having a marked but not dichotomous effect on outcome, will not hold its value in a clinical setting due to is rarity. Only until upfront genome wide pharmacogenetics becomes feasible and affordable, will elucidation of the effects of such rare SNPs be of value for the average patient.

Upfront pharmacogenetic analysis in GIST is currently not part of clinical practice. Therefore, most DNA samples were taken from residual blood samples or samples collected as part of a biobank for which patients had given informed consent. However, from some patients DNA was only available in the form of a formalin fixed frozen paraffin embedded (FFPE) pathology sample. These FFPE samples were used to obtain DNA for tumor mutation analysis, germ line DNA analysis or both. FFPE samples have been shown to be valid proxy for blood samples in case of a pharmacogenetic analysis.[25] Patients of whom DNA was taken from FFPE tissue were retained in the analysis, as almost all SNPs were in Hardy-Weinberg equilibrium. The retrospective character of patient accrual considered the cause for any deviations from the Hardy-Weinberg equilibrium.

The data for these pharmacogenetics studies were also collected retrospectively, although some patients had been treated as part of a clinical study or a treatment use trial. This implies a measure of inherent uncertainty, as patient were not checked for progressive disease at predefined intervals. Also, it required the use of the endpoint dose reduction due to toxicity since the notation of adverse events and its grade were not considered sufficiently reliable. However, as these were exploratory studies in a disease with relatively low incidence, it was deemed better to include as many patients as possible, regardless of treatment in a prospective study protocol. These exploratory studies can function as a starting point for new efforts in finding and validating genetic polymorphisms associated with TKI effect in GIST treatment. Aside from pharmacogenetics, these new studies should also include TKI serum levels.

Only in the last pharmacogenetics study (chapter 6) imatinib serum levels were related to SNPs. The three larger pharmacogenetics studies did not have this aspect, mostly due to the retrospective nature. It would have been of interest to relate imatinib serum levels to SNPs and to clinical outcome in survival and toxicity. Imatinib trough

levels have been associated to treatment effect, but studies investigating SNPs and imatinib serum levels are scarce.[26] The genetic basis for too low or too high imatinib serum levels is yet to be clarified. If that has been elucidated, patients can receive therapy at an adjusted dose from the moment it is started, instead of needing to react to serum levels after some time of treatment. This could improve treatment effect in some patients, while preventing early serious adverse events in others.

The studies in this thesis sought to find genetic biomarkers for treatment effect in GIST. Non-genetic markers for survival include the size of the primary tumor when resected and the mitotic index, with large tumors or those with a high mitotic index having a worse prognosis.[27] The Ki-67 protein (also known as MKI67)is used as a biomarker for cell proliferation in malignancies and its expression has also been associated with the malignant risk of resected GISTs. While high Ki67 expression appears to be associated with recurrence and risk group, this marker does not better predict poor prognosis than mitotic count and tumor size do.[28,29] The tumor-suppressor protein p53 could not reliably be associated to GIST risk of malignancy.[30] These studies did not assess an association with survival in case of advanced GIST, as was the focus for the pharmacogenetic studies in this thesis.

Genetic information regarding the tumor can also be found using circulating tumor DNA (ctDNA). As tumor cells undergo apoptosis and necrosis, tumor derived DNA fragments entered into the patient's circulatory system.[31] In one study, loss of heterozygosity in serum DNA was associated with GIST recurrence, but not with overall survival.[32] In all patients with metastases ctDNA is detectable. In patients without metastases, tumor burden appears to be the most important factor whether ctDNA can be detected.[33] GIST is known to have genetically heterogeneous metastases and this method could capture more secondary mutations than a histologic biopsy of a single lesion would.[34] Using ctDNA, physicians are provided with up-to-date genetic information. This could serve to discover new mutations before drug resistance is observed trough growing metastases on a CT-scan and it would avoid the need for invasive tumor biopsies.[35,36]

A distinct type of genetic biomarker are microRNA's (miRNA's). These miRNA's are small non-coding RNA molecules that are active in post-transcriptional regulation of gene expression in tumors. [37] Up- and downregulation of miRNA's influence tumor cell proliferation, apoptosis, migration and invasion. Prognostic miRNA's have been found for GIST. Metastases and reduced survival has been associated with upregulated miR-196a and downregulated miR-186.[38,39] Prolonged overall and disease-free survival has been associated with miR-1915 in GIST.[40] Imatinib resistance has also been linked to miRNA expression levels. For instance, over-expression of miR-218 can improve the sensitivity of GIST to imatinib.[41] On the other hand, low expression of miR-518a-5p

results in reduced response to imatinib.[42] The effect of miR-125a-5p overexpression on imatinib resistance has been linked to reduced expression of PTPN18, a protein that regulated cell growth an cell cycles.[40] In a GIST cell line study, altered miRNAs 221 and miRNA222 molecules resulted in effective inhibition of KIT gene expression.[43] Thus far, miRNA's have not yet been tested in a clinical setting with GIST patients.

As for cost-effectiveness studies in GIST, these have not been performed with imatinib due to the remarkable gain in survival this drug offers. There is a cost-effectiveness study in which sunitinib was compared as second line therapy with best supportive care in a Spanish setting.[44] That study calculated sunitinib extend survival by 0.71 life years at a cost of € 49.000 per gained QALY (quality-adjusted life-year). The cost-effectiveness of third line regorafenib was also assessed in comparison to imatinib rechallenge in GIST patients in Germany.[45] With 0.6 life years gained, the incremental cost-effectiveness ratio was € 21.000 per QALY gained, regorafenib appears to be cost-effective in this context, despite the small gain in survival.

General limitations to these pharmacogenetic studies arise mostly out of their retrospective character. Therapy adherence was not documented and could not be corrected for. Equally, the use of over-the-counter-drugs could have influenced the effects of the prescribed drugs. A substantial number of patients had received gastrointestinal surgery by the time TKIs were needed to treat advanced disease. As is reported for sunitinib, dosing has to be increased in case of a gastrectomy combined with a small bowel resection.[46] This is not needed if only a gastrectomy was performed.[47] While previous surgeries had been reported, these have not been corrected for.

These pharmacogenetic studies on the efficacy of imatinib and sunitinib in advanced GIST and the toxicity of imatinib in this disease found several SNPs to be associated. These were SNPs in *SLCO1B3, KDR, VEGFA, SLC22A5, IL4R, ABCG2* and in *CYP1A2*. When replicated, these SNPs may identify patients who are most at risk of unfavorable treatment effects in terms of survival and toxicity. It may select patients whom may benefit from more frequent outpatient treatment evaluation. In the end, this could be an additional tool in prolonging patient survival and improving quality of life.

Part II: Use of trabectedin in STS

Trabectedin was a new antitumor agent when the research for this thesis began. It has a unique mechanism of action involving DNA binding and influencing the tumor microenvironment. Patients with certain soft tissue sarcomas (STSs) may benefit from this agent, and its toxicity is most often tolerable (**chapter 7**). Trabectedin has to be positioned in the limited spectrum of active systemic agents in STS, and the costs and

benefits need to be weighed in light of other available drugs. Also, as a new drug, its toxicity pattern needs to be documented, as new types of adverse events have occurred.

The study on trabectedin versus ifosfamide after doxorubicin therapy in STS advanced patients (**chapter 8**) described both the survival and the cost-effectiveness analysis (CEA). The study was conceived in an effort to define the cost-effectiveness of trabectedin, so Dutch health authorities could decide on reimbursing this drug or refrain from doing so. As almost all included patients chose trabectedin treatment, too few patients chose to be in the best supportive care arm. This necessitated a revised study design. Therefore, data on ifosfamide as second line treatment for advanced STS was used from two clinical trials (European Organisation for Research and Treatment of Cancer (EORTC) trial numbers 62912 and 62953).[48,49]In the performed study, trabectedin was an active drug against leiomyosarcoma or liposarcoma (the so-called L-sarcomas). The progression free rates at 3 and 6 months were 59% and 42%, respectively. This was enough to pass the EORTC criteria in STS for a drug to be considered an active antitumor agent, with threshold at 3 and 6 months set at 39% and 14%, respectively.[50]

Trabectedin has recently been compared to best supportive care head-to-head in a randomized trial with patients with advanced STS after doxorubicin therapy. This study was presented at a conference meeting, but has not yet been published in a journal.[51] Patients with an L-sarcoma had a median PFS of 5.3 months compared to 1.4 months with best supportive care. For the group of other sarcomas (the so-called non-L-sarcomas) median PFS did not reach 2 months in either treatment group. As patients were allowed to cross-over after progressive disease, OS did not differ. A CEA using data from this study would be very interesting indeed.

The CEA of trabectedin versus ifosfamide for the treatment of L-sarcomas showed an incremental cost-effectiveness ratio (ICER) of € 80,000 per QALY gained. The trabectedin acquisition costs and the difference in survival between trabectedin and ifosfamide were the variables with the strongest impact on the ICER. The ICER of € 80,000 per QALY gained is at the top end to the often used threshold of € 80.000 per QALY, which can be found in a 2006 report entitled 'Sensible and sustainable care', written by the Council for Public Health and Health Care to the Minister of Health, Welfare and Sport.[52] This means that trabectedin may be considered to be just cost-effective in the second line treatment of advanced soft tissue sarcoma. Of note, many commonly used drugs in the Netherlands surpass this amount of money per QALY gained or have never been analyzed in this regard.

In the CEA in this chapter, ifosfamide gave longer survival for patients with non-L-sarcomas, although this difference was not significant. Treatment with ifosfamide had

less costs comparted to trabectedin, which made ifosfamide to dominate trabectedin in non-L-sarcoma.

As there is an unmet clinical need for drugs active in this group of STS, the outcome of a CEA may not be the most relevant factor in the decision to continue to prescribe trabectedin to these patients. The annual turnover of trabectedin in the Netherlands does not exceed a few million euros. Due to the small impact trabectedin has on the health care budget, the outcome of this CEA has become irrelevant for Dutch health authorities in the reimbursement decision. Nonetheless, health care professionals should still seek to treat their patients with best possible therapy, in terms of survival, safety, and, from a societal point of view, best cost-effectiveness.

Trabectedin is given through a central venous catheter and the associated adverse events were studied (**chapter 9**). In the LUMC, sterile inflammation along the catheter trajectory was observed. This was an unexpected adverse event and was not understood at first. The symptoms mimicked an infection with the erythema and pain and treated as such. Irrefutable evidence of infection such as blood culture remained absent. The erythema was most prominent along the catheter trajectory and it was related to trabectedin infusion. Porosity of the catheter was deemed the cause, with small quantities of trabectedin permeating but not leading to full trabectedin extravasation.[53] A test by PharmaMar, the manufacturer of trabectedin, failed to observe catheter porosity of a previously used catheter. This could not rule out sub lower-limit of quantification leakage. Placing the catheter deeper under the skin resolved this issue, and the inflammation along the catheter trajectory was not seen again.

Future perspectives

The future of GIST treatment will be one in which treatment will be tailored to the genetic properties of the tumor and the patient. Imatinib was hailed as the magic bullet due to its targeted therapy properties. Due to secondary mutations in GIST-metastases, drugs with another range of specific targets needed to be employed. Sunitinib and regorafenib have more targets than imatinib, and are thus able to be effective in imatinib-resistant GIST. To keep TKI therapy after imatinib as targeted as possible, future therapy will need to consider GIST resistance mutations at each step of treatment.

This can be accomplished by obtaining information on new GIST-mutation, preferably by analyzing free circulating tumor DNA from a patient's blood.[35,54] Subsequently, the choice of therapy should be aimed at what particular secondary mutation is causing disease progression. For instance, a tumor with a *KIT* exon 13 or 14 mutation can react favorably to sunitinib, whereas KIT exon 17 mutations respond better on nilotinib,

and olaratumab has shown to prolong survival in patients with a *PDGFRα* D842V mutation.[55,56] New TKIs like DCC-2816 and BLU-285 are expected to deliver prolonged anti-tumor effect in GIST due their activity against a broad set of resistance mutations in both *KIT* and *PDGFRα*.[57,58] Clinical trials with these drugs in several lines of therapy have been started, and results will be available soon.

Better understanding of miRNA's effect in GIST could also lead to the identification of new therapeutic targets. Clinical tests with miRNA's in other fields of medicine have already been initiated.[59] The use of effective GIST-specific miRNA's therapeutics will however probably take a considerable number of years of further research.

Aside from the tumor biology, germline genetic aberrations also influence a drug response and the SNPs found in this thesis need validation. The pharmacogenetic studies in this thesis used a candidate gene approach. Polymorphisms of interest are tested for an association with effect, needing a hypothesis. Lack of knowledge on a drug's mechanism of action hinders the hypothesis formation, and in the recent past the capacity for genotyping was also a restraint. However, with the advent of genome wide association studies all SNPs are genotyped, free of hypotheses. This technique is already used in GIST research and pharmacogenetics.[60] With next generation sequencing the whole genome is available for analysis.[61]

The research in this thesis found SNPs related to survival during GIST therapy of equal Hazard Ratio as the tumor mutations. Yet, SNPs analysis in GIST is not yet routinely performed as a part of clinical care. Had these studies found a SNP that would predict for poor imatinib response and very good sunitinib response, that SNP could have had a clinical impact in turning around the normally used order of TKIs. Since both drugs are from the same class of drugs, this possibility was very small.

Additional studies are needed to better understand the effects of SNPs found in these exploratory studies. Although a reported functionality was one of the selection criteria, it is unknown to what extent these SNPs influence TKI serum or intracellular levels. Cell culture studies with GIST-cells and hepatocytes would need to be designed to test whether or not these SNPs lead to the effects as hypothesized in the discussion sections of the previous chapters.

In future health care, every oncology patient who is treated with systemic therapy should be informed about the research potential of pharmacogenetics and personalized medicine, and should be asked for a DNA sample. This way, correlations with treatment outcome can be studied and may be adjusted to a patient's germline genetic make-up and in turn lead to better patient survival and quality of life.

The future of STS treatment is one that looks far better than the past. Since the dawn of this century the number of drugs available to treat patients with advanced soft

tissue sarcoma has increased more so than in the preceding decades. Doxorubicin and ifosfamide were joined by trabectedin, pazopanib, paclitaxel, dacarbazine, eribulin and gemcitabine, depending on STS subtype.[62] The clinical benefit that immunotherapy may offer is also being investigated. This will necessitate careful analysis of anti-tumor effects, the associated toxicity, as well as the cost-effectiveness of these drugs compared to one another. However, other than indirect comparisons of efficacy, many of these agents are not likely to be the tested head-to-head in a randomized trial in the near future. This is due the costs of randomized trials, costs of drugs still under patent and it is unlikely that a manufacturer is willing to risk the position of its drug. As far as the number of patients needed for sufficient study power, the past multi-center group trials have shown that studies with enough patients can be performed in low incidence malignancies as STS.

New large clinical trials will be performed no doubt, testing new agents and comparing them to drugs already available to patients. These trials should include data collection not only on survival and toxicity, but also on quality of life and on health care usage. That way, analysis of the cost-effectiveness can be performed, aiding in positioning new and old drugs in the treatment spectrum against STS. This will help in delivering patients the best therapy available and advancing the knowledge on systemic treatment options in soft tissue sarcoma.

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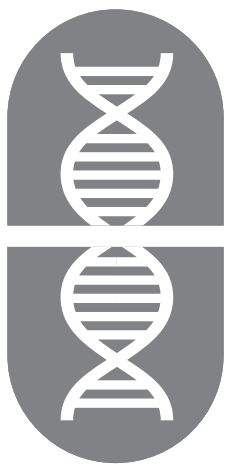
11

Summary and appendices

English summary
Nederlandse samenvatting
List of publications
Curriculum vitae
Dankwoord



English summary

This thesis on systemic treatment options in soft tissue sarcomas consists of two parts, as written in the general introduction in **chapter 1**. In part I, the pharmacogenetics of systemic gastro-intestinal stromal tumors (GIST) treatment is investigated. In part II the usage of trabectedin is soft tissue sarcomas (STS) in the Netherlands is studied.

Part I: Pharmacogenetics of systemic GIST-treatment

The development of the current systemic treatment of advanced GIST is described in **chapter 2**, with an emphasis on imatinib and sunitinib, the first and second line therapies, respectively. Also, after a description of the third line agent regorafenib, new drugs are highlighted. Those drugs may supplant an existing drug or may contribute to establish a fourth line of systemic therapy. In particular, DCC-2618 and BLU-285 hold promise, as these tyrosine kinase inhibitors are able to block kinase domains, regardless of a wide variety of possible mutations.

The core of this part of the thesis consists of three exploratory pharmacogenetic pathway analyses. DNA samples were obtained from a cohort of GIST patients from four referral center in the Netherlands. Single nucleotide polymorphisms (SNPs) were selected in genes related to pharmacokinetics and pharmacodynamics of imatinib or sunitinib. This was based on rational criteria including a minor allele frequency of 0.1 and a presumed functionality of the SNP. Together with clinical factors, these SNPs were then associated with the endpoints in each study.

The endpoints in **chapter 3** are the progression free survival (PFS) and overall survival (OS) of imatinib 400mg once daily as first line therapy in 227 patients with advanced GIST. This study in imatinib efficacy shows SNPs in angiogenesis related genes to be associated with worse PFS. These were the AA genotype in rs1570360 in *VEGFA* and the AA genotype in rs1870377 in *KDR* (also known as VEGFR2). The altered tumor microvasculature may affect imatinib function. In the pharmacokinetic pathway of imatinib, PFS was only associated with a T allele in rs4149117 in *SLCO1B3*, which encodes for a drug-influx transporter protein that has imatinib as a substrate. The other tested SNPs in the pharmacokinetic pathway (such as in *ABCB1*, *ABCG2*, *SLC22A1*, *SL22A5* or *CYP3A4*) were not associated with survival, possibly because the effects of these SNPs are absent or too small to detect. Synchronous metastases and *KIT* exon 9 mutations were also associated with worse PFS, whereas *KIT* exon 11 mutations led to longer PFS. The imatinib dosage of 400mg once daily is considered too low for patients with a *KIT* exon 9 mutation, but this was corrected for in the multivariate analysis. OS was not associated with any of the tested SNPs.

Sunitinib was the drug that was investigated in **chapter 4** , the second line therapy for advanced GIST. In 127 patients clinical factors and SNPs were associated with survival. The TT genotype in rs1056878 in *POR* was associated with PFS. This gene influences the activity of cytochrome P450 (CYP) metabolizing enzymes and thus could have an impact on sunitinib serum levels. OS was associated with a T allele in rs4149117 in *SLCO1B3,* and with the CCC-CCC alleles in a haploblock in *SLC22A5*, consisting of three rs2631367, rs2631370 and rs2631372. *SLC22A5* encodes for a drug influx transporter. The GC-GC alleles in a haploblock in *IL4R*, consisting of rs1801275 and rs1805015, for the IL4-receptor were also associated with OS. Synchronous metastases were associated with shorter survival, as was the case with imatinib efficacy. In contrast to the imatinib study, the primary etiologic GIST mutation was not associated with survival.

After efficacy, in **chapter 5** the toxicity of imatinib was investigated. In this study the clinical endpoints were the need for imatinib dose reduction and cessation of therapy due to adverse events. Imatinib has a relatively mild toxicity profile, yet concurrent multiple adverse events can lead to a dose reduction. In 315 GIST patients who were treated with imatinib 400mg once daily in the neo-adjuvant, adjuvant or palliative stage, the dose was reduced in about ten percent of patients. Most of these dose reduction occurred early in treatment and older patients usually experienced more toxicity. The A allele in rs2231137 in *ABCG2* and the CC genotype in rs762551 in *CYP1A2* were associated with the need for dose reduction, even after correction for multiple testing of 36 SNPs. *ABCG2* encodes for a drug efflux transporter, that might be impaired by this SNP. This could result in increased intracellular imatinib levels and thus increased toxicity. The enzyme encoded for by *CYP1A2* is one of the cytochrome P450 metabolizing enzymes and has reduced activity if the tested SNP is present. This could lead to increased imatinib exposure. The well-known polymorphisms in *ABCB1* such as rs1045642, rs1128503 and rs2032582 were not associated with dose reduction. Cessation of imatinib due to adverse events was not associated with any of the tested SNPs, possibly due too few events of treatment cessation.

The last study in this part of thesis also was a pharmacogenetic study, but **chapter 6** has a different design in several aspects. First, although all patients received imatinib a number of patients did not have a GIST, but had chronic myeloid leukemia instead, a hematological malignancy that responds very well to imatinib therapy. Secondly, standardized imatinib trough levels were associated with SNPs in *CYP2C8* after patients had taken imatinib for at least one month. It was assumed that after this period CYP2C8 would become a more prominent metabolizer of imatinib due to auto-inhibition of CYP3A4. However, none of the tested SNPs in *CYP2C8* were associated with standardized imatinib trough levels.

Part II: Usage of trabectedin in STS

The development of trabectedin is described in **chapter 7**, especially its use in the treatment of advanced STS. The mechanism of action of trabectedin is highlighted, as is its efficacy in different types of STS. Clearly, trabectedin will incur most benefit in patients with a leiomyosarcoma or a liposarcoma, but in case of a synovial sarcoma trabectedin may also induce an anti-tumor effect. Toxicity is usually manageable, with fatigue, elevated transaminases and bone marrow depression the most frequent adverse events.

The cost-effectiveness of trabectedin versus ifosfamide monotherapy in the second line therapy of advanced STS was studied in **chapter 8**. The original idea was to compare trabectedin to best supportive care in this setting. However, the observational phase IV study performed to gather data to this end, resulted in patients choosing trabectedin so often, that only sufficient data was collected for the trabectedin arm. Therefore, previously published EORTC data on ifosfamide in this treatment setting was obtained and used for comparison. Data on quality of life during treatment was taken from the observational study and from literature. The use of health care recourses were scored in the study and cost were taken from public sources.

In this study in **chapter 8**, trabectedin was shown to be active in patients with leiomyosarcoma or a liposarcoma, the so-called L-sarcomas. In the survival analysis, the progression free survival for L-sarcoma patients was 2.5 months longer with trabectedin compared to ifosfamide. In the group of non-L-sarcomas patients, however, ifosfamide resulted in longer survival. All these differences were not statistically significant. In the cost-effectiveness analysis, the incremental cost-effectiveness ratio of trabectedin for L-sarcoma was € 80,000 per QALY gained. The difference in survival and the drug acquisition costs had the largest impact in this result. The costs per QALY are at the top end of what is currently acceptable in the Netherlands. In non-L-sarcoma patients ifosfamide was dominant, as it resulted in longer survival at lower costs.

A trabectedin specific adverse event was described in **chapter 9**, together with other vascular access related adverse events. The new adverse event was the development of a sterile inflammation along the catheter trajectory. This was observed in the LUMC after implantation of vascular access ports, but not in other centers using the same device. Placing the catheter deeper under the skin resolved the issue.

A general discussion in **chapter 10** concludes this thesis. Comments made regarding the pharmacogenetic pathway analyses in GIST patients include the exploratory character of these studies and the relatively large numbers of patients included. Also the retrospective nature of data collection was a significant factor and it precluded

the inclusion of tyrosine kinase inhibitor serum concentration measurement into the analyses. Obviously, the SNPs associated with survival or adverse events need to be validated before clinical usage. If that would succeed, these SNPs could help in establishing personalized medicine in GIST treatment, and thus improving patient´s quality of life. Comments regarding the cost-effectiveness analysis of trabectedin versus ifosfamide include the notion that trabectedin was active in L-sarcomas, according to EORTC criteria for STS. The cost per QALY gained is at the top end of the usually accepted amount. Nonetheless, as long as there is an unmet clinical need in patients with advanced STS, the cost- effectiveness of trabectedin may not be the decisive criterion whether or not to prescribe this drug, but the desire to provide the best possible medical care will be.

Nederlandse samenvatting

Dit proefschrift over de systemische behandelopties van wekedelensarcomen bestaat uit twee delen, zoals is geschreven in de inleiding in **hoofdstuk 1**. In deel I is de farmacogenetica van systemische behandeling van gastro-intestinale stromacel tumoren (GIST) onderzocht. In deel II is het gebruik van trabectedine bij wekedelensarcoom in Nederland bestudeerd.

Deel I: Farmacogenetica van systemische GIST-behandeling

De ontwikkeling van hedendaagse systemische behandeling van gevorderde GIST is beschreven in **hoofdstuk 2**, met extra aandacht voor imatinib en sunitinib, de respectievelijk eerste- en tweedelijns behandelingen. Tevens worden, na een beschrijving van het derdelijns middel regorafenib, nieuwe middelen uitgelicht. Die middelen zouden de plek van een bestaand middel over kunnen nemen, of kunnen bijdragen aan een vierde lijn systemische behandeling. In het bijzonder bieden DCC-2618 en BLU-285 perspectief, omdat deze tyrosine kinase remmers in staat zijn meerdere kinase domeinen te blokkeren, ongeacht een brede variatie van mogelijk mutaties daarin.

De kern van dit deel van het proefschrift bestaat uit drie exploratieve farmacogenetische signaaltransductiecascade analyses. DNA monsters werden verkregen van een cohort GIST patiënten uit vier verwijscentra in Nederland. Enkel-nucleotide polymorfismen (SNPs) werden geselecteerd in genen gerelateerd aan de farmacokinetiek en farmacodynamiek van imatinib of sunitinib. Dit was gebaseerd op rationale criteria, zoals een minor allel frequentie van 0,1 en een verwachte functionaliteit van de SNP. Samen met klinische variabelen werden deze SNPs vervolgens geassocieerd met de eindpunten van iedere studie.

De eindpunten in **hoofdstuk 3** zijn de progressie vrije overleving (PFS) en totale overleving (OS) van in imatinib 400mg eens per dag als eerste lijn behandeling in 227 patiënten met gevorderde GIST. De studie met de werkzaamheid van imatinib toonde dat SNPs in angiogenese gerelateerde genen, te weten het AA genotype in rs1570360 in *VEGFA* en het AA genotype in rs1870377in *KDR* (ook bekend als VEGFR2), geassocieerd waren met slechtere PFS. De veranderde microvasculatuur van de tumor zou de werking van imatinib kunnen beïnvloeden. In het farmacokinetische signaaltransductiepad van imatinib was PFS alleen geassocieerd met een T allel in rs4149117 in *SLCO1B3*, wat codeert voor een geneesmiddel-influx transporteiwit dat imatinib als substraat heeft. De andere geteste SNPs in het farmacokinetische signaaltransductiepad van imatinib (zoals in *ABCB1*, *ABCG2*, *SLC22A1*, *SL22A5* of *CYP3A4*) waren niet geassocieerd

met overleving, mogelijk omdat het effect van deze SNPs afwezig zijn of te klein om te detecteren. Synchrone uitzaaiingen en *KIT* exon 9 mutaties werden ook geassocieerd met slechtere PFS, terwijl *KIT* exon 11 mutatie leidden tot langere PFS. De 400mg eens per dag dosering van imatinib wordt beschouwd als te laag voor patiënten met een *KIT* 9 mutatie, maar daarvoor was gecorrigeerd in de multivariate analyse. OS was niet geassocieerd met een van de geteste SNPs.

Sunitinib was het geneesmiddel dat in **hoofdstuk 4** werd onderzocht, de tweedelijns behandeling van gevorderde GIST. In 127 patiënten werden klinische variabelen en SNPs geassocieerd met overleving. Het TT genotype in rs1056878 in *POR* was geassocieerd met PFS. Dit gen beïnvloedt de activiteit van cytochroom P450 (CYP) metaboliserende enzymen en kan dus invloed hebben op de serumwaardes van sunitinib. OS was geassocieerd met een T allel in rs4149117in *SLCO1B3* en met de CCC-CCC allelen in een haploblok in *SLC22A5*, bestaande uit rs2631367, rs2631370 en rs2631372, dat codeert voor een geneesmiddel-influx transporteiwit. De GC-GC allelen in een haploblok in *IL4R*, bestaande uit rs1801275 en rs1805015, voor de IL4-receptor was ook geassocieerd met OS. Synchrone metastasen waren geassocieerd met korter overleving, zoals ook met imatinib werkzaamheid. In tegenstelling met de studie met imatinib was de primaire etiologische GIST mutatie niet geassocieerd met overleving.

Na de werkzaamheid werd in **hoofdstuk 5** de toxiciteit van imatinib onderzocht. In deze studie waren de klinische eindpunten de noodzaak om de dosering van imatinib te verminderend en het staken van de behandeling wegens bijwerkingen. Imatinib heeft een relatief mild toxiciteitsprofiel, maar meerdere bijwerkingen tegelijkertijd kunnen leiden tot een dosisvermindering. Bij 315 GIST patiënten die werden behandeld met 400mg eens per dag in de neo-adjuvant, adjuvant of palliatieve setting werd de dosis in ongeveer tien procent van de patiënten verminderd. De meeste van deze dosisverminderingen gebeurde in het begin van de behandeling en oudere patiënte hadden meestal meer bijwerkingen. Het A allel in rs2231137 in *ABCG2* en het CC genotype in rs762551 in *CYP1A2* waren geassocieerd met de noodzaak om de dosering te verminderen, zelfs na correctie voor meervoudige testen van 36 SNPs. *ABCG2* codeert voor een geneesmiddel-efflux transporteiwit, dat mogelijk minder werkt door deze SNP. Dat kan leiden tot een verhoogde intracellulaire imatinib spiegel en daarmee verhoogde toxiciteit. Het enzym dat wordt gecodeerd door *CYP1A2* is een van de cytochroom P450 metaboliserende enzymen en heeft verminderde activiteit als de geteste SNP aanwezig is. Dit kan leiden tot hogere imatinib blootstelling. De bekende polymorfismen in *ABCB1* zoals rs1045642, rs1128503 en rs2032582 waren niet geassocieerd met dosisverminderingen. Staken van de behandeling wegens bijwerkingen waren niet geassocieerd met een van de geteste SNPs, mogelijk door te weinig optreden hiervan.

Het laatste onderzoek van in dit deel van het proefschrift was ook een farmacogenetica onderzoek, maar **hoofdstuk 6** had op verschillende punten een andere opzet. Ten eerste, hoewel alle patiënten imatinib kregen had een aantal patiënten niet een GIST maar chronische myeloïde leukemie, een hematologische maligniteit die goed reageert op behandeling met imatinib. Ten tweede werden gestandaardiseerde imatinib dalspiegels geassocieerd met SNPs in *CYP2C8*, nadat patiënten imatinib gedurende tenminste één maand hadden gebruikt. De aanname was dat CYP2C8 een belangrijker metabolisator van imatinib zou worden door auto-inhibitie van CYP3A4. Echter, geen van de geteste SNPS in CYP2C8 waren geassocieerd met gestandaardiseerde imatinib dalspiegels.

Deel II: Gebruik van trabectedine bij wekedelensarcoom

De ontwikkeling van trabectedin is beschreven in **hoofdstuk 7**, met name het gebruik in de behandeling van gevorderd wekedelensarcoom. Het werkingsmechanisme van trabectedine is uitgelicht, net als de werkzaamheid bij verschillende types wekedelensarcoom. Duidelijk is dat trabectedine het meeste baat zal hebben bij patiënten met een leiomyosarcoom of een liposarcoom het meest aan trabectedine hebben, maar in geval van een synoviosarcoom zou trabectedine ook een antitumor effect kunnen bewerkstelligen. De toxiciteit is meestal te handteren, met moeheid, verhoogde leverenzymen en beenmergdepressie als meest voorkomende bijwerkingen. De kosteneffectiviteit van trabectedine ten opzichte van ifosfamide monotherapie in de tweedelijns behandeling van gevorderde wekedelensarcoom is onderzocht in **hoofdstuk 8**. De oorspronkelijk opzet was om trabectedine en best ondersteunde zorg (BSC) in deze setting te vergelijken. Echter, de observationele fase IV studie die is uitgevoerd om hiertoe data te verzamelen, resulteerde erin dat patiënten zo vaak voor trabectedine kozen, dat alleen voldoende data was verzameld van de trabectedine groep. Daarom werd eerder gepubliceerde EORTC data over ifosfamide in deze behandelgroep verkregen en gebruikt als vergelijking. Data over de kwaliteit van leven werd uit de observationele studie gehaald en uit de literatuur. Het gebruik van gezondheidszorgmiddelen was bijgehouden in de studie en kosten werd uit openbare bronnen gehaald.

In deze studie in **hoofdstuk 8** toonde aan dat trabectedine een actief middel is bij patiënten met een leiomyosarcoom of een liposarcoom, de zogeheten L-sarcomen. In de overlevingsanalyse was de progressie vrije overleving voor patiënten met L-sarcomen 2,5 maanden langer voor trabectedine vergeleken met ifosfamide. Echter, in de groep van niet-L-sarcomen patiënten leidde ifosfamide tot langere overleving.

Al deze verschillen waren statistisch niet significant. In de kosteneffectiviteitsanalyse was de incrementele kosteffectiviteit ratio van trabectedine bij L-sarcomen € 80.000 per gewonnen QALY. Het verschil in overleving en de medicijnkosten hadden de grootste invloed op dit resultaat. De kosten per gewonnen QALY zitten aan de bovengrens van wat in Nederland momenteel acceptabel is. Bij de niet-L-sarcomen patiënten was ifosfamide dominant, want het leidde tot langere overleving voor minder kosten.

Een voor trabectedine specifieke bijwerking is beschreven in **hoofdstuk 9**, samen met andere bijwerkingen gerelateerd aan de vaattoegang. De nieuwe bijwerking was het ontstaan van een steriele ontsteking langs het traject van de katheter. Dit werd gezien in het LUMC na het plaatsen van een poort voor de vaattoegang, maar niet in andere centra die hetzelfde apparaat gebruiken. Het probleem werd opgelost door de katheter dieper onder de huid te plaatsen.

Een algemene samenvatting in **hoofdstuk 10** beëindigt dit proefschrift. Opmerkingen over de farmacogenetische signaaltransductiecascade analyses met GIST-patiënten waren onder meer het exploratieve karakter van deze studies en de relatief grote aantallen geïncludeerde patiënten. Ook was de retrospectieve dataverzameling een belangrijke factor en het sloot het gebruik van bloedspiegelbepalingen van tyrosine kinase remmers concentraties in de analyses uit. Vanzelfsprekend moeten de SNPs die geassocieerd waren met overleving of bijwerkingen worden gevalideerd voor dat het kan worden gebruikt in de klinische praktijk. Als dat zou lukken, zouden deze SNPs kunnen bijdragen aan het gepersonaliseerde geneeskunde in de behandeling van GIST, en daarmee het verbeteren van de kwaliteit van leven van patiënten. Opmerkingen over de kosten-effectiviteitsanalyse van trabectedine ten opzichte van ifosfamide waren onder meer dat trabectedine volgens de EORTC criteria voor wekendelensarcomen een actief middel is bij L-sarcomen. De kosten per gewonnen QALY zitten aan de bovengrens van het algemeen geaccepteerde bedrag. Niettemin, zo lang er een klinische behoefte is bij patiënten met gevorderde wekendelensarcomen die niet anders wordt voldaan, zal kosteneffectiviteit niet de doorslaggevende factor zijn om wel of niet dit middel voor te schrijven. Het verlangen om de best mogelijke medische zorg te bieden, zal dat wel zijn.

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Curriculum vitae

Michiel Verboom was born on January 9th 1985 in the Hague. He attended the Rijnlands Lyceum Wassenaar in Wassenaar and obtained his gymnasium graduation cum laude in 2003. Subsequently, he started studying medicine at the Leiden University, receiving his doctoral degree in 2009. During 2006-2007 he interrupted his studies and spend a full year at the board of the Veerstichting as treasurer, organizing the yearly Veerstichting symposium in Leiden. During the clinical rotations, his interest in medical oncology was kindled and he performed his final scientific as well as clinical internship at the Department of Clinical Oncology at the LUMC (supervisor prof. dr. H. Gelderblom). In 2011 he graduated medicine cum laude and started with the research culminating in this thesis (supervisors prof. dr. H. Gelderblom and prof. dr. H.-J. Guchelaar). In 2015 Michiel commenced his clinical training to become an internist at the Haaglanden Medisch Centrum (opleider dr. A. Bootsma), and in 2018 continuing at the Leiden University Medical Center (opleider prof. dr. J.W. de Fijter). In 2019 he will start his training in the subspecialty of medical oncology (opleider prof. J.A.E. Portielje). Michiel is married to Caroline van Kinschot and they have a daughter named Floortje.

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