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TRANSLATIONAL INVESTIGATIONS OF NOVEL AND CURRENT ANTITUMORAL THERAPIES IN GASTROINTESTINAL STROMAL TUMORS

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To my family and Sophia

ABSTRACT

Gastrointestinal stromal tumor (GIST) is the most common human sarcoma. Its incidence is around 10-15 per million person-years, translating into 150 new cases each year in Sweden. The molecular background for the absolute majority of GIST is characterized by *gain-of-function* mutations in *KIT* or *PDGFRA* genes, both encode receptor tyrosine kinases, allowing for targeted treatment with imatinib. This has revolutionized the treatment of GIST, which is inherently radio- and chemotherapy insensitive. However, durable remissions are uncommon relating to the development of resistance.

The overall aim of the thesis was to explore novel and current treatments in GIST, as few treatment alternatives exist.

In **paper I**, we examined the functional role of DOG1 protein, a diagnostic marker, in GIST. The protein is a calcium-activated chloride channel. We determined the expression of DOG1 and found a difference between imatinib-sensitive and imatinib-resistant cell lines with regards to subcellular localization. Electrophysiological registration confirmed the modulating ability of the DOG1 activator and inhibitor. Only modest effect was seen on proliferation, DOG1 inhibition induced a shift from early apoptotic to late apoptotic cells in the imatinib-resistant cell line.

In **paper II**, we used a new potent inhibitor ($CaCC_{inh}$-A01) of DOG1. We confirmed its inhibitory effect on chloride currents using patch-clamp technique. The cell viability was reduced. Furthermore, colony formation ability was markedly decreased after incubation with $CaCC_{inh}$-A01. $CaCC_{inh}$-A01 also led to a G1-cell cycle arrest, which was not seen with $T16_{inh}$-A01 treatment. Therefore, **paper I** and **II**, confirms that DOG1 could potentially be a target for therapy.

In **paper III**, we explored the antitumoral effects of a novel polymer-based therapy (PVAC). *In vitro* experiments revealed PVAC potently induced a population of non-viable cells, in a non-linear dose-response relationship. *In vivo* PVAC inhibited tumor growth in immunocompetent mice, and an increased $CD3^+$ cell infiltration intratumorally was observed.

In **paper IV**, we explored the commonly used tyrosine kinase inhibitors imatinib, sunitinib, and nilotinib possible interaction with ATP-binding sites, in which we used murine pancreatic β-cells as ATP-sensitive K^+ (K_{ATP}) channel donors. By using patch-clamp technique, we showed that all three tyrosine kinase inhibitors decreased the channel activity. Further studies revealed an increased channel activity with imatinib in the presence of ATP and ADP.

In **paper V,** the aim was to determine the safety and efficacy of intratumorally injected allogeneic pro-inflammatory dendritic cells (ilixadencel) in patients with advanced GIST and progression on tyrosine kinase inhibitors. The study showed an acceptable safety profile, and promising radiological response was observed in two out of six patients.

To conclude, this translational thesis adds knowledge to new potential targets and novel antitumoral strategies, and increases our understanding of current treatment. Lastly, a clinical study found encouraging response in some patients and warrants further studies.

LIST OF SCIENTIFIC PAPERS

The thesis is based on the following papers, hereby denoted with Roman numbers:

I. Berglund E, Akçakaya P, Berglund D, Karlsson F, Vukojević V, Lee L, Bogdanović D, Lui WO, Larsson C, Zedenius J, **Fröbom R**, Bränström R. Functional role of the Ca^{2+}-activated Cl^- channel DOG1/TMEM16A in gastrointestinal stromal tumor cells.
Experimental Cell Research 2014;326(2):315-325.
doi: 10.1016/j.yexcr.2014.05.003

II. **Fröbom R**, Sellberg F, Xu C, Zhao A, Larsson C, Lui WO, Nilsson IL, Berglund E, Bränström R. Biochemical inhibition of DOG1/TMEM16A achieves antitumoral effects in human gastrointestinal stromal tumor cells *in vitro*.
Anticancer Research 2019;39(7):3433-3442.
doi: 10.21873/anticanres.13489

III. Sellberg F*****, **Fröbom R***, Binder C, Berglund D, Berglund E. Carbazate-activated polyvinyl alcohol (PVAC) as an antitumoral polymer.
Manuscript.

IV. **Fröbom R**, Berglund E, Aspinwall CA, Lui WO, Nilsson IL, Larsson C, Bränström R. Direct inhibition of the ATP-sensitive K^+ channel by tyrosine kinase inhibitors imatinib, sunitinib and nilotinib.
Manuscript.

V. **Fröbom R***, Berglund E*****, Berglund D, Nilsson IL, Åhlén J, Von Sivers K, Linder Stragliotto C, Suenaert P, Karlsson-Parra A, Bränström R. Phase 1 trial evaluating safety and efficacy of intratumorally administered inflammatory allogeneic dendritic cells (ilixadencel) in advanced gastrointestinal stromal tumors.
Manuscript.

***** Shared first authorship

OTHER PUBLICATIONS:

Berglund E, Alonso-Guallart P, Danton M, Sellberg F, Binder C, **Fröbom R,** Berglund D, Llore N, Sakai H, Iuga A, Ekanayake-Alper D, Reimann KA, Sachs DH, Sykes M, Griesemer A. Safety and pharmacodynamics of anti-CD2 monoclonal antibody treatment in cynomolgus macaques- an experimental study.
Transpl Int. 2019. [Epub ahead of print]

Berglund E, Daré E, Branca RM, Akcakaya P, **Fröbom R**, Berggren PO, Lui WO, Larsson C, Zedenius J, Orre L, Lehtiö J, Kim J, Bränström R. Secretome protein signature of human gastrointestinal stromal tumor cells.
Exp Cell Res 2015;336(1):158-170.

Berglund E, Ubhayasekera SJ, Karlsson F, Akcakaya P, Aluthgedara W, Ahlen J, **Fröbom R**, Nilsson IL, Lui WO, Larsson C, Zedenius J, Bergquist J. and Bränstrom R. Intracellular concentration of the tyrosine kinase inhibitor imatinib in gastrointestinal stromal tumor cells.
Anticancer Drugs 2014;25(4):415-422.

Ueda P, Rafatnia F, Bäärnhielm M, **Fröbom R**, Korzunowicz G, Lönnerbro R, Hedström AK, Eyles D, Olsson T, Alfredsson L. Neonatal vitamin D status and risk of multiple sclerosis.
Ann Neurol 2014;76(3):338-346.

Lu Y, **Fröbom R**, Lagergren J. Incidence patterns of small bowel cancer in a population-based study in Sweden: increase in duodenal adenocarcinoma.
Cancer Epidemiol 2012;36(3):158-163.

CONTENTS

LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
CaCC	Calcium-activated chloride channel
CML	Chronic myelogenous leukemia
CT	Computed tomography
DOG1	Discovered on GIST-1 (other synonyms TMEM16A, ANO1)
EGFR	Epidermal growth factor receptor
EMT	Epithelial-mesenchymal transition
ETV1	ETS Translocation Variant 1
FDA	US Food and Drug Administration
FDG-PET	18F-fluorodeoxyglucose positron emission tomography
GIST	Gastrointestinal stromal tumor
HIF1A	Hypoxia-inducible factor 1 alpha
ICC	Interstitial cell of Cajal
Ido	Indoleamine 2,3-dioxygenase
IGF	Insulin Growth Factor
KIT	c-kit, stem cell factor receptor
MAPK	Mitogen-activated protein kinase
MAX	MYC-associated factor X
mTOR	Mammalian target of rapamycin
NF1	Neurofibromin 1
NIH	National Institute of Health
OS	Overall survival
PDGFRA	Platelet-derived growth factor receptor alpha
PFS	Progression-free survival
PI-3K	Phosphoinositide-3-kinase
PIP_2	Phosphatidylinositol 4,5-bisphosphonate
PVAC	Polyvinyl alcohol carbazate
RFS	Recurrence-free survival
RTK	Receptor tyrosine kinase
SCF	Stem cell factor
SDH	Succinate dehydrogenase
STAT3	Signal transducer and activator of transcription 3
STS	Soft tissue sarcoma
TKI	Tyrosine kinase inhibitor
VEGF	Vascular endothelial growth factor

INTRODUCTION

Gastrointestinal stromal tumors (GIST) can arise anywhere in the gastrointestinal tract and is the most common type of sarcoma. Its diagnostic specificity was greatly improved two decades ago, with the finding of new diagnostic markers like KIT and DOG1. At the same time, pathogenic mutations were identified that not only increased diagnostic specificity, but also resulted in the introduction of targeted therapies. GIST was the first solid tumor with such treatment and has in many ways served as a model system for modern targeted therapies. Despite the success of the introduction of targeted treatment, resistance ultimately develops in a majority of patients, and durable remissions are rare. New treatment alternatives are, therefore, needed to address this clinical problem.

GASTROINTESTINAL STROMAL TUMORS - GIST

GIST is part of the group of mesenchymal-derived tumors, referred to as sarcomas. Sarcomas constitute approximately 1-2% of all cancer in adults (Mastrangelo *et al.*, 2012), whereas epithelial tumors are the largest group of tumors. Sarcomas constitute a heterogeneous group of tumors, with presently about 60 different types, and new types are still identified or re-classified with increasing knowledge (Jo and Fletcher, 2014). Sarcomas are generally classified as either soft tissue sarcomas, often abbreviated as STS, or as skeletal sarcomas. GIST belongs to soft tissue sarcomas. With the development of new diagnostic methods, significant advantages in differentiating and subclassifying these tumors have been made in the last two decades.

In the mid-1900s, there were difficulties in diagnosing and distinguishing between different types of mesenchymal tumors. Mesenchymal-derived tumors were, including GIST, collectively termed smooth-muscle neoplasms, and classified, depending on cellular morphology, as leiomyoma, leiomyosarcoma, leiomyoblastoma, depending on if the lesion was benign, malignant or possessed an epithelioid feature, they were even misclassified as nerve sheath tumors or schwannomas (Fletcher *et al.*, 2002). The introduction of immunohistochemistry in the 1980s did not reveal the typical immunophenotype seen in smooth muscle differentiated cells, or Schwannian cells (Mazur and Clark, 1983). Electron microscopy examination did not show ultrastructural features of smooth muscle cells, such as myofilament, therefore the terminology was changed to gastric stromal tumors instead of smooth-muscle neoplasm (Mazur and Clark, 1983). In addition, given the appearance of the cells with vacuoles, cell processes and primitive junctions rather suggested a possible cell source in the myenteric nervous system. Further efforts followed to enable differential diagnosis between GIST, leiomyomas and schwannomas. Another protein named CD34 was found to be a marker that

served this purpose (Miettinen, Virolainen and Maarit-Sarlomo-Rikala, 1995; van de Rijn, Hendrickson and Rouse, 1994). However, it was later found that this marker was only present in 60-70% of all GIST (Fletcher *et al.*, 2002).

In the late 1990s, several key findings were made that facilitated the diagnosis of GIST. Firstly, it was found that CD117 (KIT) was highly expressed in GIST and enabled improved differentiation between GIST and leiomyomatous tumors and schwannoma (Sarlomo-Rikala *et al.*, 1998). This was a shared feature with the interstitial cells of Cajal (ICC), which serves as the connection between the autonomous nervous system and gut muscle, enabling peristalsis (Huizinga *et al.*, 1995). The similar expression patterns, including vimentin and CD34, also suggested the connection between ICC and GIST (Kindblom *et al.*, 1998; Sircar *et al.*, 1999). Additionally, it was shown that KIT-deficient mice lacked functional ICCs and KIT-inhibition in ICC induced a smooth-muscle differentiation (Torihashi *et al.*, 1999; Kitamura and Hirotab, 2004). Collectively, it is today believed that GIST originates from ICCs or stem cells that typically differentiate into ICCs.

The finding of abundant KIT expression in GIST soon led to the discovery that *gain-of-function* mutation is a common event in GIST (Hirota *et al.*, 1998), which shortly after came to revolutionize the treatment of GIST, as described below in the section "Clinical management of GIST".

Histopathology

A diagnosis of GIST is difficult using only a morphological examination alone and there are several differential diagnoses that need to be considered. Several markers have been identified that aid in the diagnosis of GIST, see Table 1. The key findings described above had a profound impact on the diagnosis of GIST and confirmed these to be sensitive markers for GIST disease (Kindblom *et al.*, 1998; Hornick and Fletcher, 2002; Sarlomo-Rikala *et al.*, 1998). The majority, ~95% of GIST express KIT (Miettinen and Lasota, 2013). Gene expression analysis had revealed another interesting marker for GIST, which was named "Discovered on GIST-1" (DOG1), and found to be positive in ~98% of GIST irrespective of mutational status (West *et al.*, 2004). It was shortly thereafter introduced into the diagnostic markers and is still used today as a hallmark in the diagnosis of GIST. Interestingly, this protein is also expressed at high levels in ICCs (Hwang *et al.*, 2009). Protein kinase C theta, is expressed in about 85% of GIST and was found to stain all KIT-negative GIST specimens, making it a useful marker in the presence of KIT-negative GIST (Motegi *et al.*, 2005), as DOG1 is negative in two-thirds of KIT-negative GIST (Liegl *et al.*, 2009). KIT (CD117) and DOG1 today serve as the preferred markers in GIST diagnostics (Figure 1) and can stain almost 100% of GIST tumors (Novelli *et al.*, 2010).

Target protein	Proportion positive in GIST
DOG1	>95%
KIT (CD117)	>95%
PKC theta	85%
CD34	60-70%
Smooth-muscle actin	30-40%
S100	5%

Table 1. Protein markers expressed in GISTs. (Fletcher *et al.*, 2002; Miettinen, Virolainen and Maarit-Sarlomo-Rikala, 1995; Motegi *et al.*, 2005).

Macroscopically, GISTs are commonly well-circumscribed with a pink or tan cut surface, which might exhibit necrosis and cystic degeneration (Corless, 2014). Most GIST cases (~70%) exhibit a spindle-cell morphology, which is characterized by cells arranged in fascicles or spiral pattern. Cell-borders are difficult to distinguish depending on syncytial appearance. Epithelioid-type GIST is observed in around 20% of GIST, with rounded cells with clear cytoplasm, in contrast to spindle-cell with more eosinophilic cytoplasm. The remaining (~10-20%) part is a mixed-type, where both spindle-cell and epithelioid can be present in the same tumor (Fletcher *et al.*, 2002). With the advent of tyrosine kinase inhibitor treatment, it should be noted that the morphology and immunophenotype may change during tyrosine kinase inhibitor treatment (Pauwels *et al.*, 2005).

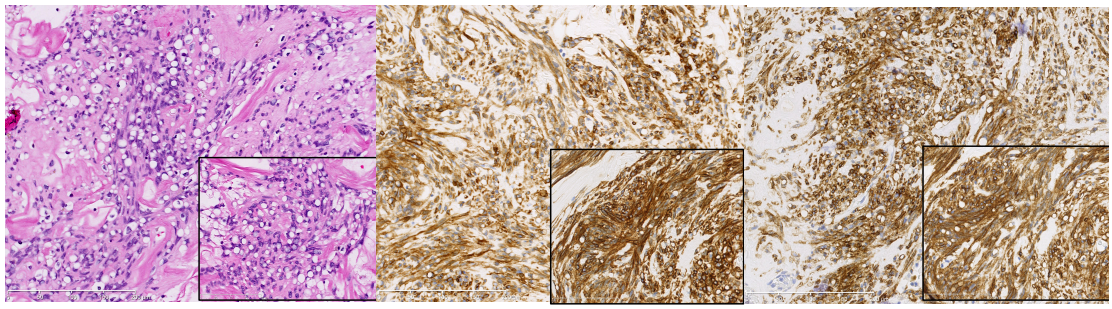

Figure 1. An example of GIST from the stomach with a spindle-shaped morphology. From left to right: haematoxylin staining (20X and 40X), immunostaining for CD117 (KIT) and DOG1. The mutational screening revealed a *KIT* exon 11 mutation.

Epidemiology

As stated above, GIST is the most common type of sarcoma, contributing to about 20% of the reported soft tissue sarcomas (Ducimetière *et al.*, 2011; Mastrangelo *et al.*, 2012; Yang *et al.*, 2019). In a meta-analysis, of 13,500 GIST patients, the incidence was found to be around 10-15 per million per year (Søreide *et al.*, 2016). However, reported incidences varied 4-5 folds between reported studies. The prevalence of GIST has been estimated to be 130 per million (Nilsson *et al.*, 2005), and will likely increase in the future as treatment efficacy improves and GIST

patients live longer with the disease. The incidence is also age-dependent. The median age at the time of diagnosis is around 65, with the age-adjusted incidence being 4-5-fold higher for people aged >50 years compared to <50 years of age (Yan *et al.*, 2008). GISTs show no distinct predilection to either gender, and the female:male ratio is around one in most reported series (Søreide *et al.*, 2016). Secondary malignancies have been reported in 20% of GIST (Cavnar *et al.*, 2019). Pediatric GISTs are uncommon and are typically not associated with *KIT* or *PDGFRA* mutations (Prakash *et al.*, 2005; Miettinen, Lasota and Sobin, 2005).

The anatomical distribution of GIST is fairly consistent across different studies. Stomach (~55%) and the small intestine (~30%) are the most common, together making up the absolute majority of localized GIST. Less frequent locations are colorectal (6%, which mostly refers to GIST tumors of the rectum) and esophagus (0.7%) (Søreide *et al.*, 2016). The predilection for certain anatomical sites is partly related to the mutational status (Corless, Barnett and Heinrich, 2011). It has previously been shown that small intestinal GISTs have a worse prognosis (Miettinen and Lasota, 2006), but after imatinib was introduced, a recent study showed that small intestine location does not affect long-term prognosis (Cavnar *et al.*, 2019).

Micro-GISTs are tumors of small size (ranging from 1 to 10 mm) that rarely become malignant, and are frequently encountered in the general population in up to 30% of patients (Corless *et al.*, 2002; Kawanowa *et al.*, 2006; Agaimy *et al.*, 2007; Abraham *et al.*, 2007). Micro-GISTs do not express mitotic markers and are frequently calcified, implicating tumor progression arrest (Corless, 2014). However, they frequently carry similar mutations in *KIT,* and sometimes also *PDGFRA,* as larger GISTs (Muenst *et al.*, 2011; Rossi *et al.*, 2010; Corless *et al.*, 2002). From this, it has been hypothesized that the gain-of-function mutations in *KIT* and *PDGFRA* are indeed early events in the tumor development but are not sufficient to cause clinically overt GIST.

Clinical presentation and work-up

GISTs vary in size, with most tumors being larger than 5 cm, and only about 10% are less than 2 cm (Søreide *et al.*, 2016). Varying degrees of symptoms occurs; while 75-80% of the patients are symptomatic at the time of diagnosis/clinical presentation, 20-25% are asymptomatic and the tumors are found on routine imaging, endoscopy, or intraoperatively. The disease might follow an indolent course with just minor symptoms such as fatigue, dysphagia, palpable mass, and anemia. However, dramatic courses with the patient presenting with massive bleeding or tumor rupture that requires emergent surgery also occurs (Etherington and DeMatteo, 2019).

The Scandinavian Sarcoma Group recommends referral to a sarcoma center for tumors that are larger than 5 cm, deep-seated tumors, or metastatic disease. Multidisciplinary workup is important and should involve surgeons, oncologists,

radiologists and pathologists among others. Generally, the preferred imaging for GIST is contrast-enhanced computer tomography (CT) of the abdomen and pelvis for staging (Casali *et al.*, 2018). GIST is characterized by a contrast-enhanced, most commonly in the periphery, tumor mass that can either be exophytic or endophytic appearance. Since GIST are commonly large, it is not unusual with tumors present outside the organ from which it originally developed (Levy *et al.*, 2003). Upon diagnosis, about 15% of patients present with metastatic disease (Cavnar *et al.*, 2019), and most common metastatic locations are liver and peritoneum, which together account for about 80% of metastases, making imaging of abdomen and pelvis plausible in the majority of cases. For rectal GISTs, magnetic resonance imaging (MRI) may provide better staging (Casali *et al.*, 2018).

Molecular basis of GIST

The majority of GISTs carry driving mutations in the *KIT* gene (c-kit, stem cell factor receptor), and a subset display mutations in the *PDGFRA* gene (platelet-derived growth-factor receptor alpha) (Hirota *et al.*, 1998; Hirota *et al.*, 2003; Heinrich *et al.*, 2003). Both genes are located on the long arm of chromosome 4 (Stenman, Eriksson and Claesson-Welsh, 1989) and encode proteins that belong to the subfamily of type III receptor tyrosine kinases (see Figure 2).

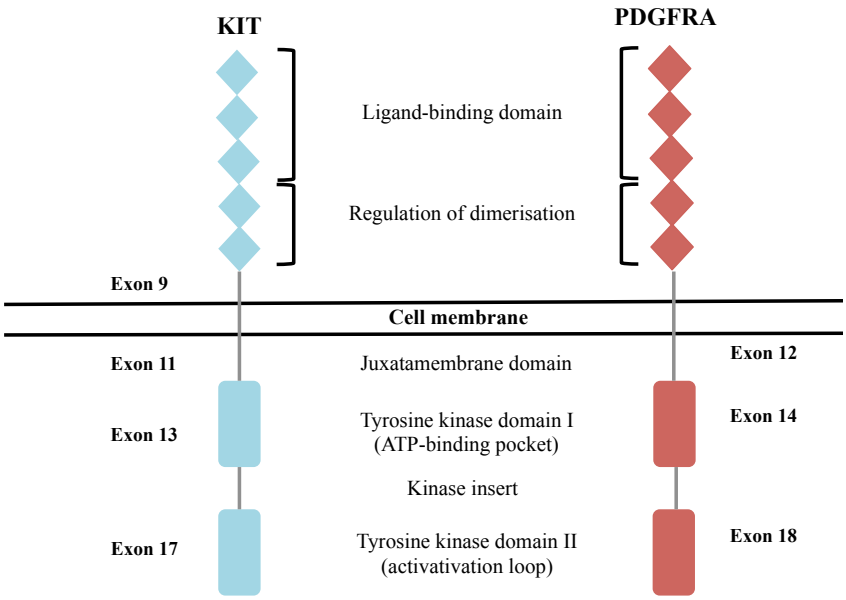

Figure 2. Structure of the type III receptor tyrosine kinases KIT and PDGFRA, with indication of the corresponding commonly mutated exons. Adapted and modified from (Joensuu, Hohenberger and Corless, 2013).

Receptor tyrosine kinases (RTK) share some common features but are different in several ways. The extracellular part differs between them to ascertain ligand specificity. In KIT and PDGFRA, it consists of five immunoglobulin-like domains for ligand recognition. The transmembrane is located in the cell membrane and the juxtamembrane domain is in close proximity intracellularly. The intracellular part contains two domains, one ATP-binding domain and one activation loop. The exons encoding for the different parts of the KIT and PDGFRA receptors are shown in Figure 2. Upon ligand binding, RTKs, which are usually present in their monomer form, dimerize and undergo autophosphorylation, during which phosphates are added on selected tyrosine residues in the dimerized pair of receptors. This, in turn, leads to the activation of downstream signaling pathways (Schlessinger and Ullrich, 1992).

Under physiological conditions, ligand binding of stem cell factor (SCF) to KIT and of platelet-derived growth factor (PDGF) to PDGFRA leads to dimerization of the receptor and subsequent activation (Blume-Jensen *et al.*, 1991; Yuzawa *et al.*, 2007). KIT is usually expressed and essential in the function of hematopoietic stem cells,

melanocytes, germ cells, mast cells and ICCs (Maeda *et al.*, 1992; Huizinga *et al.*, 1995; Ashman, 1999; Alexeev and Yoon, 2006). In contrast to the normal function of RTKs, mutated KIT/PDGFRA mutants are not affected by the presence of ligands; instead they are activated in a ligand-independent way, leading to constitutive activation. The KIT-receptor is phosphorylated in its mutated form in GIST specimens (Rubin *et al.*, 2001) and can be reset to its non-phosphorylated and thereby inactive state by imatinib (Rubin *et al.*, 2001; Tuveson *et al.*, 2001), which disrupt downstream signaling of the KIT-pathway.

A signaling pathway can be defined as a sequence of the interacting molecules – from receptor to effector function – that ultimately leads to a cellular response. In GIST, key signaling pathways occurring downstream of the driver mutations have been identified, all leading to increased cell survival and proliferation. The activated pathways include the mitogen-activated protein kinase (MAPK) pathway, the phosphatidylinositol-3-kinase (PI-3K)/Akt/mTOR-pathway, and signal transducer and activator of transcription-3 (STAT3) pathway (Figure 3) (Duensing *et al.*, 2004; Rossi *et al.*, 2006; Corless, 2014; Bauer *et al.*, 2007). Attempts to elucidate the relevance of these signaling pathways relevance have been made using biochemical inhibition of key targets in these pathways, as it could also be a therapeutic strategy in *KIT/PDGFRA* wild-type GIST. Inhibiting PI-3K leads to a more pronounced effect *in vitro* compared to MAPK inhibition (Bauer *et al.*, 2007). Combination of both PI-3K and MAPK inhibition leads to increased antitumoral activity, but might be limited by toxicity seen *in vivo* (Bosbach *et al.*, 2017).

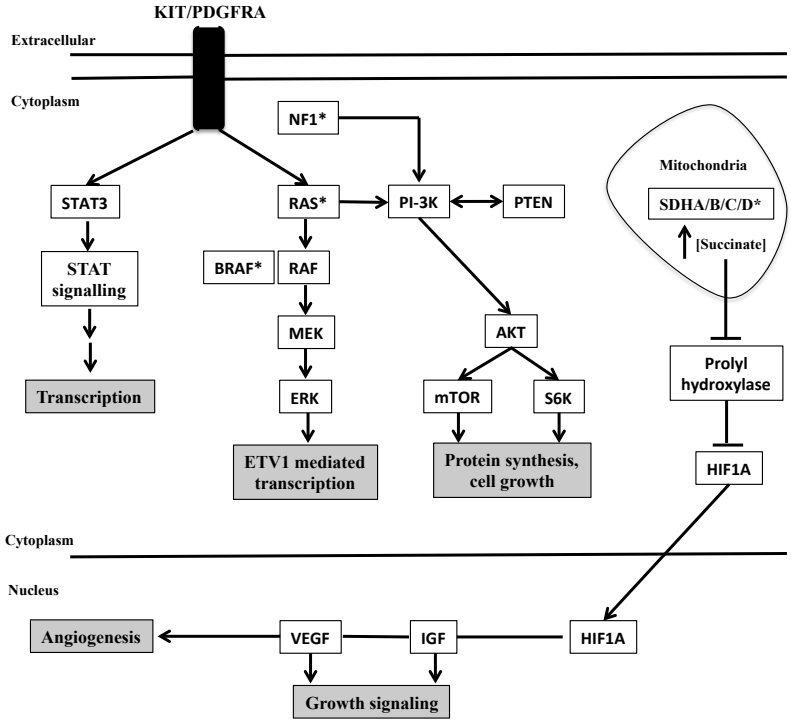

Figure 3. Major signaling pathways in GIST. *KIT* or *PDGFRA* mutation leads to a constitutively active receptor, which activates downstream signaling pathways, such as MAPK (RAF/MEK/ERK), STAT3, and PI3K/Akt pathways for cell proliferation and survival. ETV1 mediated transcription is crucial in GIST, and have been shown to be an important survival factor for GIST. Asterisks denote pathways mutated in non-*KIT/PDGFRA* mutated GIST, which activates similar pathways as *KIT*/*PDGFRA* mutated GISTs. Succinate dehydrogenase (SDH) is located in the mitochondria, consisting of four subunits A-D. SDH-deficiency leads to the accumulation of succinate and inhibition of prolyl hydroxylases, leading to stabilization of HIF1A and increase transcription of its target genes, such as *VEGF* and *IGF* for cell growth and angiogenesis. Adapted and modified from Joensuu 2013 and Corless 2014.

Approximately 80-90%, of GISTs have mutations in either *KIT* or *PDGFRA* (Corless, Barnett and Heinrich, 2011). Most commonly, *KIT* mutations occur in exon 11, which is seen in 65% of GISTs. Different types of mutation can occur, but are most frequently deletions or indels (Joensuu *et al.*, 2017). Deletion in exon 11 is associated with a poorer prognosis compared to other mutations in *KIT* (Martín et al., 2005). Exon 11 encodes the juxtamembrane domain, which normally has an auto-inhibitory function (Figure 2) (Mol *et al.*, 2004). Functionally, it is believed that in mutated the juxtamembrane domain, the autoinhibitory function is lost. Less common is the exon 9 mutations that occur in about 10% of GISTs (Lux *et al.*, 2000), encoding the extracellular domain (Figure 2). Exon 9 encodes a domain that is part of ligand-recognition, and mutation in exon 9 is believed to cause conformational change similar to that observed when SCF-ligand binds the KIT-receptor (Yuzawa *et al.*, 2007). Exon 9 frequently occurs in the small intestine (Table 2), and a higher dose of tyrosine kinase inhibition is recommended for exon 9 mutated GISTs (Casali *et al.*, 2018). Mutations in exon 13 and 17 encoding the intracellular ATP-binding site and activation loop rarely occur, but are more common as secondary mutations

causing tyrosine kinase inhibitor resistance (Table 2). *PDGFRA* mutations are less common and do not occur together with *KIT* mutations (Heinrich *et al.*, 2003). However, the sites of mutation are different, where *PDGFRA* mutation occurs predominantly in exon 14 or 18 (about 8%) encoding the intracellular domain with the ATP-binding site or activation loop, exon 18 or 14 (Figure 2) (Corless, Barnett and Heinrich, 2011). PDGFRA mutated GISTs most frequently occurs in the stomach (Table 2). Tumors with the most common mutation affecting codon 842 in exon 18 are considered to be imatinib-resistant. (Cassier *et al.*, 2012).

Genetic aberrations/syndrome	**Frequency**	**Anatomical sites**
***KIT* mutation**	**75-80%**	
Exon 8	Rare	Small intestine
Exon 9	8-10%	Small intestine, colon
Exon 11	65-67%	All sites
Exon 13	1%	All sites
Exon 17	1%	All sites
***PDGFRA* mutation**	**~10%**	
Exon 12	1%	All sites
Exon 14	Rare	Stomach
Exon 18 (D842V)	6%	Stomach, mesentery, omentum
Exon 18 (non-D842V)	2%	All sites
Wild-type (not *KIT and PDGFRA*)	**~15%**	
BRAF V600E	2%	
SDHA/B/C/D mutations	6%	Stomach and small intestine
*HRAS, NRAS, PIK3CA*mutations	1%	
Pediatric/Carney Triad	1%	Stomach
NF1	1%	Small intestine

Table 2. Molecular features, mutation frequency and preferential anatomical site of GISTs. Adapted and modified from (Corless, 2014; Corless, Barnett and Heinrich, 2011).

Around 10-15% of GISTs do not have mutations in *KIT* or *PDGFRA*, and have been classified as wild-type GIST. Morphologically they are often indistinguishable from mutated *KIT* and *PDGFRA* GIST (Hostein *et al.*, 2010). Identification of several other genetic aberrations has improved our understanding of wild-type GIST. Succinate-dehydrogenase (SDH) deficient GISTs are the most common among the wild-type GIST (Boikos *et al.*, 2016). SDH deficiency leads to increased transcription of hypoxia-inducible factor 1-alpha (HIF1A) regulated genes (Figure 3) (Corless, 2014). Pediatric and young adult GISTs are overrepresented in the SDH-deficiency group (Miettinen *et al.*, 2011), which can be found in the germline, as part of Carney-Stratakis syndrome, with germline mutations of *SDHB, SDHC* or *SDHD* (Stratakis and Carney, 2009). Mutations in neurofibromin 1 (*NF1*) have been described in about 1% of GISTs, and occur at a younger age as part of the NF1 syndrome and are almost exclusively located in the small intestine with multifocal low-risk GIST (Miettinen *et al.*, 2006; Andersson *et al.*, 2005; Gasparotto *et al.*,

2017). Finally, the *BRAF* V600E mutations have also been identified, most commonly located in the stomach and with activated MAPK-pathway (Figure 3) (Hostein *et al.*, 2010; Agaimy *et al.*, 2009).

Progression beyond *KIT/PDGFRA* mutations

Micro-GISTs have provided a model system to study additional aberrations that lead to a progression of the disease. Several pivotal findings have identified alterations that occur in a step-wise manner in order for progression to occur. ETV1 is a transcription factor that is critical for the survival and development of GIST tumors (Chi *et al.*, 2010), which is also stabilized by the MAPK pathway (Figure 3). Dual inhibition of KIT and MAPK (which suppress the expression of ETV1) leads to a durable response in mice treated with KIT and MAPK inhibition (Ran *et al.*, 2015). DNA copy number losses are, the most common being loss of chromosomal region 14q, which is observed in 60-70% of GIST (El-Rifai *et al.*, 2000). Inactivation of the cell cycle regulator and tumor suppressor MYC-associated factor X (MAX), occurs in 50% of GISTs and micro-GISTs, and is likely an early event in the progression of GIST (Schaefer *et al.*, 2017). The tumor suppressor dystrophin has also been shown to be inactivated in over 90% of metastatic GIST tumors, providing evidence for its role in metastatic behavior, and likely a late event in progression (Wang *et al.*, 2014). It is now believed that the progression after initial *KIT/PDGFRA* mutations is largely due to dysregulated cell cycle genes (Ohshima *et al.*, 2019; Schaefer, Mariño-Enríquez and Fletcher, 2017; Heinrich *et al.*, 2019).

Discovered on GIST-1 (DOG1) – role in tumors

Ion channels are proteins that allow the passage of ions to cross the membrane, both between intracellular and extracellular compartments. Signals transduction via ion channels is usually fast compared to for example RTK-mediated signaling. Ion channels are regulated, or gated, by different means such as ligands, voltage, or mechanically. During the 1990s, ion channels were found to regulate several critical processes that are hallmarks of cancer. They were shown to be involved in the regulation of cellular proliferation, cell cycle, apoptosis, invasiveness and it is now well accepted that ion channels are critical in many of the processes needed for tumorigenesis, as reviewed in (Prevarskaya, Skryma and Shuba, 2018; Leanza *et al.*, 2016).

The DOG1 protein, also known as anoctamin-1 (ANO1) or transmembrane member 16A (TMEM16A), is an ion channel that is widely expressed in the body. It belongs to the group of Ca^{2+}-activated Cl^- channels (CaCCs), which regulate several critical physiological processes such as fluid secretion, smooth-muscle contraction, gut peristalsis and nociception (Ferrera, Caputo and Galietta, 2010; Hartzell, Putzier and Arreola, 2005).

DOG1 is functionally an ion channel that opens in response to increased intracellular Ca^{2+}-concentration ($[Ca^{2+}]_i$), and is voltage-dependent (Paulino *et al.*, 2017; Caputo *et al.*, 2008). More recently, it has also been shown that phosphatidylinositol 4,5-bisphosphate (PIP_2) is needed for activation (Tembo *et al.*, 2019). In mice, knockdown of DOG1 is lethal already within the first month after birth (Rock, Futtner and Harfe, 2008). DOG1 regulates a variety of physiological functions, notably gut peristalsis that is mediated by ICCs, the likely cell-of-origin of GIST (Hwang *et al.*, 2009; Malysz *et al.*, 2017).

Emerging evidence suggests that DOG1 is involved in tumor progression in several types of cancer (Ji *et al.*, 2018). DOG1 overexpression have been observed in several tumors such as gastric cancer (Liu *et al.*, 2015), colon cancer (Sui *et al.*, 2014), lung (Jia *et al.*, 2015), head and neck (Duvvuri *et al.*, 2012), among others. The mechanism of this is not fully understood, and might be explained by *ANO1* (encodes DOG1 protein) gene located on the long arm of chromosome 11 (11q13), which is frequently amplified in tumors (Wang *et al.*, 2017). Which has spurred an investigation into its role in tumorigenesis. Furthermore, DOG1 expression has been shown to be a prognostic marker in different cancers (Ruiz *et al.*, 2012; Choi *et al.*, 2014; Li *et al.*, 2016).

DOG1 have been shown to be involved in several processes critical for cancer cells such as proliferation (Deng *et al.*, 2016), cell cycle progression (Guan *et al.*, 2016), migration and invasion (Liu *et al.*, 2012), and also in epithelial-mesenchymal transition (EMT) (Shiwarski *et al.*, 2014). Interestingly, DOG1 is associated with a protein network linking the cell membrane to the cytoskeleton (Perez-Cornejo *et al.*, 2012).

In GIST, knockdown of DOG1 inhibited tumor growth *in vivo* xenograft model, which was cell line-dependent, no significant effect was seen *in vitro* after knockdown or biochemical inhibition (Simon *et al.*, 2013). Intriguingly, the authors observed that loss of KIT expression often occurred together with loss of DOG1 expression, in GIST cell lines. However, KIT signaling was not affected by DOG1 knockdown *in vitro* (Simon et al., 2013). DOG1 has not been found to be mutated in clinical GIST specimens (Li *et al.*, 2015; Miwa *et al.*, 2008).

Several signaling pathways have been investigated for the mechanisms of how DOG1 contributes to tumor progression. Among others, DOG1 has been found to activate EGF-receptor (EGFR, also a RTK) signaling in breast cancer to promote tumor progression, and both knockdown and biochemical inhibition of DOG1 decreased EGFR signaling (Britschgi *et al.*, 2013). Further studies revealed that DOG1 directly interacted with EGFR, and lead to increased signaling due to interaction with the juxtamembrane domain that regulates EGFR activity (Bill *et al.*, 2015). This provides evidence that DOG1 can interact directly with RTKs. Combinatorial inhibition of DOG1 and EGFR inhibition resulted in improved efficacy of growth suppression *in vitro* (Bill *et al.*, 2015). In head and neck squamous cell carcinoma, DOG1 overexpression was correlated to reduced levels of the pro-apoptotic protein Bim (Godse *et al.*, 2017). Indeed,, imatinib has been shown to up-

regulate Bim in GIST, which leads to increased imatinib-induced apoptosis in GIST (Gordon and Fisher, 2010). This might support the combinatorial treatment with DOG1 inhibition and TKI treatment using imatinib. Several DOG1 modulators now exist (Namkung, Phuan and Verkman, 2011; De La Fuente *et al.*, 2008), and we addressed the role of DOG1 using these modulators in **paper I** and **II**.

CLINICAL MANAGEMENT OF GIST

The seminal findings of mutated receptor tyrosine kinases paved the way for targeted therapies in GIST, being the first solid tumor with targeted therapy. Historical data has reported consistently low response rates to conventional chemotherapies, around 0-15% (Dematteo *et al.*, 2002). Therefore, when imatinib was introduced in 2002 it revolutionized the treatment of GIST and increased the median survival from ~1.5 years (Nilsson *et al.*, 2005; DeMatteo *et al.*, 2000) to 5 years (Blanke *et al.*, 2008a).

Treatment challenges now consist of the selection of patients that would benefit from neoadjuvant and/or adjuvant treatment, and for how long the treatment should continue. Further, the majority of patients develop treatment resistance to tyrosine kinase inhibitors (TKI), and major efforts are being undertaken to decide optimal management of patients, and the development of new treatment strategies. This issue is also addressed in this work in **paper I, II**, **III** and in a clinical trial **paper V**.

Risk stratification

Risk stratification of patients is crucial for determining the optimal management of patients. Several such systems have been developed, the first was developed in 2002 consisting of two variables; tumor size and mitotic count (Fletcher *et al.*, 2002), which was introduced as the National Institute of Health (NIH) criteria, which was then modified to also include tumor site and presence of tumor rupture (Table 3) (Joensuu, 2008). The risk categories were divided into very low risk, low risk, intermediate and high risk. The modified NIH criteria were validated and particularly identified patients at high risk (Rutkowski *et al.*, 2011; Joensuu *et al.*, 2012b). Others include Armed Forces Institute of Pathology (AFIP) criteria which includes the parameters with different cutoffs in NIH modified criteria but lack tumor rupture (Miettinen and Lasota, 2006). Nomograms are also available for risk classification (Gold *et al.*, 2009). The different classification system has been shown to be somewhat similar in terms of performance (Joensuu *et al.*, 2012b).

The modified NIH criteria for risk stratification are used at Karolinska University Hospital. This risk system is good at identifying high-risk patients, and can be used to determine whether the patient should be treated with adjuvant TKI treatment or not. The mutational status is not included in the present classification system, but has an impact on treatment decisions.

Risk groups	Size (cm)	Mitotic counts	Tumor site	10-year RFS
Very low	<2	<5	Any	94.9%
Low	2.1-5	<5	Any	89.7%
Intermediate	<5	6-10	Stomach	86.9%
Intermediate	5.1-10	<5	Stomach	
High risk	>10	Any count	Any	36.2%
High risk	Any size	>10	Any	
High risk	>5	>5	Any	
High risk	<5	>5	Non-stomach	
High risk	5.1-10	<5	Non-stomach	

Table 3. The National Institute of Health (NIH) modified criteria for risk stratification. The 10-year recurrence-free survival (RFS) is based on pooled data from 10 different population-based studies. Mitotic counts are per 50 high-power fields (HPF). NIH modified criteria also includes tumor rupture, if such is present at diagnosis or intraoperatively, it is classified as High risk, regardless of other markers. (Joensuu, Hohenberger and Corless, 2013).

Surgical management of GIST

The ultimate goal of GIST surgery is radically removing the tumor while preserving as much function as possible. Neoadjuvant imatinib can be used to shrink the tumor prior to surgery in order to perform less invasive surgery. Detailed imaging is crucial in determining the surgical anatomy. Careful surgery is performed in order to prevent intraoperative tumor rupture, which is a known poor prognostic factor (Table 3) (McCarter *et al.*, 2012).

The current treatment of localized GIST is surgery. A pivotal study of imatinib as adjuvant therapy found that in the placebo arm, around 70% of patients undergoing surgery alone is cured (Dematteo *et al.*, 2009). However, adjuvant treatment with imatinib was shown to improve recurrence-free survival (RFS) in localized GIST (Dematteo *et al.*, 2009). Surgical margins are important, and the goal of surgery is to achieve negative microscopic margins, referred to as R0. Further, the macroscopical surgical margins are of prognostic significance and should be considered as well (Åhlén *et al.*, 2018).

If R0 resection is not feasible, neoadjuvant imatinib-treatment has to be considered (Figure 4), to allow for tumor shrinkage before surgery, enabling less extensive surgery while preserving function. R0-resection was possible in 80% of the GISTs operated on following neoadjuvant imatinib-treatment (Rutkowski *et al.*, 2013). The timing of surgery is dependent on the maximal tumor shrinkage, which is usually around 6-9 months of treatment duration. For this purpose, also mutational status analysis is of benefit and is recommended in all localized GISTs (Casali *et al.*, 2018), to allow selection of the optimal tyrosine kinase inhibitor and adequate dosing in cases less sensitive to imatinib. GISTs rarely metastasize to lymph nodes, therefore lymphadenectomy is not recommended. However, SDH-deficient GISTs usually have lymph engagement that might warrant lymphadenectomy (Boikos *et al.*, 2016).

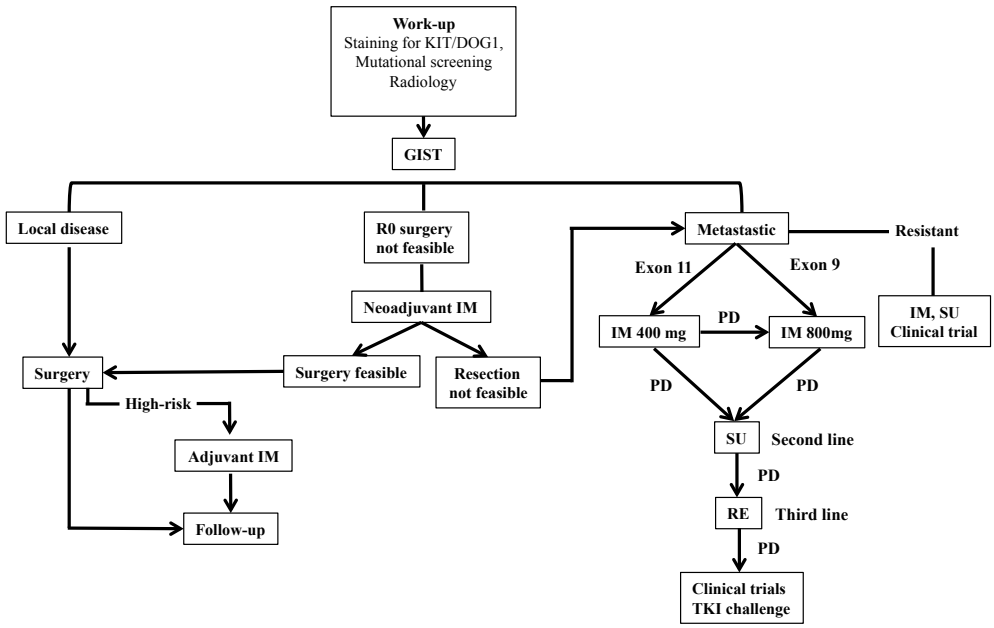

Figure 4. Schematic overview of the clinical management of GIST, as recommended by the European Society of Medical Oncology (ESMO). Abbreviations: IM, imatinib; SU, sunitinib; RE, regorafenib; PD, progressive disease; TKI, tyrosine kinase inhibitor. Imatinib is used as first treatment line, sunitinib as second line and regorafenib as third line. Adapted and modified from (Casali *et al.*, 2018).

Medical treatment

The discovery of mutations in genes coding for KIT in the late 1990s and PDGFRA at the beginning of 2000s led to the introduction of targeted therapy in GIST. However, this treatment was first used as a treatment in chronic myelogenous leukemia (CML), since that inhibition of ABL (of the BCR-ABL fusion protein present in >95% of CML) that exerted strong antitumoral activity in cell lines (Druker *et al.*, 1996), and patients (Druker *et al.*, 2001). Further, this demonstrated the feasibility of targeting specific alterations in cancer, thus increasing specificity.

At this time, gain-of-function mutations in *KIT* had been identified, sharing the molecular properties with ABL by being a tyrosine kinase. In 2000, the first GIST patient received imatinib as a treatment. The patient had rapidly progressive GIST disease, and demonstrated a dramatical tumor response (Joensuu *et al.*, 2001). The U.S. Food and Drug Administration (FDA) approved the treatment in 2002, after a pivotal clinical trial, where half of the patients displayed a partial response (Demetri *et al.*, 2002). The metabolic response was seen on [^{18}F]fluoro-2-deoxy-D-glucose, or FDG,- positron emission tomography (PET) scans (FDG-PET), which has become a useful marker for response to imatinib treatment (Van den Abbeele, 2008). Recently it was also shown that imatinib decreases glycolytic rates, and changes the tumor cells into a more oxidative phosphorylation phenotype, and combining mitochondrial

inhibition and imatinib treatment enhances the efficacy of antitumoral effect (Vitiello *et al.*, 2018).

Imatinib can prevent the oncogenic signaling that occurs as a consequence of mutated KIT or PDGFRA receptors (Buchdunger *et al.*, 2000). It acts by competitively inhibiting ATP from binding to the ATP binding site of the RTK (Figure 2), where ATP acts as a phosphate-donor for the kinase (Mol *et al.*, 2004). This is a shared mode of action for the TKI described herein, and we describe their possible interaction with ATP-binding sites on other proteins **paper IV**.

Imatinib in the adjuvant setting

In the adjuvant setting, only imatinib is approved for usage. The duration of treatment has been studied in several studies, where duration has been studied for 1-3 years (Casali *et al.*, 2015; Corless *et al.*, 2014; Joensuu *et al.*, 2016). The optimal treatment duration has been found to be 3 years, since that increases the overall survival (OS) and not only progression-free survival (PFS). Three years adjuvant imatinib is therefore recommended in Europe for high risk GIST, whereas intermediate risk tumors should be discussed in therapy conference (Casali *et al.*, 2018). The duration of treatment likely has an effect on patients' compliance with the medication, as side effects are not uncommon. Studies have evaluated both 3- and 5-years duration of the treatment, and imatinib cessation occurred in 26% (Joensuu *et al.*, 2012a) and 49% (Raut *et al.*, 2018) of patients, respectively. It also stresses the importance of patient education upon initiation of TKI treatment, since many side effects can be managed as well as the importance of assessing patients' side effects throughout treatment. The clinical dilemma with side effects during TKI treatment is partly addressed in **paper IV**.

Tyrosine kinase inhibitors in advanced/metastatic GIST

Advanced and metastatic GIST represents a major challenge, but has favorable outcomes since the introduction of TKI treatment. Historical data on survival using chemotherapy regimens was low, around 1.5 years (DeMatteo *et al.*, 2000). Today three drugs have been approved by the FDA for treating metastatic GIST. Imatinib, as discussed above is the first line treatment, followed by sunitinib as the second line treatment, and regorafenib is used as a third line treatment regimen. Mutational analysis is crucial since this affects the treatment response, however, heterogeneous mutational status is common, and may differ even in the same lesion and at different locations (Liegl *et al.*, 2008; Antonescu *et al.*, 2005).

Imatinib has been shown to control the disease in over 80% of GIST patients of cases short term (Demetri *et al.*, 2002). After the pivotal trials of imatinib as a treatment for advanced GIST, it was found that 400 mg imatinib once a day was comparable to 400 mg twice a day (Blanke *et al.*, 2008b; Verweij *et al.*, 2004). Today it is known that around 20% with advanced disease treated with imatinib have a survival exceeding

10 years (Heinrich *et al.*, 2017; Casali *et al.*, 2017), but the long-term follow up failed to identify possible prognostic factors (Casali *et al.*, 2017). A trial investigated whether imatinib treatment could be discontinued after three years of stable disease, in which two-year PFS was 80% in the continuation group compared to 16% in discontinuation group (Le Cesne *et al.*, 2010). This indicates that imatinib cannot eradicate all tumor cells, and patients should receive imatinib if it is tolerable. Most patients do develop resistance against imatinib within around 18 months (Blanke *et al.*, 2008b). The most common cause of resistance is secondary mutations in exons encoding the intracellular domains of the receptor (Debiec-Rychter *et al.*, 2005; Antonescu *et al.*, 2005), thereby interfering with imatinib's binding.

Sunitinib is a TKI with activity against several RTKs such as KIT, PDGFRA, VEGFR and FLT-3 (Abrams *et al.*, 2003). In a phase III trial evaluating sunitinib as a second-line treatment upon progression using imatinib, it was shown that sunitinib prolonged both the progression-free survival from 1.4 to 6.8 months, and overall survival (PFS and OS, respectively) (Demetri *et al.*, 2006). Sunitinib was also shown to be effective in GIST with *KIT* mutations in ATP-binding sites (exon 13 and 14, see Figure 2) (Heinrich *et al.*, 2008). Long-term follow up of the phase III trial revealed no difference in OS, which was attributable to the crossover design in the initial study (Demetri *et al.*, 2012). Regorafenib is a TKI with multiple targets, but notably for KIT, RAF, BRAF and PDGFRA (von Mehren and Joensuu, 2018). Regorafenib was approved as a third-line agent after a pivotal study demonstrating a PFS of 4.8 months compared to 0.9 in the placebo-arm in patients progressing on imatinib and sunitinib (Demetri *et al.*, 2013).

Other TKIs have been used in trials, but not demonstrated superior benefits compared to current lines. As described in the "Molecular Basis of GIST" section, there is a theoretical rationale for exploring several types of inhibitors such as ETV1, mTOR inhibitors, cell cycle inhibitors, which are investigated in clinical trials (clinicaltrials.gov; accessed 2019/11/03). In **paper I, II** and **III**, we investigated novel compounds for antitumoral activity in GIST *in vitro.* While **paper V**, assess the safety and efficacy of immunotherapy in GIST.

Immunotherapy in GIST

Immunotherapy has established itself as a treatment within modern oncology. After seminal studies during the last century, it is now appreciated that the immune system is capable of both eliminating tumor cells and promoting tumor growth through what is called immunoediting (Schreiber, Old and Smyth, 2011). Most of this evidence comes from animal studies. In humans, patients on immunosuppressive medication (such as transplanted patients) and acquired immunodeficiency (such as AIDS), displays an increased risk of developing certain cancers (Grulich *et al.*, 2007). Moreover, it has been shown that immune function and profiles are prognostic factors in several different cancer types (Clark *et al.*, 1989; Pagès *et al.*, 2005; Zhang *et al.*,

2003), suggesting a role of the immune system in disease control. Several therapies have been developed to enhance the immune system function and break the tolerance that exists within the tumors.

In GIST, the immunological profile has been found to skew towards an immunosuppressive state, i.e. an immunological tolerance within the microenvironment. Immune cells creating an immunosuppressive environment were found to be relatively more common than immune cells that can mediate a response against the tumors (van Dongen *et al.*, 2010). Mutational status in GIST also affects the immunological environment in GIST. *PDGFRA* being more immunogenic active with more cytolytic immune cells that could possibly mediate tumor killing compared to *KIT* mutated GISTs (Vitiello *et al.*, 2019). Certain immune cells such as NK-cells and $CD3^+$ T-cells are associated with longer PFS (Rusakiewicz *et al.*, 2013). Furthermore, TKI treatment can alter the immunological environment within tumors. For example, imatinib treatment can activate T-cells, by increasing the ratio between cytotoxic ($CD8^+$ T-cells) and regulatory T-cells and also induce apoptosis in regulatory T-cells, by downregulating indoleamine 2,3-dioxygenase (Ido), which is an immunosuppressive enzyme (Balachandran *et al.*, 2011). There is a reason to believe that immunotherapy might be beneficial. Currently, there are six (including **paper V**) studies evaluating immunotherapeutics in GIST (clinicaltrials.gov accessed on 2019-11-01). Immune checkpoint inhibitors (such as PD-1 and CTLA-4 inhibitors) are the most common, which disrupt the immune checkpoint that activates immune cells for the antitumoral response (Pardoll, 2012). Interestingly, one trial is evaluating Ido-inhibition combined with imatinib, again emphasizing the role of preclinical studies to guide clinical studies.

AIMS

The clinical dilemmas addressed in this thesis work are 1) the few treatment alternatives that exist after TKI treatment failure, and 2) side-effect that reduces compliance for TKI treatment.

Specific aims of the described papers:

Paper I: Determine the functional role of DOG1 in GIST

Paper II: Explore a more potent DOG1 inhibitor for antitumor effects in GIST

Paper III: Explore possible antitumoral effects of PVAC

Paper IV: Determine the effects of commonly used TKIs possible interaction with the ATP-binding site of ATP-sensitive potassium channel

Paper V: Determine the safety and efficacy of intratumorally injected pro-inflammatory allogeneic dendritic cells in advanced GIST disease

MATERIAL AND METHODS

This chapter contains a short description of each method that has been used in this thesis work, more detailed descriptions of the methodology are given in the papers.

CELL LINES FOR *IN VITRO* EXPERIMENTS

In **paper I**, two well-established human GIST cell lines were used: GIST882 (imatinib-sensitive) and GIST48 (imatinib-resistant). Both of these have been validated for authenticity in our research group (Berglund *et al.*, 2013; Berglund *et al.*, 2014). In **paper II**, human GIST cell lines GIST-T1 and GIST882 were used. In **paper III**, GIST-T1, A375 (human melanoma) and B16.F10 (murine melanoma) were used. In **paper IV**, MIN6m9 (murine β-cell) was used as an ATP-sensitive potassium channel (K_{ATP}) source. Maintenance of cells is described in **paper I-IV.**

ANIMAL MODEL

In **paper III**, two different mouse strains, C57BL/6J and the athymic nude mice Crl:NU(NCr)-FOXn1^{nu}, were used to determine the antitumoral effects of PVAC. The study was reviewed and approved by the Ethical Committee (Dnr N37/15).

PATIENTS IN THE CLINICAL STUDY

In **paper V**, six patients were included into a trial examining the safety and efficacy of a new advanced therapy medicinal product (ATMP), Ilixadencel. The trial was reviewed and approved by both the Ethics committee (Dnr 2015/1619-31) and the Medical Product Agency (Läkemedelsverket) (Dnr 5.1 2015/77670). The trial was conducted in accordance with the Helsinki declaration.

MATERIAL

Compounds

DOG1 activator and inhibitors (Paper I and II)

E-act (3,4,5-Trimethoxy-N-(2-methoxyethyl)-N-(4-phenyl-2-thiazolyl)-benzamide) was used as DOG1 activator, and **$CaCC_{inh}$-A01** 6-(1,1-Dimethylethyl)-2-[(2-furanylcarbonyl)amino]-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid and **T16inh-A01** (2-[(5-Ethyl-1,6-dihydro-4-methyl-6-oxo-2-pyrimidinyl)thio]-N-[4-(4-methoxyphenyl)-2-thiazolyl]-acetamide) were used as DOG1 inhibitors. All compounds were dissolved in dimethyl sulfoxide (DMSO). All compounds were purchased from either Sigma-Aldrich (Saint Louis, MO, USA) or Merck (Billerica, MA, USA).

PVAC (Paper III)

PVAC is a polymeric molecule which consists of a polyvinyl alcohol backbone, with carbazate moieties groups at some hydroxyl moieties. The compound PVAC was synthesized by Specific Polymers (Castries, France) and dissolved in purified water (Milli-Q purification system, Millipore, MA, USA) or culture medium prior to use. As controls, we used polyvinyl alcohol (Sigma-Aldrich), which has the same molecular weight as the PVAC, and ethyl-carbazate (Sigma-Aldrich), which is a low-molecular weight carbazate compound. Both of these compounds were dissolved in deionized and purified water.

Tyrosine kinase inhibitors (TKIs)

Imatinib mesylate was used in **paper I** and **IV**, while sunitinib malate and nilotinib were used in **paper IV**. Imatinib mesylate was dissolved in deionized and purified water, while sunitinib malate and nilotinib were dissolved in DMSO. Imatinib mesylate and nilotinib were a gift from Novartis (Basel, Switzerland). Sunitinib malate was purchased from Sigma-Aldrich.

Ilixadencel

Ilixadencel is a cell product produced from healthy blood donors according to Good Manufacturing Practice. Its production has been described in detail (Karlsson-Parra *et al.*, 2018), and was produced at Cancer Center Karolinska (Karolinska University Hospital, Sweden) or at BioNTech (Idar-Oberstein, Germany). The product was provided by Immunicum AB (Stockholm, Sweden).

EXPERIMENTAL METHODS

Immunocytochemistry, immunofluorescence and immunohistochemistry (Paper I and III)

Immunocytochemistry (ICC) and immunofluorescence (IF) are commonly used methods to study the expression and subcellular localization of specific protein on single layers of cells, while immunohistochemistry is used to study the expression of a specific protein in tissue sections. All these methods are based on the interaction between antibodies and antigens. The difference between ICC and IF is the detection method; ICC is detected by chromogenic substrate, while the IF is detected by the secondary antibody conjugated with a fluorophore. In **paper I**, ICC was used to verify the protein expression of CD117 (KIT) and DOG1 in GIST cell lines, and IF was used to determine the subcellular localization of these two proteins. In **paper III**, immunohistochemistry was used to stain CD3 (T-cell co-receptor) and Ki-67 (proliferation marker).

Electrophysiology (Paper I, II and IV)

The patch-clamp technique can be used to study electrophysiological events, and allow direct recording of single ion channel currents (Hamill *et al.*, 1981). We have used the method to study effect of different modulators on Cl^- and K^+ currents. Different experimental setups can be used (Figure 5), in this thesis whole-cell and excised inside-out patches were used.

In **paper I** and **II**, whole-cell currents were measured using optimized solution (specified in papers) for the DOG1 channel, the pipette was in direct contact with the cell's cytoplasm (right in Figure 5). And in **paper IV**, we used inside-out excised patches (left in Figure 5) to record single channel currents of K_{ATP} channel upon exposure to tyrosine kinase inhibitors.

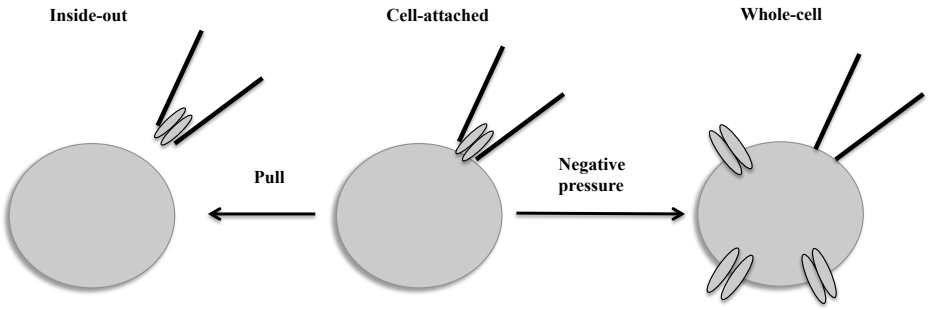

Figure 5. Different modes of patch-clamp technique used in this thesis work. Cell-attached is the first step for both modes, after this pulling part of the cell membrane will yield an inside-out mode, while applying negative pressure will yield whole-cell mode recording.

In brief, glass pipettes were made of certain size. For **paper I** and **II**, using a robotic system, the pipette was inserted into the cells and a seal was achieved that creates an isolated measurement system. For **paper IV,** glass pipettes were pulled and attached to the cellular membrane, after which it was quickly drawn away, allowing single-channel recording.

Cell proliferation, viability and colony-forming ability (Paper I, II and III)

Several methods were performed in **paper I-III** to determine possible antitumoral effects of various compounds. Assays based on cellular metabolism have been used to measure cytotoxicity, cell viability and proliferation. It is important to remember that, in certain settings, metabolic alterations from the drug studied might influence the results of these assays.

WST-1 assay

The commercially available WST-1 assay was used to assess proliferation in **paper I.** WST-1 is a colorimetric assay that enables measurement of cell proliferation and viability. This assay is based on the formation of formazan from tetrazolium salt WST-1 that is catalyzed by mitochondrial dehydrogenases, and thus the amount of formazan corresponds to the number of viable/metabolically active cells.

CellTiter-Glo assay

CellTiter-Glo assay is an ATP-dependent cell lytic luminescence method to assess cell viability. It employs a recombinant luciferase that catalyzes the reaction from luciferin to oxyluciferin, which is an ATP-dependent reaction that generates a luminescent signal for detection. The amount of ATP correlates to the number of metabolically active cells in the system. The assay also inhibits enzymes that would interfere with ATP-measurement (ATPases). This assay was used in **paper I** and **III**.

Colony formation assay

Colony formation assay, or clonogenic assay, measures the ability for single cells to divide after exposure to different compounds. In order to achieve this, cell suspension was diluted to desirable concentration (1,000 cells/well in 12-well plates; for reference, 2,500-10,000 cells/well were seeded in 96-well plates, which is 10-fold smaller (area) than 12-well plates). Cells were treated with DOG1 inhibitors, and no medium change or drugs were added during the 10-14 days incubation period. Cells were stained, and colonies were quantified. The assay was performed in **paper II**.

Flow cytometry (Paper I, II and III)

Flow cytometry is a method that has several applications and is used both in research and clinical setting. In this work, single cells were analyzed, but flow cytometry can also be used to study particles. The cells travel along with the fluidics system and enter through a laser beam. From this, different cellular characteristics can be determined, such as size and internal complexity (which generally relates to granularity). The laser beam will also excite fluorescent probes present on the particles or cells and record the returning emission. The flow cytometer can differentiate between many different emission signals simultaneously, allowing for the identification of cells based on, for example, protein expression.

Annexin V/7-AAD or PI (Paper I and III)

Apoptosis is a highly orchestrated sequence of events that occurs inside the cell, which is also called programmed cell death. One of the early events during apoptosis is the translocation of phosphatidylserine from the inner to the outer surface of plasma membrane, exposing itself to the external cellular environment (Vermes *et al.*, 1995). Annexin V has a very high binding affinity for phosphatidylserine and binds to apoptotic cells with exposed phosphatidylserine. Annexin V can be conjugated to a fluorochrome, such as phycoerythrin, for detection. 7-aminoactinomycin D (7-AAD) and propidium iodide (PI) are indirect markers of late apoptotic/necrotic cell populations, indicating that cells have permeabilized cell membranes. By co-staining annexin V and 7-AAD or PI, we could distinguish early and late apoptotic/necrotic cells. This finding also serves as an excellent method to validate findings from the cellular viability assays described above. Since the status of each cell is recorded separately, it is easy to determine the percentage of cells that are affected by a certain treatment

Cell Cycle analysis (Paper II and III)

The approach for analyzing cell cycle events varies, and in this study, we used univariate analysis of DNA content that was stained with PI (Pozarowski and Darzynkiewicz, 2004). The histogram generated yields proportion of cells in each phase of the cell cycle (G1, S and G2/M), where x-axis indicates the DNA content and y-axis the number of events. In this assay, cells were fixed with ethanol prior to PI staining. The staining solution contains RNAse A that prevents the binding of PI to RNAs. Fragmented DNA, as an indicator of apoptosis, can also be detected, which is referred to as the sub-G1 phase (Riccardi and Nicoletti, 2006).

Animal experiments (Paper III)

The animal experiments were performed by Adlego AB Biomedical, at the Astrid Fagreaus Laboratory (Solna, Sweden). The experimental setup was inoculating either B16.F10 cells into C57BL/6J and MDA-MB-231 cells into athymic nude mice. After the tumor reached a certain volume, mice were randomized into three groups to reduce potential confounding factors, to receive low-dose PVAC, high-dose PVAC or vehicle intratumorally. The B16.F10 inoculated mice were treated three times/week, and the MDA-MB-231 inoculated mice were treated twice/week. Tumor size and animal health were tracked over time and animals were euthanized at either volume indication or study length. Tumors were excised and sent for histopathological evaluation.

STUDY DESIGN OF THE CLINICAL STUDY (PAPER V)

The trial in **paper V** was a single-center trial conducted at Karolinska University Hospital, and was designed as an open-label and single-armed phase 1 trial. The investigational product was ilixadencel. The main inclusion criteria were progressive, while on tyrosine kinase inhibitor treatment, advanced or metastatic GIST disease. For detailed inclusion and exclusion criteria, see **paper V.**

The trial was registered on www.clinicaltrials.gov, identifier: NCT02686944.

RESULTS AND DISCUSSION

PAPER I AND II – DOG1 MODULATION IN GIST

We looked at the functional consequences of DOG1 modulation, by using biochemical inhibition with two inhibitors ($T16_{inh}$-A01 and $CaCC_{inh}$-A01) and the activator E-act. The two studies were conducted sequentially. Shortly after **paper I** was completed, a study found a novel mechanism that DOG1 channel activity inhibition was not needed for antitumoral effects; rather, it was found that DOG1 inhibitors which also degraded the protein were necessary for maximum antitumoral effects (Bill *et al.*, 2014). Therefore, we investigated the DOG1-degradable CaCCinh-A01 in **paper II** and compared it to $T16_{inh}$-A01.

In **paper I**, we first confirmed DOG1 expression in GIST48 and GIST882 cells. By employing confocal laser scanning microscopy, we showed that subcellular localization of DOG1 varied between the two cell lines. In GIST882, DOG1 was predominantly expressed on the plasma membrane, whereas in GIST48 DOG1 was expressed in close proximity to the cell nuclei. It was reported that the spatial distribution of DOG1 was strictly confined to the apical part of epithelial cells in the airway lumen, where it regulates fluid secretion (Scudieri *et al.*, 2012), suggesting that a defined DOG1 expression pattern might have functional consequences.

We then assessed the effects on Cl^- currents using the patch-clamp technique and whole-cell configuration. The modulator E-act activated Cl^- currents in the presence of high $[Ca^{2+}]_i$, while both $T16_{inh}$-A01 and $CaCC_{inh}$-A01 inhibited. At the maximum concentration tested, $T16_{inh}$-A01 (GIST882) inhibited Cl^- currents more than $CaCC_{inh}$-A01 (GIST-T1), which might be explained by different cell lines. As for specificity, it should be noted that knockdown of DOG1 in GIST-T1 resulted in a decrease of chloride currents by 90-96% (Simon *et al.*, 2013), suggesting an important role of DOG1 in regulating Cl^- currents across cell membrane in GIST. In addition, we observed increased channel activity in the presence of high $[Ca^{2+}]_i$, supporting that DOG1 is a Ca^{2+}-activated Cl^- channel.

To explore the functional consequences of DOG1 modulation, GIST48 and GIST882 cells were treated with E-act or $T16_{inh}$-A01, and assessed for proliferation rates at different time points **(paper I)**. Even though there were significant changes upon the treatment, the absolute numbers were low compared to control. We, therefore, interpreted the effect on proliferation as minor. In **paper II**, $CaCC_{inh}$-A01 and $T16_{inh}$-01 treated GIST882 and GIST-T1 cells were assessed for cell viability at different time points. $CaCC_{inh}$-A01 reduced cell viability by approximately 30% in both GIST-T1 and GIST882 cells. The potency of $T16_{inh}$-A01 to reduce cell viability was different between the cell lines, where it was most potent in GIST-T1 and less so (~10% reduction) in GIST882.

In **paper I**, cells were assessed for the apoptotic markers annexin V (early apoptosis marker) and 7-AAD (late apoptotic or necrotic cells), by flow cytometric analysis. In GIST882, only

small cell population differences were observed. In GIST48 cells, the population of cells in the early apoptotic phase shifted to late apoptotic cells with $T16_{inh}$-A01 treatment, without markedly affecting the double-negative cells.

In **paper II**, we also investigated the long-term effect of DOG1 inhibition in GIST-T1 cells using colony formation assay. A marked decrease in colony-forming ability was seen, in a dose-dependent manner, in $CaCC_{inh}$-A01 treated cells, while no effect was seen in $T16_{inh}$-A01 treated cells.

Finally, in **paper II**, cell cycle analysis was performed to investigate whether $CaCC_{inh}$-A01 and $T16_{inh}$-A01 altered the distribution of cells in each cell cycle phase. The effect on the cell cycle varied between the DOG1 inhibitors. In both cell lines, CaCCinh-A01 induced a G1-cell cycle arrest that was dose-dependent, leading to 6-10% increase in cells in G1-phase, which is in agreement with reports from epithelial tumors treated with DOG1 inhibitors (Bill *et al.*, 2014; Guan *et al.*, 2016). In addition, G1-cell cycle arrest is also seen in *DOG1* knockdown (Deng *et al.*, 2016; Sui *et al.*, 2014). $T16_{inh}$-A01 did not alter the distribution of cells at either concentration.

Key findings

- DOG1 localization varies between the cell lines on the subcellular level.
- DOG1 channel activity is voltage- and Ca^{2+} dependent, and can be modulated by biochemical inhibitors.
- DOG1 modulation leads to antitumoral effects in GIST. More pronounced effects are seen with $CaCC_{inh}$-A01, leading to both decreased cellular viability, cell cycle arrest and decreased the ability to form colonies. The effects of $T16_{inh}$-A01 was cell-line dependent, and decreased cell viability and lead to a shift of early apoptotic into late apoptotic cells in imatinib-resistant cell line.

Significance of the findings

The outlined effects of DOG1 inhibition in **paper II** warrant further studies, as it might be a suitable target for therapy in GIST. The findings are in agreement with what has been shown in epithelial tumors, and that DOG1 degradable-inhibitors ($CaCC_{inh}$-A01) are more potent than DOG1 non-degradable inhibitors ($T16_{inh}$-A01). To proceed, it is essential to confirm these findings in animal models, which have thus far not included biochemical inhibition. Evidently, DOG1 interacts with RTKs (Britschgi *et al.*, 2013; Bill *et al.*, 2015), and DOG1 should be examined for such interactions. The finding that DOG1 localization varies in cell lines with different imatinib sensitivity should be further examined, since it is known that nuclear KIT receptors exist in imatinib-resistant cell lines (Hsueh *et al.*, 2019).

PAPER III – PVAC AS AN ANTITUMORAL AGENT

In this paper, we evaluated the antitumor effect of a novel compound PVAC *in vitro* and *in vivo*. In a first step, GIST-T1 cells were used to determine the effect of PVAC on cell viability using CellTiter Glo assay. We observed a non-linear dose-response relationship on cell viability. This kind of surprising finding led us to investigate whether it was seeding density-dependent. Indeed, we showed that high cell density had lower cell viability after 48 h of PVAC treatment, suggesting that the effect of PVAC was both time and seeding density-dependent. However, the dose-response relationship was non-linear; this might be due to multiple binding sites on the PVAC molecule with an optimal ratio between PVAC and cells needed to exert its effect.

The PVAC polymer molecule consists of polyvinyl alcohol as a backbone with coupled carbazate groups. The molar ratio between the two constituents is around 5:1. We therefore used polyvinyl alcohol and ethyl-carbazate (carbazate with an ethyl group added to it) as negative controls. Our results showed that the two compounds had no effect on cellular viability, indicating that PVAC is necessary to achieve the antitumoral effects.

We further assessed the effect of PVAC on apoptosis using flow cytometric analysis of annexin V and 7-AAD or PI-stained cells in A375, B16 and GIST-T1 cell lines. In all three cell lines, PVAC treatment potently induced late apoptotic/necrotic cell populations in a non-linear dose-response relationship. In peripheral mononuclear blood cells, it has been shown that lower concentrations of different polymers induce early apoptotic cells (as defined by annexin V positivity) more potently than higher doses (Jeong *et al.*, 2017). Morphological inspection of A375 cells corresponded to the observations in flow cytometry. We also observed cross-linked cell debris, which we believe is an effect of PVAC. Flow cytometric analysis of cell cycle in A375 cells was only affected in highest-dose.

The *in vitro* results led us to speculate whether PVACs effect was similar to that of other investigated polymer-based agents, with a membrane-disrupting mechanism, which had shown that antitumoral activity increased with increasing hydrophobicity were more hemolytic (Takahashi *et al.*, 2019). However, we found that PVAC molecule was more hydrophilic than hydrophobic, and that this likely does not explain the cellular toxicity seen. Moreover, PVAC has been shown to reduce hemolysis in red blood cells (Sellberg, Unpublished data 2019), which might suggest a membrane-interaction mechanism. Possibly, since the carbazate targets biological electrophiles such as carbonyls, it might be hypothesized that reactive carbonyl species (RCS) formed in the cell membrane due to oxidative stress (Fritz and Petersen, 2013), might be the target for PVAC and through that mechanism target the membrane. However, we were not able to confirm it in the present study.

To determine *in vivo* efficacy, we inoculated B16.F10 (melanoma) cells into immunocompetent mice and MDA-MB-231 (breast cancer) into athymic nude mice, and treated the mouse models with low-dose (close to optimum dose in *in vitro* studies) or high-

dose (about 20-fold higher) of PVAC. We found a significant inhibition of tumor growth in the melanoma mouse model. Furthermore, immunostaining of the excised tumors revealed an increase of $CD3^+$ cell infiltration, but no difference in Ki-67 staining. In the breast cancer mouse model, no inhibition of tumor growth was observed, however, pathologist evaluation reported increased leukocyte infiltration and decreased stromal tissue that could be a therapeutic effect. In addition, excised tumors were RNA sequenced and some genes were related to the immune system, which will be validated in follow-up studies.

Key findings

- *In vitro* PVAC displays a non-linear, seeding- and time-dependent antitumoral effect, with effects on cellular viability. PVAC constituents did not exert antitumoral effects at relevant concentrations.
- Tumor growth inhibition occurred in the melanoma mouse model after PVAC treatment, with an increased intratumoral CD3+ cell infiltration.
- PVAC treatment appeared safe in mice, with no obvious toxicity reported.

Significance of findings

This work characterizes the antitumoral effects of PVAC, a novel antitumoral agent, in GIST melanoma and breast cancer cell lines. We see this compound as a potential toolbox, we used one structure of PVAC in this study, but it can be modulated in several ways, such as the molar ratio of carbazate groups and size of PVAC. We find the increased infiltration of $CD3^+$ cells promising. As major efforts are being put in to develop agents that can trigger immune response. However, further analysis of these cells is needed to draw any firm conclusion.

Little is known about using polymers as antitumoral agent, but several possible advantages exist in large molecule antitumoral compounds. One of the most notable examples is the enhanced retention and permeability effect (EPR), which describes the phenomenon that large molecules accumulate within the tumor (Maeda, 2017). This effect has been validated using PVA in mice, which were shown to accumulate in tumors in a size-dependent manner (Tabata, Murakami and Ikada, 1998). In addition, PVA was used as the backbone in a molecule targeting melanoma and neuroblastoma, with promising findings *in vitro* and *in vivo* findings, where the exact mechanism remained obscure as well (Raffaghello *et al.*, 2006). Others have, however, demonstrated membrane-disrupting mechanisms (Park *et al.*, 2018; Takahashi *et al.*, 2019).

PAPER IV – DIRECT INTERACTION BETWEEN TYROSINE KINASE INHIBITORS AND K_{ATP} CHANNEL

In this work, we investigated the effect of tyrosine kinase inhibitors on the K_{ATP} channel, since this ion channel also contains an ATP-binding site. MIN6m9 murine pancreatic β-cell line was used as a K_{ATP} channel source. Imatinib, nilotinib, and sunitinib were the TKI used. Concentrations were considered to be biologically relevant, as we previously have determined intracellular imatinib concentrations in clinical GIST specimens, which showed concentrations around ~10-70 μM (Berglund *et al.*, 2014).

The inside-out configuration of the patch-clamp technique was used to study the direct effect of TKIs on the K_{ATP} channel. In this mode, cellular metabolism and other factors are omitted to allow the recording of single-channel. To determine the presence of K_{ATP} channels in the membrane patch, we started the recording by exposing the patch to ATP, a known inhibitor, which decreased channel activity as expected. The inhibitory capacity, to achieve 50% channel activity inhibition, of imatinib was estimated to be 9.4 μM, as determined by exposing the K_{ATP} channel to various concentrations ranging from 1-100 μM. We found no statistical difference in single K_{ATP} channel kinetics when exposed to imatinib compared to the control solution.

Next, we investigated whether sunitinib and nilotinib altered K_{ATP} channel activity. All TKIs used reduced the K_{ATP} channel activity. Sunitinib belongs to type I group of TKI, which binds ATP-site as mode of action; whereas imatinib and nilotinib belong to type II TKI, which binds both the ATP-binding site as well as an allosteric site adjacent to the ATP binding pocket (Liu and Gray, 2006). In large screenings, type II inhibitors are typically, but not always, more selective compared to type I. For the specific inhibitors, they were rated as (based on selectivity score (defined as inhibited protein kinases divided by tested protein kinases) imatinib < nilotinib < sunitinib (Davis *et al.*, 2011).

To further confirm the direct interaction with K_{ATP} channel, we recorded other type of ion channels such as large-conductance voltage and Ca^{2+}-activated K-channels (K_{BK}) and small-conductance K-channels (K_{SK}). Unlike K_{ATP}, no effect on the channel activity by TKI was observed, indicating that it is an on-target effect interfering with ATP-binding site on K_{ATP} channel.

In normal β-cells, it is generally considered that the ratio between ATP and ADP is the main determinant for the channel activity; where ATP inhibits channel activity and ADP activates it. We, therefore, used an experimental setup where isolated membrane patches were exposed to ADP and ATP (ratio 1:1), with subsequent exposure to imatinib. These experiments revealed an increase in K_{ATP} channel activity upon imatinib exposure. This is likely a finding that needs to be further validated using whole-cell mode on patch-clamp, to confirm its activating ability.

Key findings

- Tyrosine kinase inhibitors, when exposed alone, directly inhibits K_{ATP} channels.
- In the presence of physiological ratio of ATP and ADP, imatinib increased K_{ATP} channel activity.
- No effect was seen on other type of channels, such as K_{BK} and K_{SK}.

Significance of the findings

K_{ATP} is omnipresent in the body and is involved in a wide variety of physiological functions and is expressed in pancreatic β-cell, muscle, and heart among others. The finding that TKI directly interacts with the K_{ATP} channel, might explain some of the side effects seen with K_{ATP}-interacting drugs such as headaches, gastrointestinal motility problems, fluid retention (Jahangir and Terzic, 2005) The used tyrosine kinase inhibitors represent both type I and type II inhibitors, which generally have different selectivity for the target. We show that both are capable of interacting with the K_{ATP} channel.

PAPER V – CELL-BASED IMMUNOTHERAPY IN GIST – PHASE 1 TRIAL

In this trial, the safety and efficacy of ilixadencel administration were evaluated. The general inclusion criteria were patients with advanced unresectable or metastatic progressing GIST while on second or later line (Figure 4). The decision to include patients was made at a multidisciplinary treatment conference.

Six patients were enrolled in the study and received two doses of ilixadencel; a seventh patient was screened but did not meet inclusion criteria related to abnormal hematological parameters. The mean age of the included patients was 57 years. The line of treatment was second (50%), third (16.7%) or fourth (33.3%). Four of the patients had had metastatic disease, and two patients had locally advanced that were deemed unresectable.

Treatment safety was monitored throughout the study, by both clinical examination and blood tests (including cell counts, kidney and liver function tests, endocrine, inflammation). In total, 19 adverse events occurred during the study, of which six were interpreted as probably or possibly caused by ilixadencel. The most common was fever and chills (three reported), abdominal pain (two reported), discomfort at the injection site (one reported). Fever and chills are interpreted as related to the inflammatory response that occurs. All adverse events had resolved before the study end. No clinically significant laboratory abnormalities were found to be related to ilixadencel treatment. Since the cells are allogeneic, i.e. from another individual (donor) than the recipient, we evaluated alloimmunization. Three patients developed donor-specific antibodies indicative of alloimmunization. These findings confirm the acceptable safety profile from two earlier trials using ilixadencel (Laurell *et al.*, 2017; Rizell *et al.*, 2019).

The tumor response was evaluated by radiological examination at three-month intervals. The criteria used in the study were RECIST 1.1 or Choi criteria. Four patients had progressive disease on first follow up after 3 months (one at 2 months due to general health deterioration). However, two patients exhibited a partial response 6 months after ilixadencel determined by Choi criteria, which is the preferred criteria for evaluating GIST tumors (Benjamin *et al.*, 2007). The duration of partial response was 3 month and 6 months (ongoing response at the end of the study). Using RECIST 1.1 criteria, the best response of stable disease occurred in two patients that remained for 9 and 12 months (ongoing at the end of study). The PFS was 4.0 months.

Key findings

- Ilixadencel is a safe product in patients with advanced GIST.
- Promising response in two out of six patients warrants further studies.

Significance of the findings

To our knowledge, this is the first trial of cell-based immunotherapy in GIST. Immunotherapeutic treatment of GIST is likely beneficial, since the microenvironment in GIST is generally immunosuppressive (Rusakiewicz *et al.*, 2013; van Dongen *et al.*, 2010). Concurrent treatment with TKIs have been shown to influence the immune system, and this should be further studied and which combination would synergize most with immunotherapy. Although limited by the few study participants, the response rate, given that the response rate is usually around 10% in second-line and following TKIs (Mei *et al.*, 2018), is encouraging for future studies.

CONCLUDING REMARKS

In this translational work, several potential therapeutic targets have been identified, as well as increased understanding of current treatments. Furthermore, the safety and efficacy of cell-based immunotherapy in a clinical setting in GIST have been addressed.

In conclusion:

- Biochemical inhibition of DOG1 is a potential pharmacological target in GIST, owing to its abundance in GIST. Further studies should assess its usefulness in an *in vivo* setting and evaluate side effects that might occur.
- PVAC shows promising antitumor activity in vitro and in vivo, suggesting a novel agent for GIST treatment. The mechanism of action remains to be elucidated in future studies, this provides a proof-of-concept that activated polymers can lead to antitumoral effects *in vitro* and *in vivo*.
- Tyrosine kinase inhibitor interferes with ATP binding site on a commonly expressed ion channel, namely the ATP-sensitive K^+ (K_{ATP}) channel. This finding adds important knowledge about TKIs, and may as well explain some of the side effects seen in GIST patients during TKI therapy.
- Ilixadencel has an acceptable safety profile in patients with advanced GIST, and promising results seen in two patients warrant further studies. By using treatment with the intention to break the intratumoral tolerance might lead to improved clinical outcomes.

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REFERENCES

Abraham, S. C., Krasinskas, A. M., Hofstetter, W. L., Swisher, S. G. and Wu, T. T. (2007) '"Seedling" mesenchymal tumors (gastrointestinal stromal tumors and leiomyomas) are common incidental tumors of the esophagogastric junction', *Am J Surg Pathol,* 31(11), pp. 1629-35.

Abrams, T. J., Lee, L. B., Murray, L. J., Pryer, N. K. and Cherrington, J. M. (2003) 'SU11248 inhibits KIT and platelet-derived growth factor receptor beta in preclinical models of human small cell lung cancer', *Mol Cancer Ther,* 2(5), pp. 471-8.

Agaimy, A., Terracciano, L. M., Dirnhofer, S., Tornillo, L., Foerster, A., Hartmann, A. and Bihl, M. P. (2009) 'V600E BRAF mutations are alternative early molecular events in a subset of KIT/PDGFRA wild-type gastrointestinal stromal tumours', *J Clin Pathol,* 62(7), pp. 613-6.

Agaimy, A., Wünsch, P. H., Hofstaedter, F., Blaszyk, H., Rümmele, P., Gaumann, A., Dietmaier, W. and Hartmann, A. (2007) 'Minute gastric sclerosing stromal tumors (GIST tumorlets) are common in adults and frequently show c-KIT mutations', *Am J Surg Pathol,* 31(1), pp. 113-20.

Alexeev, V. and Yoon, K. (2006) 'Distinctive role of the cKit receptor tyrosine kinase signaling in mammalian melanocytes', *J Invest Dermatol,* 126(5), pp. 1102-10.

Andersson, J., Sihto, H., Meis-Kindblom, J. M., Joensuu, H., Nupponen, N. and Kindblom, L. G. (2005) 'NF1-associated gastrointestinal stromal tumors have unique clinical, phenotypic, and genotypic characteristics', *Am J Surg Pathol,* 29(9), pp. 1170-6.

Antonescu, C. R., Besmer, P., Guo, T., Arkun, K., Hom, G., Koryotowski, B., Leversha, M. A., Jeffrey, P. D., Desantis, D., Singer, S., Brennan, M. F., Maki, R. G. and DeMatteo, R. P. (2005) 'Acquired resistance to imatinib in gastrointestinal stromal tumor occurs through secondary gene mutation', *Clin Cancer Res,* 11(11), pp. 4182-90.

Ashman, L. K. (1999) 'The biology of stem cell factor and its receptor C-kit', *Int J Biochem Cell Biol,* 31(10), pp. 1037-51.

Balachandran, V. P., Cavnar, M. J., Zeng, S., Bamboat, Z. M., Ocuin, L. M., Obaid, H., Sorenson, E. C., Popow, R., Ariyan, C., Rossi, F., Besmer, P., Guo, T., Antonescu, C. R., Taguchi, T., Yuan, J., Wolchok, J. D., Allison, J. P. and DeMatteo, R. P. (2011) 'Imatinib potentiates antitumor T cell responses in gastrointestinal stromal tumor through the inhibition of Ido', *Nat Med,* 17(9), pp. 1094-100.

Bauer, S., Duensing, A., Demetri, G. D. and Fletcher, J. A. (2007) 'KIT oncogenic signaling mechanisms in imatinib-resistant gastrointestinal stromal tumor: PI3-kinase/AKT is a crucial survival pathway', *Oncogene,* 26(54), pp. 7560-8.

Benjamin, R. S., Choi, H., Macapinlac, H. A., Burgess, M. A., Patel, S. R., Chen, L. L., Podoloff, D. A. and Charnsangavej, C. (2007) 'We should desist using RECIST, at least in GIST', *J Clin Oncol,* 25(13), pp. 1760-4.

Berglund, E., Berglund, D., Akcakaya, P., Ghaderi, M., Daré, E., Berggren, P. O., Köhler, M., Aspinwall, C. A., Lui, W. O., Zedenius, J., Larsson, C. and Bränström, R. (2013) 'Evidence for Ca(2+)-regulated ATP release in gastrointestinal stromal tumors', *Exp Cell Res,* 319(8), pp. 1229-38.

Berglund, E., Ubhayasekera, S. J., Karlsson, F., Akcakaya, P., Aluthgedara, W., Ahlen, J., Frobom, R., Nilsson, I. L., Lui, W. O., Larsson, C., Zedenius, J., Bergquist, J. and Branstrom, R. (2014) 'Intracellular concentration of the tyrosine kinase inhibitor imatinib in gastrointestinal stromal tumor cells', *Anticancer Drugs,* 25(4), pp. 415-22.

Bill, A., Gutierrez, A., Kulkarni, S., Kemp, C., Bonenfant, D., Voshol, H., Duvvuri, U. and Gaither, L. A. (2015) 'ANO1/TMEM16A interacts with EGFR and correlates with sensitivity to EGFR-targeting therapy in head and neck cancer', *Oncotarget,* 6(11), pp. 9173-88.

Bill, A., Hall, M. L., Borawski, J., Hodgson, C., Jenkins, J., Piechon, P., Popa, O., Rothwell, C., Tranter, P., Tria, S., Wagner, T., Whitehead, L. and Gaither, L. A. (2014) 'Small molecule-facilitated degradation of ANO1 protein: a new targeting approach for anticancer therapeutics', *J Biol Chem,* 289(16), pp. 11029-41.

Blanke, C. D., Demetri, G. D., von Mehren, M., Heinrich, M. C., Eisenberg, B., Fletcher, J. A., Corless, C. L., Fletcher, C. D., Roberts, P. J., Heinz, D., Wehre, E., Nikolova, Z. and Joensuu, H. (2008a) 'Long-term results from a randomized phase II trial of standard- versus higher-dose imatinib mesylate for patients with unresectable or metastatic gastrointestinal stromal tumors expressing KIT', *J Clin Oncol,* 26(4), pp. 620-5.

Blanke, C. D., Rankin, C., Demetri, G. D., Ryan, C. W., von Mehren, M., Benjamin, R. S., Raymond, A. K., Bramwell, V. H., Baker, L. H., Maki, R. G., Tanaka, M., Hecht, J. R., Heinrich, M. C., Fletcher, C. D., Crowley, J. J. and Borden, E. C. (2008b) 'Phase III randomized, intergroup trial assessing imatinib mesylate at two dose levels in patients with unresectable or metastatic gastrointestinal stromal tumors expressing the kit receptor tyrosine kinase: S0033', *J Clin Oncol,* 26(4), pp. 626-32.

Blume-Jensen, P., Claesson-Welsh, L., Siegbahn, A., Zsebo, K. M., Westermark, B. and Heldin, C. H. (1991) 'Activation of the human c-kit product by ligand-induced dimerization mediates circular actin reorganization and chemotaxis', *EMBO J,* 10(13), pp. 4121-8.

Boikos, S. A., Pappo, A. S., Killian, J. K., LaQuaglia, M. P., Weldon, C. B., George, S., Trent, J. C., von Mehren, M., Wright, J. A., Schiffman, J. D., Raygada, M., Pacak, K., Meltzer, P. S., Miettinen, M. M., Stratakis, C., Janeway, K. A. and Helman, L. J. (2016) 'Molecular Subtypes of KIT/PDGFRA Wild-Type Gastrointestinal Stromal Tumors: A Report From the National Institutes of Health Gastrointestinal Stromal Tumor Clinic', *JAMA Oncol,* 2(7), pp. 922-8.

Bosbach, B., Rossi, F., Yozgat, Y., Loo, J., Zhang, J. Q., Berrozpe, G., Warpinski, K., Ehlers, I., Veach, D., Kwok, A., Manova, K., Antonescu, C. R., DeMatteo, R. P. and Besmer, P. (2017) 'Direct engagement of the PI3K pathway by mutant KIT dominates oncogenic signaling in gastrointestinal stromal tumor', *Proc Natl Acad Sci U S A,* 114(40), pp. E8448-E8457.

Britschgi, A., Bill, A., Brinkhaus, H., Rothwell, C., Clay, I., Duss, S., Rebhan, M., Raman, P., Guy, C. T., Wetzel, K., George, E., Popa, M. O., Lilley, S., Choudhury, H., Gosling, M., Wang, L., Fitzgerald, S., Borawski, J., Baffoe, J., Labow, M., Gaither, L. A. and Bentires-Alj, M. (2013) 'Calcium-activated chloride channel ANO1 promotes breast cancer progression by activating EGFR and CAMK signaling', *Proc Natl Acad Sci U S A,* 110(11), pp. E1026-34.

Buchdunger, E., Cioffi, C. L., Law, N., Stover, D., Ohno-Jones, S., Druker, B. J. and Lydon, N. B. (2000) 'Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors', *J Pharmacol Exp Ther,* 295(1), pp. 139-45.

Caputo, A., Caci, E., Ferrera, L., Pedemonte, N., Barsanti, C., Sondo, E., Pfeffer, U., Ravazzolo, R., Zegarra-Moran, O. and Galietta, L. J. (2008) 'TMEM16A, a membrane

protein associated with calcium-dependent chloride channel activity', *Science,* 322(5901), pp. 590-4.

Casali, P. G., Abecassis, N., Aro, H. T., Bauer, S., Biagini, R., Bielack, S., Bonvalot, S., Boukovinas, I., Bovee, J. V. M. G., Brodowicz, T., Broto, J. M., Buonadonna, A., De Álava, E., Dei Tos, A. P., Del Muro, X. G., Dileo, P., Eriksson, M., Fedenko, A., Ferraresi, V., Ferrari, A., Ferrari, S., Frezza, A. M., Gasperoni, S., Gelderblom, H., Gil, T., Grignani, G., Gronchi, A., Haas, R. L., Hassan, B., Hohenberger, P., Issels, R., Joensuu, H., Jones, R. L., Judson, I., Jutte, P., Kaal, S., Kasper, B., Kopeckova, K., Krákorová, D. A., Le Cesne, A., Lugowska, I., Merimsky, O., Montemurro, M., Pantaleo, M. A., Piana, R., Picci, P., Piperno-Neumann, S., Pousa, A. L., Reichardt, P., Robinson, M. H., Rutkowski, P., Safwat, A. A., Schöffski, P., Sleijfer, S., Stacchiotti, S., Sundby Hall, K., Unk, M., Van Coevorden, F., van der Graaf, W. T. A., Whelan, J., Wardelmann, E., Zaikova, O., Blay, J. Y. and EURACAN, E. G. C. a. (2018) 'Gastrointestinal stromal tumours: ESMO-EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up', *Ann Oncol,* 29(Suppl 4), pp. iv68-iv78.

Casali, P. G., Le Cesne, A., Poveda Velasco, A., Kotasek, D., Rutkowski, P., Hohenberger, P., Fumagalli, E., Judson, I. R., Italiano, A., Gelderblom, H., Adenis, A., Hartmann, J. T., Duffaud, F., Goldstein, D., Broto, J. M., Gronchi, A., Dei Tos, A. P., Marréaud, S., van der Graaf, W. T., Zalcberg, J. R., Litière, S. and Blay, J. Y. (2015) 'Time to Definitive Failure to the First Tyrosine Kinase Inhibitor in Localized GI Stromal Tumors Treated With Imatinib As an Adjuvant: A European Organisation for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group Intergroup Randomized Trial in Collaboration With the Australasian Gastro-Intestinal Trials Group, UNICANCER, French Sarcoma Group, Italian Sarcoma Group, and Spanish Group for Research on Sarcomas', *J Clin Oncol,* 33(36), pp. 4276-83.

Casali, P. G., Zalcberg, J., Le Cesne, A., Reichardt, P., Blay, J. Y., Lindner, L. H., Judson, I. R., Schöffski, P., Leyvraz, S., Italiano, A., Grünwald, V., Pousa, A. L., Kotasek, D., Sleijfer, S., Kerst, J. M., Rutkowski, P., Fumagalli, E., Hogendoorn, P., Litière, S., Marreaud, S., van der Graaf, W., Gronchi, A., Verweij, J. and European Organisation for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group, I. a. S. G., and Australasian Gastrointestinal Trials Group (2017) 'Ten-Year Progression-Free and Overall Survival in Patients With Unresectable or Metastatic GI Stromal Tumors: Long-Term Analysis of the European Organisation for Research and Treatment of Cancer, Italian Sarcoma Group, and Australasian Gastrointestinal Trials Group Intergroup Phase III Randomized Trial on Imatinib at Two Dose Levels', *J Clin Oncol,* 35(15), pp. 1713-1720.

Cassier, P. A., Fumagalli, E., Rutkowski, P., Schöffski, P., Van Glabbeke, M., Debiec-Rychter, M., Emile, J. F., Duffaud, F., Martin-Broto, J., Landi, B., Adenis, A., Bertucci, F., Bompas, E., Bouché, O., Leyvraz, S., Judson, I., Verweij, J., Casali, P., Blay, J. Y., Hohenberger, P. and Cancer, E. O. f. R. a. T. o. (2012) 'Outcome of patients with platelet-derived growth factor receptor alpha-mutated gastrointestinal stromal tumors in the tyrosine kinase inhibitor era', *Clin Cancer Res,* 18(16), pp. 4458-64.

Cavnar, M. J., Seier, K., Curtin, C., Balachandran, V. P., Coit, D. G., Yoon, S. S., Crago, A. M., Strong, V. E., Tap, W. D., Gönen, M., Antonescu, C. R., Brennan, M. F., Singer, S. and DeMatteo, R. P. (2019) 'Outcome of 1000 Patients With Gastrointestinal Stromal Tumor (GIST) Treated by Surgery in the Pre and Post-imatinib Eras', *Ann Surg*.

Chi, P., Chen, Y., Zhang, L., Guo, X., Wongvipat, J., Shamu, T., Fletcher, J. A., Dewell, S., Maki, R. G., Zheng, D., Antonescu, C. R., Allis, C. D. and Sawyers, C. L. (2010) 'ETV1 is a lineage survival factor that cooperates with KIT in gastrointestinal stromal tumours', *Nature,* 467(7317), pp. 849-53.

Choi, E. J., Yun, J. A., Jabeen, S., Jeon, E. K., Won, H. S., Ko, Y. H. and Kim, S. Y. (2014) 'Prognostic significance of TMEM16A, PPFIA1, and FADD expression in invasive ductal carcinoma of the breast', *World J Surg Oncol,* 12, pp. 137.

Clark, W. H., Elder, D. E., Guerry, D., Braitman, L. E., Trock, B. J., Schultz, D., Synnestvedt, M. and Halpern, A. C. (1989) 'Model predicting survival in stage I melanoma based on tumor progression', *J Natl Cancer Inst,* 81(24), pp. 1893-904.

Corless, C. L. (2014) 'Gastrointestinal stromal tumors: what do we know now?', *Mod Pathol,* 27 Suppl 1, pp. S1-16.

Corless, C. L., Ballman, K. V., Antonescu, C. R., Kolesnikova, V., Maki, R. G., Pisters, P. W., Blackstein, M. E., Blanke, C. D., Demetri, G. D., Heinrich, M. C., von Mehren, M., Patel, S., McCarter, M. D., Owzar, K. and DeMatteo, R. P. (2014) 'Pathologic and molecular features correlate with long-term outcome after adjuvant therapy of resected primary GI stromal tumor: the ACOSOG Z9001 trial', *J Clin Oncol,* 32(15), pp. 1563-70.

Corless, C. L., Barnett, C. M. and Heinrich, M. C. (2011) 'Gastrointestinal stromal tumours: origin and molecular oncology', *Nat Rev Cancer,* 11(12), pp. 865-78.

Corless, C. L., McGreevey, L., Haley, A., Town, A. and Heinrich, M. C. (2002) 'KIT mutations are common in incidental gastrointestinal stromal tumors one centimeter or less in size', *Am J Pathol,* 160(5), pp. 1567-72.

Davis, M. I., Hunt, J. P., Herrgard, S., Ciceri, P., Wodicka, L. M., Pallares, G., Hocker, M., Treiber, D. K. and Zarrinkar, P. P. (2011) 'Comprehensive analysis of kinase inhibitor selectivity', *Nat Biotechnol,* 29(11), pp. 1046-51.

De La Fuente, R., Namkung, W., Mills, A. and Verkman, A. S. (2008) 'Small-molecule screen identifies inhibitors of a human intestinal calcium-activated chloride channel', *Mol Pharmacol,* 73(3), pp. 758-68.

Debiec-Rychter, M., Cools, J., Dumez, H., Sciot, R., Stul, M., Mentens, N., Vranckx, H., Wasag, B., Prenen, H., Roesel, J., Hagemeijer, A., Van Oosterom, A. and Marynen, P. (2005) 'Mechanisms of resistance to imatinib mesylate in gastrointestinal stromal tumors and activity of the PKC412 inhibitor against imatinib-resistant mutants', *Gastroenterology,* 128(2), pp. 270-9.

Dematteo, R. P., Ballman, K. V., Antonescu, C. R., Maki, R. G., Pisters, P. W., Demetri, G. D., Blackstein, M. E., Blanke, C. D., von Mehren, M., Brennan, M. F., Patel, S., McCarter, M. D., Polikoff, J. A., Tan, B. R., Owzar, K. and Team, A. C. o. S. O. G. A. I. A. G. S. (2009) 'Adjuvant imatinib mesylate after resection of localised, primary gastrointestinal stromal tumour: a randomised, double-blind, placebo-controlled trial', *Lancet,* 373(9669), pp. 1097-104.

Dematteo, R. P., Heinrich, M. C., El-Rifai, W. M. and Demetri, G. (2002) 'Clinical management of gastrointestinal stromal tumors: before and after STI-571', *Hum Pathol,* 33(5), pp. 466-77.

DeMatteo, R. P., Lewis, J. J., Leung, D., Mudan, S. S., Woodruff, J. M. and Brennan, M. F. (2000) 'Two hundred gastrointestinal stromal tumors: recurrence patterns and prognostic factors for survival', *Ann Surg,* 231(1), pp. 51-8.

Demetri, G. D., Garrett, C. R., Schöffski, P., Shah, M. H., Verweij, J., Leyvraz, S., Hurwitz, H. I., Pousa, A. L., Le Cesne, A., Goldstein, D., Paz-Ares, L., Blay, J. Y., McArthur, G. A., Xu, Q. C., Huang, X., Harmon, C. S., Tassell, V., Cohen, D. P. and Casali, P. G. (2012) 'Complete longitudinal analyses of the randomized, placebo-controlled, phase III trial of

sunitinib in patients with gastrointestinal stromal tumor following imatinib failure', *Clin Cancer Res,* 18(11), pp. 3170-9.

Demetri, G. D., Reichardt, P., Kang, Y. K., Blay, J. Y., Rutkowski, P., Gelderblom, H., Hohenberger, P., Leahy, M., von Mehren, M., Joensuu, H., Badalamenti, G., Blackstein, M., Le Cesne, A., Schöffski, P., Maki, R. G., Bauer, S., Nguyen, B. B., Xu, J., Nishida, T., Chung, J., Kappeler, C., Kuss, I., Laurent, D., Casali, P. G. and investigators, G. s. (2013) 'Efficacy and safety of regorafenib for advanced gastrointestinal stromal tumours after failure of imatinib and sunitinib (GRID): an international, multicentre, randomised, placebo-controlled, phase 3 trial', *Lancet,* 381(9863), pp. 295-302.

Demetri, G. D., van Oosterom, A. T., Garrett, C. R., Blackstein, M. E., Shah, M. H., Verweij, J., McArthur, G., Judson, I. R., Heinrich, M. C., Morgan, J. A., Desai, J., Fletcher, C. D., George, S., Bello, C. L., Huang, X., Baum, C. M. and Casali, P. G. (2006) 'Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial', *Lancet,* 368(9544), pp. 1329-38.

Demetri, G. D., von Mehren, M., Blanke, C. D., Van den Abbeele, A. D., Eisenberg, B., Roberts, P. J., Heinrich, M. C., Tuveson, D. A., Singer, S., Janicek, M., Fletcher, J. A., Silverman, S. G., Silberman, S. L., Capdeville, R., Kiese, B., Peng, B., Dimitrijevic, S., Druker, B. J., Corless, C., Fletcher, C. D. and Joensuu, H. (2002) 'Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors', *N Engl J Med,* 347(7), pp. 472-80.

Deng, L., Yang, J., Chen, H., Ma, B., Pan, K., Su, C., Xu, F. and Zhang, J. (2016) 'Knockdown of TMEM16A suppressed MAPK and inhibited cell proliferation and migration in hepatocellular carcinoma', *Onco Targets Ther,* 9, pp. 325-33.

Druker, B. J., Talpaz, M., Resta, D. J., Peng, B., Buchdunger, E., Ford, J. M., Lydon, N. B., Kantarjian, H., Capdeville, R., Ohno-Jones, S. and Sawyers, C. L. (2001) 'Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia', *N Engl J Med,* 344(14), pp. 1031-7.

Druker, B. J., Tamura, S., Buchdunger, E., Ohno, S., Segal, G. M., Fanning, S., Zimmermann, J. and Lydon, N. B. (1996) 'Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells', *Nat Med,* 2(5), pp. 561-6.

Ducimetière, F., Lurkin, A., Ranchère-Vince, D., Decouvelaere, A. V., Péoc'h, M., Istier, L., Chalabreysse, P., Muller, C., Alberti, L., Bringuier, P. P., Scoazec, J. Y., Schott, A. M., Bergeron, C., Cellier, D., Blay, J. Y. and Ray-Coquard, I. (2011) 'Incidence of sarcoma histotypes and molecular subtypes in a prospective epidemiological study with central pathology review and molecular testing', *PLoS One,* 6(8), pp. e20294.

Duensing, A., Medeiros, F., McConarty, B., Joseph, N. E., Panigrahy, D., Singer, S., Fletcher, C. D., Demetri, G. D. and Fletcher, J. A. (2004) 'Mechanisms of oncogenic KIT signal transduction in primary gastrointestinal stromal tumors (GISTs)', *Oncogene,* 23(22), pp. 3999-4006.

Duvvuri, U., Shiwarski, D. J., Xiao, D., Bertrand, C., Huang, X., Edinger, R. S., Rock, J. R., Harfe, B. D., Henson, B. J., Kunzelmann, K., Schreiber, R., Seethala, R. S., Egloff, A. M., Chen, X., Lui, V. W., Grandis, J. R. and Gollin, S. M. (2012) 'TMEM16A induces MAPK and contributes directly to tumorigenesis and cancer progression', *Cancer Res,* 72(13), pp. 3270-81.

El-Rifai, W., Sarlomo-Rikala, M., Andersson, L. C., Knuutila, S. and Miettinen, M. (2000) 'DNA sequence copy number changes in gastrointestinal stromal tumors: tumor progression and prognostic significance', *Cancer Res,* 60(14), pp. 3899-903.

Etherington, M. S. and DeMatteo, R. P. (2019) 'Tailored management of primary gastrointestinal stromal tumors', *Cancer*.

Ferrera, L., Caputo, A. and Galietta, L. J. (2010) 'TMEM16A protein: a new identity for Ca(2+)-dependent Cl^- channels', *Physiology (Bethesda),* 25(6), pp. 357-63.

Fletcher, C. D., Berman, J. J., Corless, C., Gorstein, F., Lasota, J., Longley, B. J., Miettinen, M., O'Leary, T. J., Remotti, H., Rubin, B. P., Shmookler, B., Sobin, L. H. and Weiss, S. W. (2002) 'Diagnosis of gastrointestinal stromal tumors: A consensus approach', *Hum Pathol,* 33(5), pp. 459-65.

Fritz, K. S. and Petersen, D. R. (2013) 'An overview of the chemistry and biology of reactive aldehydes', *Free Radic Biol Med,* 59, pp. 85-91.

Gasparotto, D., Rossi, S., Polano, M., Tamborini, E., Lorenzetto, E., Sbaraglia, M., Mondello, A., Massani, M., Lamon, S., Bracci, R., Mandolesi, A., Frate, E., Stanzial, F., Agaj, J., Mazzoleni, G., Pilotti, S., Gronchi, A., Dei Tos, A. P. and Maestro, R. (2017) 'Quadruple-Negative GIST Is a Sentinel for Unrecognized Neurofibromatosis Type 1 Syndrome', *Clin Cancer Res,* 23(1), pp. 273-282.

Godse, N. R., Khan, N., Yochum, Z. A., Gomez-Casal, R., Kemp, C., Shiwarski, D. J., Seethala, R. S., Kulich, S., Seshadri, M., Burns, T. F. and Duvvuri, U. (2017) 'TMEM16A/ANO1 Inhibits Apoptosis Via Downregulation of Bim Expression', *Clin Cancer Res,* 23(23), pp. 7324-7332.

Gold, J. S., Gönen, M., Gutiérrez, A., Broto, J. M., García-del-Muro, X., Smyrk, T. C., Maki, R. G., Singer, S., Brennan, M. F., Antonescu, C. R., Donohue, J. H. and DeMatteo, R. P. (2009) 'Development and validation of a prognostic nomogram for recurrence-free survival after complete surgical resection of localised primary gastrointestinal stromal tumour: a retrospective analysis', *Lancet Oncol,* 10(11), pp. 1045-52.

Gordon, P. M. and Fisher, D. E. (2010) 'Role for the proapoptotic factor BIM in mediating imatinib-induced apoptosis in a c-KIT-dependent gastrointestinal stromal tumor cell line', *J Biol Chem,* 285(19), pp. 14109-14.

Grulich, A. E., van Leeuwen, M. T., Falster, M. O. and Vajdic, C. M. (2007) 'Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis', *Lancet,* 370(9581), pp. 59-67.

Guan, L., Song, Y., Gao, J. and Wang, K. (2016) 'Inhibition of calcium-activated chloride channel ANO1 suppresses proliferation and induces apoptosis of epithelium originated cancer cells', *Oncotarget,* 7(48), pp. 78619-78630.

Hamill, O. P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F. J. (1981) 'Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches', *Pflugers Arch,* 391(2), pp. 85-100.

Hartzell, C., Putzier, I. and Arreola, J. (2005) 'Calcium-activated chloride channels', *Annu Rev Physiol,* 67, pp. 719-58.

Heinrich, M. C., Corless, C. L., Duensing, A., McGreevey, L., Chen, C. J., Joseph, N., Singer, S., Griffith, D. J., Haley, A., Town, A., Demetri, G. D., Fletcher, C. D. and Fletcher, J. A. (2003) 'PDGFRA activating mutations in gastrointestinal stromal tumors', *Science,* 299(5607), pp. 708-10.

Heinrich, M. C., Maki, R. G., Corless, C. L., Antonescu, C. R., Harlow, A., Griffith, D., Town, A., McKinley, A., Ou, W. B., Fletcher, J. A., Fletcher, C. D., Huang, X., Cohen, D. P., Baum, C. M. and Demetri, G. D. (2008) 'Primary and secondary kinase genotypes correlate

with the biological and clinical activity of sunitinib in imatinib-resistant gastrointestinal stromal tumor', *J Clin Oncol,* 26(33), pp. 5352-9.

Heinrich, M. C., Patterson, J., Beadling, C., Wang, Y., Debiec-Rychter, M., Dewaele, B., Corless, C. L., Duensing, A., Raut, C. P., Rubin, B., Ordog, T., van de Rijn, M., Call, J., Mühlenberg, T., Fletcher, J. A. and Bauer, S. (2019) 'Genomic aberrations in cell cycle genes predict progression of', *Clin Sarcoma Res,* 9, pp. 3.

Heinrich, M. C., Rankin, C., Blanke, C. D., Demetri, G. D., Borden, E. C., Ryan, C. W., von Mehren, M., Blackstein, M. E., Priebat, D. A., Tap, W. D., Maki, R. G., Corless, C. L., Fletcher, J. A., Owzar, K., Crowley, J. J., Benjamin, R. S. and Baker, L. H. (2017) 'Correlation of Long-term Results of Imatinib in Advanced Gastrointestinal Stromal Tumors With Next-Generation Sequencing Results: Analysis of Phase 3 SWOG Intergroup Trial S0033', *JAMA Oncol,* 3(7), pp. 944-952.

Hirota, S., Isozaki, K., Moriyama, Y., Hashimoto, K., Nishida, T., Ishiguro, S., Kawano, K., Hanada, M., Kurata, A., Takeda, M., Muhammad Tunio, G., Matsuzawa, Y., Kanakura, Y., Shinomura, Y. and Kitamura, Y. (1998) 'Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors', *Science,* 279(5350), pp. 577-80.

Hirota, S., Ohashi, A., Nishida, T., Isozaki, K., Kinoshita, K., Shinomura, Y. and Kitamura, Y. (2003) 'Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumors', *Gastroenterology,* 125(3), pp. 660-7.

Hornick, J. L. and Fletcher, C. D. (2002) 'Immunohistochemical staining for KIT (CD117) in soft tissue sarcomas is very limited in distribution', *Am J Clin Pathol,* 117(2), pp. 188-93.

Hostein, I., Faur, N., Primois, C., Boury, F., Denard, J., Emile, J. F., Bringuier, P. P., Scoazec, J. Y. and Coindre, J. M. (2010) 'BRAF mutation status in gastrointestinal stromal tumors', *Am J Clin Pathol,* 133(1), pp. 141-8.

Hsueh, Y. S., Chang, H. H., Shan, Y. S., Sun, H. S., Fletcher, J. A., Li, C. F. and Chen, L. T. (2019) 'Nuclear KIT induces a NFKBIB-RELA-KIT autoregulatory loop in imatinib-resistant gastrointestinal stromal tumors', *Oncogene,* 38(38), pp. 6550-6565.

Huizinga, J. D., Thuneberg, L., Klüppel, M., Malysz, J., Mikkelsen, H. B. and Bernstein, A. (1995) 'W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity', *Nature,* 373(6512), pp. 347-9.

Hwang, S. J., Blair, P. J., Britton, F. C., O'Driscoll, K. E., Hennig, G., Bayguinov, Y. R., Rock, J. R., Harfe, B. D., Sanders, K. M. and Ward, S. M. (2009) 'Expression of anoctamin 1/TMEM16A by interstitial cells of Cajal is fundamental for slow wave activity in gastrointestinal muscles', *J Physiol,* 587(Pt 20), pp. 4887-904.

Jahangir, A. and Terzic, A. (2005) 'K(ATP) channel therapeutics at the bedside', *J Mol Cell Cardiol,* 39(1), pp. 99-112.

Jeong, H., Hwang, J., Lee, H., Hammond, P. T., Choi, J. and Hong, J. (2017) 'In vitro blood cell viability profiling of polymers used in molecular assembly', *Sci Rep,* 7(1), pp. 9481.

Ji, Q., Guo, S., Wang, X., Pang, C., Zhan, Y., Chen, Y. and An, H. (2018) 'Recent advances in TMEM16A: Structure, function, and disease', *J Cell Physiol*.

Jia, L., Liu, W., Guan, L., Lu, M. and Wang, K. (2015) 'Inhibition of Calcium-Activated Chloride Channel ANO1/TMEM16A Suppresses Tumor Growth and Invasion in Human Lung Cancer', *PLoS One,* 10(8), pp. e0136584.

Jo, V. Y. and Fletcher, C. D. (2014) 'WHO classification of soft tissue tumours: an update based on the 2013 (4th) edition', *Pathology,* 46(2), pp. 95-104.

Joensuu, H. (2008) 'Risk stratification of patients diagnosed with gastrointestinal stromal tumor', *Hum Pathol,* 39(10), pp. 1411-9.

Joensuu, H., Eriksson, M., Sundby Hall, K., Hartmann, J. T., Pink, D., Schütte, J., Ramadori, G., Hohenberger, P., Duyster, J., Al-Batran, S. E., Schlemmer, M., Bauer, S., Wardelmann, E., Sarlomo-Rikala, M., Nilsson, B., Sihto, H., Monge, O. R., Bono, P., Kallio, R., Vehtari, A., Leinonen, M., Alvegård, T. and Reichardt, P. (2012a) 'One vs three years of adjuvant imatinib for operable gastrointestinal stromal tumor: a randomized trial', *JAMA,* 307(12), pp. 1265-72.

Joensuu, H., Eriksson, M., Sundby Hall, K., Reichardt, A., Hartmann, J. T., Pink, D., Ramadori, G., Hohenberger, P., Al-Batran, S. E., Schlemmer, M., Bauer, S., Wardelmann, E., Nilsson, B., Sihto, H., Bono, P., Kallio, R., Junnila, J., Alvegård, T. and Reichardt, P. (2016) 'Adjuvant Imatinib for High-Risk GI Stromal Tumor: Analysis of a Randomized Trial', *J Clin Oncol,* 34(3), pp. 244-50.

Joensuu, H., Hohenberger, P. and Corless, C. L. (2013) 'Gastrointestinal stromal tumour', *Lancet,* 382(9896), pp. 973-83.

Joensuu, H., Roberts, P. J., Sarlomo-Rikala, M., Andersson, L. C., Tervahartiala, P., Tuveson, D., Silberman, S., Capdeville, R., Dimitrijevic, S., Druker, B. and Demetri, G. D. (2001) 'Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor', *N Engl J Med,* 344(14), pp. 1052-6.

Joensuu, H., Vehtari, A., Riihimäki, J., Nishida, T., Steigen, S. E., Brabec, P., Plank, L., Nilsson, B., Cirilli, C., Braconi, C., Bordoni, A., Magnusson, M. K., Linke, Z., Sufliarsky, J., Federico, M., Jonasson, J. G., Dei Tos, A. P. and Rutkowski, P. (2012b) 'Risk of recurrence of gastrointestinal stromal tumour after surgery: an analysis of pooled population-based cohorts', *Lancet Oncol,* 13(3), pp. 265-74.

Joensuu, H., Wardelmann, E., Sihto, H., Eriksson, M., Sundby Hall, K., Reichardt, A., Hartmann, J. T., Pink, D., Cameron, S., Hohenberger, P., Al-Batran, S. E., Schlemmer, M., Bauer, S., Nilsson, B., Kallio, R., Junnila, J., Vehtari, A. and Reichardt, P. (2017) 'Effect of KIT and PDGFRA Mutations on Survival in Patients With Gastrointestinal Stromal Tumors Treated With Adjuvant Imatinib: An Exploratory Analysis of a Randomized Clinical Trial', *JAMA Oncol,* 3(5), pp. 602-609.

Karlsson-Parra, A., Kovacka, J., Heimann, E., Jorvid, M., Zeilemaker, S., Longhurst, S. and Suenaert, P. (2018) 'Ilixadencel - an Allogeneic Cell-Based Anticancer Immune Primer for Intratumoral Administration', *Pharm Res,* 35(8), pp. 156.

Kawanowa, K., Sakuma, Y., Sakurai, S., Hishima, T., Iwasaki, Y., Saito, K., Hosoya, Y., Nakajima, T. and Funata, N. (2006) 'High incidence of microscopic gastrointestinal stromal tumors in the stomach', *Hum Pathol,* 37(12), pp. 1527-35.

Kindblom, L. G., Remotti, H. E., Aldenborg, F. and Meis-Kindblom, J. M. (1998) 'Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal', *Am J Pathol,* 152(5), pp. 1259-69.

Kitamura, Y. and Hirotab, S. (2004) 'Kit as a human oncogenic tyrosine kinase', *Cell Mol Life Sci,* 61(23), pp. 2924-31.

Laurell, A., Lönnemark, M., Brekkan, E., Magnusson, A., Tolf, A., Wallgren, A. C., Andersson, B., Adamson, L., Kiessling, R. and Karlsson-Parra, A. (2017) 'Intratumorally

injected pro-inflammatory allogeneic dendritic cells as immune enhancers: a first-in-human study in unfavourable risk patients with metastatic renal cell carcinoma', *J Immunother Cancer,* 5, pp. 52.

Le Cesne, A., Ray-Coquard, I., Bui, B. N., Adenis, A., Rios, M., Bertucci, F., Duffaud, F., Chevreau, C., Cupissol, D., Cioffi, A., Emile, J. F., Chabaud, S., Pérol, D., Blay, J. Y. and Group, F. S. (2010) 'Discontinuation of imatinib in patients with advanced gastrointestinal stromal tumours after 3 years of treatment: an open-label multicentre randomised phase 3 trial', *Lancet Oncol,* 11(10), pp. 942-9.

Leanza, L., Managò, A., Zoratti, M., Gulbins, E. and Szabo, I. (2016) 'Pharmacological targeting of ion channels for cancer therapy: In vivo evidences', *Biochim Biophys Acta,* 1863(6 Pt B), pp. 1385-97.

Levy, A. D., Remotti, H. E., Thompson, W. M., Sobin, L. H. and Miettinen, M. (2003) 'Gastrointestinal stromal tumors: radiologic features with pathologic correlation', *Radiographics,* 23(2), pp. 283-304, 456; quiz 532.

Li, J., Zhang, H., Lu, Y., Chen, Z. and Su, K. (2015) 'Presence of PDGFRA and DOG1 mutations in gastrointestinal stromal tumors among Chinese population', *Int J Clin Exp Pathol,* 8(5), pp. 5721-6.

Li, Q., Zhi, X., Zhou, J., Tao, R., Zhang, J., Chen, P., Røe, O. D., Sun, L. and Ma, L. (2016) 'Circulating tumor cells as a prognostic and predictive marker in gastrointestinal stromal tumors: a prospective study', *Oncotarget,* 7(24), pp. 36645-36654.

Liegl, B., Hornick, J. L., Corless, C. L. and Fletcher, C. D. (2009) 'Monoclonal antibody DOG1.1 shows higher sensitivity than KIT in the diagnosis of gastrointestinal stromal tumors, including unusual subtypes', *Am J Surg Pathol,* 33(3), pp. 437-46.

Liegl, B., Kepten, I., Le, C., Zhu, M., Demetri, G. D., Heinrich, M. C., Fletcher, C. D., Corless, C. L. and Fletcher, J. A. (2008) 'Heterogeneity of kinase inhibitor resistance mechanisms in GIST', *J Pathol,* 216(1), pp. 64-74.

Liu, F., Cao, Q. H., Lu, D. J., Luo, B., Lu, X. F., Luo, R. C. and Wang, X. G. (2015) 'TMEM16A overexpression contributes to tumor invasion and poor prognosis of human gastric cancer through TGF-β signaling', *Oncotarget,* 6(13), pp. 11585-99.

Liu, W., Lu, M., Liu, B., Huang, Y. and Wang, K. (2012) 'Inhibition of Ca(2+)-activated Cl(-) channel ANO1/TMEM16A expression suppresses tumor growth and invasiveness in human prostate carcinoma', *Cancer Lett,* 326(1), pp. 41-51.

Liu, Y. and Gray, N. S. (2006) 'Rational design of inhibitors that bind to inactive kinase conformations', *Nat Chem Biol,* 2(7), pp. 358-64.

Lux, M. L., Rubin, B. P., Biase, T. L., Chen, C. J., Maclure, T., Demetri, G., Xiao, S., Singer, S., Fletcher, C. D. and Fletcher, J. A. (2000) 'KIT extracellular and kinase domain mutations in gastrointestinal stromal tumors', *Am J Pathol,* 156(3), pp. 791-5.

Maeda, H. (2017) 'Polymer therapeutics and the EPR effect', *J Drug Target,* 25(9-10), pp. 781-785.

Maeda, H., Yamagata, A., Nishikawa, S., Yoshinaga, K., Kobayashi, S. and Nishi, K. (1992) 'Requirement of c-kit for development of intestinal pacemaker system', *Development,* 116(2), pp. 369-75.

Malysz, J., Gibbons, S. J., Saravanaperumal, S. A., Du, P., Eisenman, S. T., Cao, C., Oh, U., Saur, D., Klein, S., Ordog, T. and Farrugia, G. (2017) 'Conditional genetic deletion of Ano1

in interstitial cells of Cajal impairs Ca', *Am J Physiol Gastrointest Liver Physiol,* 312(3), pp. G228-G245.

Martín, J., Poveda, A., Llombart-Bosch, A., Ramos, R., López-Guerrero, J. A., García del Muro, J., Maurel, J., Calabuig, S., Gutierrez, A., González de Sande, J. L., Martínez, J., De Juan, A., Laínez, N., Losa, F., Alija, V., Escudero, P., Casado, A., García, P., Blanco, R., Buesa, J. M. and Research, S. G. f. S. (2005) 'Deletions affecting codons 557-558 of the c-KIT gene indicate a poor prognosis in patients with completely resected gastrointestinal stromal tumors: a study by the Spanish Group for Sarcoma Research (GEIS)', *J Clin Oncol,* 23(25), pp. 6190-8.

Mastrangelo, G., Coindre, J. M., Ducimetière, F., Dei Tos, A. P., Fadda, E., Blay, J. Y., Buja, A., Fedeli, U., Cegolon, L., Frasson, A., Ranchère-Vince, D., Montesco, C., Ray-Coquard, I. and Rossi, C. R. (2012) 'Incidence of soft tissue sarcoma and beyond: a population-based prospective study in 3 European regions', *Cancer,* 118(21), pp. 5339-48.

Mazur, M. T. and Clark, H. B. (1983) 'Gastric stromal tumors. Reappraisal of histogenesis', *Am J Surg Pathol,* 7(6), pp. 507-19.

McCarter, M. D., Antonescu, C. R., Ballman, K. V., Maki, R. G., Pisters, P. W., Demetri, G. D., Blanke, C. D., von Mehren, M., Brennan, M. F., McCall, L., Ota, D. M., DeMatteo, R. P. and Team, A. C. o. S. O. G. A. I. A. G. S. (2012) 'Microscopically positive margins for primary gastrointestinal stromal tumors: analysis of risk factors and tumor recurrence', *J Am Coll Surg,* 215(1), pp. 53-9; discussion 59-60.

Mei, L., Du, W., Idowu, M., von Mehren, M. and Boikos, S. A. (2018) 'Advances and Challenges on Management of Gastrointestinal Stromal Tumors', *Front Oncol,* 8, pp. 135.

Miettinen, M., Fetsch, J. F., Sobin, L. H. and Lasota, J. (2006) 'Gastrointestinal stromal tumors in patients with neurofibromatosis 1: a clinicopathologic and molecular genetic study of 45 cases', *Am J Surg Pathol,* 30(1), pp. 90-6.

Miettinen, M. and Lasota, J. (2006) 'Gastrointestinal stromal tumors: pathology and prognosis at different sites', *Semin Diagn Pathol,* 23(2), pp. 70-83.

Miettinen, M. and Lasota, J. (2013) 'Gastrointestinal stromal tumors', *Gastroenterol Clin North Am,* 42(2), pp. 399-415.

Miettinen, M., Lasota, J. and Sobin, L. H. (2005) 'Gastrointestinal stromal tumors of the stomach in children and young adults: a clinicopathologic, immunohistochemical, and molecular genetic study of 44 cases with long-term follow-up and review of the literature', *Am J Surg Pathol,* 29(10), pp. 1373-81.

Miettinen, M., Virolainen, M. and Maarit-Sarlomo-Rikala (1995) 'Gastrointestinal stromal tumors--value of CD34 antigen in their identification and separation from true leiomyomas and schwannomas', *Am J Surg Pathol,* 19(2), pp. 207-16.

Miettinen, M., Wang, Z. F., Sarlomo-Rikala, M., Osuch, C., Rutkowski, P. and Lasota, J. (2011) 'Succinate dehydrogenase-deficient GISTs: a clinicopathologic, immunohistochemical, and molecular genetic study of 66 gastric GISTs with predilection to young age', *Am J Surg Pathol,* 35(11), pp. 1712-21.

Miwa, S., Nakajima, T., Murai, Y., Takano, Y. and Sugiyama, T. (2008) 'Mutation assay of the novel gene DOG1 in gastrointestinal stromal tumors (GISTs)', *J Gastroenterol,* 43(7), pp. 531-7.

Mol, C. D., Dougan, D. R., Schneider, T. R., Skene, R. J., Kraus, M. L., Scheibe, D. N., Snell, G. P., Zou, H., Sang, B. C. and Wilson, K. P. (2004) 'Structural basis for the

autoinhibition and STI-571 inhibition of c-Kit tyrosine kinase', *J Biol Chem,* 279(30), pp. 31655-63.

Motegi, A., Sakurai, S., Nakayama, H., Sano, T., Oyama, T. and Nakajima, T. (2005) 'PKC theta, a novel immunohistochemical marker for gastrointestinal stromal tumors (GIST), especially useful for identifying KIT-negative tumors', *Pathol Int,* 55(3), pp. 106-12.

Muenst, S., Thies, S., Went, P., Tornillo, L., Bihl, M. P. and Dirnhofer, S. (2011) 'Frequency, phenotype, and genotype of minute gastrointestinal stromal tumors in the stomach: an autopsy study', *Hum Pathol,* 42(12), pp. 1849-54.

Namkung, W., Phuan, P. W. and Verkman, A. S. (2011) 'TMEM16A inhibitors reveal TMEM16A as a minor component of calcium-activated chloride channel conductance in airway and intestinal epithelial cells', *J Biol Chem,* 286(3), pp. 2365-74.

Nilsson, B., Bümming, P., Meis-Kindblom, J. M., Odén, A., Dortok, A., Gustavsson, B., Sablinska, K. and Kindblom, L. G. (2005) 'Gastrointestinal stromal tumors: the incidence, prevalence, clinical course, and prognostication in the preimatinib mesylate era--a population-based study in western Sweden', *Cancer,* 103(4), pp. 821-9.

Novelli, M., Rossi, S., Rodriguez-Justo, M., Taniere, P., Seddon, B., Toffolatti, L., Sartor, C., Hogendoorn, P. C., Sciot, R., Van Glabbeke, M., Verweij, J., Blay, J. Y., Hohenberger, P., Flanagan, A. and Dei Tos, A. P. (2010) 'DOG1 and CD117 are the antibodies of choice in the diagnosis of gastrointestinal stromal tumours', *Histopathology,* 57(2), pp. 259-70.

Ohshima, K., Fujiya, K., Nagashima, T., Ohnami, S., Hatakeyama, K., Urakami, K., Naruoka, A., Watanabe, Y., Moromizato, S., Shimoda, Y., Serizawa, M., Akiyama, Y., Kusuhara, M., Mochizuki, T., Sugino, T., Shiomi, A., Tsubosa, Y., Uesaka, K., Terashima, M. and Yamaguchi, K. (2019) 'Driver gene alterations and activated signaling pathways toward malignant progression of gastrointestinal stromal tumors', *Cancer Sci*.

Pagès, F., Berger, A., Camus, M., Sanchez-Cabo, F., Costes, A., Molidor, R., Mlecnik, B., Kirilovsky, A., Nilsson, M., Damotte, D., Meatchi, T., Bruneval, P., Cugnenc, P. H., Trajanoski, Z., Fridman, W. H. and Galon, J. (2005) 'Effector memory T cells, early metastasis, and survival in colorectal cancer', *N Engl J Med,* 353(25), pp. 2654-66.

Pardoll, D. M. (2012) 'The blockade of immune checkpoints in cancer immunotherapy', *Nat Rev Cancer,* 12(4), pp. 252-64.

Park, N. H., Cheng, W., Lai, F., Yang, C., Florez de Sessions, P., Periaswamy, B., Wenhan Chu, C., Bianco, S., Liu, S., Venkataraman, S., Chen, Q., Yang, Y. Y. and Hedrick, J. L. (2018) 'Addressing Drug Resistance in Cancer with Macromolecular Chemotherapeutic Agents', *J Am Chem Soc,* 140(12), pp. 4244-4252.

Paulino, C., Kalienkova, V., Lam, A. K. M., Neldner, Y. and Dutzler, R. (2017) 'Activation mechanism of the calcium-activated chloride channel TMEM16A revealed by cryo-EM', *Nature,* 552(7685), pp. 421-425.

Pauwels, P., Debiec-Rychter, M., Stul, M., De Wever, I., Van Oosterom, A. T. and Sciot, R. (2005) 'Changing phenotype of gastrointestinal stromal tumours under imatinib mesylate treatment: a potential diagnostic pitfall', *Histopathology,* 47(1), pp. 41-7.

Perez-Cornejo, P., Gokhale, A., Duran, C., Cui, Y., Xiao, Q., Hartzell, H. C. and Faundez, V. (2012) 'Anoctamin 1 (Tmem16A) Ca2+-activated chloride channel stoichiometrically interacts with an ezrin-radixin-moesin network', *Proc Natl Acad Sci U S A,* 109(26), pp. 10376-81.

Pozarowski, P. and Darzynkiewicz, Z. (2004) 'Analysis of cell cycle by flow cytometry', *Methods Mol Biol,* 281, pp. 301-11.

Prakash, S., Sarran, L., Socci, N., DeMatteo, R. P., Eisenstat, J., Greco, A. M., Maki, R. G., Wexler, L. H., LaQuaglia, M. P., Besmer, P. and Antonescu, C. R. (2005) 'Gastrointestinal stromal tumors in children and young adults: a clinicopathologic, molecular, and genomic study of 15 cases and review of the literature', *J Pediatr Hematol Oncol,* 27(4), pp. 179-87.

Prevarskaya, N., Skryma, R. and Shuba, Y. (2018) 'Ion Channels in Cancer: Are Cancer Hallmarks Oncochannelopathies?', *Physiol Rev,* 98(2), pp. 559-621.

Raffaghello, L., Zuccari, G., Carosio, R., Orienti, I. and Montaldo, P. G. (2006) 'In vitro and in vivo antitumor activity of the novel derivatized polyvinyl alcohol-based polymer P10(4)', *Clin Cancer Res,* 12(11 Pt 1), pp. 3485-93.

Ran, L., Sirota, I., Cao, Z., Murphy, D., Chen, Y., Shukla, S., Xie, Y., Kaufmann, M. C., Gao, D., Zhu, S., Rossi, F., Wongvipat, J., Taguchi, T., Tap, W. D., Mellinghoff, I. K., Besmer, P., Antonescu, C. R. and Chi, P. (2015) 'Combined inhibition of MAP kinase and KIT signaling synergistically destabilizes ETV1 and suppresses GIST tumor growth', *Cancer Discov,* 5(3), pp. 304-15.

Raut, C. P., Espat, N. J., Maki, R. G., Araujo, D. M., Trent, J., Williams, T. F., Purkayastha, D. D. and DeMatteo, R. P. (2018) 'Efficacy and Tolerability of 5-Year Adjuvant Imatinib Treatment for Patients With Resected Intermediate- or High-Risk Primary Gastrointestinal Stromal Tumor: The PERSIST-5 Clinical Trial', *JAMA Oncol,* 4(12), pp. e184060.

Riccardi, C. and Nicoletti, I. (2006) 'Analysis of apoptosis by propidium iodide staining and flow cytometry', *Nat Protoc,* 1(3), pp. 1458-61.

Rizell, M., Sternby Eilard, M., Andersson, M., Andersson, B., Karlsson-Parra, A. and Suenaert, P. (2019) 'Phase 1 Trial With the Cell-Based Immune Primer Ilixadencel, Alone, and Combined With Sorafenib, in Advanced Hepatocellular Carcinoma', *Front Oncol,* 9, pp. 19.

Rock, J. R., Futtner, C. R. and Harfe, B. D. (2008) 'The transmembrane protein TMEM16A is required for normal development of the murine trachea', *Dev Biol,* 321(1), pp. 141-9.

Rossi, F., Ehlers, I., Agosti, V., Socci, N. D., Viale, A., Sommer, G., Yozgat, Y., Manova, K., Antonescu, C. R. and Besmer, P. (2006) 'Oncogenic Kit signaling and therapeutic intervention in a mouse model of gastrointestinal stromal tumor', *Proc Natl Acad Sci U S A,* 103(34), pp. 12843-8.

Rossi, S., Gasparotto, D., Toffolatti, L., Pastrello, C., Gallina, G., Marzotto, A., Sartor, C., Barbareschi, M., Cantaloni, C., Messerini, L., Bearzi, I., Arrigoni, G., Mazzoleni, G., Fletcher, J. A., Casali, P. G., Talamini, R., Maestro, R., Maestra, R. and Dei Tos, A. P. (2010) 'Molecular and clinicopathologic characterization of gastrointestinal stromal tumors (GISTs) of small size', *Am J Surg Pathol,* 34(10), pp. 1480-91.

Rubin, B. P., Singer, S., Tsao, C., Duensing, A., Lux, M. L., Ruiz, R., Hibbard, M. K., Chen, C. J., Xiao, S., Tuveson, D. A., Demetri, G. D., Fletcher, C. D. and Fletcher, J. A. (2001) 'KIT activation is a ubiquitous feature of gastrointestinal stromal tumors', *Cancer Res,* 61(22), pp. 8118-21.

Ruiz, C., Martins, J. R., Rudin, F., Schneider, S., Dietsche, T., Fischer, C. A., Tornillo, L., Terracciano, L. M., Schreiber, R., Bubendorf, L. and Kunzelmann, K. (2012) 'Enhanced expression of ANO1 in head and neck squamous cell carcinoma causes cell migration and correlates with poor prognosis', *PLoS One,* 7(8), pp. e43265.

Rusakiewicz, S., Semeraro, M., Sarabi, M., Desbois, M., Locher, C., Mendez, R., Vimond, N., Concha, A., Garrido, F., Isambert, N., Chaigneau, L., Le Brun-Ly, V., Dubreuil, P., Cremer, I., Caignard, A., Poirier-Colame, V., Chaba, K., Flament, C., Halama, N., Jäger, D., Eggermont, A., Bonvalot, S., Commo, F., Terrier, P., Opolon, P., Emile, J. F., Coindre, J. M., Kroemer, G., Chaput, N., Le Cesne, A., Blay, J. Y. and Zitvogel, L. (2013) 'Immune infiltrates are prognostic factors in localized gastrointestinal stromal tumors', *Cancer Res,* 73(12), pp. 3499-510.

Rutkowski, P., Bylina, E., Wozniak, A., Nowecki, Z. I., Osuch, C., Matlok, M., Switaj, T., Michej, W., Wroński, M., Głuszek, S., Kroc, J., Nasierowska-Guttmejer, A. and Joensuu, H. (2011) 'Validation of the Joensuu risk criteria for primary resectable gastrointestinal stromal tumour - the impact of tumour rupture on patient outcomes', *Eur J Surg Oncol,* 37(10), pp. 890-6.

Rutkowski, P., Gronchi, A., Hohenberger, P., Bonvalot, S., Schöffski, P., Bauer, S., Fumagalli, E., Nyckowski, P., Nguyen, B. P., Kerst, J. M., Fiore, M., Bylina, E., Hoiczyk, M., Cats, A., Casali, P. G., Le Cesne, A., Treckmann, J., Stoeckle, E., de Wilt, J. H., Sleijfer, S., Tielen, R., van der Graaf, W., Verhoef, C. and van Coevorden, F. (2013) 'Neoadjuvant imatinib in locally advanced gastrointestinal stromal tumors (GIST): the EORTC STBSG experience', *Ann Surg Oncol,* 20(9), pp. 2937-43.

Sarlomo-Rikala, M., Kovatich, A. J., Barusevicius, A. and Miettinen, M. (1998) 'CD117: a sensitive marker for gastrointestinal stromal tumors that is more specific than CD34', *Mod Pathol,* 11(8), pp. 728-34.

Schaefer, I. M., Mariño-Enríquez, A. and Fletcher, J. A. (2017) 'What is New in Gastrointestinal Stromal Tumor?', *Adv Anat Pathol,* 24(5), pp. 259-267.

Schaefer, I. M., Wang, Y., Liang, C. W., Bahri, N., Quattrone, A., Doyle, L., Mariño-Enríquez, A., Lauria, A., Zhu, M., Debiec-Rychter, M., Grunewald, S., Hechtman, J. F., Dufresne, A., Antonescu, C. R., Beadling, C., Sicinska, E. T., van de Rijn, M., Demetri, G. D., Ladanyi, M., Corless, C. L., Heinrich, M. C., Raut, C. P., Bauer, S. and Fletcher, J. A. (2017) 'MAX inactivation is an early event in GIST development that regulates p16 and cell proliferation', *Nat Commun,* 8, pp. 14674.

Schlessinger, J. and Ullrich, A. (1992) 'Growth factor signaling by receptor tyrosine kinases', *Neuron,* 9(3), pp. 383-91.

Schreiber, R. D., Old, L. J. and Smyth, M. J. (2011) 'Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion', *Science,* 331(6024), pp. 1565-70.

Scudieri, P., Caci, E., Bruno, S., Ferrera, L., Schiavon, M., Sondo, E., Tomati, V., Gianotti, A., Zegarra-Moran, O., Pedemonte, N., Rea, F., Ravazzolo, R. and Galietta, L. J. (2012) 'Association of TMEM16A chloride channel overexpression with airway goblet cell metaplasia', *J Physiol,* 590(23), pp. 6141-55.

Shiwarski, D. J., Shao, C., Bill, A., Kim, J., Xiao, D., Bertrand, C. A., Seethala, R. S., Sano, D., Myers, J. N., Ha, P., Grandis, J., Gaither, L. A., Puthenveedu, M. A. and Duvvuri, U. (2014) 'To "grow" or "go": TMEM16A expression as a switch between tumor growth and metastasis in SCCHN', *Clin Cancer Res,* 20(17), pp. 4673-88.

Simon, S., Grabellus, F., Ferrera, L., Galietta, L., Schwindenhammer, B., Muhlenberg, T., Taeger, G., Eilers, G., Treckmann, J., Breitenbuecher, F., Schuler, M., Taguchi, T., Fletcher, J. A. and Bauer, S. (2013) 'DOG1 regulates growth and IGFBP5 in gastrointestinal stromal tumors', *Cancer Res,* 73(12), pp. 3661-70.

Sircar, K., Hewlett, B. R., Huizinga, J. D., Chorneyko, K., Berezin, I. and Riddell, R. H. (1999) 'Interstitial cells of Cajal as precursors of gastrointestinal stromal tumors', *Am J Surg Pathol,* 23(4), pp. 377-89.

Stenman, G., Eriksson, A. and Claesson-Welsh, L. (1989) 'Human PDGFA receptor gene maps to the same region on chromosome 4 as the KIT oncogene', *Genes Chromosomes Cancer,* 1(2), pp. 155-8.

Stratakis, C. A. and Carney, J. A. (2009) 'The triad of paragangliomas, gastric stromal tumours and pulmonary chondromas (Carney triad), and the dyad of paragangliomas and gastric stromal sarcomas (Carney-Stratakis syndrome): molecular genetics and clinical implications', *J Intern Med,* 266(1), pp. 43-52.

Sui, Y., Sun, M., Wu, F., Yang, L., Di, W., Zhang, G., Zhong, L., Ma, Z., Zheng, J., Fang, X. and Ma, T. (2014) 'Inhibition of TMEM16A expression suppresses growth and invasion in human colorectal cancer cells', *PLoS One,* 9(12), pp. e115443.

Søreide, K., Sandvik, O. M., Søreide, J. A., Giljaca, V., Jureckova, A. and Bulusu, V. R. (2016) 'Global epidemiology of gastrointestinal stromal tumours (GIST): A systematic review of population-based cohort studies', *Cancer Epidemiol,* 40, pp. 39-46.

Tabata, Y., Murakami, Y. and Ikada, Y. (1998) 'Tumor accumulation of poly(vinyl alcohol) of different sizes after intravenous injection', *J Control Release,* 50(1-3), pp. 123-33.

Takahashi, H., Yumoto, K., Yasuhara, K., Nadres, E. T., Kikuchi, Y., Taichman, R. S. and Kuroda, K. (2019) 'Anticancer polymers designed for killing dormant prostate cancer cells', *Sci Rep,* 9(1), pp. 1096.

Tembo, M., Wozniak, K. L., Bainbridge, R. E. and Carlson, A. E. (2019) 'Phosphatidylinositol 4,5-bisphosphate (PIP', *J Biol Chem,* 294(33), pp. 12556-12564.

Torihashi, S., Nishi, K., Tokutomi, Y., Nishi, T., Ward, S. and Sanders, K. M. (1999) 'Blockade of kit signaling induces transdifferentiation of interstitial cells of cajal to a smooth muscle phenotype', *Gastroenterology,* 117(1), pp. 140-8.

Tuveson, D. A., Willis, N. A., Jacks, T., Griffin, J. D., Singer, S., Fletcher, C. D., Fletcher, J. A. and Demetri, G. D. (2001) 'STI571 inactivation of the gastrointestinal stromal tumor c-KIT oncoprotein: biological and clinical implications', *Oncogene,* 20(36), pp. 5054-8.

van de Rijn, M., Hendrickson, M. R. and Rouse, R. V. (1994) 'CD34 expression by gastrointestinal tract stromal tumors', *Hum Pathol,* 25(8), pp. 766-71.

Van den Abbeele, A. D. (2008) 'The lessons of GIST--PET and PET/CT: a new paradigm for imaging', *Oncologist,* 13 Suppl 2, pp. 8-13.

van Dongen, M., Savage, N. D., Jordanova, E. S., Briaire-de Bruijn, I. H., Walburg, K. V., Ottenhoff, T. H., Hogendoorn, P. C., van der Burg, S. H., Gelderblom, H. and van Hall, T. (2010) 'Anti-inflammatory M2 type macrophages characterize metastasized and tyrosine kinase inhibitor-treated gastrointestinal stromal tumors', *Int J Cancer,* 127(4), pp. 899-909.

Vermes, I., Haanen, C., Steffens-Nakken, H. and Reutelingsperger, C. (1995) 'A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V', *J Immunol Methods,* 184(1), pp. 39-51.

Verweij, J., Casali, P. G., Zalcberg, J., LeCesne, A., Reichardt, P., Blay, J. Y., Issels, R., van Oosterom, A., Hogendoorn, P. C., Van Glabbeke, M., Bertulli, R. and Judson, I. (2004) 'Progression-free survival in gastrointestinal stromal tumours with high-dose imatinib: randomised trial', *Lancet,* 364(9440), pp. 1127-34.

Vitiello, G. A., Bowler, T. G., Liu, M., Medina, B. D., Zhang, J. Q., Param, N. J., Loo, J. K., Goldfeder, R. L., Chibon, F., Rossi, F., Zeng, S. and DeMatteo, R. P. (2019) 'Differential immune profiles distinguish the mutational subtypes of gastrointestinal stromal tumor', *J Clin Invest,* 129(5), pp. 1863-1877.

Vitiello, G. A., Medina, B. D., Zeng, S., Bowler, T. G., Zhang, J. Q., Loo, J. K., Param, N. J., Liu, M., Moral, A. J., Zhao, J. N., Rossi, F., Antonescu, C. R., Balachandran, V. P., Cross, J. R. and DeMatteo, R. P. (2018) 'Mitochondrial Inhibition Augments the Efficacy of Imatinib by Resetting the Metabolic Phenotype of Gastrointestinal Stromal Tumor', *Clin Cancer Res,* 24(4), pp. 972-984.

von Mehren, M. and Joensuu, H. (2018) 'Gastrointestinal Stromal Tumors', *J Clin Oncol,* 36(2), pp. 136-143.

Wang, H., Zou, L., Ma, K., Yu, J., Wu, H., Wei, M. and Xiao, Q. (2017) 'Cell-specific mechanisms of TMEM16A Ca', *Mol Cancer,* 16(1), pp. 152.

Wang, Y., Marino-Enriquez, A., Bennett, R. R., Zhu, M., Shen, Y., Eilers, G., Lee, J. C., Henze, J., Fletcher, B. S., Gu, Z., Fox, E. A., Antonescu, C. R., Fletcher, C. D., Guo, X., Raut, C. P., Demetri, G. D., van de Rijn, M., Ordog, T., Kunkel, L. M. and Fletcher, J. A. (2014) 'Dystrophin is a tumor suppressor in human cancers with myogenic programs', *Nat Genet,* 46(6), pp. 601-6.

West, R. B., Corless, C. L., Chen, X., Rubin, B. P., Subramanian, S., Montgomery, K., Zhu, S., Ball, C. A., Nielsen, T. O., Patel, R., Goldblum, J. R., Brown, P. O., Heinrich, M. C. and van de Rijn, M. (2004) 'The novel marker, DOG1, is expressed ubiquitously in gastrointestinal stromal tumors irrespective of KIT or PDGFRA mutation status', *Am J Pathol,* 165(1), pp. 107-13.

Yan, B. M., Kaplan, G. G., Urbanski, S., Nash, C. L. and Beck, P. L. (2008) 'Epidemiology of gastrointestinal stromal tumors in a defined Canadian Health Region: a population-based study', *Int J Surg Pathol,* 16(3), pp. 241-50.

Yang, Z., Zheng, R., Zhang, S., Zeng, H., Li, H. and Chen, W. (2019) 'Incidence, distribution of histological subtypes and primary sites of soft tissue sarcoma in China', *Cancer Biol Med,* 16(3), pp. 565-574.

Yuzawa, S., Opatowsky, Y., Zhang, Z., Mandiyan, V., Lax, I. and Schlessinger, J. (2007) 'Structural basis for activation of the receptor tyrosine kinase KIT by stem cell factor', *Cell,* 130(2), pp. 323-34.

Zhang, L., Conejo-Garcia, J. R., Katsaros, D., Gimotty, P. A., Massobrio, M., Regnani, G., Makrigiannakis, A., Gray, H., Schlienger, K., Liebman, M. N., Rubin, S. C. and Coukos, G. (2003) 'Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer', *N Engl J Med,* 348(3), pp. 203-13.

Åhlén, J., Karlsson, F., Wejde, J., Nilsson, I. L., Larsson, C. and Bränström, R. (2018) 'Wide Surgical Margin Improves the Outcome for Patients with Gastrointestinal Stromal Tumors (GISTs)', *World J Surg,* 42(8), pp. 2512-2521.