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Imatinib versus gastro intestinal stromal tumors

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Abstract

This thesis is about whether imatinib, a tyrosine kinase inhibitor, is successful enough as a treatment for gastro intestinal stromal tumor (GIST) patients. Of all GISTs 70-80% harbor mutations in the receptor tyrosine kinase KIT, 5-8% in the platelet derived growth factor α tyrosine kinase (PDGFRA) and 12-15% do not harbor any KIT- or PDGFRA mutations (wild-type GISTs). The KIT- or PDGFRA mutations are oncogenic driver mutations, that lead to constant activation of the downstream pathways, which leads to GIST progression. Imatinib inhibits signaling of mutated KIT and mutated PDGFRA in GISTs. Overall imatinib can prevent GIST progression in more than 70% of all GISTs. The problem is that imatinib cannot prevent progression in wild-type GISTs and can only prevent progression in some PDGFRA-mutated GISTs. On top of this imatinib-sensitive GISTs develop drug resistance, probably because imatinib does not actively eradicate all GIST cells. There are many ideas of how to improve treatment of GIST patients, but the most promising for imatinib-resistant GISTs are inhibitors of downstream components of the KIT- or PDGFRA pathway. These promising new drugs do not only prevent GIST progression, but also introduce apoptosis.

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Introduction:

Before the year 2000 prognosis of gastro intestinal stromal tumor (GIST) patients was poor. Surgery was the only option. It appeared that in most cases GIST patients have oncogenic driver mutations in either tyrosine kinase receptor protein KIT or platelet derived growth factor receptor α (PDGFRA). Due to these mutations downstream signaling pathways are now constantly activated, which leads to uncontrolled cell survival, proliferation and differentiation. (Corless et al., 2011)

In the last decade promising kinase inhibitors were found. Imatinib, also known as Glivec, can control GIST progression in more than 70% of all GIST patients and prognosis increased a lot, especially for patients with KIT-mutated GIST. However, like most drugs, imatinib also has its limitations. Prognosis for GIST patients with PDGFRA- or wild-type mutations remains poor and within a certain period of time the tumor develops drug resistance. This leads to the following question: is imatinib successful enough as a treatment for gastro intestinal stromal tumors? (Corless et al., 2011)

In this thesis I will first give a short introduction about GISTs (chapter 1). Then I will tell something about the discovery of imatinib, its function and its clinical application (chapter 2). In chapter 3 I will discuss whether treatment of imatinib is enough and discuss its limitations. Finally, in chapter 4, I discuss possibilities to improve treatment of GIST patients.

Chapter 1 - Gastrointestinal stromal tumors

§ 1.1 - Description of the disease:

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumours of the gastrointestinal tract. GISTs start locally, mostly in the stomach. After that they spread diffusely towards liver or serosal surfaces of the abdomen. Finally it can spread to distant sites, such as lung and bone. (Corless et al., 2011)

§ 1.2 - Interstitial cells of Cajas as the cells of origin of GIST

The interstitial cells of Cajal (ICC) are found in the gastrointestinal tract, were they create the basal electrical rhythm leading to contraction of the smooth muscle for peristaltic movement. (Corless et al., 2011)

ICC are thought to be the cells of origin of GIST. In several cases GIST patients showed ICC hyperplasia. A study by O'Riain et al. showed that ICC hyperplasia developed after knock-in of mutated KIT. (Riain et al., 2005)

§ 1.3 - KIT

The KIT protein is a receptor tyrosine kinases (RTKs). RTKs are divided in approximately 20 different classes. KIT is a member of the class III RTKs, also known as the PDGF receptor family. Members of this family mostly have an important role in cell growth, cell proliferation and cellular differentiation and mutations in these RTKs are often involved in cancers. Another member of this family is the platelet-derived growth factor receptor (PDGFR), which will be discussed later on. The KIT protein normally becomes activated by a ligand called stem cell factor (SCF). After activation of the tyrosine kinase, downstream pathways get activated. This activation leads to cell survival, proliferation and differentiation (figure 1). (Hirota et al., 1998)

This SCF/KIT pathway is crucial for hematopoiesis. It plays a vital role in the development of primitive hematopoietic cells such as stem and progenitor cells. This signaling pathway has also shown to be necessary for development of ICC in the gastrointestinal tract. (Hirota et al., 1998)

KIT is associated with a lot of human malignancies such as melanomas, testicular seminoma, mast cell disease, acute myeloid leukemia and gastro intestinal stromal tumors. It appears that 70-80% of GISTs harbor a mutation in the KIT gene. These mutations lead to constitutive activation of this kinase and this is why it is such an important therapeutic target (figure 1). (Hirota et al., 1998)

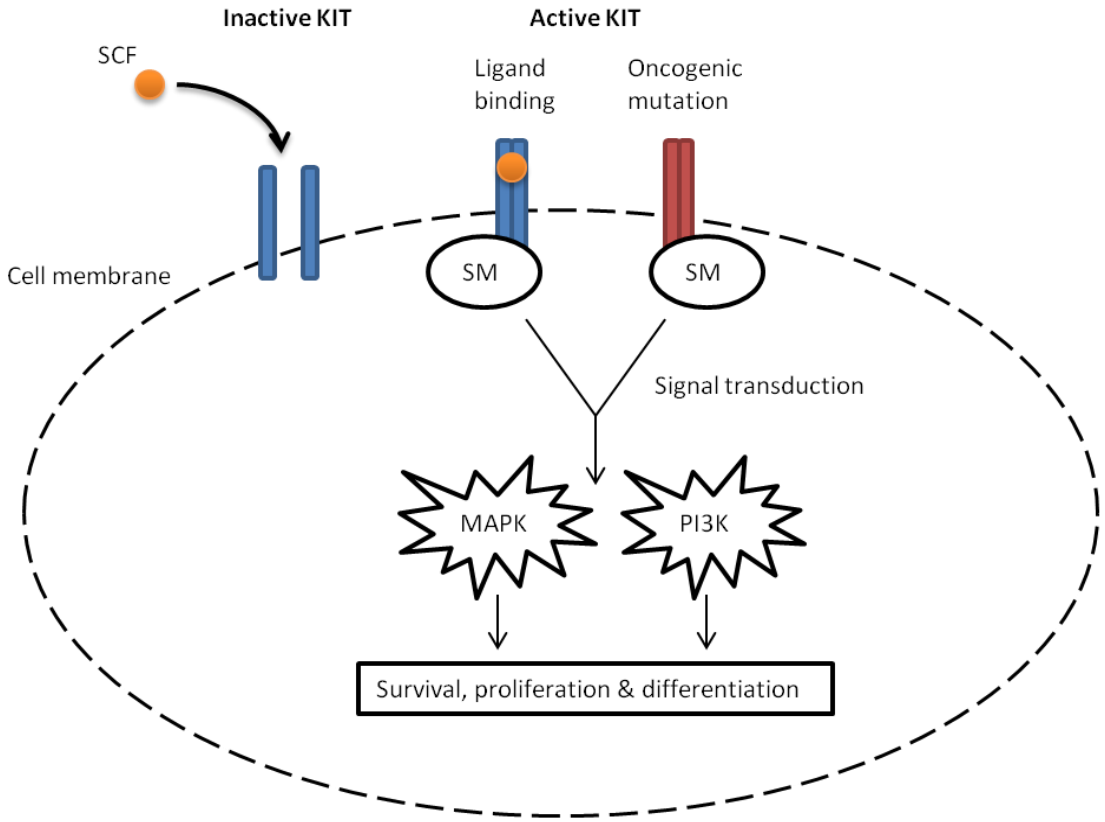

Figure 1 Ligand SCF binds to the KIT protein, leading to its phosphorylation at key amino acid residues. These residues are binding sites for signaling molecules (SM) within the cell. In this way signal transduction is initiated to downstream components. Important signaling cascades that are activated by this SCF/KIT pathway are mitogen-activated protein kinases (MAPK) and phosphatidylinositol 3-kinase (PI3K) signaling. Activation of these signaling pathways leads to cell survival, proliferation and differentiation.

Two-thirds of all GISTs harbor mutations in exon 11, which encodes the juxtamembrane domain. Normally this domain prevents the kinase activation loop from going into an active conformation, but because of this mutation the kinase activation loop becomes constantly active. (Corless et al., 2011)

7-10% of GISTs harbor mutations in exon 9. These mutations are thought to cause the KIT receptor to undergo a conformational change, which is similar to the conformational change that the KIT receptor undergoes when binding the SCF ligand. (Corless et al., 2011)

There are also mutations found in exons 8, 13 and 17, which are uncommon mutations. (Corless et al., 2011)

§ 1.4 - Other causes of GIST

Mutations that lead to GIST are divided into three groups. First of all you have the KIT mutation, which is present in 70 – 80% of the GISTs. Secondly there is the platelet-derived growth factor receptor-α (PDGFRA) mutation, which is present in 5 – 8% of the GISTs, mostly in exon 18. This mutation process is similar to the KIT mutation process as shown in figure 1. In some cases there is neither a mutation in KIT nor in PDGFRA. In these cases the GIST is divined as wild-type. 12 – 15% of the GISTs are wild-type. More about PDGFRA mutations and wild-type mutations will be discussed in chapter 3. An overview of the different mutations that lead to GIST are shown in table 1. (Corless et al., 2011)

Genetic type	Relative frequency	Anatomic distribution	Germline examples
KIT mutation (relative frequency 75–80%)			
Exon 8	Rare	Small bowel	One kindred
Exon 9 insertion AY502-503	10%	Small bowel and colon	None
Exon 11 (deletions, single nucleotide substitutions and insertions)	67%	All sites	Several kindreds
Exon 13 K642E	1%	All sites	Two kindreds
Exon 17 D820Y, N822K and Y823D	1%	All sites	Five kindreds
PDGFRA mutation (relative frequency 5–8%)			
Exon 12 (such as V561D)	1%	All sites	Two kindreds
Exon 14 N659K	<1%	Stomach	None
Exon 18 D842V	5%	Stomach, mesentery and omentum	None
Exon 18 (such as deletion of amino acids IMHD 842–846)	1%	All sites	One kindred
KIT and PDGFRA wild-type (relative frequency 12–15%)			
BRAF V600E	~7–15%		
SDHA, *SDHB*, *SDHC* and *SDHD* mutations	~2%	Stomach and small bowel	Carney–Stratakis
HRAS and *NRAS* mutation	<1%		
Sporadic paediatric GISTs	~1%	Stomach	Not heritable
GISTs as part of the Carney triad	~1%	Stomach	Not heritable
NF1-related	Rare	Small bowel	Numerous

GIST, gastrointestinal stromal tumour; NF1, neurofibromatosis type I; *PDGFRA*, platelet-derived growth factor receptor-α; SDH, succinate dehydrogenase.

Table 1 An overview of different mutations that lead to GIST. (Corless, Barnett, & Heinrich, 2011)

Although these mutations play an important role in the development of GISTs, there are other genetic events that are important in their clinical progression. So it seems that two-thirds of GISTs demonstrate partial loss or monosomy of chromosome 14. Another example is chromosome 22. In 50% of the GISTs, loss of the long arm of this chromosome was observed. (Debiec-Rychter et al., 2001)

Losses on chromosomes 1, 9, 10, 11, 13, 15 and 17 have also been observed, though these are less common. Also, gains on chromosomes 3, 8 and 17 have been observed in GISTs. (Corless et al., 2011)

The exact meaning of these gains and losses on chromosomes remains unclear.

Chapter 2 - Imatinib

§ 2.1 - Discovery of imatinib

As told in the introduction, prognosis of GIST patients before the year 2000 was poor. The response rate to chemotherapy was less than 5% and median survival for patients with advanced GIST was approximately 18 months. (Corless et al., 2011)

In 1996 nature publishes a paper that lays the foundations to make an end to this poor prognosis for GIST patients called: “effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells”. This paper describes the discovery of imatinib. (Druker et al., 1996)

Chronic myelogenous leukemia (CML) is a malignancy of pluripotent hematopoietic stem cells. Back in those days the only treatment for CML was bone marrow transplantation, but only 20-25% of the patients were eligible for such a treatment. (Druker et al., 1996)

For CML it was known that translocation between chromosomes 9 and 22 generated a Bcr-Abl fusion protein which is seen in 95% of the CML patients. Tyrosine kinase activity of this Bcr-Abl protein is essential for the transformation of a “normal cell” to a CML cell. That is why they searched for an inhibitor for this particular protein and they found one. They found that a compound called CGP 57148 (later called imatinib) selectively inhibits the proliferation of Bcr-Abl expressing cells in vitro and in vivo by competition for the ATP binding site of the tyrosine kinase domain. In colony-forming assays of peripheral blood or bone marrow from patients with CML, there was a decrease of 92-98% in the number of Bcr-Abl colonies formed and there was no inhibition of normal colony formation. (Druker et al., 1996)

Later it was observed that Abl shows structural similarity with KIT and that imatinib can inhibit proliferation of cells that express activated KIT-mutant isoforms. After this, it was demonstrated that imatinib was also effective against a KIT-mutant GIST cell line. (Corless et al., 2011)

§ 2.2 - Interaction with KIT

Imatinib stabilizes KIT in the inactive conformation by binding to its ATP-binding site. In this way it competitively inhibits ATP binding. This results in a strong anti-proliferative effect, leading some cells to undergo apoptosis through a mechanism that is dependent on histone H2AX. However, most cells simply stop proliferating through nuclear p27-mediated exit from the cell cycle, as well as by upregulation of autophagy. (Corless et al., 2011)

§ 2.3 - Clinical use of imatinib

As described above imatinib inhibits proliferation of CML cells and GIST cells. Imatinib inhibits proliferation of these tumor cells by inhibiting the tyrosine kinases that are hyperactive in these tumors. In CML this is the Bcr-Abl protein and in GISTs this mostly is the KIT protein. Imatinib can also inhibit the PDGFRA (platelet derived growth factor receptor α) tyrosine kinase activity. (Novartis, 2012)

By inhibiting these tyrosine kinases imatinib can be used as a treatment for patients with CML, GIST, relapsed or refractory Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ALL), myelodysplastic/myeloproliferative diseases associated with PDGFR gene re-arrangements, aggressive systemic mastocytosis (ASM), hypereosinophilic syndrome (HES), chronic eosinophilic leukemia (CEL) or dermatofibrosarcoma protuberans (DFSP). (Novartis, 2012)

Chapter 3 - Is treatment with imatinib enough?

§ 3.1 - Imatinib in 2011

Nowadays imatinib achieves disease control in 70-85% of patients with advanced KIT-mutated GISTs. In 2011 a study done by von Mehren, M. et al. showed out that the median survival for patients with advanced KIT-mutated GISTs who are treated with imatinib is 5 years, with 34% of the patients surviving more than 9 years. (Von Mehren et al., 2011)

So, median survival for GIST patients has improved a lot with imatinib treatment. There are, however, two big problems that have to be dealt with. First of all imatinib only works well on KIT-mutated GIST, but as shown in table 1 there are other mutations that are associated with GIST development. In addition, imatinib has no effect on wild-type GISTs and only little effect on PDGFRA-mutated GISTs. More about these mutations in paragraph 3.2 and 3.3. Secondly, imatinib fails to eradicate all GIST cells, especially mature GIST cells and GIST stem cells. Only 3-5% of patients that are treated with imatinib, show a complete response. Because of the survival of a certain group of cells, GISTs in the vast majority of patients develop drug resistance. (Corless et al., 2011) More about this in paragraph 3.4.

§ 3.2 - PDGFRA mutations that lead to GIST

Some GISTs that lack KIT mutations have high levels of phosphorylation of platelet-derived growth factor receptor α (PDGFRA). As described earlier, PDGFRA is a class III RTK, with some homology to KIT. This kinase becomes activated by the ligand PDGFA and the downstream pathways, which are then activated, are identical to those in KIT-mutant GISTs. (Corless et al., 2005)

Surprisingly, in comparison with KIT-mutant tumors, PDGFRA-mutant GISTs more often have an epithelioid morphology, they have low KIT expression, they arise almost exclusively in the stomach (KIT-mutant tumors arise at a lot of distinct sites along the gastrointestinal tract) and they show different gene expression profiles, which means that they represent a distinct subset of GISTs. (Corless et al., 2005)

PDGFRA is constantly activated in GISTs that harbor mutations in the PDGFRA juxtamembrane domain (encoded by exon 12), the tyrosine kinase domain (encoded by exon 14) or the activation loop (encoded by exon 18). In PDGFRA-mutated GISTs exon 18 mutations are more common (89,6%) than mutations in exon 12 (9,3%) or exon 14 (3.7%). See table 1 for the relative frequencies. (Corless et al., 2005)

When comparing these frequencies to the frequencies of mutations in KIT-mutated GISTs, there are a few differences. First of all two-thirds of all KIT-mutated GISTs harbor mutations in the juxtamembrane domain (exon 11), while only 9.3% of PDGFRA mutations are found in this region

(exon 12). Secondly mutations in the activation loop (exon 17) are rare in KIT-GISTs (less than 1%), but are very common in PDGFRA-mutant tumors (89.6% exon 18). (Corless et al., 2005)

Thus, the PDGFRA downstream signaling pathway is identical to the KIT downstream signaling pathway, but due to different mutations, most PDGFRA-mutated GISTs do not respond to imatinib treatment. The most common mutation that leads to imatinib resistance in PDGFRA-mutated GISTs are probably substitution mutations that affect codon 842 of the activation loop. The D842V mutation is the most common mutation in PDGFRA-mutated GISTs (62.6%) and is a homologue of the D816V mutation in KIT, which is well established to be resistant to imatinib in vitro. These mutations reduce the accessibility of the ATP-binding pocket, which makes it impossible for imatinib to bind. (Corless et al., 2005)

Currently, scientist are looking for new kinase inhibitors that can effectively target this D842V and a few possible candidates have already been found. The second-generation tyrosine kinase inhibitor (TKI) called dasatanib or the IPI-504 inhibitor against stabilizing heat shock protein 90 (HSP90) are two examples. More about second generation TKIs and HSP90 in paragraphs 4.2 and 4.3. (Corless et al., 2005) (Dewaele et al., 2008)

§ 3.3 - Wild-type mutations that lead to GIST

As described in paragraph 3.4, wild-type GISTs are GISTs in which no KIT or PDGFRA mutation is found. Wild-type mutations are found in 12-15% of the GIST patients. However, in pediatric GIST patients, only 15% of tumors show evidence of a KIT- or a PDGFRA-mutation, which means that 85% of these patients have a wild-type GIST. (Tarn et al., 2008)

Clinically these wild-type GISTs are indistinguishable from KIT-mutant or PDGFRA-mutant GISTs, as they have an identical morphology, express high levels of KIT and occur anywhere in the gastrointestinal tract. KIT is also activated, but the mechanism of this activation is unclear. However, recent studies have revealed that wild-type GISTs can be considered as a heterogeneous group and display a variety of other oncogenic mutations. (Corless et al., 2011)

As can be observed in table 1, most of these mutations are very uncommon. The BRAF V600E mutation, however, is present in 7-15% of all wild-type GISTs and might therefore be an interesting target. Figure 2 gives an overview of the role of some of these mutated compartments that lead to wild-type GIST.

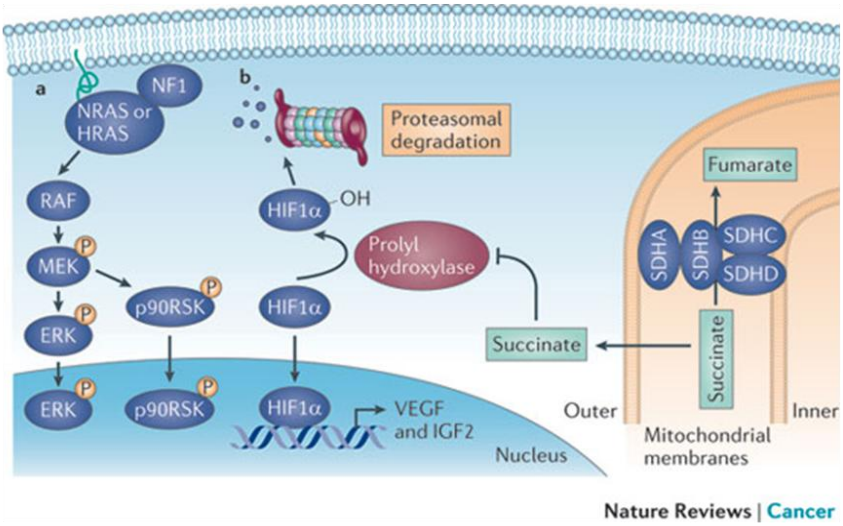

Figure 2 Mutations in neurofibromin 1 (NF1), RAS or BRAF can all increase signalling through the MAPK cascade (part **a**), leading to changes in gene expression (figure 1). The succinate dehydrogenase (SDH) complex is comprised of four subunits (part **b**). Loss of SDH complex activity owing to mutational inactivation of any of the SDH subunits leads to the cytoplasmic accumulation of succinate, which downregulates prolyl hydroxlase. This enzyme is an important negative regulator of hypoxia-inducible factor 1α (HIF1α): by hydroxylating HIF1α, prolyl hydroxlase promotes the proteasomal degradation of HIF1α. Therefore, increased succinate levels lead to increased levels of HIF1α, which can enter the nucleus and activate the transcription of vascular endothelial growth factor (VEGF) and insulin-like growth factor 2 (IGF2). (Corless, Barnett, & Heinrich, 2011)

As shown in the figure above, mutations in wild-type GISTs are mostly found in intracellular proteins. Because imatinib is an inhibitor that only works on KIT, PDGFRA and BCR-ABL tyrosine kinases, it is not suitable as a treatment for wild-type GISTs. That is why surgery is still the only effective therapy, but this is not the whole solution. With surgery you can never be sure that the whole tumor is removed and, in addition, the tumor can already have metastasized. There is still limited benefit of available drug therapies, which is the reason why prognosis for wild-type GIST patients remains poor. (Tarn et al., 2008)

However, a potential target has been found. It appears that the expression of insulin-like growth factor 1 receptor (IGF1R) is significantly higher in wild-type GISTs than in KIT- or PDGFRA-mutated GISTs. This receptor is present in half of all wild-type-mutated GISTs and it is identified as an alternative regulator of cellular proliferation and survival. (Tarn et al., 2008)

IGF1R is part of the IGF (insulin growth factor)-signaling pathway. IGF1R is activated by two different ligands: IGF-1 and IGF-2. IGF1R is the receptor, which interacts with both ligand IGF-1 and IGF-2. If one of these ligands bind, the IGF1R becomes phosphorylated, leading to activation of the MAPK and PI3K cascades, which leads to tumor cell survival, proliferation and differentiation. The difference between the KIT- and the IGF1R signaling pathway is that the molecular mechanism behind overexpression of KIT is well understood, while the molecular mechanism behind overexpression of IGF1R is not. (Tarn et al., 2008)

Nevertheless, IGF1R overexpression is detected in many types of cancer, including wild-type GISTs and this makes it an interesting target. That is why Chi Tarn et al. studied the effect of a tyrosine kinase inhibitor called NVP-AEW541 and they found positive results. This inhibitor showed to have significant inhibitory effects on IGF1R phosphorylation and on GIST cell proliferation in vitro, regardless of the KIT mutational status and IGF1R expression levels. Furthermore, they showed that

you can knock down IGF1R expression by RNAi silencing, which can induce cytotoxicity, even in the presence of activated KIT. (Tarn et al., 2008)

Besides this NVP-AEW541 inhibitor and RNAi silencing, there are other agents available for the inhibition of IGF1R signaling in cancer cells. There are IGF-1- and IGF-2-neutralizing antibodies as well as monoclonal antibodies and small tyrosine kinase inhibitors of the receptor itself. (Miller et al., 2005)

§ 3.4 - Drug resistance

So imatinib does not work so well on PDGFRA-mutated and wild-type GISTs, but it is effective against KIT-mutated GISTs. Though how effective is it? It is shown that imatinib does induce cell dead and the tumor does shrink after imatinib treatment. It is also shown that imatinib can improve prognosis for KIT-mutated GIST patients with years. The problem is that only 3-5% of patients that are treated with imatinib show complete response and the cells that survive can then develop drug resistance. (Corless et al., 2011)

Imatinib can trigger GIST cell apoptosis through up-regulation of the soluble histone H2AX (when a GIST cell fails to control the amount of free histones, this can have deleterious cellular consequences). (Liu et al., 2007) GIST stem cells and/or GIST progenitor cells however, manage to avoid this apoptosis by their low proliferation rate and by expressing genes that are associated with a different genotype. (Corless et al., 2011)

Though, most cells treated with imatinib just become quiescent through p27-mediated exit from the cell cycle (which makes the cell cycle stop at G1) and by up-regulation of autophagy. (Corless et al., 2011)

Thus, overall, imatinib does not eliminate enough GIST cells, which leads to drug resistance and continuous tumor cell growth.

In literature they give two types of imatinib resistance: primary and secondary. Primary resistance is defined as progression within the first 6 months of treatment. This form of resistance occurs in approximately 10% of patients with GIST. Interestingly, tumor response to imatinib appears to correlate with the underlying kinase genotype. The probability of primary resistance to imatinib for KIT exon 11, KIT exon 9 and wild-type GISTs is 5%, 16% and 23%, respectively. (Corless et al., 2011)

The high primary resistance in wild-type GISTs is probably because of the presence of the IGF1R (paragraph 3.3), which can replace KIT in its function for continuous tumor growth. (Tarn et al., 2008)

The most common PDGFRA mutation, D842V, is very resistant to imatinib. This mutation, already described in paragraph 3.2, stimulates the active conformation of the kinase domain and makes it almost impossible for imatinib to bind. This is the main reason why PDGFRA is so resistant to imatinib. (Corless et al., 2005)

The majority of GIST patients that do not have primary resistance, will eventually develop secondary resistance. This resistance mostly arises through an acquired mutation in KIT (67%) or PDGFRA. Such a secondary mutation was observed in 80% of all drug resistant GISTs. Remarkably such a mutation occurs almost exclusively in the same gene and allele as the primary oncogenic driver mutation. (Corless et al., 2011)

Although primary mutations that activate KIT are mostly in the juxtamembrane domain (exon 11) (table 1), secondary mutations mostly occur in two regions of the KIT kinase domain itself (which is targeted by imatinib). The first region is the ATP-binding pocket (encoded by exons 13 and 14). Mutations in this region directly interfere with drug binding and make it impossible for imatinib to bind the KIT kinase. The second region is the activation loop. Mutations in this region can stabilize KIT in the active formation and thereby hinder imatinib interaction. Another problem about these secondary mutations is that they show great heterogeneity. (Corless et al., 2011)

Chapter 4 - How to improve treatment of GIST?

If today's treatment with imatinib is not enough, what should be done to improve treatment of GIST patients? In paragraphs 3.2 and 3.3 the limited response of PDGFRA-mutated- and wild-type GISTs to imatinib has been discussed. It appeared that PDGFRA-mutations that are imatinib resistant are mostly found in codon 842 (D842V). That is why a new kinase inhibitor against this D842V might be the solution to circumvent imatinib resistance. In wild-type GISTs it appeared that there is high expression of IGF1R and new inhibitors of this IGF-signaling pathway are possibly the solution to imatinib resistance in half of all wild-type GISTs. These are two examples of new targets in GISTs. More new targets will be discussed in paragraph 4.3. Maybe GIST treatment might also be improved by using a higher dose of imatinib or by combining imatinib with other (already existing) drugs. These two options are described in paragraphs 4.1 and 4.4. But what if it is too late for these treatments and the GIST has already become imatinib resistant? Then second generation TKIs might be needed. These will be discussed in paragraph 4.2.

§ 4.1 - Higher dose of imatinib

When a GIST shows drug resistance, it is first recommended to increase the dose of imatinib. The result of applying a higher dose is prolonged disease control for a median time of 5 months and in some cases (20-30%) for even a year or more. Overall imatinib is well tolerated, so this does not have many side effects. (Blanke et al., 2008)

Some experts have proposed that a higher dose of imatinib should always be given to GIST patients. Today's treatment is 400 mg daily, but it is suggested that a daily dose of 800 mg would have a better effect on GISTs with KIT exon 9 mutations. (MetaGIST, 2010)

§ 4.2 - Second generation TKIs

Whether a higher dose of imatinib results in prolonged disease control for a period of 5 months or a year or more, one thing is certain: progression of the GIST will inevitably occur. When this happens a switch to alternative KIT and PDGFRA tyrosine kinase inhibitors (TKIs) has to be made. The possible options are listed in table 2. (Corless et al., 2011)

Drug	Targets	Trial information
Tyrosine kinase inhibitors		
Imatinib	KIT and PDGFRA	FDA approved
Sunitinib	KIT, PDGFRA and VEGFR	FDA approved
Nilotinib	KIT and PDGFRA	Phase III (ClinicalTrials.gov ID: NCT00785785)
Dasatanib	KIT and PDGFRA	Phase II (NCT00568750)
Sorafenib	KIT, PDGFRA and VEGFR	Phase II (NCT01091207)
Regorafenib	KIT, PDGFRA and VEGFR	Phase III (NCT01271712)
Vatalanib	KIT, PDGFRA and VEGFR	Phase II (NCT00117299)
Masitinib (AB1010)	KIT and PDGFRA	Phase III (NCT00812240)
Pazopanib	KIT, PDGFRA and VEGFR	Phase II (NCT01323400)
Crenolanib	PDGFRA	Phase II (NCT01243346)
HSP90 inhibitors		
STA-9090	HSP90	Phase II (NCT01039519)
AT-13387	HSP90	Phase II (NCT01294202)
AUY922	HSP90	Phase II (NCT01404650)
Monoclonal antibodies		
IMC-3G3 (Olaratumab)	PDGFRA	Phase II (NCT01316263)
Bevacizumab	VEGFR	Phase III (NCT00324987)
mTOR inhibitor		
Everolimus	mTOR	Phase II (NCT00510354)
Other		
Perifosine	AKT (PI3K pathway)	Phase II (NCT00455559)

FDA, US Food and Drug Administration; GISTs, gastrointestinal stromal tumours; HSP90, heat shock protein 90; PDGFRA, platelet-derived growth factor receptor-α; VEGFR, vascular endothelial growth factor receptor.

Table 2 New drugs to fight GISTs. (Corless, Barnett, & Heinrich, 2011)

As indicated in table 2 most TKIs are still in trial phase. Sunitinib is the only US Food and Drug Administration (FDA) approved drug to serve as an alternative for imatinib. What is also prominent is that most alternatives, including sunitinib, also target VEGFR (vascular endothelial growth factor receptor). By targeting VEGFR, these TKIs inhibit angiogenesis in the tumor and thereby tumor growth. (Corless et al., 2011)

The FDA has approved sunitinib only as a treatment for GIST patients with progression on imatinib. Despite the approval of sunitinib, the range of activity against secondary imatinib-resistant kinase mutations in KIT is still suboptimal. As shown in figure 3, sunitinib is effective against the KIT ATP-binding pocket mutations, but no effect is shown against the activation loop mutations. So a number of GISTs that are resistant to imatinib are also resistant to sunitinib. On top of this, it seemed that GISTs that only had a KIT ATP-binding pocket mutation and were sensitive to sunitinib, developed an activation loop mutation, which made them resistant to the drug. (Nishida et al., 2009)

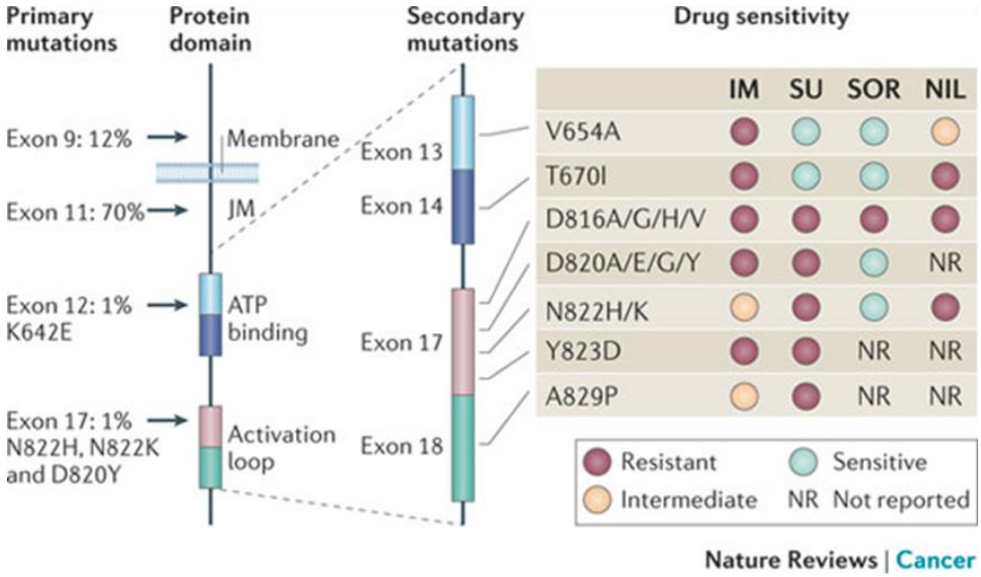

Figure 3 A comparison of the relative in vitro potency of imatinib (IM), sunitinib (SU), sorafenib (SOR) and nilotinib (NIL) versus secondary mutations that are associated with resistance to imatinib is shown. JM, juxtamembrane. (Corless, Barnett, & Heinrich, 2011)

As can be observed in figure 3, sorafenib might be a more effective tyrosine kinase inhibitor for imatinib resistant tumors than sunitinib. In phase 2 clinical trials, it provided 10-month progression-free survival. This looks very promising, but sorafenib will first have to pass the phase III trial which is currently underway. (Corless et al., 2011)

Thus, promising second generation tyrosine kinase inhibitors are being developed, though one big problem remains. Imatinib and other TKIs can possibly extend the time displayed on the ticking time bomb, but in the end the bomb will explode, in other words GIST progression can be paused, but cannot be stopped. In order to cure patients from GISTs, it seems that more actively GIST cell death needs to be induced and because imatinib barely kills GIST cells, new drugable targets have to be found.

§ 4.3 - New targets

A new kinase inhibitor against D842V might be the solution to imatinib resistant PDGFRA-mutated GISTs. Inhibitors of the IGF-signaling pathway might bring a solution to imatinib resistant wild-type GISTs. What other treatment options can be envisioned? In this paragraph some new drugs that can target imatinib-resistant GISTs will be discussed.

It appears that the expression of KIT in imatinib-resistant GISTs is three- to six fold higher than in imatinib-sensitive GISTs. This indicates that the KIT signaling pathway remains a useful therapeutic target at the time of GIST clinical progression. There are some novel inhibitors of KIT that show promising preclinical activity against certain imatinib-resistant mutations in GIST, but none of these inhibitors are expected to be effective in inhibiting all the known imatinib-resistant mutations. The reason for this is the heterogeneity of imatinib-resistant mutations within individual patients. (Bauer et al., 2007)

Figure 4 gives an overview of the signaling pathways that become activated in KIT or PDGFRA mutated GISTs. PI3-K and MAPK are two new promising downstream therapeutic targets in imatinib-resistant GISTs. Agents versus PI3-K and MAPK are expected to be effective in inhibiting all the known imatinib-resistant mutations, because they target downstream components of KIT and PDGFRA. (Bauer et al., 2007)

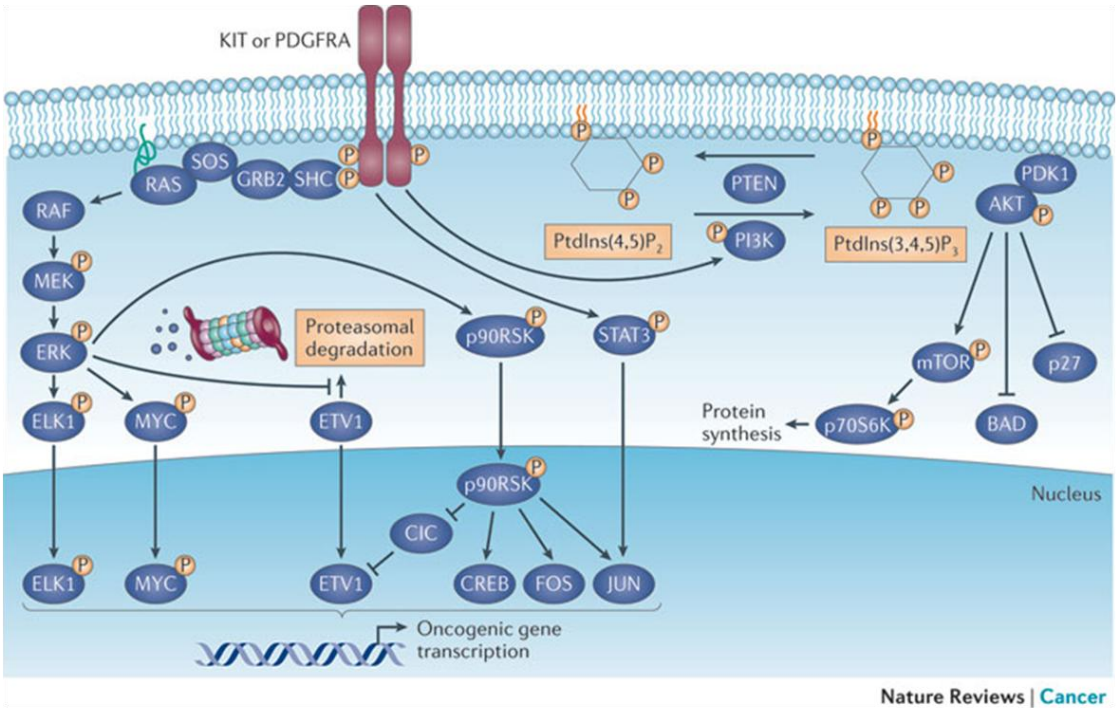

Figure 4 Oncogenic signaling in KIT- and PDGFRA-mutant GISTs. On the left there is the MAPK cascade which leads to changes in gene transcription through ELK1 and MYC. This cascade also activates P90SK which is an important protein for regulation of gene transcription. On the right there is the PI3-K cascade. PI3-K becomes activated by KIT and this leads to phosphorylation of AKT, which eventually leads to alternation in protein translation, metabolism and apoptosis. (Corless, Barnett, & Heinrich, 2011)

S. Bauer et al. put the possible new therapeutic targets PI3-K and MAPK to the test. In imatinib-resistant GISTs, PI3-kinase inhibition resulted in strong inhibition of proliferation (40-75%) as well as increased apoptosis (three- to fourfold). MAPK inhibition resulted in little inhibition of proliferation (5-20%) and no increase in apoptosis. From this they conclude that MAPK might be less suitable than PI3-K for therapeutic targeting in imatinib-resistant GIST. (Bauer et al., 2007)

Another interesting new target is heat shock protein 90 (HSP90). HSP90 is a protein that stabilizes a lot of active oncogenic proteins, such as KIT and PDGFRA. In this way such oncoproteins are protected from proteasome-mediated degradation. KIT activation seems to depend on HSP90 and inhibition of this protein causes degradation of wild-type-KIT and an imatinib-resistant KIT D816V mutant. As described in paragraph 3.2, this mutant is a homologue of the D842V mutation, which is the most common mutation in PDGFRA-mutated imatinib-resistant GISTs. (Bauer et al., 2006)

Inhibition of HSP90 is very efficient for removing active KIT proteins. In another study by S. Bauer et al. they found that after 60 minutes treatment with 17-allylamino-18-demethoxy-geldanamycin (17-AAG) there was nearly a complete loss of KIT expression, but after 6 hours KIT expression was increased again. This is probably because only a minor subset of oncogenic KIT proteins are activated during these 60 minutes. Treatment with 17-AAG for a longer period of time might therefore increase the long-term efficiency. (Sebastian Bauer et al., 2006)

A more recent study done by Floris G et al. found that another HSP90 inhibitor called IPI-493 is also very efficient in human GISTs with heterogenous KIT mutations. (Floris et al. 2011)

HSP90 inhibition also reduces KIT expression in imatinib-sensitive GISTs. When combining imatinib treatment with HSP90 inhibition, apoptosis of imatinib-sensitive GIST cells increased three- to tenfold. Therefore it might be possible to combine HSP90 with imatinib to improve prognosis for imatinib-sensitive GIST patients as well. (Bauer et al., 2006)

Thus, after GISTs become imatinib resistant, it might be wise to target downstream components, because the mutations that lead to imatinib resistance mostly occur in the KIT or PDGFRA protein and show great heterogeneity. However, it would be even better if it was possible to prevent mutations that lead to imatinib resistance, for example by combining imatinib with drugs that can eliminate the remaining tumor cells.

§ 4.4 - Combination therapy

Imatinib is able to control over 70% of all GISTs. The problem is that some of these GIST cells survive imatinib treatment and develop resistance. As mentioned in earlier paragraphs it might be possible to combine imatinib with other drugs in order to improve prognosis for imatinib-sensitive GIST patients. Combined treatment can possibly lead to better disease control and what is more important, it might eliminate the cells that survive imatinib treatment and thereby prevent imatinib resistance.

For what I read, I think the most suitable candidates for combination therapy with imatinib are the ones that can induce apoptosis. When more cells are killed, prognosis will likely improve, because it would take longer for the GIST to develop drug resistance. Two apoptosis inducing candidates are PI3K inhibitors and HSP90 inhibitors that were described before.

In a study by Anu Gupta et al. it is shown that when imatinib is combined with ATG depletion (ATG is important in the autophagy survival pathway) or with chloroquine or quinacrine (toxic agents) treatment, more GIST cells are killed compared to uncombined imatinib treatment. (Gupta et al., 2010)

When c-KIT becomes inhibited by imatinib, PI3K- and MAPK signaling pathways are inhibited as well (figure 1). Inhibition of these pathways leads to upregulation of the proapoptotic factor BIM. (Gordon et al., 2010) One way in which BIM induces apoptosis is by inhibiting the Bcl-2 protein. Bcl-2 prevents cells from going into apoptosis. A preclinical study by Reynoso D et al. shows that Bcl-2 inhibition by ABT-737 in combination with imatinib treatment also results in increased apoptosis of GIST cells. (Reynoso et al., 2011)

The death receptor protein Fas is highly expressed on GIST cells. A study by B Rikhof et al. shows that treatment with a low dose of MegaFasL (which is a Fas ligand) leads to increased apoptosis of GIST cells. On top of this MegaFasL increases the apoptotic effect of imatinib. Because Fas is highly expressed on GIST cells, antibody treatment against this Fas receptor might also induce GIST cell dead. (Rikhof et al. 2008)

So, there are a lot of different studies that indicate that combination therapy with imatinib can improve GIST treatment.

Discussion

The goal of this paper was to find out whether imatinib is successful enough as a single drug treatment for gastro intestinal stromal tumors. I believe I found my answer. Because imatinib achieves disease control in more than 70% of all GIST-patients (Corless et al., 2011), I think imatinib is very useful to pause GIST progression, but imatinib is no curing GIST patients. Moreover, its control of PDGFRA- and wild-type GISTs is insufficient and GIST cells in the vast majority of patients develop drug resistance after which GIST progression simply carries on from where it was paused. (Corless et al., 2011)

Giving a higher dose of imatinib will not solve the problem, but will most likely improve prognosis for imatinib-sensitive GIST patients. A higher dose results in prolonged disease control for a median time of 5 months and in some cases (20-30%) for even a year or more. (Blanke et al., 2008) On top of this it is thought that prognosis for KIT exon 9 mutated GIST patients will improve when treated with a higher dose. (MetaGIST, 2010) Because imatinib is well tolerated, it is at least worth trying.

I think second generation tyrosine kinase inhibitors (TKIs) might improve prognosis for GIST patients, especially when they also target VEGF. With these TKI's more GIST cells might be eliminated. It is difficult to say something about the effectivity however, because they are now only given when GISTs become imatinib resistant. In the end I do not think these new TKI's are going to be the cure for GIST patients, because for successful treatment, I think elimination of all GIST cells is needed and I do not believe that these second generation TKI's are capable to do so.

In my opinion, new targets have to be found to really cure GIST patients. The PDGFRA D842V mutation is the most common mutation in PDGFRA-mutated GISTs and causes imatinib-resistance. Kinase inhibitors that can effectively target this D842V are therefore interesting. Until a successful treatment for PDGFRA-mutated GIST patients is found, I think these patients deserve treatment with imatinib, because it is thought that GISTs in more than one third of these patients are imatinib-sensitive. (Corless et al., 2005)

Patients that suffer from wild-type GISTs with other mutations should not be forgotten. Although only a minority (12-15%) of all GIST patients suffer from these mutations, these patients have no other available treatment than surgery and prognosis for these patients is poor. I think this is a serious issue considering that 85% of all pediatric GIST patients harbor a wild-type mutation. In my opinion, patients with a wild-type GIST mutations have the right to get imatinib treatment as a trial, because you never know whether imatinib will or will not work in individual patients, but alternatives have to be found. IGF1R signaling inhibition seems to be interesting. (Tarn et al., 2008)

For imatinib-resistant KIT- and PDGFRA-mutated GISTs, there are other possible new targets. Because secondary mutations that make a KIT- or PDGFRA-mutated GIST imatinib-resistant mostly occur in the KIT- or PDGFRA protein itself and because these mutations show great heterogeneity, new targets should probably be found in downstream components of the KIT and PDGFRA signaling pathways. For inhibition of proliferation and in order to increase apoptosis of imatinib-resistant GIST cells, inhibitors of the MAPK- and the PI3-K cascade (Bauer et al., 2007), as well as inhibitors for stabilizing protein HSP90, seem to be appropriate candidates. (Bauer et al., 2006)

Whether imatinib can successfully be combined with other drugs to enhance GIST cell death, so that it might become a curable disease, remains unclear. However, a lot of studies have been done to improve imatinib treatment. Apoptosis can be induced by HSP90- or PI3K inhibition, by ATG depletion, with toxic agents like chloroquine and quinacrine, by upregulation of BIM or downregulation of Bcl-2, by targeting the Fas death receptor and maybe in some other ways.

In conclusion, I think imatinib is very successful as a treatment for GIST patients, however it is not successful enough. I think treatment is truly successful when it cures a patient and in this case that means elimination of all tumor cells. This goal can only be reached step by step and I think the most interesting first step is induction of apoptosis by targeting downstream components and by combining imatinib with apoptosis inducing drugs.

Afterword

When I decided to choose a targeted anticancer drug as a subject for my paper, I did not expect it to be so interesting at all. However, it became more and more interesting as I started to read and write about it. When leaving high school I believed I was the one to find the cure for cancer. From what I learned at the university, I started to realize this was going to be a difficult job. This paper made me realize that it is impossible and that I was very naïve. There are so much different types of cancer and each single one of them has its own mutations and properties, which makes the mystery of cancer much more complicated then I had ever imagined. That is why I respect those who spend everyday life, trying to solve this mystery.

I look forward to the oncology research course that I am allowed to follow for the coming six weeks and I want to thank Steven de Jong for his supervision.

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