CD117/c-kit in Cancer Stem Cell-Mediated Progression and Therapeutic Resistance

Brittni M. Foster,[1,†] Danish Zaidi,[1,†] Tyler R. Young,[1] Mary E. Mobley,[1] Bethany A. Kerr[1,2,*]

[1] Department of Cancer Biology, Wake Forest University School of Medicine, Winston-Salem, NC 27157

[2] Wake Forest Baptist Comprehensive Cancer Center, Winston-Salem, NC 27157

[†]These authors contributed equally to this work.

*Corresponding Author: Bethany Kerr, Ph.D., Wake Forest University School of Medicine, Medical Center Blvd, Winston-Salem, NC, 27157. Telephone: 336-716-0320; Fax: 336-716-0255; Twitter: @BethanyKerrLab; e mail: bkerr@wakehealth.edu

Abstract Word Count:

Tables: 1

Figures: 3

Supplementary Tables: 3

ABSTRACT

Metastasis is the primary cause of cancer patient morbidity and mortality but due to persisting gaps in our knowledge, it remains untreatable. Metastases often occur as patients' tumors progress or recur after initial therapy. Tumor recurrence at the primary site may be driven by a cancer stem-like cell or tumor progenitor cell, while recurrence at a secondary site is driven by metastatic cancer stem cells or metastasis-initiating cells. Ongoing efforts are aimed at identifying and characterizing these stem-like cells driving recurrence and metastasis. One potential marker for the cancer stem-like cell subpopulation is CD117/c-kit, a tyrosine kinase receptor associated with cancer progression and normal stem cell maintenance. In our analyses, CD117 was expressed in several tissues and was highly expressed in bone marrow progenitor cells. Also, we uncovered that CD117 gene amplifications and mutations occurred in multiple cancers. Further, activation of CD117 by its ligand stem cell factor (SCF; kit ligand) in the progenitor cell niche stimulates several signaling pathways driving proliferation, survival, and migration. These signaling pathways were commonly altered in patients with CD117 amplifications and mutations. Here, we examine evidence that the SCF/CD117 signaling axis controls cancer progression through the regulation of stemness and resistance to tyrosine kinase inhibitors.

Keywords: CD117/c-kit; cancer progression; cancer stem cell; tumor-initiating cell; metastasis; tyrosine kinase inhibitor; SCF

Abbreviations: AML: acute myeloid leukemia; CML: chronic myeloid leukemia; CTC: circulating tumor cell; CSC: cancer stem cell; DTC: disseminated tumor cell; GIST: gastrointestinal stromal tumor; HSC: hematopoietic stem cell; SCF: stem cell factor; TKI: tyrosine kinase inhibitor

1. Introduction

Primary tumors, when caught early, can be treated and in some cases the cancer is considered cured. A subset of patients will experience a recurrence of the primary tumor in the same site and it is hypothesized that this is due to remaining therapeutic resistant cells called cancer stem cells (CSCs). The CSC theory postulates that a subpopulation of tumor cells remaining after resection drive recurrence, while tumor cells surviving the circulation and arresting at metastatic sites driving tumor growth are metastatic CSC [1]. In either case, CSCs are capable of self-renewal and asymmetric division and may be able to recapitulate the initial tumor heterogeneity. Further, these CSC are more resistant to most treatments [1]–[8].

Cancer progression and therapeutic resistance are directly related to metastasis, the main cause of cancer-related death. Currently there are no interventions to prevent metastasis or, in many cases, to treat the metastatic tumor. Thus, there is a need to understand how cells enter and survive the circulation and then develop into overt metastases in another niche or home. One current hypothesis in the field is that a subset of tumor cells control metastasis; while in the circulation these cells are called circulating tumor cells (CTCs) and when in the metastatic niche, disseminated tumor cells (DTCs). Although approximately 3.2×10^6 cells/g tissue are shed from tumors daily, <0.01% develop into metastases [9],[10]. Thus, not all CTCs and DTCs can form a micro- or macrometastases, as many cells remain dormant within the metastatic tissue and many do not survive the shear stresses, oxygen tension changes, and other dangers of the circulation. Growth of the metastatic tumor and recapitulation of the primary tumor heterogeneity in a secondary site are driven by metastatic CSCs [11],[12]. Asymmetric division of CSCs allows for the maintenance of the CSC population as well as expansion of cells representing the full spectrum of the original heterogenic tumor.

Several markers for CTCs and CSCs have been postulated in the literature [13]. We and others have demonstrated that CD117 is expressed in aggressive cancers, on CTCs, and in recurrent and resistant tumors [14]–[17]. This review will examine the evidence that CD117 and its activation in CSCs may control tumor progression and therapeutic resistance.

2. Biology of the CD117 Receptor

The CD117 gene, officially known as “v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homologue,” is also more commonly known as c-kit, kit, or stem cell factor receptor. The CD117 gene consists of a single copy located on chromosome 4 (4q12) encompassing ~88 kBases (kb) (base pairs 54,657,927 to 54,740,714) and spanning 21 exons producing a transcript of 5.23 kb. The cDNA of CD117 encodes a 976 amino acid protein of 145 kDa. The resultant protein is a type III receptor tyrosine kinase containing an extracellular domain with 5 Ig-like loops, a 23 amino acid highly hydrophobic transmembrane domain, and an intracellular domain with tyrosine kinase activity split by a kinase insert in an ATP-binding region and in the phosphotransferase domain [18]–[20]. The CD117 protein contains 10 known glycosylation sites and is largely conserved between species with the human protein having ~83% homology to mouse and ~68% homology to chicken [21]. CD117, a proto-oncogene, is also homologous to CSF-1R, PDGFRβ, PDGFRα, and FLT3 resulting in significant inhibitor overlap [22]. This tyrosine kinase family is defined by their extracellular binding domains which consists of an extracellular ligand binding domain with five immunoglobulin-like regions, a transmembrane domain, a juxtamembrane domain and an intracellular kinase domain which is separated by a short kinase insert [22],[23]. Receptor tyrosine kinases are an important piece in cell signaling and are responsible for maintaining cell functions such as cell survival, metabolism, cell growth and progression, proliferation, apoptosis, cell migration, and cell differentiation [24]–[26]. These are important in understanding the biology of cancer cells.

2.1 CD117 Splice Variants

It has been demonstrated that CD117 of both mice and humans is expressed as two different isoforms, caused by alternative splicing, with only 4 amino acids differing (glycine, asparagine, asparagine, lysine, abbreviated as GNNK). These amino acids are either present or absent upstream of CD117's transmembrane domain. Several studies demonstrated that these splice variants can affect different signal transduction pathways and their effects on tumorigenicity depending on the cell type, constitutive tyrosine phosphorylation, and association with phosphatidylinositol 3-kinase (PI3-K) [27],[28]. In 1999, a study demonstrated the isoform GNNK^{-} transformed NIH3T2 fibroblasts caused tumorigenicity in nude mice [29]. Another study from 2003 showed a great level of expression of the GNNK^{-} isoform in testicular germline cell tumors compared to the normal testis which had a higher expression of GNKK^{+} CD117 receptor [30]. While GNNK^{-} shows a higher affinity for SCF, CD117's ligand as well as faster phosphorylation kinetics the GNNK- isoform is the dominating isoform in normal tissue such as bone marrow and melanocytes. In other studies, the ratio of GNNK^{+} to GNNK^{-} are the same in both cancers and normal tissues [31]. Further studies are required to understand the physiological and oncogenic roles of these isoforms.

2.2 Common CD117 Oncogenic Mutations

CD117 develops an overactivating or ligand-independent constitutive mutation to become oncogenic. Overactivation of CD117 cause alterations in the signaling pathways upregulating proliferation, cell survival, migration, and differentiation. Gain of function mutations have been linked to several malignancies including acute myeloid leukemia, gastrointestinal stromal tumor, mast cell leukemia, melanoma, and testicular cancer [32]. These mutations are shown to occur in the tyrosine kinase domain 1 (TK1, exon 17) in addition to the juxtadomain region (JM, exon 11). Less common mutations have been shown to occur in the extracellular domain (exons 2, 8, and 9) as well as tyrosine kinase domain 2 (TK2, exons 13 and 14) [33]. These mutations can occur in a variety of ways such as point mutations, frame deletions, and internal tandem repeats but rarely do we find more than one mutation of CD117 found in tumors. A list of mutations are further reviewed here [31],[34],[35].

3. CD117 Expression in Normal Stem Cells

Stem cells are defined by the National Institutes of Health as those that can divide for an indefinite period of time to develop specialized cells and organs [36]. These cells possess an ability to continuously self-renew and differentiate into unique cell types based upon their progenitor cells, allowing for tissue homeostasis and regeneration [37],[38]. This is made possible by asymmetrical cell division, whereby one daughter cell is identical to its mother, and the other daughter cell has continued potential for differentiation [39]–[41]. Stem cells express various markers dependent upon their resident tissues, but also express a few common markers including CD117$^+$, Lin$^-$, Sca1$^+$ (for mice), CD133$^+$, CD44$^+$, and CD34$^+$. The expression of CD117 in tissues and stem cell niches are show in Figure 1. CD117 is expressed, for example, on stem cell in the murine prostate. A single CD117 positive cell which was also Lin$^-$ Sca-1$^+$ CD133$^+$ CD44$^+$ regenerated an entire secreting prostate when mixed with urogenital mesenchymal cells and implanted on the renal capsule. Thus, this CD117 expressing cell was considered a prostate stem cell in adult tissue [42]. While each tissue contains a subpopulation of stem cells, the largest reservoir of stem cells in the body is the bone marrow.

Within the bone marrow there are several stem cell populations, but most prevalent are hematopoietic stem cells (HSC) [43],[44]. HSCs are pluripotent cells defined by their ability to proliferate and self-renew into all of the hematopoietic cell lineages throughout the organism's life-time [45]. These cells can also differentiate into endothelial cells [46]. CD117 plays an important role in the HSCs stemness, such as the ability to proliferate and differentiate [47]. Immature HSCs expresses CD34 in addition to CD117. As the cells mature and differentiate, they begin to lose the expression of CD117 along with their stemness.

Outside the bone marrow, CD117 is required for hematopoiesis in the spleen and liver niches. CD117 deletion in the spleen or bone marrow leads to a loss of the lymphocyte and erythrocyte lineages, while platelet numbers remained the same.[48] Thus, CD117 expression is required for several branches of hematopoietic cell differentiation.

4. SCF Expression in Stem Cell Niches

CD117's sole ligand SCF, also known as mast cell growth factor, kit ligand (KL), or steel factor, is a hematopoietic cytokine derived from bone marrow that is widely expressed [46],[49]. This ligand is a glycosylated, non-covalent homodimer and is expressed at variable concentrations throughout the body. SCF exists either as a soluble secreted form (sSCF) or a membrane bound form (mSCF) depending on whether the region containing exon 6 is spliced, which leads to the released soluble form [20],[43],[50]. Both isoforms are bioactive but vary in their effectiveness in activating CD117 [51].

SCF plays an important role in stimulating mature and primitive HSCs maintaining survival, promoting proliferation, and regulating growth and development of HSCs [20],[52]–[54]. SCF is expressed in niche cells controlling $CD117^{+}$ HSCs from mid gestation through adulthood [55]. Bone marrow niche cells express SCF including perivascular cells, endothelial cells, pericytes, mesenchymal stem cells, megakaryocytes, and stromal cells [56],[57]. Additionally, osteoblasts express SCF and control CD117-expressing HSC numbers near trabeculae [58]. Further, osteocytes, chondrocytes, and adipocytes differentiating from mesenchymal stem cells also express SCF [59]. SCF is expressed by megakaryocytes and osteoblasts and is capable of enhancing the differentiation of megakaryocytes and osteoclasts [60]. SCF deletion in endothelial cells or pericytes leads to HSC depletion in bone marrow [56],[59].

Outside the bone marrow, SCF is expressed in the spleen and liver to support extramedullary hematopoiesis [61],[62]. Within the spleen, SCF is expressed by red pulp endothelial cells and perivascular stromal cells and in the white pulp by central arteriolar cells and rare stromal cells. Extramedullary hematopoiesis increased the numbers of these SCF-expressing cells throughout the spleen. However, the $CD117^{+}$ HSCs were only located in the red pulp of the normal spleen [63]. Thus, SCF controls $CD117^{+}$ cell mobilization and homing to stem cell niches.

5. CD117 Activated Signaling Pathways

Activation of CD117 occurs when a SCF dimer binds with its extracellular domain. Inactive CD117 is found on the cell surface as a monomer; while SCF exists extracellularly as a dimer [32]. Upon binding of SCF, the CD117 receptor forms a homodimer causing autophosphorylation among specific tyrosine residues in the catalytic intracellular domain [22],[64]. CD117 phosphorylation triggers several signal transduction pathways including; JAK/STAT, WNT, NOTCH, RAS/MAP kinase pathway, PI3 kinase, PLC-y pathway, and SRC pathway (Figure 2). Cell survival, proliferation, differentiation, and migration occur once CD117 is activated requiring overlap of these pathways [24],[34],[47],[53],[65],[66]. CD117 is then rapidly ubiquitinated by SOCS6 after autophosphorylation resulting in internalization and degradation. The downstream pathways are discussed in detail below.

5.1 JAK/STAT Pathway

The JAK/STAT pathway plays a significant role in cell proliferation and differentiation in both murine and human cells. SCF binding induces rapid activation of JAK2 and stimulates the phosphorylation of STATS1/2/5. Once STATs are phosphorylated they translocate to the nucleus where they regulate transcription of target genes responsible for cell proliferation [67],[68].

5.2 RAS/MAP kinase pathway

Activation of the RAS/MAP kinase cascade occurs when activated CD117 recruits adaptor proteins containing a SH-2 domain such as GRB2, Shc, and SHP2. Grb2 will bind directly to CD117 at the phosphorylated Y703 and Y936 residues or indirectly to Shc or SHP2 [19],[69]. Once bound the GRB2 will associate with *sos* (Son-of-sevenless), a guanine nucleotide exchange factor, and this complex activates the G-protein Ras [19],[70]. Activation of Ras leads to the activation of Raf-1 which will activate MEK. MEK1/2 phosphorylates ERK1/2 which will phosphorylate and activates several transcription factors. The result of the activation of the RAS/MAP kinase cascade is regulation of cell proliferation, apoptosis, differentiation, adhesion, and mobility [53],[71],[72].

5.3 PI3-Kinase/Akt Signaling Pathway

PI3 kinase pathway is responsible for Akt and mTOR activity. This pathway has been shown to be activated by directly interacting with CD117 at Tyr-721 or indirectly by binding to the scaffold protein Gab2 which contacts the adapter protein Grb2 [24],[73]. The PI3-K pathway is the main pathway responsible for cell survival. Akt interacts with the pro-apoptotic factor BAD and causes inactivation leading to cells survival. Further, CD117 phosphorylation and activation of the PI3 kinase and SRC pathways contributes to SCF-mediated cell motility [74].

5.4 SRC Family Kinase Pathways

The GNKK$^-$ isoform of CD117 has displayed a stronger activation of SRC and SRC family kinases (SFK). These kinases can interact with several tyrosine residues on CD117 but only Tyr568 is required for activation. SCF can activate SFK specifically Lyn, Fyn, and PLCγ. Lyn activation has been shown to increase the activity of cyclin dependent kinase 2 (CDK2) as well as phosphorylation of Rb to promote cell proliferation [75],[76]. While Lyn has been shown to promote cell proliferation it was also demonstrated that Lyn can negatively regulate PI3-kinase/AKT pathway, although the underlying mechanism is still unknown [77]. While Lyn can negatively regulate the PI3-kinase pathway Fyn is able to phosphorylate Akt downstream. Fyn has also been shown to play a role in activating PLCγ when interacting with the truncated form of CD117 (tr-KIT) through mouse oocyte activation [77],[78].

5.5 PLCγ Pathway

Several studies show different docking sites for PLCγ. PLCγ can associate with the phosphorylated Tyr728, Tyr730, Tyr 936 and Tyr900 residues of CD117 [79]–[81]. PIP2 is hydrolyzed by PLCγ to generate DAG and IP3. DAG activates PKC by binding while IP3 causes the releases of $Ca2^{+}$. PKC has a role in cell survival, proliferation and adhesion [24],[82]. Thus, activation of the SCF/CD117 signaling axis can drive cell survival, proliferation, and motility; important steps in cancer progression.

6. CD117 Regulation of Cancer Progression

Overactivation of CD117 is the primary mutation seen in several cancer types such as gastrointestinal tumors (GIST), mastocytosis, acute myelogenous leukemia (AML), and melanoma [22],[24],[83]. Recent studies and clinical trials suggested that CD117 can be used effectively for prognosis, particularly for predicting cancer metastasis and response to chemotherapy. Biomarkers involving CD117 were identified and studied across various tumor cell types [84],[85]. In a single study, CD117 was expressed in 21% of breast cancers, 17% of colorectal cancers, 35% of sarcomas, 36% of renal cell carcinomas, 17% ovarian cancers, and 17% of hepatocellular tumors. While insignificant, there was a trend towards worse prognosis in these patients [86]. Further, 63% of AML patients had CD117 mutations, while 89-100% of GIST patient expressed CD117 [32]. Figure 3 shows CD117 (*KIT* gene) amplification and mutation in several cancers using datasets available through cBioPortal [87],[88]. Complete amplification, mutation, deletion and alterations for the CD117 *KIT* gene and the SCF *KITLG* genes are available in Tables S1 and S2, respectively. Genetic variants of CD117 (as a result of exon deletions) identified poor prognosis in GIST patients following primary tumor resection [89]–[91]. A 2012 study of resected tumors from 38 patients prior to treatment with imatinib found that 63% of tumors had mutations located on CD117 [92]. A 2017 study found that CD117 was expressed in 88% of surveyed cases where GIST had metastasized to bone, with the most common mutations in exon 11 and 13 [93]. These activating mutations, particularly in exon 11, have been confirmed in similar studies analyzing GIST patients [94],[95].

Beyond GIST, in patients with primary ovarian high-grade serous carcinoma, high expression of CD117 suggested shorter disease free survival and peritoneal metastasis [96]. This resulted from the tumorigenic and chemoresistant nature of ovarian cancer cells with CD117$^+$ phenotypes [97],[98]. Further, recent studies found that CD117$^+$ cells in the circulation are predictive of advanced prostate cancer, with a positive correlation between CD117 expression and Gleason scores [14],[99]. A 2008 study suggested a trend of increased expression of CD117 during prostate cancer metastasis to bone; a follow-up study in 2015 by the same lab found a novel pathway linking CD117 expression with BRCA2 downregulation that induced bone metastasis of prostate cancer [100]–[102]. Co-expression of CD117 and associated stem cell factors and ligands in breast carcinomas and small cell lung cancers have also been identified as playing a role in autocrine growth and tumor cell proliferation [103],[104]. Activating mutations and overexpression of the proto-oncogene CD117 are therefore important factors in considering tumor growth and metastasis in multiple solid tumors that develop outside the bone microenvironment.

These findings are not consistent across all cancers, and the expression of CD117 may impact myeloid/erythroid-derived cancers differently than it does solid tumors. For example, the opposite is true in multiple myelomas, which originate in the bone marrow. CD117 expression in malignant plasma cells has been linked to improved prognosis in patients with multiple myeloma [105]–[107]. This suggests a more complicated relationship between CD117 expression and cancer prognosis than initially suspected. In short, while the prognostic value of CD117 appears promising, it remains an area in need of further study [108].

Complementing the role of CD117, SCF may also play a role in cancer progression. Particularly high levels of SCF are found in the bone marrow, one location for metastasis and thus, a SCF gradient may be one driver of bone metastasis. Bone marrow stromal cells and prostate cancer cells express both membrane and soluble SCF; however, BMSC express much higher levels of the soluble SCF. Once exposed to bone marrow, which is high in SCF, PC3 cells started to express CD117 [16], indicating that the bone microenvironment might induce CD117 expression leading to over metastasis. Further, SCF production by hypoxic tissues induces $CD117^+$ myeloid cell mobilization and homing [109]. Thus, interplay between SCF and CD117 may drive cancer progression and metastasis.

7. CD117 Regulation of Cancer Cell "Stemness"

Studies suggest that CD117 plays an important role in cell differentiation and survival, particularly in its impact on CSCs. In a study on non-small cell lung cancer patients, tumor cells positively expressing CD117 exhibited CSC characteristics such as self-renewal and chemoresistance [110]. Similar characteristics are seen in $CD117^+$ ovarian tumor cells in which CD117 expression is related to the "stemness" of particular cancer cells [98],[111]. Beyond cancer, healthy and developing T-cells and B-cells gradually lose expression of CD117 as they differentiate and mature (thereby losing their "stemness"), further suggesting that CD117 signaling is needed to keep cell plasticity [53],[112],[113].

Activation of CD117 in cancer leads to activation of many downstream signaling pathways such as RAS/ERK, PI3-kinase, SRC, JAK/STAT, WNT, and NOTCH, and activation of these pathways are known to induce "stemness" or a stem-like phenotype. For example, the tyrosine kinase Src has been shown to interact with motifs on Akt-mTOR in acute myeloid leukemia (AML) cells, a process which upregulates signaling and stemness in AML [114]–[116]. In 2010, a study of human colon carcinoma and synovial sarcoma cell lines found that Ras/ERK pathways contributed in part to both the maintenance and acquisition of stemness in tumors [117]. The associations of CD117 *KIT* gene mutations with mutated signaling pathways genes are shown in Table S3 for prostate cancer as an example. As such, cells exhibiting "stemness" are those that share some, or all, properties of stem cells [118],[119]. In fact, $CD117^{+}$ prostate cancer cells may be CSC that express potential CSC markers Sox2, and Oct4. The cells can also generate tumors in serial tumor initiation experiments, a requirement for the classification as a CSC [15]. This ability to control "stemness" indicates that CD117 may be a marker for CSC.

8. CD117 Resistance to Tyrosine Kinase Inhibitors

Tyrosine kinase inhibitors (TKIs) are being tested in a variety of cancers expressing CD117 and other related tyrosine kinase receptors (Table 1). In particular, the TKI imatinib (Gleevec) is a standard treatment that has demonstrated specificity for inhibiting CD117, among other tyrosine kinases such as BCR-ABL [120],[121]. Early studies on imatinib *in vitro* and in human patients with GIST confirmed the role of CD117 in cancer metastasis. In these studies, imatinib was well tolerated and effective at targeting the tyrosine kinase domain of CD117 [122]–[124]. Imatinib's inhibitory effects on CD117 (coupled with its inhibition of indoleamine 2,3-dioxygenase, an immunosuppressive enzyme) have made it a first-line chemotherapeutic agent [125]–[127]. However, developing resistance to imatinib is not uncommon [128]. Unresectable metastatic imatinib-resistant GISTs led to the development of related TKIs such as sunitinib and regorafenib [129]–[132]. Tumor microenvironment SCF induces imatinib resistance by competing for binding site with a higher affinity for CD117 [133]. Imatinib induces M2 polarization of tumor associated macrophages and CD117 is upregulated in tumor with depleted of macrophages [134]. CD117 mutations in GIST are responsible for resistance to TKI treatment. 14% of GIST patients are initially resistant to imatinib and 50% develop resistance within 2 years of treatment. For most patients, sunitinib will then be used and effective unless one mutations, D816H/V is present which is resistant to both TKIs. Imatinib works better on inactive CD117 and prevents activation, but doesn't bind to activated CD117 [135]. Failure of imatinib in treatment of chronic myeloid leukemia

(CML), which primarily inhibits BCR-ABL in this cancer cell line, led to the development of nilotinib as a second-line treatment, a drug that also exhibits anti-CD117 properties [136]–[138].

Clinical trials of imatinib and related TKIs are ongoing, with researchers studying effects on various cancer cell lines. Phase 3 randomized trials found that nilotinib was unsuccessful as either first-line therapy for GIST or as second-line therapy for imatinib-resistant GIST, relegating its use mainly to CML [139],[140]. In clinical trials of patients with AIDS-associated Karposi's sarcoma, imatinib has demonstrated clinical benefit through its inhibition of both CD117 and platelet-derived growth factor (PDGF) [141]–[143]. Imatinib has also been shown to effectively treat melanoma that possesses an amplified or mutated CD117 oncogene [144],[145]. The anti-angiogenesis properties of TKIs such as imatinib, sunitinib, and pazopanib (all of which also target CD117) have been posited as promising therapies for epithelial ovarian cancer, with clinical trials demonstrating efficacy and tolerability in all three drugs [146],[147]. To date, TKIs remain a focus of study, with both pilot and large scale clinical trials reporting data on their potential benefits in metastatic melanoma, fibromatosis, and neuroendocrine tumors [148]–[152].

9. The Future of SCF/CD117 Signaling Axis in Cancer Treatment

While there is continued study of the early generations of TKIs despite their broad reactivity and off-target effects, research continues to develop inhibitors specific for each individual kinase expressed on cancer cells. Ongoing studies into TKI treatment efficacy requires new tools for studying their effects *in vivo* and *in vitro*. Prior to phase 1 clinical trials, most treatments are tested in animal models and on human cell lines. Newer patient derived xenografts are allowing for testing in primary human samples, while metastasis-on-chip models [153],[154] permit high throughput screening of candidate compounds. The ability to directly target SCF-secreting or CD117-expressing cells may improve patient treatment as CD117 activation and signaling is upregulated in a variety of tumors. Continued examination is also needed to define which tumor types and subset of patients would benefit from CD117 inhibition. A further understanding of the role of CD117 in cancer progression is necessary for determining which patients should be treated and how the receptor can be targeted.

Further, information on CD117's role in cancer progression would validate its use as a potential biomarker of CSCs in tissues and CTCs in the bloodstream. While multiple studies describe CD117-expressing cells as potential CTCs or metastatic tumor-initiating cells, the inability to properly isolate CTCs has prevented characterization of these two populations. Tracking CD117-expressing cells in a liquid biopsy would allow for definitive data confirming CD117 as a CTC marker in a variety of cancers and provide a way to evaluate patients in future CD117 inhibitor testing. Multiple labs have been developing microfluidic chips to isolate and quantify CTCs based on cell size, electromagnetic changes or cell surface marker expression [155],[156], which could be used for CD117. Further, the ability to enumerate CD117 expressing cells in tumor and the circulation could lead to improved tracking of response to treatment and therapeutic resistance in patients treated with TKIs. More recently, inhibitors specifically targeting CD117 were developed and tested *in vitro* in preventing cancer cell proliferation and migration [15],[157]. Further studies are needed to examine the effects of CD117-targeting *in vivo* and in phase 1 clinical trials. Further, combinatory targeting of CD117 with its downstream pathways may have improved efficacy. By targeting the CD117^{+} CSC population, in combination with conventional treatments working on the non-CSC population, cancer may finally be cured.

Methods

Genomic datasets available on the EMBL-European Bioinformatics Institute Gene Expression Atlas (for normal tissues) and cBioPortal (for cancerous tissues) were mined for expression of CD117 (*KIT*) and SCF (*KITLG*) mutations. The expression level (TPM) was exported or the % of patients with mutations or amplifications recorded for each dataset. CD117 (*KIT*) was queried with the signaling pathway genes in cBioPortal and the co-occurrence and *p* values recorded for each source dataset. CD117 expression (TPM value) and mean percentage ±SEM of CD117 mutations or amplifications were graphed using GraphPad Prism 7.0.

Supplementary Materials

The following are available online Table S1: Mutations and Amplifications of CD117/KIT in Cancer Datasets, Table S2: Mutations and Amplifications of SCF/KITLG in Cancer Datasets and Table S3: Mutations and Amplifications of CD117/KIT and Associated Signaling Pathways in Cancer Datasets.

Acknowledgements

Mr. Zaidi was supported by the Dubie Holliman Heart and Cancer Fund Medical Student Research Program. Ms. Young was supported by a Stand Up to Cancer's and PBS's Emperor Science Award. Dr. Kerr was supported by a NIH/NCI Pathway to Independence Award (R00 CA175291).

Author Contributions

T.R.Y. and M.E.M. collected the gene expression data. B.A.K. analyzed the gene expression data. D.Z. and B.M.F. wrote the paper. B.M.F., D.Z., T.R.Y., M.E.M. and B.A.K. edited the paper.

Conflicts of Interest

The authors declare no conflict of interest.

References

1 Lobo, N. A.; Shimono, Y.; Qian, D.; Clarke, M. F. The Biology of Cancer Stem Cells. *Annu. Rev. Cell Dev. Biol.* **2007**, *23* (1), 675–699 DOI: 10.1146/annurev.cellbio.22.010305.104154.

2 Clarke, M. F.; Dick, J. E.; Dirks, P. B.; Eaves, C. J.; Jamieson, C. H. M.; Jones, D. L.; Visvader, J.; Weissman, I. L.; Wahl, G. M. Cancer stem cells--perspectives on current status and future directions: AACR Workshop on cancer stem cells. *Cancer Res.* **2006**, *66* (19), 9339–9344 DOI: 10.1158/0008-5472.CAN-06-3126.

3 Kreso, A.; Dick, J. E. Evolution of the cancer stem cell model. *Cell Stem Cell* **2014**, *14* (3), 275–291 DOI: 10.1016/j.stem.2014.02.006.

4 Moltzahn, F. R.; Volkmer, J. P.; Rottke, D.; Ackermann, R. “Cancer stem cells”-lessons from Hercules to fight the Hydra. *Urol. Oncol.* **2008**, *26* (6), 581–589 DOI: S1078-1439(08)00164-6 [pii] 10.1016/j.urolonc.2008.07.009.

5 Nguyen, L. V.; Vanner, R.; Dirks, P.; Eaves, C. J. Cancer stem cells: an evolving concept. *Nat. Rev. Cancer* **2012**, *12* (2), 133 DOI: 10.1038/nrc3184.

6 Valent, P.; Bonnet, D.; De Maria, R.; Lapidot, T.; Copland, M.; Melo, J. V.; Chomienne, C.; Ishikawa, F.; Schuringa, J. J.; Stassi, G.; et al. Cancer stem cell definitions and terminology: the devil is in the details. *Nat. Rev. Cancer* **2012**, *12* (11), 767–775 DOI: 10.1038/nrc3368.

7 Mitra, S. S.; He, J. Q.; Esparza, R.; Hutter, G.; Cheshier, S. H.; Weissman, I. Introduction: Cancer Stem Cells. In *Cancer Stem Cells*; Liu, H., Lathia, J. D., Eds.; Elsevier, 2016; pp 3–24.

8 Kyjacova, L.; Hubackova, S.; Krejcikova, K.; Strauss, R.; Hanzlikova, H.; Dzijak, R.; Imrichova, T.; Simova, J.; Reinis, M.; Bartek, J.; et al. Radiotherapy-induced plasticity of prostate cancer mobilizes stem-like non-adherent, Erk signaling-dependent cells. *Cell*

Death Differ. **2015**, *22* (6), 898–911 DOI: 10.1038/cdd.2014.97.

9 Schilling, D.; Todenhöfer, T.; Hennenlotter, J.; Schwentner, C.; Fehm, T.; Stenzl, A. Isolated, disseminated and circulating tumour cells in prostate cancer. *Nat. Rev. Urol.* **2012**, *9* (8), 448–463 DOI: 10.1038/nrurol.2012.136.

10 Butler, T. P.; Gullino, P. M. Quantitation of cell shedding into efferent blood of mammary adenocarcinoma. *Cancer Res.* **1975**, *35* (3), 512–516.

11 Chopra, A. S.; Liu, X.; Liu, H. Cancer Stem Cells: Metastasis and Evasion from the Host Immune System. In *Cancer Stem Cells*; Liu, H., Lathia, J. D., Eds.; Elsevier, 2016; pp 341–366.

12 van der Toom, E. E.; Verdone, J. E.; Pienta, K. J. Disseminated tumor cells and dormancy in prostate cancer metastasis. *Curr. Opin. Biotechnol.* **2016**, *40*, 9–15 DOI: 10.1016/j.copbio.2016.02.002.

13 Harris, K. S.; Kerr, B. A. Prostate Cancer Stem Cell Markers Drive Progression, Therapeutic Resistance, and Bone Metastasis. *Stem Cells Int.* **2017**, *2017*, 8629234 DOI: 10.1155/2017/8629234.

14 Kerr, B. A.; Miocinovic, R.; Smith, A. K.; West, X. Z.; Watts, K. E.; Alzayed, A. W.; Klink, J. C.; Mir, M. C.; Sturey, T.; Hansel, D. E.; et al. $CD117^+$ cells in the circulation are predictive of advanced prostate cancer. *Oncotarget* **2015**, *6* (3), 1889–1897 DOI: 10.18632/oncotarget.2796.

15 Harris, K. S.; Foster, B. M.; Shi, L.; Mobley, M. E.; Elliot, P.; Kerr, B. A. CTC Marker CD117/c-kit Represents a Prostate Cancer Stem-Like Subpopulation Driving Progression, Migration, and TKI Resistance. *bioRxiv* **2018** DOI: 10.1101/256107.

16 Wiesner, C.; Nabha, S. M.; Dos Santos, E. B.; Yamamoto, H.; Meng, H.; Melchior, S. W.; Bittinger, F.; Thüroff, J. W.; Vessella, R. L.; Cher, M. L.; et al. C-kit and its ligand stem cell factor: potential contribution to prostate cancer bone metastasis. *Neoplasia* **2008**, *10* (9), 996–1003 DOI: 10.1593/neo.08618.

17 Landuzzi, L.; De Giovanni, C.; Nicoletti, G.; Rossi, I.; Ricci, C.; Astolfi, A.; Scopece, L.; Scotlandi, K.; Serra, M.; Bagnara, G. P.; et al. The metastatic ability of Ewing's sarcoma cells is modulated by stem cell factor and by its receptor c-kit. *Am. J. Pathol.* **2000**, *157* (6), 2123–2131 DOI: 10.1016/S0002-9440(10)64850-X.

18 Furitsu, T.; Tsujimura, T.; Tono, T.; Ikeda, H.; Kitayama, H.; Koshimizu, U.; Sugahara, H.; Butterfield, J. H.; Ashman, L. K.; Kanayama, Y.; et al. Identification of mutations in the coding sequence of the proto-oncogene c-kit in a human mast cell leukemia cell line causing ligand-independent activation of c-kit product. *J. Clin. Invest.* **1993**, *92* (4), 1736–1744 DOI: 10.1172/JCI116761.

19 Kitamura, Y.; Hirota, S. Kit as a human oncogenic tyrosine kinase. *Cell. Mol. Life Sci.* **2004**, *61* (23), 2924–2931 DOI: 10.1007/s00018-004-4273-y.

20 Ashman, L. K. The biology of stem cell factor and its receptor C-kit. *Int.J.Biochem.Cell Biol.* **1999**, *31* (1357–2725), 1037–1051.

21 Lammie, A.; Drobnjak, M.; Gerald, W.; Saad, A.; Cote, R.; Cordon-Cardo, C. Expression of c-kit and kit ligand proteins in normal human tissues. *J. Histochem. Cytochem.* **1994**, *42* (11), 1417–1425 DOI: 10.1177/42.11.7523489.

22 Ke, H.; Kazi, J. U.; Zhao, H.; Sun, J. Germline mutations of KIT in gastrointestinal stromal tumor (GIST) and mastocytosis. *Cell Biosci.* **2016**, *6* (1) DOI: 10.1186/s13578-016-0120-8.

23 Agarwal, S.; Kazi, J. U.; Rönnstrand, L. Phosphorylation of the activation loop tyrosine 823 in c-Kit is crucial for cell survival and proliferation. *J. Biol. Chem.* **2013**, *288* (31), 22460–22468 DOI: 10.1074/jbc.M113.474072.

24 Stankov, K.; Popovic, S.; Mikov, M. C-KIT signaling in cancer treatment. *Curr. Pharm. Des.* **2014**, *20* (17), 2849–2880 DOI: 10.2174/13816128113199990593.

25 Wheeler, D. L.; Yarden, Y. *Receptor tyrosine kinases: Family and subfamilies*; 2015.

26 Wheeler, D. L.; Yarden, Y. *Receptor tyrosine kinases: Structure, functions and role in*

human disease; 2015.

27 Voytyuk, O.; Lennartsson, J.; Mogi, A.; Caruana, G.; Courtneidge, S.; Ashman, L. K.; Rö, L. Src Family Kinases Are Involved in the Differential Signaling from Two Splice Forms of c-Kit*. **2003** DOI: 10.1074/jbc.M211726200.

28 Chan, E. C.; Bai, Y.; Bandara, G.; Simakova, O.; Brittain, E.; Scott, L.; Dyer, K. D.; Klion, A. D.; Maric, I.; Gilfillan, A. M.; et al. KIT GNNK splice variants: Expression in systemic mastocytosis and influence on the activating potential of the D816V mutation in mast cells. DOI: 10.1016/j.exphem.2013.05.005.

29 Caruana, G.; Cambareri, A. C.; Ashman, L. K. Isoforms of c-KIT differ in activation of signalling pathways and transformation of NIH3T3 Fibroblasts.

30 Sakuma, Y.; Sakurai, S.; Oguni, S.; Hironaka, M.; Saito, K. Alterations of the c-kit gene in testicular germ cell tumors. *Cancer Sci.* **2003**, *94* (6), 486–491 DOI: 10.1111/j.1349-7006.2003.tb01470.x.

31 Crosier, P. S.; Ricciardi, S. T.; Hall, L. R.; Vitas, M. R.; Clark, S. C.; Crosier, K. E. Expression of Isoforms of the Human Receptor Tyrosine Kinase c-kit in Leukemic Cell Lines and Acute Myeloid Leukemia.

32 Longley, B. J.; Reguera, M. J.; Ma, Y. Classes of c-KIT activating mutations: proposed mechanisms of action and implications for disease classification and therapy. *Leuk. Res.* **2001**, *25* (7), 571–576 DOI: 10.1016/S0145-2126(01)00028-5.

33 Sanlorenzo, M.; Vujic, I.; Posch, C.; Ma, J.; Lin, K.; Lai, K.; Oses-Prieto, J. A.; Chand, S.; Rodriguez-Peralto, J. L.; Burlingame, A.; et al. Oncogenic KIT mutations in different exons lead to specific changes in melanocyte phospho-proteome HHS Public Access. *J Proteomics* **2016**, *144*, 140–147 DOI: 10.1016/j.jprot.2016.05.019.

34 Cardoso, H. J.; Figueira, M. I.; Socorro, S. The stem cell factor (SCF)/c-KIT signalling in testis and prostate cancer. *J. Cell Commun. Signal.* **2017**, 1–11 DOI: 10.1007/s12079-017-0399-1.

35 Miettinen, M.; Lasota, J. KIT (CD117): a review on expression in normal and neoplastic tissues, and mutations and their clinicopathologic correlation. *Appl. Immunohistochem. Mol. Morphol.* **2005**, *13* (3), 205–220 DOI: 10.1097/01.pai.0000173054.83414.22.

36 Stem Cell Basics I. | stemcells.nih.gov.

37 Burns, C. E.; Zon, L. I. Portrait of a stem cell. *Dev. Cell* **2002**, *3* (5), 612–613.

38 Durand, C.; Charbord, P. *Stem Cell Biology and Regenerative Medicine*; River Publishers, 2015.

39 Lin, H.; Schagat, T. Neuroblasts: a model for the asymmetric division of stem cells. *Trends Genet.* **1997**, *13* (1), 33–39.

40 Petritsch, C.; Shen, X. Asymmetric Division of Cancer Stem Cells. In *Cancer Stem Cells*; 2016; pp 285–315.

41 Loeffler, D.; Schroeder, T. Asymmetric cell division of hematopoietic stem cells. *Exp. Hematol.* **2015**, *43* (9), S77.

42 Leong, K. G.; Wang, B. E.; Johnson, L.; Gao, W. Q. Generation of a prostate from a single adult stem cell. *Nature* **2008**, *456* (7223), 804–808 DOI: nature07427 [pii] 10.1038/nature07427.

43 Ashman, B. L. K.; Cambareri, A. C.; To, L. B.; Levinsky, R. J.; Juttner, C. A. Expression of the YB5.BS Antigen. **2017**, *78* (1), 30–37.

44 Acar, M.; Kocherlakota, K. S.; Murphy, M. M.; Peyer, J. G.; Oguro, H.; Inra, C. N.; Jaiyeola, C.; Zhao, Z.; Luby-Phelps, K.; Morrison, S. J. Deep imaging of bone marrow shows non-dividing stem cells are mainly perisinusoidal. *Nature* **2015**, *526* (7571), 126–130 DOI: 10.1038/nature15250.

45 Ema, H.; Takano, H.; Sudo, K.; Nakauchi, H. In vitro self-renewal division of hematopoietic stem cells. *J. Exp. Med.* **2000**, *192* (9), 1281–1288 DOI: 10.1084/jem.192.9.1281.

46 Matsui, J.; Wakabayashi, T.; Asada, M.; Yoshimatsu, K.; Okada, M. Stem Cell Factor/c-kit

Signaling Promotes the Survival, Migration, and Capillary Tube Formation of Human Umbilical Vein Endothelial Cells. *J. Biol. Chem.* **2004**, *279* (18), 18600–18607 DOI: 10.1074/jbc.M311643200.

47 Linnekin, D. Early signaling pathways activated by c-Kit in hematopoietic cells. *Int. J. Biochem. Cell Biol.* **1999**, *31* (10), 1053–1074 DOI: 10.1016/S1357-2725(99)00078-3.

48 Kimura, Y.; Ding, B.; Imai, N.; Nolan, D. J.; Butler, J. M.; Rafii, S. c-Kit-mediated functional positioning of stem cells to their niches is essential for maintenance and regeneration of adult hematopoiesis. *PLoS One* **2011**, *6* (10), e26918 DOI: 10.1371/journal.pone.0026918.

49 Reber, L.; Da Silva, C. A.; Frossard, N. Stem cell factor and its receptor c-Kit as targets for inflammatory diseases. *Eur J Pharmacol* **2006**, *533* (1–3), 327–340 DOI: S0014-2999(05)01400-7 [pii] 10.1016/j.ejphar.2005.12.067.

50 Miyazawa, K.; Williams, D. a; Gotoh, A.; Nishimaki, J.; Broxmeyer, H. E.; Toyama, K. Membrane-bound Steel factor induces more persistent tyrosine kinase activation and longer life span of c-kit gene-encoded protein than its soluble form. *Blood* **1995**, *85* (3), 641–649.

51 Longley, B. J.; Tyrrell, L.; Ma, Y.; Williams, D. A.; Halaban, R.; Langley, K.; Lu, H. S.; Schechter, N. M. Chymase cleavage of stem cell factor yields a bioactive, soluble product. *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94* (17), 9017–9021.

52 Clarke, K.; Basserb, R. L. The Role of Platelet Growth Factors in Cancer Therapy. **1996**, *14*, 274–280.

53 Liang, J.; Wu, Y. L.; Chen, B. J.; Zhang, W.; Tanaka, Y.; Sugiyama, H. The C-Kit receptor-mediated signal transduction and tumor-related diseases. *Int. J. Biol. Sci.* **2013**, *9* (5), 435–443 DOI: 10.7150/ijbs.6087.

54 Burck, E.; Santos, D.; Yamamoto, H.; Bittinger, F.; Thüroff, J. W.; Vessella, R. L.; Cher, M. L. C-Kit and Its Ligand Stem Cell Factor : Potential Contribution to Prostate Cancer

Bone Metastasis 1. *Neoplasia* **2008**, *10* (9), 996–1003 DOI: 10.1593/neo.08618.

55 Sasaki, T.; Mizuochi, C.; Horio, Y.; Nakao, K.; Akashi, K.; Sugiyama, D. Regulation of hematopoietic cell clusters in the placental niche through SCF/Kit signaling in embryonic mouse. *Development* **2010**, *137* (23).

56 Ding, L.; Saunders, T. L.; Enikolopov, G.; Morrison, S. J. Endothelial and perivascular cells maintain haematopoietic stem cells. *Nature* **2012**, *481* (7382), 457–462 DOI: 10.1038/nature10783.

57 Broudy, V. C. Stem cell factor and hematopoiesis. *Blood* **1997**, *90* (4), 1345–1364.

58 Calvi, L. M.; Link, D. C. The hematopoietic stem cell niche in homeostasis and disease. *Blood* **2015**, *126* (22), 2443–2451 DOI: 10.1182/blood-2015-07-533588.

59 Asada, N.; Kunisaki, Y.; Pierce, H.; Wang, Z.; Fernandez, N. F.; Birbrair, A.; Ma'ayan, A.; Frenette, P. S. Differential cytokine contributions of perivascular haematopoietic stem cell niches. *Nat. Cell Biol.* **2017**, *19* (3), 214–223 DOI: 10.1038/ncb3475.

60 Kacena, M. A.; Gundberg, C. M.; Horowitz, M. C. A reciprocal regulatory interaction between megakaryocytes, bone cells, and hematopoietic stem cells. *Bone* **2006**, *39* (5), 978–984 DOI: 10.1016/j.bone.2006.05.019.

61 Inra, C. N.; Zhou, B. O.; Acar, M.; Murphy, M. M.; Richardson, J.; Zhao, Z.; Morrison, S. J. A perisinusoidal niche for extramedullary haematopoiesis in the spleen. *Nature* **2015**, *527* (7579), 466–471 DOI: 10.1038/nature15530.

62 Kollet, O.; Shivtiel, S.; Chen, Y.-Q.; Suriawinata, J.; Thung, S. N.; Dabeva, M. D.; Kahn, J.; Spiegel, A.; Dar, A.; Samira, S.; et al. HGF, SDF-1, and MMP-9 are involved in stress-induced human CD34+ stem cell recruitment to the liver. *J. Clin. Invest.* **2003**, *112* (2), 160–169 DOI: 10.1172/JCI17902.

63 Inra, C. N.; Zhou, B. O.; Acar, M.; Murphy, M. M.; Richardson, J.; Zhao, Z.; Morrison, S. J. A perisinusoidal niche for extramedullary haematopoiesis in the spleen. *Nature* **2015**, *527* (7579), 466–471 DOI: 10.1038/nature15530.

64 Blume-Jensen, P.; Claesson-Welsh, L.; Siegbahn, A.; Zsebo, K. M.; Westermark, B.; Heldin, C. H. Activation of the human c-kit product by ligand-induced dimerization mediates circular actin reorganization and chemotaxis. *EMBO J* **1991**, *10* (13), 4121–4128.

65 Blechman, J. M.; Lev, S.; Givol, D.; Yarden, Y. Structure-function analyses of the kit receptor for the steel factor. *Stem Cells* **1993**, *11 Suppl 2*, 12–21.

66 Hsu, Y.; Wu, G.; Mendiaz, E. a; Syed, R.; Wypych, J.; Toso, R.; Mann, M. B.; Boone, T. C.; Narhi, L. O.; Lu, H. S.; et al. The Majority of Stem Cell Factor Exists as Monomer under Physiological Conditions. *J. Biol. Chem.* **1997**, *272* (10), 6406–6415.

67 Weiler, S. R.; Mou, S.; Deberry, C. S.; Keller, J. R.; Ruscetti, F. W.; Ferris, D. K.; Longo, D. L.; Linnekin, D. JAK2 Is Associated With the c-kit Proto-oncogene Product and Is Phosphorylated in Response to Stem Cell Factor.

68 MOUb, S.; Deberry, C. S.; Weiler, S. R.; KELLERb, J. R.; Rusce, F. W.; Longo, D. L. Stem Cell Factor, the JAK-STAT Pathway and Signal Transduction. *27*, 439–444.

69 Thömmes, K.; Lennartsson, J.; Carlberg, M.; Rönnstrand, L. Identification of Tyr-703 and Tyr-936 as the primary association sites for Grb2 and Grb7 in the c-Kit/stem cell factor receptor. *Biochem. J* **1999**, *341 (Pt 1*, 211–216.

70 Duronio, V.; Welham, M. J.; Abraham, S.; Dryden, P.; Schrader, J. W. p21ras activation via hemopoietin receptors and c-kit requires tyrosine kinase activity but not tyrosine phosphorylation of p21ras {GTPase-activating} protein. *Proc. Natl. Acad. Sci.* **1992**, *89* (5), 1587–1591.

71 Yasuda, T.; Kurosaki, T. Regulation of lymphocyte fate by Ras/ERK signals. *Cell Cycle* **2008**, *7* (23), 3634–3640 DOI: 10.4161/cc.7.23.7103.

72 Kuang, D.; Zhao, X.; Xiao, G.; Ni, J.; Feng, Y.; Wu, R.; Wang, G. Stem cell factor/c-kit signaling mediated cardiac stem cell migration via activation of p38 MAPK. *Basic Res. Cardiol.* **2008**, *103* (3), 265–273 DOI: 10.1007/s00395-007-0690-z.

73 Sun, J.; Pedersen, M.; Rönnstrand, L. Gab2 is involved in differential phosphoinositide 3-kinase signaling by two splice forms of c-Kit. *J. Biol. Chem.* **2008**, *283* (41), 27444–27451 DOI: 10.1074/jbc.M709703200.

74 Ueda, S.; Mizuki, M.; Ikeda, H.; Tsujimura, T.; Matsumura, I.; Nakano, K.; Daino, H.; Honda, Z.-I.; Sonoyama, J.; Shibayama, H.; et al. Critical roles of c-Kit tyrosine residues 567 and 719 in stem cell factor–induced chemotaxis: contribution of src family kinase and PI3-kinase on calcium mobilization and cell migration.

75 Linnekin, D.; DeBerry, C. S.; Mou, S. Lyn associates with the juxtamembrane region of c-Kit and is activated by stem cell factor in hematopoietic cell lines and normal progenitor cells. *J. Biol. Chem.* **1997**, *272* (43), 27450–27455 DOI: 10.1074/JBC.272.43.27450.

76 Saleem, M.; Babaei, A.; Press, D. Receptor tyrosine kinase (c-Kit) inhibitors : a potential therapeutic target in cancer cells. **2016**, 2443–2459 DOI: 10.2147/DDDT.S89114.

77 Shivakrupa, $ R; Linnekin, D. Lyn contributes to regulation of multiple Kit-dependent signaling pathways in murine bone marrow mast cells. **2004** DOI: 10.1016/j.cellsig.2004.06.004.

78 Sette, C.; Paronetto, M. P.; Barchi, M.; Bevilacqua, A.; Geremia, R.; Rossi, P. Tr-kit-induced resumption of the cell cycle in mouse eggs requires activation of a Src-like kinase. *EMBO J.* **2002**, *21* (20), 5386–5395 DOI: 10.1093/EMBOJ/CDF553.

79 Phane Maddens, S.; Charruyer, A.; Plo, I.; Dubreuil, P.; Berger, S.; Salles, B.; Laurent, G.; Jaffré, J.-P. Kit signaling inhibits the sphingomyelin-ceramide pathway through PLC 1: implication in stem cell factor radioprotective effect.

80 Lennartsson, J.; Wernstedt, C.; Engström, U.; Hellman, U.; Rönnstrand, L. Identification of Tyr900 in the kinase domain of c-Kit as a Src-dependent phosphorylation site mediating interaction with c-Crk. DOI: 10.1016/S0014-4827(03)00206-4.

81 Gommerman, J. L.; Sittaro, D.; Klebasz, N. Z.; Williams, D. A.; Berger, S. A. Differential stimulation of c-Kit mutants by membrane-bound and soluble Steel Factor correlates with

leukemic potential.

82 Lennartsson, J.; Jelacic, T.; Linnekin, D.; Shivakrupa, R. Normal and Oncogenic Forms of the Receptor Tyrosine Kinase Kit. *Stem Cells* **2005**, *23* (1), 16–43 DOI: 10.1634/stemcells.2004-0117.

83 Longley, B. J.; Reguera, M. J.; Ma, Y. Classes of c-KIT activating mutations: proposed mechanisms of action and implications for disease classification and therapy. *Leuk Res* **2001**, *25* (7), 571–576 DOI: S0145-2126(01)00028-5 [pii].

84 Tay, C. M.; Ong, C. W.; Lee, V. K. M.; Pang, B. {KIT} gene mutation analysis in solid tumours: biology, clincial applications and trends in diagnostic reporting. *Pathology* **2013**, *45* (2), 127–137.

85 Medinger, M.; Kleinschmidt, M.; Mross, K.; Wehmeyer, B.; Unger, C.; Schaefer, H.-E. E.; Weber, R.; Azemar, M. c-kit (CD117) expression in human tumors and its prognostic value: an immunohistochemical analysis. *Pathol. Oncol. Res.* **2010**, *16* (3), 295–301 DOI: 10.1007/s12253-010-9247-9.

86 Medinger, M.; Kleinschmidt, M.; Mross, K.; Wehmeyer, B.; Unger, C.; Schaefer, H. E.; Weber, R.; Azemar, M. c-kit (CD117) expression in human tumors and its prognostic value: an immunohistochemical analysis. *Pathol Oncol Res* **2010**, *16* (3), 295–301 DOI: 10.1007/s12253-010-9247-9.

87 Cerami, E.; Gao, J.; Dogrusoz, U.; Gross, B. E.; Sumer, S. O.; Aksoy, B. A.; Jacobsen, A.; Byrne, C. J.; Heuer, M. L.; Larsson, E.; et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* **2012**, *2* (5), 401–404 DOI: 10.1158/2159-8290.CD-12-0095.

88 Gao, J.; Aksoy, B. A.; Dogrusoz, U.; Dresdner, G.; Gross, B.; Sumer, S. O.; Sun, Y.; Jacobsen, A.; Sinha, R.; Larsson, E.; et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci. Signal.* **2013**, *6* (269), pl1 DOI: 10.1126/scisignal.2004088.

89 Hou, Y.-Y.; Grabellus, F.; Weber, F.; Zhou, Y.; Tan, Y.-S.; Li, J.; Shen, K.-T.; Qin, J.; Sun, Y.-H.; Qin, X.-Y.; et al. Impact of {KIT} and {PDGFRA} gene mutations on prognosis of patients with gastrointestinal stromal tumors after complete primary tumor resection. *J. Gastrointest. Surg.* **2009**, *13* (9), 1583–1592.

90 Andersson, J.; Bümming, P.; Meis-Kindblom, J. M.; Sihto, H.; Nupponen, N.; Joensuu, H.; Odén, A.; Gustavsson, B.; Kindblom, L.-G.; Nilsson, B. Gastrointestinal stromal tumors with {KIT} exon 11 deletions are associated with poor prognosis. *Gastroenterology* **2006**, *130* (6), 1573–1581.

91 Wozniak, A.; Rutkowski, P.; Piskorz, A.; Ciwoniuk, M.; Osuch, C.; Bylina, E.; Sygut, J.; Chosia, M.; Rys, J.; Urbanczyk, K.; et al. Prognostic value of {KIT/PDGFRA} mutations in gastrointestinal stromal tumours ({GIST)}: Polish Clinical {GIST} Registry experience. *Ann. Oncol.* **2012**, *23* (2), 353–360.

92 Søreide, K.; Sandvik, O. M.; Søreide, J. A.; Gudlaugsson, E.; Mangseth, K.; Haugland, H. K. Tyrosine-kinase mutations in {c-KIT} and {PDGFR-alpha} genes of imatinib na{"\i}ve adult patients with gastrointestinal stromal tumours ({GISTs}) of the stomach and small intestine: relation to tumour-biological risk-profile and long-term outcome. *Clin. Transl. Oncol.* **2012**, *14* (8), 619–629.

93 Kosemehmetoglu, K.; Kaygusuz, G.; Fritchie, K.; Aydin, O.; Yapicier, O.; Coskun, O.; Karatayli, E.; Boyacigil, S.; Guler, G.; Dervisoglu, S.; et al. Clinical and pathological characteristics of gastrointestinal stromal tumor (GIST) metastatic to bone. *Virchows Arch.* **2017** DOI: 10.1007/s00428-017-2138-7.

94 Penzel, R.; Aulmann, S.; Moock, M.; Schwarzbach, M.; Rieker, R. J.; Mechtersheimer, G. The location of {KIT} and {PDGFRA} gene mutations in gastrointestinal stromal tumours is site and phenotype associated. *J. Clin. Pathol.* **2005**, *58* (6), 634–639.

95 Burger, K.; den Bakker, M. A.; Kros, J. M.; de Bruin, A. M.; Oosterhuis, W.; van den Ingh, H. F. G. M.; van der Harst, E.; de Schipper, H. P.; Wiemer, E. A. C.; Nooter, K. Activating

mutations in {c-KIT} and {PDGFR$α$} are exclusively found in gastrointestinal stromal tumors and not in other tumors overexpressing these imatinib mesylate target genes. *Cancer Biol. Ther.* **2005**, *4* (11), 1270–1274.

96 Stemberger-Papić, S.; Vrdoljak-Mozetic, D.; Ostojić, D. V.; Rubesa-Mihaljević, R.; Krigtofić, I.; Brncić-Fisher, A.; Kragević, M.; Eminović, S. Expression of {CD133} and {CD117} in 64 Serous Ovarian Cancer Cases. *Coll. Antropol.* **2015**, *39* (3), 745–753.

97 Burgos-Ojeda, D.; Rueda, B. R.; Buckanovich, R. J. Ovarian cancer stem cell markers: prognostic and therapeutic implications. *Cancer Lett.* **2012**, *322* (1), 1–7.

98 Luo, L.; Zeng, J.; Liang, B.; Zhao, Z.; Sun, L.; Cao, D.; Yang, J.; Shen, K. Ovarian cancer cells with the {CD117} phenotype are highly tumorigenic and are related to chemotherapy outcome. *Exp. Mol. Pathol.* **2011**, *91* (2), 596–602.

99 Foroozan, M.; Roudi, R.; Abolhasani, M.; Gheytanchi, E.; Mehrazma, M. Clinical significance of endothelial cell marker CD34 and mast cell marker CD117 in prostate adenocarcinoma. *Pathol. - Res. Pract.* **2017** DOI: 10.1016/j.prp.2017.04.027.

100 Wiesner, C.; Nabha, S. M.; Dos Santos, E. B.; Yamamoto, H.; Meng, H.; Melchior, S. W.; Bittinger, F.; Thüroff, J. W.; Vessella, R. L.; Cher, M. L.; et al. C-kit and its ligand stem cell factor: potential contribution to prostate cancer bone metastasis. *Neoplasia* **2008**, *10* (9), 996–1003.

101 Mainetti, L. E.; Zhe, X.; Diedrich, J.; Saliganan, A. D.; Cho, W. J.; Cher, M. L.; Heath, E.; Fridman, R.; Kim, H.-R. C.; Bonfil, R. D. Bone-induced c-kit expression in prostate cancer: a driver of intraosseous tumor growth. *Int. J. cancer* **2015**, *136* (1), 11–20 DOI: 10.1002/ijc.28948.

102 Atala, A. Re: {Bone-Induced} c-Kit Expression in Prostate Cancer: A Driver of Intraosseous Tumor Growth. *J. Urol.* **2015**, *194* (1), 260.

103 Hines, S. J.; Organ, C.; Kornstein, M. J.; Krystal, G. W. Coexpression of the c-kit and stem cell factor genes in breast carcinomas. *Cell Growth Differ.* **1995**, *6* (6), 769–779.

104 Krystal, G. W.; Hines, S. J.; Organ, C. P. Autocrine growth of small cell lung cancer mediated by coexpression of c-kit and stem cell factor. *Cancer Res.* **1996**, *56* (2), 370–376.

105 Schmidt-Hieber, M.; Perez-Andres, M.; Paiva, B.; Flores-Montero, J.; Perez, J. J.; Gutierrez, N. C.; Vidriales, M.-B.; Matarraz, S.; San Miguel, J. F.; Orfao, A. {CD117} expression in gammopathies is associated with an altered maturation of the myeloid and lymphoid hematopoietic cell compartments and favorable disease features. *Haematologica* **2010**, *96* (2), 328–332.

106 Bataille, R.; Pellat-Deceunynck, C.; Robillard, N.; Avet-Loiseau, H.; Harousseau, J.-L.; Moreau, P. {CD117} (c-kit) is aberrantly expressed in a subset of {MGUS} and multiple myeloma with unexpectedly good prognosis. *Leuk. Res.* **2008**, *32* (3), 379–382.

107 Pan, Y.; Wang, H.; Tao, Q.; Zhang, C.; Yang, D.; Qin, H.; Xiong, S.; Tao, L.; Wu, F.; Zhang, J.; et al. Absence of both {CD56} and {CD117} expression on malignant plasma cells is related with a poor prognosis in patients with newly diagnosed multiple myeloma. *Leuk. Res.* **2016**, *40*, 77–82.

108 Zhao, F.; Chen, Y.; Wu, Q.; Wang, Z.; Lu, J. Prognostic value of {CD117} in cancer: a meta-analysis. *Int. J. Clin. Exp. Pathol.* **2014**, *7* (3), 1012–1021.

109 Kuonen, F.; Laurent, J.; Secondini, C.; Lorusso, G.; Stehle, J.-C.; Rausch, T.; Faes-Van't Hull, E.; Bieler, G.; Alghisi, G.-C.; Schwendener, R.; et al. Inhibition of the Kit ligand/c-Kit axis attenuates metastasis in a mouse model mimicking local breast cancer relapse after radiotherapy. *Clin. Cancer Res.* **2012**, *18* (16), 4365–4374 DOI: 10.1158/1078-0432.CCR-11-3028.

110 Sakabe, T.; Azumi, J.; Haruki, T.; Umekita, Y.; Nakamura, H.; Shiota, G. {CD117} expression is a predictive marker for poor prognosis in patients with non-small cell lung cancer. *Oncol. Lett.* **2017**, *13* (5), 3703–3708.

111 Foster, R.; Buckanovich, R. J.; Rueda, B. R. Ovarian cancer stem cells: working towards

the root of stemness. *Cancer Lett.* **2013**, *338* (1), 147–157.

112 Ray, P.; Krishnamoorthy, N.; Oriss, T. B.; Ray, A. Signaling of c-kit in dendritic cells influences adaptive immunity. *Ann. N. Y. Acad. Sci.* **2010**, *1183* (1), 104–122 DOI: 10.1111/j.1749-6632.2009.05122.x.

113 Vanegas, N.-D. P.; Vernot, J.-P. Loss of quiescence and self-renewal capacity of hematopoietic stem cell in an in vitro leukemic niche. *Exp. Hematol. Oncol.* **2017**, *6* (1), 2 DOI: 10.1186/s40164-016-0062-1.

114 Li, X.-Y.; Jiang, L.-J.; Chen, L.; Ding, M.-L.; Guo, H.-Z.; Zhang, W.; Zhang, H.-X.; Ma, X.-D.; Liu, X.-Z.; Xi, X.-D.; et al. {RIG-I} modulates Src-mediated {AKT} activation to restrain leukemic stemness. *Mol. Cell* **2014**, *53* (3), 407–419.

115 {RIG-I} inhibits {SRC-mediated} {AKT/mTOR} signaling and stemness in {AML}. *Cancer Discov.* **2014**, *4* (3), OF19.

116 Jiang, T.; Qiu, Y. Interaction between Src and a C-terminal Proline-rich Motif of Akt Is Required for Akt Activation. *J. Biol. Chem.* **2003**, *278* (18), 15789–15793.

117 Tabu, K.; Kimura, T.; Sasai, K.; Wang, L.; Bizen, N.; Nishihara, H.; Taga, T.; Tanaka, S. Analysis of an alternative human {CD133} promoter reveals the implication of {Ras/ERK} pathway in tumor stem-like hallmarks. *Mol. Cancer* **2010**, *9*, 39.

118 Ivanovic, Z.; Vlaski-Lafarge, M. Evolutionary Origins of Stemness. In *Anaerobiosis and Stemness*; 2016; pp 177–209.

119 Cai, J.; Weiss, M. L.; Rao, M. S. In search of ``stemness''. *Exp. Hematol.* **2004**, *32* (7), 585–598.

120 Iqbal, N.; Iqbal, N. Imatinib: a breakthrough of targeted therapy in cancer. *Chemother. Res. Pr.* **2014**, *2014*, 357027.

121 Al-Hadiya, B. M. H.; Bakheit, A. H. H.; Abd-Elgalil, A. A. Imatinib mesylate. *Profiles Drug Subst. Excip. Relat. Methodol.* **2014**, *39*, 265–297.

122 Joensuu, H.; Roberts, P. J.; Sarlomo-Rikala, M.; Andersson, L. C.; Tervahartiala, P.;

Tuveson, D.; Silberman, S. L.; Capdeville, R.; Dimitrijevic, S.; Druker, B.; et al. Effect of the Tyrosine Kinase Inhibitor {STI571} in a Patient with a Metastatic Gastrointestinal Stromal Tumor. *N. Engl. J. Med.* **2001**, *344* (14), 1052–1056.

123 van Oosterom, A. T.; Judson, I.; Verweij, J.; Stroobants, S.; di Paola, E.; Dimitrijevic, S.; Martens, M.; Webb, A.; Sciot, R.; Van Glabbeke, M.; et al. Safety and efficacy of imatinib ({STI571}) in metastatic gastrointestinal stromal tumours: a phase {I} study. *Lancet* **2001**, *358* (9291), 1421–1423.

124 Tuveson, D. A.; Willis, N. A.; Jacks, T.; Griffin, J. D.; Singer, S.; Fletcher, C. D.; Fletcher, J. A.; Demetri, G. D. {STI571} inactivation of the gastrointestinal stromal tumor {c-KIT} oncoprotein: biological and clinical implications. *Oncogene* **2001**, *20* (36), 5054–5058.

125 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.; et al. Imatinib potentiates antitumor {T} cell responses in gastrointestinal stromal tumor through the inhibition of Ido. *Nat. Med.* **2011**, *17* (9), 1094–1100.

126 Seifert, A. M.; Zeng, S.; Zhang, J. Q.; Kim, T. S.; Cohen, N. A.; Beckman, M. J.; Medina, B. D.; Maltbaek, J. H.; Loo, J. K.; Crawley, M. H.; et al. {PD-1/PD-L1} Blockade Enhances T-cell Activity and Antitumor Efficacy of Imatinib in Gastrointestinal Stromal Tumors. *Clin. Cancer Res.* **2017**, *23* (2), 454–465.

127 Seifert, A. M.; Kim, T. S.; Greer, J. B.; Cohen, N. A.; Beckman, M. J.; Santamaria-Barria, J. A.; Zeng, S.; Crawley, M. H.; Green, B. L.; DeMatteo, R. P. {PD-1/PD-L1} Blockade Enhances the Efficacy of Imatinib in Gastrointestinal Stromal Tumor ({GIST}). *J. Am. Coll. Surg.* **2014**, *219* (3), S129.

128 Edris, B.; Willingham, S. B.; Weiskopf, K.; Volkmer, A. K.; Volkmer, J.-P.; Mühlenberg, T.; Montgomery, K. D.; Contreras-Trujillo, H.; Czechowicz, A.; Fletcher, J. A.; et al. {Anti-KIT} monoclonal antibody inhibits imatinib-resistant gastrointestinal stromal tumor growth. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110* (9), 3501–3506.

129 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.; et al. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. *Lancet* **2006**, *368* (9544), 1329–1338.

130 Demetri, G. D.; Reichardt, P.; Kang, Y.-K.; Blay, J.-Y.; Rutkowski, P.; Gelderblom, H.; Hohenberger, P.; Leahy, M.; von Mehren, M.; Joensuu, H.; et al. 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* **2013**, *381* (9863), 295–302.

131 Parikh, P. M.; Gupta, S. Management of gastrointestinal stromal tumor: the imatinib era and beyond. *Indian J. Cancer* **2013**, *50* (1), 31–40.

132 Demetri, G. D.; Heinrich, M. C.; Fletcher, J. A.; Fletcher, C. D. M.; den Abbeele, A. D.; Corless, C. L.; Antonescu, C. R.; George, S.; Morgan, J. A.; Chen, M. H.; et al. Molecular target modulation, imaging, and clinical evaluation of gastrointestinal stromal tumor patients treated with sunitinib malate after imatinib failure. *Clin. Cancer Res.* **2009**, *15* (18), 5902–5909.

133 Calipel, A.; Landreville, S.; De La Fouchardière, A.; Mascarelli, F.; Rivoire, M.; Penel, N.; Mouriaux, F. Mechanisms of resistance to imatinib mesylate in KIT-positive metastatic uveal melanoma. *Clin. Exp. Metastasis* **2014**, *31* (5), 553–564 DOI: 10.1007/s10585-014-9649-2.

134 Cavnar, M. J.; Zeng, S.; Kim, T. S.; Sorenson, E. C.; Ocuin, L. M.; Balachandran, V. P.; Seifert, A. M.; Greer, J. B.; Popow, R.; Crawley, M. H.; et al. KIT oncogene inhibition drives intratumoral macrophage M2 polarization. *J. Exp. Med.* **2013**, *210* (13), 2873–2886 DOI: 10.1084/jem.20130875.

135 Gajiwala, K. S.; Wu, J. C.; Christensen, J.; Deshmukh, G. D.; Diehl, W.; DiNitto, J. P.; English, J. M.; Greig, M. J.; He, Y.-A.; Jacques, S. L.; et al. KIT kinase mutants show

unique mechanisms of drug resistance to imatinib and sunitinib in gastrointestinal stromal tumor patients. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106* (5), 1542–1547 DOI: 10.1073/pnas.0812413106.

136 Saglio, G.; Kim, D.-W.; Issaragrisil, S.; le Coutre, P.; Etienne, G.; Lobo, C.; Pasquini, R.; Clark, R. E.; Hochhaus, A.; Hughes, T. P.; et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. *N. Engl. J. Med.* **2010**, *362* (24), 2251–2259.

137 Kantarjian, H. M.; Hochhaus, A.; Saglio, G.; De Souza, C.; Flinn, I. W.; Stenke, L.; Goh, Y.-T.; Rosti, G.; Nakamae, H.; Gallagher, N. J.; et al. Nilotinib versus imatinib for the treatment of patients with newly diagnosed chronic phase, Philadelphia chromosome-positive, chronic myeloid leukaemia: 24-month minimum follow-up of the phase 3 randomised {ENESTnd} trial. *Lancet Oncol.* **2011**, *12* (9), 841–851.

138 Reichardt, P.; Blay, J.-Y.; Gelderblom, H.; Schlemmer, M.; Demetri, G. D.; Bui-Nguyen, B.; McArthur, G. A.; Yazji, S.; Hsu, Y.; Galetic, I.; et al. Phase {III} study of nilotinib versus best supportive care with or without a {TKI} in patients with gastrointestinal stromal tumors resistant to or intolerant of imatinib and sunitinib. *Ann. Oncol.* **2012**, *23* (7), 1680–1687.

139 Blay, J.-Y.; Shen, L.; Kang, Y.-K.; Rutkowski, P.; Qin, S.; Nosov, D.; Wan, D.; Trent, J.; Srimuninnimit, V.; Pápai, Z.; et al. Nilotinib versus imatinib as first-line therapy for patients with unresectable or metastatic gastrointestinal stromal tumours ({ENESTg1)}: a randomised phase 3 trial. *Lancet Oncol.* **2015**, *16* (5), 550–560.

140 Kanda, T.; Ishikawa, T.; Takahashi, T.; Nishida, T. Nilotinib for treatment of gastrointestinal stromal tumors: out of the equation? *Expert Opin. Pharmacother.* **2013**, *14* (13), 1859–1867.

141 Koon, H. B.; Krown, S. E.; Lee, J. Y.; Honda, K.; Rapisuwon, S.; Wang, Z.; Aboulafia, D.; Reid, E. G.; Rudek, M. A.; Dezube, B. J.; et al. Phase {II} trial of imatinib in {AIDS-associated} Kaposi's sarcoma: {AIDS} Malignancy Consortium Protocol 042. *J. Clin.*

Oncol. **2014**, *32* (5), 402–408.

142 Koon, H. B.; Bubley, G. J.; Pantanowitz, L.; Masiello, D.; Smith, B.; Crosby, K.; Proper, J.; Weeden, W.; Miller, T. E.; Chatis, P.; et al. Imatinib-induced regression of {AIDS-related} Kaposi's sarcoma. *J. Clin. Oncol.* **2005**, *23* (5), 982–989.

143 Scheinfeld, N.; Schienfeld, N. A comprehensive review of imatinib mesylate (Gleevec) for dermatological diseases. *J. Drugs Dermatol.* **2006**, *5* (2), 117–122.

144 Hodi, F. S.; Corless, C. L.; Giobbie-Hurder, A.; Fletcher, J. A.; Zhu, M.; Marino-Enriquez, A.; Friedlander, P.; Gonzalez, R.; Weber, J. S.; Gajewski, T. F.; et al. Imatinib for melanomas harboring mutationally activated or amplified {KIT} arising on mucosal, acral, and chronically sun-damaged skin. *J. Clin. Oncol.* **2013**, *31* (26), 3182–3190.

145 Carvajal, R. D. Another option in our {KIT} of effective therapies for advanced melanoma. *J. Clin. Oncol.* **2013**, *31* (26), 3173–3175.

146 Jackson, A. L.; Eisenhauer, E. L.; Herzog, T. J. Emerging therapies: angiogenesis inhibitors for ovarian cancer. *Expert Opin. Emerg. Drugs* **2015**, *20* (2), 331–346.

147 Leone Roberti Maggiore, U.; Valenzano Menada, M.; Venturini, P. L.; Ferrero, S. The potential of sunitinib as a therapy in ovarian cancer. *Expert Opin. Investig. Drugs* **2013**, *22* (12), 1671–1686.

148 Mahipal, A.; Tijani, L.; Chan, K.; Laudadio, M.; Mastrangelo, M. J.; Sato, T. A pilot study of sunitinib malate in patients with metastatic uveal melanoma. *Melanoma Res.* **2012**, *22* (6), 440–446.

149 Penel, N.; Le Cesne, A.; Bui, B. N.; Perol, D.; Brain, E. G.; Ray-Coquard, I.; Guillemet, C.; Chevreau, C.; Cupissol, D.; Chabaud, S.; et al. Imatinib for progressive and recurrent aggressive fibromatosis (desmoid tumors): an {FNCLCC/French} Sarcoma Group phase {II} trial with a long-term follow-up. *Ann. Oncol.* **2011**, *22* (2), 452–457.

150 Chugh, R.; Wathen, J. K.; Patel, S. R.; Maki, R. G.; Meyers, P. A.; Schuetze, S. M.; Priebat, D. A.; Thomas, D. G.; Jacobson, J. A.; Samuels, B. L.; et al. Efficacy of imatinib

in aggressive fibromatosis: Results of a phase {II} multicenter Sarcoma Alliance for Research through Collaboration ({SARC}) trial. *Clin. Cancer Res.* **2010**, *16* (19), 4884–4891.

151 Koch, C. A.; Gimm, O.; Vortmeyer, A. O.; Al-Ali, H. K.; Lamesch, P.; Ott, R.; Kluge, R.; Bierbach, U.; Tannapfel, A. Does the expression of c-kit ({CD117}) in neuroendocrine tumors represent a target for therapy? *Ann. N. Y. Acad. Sci.* **2006**, *1073*, 517–526.

152 Kostoula, V.; Khan, K.; Savage, K.; Stubbs, M.; Quaglia, A.; Dhillon, A.; Hochhauser, D.; Caplin, M. Expression of c-kit ({CD117}) in neuroendocrine tumours - a target for therapy? *Oncol. Rep.* **2005**.

153 Skardal, A.; Devarasetty, M.; Forsythe, S.; Atala, A.; Soker, S. A reductionist metastasis-on-a-chip platform for in vitro tumor progression modeling and drug screening. *Biotechnol. Bioeng.* **2016**, *113* (9), 2020–2032 DOI: 10.1002/bit.25950.

154 Skardal, A.; Devarasetty, M.; Soker, S.; Hall, A. R. In situ patterned micro 3D liver constructs for parallel toxicology testing in a fluidic device. *Biofabrication* **2015**, *7* (3), 31001 DOI: 10.1088/1758-5090/7/3/031001.

155 Kozminsky, M.; Nagrath, S. Circulating Tumor Cells, Cancer Stem Cells, and Emerging Microfluidic Detection Technologies With Clinical Applications. In *Cancer Stem Cells*; Elsevier, 2016; pp 473–497.

156 Srinivasaraghavan, V.; Strobl, J.; Agah, M. Microelectrode bioimpedance analysis distinguishes basal and claudin-low subtypes of triple negative breast cancer cells. *Biomed. Microdevices* **2015**, *17* (4), 80 DOI: 10.1007/s10544-015-9977-2.

157 Na, Y. J.; Baek, H. S.; Ahn, S. M.; Shin, H. J.; Chang, I.-S.; Hwang, J. S. [4-t-Butylphenyl]-N-(4-imidazol-1-yl phenyl)sulfonamide (ISCK03) inhibits SCF/c-kit signaling in 501mel human melanoma cells and abolishes melanin production in mice and brownish guinea pigs. *Biochem. Pharmacol.* **2007**, *74* (5), 780–786 DOI: 10.1016/J.BCP.2007.05.028.

158 Drugbank Database.

159 Juurikivi, A.; Sandler, C.; Lindstedt, K. A.; Kovanen, P. T.; Juutilainen, T.; Leskinen, M. J.; Mäki, T.; Eklund, K. K. Inhibition of c-kit tyrosine kinase by imatinib mesylate induces apoptosis in mast cells in rheumatoid synovia: a potential approach to the treatment of arthritis. *Ann. Rheum. Dis.* **2005**, *64* (8), 1126–1131 DOI: 10.1136/ard.2004.029835.

160 Galanis, A.; Levis, M. Inhibition of c-Kit by tyrosine kinase inhibitors. *Haematologica* **2015**, *100* (3), e77-9 DOI: 10.3324/haematol.2014.117028.

161 Di Gion, P.; Kanefendt, F.; Lindauer, A.; Scheffler, M.; Doroshyenko, O.; Fuhr, U.; Wolf , J.; Jaehde, U. Clinical Pharmacokinetics of Tyrosine Kinase Inhibitors. *Clin. Pharmacokinet.* **2011**, *50* (9), 551–603 DOI: 10.2165/11593320-000000000-00000.

162 Hu, S.; Niu, H.; Minkin, P.; Orwick, S.; Shimada, A.; Inaba, H.; Dahl, G. V. H.; Rubnitz, J.; Baker, S. D. Comparison of antitumor effects of multitargeted tyrosine kinase inhibitors in acute myelogenous leukemia. *Mol. Cancer Ther.* **2008**, *7* (5), 1110–1120 DOI: 10.1158/1535-7163.MCT-07-2218.

163 Andersson, P.; Euler, M. Von; Beckert, M. Comparable pharmacokinetics of 85 mg RightSize nilotinib (XS003) and 150 mg Tasigna in healthy volunteers using a hybrid nanoparticle-based formulation platform for protein kinase inhibitors. *J. Clin. Oncol.* **2014**, *32* (15_suppl), e13551–e13551 DOI: 10.1200/jco.2014.32.15_suppl.e13551.

164 Wong, S.-F. New dosing schedules of dasatinib for CML and adverse event management. *J. Hematol. Oncol.* **2009**, *2* (1), 10 DOI: 10.1186/1756-8722-2-10.

165 Santos, F. P. S.; Ravandi, F. Advances in treatment of chronic myelogenous leukemia--new treatment options with tyrosine kinase inhibitors. *Leuk. Lymphoma* **2009**, *50 Suppl 2* (0 2), 16–26 DOI: 10.3109/10428190903383427.

166 Chen, Y.; Tortorici, M. A.; Garrett, M.; Hee, B.; Klamerus, K. J.; Pithavala, Y. K. Clinical Pharmacology of Axitinib. *Clin. Pharmacokinet.* **2013**, *52* (9), 713–725 DOI: 10.1007/s40262-013-0068-3.

167 Bellesoeur, A.; Carton, E.; Alexandre, J.; Goldwasser, F.; Huillard, O. Axitinib in the treatment of renal cell carcinoma: design, development, and place in therapy. *Drug Des. Devel. Ther.* **2017**, *11*, 2801–2811 DOI: 10.2147/DDDT.S109640.

168 Masitinib (also known as Kinavet® and Masivet®) – MSAA: The Multiple Sclerosis Association Of America https://mymsaa.org/publications/msresearch-update-2017/masitinib (accessed Jan 18, 2018).

169 Bellamy, F.; Bader, T.; Moussy, A.; Hermine, O. Pharmacokinetics of masitinib in cats. *Vet. Res. Commun.* **2009**, *33* (8), 831–837 DOI: 10.1007/s11259-009-9231-6.

170 Dubreuil, P.; Letard, S.; Ciufolini, M.; Gros, L.; Humbert, M.; Castéran, N.; Borge, L.; Hajem, B.; Lermet, A.; Sippl, W.; et al. Masitinib (AB1010), a Potent and Selective Tyrosine Kinase Inhibitor Targeting KIT. *PLoS One* **2009**, *4* (9), e7258 DOI: 10.1371/journal.pone.0007258.

171 Deng, Y.; Sychterz, C.; Suttle, A. B.; Dar, M. M.; Bershas, D.; Negash, K.; Qian, Y.; Chen, E. P.; Gorycki, P. D.; Ho, M. Y. K. Bioavailability, metabolism and disposition of oral pazopanib in patients with advanced cancer. *Xenobiotica* **2013**, *43* (5), 443–453 DOI: 10.3109/00498254.2012.734642.

172 Pazopanib HCl (GW786034 HCl) | VEGFR inhibitor | Read Reviews & Product Use Citations http://www.selleckchem.com/products/Pazopanib-Hydrochloride.html (accessed Jan 18, 2018).

173 Yancey, M. F.; Merritt, D. A.; Lesman, S. P.; Boucher, J. F.; Michels, G. M. Pharmacokinetic properties of toceranib phosphate (Palladia, SU11654), a novel tyrosine kinase inhibitor, in laboratory dogs and dogs with mast cell tumors. *J. Vet. Pharmacol. Ther.* **2010**, *33* (2), 162–171 DOI: 10.1111/j.1365-2885.2009.01133.x.

174 Halsey, C. H.; Gustafson, D. L.; Rose, B. J.; Wolf-Ringwall, A.; Burnett, R. C.; Duval, D. L.; Avery, A. C.; Thamm, D. H. Development of an in vitro model of acquired resistance to toceranib phosphate (Palladia®) in canine mast cell tumor. *BMC Vet. Res.* **2014**, *10* (1),

105 DOI: 10.1186/1746-6148-10-105.

175 Yakes, F. M.; Chen, J.; Tan, J.; Yamaguchi, K.; Shi, Y.; Yu, P.; Qian, F.; Chu, F.; Bentzien, F.; Cancilla, B.; et al. Cabozantinib (XL184), a novel MET and VEGFR2 inhibitor, simultaneously suppresses metastasis, angiogenesis, and tumor growth. *Mol. Cancer Ther.* **2011**, *10* (12), 2298–2308 DOI: 10.1158/1535-7163.MCT-11-0264.

176 Zhao, J.; Quan, H.; Xu, Y.; Kong, X.; Jin, L.; Lou, L. Flumatinib, a selective inhibitor of BCR-ABL/PDGFR/KIT, effectively overcomes drug resistance of certain KIT mutants. *Cancer Sci.* **2014**, *105* (1), 117–125 DOI: 10.1111/cas.12320.

177 Luo, H.; Quan, H.; Xie, C.; Xu, Y.; Fu, L.; Lou, L. HH-GV-678, a novel selective inhibitor of Bcr-Abl, outperforms imatinib and effectively overrides imatinib resistance. *Leukemia* **2010**, *24* (10), 1807–1809 DOI: 10.1038/leu.2010.169.

178 Petryszak, R.; Keays, M.; Tang, Y. A.; Fonseca, N. A.; Barrera, E.; Burdett, T.; Füllgrabe, A.; Fuentes, A. M.-P.; Jupp, S.; Koskinen, S.; et al. Expression Atlas update—an integrated database of gene and protein expression in humans, animals and plants. *Nucleic Acids Res.* **2016**, *44* (D1), D746–D752 DOI: 10.1093/nar/gkv1045.

Drug Name	Trade Name	Select targets (other than CD117)	Bioavailability	Specificity for CD117	References
Imatinib	Gleevec/Glivec, STI571	BCR-Abl, RET, PDGF-R	98%	0.1 μM	[158]–[160]
Sunitinib	Sutent, SU11248	JAK/STAT, PDGF-R, Ras/MAPK, VEGFR	50% (fasting)	26 nM	[158],[160]–[162]
Nilotinib	Tasigna	BCR-Abl, Lck	30%	N.A.	[158],[163]
Dasatinib	Sprycel	BCR-Abl, Src	14-34%	13 nM	[158],[160],[164],[165]
Axitinib	Inlyta	BCR-Abl, PDGFR, VEGFR	58%	1.7 nM	[158],[166],[167]
Masitinib	Masivet, Kinavet	FGFR, PDGFR	60% (animals)	200±40 nM	[168]–[170]
Pazopanib	Votrient	FGFR, PDGFR, VEGFR	14-39%	146 nM	[158],[160],[171],[172]
Toceranib	Palladia	PDGFR, VEGFR	77%	<10 nM	[173],[174]
Cabozantinib	XL184	VEGFR, c-Met	74-93%	4.6 nM	[175]
Flumatinib	HH-GV-678	c-Abl, PDGFR	N.A.	2.66 μM	[176],[177]

Table 1. Specificity of Tyrosine Kinase Inhibitors for CD117. N.A. indicates not available.

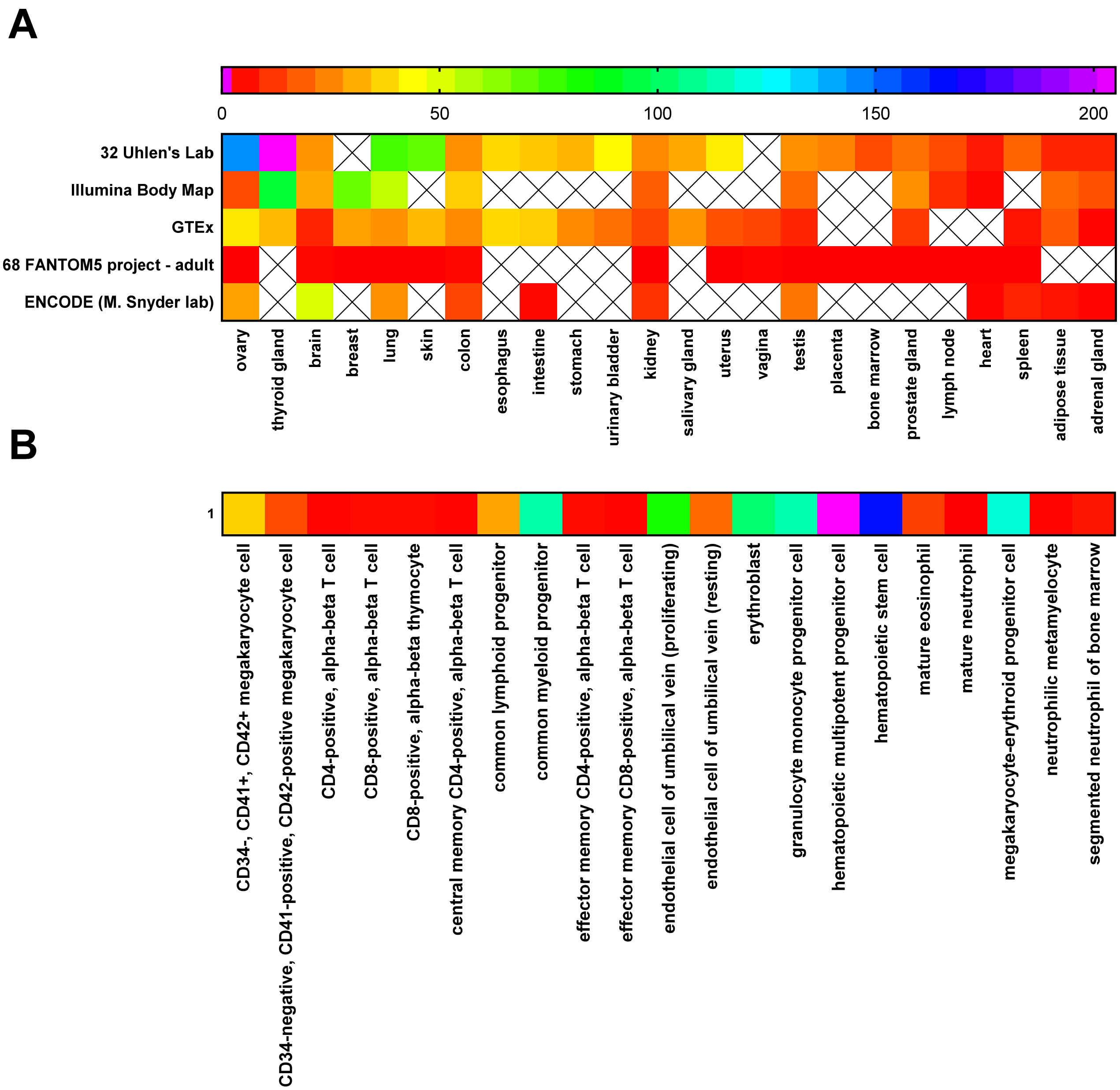

A
0
50
100
150
200
32 Uhlen's Lab
Illumina Body Map
GTEx
68 FANTOM5 project - adult
ENCODE (M. Snyder lab)
ovary
thyroid gland
brain
breast
lung
skin
colon
esophagus
intestine
stomach
urinary bladder
kidney
salivary gland
uterus
vagina
testis
placenta
bone marrow
prostate gland
lymph node
heart
spleen
adipose tissue
adrenal gland
B
1
CD34-, CD41+, CD42+ megakaryocyte cell
CD34-negative, CD41-positive, CD42-positive megakaryocyte cell
CD4-positive, alpha-beta T cell
CD8-positive, alpha-beta T cell
CD8-positive, alpha-beta thymocyte
central memory CD4-positive, alpha-beta T cell
common lymphoid progenitor
common myeloid progenitor
effector memory CD4-positive, alpha-beta T cell
effector memory CD8-positive, alpha-beta T cell
endothelial cell of umbilical vein (proliferating)
endothelial cell of umbilical vein (resting)
erythroblast
granulocyte monocyte progenitor cell
hematopoietic multipotent progenitor cell
hematopoietic stem cell
mature eosinophil
mature neutrophil
megakaryocyte-erythroid progenitor cell
neutrophilic metamyelocyte
segmented neutrophil of bone marrow

Figure 1

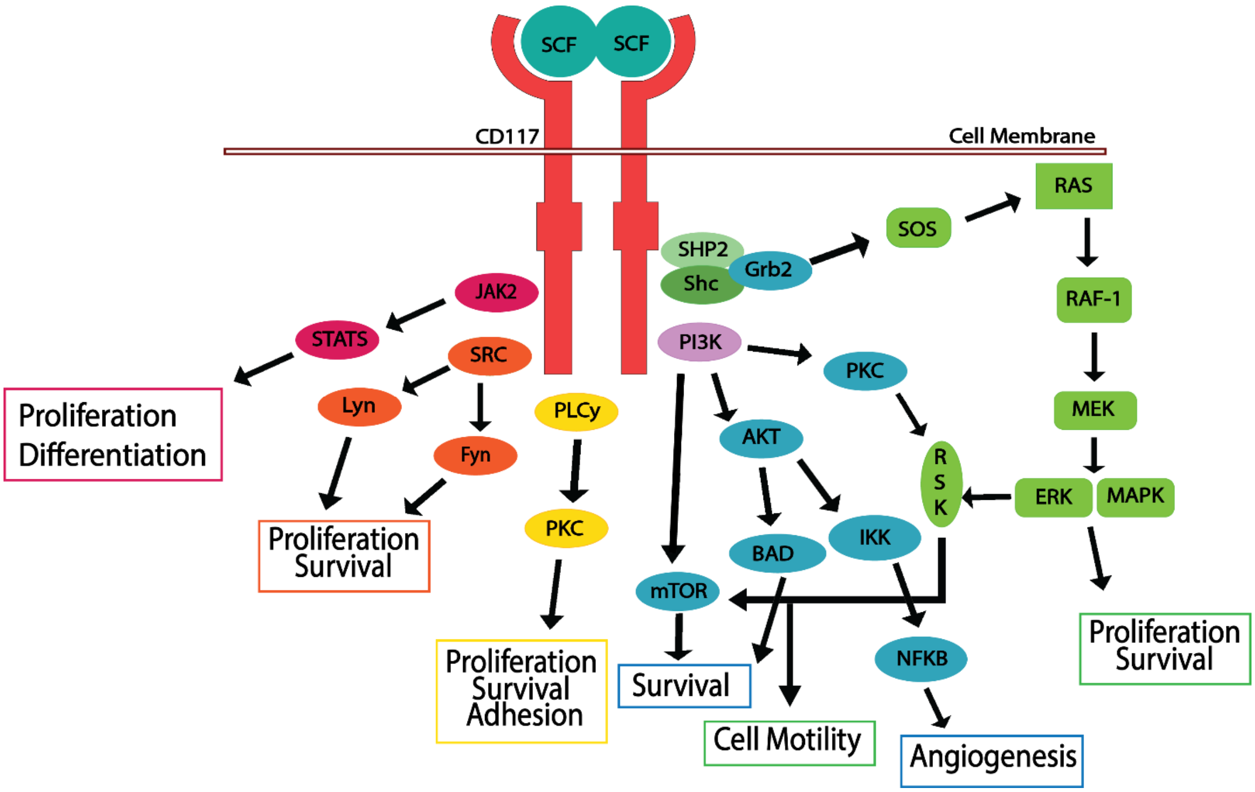
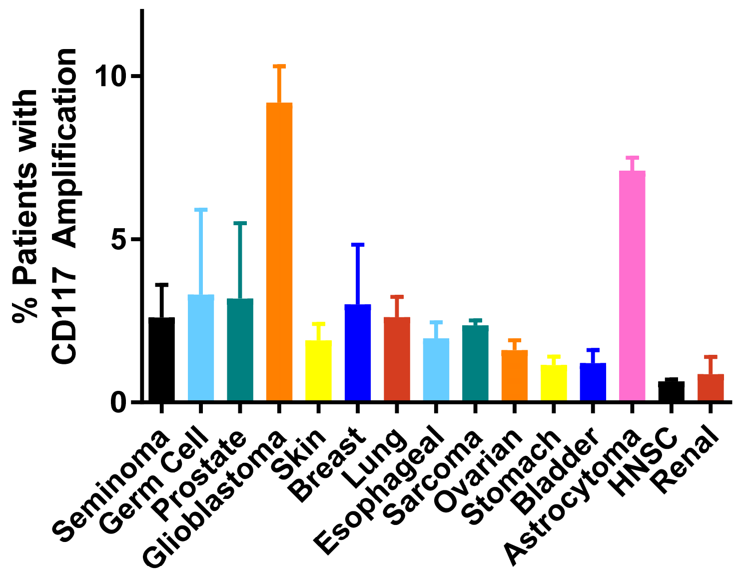

SCF
SCF
CD117
Cell Membrane
RAS
SOS
SHP2
Shc
Grb2
RAF-1
JAK2
STATS
SRC
PI3K
PKC
Proliferation Differentiation
Lyn
PLCγ
MEK
AKT
R S K
Fyn
ERK
MAPK
Proliferation Survival
PKC
IKK
BAD
mTOR
NFKB
Proliferation Survival
Proliferation Survival Adhesion
Survival
Cell Motility
Angiogenesis

Figure 2

A


% Patients with
CD117 Amplification
10
5
0
Seminoma
Germ Cell
Prostate
Glioblastoma
Skin
Breast
Lung
Esophageal
Sarcoma
Ovarian
Stomach
Bladder
Astrocytoma
HNSC
Renal

B

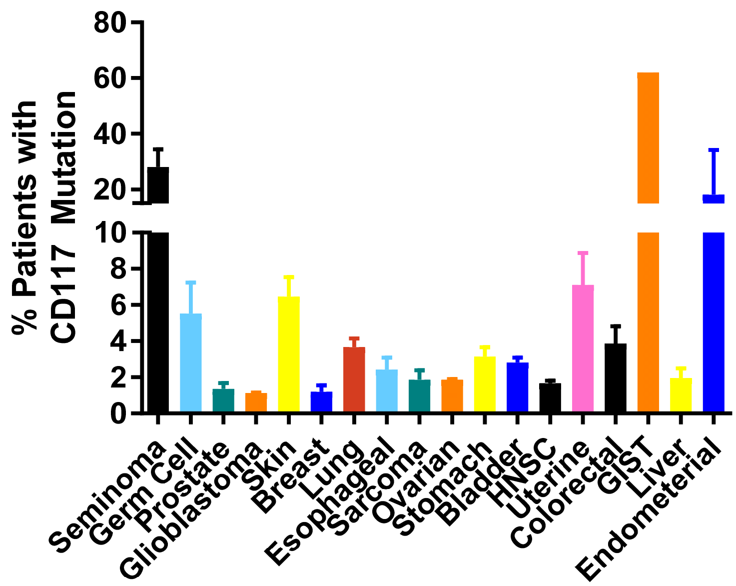

% Patients with
CD117 Mutation
80
60
40
20
10
8
6
4
2
0
Seminoma
Germ Cell
Prostate
Glioblastoma
Skin
Breast
Lung
Esophageal
Sarcoma
Ovarian
Stomach
Bladder
HNSC
Uterine
Colorectal
GIST
Liver
Endometerial

Figure 3

Figure Legends

Figure 1. CD117 is expressed in normal tissues. CD117 Expression in (A) normal tissues and (B) bone marrow progenitor cells using data mined from the EMBL-European Bioinformatics Institute Gene Expression Atlas [178].

Figure 2. CD117 activation stimulates multiple signaling pathways. SCF ligand binding to the CD117 receptor induced dimerization and downstream signaling resulting in proliferation, differentiation, survival, adhesion, motility and angiogenesis.

Figure 3. CD117 is amplified or mutated in a variety of cancers. Genomic datasets in cBioPortal [87],[88] were examined for amplifications (A) or mutations (B) of the CD117 *KIT* gene. The mean percentage of patients with each cancer type with amplifications or mutations ±SEM are shown.