AMERICAN UNIVERSITY OF BEIRUT

MODELING CHRONIC MYELOID LEUKEMIA IN *DROSOPHILA MELANOGASTER*: INSIGHTS ON PATHOGENESIS AND THERAPEUTIC APPROACHES

by
AMANI YOUSSEF AL OUTA

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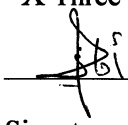

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AN ABSTRACT OF THE DISSERTATION OF

Amani Youssef Al Outa for Doctor of Philosophy
Major: Biomedical Sciences

Title: Modeling Chronic Myeloid Leukemia in *Drosophila melanogaster*: Insights on Pathogenesis and Therapeutic Approaches

Background: Chronic myeloid leukemia (CML) is caused by a balanced chromosomal translocation resulting in the formation of *BCR-ABL1* fusion gene encoding a constitutively active BCR-ABL1 tyrosine kinase, which activates a myriad of signal transduction pathways leading to malignant transformation. Although tyrosine kinase inhibitors (TKIs) have revolutionized CML therapy and became the standard treatment of CML; they are non-curative and some mutations have proven elusive particularly the T315I mutation. The fruit fly *Drosophila melanogaster* is an established efficient *in vivo* model to study human diseases including cancer. The targeted expression of chimeric human/fly and full human BCR-ABL1 in *Drosophila* eyes has been shown to result in detrimental effects. Hence, a well-established fruit fly CML model is crucial for getting better insights on the disease pathogenesis and alternative therapeutic approaches.

Methods: In this study, we generated transgenic fly lines carrying the full human *BCR-ABL1*^{p210} and the first and second generation TKI resistant *BCR-ABL1*$^{p210/T315I}$ fusion oncogenes using Phi-C31 mediated site-specific transgenesis. The binary GAL4-UAS system was used for spatial and temporal control over transgene expression in different *Drosophila* tissues including eyes, hemocytes, wings, imaginal discs and ubiquitous expression. For analysis of eye phenotypes, flies were fixed, critically point dried, gold-coated and visualized using scanning electron microscope (SEM). A grading scale that describes the different levels of severity of the eye phenotype was used for quantification. For drug studies, TKIs (imatinib, nilotinib, dasatinib and ponatinib) were mixed with fly food and larvae were allowed to feed on the mixture. Eclosing adult flies were monitored for changes in ommatidial architecture in the posterior end of the eye which showed a unique defect upon BCR-ABL1 expression. One researcher coded the SEM images and another researcher was blinded to the data and used Image J to measure the area of the posterior eye defect. Western Blot was used to verify transgene expression and phosphorylation status. Immunofluorescence was used to detect ELAV expression as a neuronal marker in dissected *Drosophila* eye discs. We have also aimed to test this model for genetic screening by designing a small scale RNA interference (RNAi) based genetic screen coupled to GAL4 system to control the knockdown of specific genes in BCR-ABL1 expressing tissues.

Results: In all tested tissues, the transformative potential of BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ was clearly revealed leading to eyes and wings developmental defects as well as increased number of circulating hemocytes and lethality upon ubiquitous transgenes expression. Interestingly, in all scenarios BCR-ABL1$^{p210/T315I}$ expression resulted in more severe phenotypes than its wild type counterpart BCR-ABL1^{p210} reflecting a greater oncogenic potential of the mutant. We then assessed the efficacy of the currently used TKIs focusing on a particular eye defect in the posterior end of the adult *Drosophila* compound eye. Treatment of BCR-ABL1^{p210} expressing flies with potent TKIs (dasatinib and ponatinib) resulted in rescue of ommatidial loss and restoration of normal development. We have also tested the ability of these potent drugs in reversing an engrailed (expressed in posterior part of all imaginal discs) driven lethality phenotype upon BCR-ABL^{p210} and BCR-ABL1$^{p210/T315I}$ expression. Dasatinib and ponatinib partially rescued BCR-ABL1^{p210} induced pupal lethality and allowed the eclosure of adult flies. On the other hand, ponatinib showed the potential to suppress larval lethality induced by BCR-ABL1 $^{p210/T315I}$ expression and allowed the development of pupae.

Conclusion: Taken together, this work established a CML tailored BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ fly model which can be further developed to test new compounds as well as can be designed specifically for high throughput drug screening for identifying potential drug hits from a wide range of drug libraries that could help in speeding up drug discovery in CML field. The model also serves as a platform for genetic screening for deciphering potential genes that can aggravate or mitigate BCR-ABL1 mediated phenotypes; hence might provide better insights on the pathogenetic interactions of the fusion gene BCR-ABL1 and its mutants and eventually nominate new potentially specific therapeutic targets.

CONTENTS

ILLUSTRATIONS

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LIST OF ABBREVIATIONS

ABL1	Abelson murine leukemia viral oncogene homolog 1
AKT	Protein kinase B
ALK	Anaplastic lymphoma kinase
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
A-MULV	Abelson murine leukemia virus
AP	Acute phase
APC	Adenomatous polyposis coli
Ask1	Apoptosis signal-regulating kinase 1
ATP	Adenosine triphosphate
Bad	BCL2 antagonist of cell death
BC	Blast crisis
BCR	Breakpoint cluster region
CagA	Cytotoxin-associated gene A
CML	Chronic myeloid leukemia
CNL	Chronic neutrophilic leukemia
CP	Chronic phase
CRK	CT10 regulator of Kinase
CSCs	Cancer stem cells
dAbl	*Drosophila* abelson kinase
Dbl	Duffy binding-like domain
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
Dpp	Decapentaplegic
EGFR	Epidermal growth factor receptor
Ena	Enabled
FDA	Food and drug administration
FGFR	Fibroblast growth factor receptor
FoxO	Forkhead box O
GDP	Guanidine diphosphate
GRB2	Growth factor receptor-bound protein 2
GTP	Guanidine triphosphate
Hh	Hedgehog
hr	hour
HSCs	Haematopoietic stem cells
IFN-α	Interferon-alpha
IMD	Immunodeficiency
JAK	Janus-activated kinase

JNK	Jun N-terminal kinase
LSCs	Leukemic stem cells
MAP	Mitogen-activated protein
MAPK	Mitogen-activated protein kinase
Mdm2	Mouse double minute 2 homolog
MMR	Major molecular response
PBS	Phosphate buffered saline
PDGF	Platelet-derived growth factor
PDGFR	Platelet-derived growth factor receptor
PH	Pleckstrin homology
Ph	Philadelphia
PI3K	Phosphoinositide 3-kinase
RNAi	RNA interference
SEM	Scanning electron microscopy
SFKs	Src family kinases
SOS	Son of sevenless
Src	Rous sarcoma oncogene
STAT	Signal transduction and activator of transcription
TKIs	Tyrosine kinase inhibitors
UAS	Upstream activating sequence
VEGFR	Vascular endothelial growth factor receptor
Wg	Wingless

CHAPTER I

INTRODUCTION

A. Chronic Myeloid Leukemia (CML)

1. Historical Perspective

CML was the first leukemia type to be characterized whereby back in 1845 the findings of physicians David Craigie and Hughes Bennett on patients suffering from fever, splenomegaly, and leukocytosis were published in the Edinburgh Medical and Surgical Journal (Goldman, 2010). While simultaneously several reports were emerging describing patients with similar symptoms, an important milestone was reached in 1872 by Ernst Neuman who pinpointed the origin of leukemia to be the bone marrow. In almost 100 years later, Nowell and Hungerford described an aberrant chromosome in the karyotype of leukemia patients which was later designated the term "Philadelphia (Ph) chromosome". Further description of this cytogenetic abnormality was accomplished by Rowley in 1973 who revealed that the Ph chromosome is the result of a reciprocal translocation between chromosomes 9 and 22 (Rowley, 1973). Later it was discovered that the translocation occurs from *ABELSON* (*ABL1*) gene on chromosome 9 to the *BREAKPOINT CLUSTER* (*BCR*) gene on chromosome 22 shaping the *BCR-ABL1* fusion gene on the Ph chromosome (Goldman, 2010) (Figure 1).

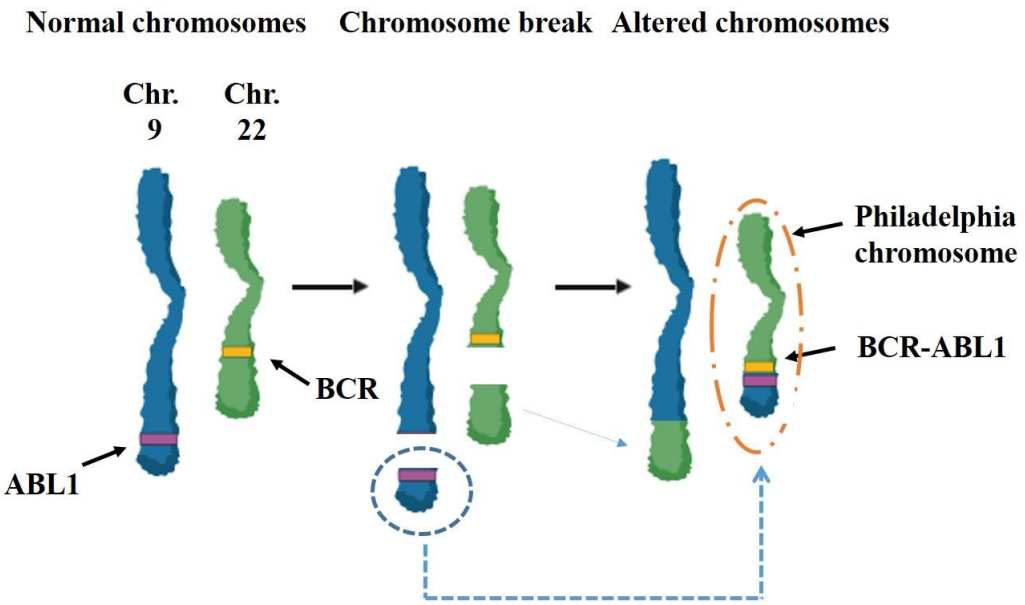


Figure 1. Schematic representation of the Philadelphia chromosome formation. The fusion of *ABL1* gene on chromosome 9 with *BCR* gene on chromosome 22 is depicted leading to the formation of Philadelphia chromosome harboring the fusion gene *BCR-ABL1*.

2. Molecular Biology and Pathophysiology of CML

CML is a clonal myeloproliferative neoplasm that clinically manifests in three different stages: chronic, accelerated and blast crisis (BC) (Savage, Szydlo, & Goldman, 1997; Spiers, 1977). Lasting for years; the chronic phase (CP) is a long phase characterized by mature cells and myeloid precursors accumulation in the bone marrow, blood, and extramedullary areas. The accelerated phase (AP) lasts for a shorter period of time, about 4-6 months, whereby the patient is heavily burdened with precursor/progenitor cells. The final phase is the BC which lasts for a very short period of time and is marked by the presence of blast cells with halted differentiation (H. M. Kantarjian et al., 1987).

The reciprocal translocation t(9;22)(q34;q11) taking place in CML, involves the long arms of chromosomes 9 and 22 resulting in the juxtaposition of 5' end of *BCR gene* and the 3' end of *ABL1* gene. The recombination is highly variable but usually comprises *BCR* introns 13 or 14 fusion with a 140 kilobase (kb) *ABL1* region occurring between exons 1b and 2. e13a2 (*BCR* exon 13 and *ABL1* exon 2) or e14a2 junctions form the two major *BCR-ABL1* transcripts which arise from mRNA splicing regardless of the breakpoint position (M. W. N. Deininger, Goldman, & Melo, 2000) . Both transcripts are expressed as a 210-kD BCR-ABL1 protein, known as p210, harboring a tyrosine kinase with constitutive activity that is indispensable for leukemic cells survival (Ben-Neriah, Daley, Mes-Masson, Witte, & Baltimore, 1986; Valent, 2008). As a matter of fact, the CP in CML can be sufficiently insinuated by the Ph chromosome alone and this was proven through the transplantation of murine bone marrow cells which are retrovirally infected with BCR-ABL1 in lethally irradiated mice (Daley, Van Etten, & Baltimore, 1990). BCR-ABL1^{p210} is not the only fusion protein that is formed; P190 and P230 are the other two fusion products that are produced based on different breakpoint locations in BCR that fuse with ABL1 exon a2. Interestingly, each of these BCR-ABL1 products has a different transforming capacity resulting in a different type of leukemia (Melo, 1996). Most of CML patients and about one-third of Ph^+ acute lymphoblastic leukemia (ALL) patients harbor the 210-kd BCR-ABL1. The p-190 kD fusion protein predominates in the remaining Ph^+ ALL patients and rarely in CML patients (Chan et al., 1987; Suryanarayan et al., 1991). The fusion that produces the p-

230 protein is believed to be a rare case that takes place in chronic neutrophilic leukemia (CNL) (Pane et al., 1996).

3. BCR and ABL1 Genes: The Translocation Partners

Human *ABL1* gene, a ubiquitously expressed 145 kD nonreceptor tyrosine kinase, is the homologue of *v-ABL* oncogene present in the Abelson murine leukemia virus (A-MULV) (Abelson & Rabstein, 1970; Laneuville, 1995). Interaction with a myriad of known proteins is carried through several structural domains in ABL1. The NH2 terminus carries three Src homology domains (SH1-SH3), with SH1 possessing the tyrosine kinase function (Cohen, Ren, & Baltimore, 1995). Interaction between ABL1 and SH3 domains in other proteins such as CRK is accomplished through proline-rich sequences in ABL1 (Feller, Knudsen, & Hanafusa, 1994). The 3' end carries the DNA (Kipreos & Wang, 1992) and actin (McWhirter & Wang, 1993) binding motifs as well as the nuclear binding signals (Van Etten, Jackson, & Baltimore, 1989). ABL1 normally shuttles between the nucleus and cytoplasm to mediate various cellular functions such as cell cycle regulation (Kipreos & Wang, 1990; Sawyers, McLaughlin, Goga, Havlik, & Witte, 1994), the cells response to genotoxic stress (Yuan et al., 1999) and relaying of information related to the cellular surrounding via integrin signaling (Lewis & Schwartz, 1998). Just like *ABL1*, *BCR* gene encodes for a ubiquitously expressed protein which is cytoplasmic (Maru & Witte, 1991) with well-studied structural domains. The N-terminus encodes a serine-threonine kinase and a coiled-coil domain which permits dimer formation *in vivo* (McWhirter, Galasso, &

Wang, 1993; Reuther, Fu, Cripe, Collier, & Pendergast, 1994). Pleckstrin-homology (PH) and Dbl-like domains fall in the middle region and play a role in exchanging guanidine triphosphate (GTP) for guanidine diphosphate (GDP) when Rho guanidine exchange factors are involved (Denhardt, 1996).

4. Mechanisms of BCR-ABL1-Mediated Malignancy

The fusion of *BCR* and *ABL1* grants the tyrosine kinase a diversity of protein-protein interacting domains. Normally, ABL1 exerts its physiological roles by alternating between the cytoplasm and nucleus, however upon fusion with BCR this property is lost and ABL1 becomes confined to the cytoplasm interacting with a myriad of proteins and activating the oncogenic pathway (Cilloni & Saglio, 2012). Several features in BCR-ABL1 play a role in its transforming capacity: SH1, SH2, and actin-binding domains are considered the culprits in ABL1 while in BCR they involve signals driven by the coiled-coil domain (McWhirter & Wang, 1993), the tyrosine 177 residue (Pendergast et al., 1993), and the phosphoserine-threonine rich sequences (Pendergast, Muller, Havlik, Maru, & Witte, 1991). BCR N-terminal coiled-coil domain allows for BCR-ABL1 dimerization and insinuates trans-autophosphorylation events, thereby, activating the kinase domain and granting docking sites for intermediary adaptor proteins such as GRB2 (Thomas O'Hare, Deininger, Eide, Clackson, & Druker, 2011). GRB2 recruits Son of Sevenless (SOS) then the whole complex (BCR-ABL1/GRB2/SOS) activates Ras (Pendergast et al., 1993; Puil et al., 1994) and GRB2-associated binding protein 2 (GAB2) (Sattler et al., 2002) culminating in the constitutive activation of Ras pathway

and, therefore, activation of mitogen-activated protein (MAP), extracellular signal-regulated kinase (ERK)1/2 (MEK), and MAPK proteins; collectively leading to altered cellular proliferation (Figure 2).

The BCR-ABL1/GRB2/SOS complex can also activate the PI3K/AKT pathway. PI3K activates AKT kinase, the kinase responsible for a series of phosphorylation events of players in the apoptotic pathway such as caspase 9, Mdm2, Bad, and Ask1 (Franke, Kaplan, & Cantley, 1997). PI3K/AKT pathway can also be activated by BCR-ABL through CRKL and CRK; collectively fueling the extended survival and expansion of the leukemic clone of cells (Cilloni & Saglio, 2012) (Figure 2).

Another crucial role in BCR-ABL1 mediated leukemogenesis is played by transcription factors such as Signal transduction and activator of transcription (STAT) which renders leukemic cells cytokine-independent. STAT5 can be directly phosphorylated and activated by BCR-ABL1 or can be indirectly activated through phosphorylation by Janus-activated kinase (JAK2) (Ilaria & Van Etten, 1996) (Figure 2).

In addition to the activation of these pathways, BCR-ABL1 affects the cytoskeletal properties of hematopoietic cells leading to increased motility and disrupted adhesion properties (Bazzoni, Carlesso, Griffin, & Hemler, 1996; Gordon, Dowding, Riley, Goldman, & Greaves, 1987). The disruption in cytoskeletal functions of CML cells contributes to the progression of the disease through enabling their release from the bone marrow prematurely and accumulation in other hematopoietic tissues (Li

et al., 2007). It was shown that BCR-ABL1 can alter the interaction between β1-integrin and the actin cytoskeleton leading to disrupted function of integrin in CML progenitor cells and, hence, plays a role in imparting the altered adhesion and increased motility phenotype (Bhatia, Munthe, & Verfaillie, 1999)

The above described activation of different signaling pathways by BCR-ABL1 converges into imposing loss of proliferation control and leukemic clone expansion (Cilloni & Saglio, 2012) with the tyrosine kinase activity of BCR-ABL1 being responsible for initiating its transformative phenotype (Lugo, Pendergast, Muller, & Witte, 1990).

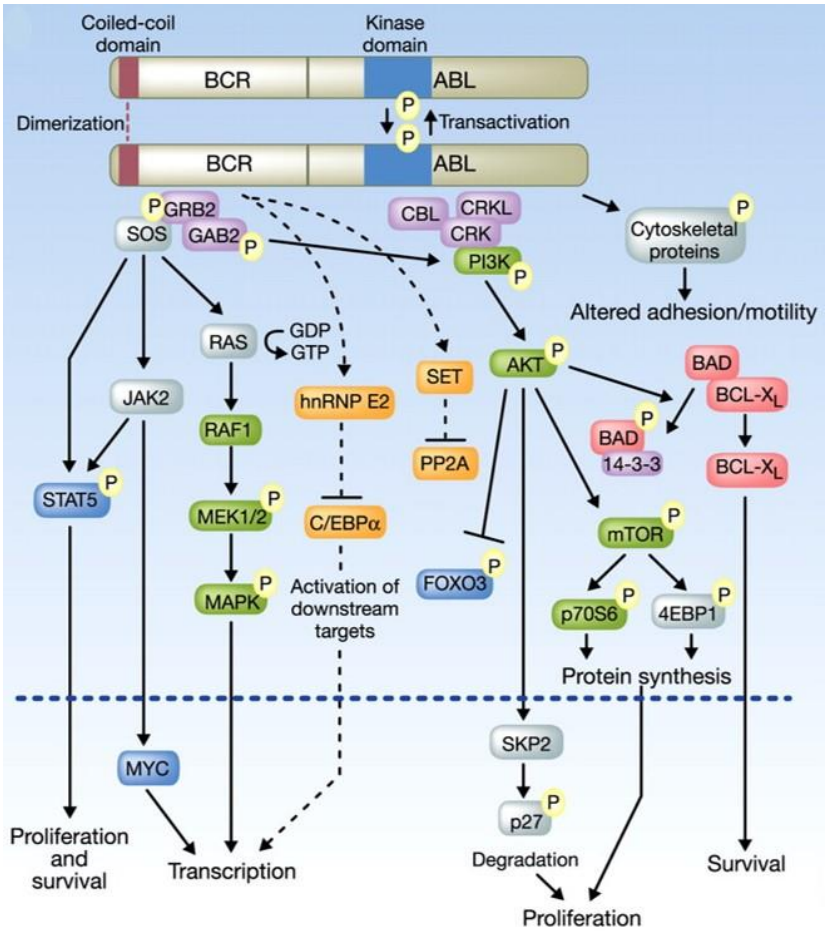


Figure 2. **BCR-ABL1 signaling network in CML.** Dimerization via BCR coiled-coil domain triggers autophosphorylation and activation of the kinase in BCR-ABL forming docking sites for adaptor molecules (GRB2) as well. Several pathways are activated which converge into increased proliferation, survival, inhibition of apoptosis, and altered cytoskeletal properties of CML cells (Thomas O'Hare et al., 2011).

5. CML Treatment

a. History

Historically, treatment strategies for combating CML relied on cytoreductive agents (hydroxyurea, arsenic, and busulfan) which were only palliative. Treatment in

the nineteenth century relied mainly on arsenicals until the implementation of radiotherapy in the 1900s followed by busulfan and hydroxyurea (Bolin, Robinson, Sutherland, & Hamman, 1982) in the 1960s. However, the above mentioned treatment approaches failed to alter the disease progression and the only effective paradigm included allogeneic stem cell transplantation which was limited by increased risks of morbidity and mortality. Patients were not suitable candidates for transplantation were given interferon alpha (IFN-α) which was proven to act as an anti-cancer drug with pro-apoptotic (Chawla-Sarkar et al., 2003) and immunomodulatory effects (Biron, 2001). IFN-α, as a single therapy (Allan, Shepherd, & Richards, 1995) or combined with anti-cancer drugs such as cytarabine (Chen et al., 2011), showed higher efficacy than busulfan and hydroxyurea for its ability to establish durable cytogenetic responses and increased survival. However, IFN-α exhibited a toxicity profile (Chen et al., 2011) which limited its use and necessitated the research into safer and more effective therapeutic approaches for CML (Figure 3).

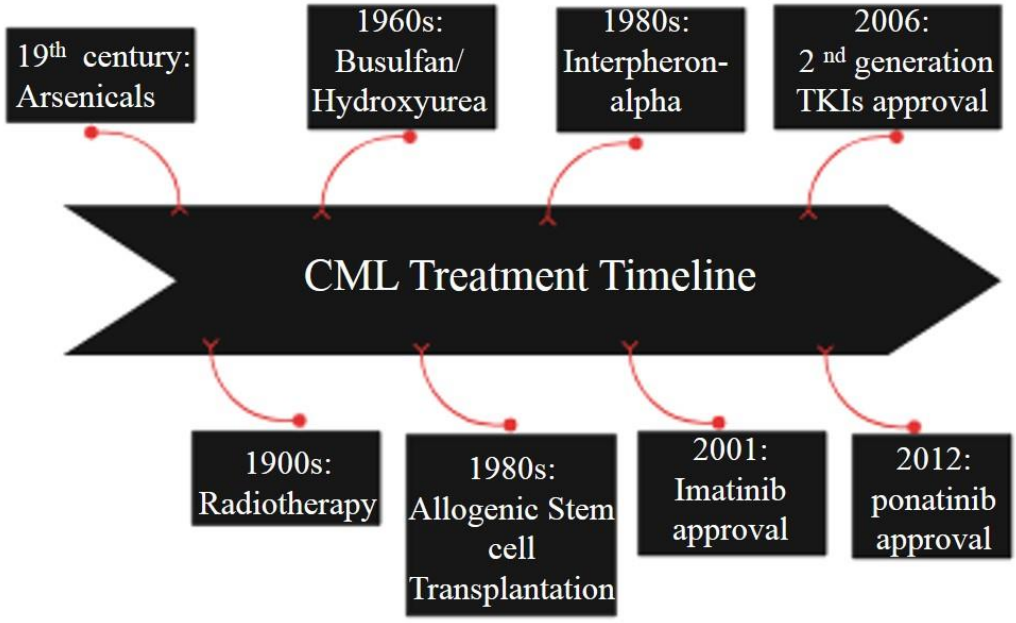


Figure 3. CML treatment timeline. CML treatment progression with time starting with palliative therapies (arsenicals, hydroxyurea, and busulfan) followed by radiotherapy, interferon–alpha, and allogenic stem cell transplantation which were limited by their side effects and ending with the era of targeted therapy with TKIs. Years indicated refer to the introduction of the drug for use by patients.

b. The Era of Tyrosine Kinase Inhibitors (TKIs)

It was not until the discovery of TKIs that CML treatment was revolutionized. TKIs competitively inhibit the Adenosine triphosphate (ATP) binding site in the BCR-ABL1 kinase domain and, hence, block the phosphorylation of proteins which play roles in BCR-ABL1 signal transduction cascade (Figure 4); culminating in apoptosis and inhibition of proliferation of CML cells (Cornelison, Kantarjian, Cortes, & Jabbour, 2011). TKIs, with their prominent efficacy, shaped a new era for CML patients and altered the deadly nature of CML, making it a more controlled type of leukemia (Baccarani et al., 2013).

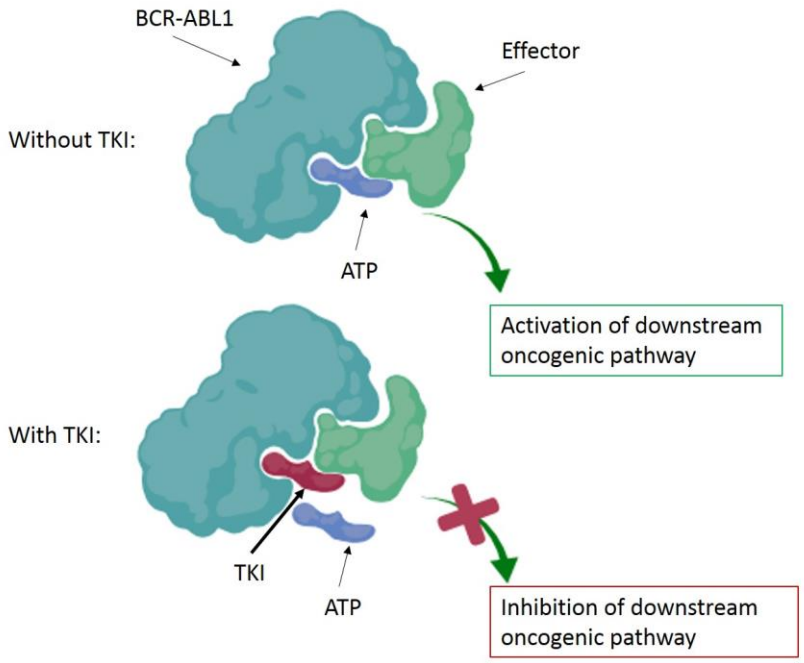


Figure 4. **Schematic representation of the mode of action of TKIs**. In the absence of TKI, ATP occupies the kinase domain allowing for activation of downstream oncogenic pathways. In the presence of TKI, ATP is competitively inhibited thereby blocking the oncogenic pathway (Created with Biorender.com).

i. First-generation TKI: Imatinib

Imatinib development dates back to the late 1980s; it belongs to the 2-phenylaminopyrimidine class and is the fruit of tedious high-throughput screens of drug libraries done to identify kinase inhibitors (Buchdunger et al., 1996; Druker & Lydon, 2000). It binds to the inactive form of BCR-ABL1 kinase and, hence, inhibits ATP binding (Druker et al., 2006) and was granted the USA Food and Drug Administration (FDA) approval in 2001 (Iqbal & Iqbal, 2014). Imatinib (STI571) was developed as a signal transduction inhibitor whereby in pre-clinical models it was proven to inhibit the autophosphorylation, in sub-micromolar concentrations, of the platelet-derived growth

factor receptor (PDGFR), Kit receptor, and v-ABL as well as hindered inositol formation by platelet-derived growth factor (PDGF), c-Fos mRNA expression, and MAP Kinase activation in cells (Buchdunger et al., 1996; Buchdunger et al., 1995). The *in vivo* and *in vitro* efficacy of imatinib was tested in pre-clinical models demonstrating its inhibitory effect on cellular proliferation and tumorigenesis, however, also pointing to the need for continuous inhibition of the kinase activity for optimal biological efficacy (Carroll et al., 1997; M. W. Deininger, Goldman, Lydon, & Melo, 1997; Druker et al., 1996; le Coutre et al., 1999). As a matter of fact treatment of nude mice injected with BCR-ABL-positive human leukemic cell lines, bearing mice three times/day with imatinib was superior to once or twice/day administration and resulted in 87%-100% of mice being cured (le Coutre et al., 1999). With a tolerable animal toxicity profile, imatinib entered clinical studies. Phase I study tested imatinib in CP CML patients who failed IFN-α treatment (Druker, Talpaz, et al., 2001); and, then, was extended to include patients in lymphoid and myeloid BC as well as those with relapsed or refractory Ph^+ ALL (Druker, Sawyers, et al., 2001). Collectively, phase I and phase II studies shaped imatinib as a well-tolerated effective single-agent TKI in CP CML patients who have failed IFN-α therapy, CML BC patients as well as those with Ph^+ ALL. The success of these two phases led to a multicenter, international, open-label, phase III randomized study The International Randomized Study of Interferon and STI571 (IRIS) whereby imatinib was compared to IFN-α plus low-dose cytarabine. In the context of tolerability, hematologic and cytogenetic responses and the chance of progression to AP or BC CML in newly diagnosed chronic-phase CML patients;

imatinib showed superiority over IFN-α plus low-dose cytarabine (S. G. O'Brien et al., 2003). Follow-up studies of patients randomized to imatinib arm of IRIS study who continued imatinib showed excellent overall survival rates (85%), durable hematologic, and cytogenetic responses and low chances of progression to BC (Druker et al., 2006; Hochhaus et al., 2009; S. O'Brien, Guilhot, & Goldman; S. G. O'Brien et al., 2003).

However, imatinib success in increasing the overall survival rates of CML patients was outshined by cases of relapse or lack of response. Attempts to identify causes of treatment failure pointed to several factors which were either BCR-ABL1-dependent or -independent. Pharmacokinetics variability, clonal evolution, overexpression of genes responsible for imatinib transport, overexpression of tyrosine kinases such as the Src family kinases (SFKs), and stem cell quiescence are all considered BCR-ABL1 independent mechanisms of resistance. On the other hand, *BCR-ABL1* gene amplification and most importantly point mutations in the BCR-ABL1 kinase domain which hinder imatinib binding are considered BCR-ABL1-dependent mechanisms (Quintás-Cardama, Kantarjian, & Cortes, 2009). Resistance due to mutations remains the dominant factor harboring about 40-90% of CML cases with imatinib resistance (Branford & Hughes, 2006; Corbin, La Rosee, Stoffregen, Druker, & Deininger, 2003; Gorre et al., 2001; Hochhaus et al., 2002; Jabbour et al., 2006; Lahaye et al., 2005; Lowenberg, 2003; Shah et al., 2002). The first BCR-ABL1 point mutation imparting imatinib resistance was identified by Sawyers and colleagues when analyzing nine imatinib-resistant CML patients (Gorre et al., 2001). Analysis revealed a point mutation substituting threonine with isoleucine at position 315 (T315I) in ABL

kinase domain in six out of nine patients. Later on, several studies identified different affected residues with 60–70% of all mutations occurring at residues Gly250, Tyr253, Glu255, Thr315, Met351, and Phe359 (Branford et al., 2002; Shah et al., 2002). Resistance to imatinib created a crucial hurdle for researchers which prompted research into second generation TKIs which can overcome imatinib-resistant mutations.

ii. Second-generation TKIs: Dasatinib and Nilotinib

Dasatinib (BMS-354825) was the first TKI to be clinically tested after imatinib and was identified through a screen of a series of substituted 2-(aminopyridyl) - and 2-(aminopyrimidinyl) thiazole-5-carboxamides demonstrating potent ABL/Src kinase inhibition accompanied by inhibition of proliferation upon testing in preclinical models (Lombardo et al., 2004). Unlike imatinib, dasatinib can bind to the active conformation of ABL kinase which is in close resemblance to that of SFKs in addition to the inactive conformation (Tokarski et al., 2006). Src and SFKs are not far away from the oncogenic signaling initiated by BCR-ABL1; as a matter of fact, BCR-ABL1 is suggested to activate its signaling pathway through activation of non-receptor tyrosine kinases such as SFKs which include Fyn, Lyn, and Hck.

The pharmacodynamics properties of dasatinib demonstrate its high potency whereby compared to imatinib, it is 325 times more potent (T. O'Hare et al., 2005). Dasatinib inhibits the tyrosine kinase activity of BCR-ABL1, Src, and SFKs including Fgr, Fyn, Hck, Lck, Lyn, and Yes (Das et al., 2006) in addition to KIT, PDGFR, and ephrin receptor tyrosine kinases (Huang et al., 2007; Lombardo et al., 2004; Rix et al., 2007).

Moreover, dasatinib ability to bind to both conformations of the kinase (active and inactive) (Lombardo et al., 2004; T. O'Hare et al., 2005; Tokarski et al., 2006) granted it the ability to inhibit a range of imatinib-resistant mutations (T. O'Hare et al., 2005; Redaelli et al., 2009; Shah et al., 2007; Shah et al., 2004) which acted by destabilizing imatinib's preferred conformation (inactive) (T. O'Hare et al., 2005; Shah et al., 2007; Tokarski et al., 2006). Dasatinib was approved by the FDA in June 2006, after success in phase I and phase II studies (J. Cortes et al., 2007; Talpaz et al., 2006), for the treatment of resistant or intolerant cases of imatinib and Ph^+ ALL. Later, the DASISION (DASatinib versus Imatinib Study In treatment-Naive CML patients) trial which compared first-line imatinib 400 mg once daily versus first-line dasatinib 100 mg once daily revealed deeper and faster cytogenetic and molecular responses with dasatinib in patients newly diagnosed for CP-CML (H. Kantarjian et al., 2010).

Another drug that was designed to overcome imatinib-resistant mutations is nilotinib. Compared to imatinib, nilotinib exhibits at least 10-50 fold higher potency and is more selective for BCR-ABL1 tyrosine kinase inhibition than imatinib (Weisberg et al., 2005). Nilotinib is selective for inhibition of BCR-ABL1, Kit, and PDGFR tyrosine kinases (Weisberg et al., 2005). The Evaluating Nilotinib Efficacy and Safety in Clinical Trials–Newly Diagnosed Patients (ENESTnd) study, compared nilotinib (300 mg or 400 mg twice daily) to that of imatinib (400 mg once daily) on the level of efficacy and safety in patients who are newly diagnosed Ph^+ CP-CML and considered the primary end point as the rate of major molecular response (MMR) at 12 months. Hereby, patients on nilotinib showed significantly higher rates of MMR than those on

imatinib. Moreover, nilotinib showed a greater disease control over imatinib whereby fewer patients progressed to the crisis or AP compared to imatinib. However; despite the increased spectrum of mutations targeted by second generation TKIs, both dasatinib and nilotinib could not target the famous T315I gatekeeper mutation. This necessitated the search for drugs that could handle this elusive mutation which yielded ponatinib as a third-generation TKI.

iii. Third-generation TKI: Ponatinib

Ponatinib is a potent TKI that targets a myriad of BCR-ABL1 kinase domain mutations including the T315I mutation (J. E. Cortes et al., 2012). Its spectrum of kinases inhibition involves: BCR-ABL1 in addition to Src and members of the vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor receptor (FGFR), and PDGFR families of receptor tyrosine kinases (T. O'Hare et al., 2009). In the PACE (Ponatinib in patients with CML or Ph^+ ALL) study ponatinib revealed significant anti-leukemic activity, documented efficacy against patients harboring the T315I mutation and no single BCR-ABL1 kinase mutation showed resistance to ponatinib (J. E. Cortes et al., 2013). The major concern with ponatinib was an arterial thrombotic side effect which prompted additional follow-up studies that revealed a high frequency of venous adverse side effects and, subsequently, lead to the withdrawal of ponatinib from the market. However, withdrawal created a major problem for patients who cannot take any other TKI, therefore, ponatinib was re-introduced to the market but under strict safety measures. Ponatinib indications included CML and Ph^+ ALL patients having the T315I

mutation (Gainor & Chabner, 2015). Studies done to elucidate the mechanism by which T315I altered the ABL1 kinase domain and imparted resistance to first and second generation TKIs revealed that T315I changed the three-dimensional topography of the BCR-ABL1 ATP pocket and, hence, decreased the sensitivity to imatinib. Moreover, the mutation with its bulky isoleucine side chain results in a missing hydrogen bond that is usually formed with the side chain hydroxyl group of Thr315 and which is critical for imatinib binding (Corbin, Buchdunger, Pascal, & Druker, 2002; Schindler et al., 2000). However, Pricl *et al*. demonstrated that the leading cause behind imatinib inability to bind is a domino effect of a series of structural rearrangements which take place to make room for the mutant and involving several points of contact with the drug (Pricl, Fermeglia, Ferrone, & Tamborini, 2005). At the same time, large scale molecular dynamics simulation demonstrated that the hindrance of imatinib interaction with residues E286 and M290 is the reason imatinib fails to bind to the mutated T315I (Lee et al., 2008). Interestingly, this explanation could be extended to nilotinib failure to target T315I (von Bubnoff et al., 2006) since nilotinib has a close interaction with E286 too (Weisberg et al., 2006). As for dasatinib, it is not precise whether this explanation can be applied however breakdown of interactions due to T315I induced conformational changes is possible (Lee et al., 2008). Levinson *et al*. went further to explain dasatinib failure with inability to form an active Src site due to T315I changes in E286 residue (Levinson et al., 2006). Ponatinib was structurally designed and developed to preclude the necessity for the hydrogen bond formation and, hence, overcoming the binding

obstacle; with a triple carbon-carbon bond ponatinib is capable to accommodate for the bulky isoleucine side chain (T. O'Hare et al., 2009).

Despite the important clinical outcomes of TKIs several patients fail to reap the whole benefits and that is because of issues of resistance, side effects, and adherence to treatment. Lack of adherence particularly shapes a common response by patients to lifelong TKI treatments and it was estimated that the risk for poor outcomes increases three times with noncompliance in CP-CML subjects (Noens et al., 2009). Adverse events associated with TKIs represent one of the reasons of poor drug compliance. Moreover, the management of side effects often necessitates supportive care or additional medications. Side effects include: myelosuppression, edema, pleural effusion, altered liver enzymes levels, skin toxicity, and gastrointestinal disturbances (de Almeida, Fogliatto, & Couto, 2014). The financial burden on the patient is another limitation to consider which sometimes delays the administration of the drug and also pools into the reasons of decreased drug compliance.

6. CML Models

a. *In Vitro* Models

i. Immortalized Hematopoietic Cell Lines

Hematopoietic cell lines expressing the Ph chromosome represent pre-clinical continuous high-throughput tools for initial validation of therapeutic targets and drug screening. Other important applications of CML cell lines include: genome wide RNA interference (RNAi) screening for TKIs resistant genes (Ma, Roderick, Kelliher, &

Green, 2016) as well as reporter gene assays for tracking the specificity of activated signaling pathways (Correa et al., 2012). One major concern with their use is that they are often derived from CML patients in the BC phase which carry additional mutations on top of BCR-ABL1. Moreover, the excessive culturing in different research labs across the world produces cell lines with different characteristics that are not conclusive of the events taking place *in vivo* (Tang, 2019). However, the gene expression analysis of forty cell lines showed that despite numerous cell passages, pathogenic critical pathways are conserved to a certain extent (Andersson et al., 2005). Examples of some commercially available CML cell lines include: K-562, KU-812, LAMA-84, JK-1, and TK-6 (Clarke & Holyoake, 2017).

ii. Primary Patient CML Cells

Primary patient cells represent a crucial pre-clinical *in vitro* tool that merges between variability in patients' biology and *in vitro* assays required for decoding disease mechanisms and response to therapies. The primary limitation that exists is the inability to freely manipulate these cells as much as what can be done in immortalized cell lines which limits the applicable assays in this context. However, approaches including colony forming assays, cell cycle and apoptosis flow cytometry, polymerase chain reaction, and western blotting are still applicable and, hence, can aid in the understanding of a myriad of underlying disease mechanisms and responses to treatments (Clarke & Holyoake, 2017). Studies done using primary patient CML cells aided researchers understand important pathways that help maintain leukemic stem cells

(LSCs) (Corbin et al., 2011) and highlighted the resistance of these cells to TKI-initiated apoptosis (Corbin et al., 2011), thereby, solidifying the targeting of additional pathways besides BCR-ABL1 inhibition as an efficient approach for LSC eradication (Clarke & Holyoake, 2017).

b. *In Vivo* Models

Despite the fact that *in vitro* models are substantial for any pre-clinical assessment of therapeutic approaches; *in vivo* models remain pivotal in terms of providing the physiological microenvironment, cell to cell interactions, and surrounding signals that take place whilst studying a specific research question. In CML, mice are the *in vivo* animals employed in research labs and they segregate into three different subtypes:

i. Retroviral Transduction/Transplantation Model

The retroviral transduction and transplantation model relies on the infection of 5-Fluorouracil treated mouse bone marrow cells with BCR-ABL1 encoded in a retrovirus. Then the transduced cells are transplanted into irradiated syngeneic recipients (Daley et al., 1990; Kelliher, McLaughlin, Witte, & Rosenberg, 1990; Zhang & Li, 2016). The recipient mice eventually develop a myeloproliferative CML-like pathology. This model is effective in studying effects of gene deletions and co-expression (Hao & Ren, 2000) as well as changes imposed by BCR-ABL1 mutant

variants (Miething et al., 2006) and constitutes a platform for studying CML LSCs (Peng & Li, 2016).

ii. SCLtTA/BCR-ABL1 Transgenic Model

This model involves a tetracycline-regulated transactivator (tTA) under the control of the murine stem cell leukemia gene 3' enhancer (SCL) that controls p210 BCR-ABL1 expression. When tetracycline is removed from the drinking water the expression of p210 is specifically driven in the stem and progenitor hematopoietic cells. Mice then develop neutrophilia, invasion of myeloid cells to other organs such as liver, lungs and spleen and leukocytosis. Unlike the retroviral mouse model, this model is characterized by a slower onset of disease which mimics the natural course of CML pathology in humans and, thereby, might allow more easily to track events taking place right after BCR-ABL1 expression (Koschmieder et al., 2005).

iii. Xenograft Model

In the xenograft mouse model, immunodeficient mice are engrafted with human malignant cells and subsequently the Ph^+ engraftment ability is assessed (Ito et al., 2002; Shultz et al., 2005). Most commonly $CD34^+$ cells are used for assessment of their engraftment ability in sub-lethally irradiated mice. Usually the degree of engraftment relies on the patient phase from which the cells are derived with higher rates seen with BC cells as compared to CP cells (Dazzi et al., 1998; J. C. Wang et al., 1998).

Conclusively, in CML and just like in any other cancer field that is thirsty for discovering new better therapeutic approaches and expanding the knowledge on the disease, research is faced by a slowdown particularly because *in vitro* approaches most of the time cannot reproduce the *in vivo* story and when data from *in vitro* studies is moved to be validated in mice it often results in invalid conclusions. While mice remain invaluable as *in vivo* models, their high cost and time-consuming processes limit their use as initial high throughput screening whole animals. *Drosophila melanogaster* emerges in this aspect to provide an *in vivo* whole animal with a low cost and fast generation time. In this context, establishing a *Drosophila* CML model in the field puts us in a position to use both genetic screening and drug screening approaches to broaden our knowledge on CML as well as to offer potentially new therapeutic approaches which could be validated more confidently in mice models, hence, the cooperation between both models for faster and efficient research in CML.

B. *Drosophila melanogaster* Development

The use of *Drosophila* dates back to over a century ago when Thomas Hunt Morgan the "father ''of *Drosophila* decided to refine Gregor Mendel's theory of inheritance. The first lab ever to start using *Drosophila* in research was documented to be William Castle's group at Harvard in 1901. Morgan's work was granted the Nobel Prize in Physiology or Medicine in1933 "*for his discoveries concerning the role played by the chromosome in heredity*" (Jennings, 2011). Since then, the research field integrated *Drosophila* as an instrumental *in vivo* model for various diseases including

cancer especially that about 75% of disease-causing human genes have orthologs in *Drosophila* (Reiter, Potocki, Chien, Gribskov, & Bier, 2001a). Different fly tissues have been successfully used to model diseases and understand biological processes such as fly wings (Fang, Soares, Teng, Geary, & Bonini, 2012), eyes (F. D. Karim et al., 1996), hematopoietic organs (Evans, Liu, & Banerjee, 2014; Gold & Brückner, 2014; McGuire, Deshazer, & Davis, 2005), nervous system and, brain (Branson, Robie, Bender, Perona, & Dickinson, 2009; Fossgreen et al., 1998; Inagaki, Kamikouchi, & Ito, 2010; McGuire et al., 2005).

1. Drosophila Life Cycle

Drosophila, the fruit fly or vinegar fly, is a species of the family *Drosophiladea* widely used as a research model. It is ectothermic whereby its development period is highly dependent on its surrounding temperature. *Drosophila* life cycle comprises of an egg stage, L1 to L3 larval stages, pupal stage, and finally the adult stage (Figure 5). The fastest development from egg to adult occurs at 29°C (7 days); this period increases with the decrease in temperature whereby it becomes 8.5 days at 25°C (optimal condition), and 19 days at 18°C (Ashburner, 1989). At 25°C, its life span from egg to death is around 50 days (Linford, Bilgir, Ro, & Pletcher, 2013). After a female fly lays a fertilized egg on the surface of food, embryogenesis proceeds rapidly and is completed in 24 hours after fertilization of the oocyte by the male sperm (Hales, Korey, Larracuente, & Roberts, 2015). Following this comes the larval stage which proceeds through 4 days from the first instar to second and finally third instar

stage. The larvae undergo molting and rapid growth of differentiated cells (Widmann, Eichler, Selcho, Thum, & Pauls, 2018). During larval stages, precursor structures are formed known as imaginal discs which undergo transformation during the following pupal stage into the adult structures (Widmann et al., 2018). The pupal stage lasts around 4 days during which metamorphosis takes place, a process of lysis of the imaginal discs and formation of new structures such as head, wings, legs, eyes, and reproductive apparatus. After completion of the pupal stage, the fly ecloses and enters the adult stage.

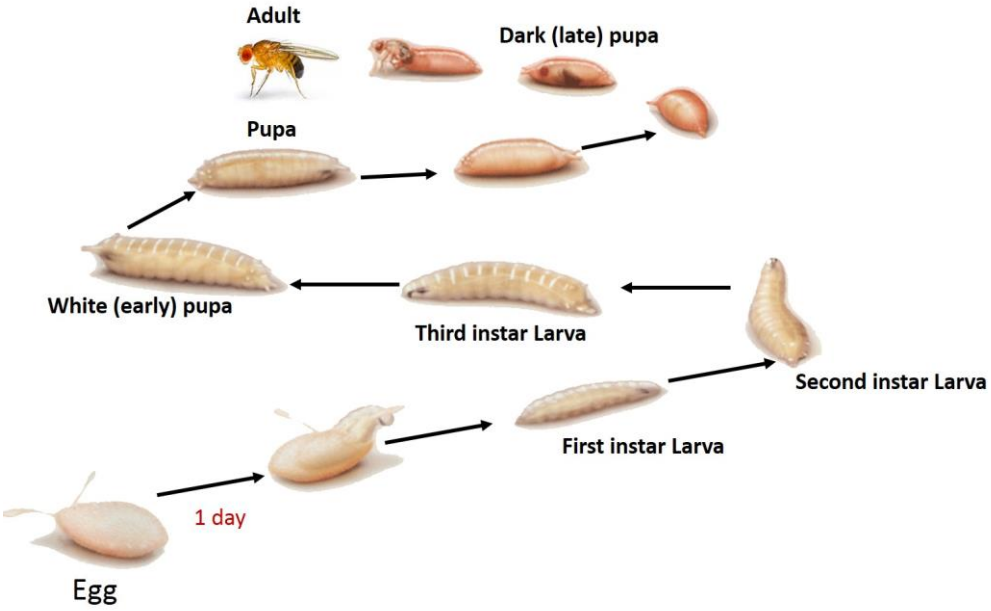


Figure 5. *Drosophila melanogaster* life cycle at 25°C. The development of *Drosophila* from an embryo all the way to an eclosed adult is depicted. Each arrow represents one day.

2. Drosophila Hematopoietic System

Being an ectotherm, *Drosophila* harbors an open circulatory system with low hydrostatic pressure characterized by the presence of a simple tube-like heart (also termed dorsal vessel) and interstitial fluid known as "hemolymph". The hemolymph is pumped from the posterior region to the anterior of the fly body by the cardiac tube and it carries nutrients, metabolites, hormones, peptides, and hemocytes (Rotstein & Paululat, 2016) (Figure 6).

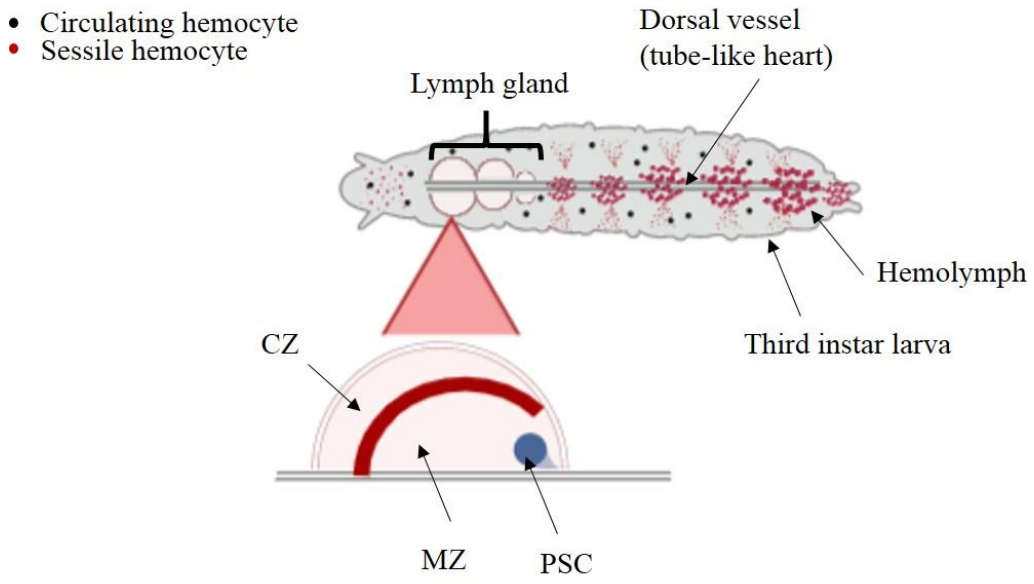


Figure 6. Components of *Drosophila* larva hematopoietic system. A third instar *Drosophila* larva is shown with circulating hemocytes in the hemolymph depicted as black dots and sessile hemocytes as red dots. The different zones in the primary lobe of the lymph gland are shown. To the periphery lies the cortical zone (CZ), at the center is

the medullary zone (MZ) forming a compact cellular layer and finally the posterior signaling center (PSC) which has no distinctive structure. (Created with Biorender.com)

a. Types of Hemocytes

Major cellular immune functions in *Drosophila* are orchestrated by at least three types of terminally differentiated hemocytes which are in close resemblance to the vertebrate myeloid lineage: plasmatocytes, crystal cells, and lamellocytes (Figure 7) (Evans, Hartenstein, & Banerjee, 2003). *Drosophila* larvae hemocytes are housed in three main compartments: the hemolymph, subepithelial patches (sessile hemocytes), and in lymph glands (Márkus et al., 2009). Accounting for 90-95% of the circulating hemocytes, plasmatocytes are considered the main representative hemocytes. They are available throughout all developmental stages with a phagocytic activity for removal of apoptotic debris (Tepass, Fessler, Aziz, & Hartenstein, 1994) and microbes (Elrod-Erickson, Mishra, & Schneider, 2000; Tahir M. Rizki & Rizki, 1984) thereby resembling mammalian macrophages/monocytes (Evans et al., 2003; Kocks et al., 2005; Tepass et al., 1994). Plasmatocytes are capable of secreting antimicrobial peptides (AMPs), and hence, mediate *Drosophila* humoral response (Dimarcq et al., 1997; Shandala et al., 2011). The integration of the systemic responses and the maintenance of the organismal homeostasis necessitates the presence of a cross-talk between plasmatocytes and organs acting as barriers between the fly and its surrounding environment. Reported cross-talks include for example those of plasmatocytes with the gut (Ayyaz, Li, & Jasper, 2015) and the fat body as well as the visceral muscles (Irving et al., 2005; Shia et al., 2009; Woodcock et al., 2015; Yang & Hultmark, 2016). The

remaining ~5% of the circulating hemocytes are platelet-like cells known as crystal cells which are non-phagocytic harboring crystalline inclusions (Lanot, Zachary, Holder, & Meister, 2001; SHRESTHA & GATEFF, 1982) that execute melanization responses such as those required for wound healing (Ramet, Lanot, Zachary, & Manfruelli, 2002) and innate immunity (De Gregorio et al., 2002). Crystal cells harbor prophenoloxidase which is the essential enzyme required for melanin synthesis (M. T. Rizki & Rizki, 1959). Both crystal cells and plasmatocytes play an important role during clotting response by secreting hemolectin, a protein that has been reported to harbor conserved domains available in human von Willebrand factor, coagulation factor V/VIII as well as complement factors (Akira Goto, Kadowaki, & Kitagawa, 2003; A. Goto et al., 2001; Scherfer et al., 2004). The third type of hemocytes is known as lamellocytes which are cryptic stress-induced cells that are rare in normal conditions and are induced in huge numbers for encapsulation of large foreign particles such as eggs of parasitic wasps during immune challenges (T. M. Rizki & Rizki, 1992). Lamellocytes are also involved in melanotic tumor or pseudotumor formation in *Drosophila* which are black melanotic spots that were reported about sixty years ago to involve hemocytes (Claudio, 1958; Oftedal, 1953; M. T. Rizki, 1960). Generally, these tumours are called “melanotic masses” and for description of specific phenotypes they are termed “melanotic nodules” or “melanizations” since they are mostly non-invasive and have tumorous overgrowth only in some cases (Minakhina & Steward, 2006). Genetic backgrounds with mutations or expression of genes that lead to abnormal signaling such as those in Wingless (Wg), JAK/STAT, Toll, and Jun N-terminal kinase (JNK) are coupled to increased numbers of

lamellocytes and formation of melanotic tumours (Zettervall et al., 2004). For example, the constitutive activation of Toll and JAK-STAT pathways in Toll-gain-of-function/ cactus-loss-of-function and hopscotch Tumorous-lethal (hopTum-1) mutants respectively, has been reported to result in the above described phenotype (Lemaitre et al., 1995; Luo, Hanratty, & Dearolf, 1995; Qiu, Pan, & Govind, 1998).

Aside from the pool of circulating hemocytes, the majority of hemocytes are described to localize to cuticular epidermis forming clusters known as sessile hemocytes (Kurucz et al., 2007; Lanot et al., 2001; Makhijani, Alexander, Tanaka, Rulifson, & Bruckner, 2011) which have been reported to act as true hematopoietic compartments feeding the pool of circulating hemocytes with lamellocytes during immune challenges. These lamellocytes are thought to originate from first generation larval plasmatocytes (Viktor Honti et al., 2010; Márkus et al., 2009; Stofanko, Kwon, & Badenhorst, 2010).

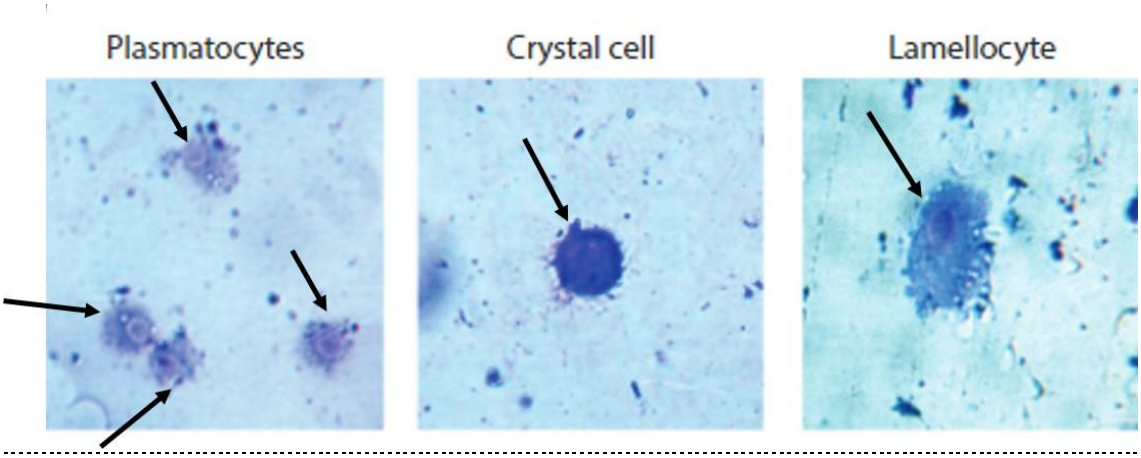


Figure 7. Third instar larvae hemocytes with Giemsa-Rosenfeld staining. The left panel shows plasmatocytes which are round in shape and harbor irregular margins. The middle panel is a crystal cell with a darkly stained crystals containing cytoplasm. The right panel shows a lamellocyte with dark nucleus (Rajak, Dutta, & Roy, 2015).

b. Lymph Gland

The lymph gland lies approximately one third of the larval length from the anterior end towards the dorsal side beneath the brain (Figure 6) (Reimels & Pfleger, 2016). It develops from a set of cell clusters that arises from the cardiogenic mesoderm along with the heart-like tube, the dorsal vessel as well as nephrocyte-like pericardial cells (S.-H. Jung, C. J. Evans, C. Uemura, & U. Banerjee, 2005; Astrid Rugendorff, Amelia Younossi-Hartenstein, & Volker Hartenstein, 1994). A single precursor cell in the cardiogenic mesoderm can lead to the formation of the dorsal vessel and lymph gland (Lolitika Mandal, Utpal Banerjee, & Volker Hartenstein, 2004). This resembles the mammalian hemangioblast, which can develop into both the blood and vascular cells (S. H. Jung, C. J. Evans, C. Uemura, & U. Banerjee, 2005; A. Rugendorff, A. Younossi-Hartenstein, & V. Hartenstein, 1994). It is not until the late-second to early third instar stage that the lymph gland appears as a distinct organ with the primary lobes discernable as specific structures containing variable zones. Located towards the periphery of the primary lobe is the cortical zone (CZ) which appears as a collection of cells that are loosely packed and granular in appearance. On the contrary, the cells towards the center of the primary lobe forming the medullary zone (MZ) are compact in organization. The third zone, the posterior signaling center (PSC) constitutes fifty cells during the third-instar stage but is not structurally distinctive. In addition to the architectural variation, each zone expresses its own collection of markers which is

indicative of the nature of the residing hematopoietic population (S.-H. Jung et al., 2005). For example, the medullary zone, supporting hematopoietic progenitor cells, expresses E-cadherin (H. Agaisse, U.-M. Petersen, M. Boutros, B. Mathey-Prevot, & N. Perrimon, 2003), Domeless (Bourbon et al., 2002), and Unpaired (Hervé Agaisse et al., 2003) which are pro-hemocyte markers. While the cortical zone expresses mature hemocyte markers such as Hemolectin (Akira Goto et al., 2003), Peroxidasin (Nelson et al., 1994), and Lozenge. On the other hand, the PSC expresses a set of markers such as Antennapedia (Mandal, Martinez-Agosto, Evans, Hartenstein, & Banerjee, 2007) and Collier (Michèle Crozatier, Ubeda, Vincent, & Meister, 2004) and mediates signaling pathways such as the JAK/STAT and hedgehog (Hh) to maintain the progenitor state of the hemocytes in the medullary zone (Krzemień et al., 2007; Mandal et al., 2007). It also plays a role in inducing lamellocyte differentiation (Michèle Crozatier et al., 2004).

c. Transient and Definitive Hematopoiesis

Hematopoiesis is the process whereby self-renewing multipotent hematopoietic stem cells (HSCs) differentiate into different types of blood lineages. In vertebrates, hematopoiesis occurs through the primitive and definitive waves which are spatially and temporally distinct (Galloway & Zon, 2003). The primitive wave is a transient wave that supports embryonic development through production of erythrocytes by extraembryonic yolk sac or any other equivalent site (Palis & Yoder, 2001). In the embryonic stage, HSCs originate in aorta/gonad/mesonephros (AGM) region of the embryo proper and home to hematopoietic organs such as the bone marrow and fetal

liver in mammals (Godin & Cumano, 2002). Definitive hematopoiesis is a *de novo* lifelong wave that gives rise to all blood cell types in mature organisms.

In *Drosophila* similar waves also take place to provide the organism with the needed hematopoietic components throughout its life cycle. Each stage comprises a distinct set of hemocytes and hereby, as in vertebrates, hematopoiesis in *Drosophila* is generally described to be ruled by two successive waves known as the embryonic and larval hematopoiesis (M. Crozatier & Meister, 2007) .The first wave of hematopoiesis is embryonic hematopoiesis and gives rise to hemocytes that originate from the head (procephalic) mesoderm and then migrate throughout the whole embryo (Tepass et al., 1994). The second wave is larval hematopoiesis whereby lymph glands, which are mesodermal in origin, differentiate towards the end of embryogenesis along the anterior region of the dorsal vessel (A. Rugendorff et al., 1994), and become active hematopoietic sites (Lanot et al., 2001). Lymph glands remain intact housing hemocytes which are dispersed into the larval hemolymph only under stressful incidences as in infection and injury (H. Agaisse, U. M. Petersen, M. Boutros, B. Mathey-Prevot, & N. Perrimon, 2003; T. M. Rizki & Rizki, 1992; Sorrentino, Carton, & Govind, 2002). Under normal conditions, the lymph gland undergoes disintegration in the early pupa (Lanot et al., 2001) to release hemocytes that constitute altogether with embryonic hemocytes (Holz, Bossinger, Strasser, Janning, & Klapper, 2003; V. Honti, Csordas, Kurucz, Markus, & Ando, 2014) what was thought to be the only sources of the adult fly hemocytes pool. However, recently Gosh et al. (2015) refuted the notion that adult flies immune challenges rely only on pre-formed life-long embryonic and larval

hemocytes by documenting that adult flies harbor active hematopoietic clusters along the dorsal side of the fly abdomen which house progenitor cells capable of differentiating into plasmatocytes and crystal cells (Ghosh, Singh, Mandal, & Mandal, 2015).

Hereby, we have described the hematopoietic system in *Drosophila*, one of the important systems employed in the fruit fly research studies when considering the effects of oncogene expression especially their leukemogenic properties. However, other *Drosophila* systems are also used to create models of diseases regardless of the presence of the true organ of the disease. One such example is the *Drosophila* eyes.

3. Drosophila Eyes Development

In a manner similar to what takes place during the vertebrate eye field establishment, the *Drosophila* eye disc initiates from an ectoderm-derived infolding whereby Hh and Decapentaplegic (Dpp) cooperate to yield two eye fields (Figure 8) (T. Chang, Mazotta, Dumstrei, Dumitrescu, & Hartenstein, 2001). Cellular proliferation in this epithelial layer then takes place under the control of Notch and epidermal growth factor receptor (EGFR) pathways which initiate complex signaling pathways for shaping the eye field (T. Chang, Shy, & Hartenstein, 2003; Kenyon, Ranade, Curtiss, Mlodzik, & Pignoni, 2003; Kumar & Moses, 2001). Eye differentiation initiates during third instar larva and early pupal life (Wolff & Ready, 1993) to form the adult *Drosophila* compound eye comprising 700 to 750 ommatidia, which are unit eyes harboring eight photoreceptor neurons, four non-neuronal cone cells, and two pigment

cells. Pigment cells optically shield the cores of neighboring ommatidia, hence prohibit the passage of light between ommatidia. This highly sensitive structure necessitates accurate interplay among cell movement, proliferation, death, and signaling which results in a neat and highly ordered ommatidial arrangement (Ross Cagan, 2009). The process of retinal differentiation is associated with what is known as a morphogenetic furrow, an indentation in the epithelial layer initiated by Hh, allowing for transient cell cycle arrest and cell shape alterations. This event takes place every 90-120 minutes sweeping anteriorly across the eye disc and leaving behind it rows of highly arranged and differentiated ommatidia (Ready, Hanson, & Benzer, 1976; Wolff & Ready, 1993). Precisely, anterior to the furrow cells proliferate to enlarge the eye field, the furrow itself is cells in cell cycle arrest and behind it cell specification takes place (R. Cagan, 2009).

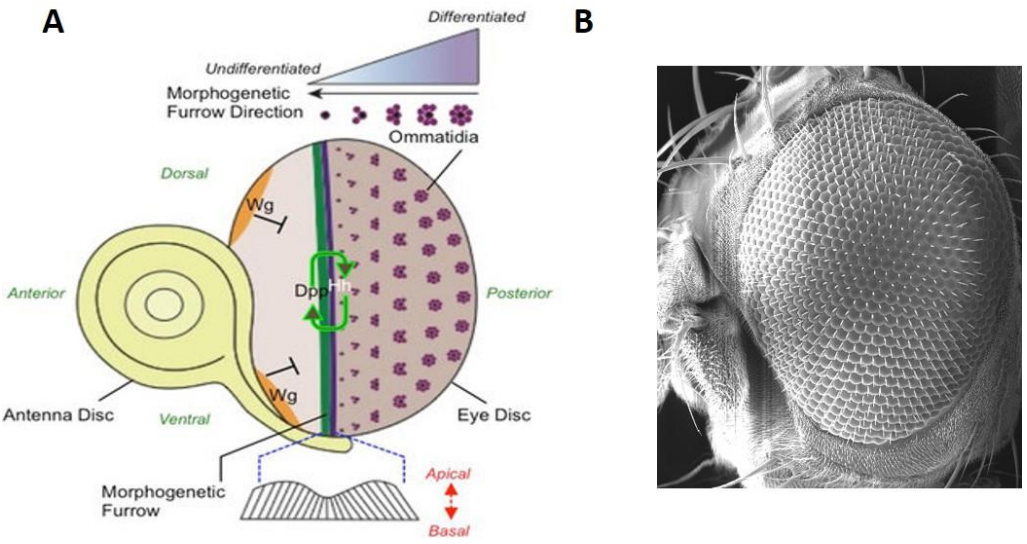


Figure 8. Eye development in *Drosophila*. (A) Eye development during third instar larval stage with Hh and Dpp initiating the process whereby a morphogenetic furrow that sweeps the eye disc anteriorly leads to ommatidial development behind it. **(B)** An adult *Drosophila* compound eye showing highly arranged ommatidial facets. Modified from Dash *et al.* (Dash, Siddam, Barnum, Janga, & Lachke, 2016).

C. *Drosophila* as a Cancer Model

Recently, *Drosophila* has been used as a cancer model after a century of elucidating the power of the fruit fly particularly in simplifying interactions between genes, cells, and tissues combined along with modern genetic analysis technologies. Moreover, the recognition that cancer cell lines exhibit shortcomings when trying to extract data for clinical relevance (Gillet, Varma, & Gottesman, 2013) and that *in vivo* mammalian models are expensive, time consuming, and could not provide facile genetic tools for studying the knottiness of tumorigenesis; pushed curious researchers to look up for models that provide time-efficiency and are inexpensive whilst securing the *in vivo* complexity of physiological interactions. Interestingly, fruit flies have a rapid life cycle,

are inexpensive, and large quantities can be kept in a laboratory and most importantly have simple signaling pathways which can be used to study fundamental processes; collectively this can ensure rapid and timely performance of experiments especially those related to cancer which otherwise take several months to years in mice models (Warr, Shaw, Azim, Piper, & Parsons, 2018). Now, the question that arises at this point: Do fruit flies develop cancer? Basically, they do not because of their limited life span however; cancer-related genes and biological processes are conserved between human and flies (Reiter, Potocki, Chien, Gribskov, & Bier, 2001b). Moreover, the ectopic expression or interference with cancer-related genes in *Drosophila* tissues often leads to phenotypes revealing the basic hallmarks of cancer such as genomic instability, evasion of apoptosis, sustained proliferation, and cell invasion and metastasis (Hanahan & Weinberg, 2000, 2011). Therefore, a stream-lined genome combined with low levels of redundancy and high levels of homology (to about 75% of human genes) ensures the ease of applying large-scale genetic screens where hits often describe the gene function which can be translated to higher organisms as humans. Notch, Hh, and Salvador/Warts/Hippo are all examples of genes identified through such screens and which later appeared to play a role in tumorigenesis in humans (Gonzalez, 2013).

1. Cancer Through Drosophila Eyes

The first thing that comes to one's mind in this perspective is that *Drosophila* eyes can be useful for modeling ocular tumors only. While the fly has been useful in this paradigm (Bennett, Lyulcheva, & Cobbe, 2015), researchers went way beyond

ocular tumors to model several cancers ranging from solid to liquid tumors in *Drosophila* eyes! The question that hunts every researcher in this context, as with all cancer models, is to what extent can this model recapitulate the real human tumor?

Drosophila eyes specifically served in high-throughput genetic screening and dissection of molecular interactions (B. J. Thomas & Wassarman, 1999). Eye development in fruit fly necessitates the cooperation of about two-thirds of *Drosophila* genes most of which play roles in general cellular functions (Thaker & Kankel, 1992). This particular characteristic allows for exploiting phenotypic eye assessment for describing gene roles in other tissues. Studies done in *Drosophila* eyes fall into the whole spectrum of important biological processes; ranging from cellular proliferation, differentiation, and tissue patterning to apoptosis (F. D. Karim et al., 1996). The fly eye has been used as an *in vivo* test tube to identify several interactions of *Drosophila* and human cancer-related genes such as characterization of Ras pathway (Fortini, Simon, & Rubin, 1992; Felix D Karim & Rubin, 1998), upstream regulators (Grzeschik, Parsons, Allott, Harvey, & Richardson, 2010) and new players (Poon, Lin, Zhang, & Harvey, 2011) in the Hippo pathway, and the role of glycogen synthase kinase 3β (GSK3β) in Adenomatous polyposis coli (APC) and beta-catenin binding (Rao, Makhijani, & Shashidhara, 2008). Studies involving the assessment of the rough eye phenotype produced upon ectopic expression of cancer-related genes were also pivotal in deciphering important cancer clues. As an example the expression of human Anaplastic lymphoma kinase (ALK) and its mutants which are associated with neuroblastoma aided in identifying which mutants are ligand-dependent and which are independent;

hence, hinting to the ones that can be targeted with ALK inhibitors (Chand et al., 2013; Martinsson et al., 2011). Another study utilized *Drosophila* rough eye phenotype and its reversal as an endpoint to identify that cytotoxin-associated gene A (*CagA*) functions as the *Drosophila* GRB2-associated binding protein (GAB) adaptor protein whereby *CagA* rescued ommatidial development in the absence of Daughter of Sevenless (GAB homologue) (Botham, Wandler, & Guillemin, 2008). As a matter of fact, *Drosophila* eyes assays were applied for studies beyond basic research and a sound example here is the multiple endocrine neoplasia (MEN2) model whereby the mis-expression of activated or wild-type *Drosophila* Ret ortholog (dRet) in *Drosophila* eyes resulted in a rough eye phenotype (Read et al., 2005) that was exploited for genetic and chemical screening. While the genetic screening identified important genetic regulators (Read, Bach, & Cagan, 2004), the chemical screening validated the *in vivo* effect of vandetanib, an anilinoquinazoline compound, and provided the first evidence of its effect in an intact epithelial layer whereby vandetanib nearly completely rescued ommatidial development in Ret expressing *Drosophila* eyes (Vidal, Wells, Ryan, & Cagan, 2005).

2. Drosophila as a Leukemia Model

When touching on the subject of leukemia, HSCs directly come into the picture due to the fact that this concept has been adopted by scientists to explain the root of cancer including leukemia (Dick, 2008) . In adult mammals and mice, the bone marrow is the organ that houses HSCs which give rise to the lymphoid and myeloid lineage. The

surrounding microenvironment of HSCs has been coined the term “the niche” (L. D. Wang & Wagers, 2011) and supports HSCs self-renewal and pluripotency to differentiate into different types of cells. This clearly demonstrates that the nature of communication between HSCs and the niche at the level of genetics and cellular mechanisms is crucial for optimal functioning of HSCs. Studies have shown that the disruption of this conveyance between HSCs and the niche can lead to blood cancers like acute myeloid leukemia (AML) (Oh & Humphries, 2012). To better understand the mechanisms of the underlying processes behind HSCs and niche relationship, researchers should deal with a simplified version of the structural and cellular complexity of mammalian bone marrow; and here is where *Drosophila* PSC comes into the picture. As discussed earlier, the PSC represents a primitive niche that orchestrates the fate of pro-hemocytes and provides the necessary signaling for maintenance of pro-hemocytes characteristics (M. Crozatier & Meister, 2007). The lymph gland primary lobes as well represent a simplified version of the mammalian bone marrow niche since they capture much of the molecular mechanisms that function in the mammalian aorta-gonadal-mesonephros mesoderm (L. Mandal, U. Banerjee, & V. Hartenstein, 2004). On the other hand, these primitive niche versions come at the expense of a limited number of differentiated cells as well as the total absence of the lymphoid lineage; however, an optimistic approach is that this adds simplicity to the system and allows for easier understanding of basic communication processes between HSCs and the niche (M. Crozatier & Vincent, 2011). Another approach for studying leukemogenesis in flies is looking for the presence of what is known as “melanotic tumors” or *Drosophila*

leukemia which were historically used as an assay to pinpoint genes regulating hemocytes development and playing a role in leukemogenesis (Gateff, 1978, 1994; Sang & Burnet, 1963; Sparrow, 1974; Watson, Justice, & Bryant, 1994). Those melanotic masses are easily observed under the transparent cuticle of the larva and studies have shown that they comprise of melanized aggregating hemocytes that might also attach to other tissues (Minakhina & Steward, 2006; R. M. Rizki & Rizki, 1979). They mostly signify increased blood cells proliferation, lamellocytes differentiation or enlarged and precociously rupturing lymph glands. Nonetheless, deregulation of the hematopoietic system is not the sole contributor to melanotic tumor formation, disruption of immune system pathways is another culprit. Consequently, here comes the role of critically following up on selected gene hits when using melanotic tumor assays to characterize if they fall under immunity, homeostasis or cancer umbrella (Boulet, Miller, Vandel, & Waltzer, 2018).

Perhaps the most ill-famed gene identified through melanotic tumor assays to be linked to human cancers is the gene *hopscotch* (*Hop*) which encodes the *Drosophila* homologue of mammalian JAK kinase involved in the JAK/STAT pathway (Amoyel, Anderson, & Bach, 2014). Several studies came out then identifying the network of Hop activity that contributes to melanotic tumor formation. One such study is by Anderson *et al.* who demonstrated that the Hippo pathway is activated by Hop and leads to melanotic tumor formation mediated by peripheral hemocytes proliferation; this was studied through a deficiency screen for modifiers of the mutation *tumorous-lethal* (Anderson, Bailetti, Rodkin, De, & Bach, 2017), an allele of *hop* (*hop Tum-l*)*hop* $^{Tum\text{-}l}$

which was associated more than 40 years ago as with the formation of melanotic tumors (Corwin & Hanratty, 1976; Hanratty & Ryerse, 1981). Another study by Terriente-Felix *et al*. demonstrated the tumorigenic cross talk between p38 MAP Kinase and JAK-STAT that leads to lymph gland hypertrophy in a *Drosophila* myeloproliferative neoplasm model (Terriente-Felix, Perez, Bray, Nebreda, & Milan, 2017).

As for studies involving the expression of human leukemogenic proteins, perhaps the first site that comes to mind is the expression in hematopoietic system; however "ectopic expression" of these proteins has been done in some *Drosophila* studies. The logic behind expressing a leukemogenic protein in a non-hematopoietic tissue is the ease of tissue accessibility, the previous knowledge of the tissue of expression as well as the availability of screening tools (Boulet et al., 2018). Interestingly, the first oncogene to be studied in *Drosophila* was *BCR-ABL1* by Fogerty *et al.* (Fogerty et al., 1999). This was followed with AML and other models highlighted in Table 1 along with the transgene expressed and the phenotypes obtained. It is noteworthy to mention that these studies all expressed the human oncoprotein responsible for the leukemia type in hematopoietic or non-hematopoietic tissues. However, none of these studies reported using the model as a drug screening model.

Leukemia type	Transgene expressed	Phenotypes	Reference
Chronic myeloid leukemia(CML) /Ph^+ acute lymphoblastic leukemia (ALL)	Chimeric human/fly *BCR-ABL1* P210 and P185	- BCR-ABL1 causes CNS and eye defects and increases the phosphorylation of the *Drosophila* abl substrate Enabled (Ena).	(Fogerty et al., 1999)
	Chimeric human/fly *BCR-ABL1* P210 and P185	-BCR-ABL1 expression during embryonic development leads to lethality and disruption of morphogenesis (disruption of head involution, segment grooves and dorsal closure).	(T. L. Stevens et al., 2008)
	Human *BCR-ABL1^{P210}*	-BCR-ABL1 expression in *Drosophila* eyes results in altered differentiation and interferes with dAbl signaling -Expression in medullary zone of lymph gland results in increased circulating hemocytes	(Bernardoni et al., 2019)
	Human *BCR-ABL1^{P210}* and T315I	-T315I resulted in a more severe rough eye phenotype -The model was validated for drug screening by feeding flies TKIs	(Al Outa et al., 2019)
ALL/AML	Mixed lineage leukemia (*MLL*), *MLL-AF9*, and *MLL-AF4*	- MLL-AF9, and MLL-AF4 cause larval/pupal lethality upon expression in blood lineage and during early and late development -The fusions showed differing effects on proliferation and chromosome condensation in larval brain	(Muyrers-Chen et al., 2004)
AML	Human *AML1-ETO*	-AML1-ETO acts as a transcriptional repressor of Lozenge target genes in *Drosophila* eyes.	(Wildonger & Mann, 2005)
	Human *AML1-ETO*	-Expression of human AML1-ETO in Lz^+ blood cells inhibited the differentiation of crystal cells, and induced an increase in circulating Lz^+ progenitors.	(Osman et al., 2009)

		- Identification of calpain B as required for AML1-ETO activity in *Drosophila* hemocytes	
	Human *AML1-ETO*	- Expression in majority of circulating hemocytes using (*hml*-Gal4) increased hemocytes count along with expansion of hemocytes progenitors.	(Sinenko et al., 2010)
	Human Myeloid leukemia factor (MLF1)	- *Drosophila mlf* appeared to play a role in RUNX1-ETO stabilization - Human MLF1 expressed under the control of *lz*-Gal4 reversed *mlf*-associated crystal cell defects	(Bras et al., 2012)
	Human *AML1-ETO*	- *In vivo* RNAi in *Drosophila* expressing human AML1-ETO identifies Pontin/RUVBL1 as a gene responsible for AML1-ETO-induced lethality and blood cell proliferation.	(Breig et al., 2014)
	Human *NUP98-HOXA9 (NA9*)	-Expression of NA9 in *Drosophila* cortical zone of lymph gland and circulating hemocytes results in increased cellular proliferation and enlargement of posterior signaling center.	(Baril et al., 2017)
Human T-cell lymphotropic virus type 1 (HTLV-1)-induced adult T-cell leukemia/ lymphoma	*HTLV-1* Tax transactivator (*Tax-1*)	- Expression of Tax-1 in *Drosophila* eyes under the control of GMR-GAL4 results in a rough eye phenotype - Expression of Tax-1 in *Drosophila* hemocytes under the control of peroxidasin-GAL4 results in increased hemocytes number - Relish of the IMD pathway reversed the rough eye phenotype through an RNAi-based screen.	(M. Shirinian et al., 2015)

Table 1. Summary of studies expressing human leukemogenic proteins in *Drosophila melanogaster.* (Al Outa and Abubaker *et al*., review in preparation).

D. *Drosophila* as an Anti-cancer Drug Screening Model

1. The Traditional Cancer Drug Discovery Process

In the pipeline of anti-cancer drug discovery process, two major approaches are followed: the "target-based" and the "phenotypic". In the target-based, the criteria for selecting drug hits involve compounds that show efficacy against specific targets that are employed in cancer initiation and progression. The effects on the targets is evaluated by combining small molecule libraries screening, protein structure-based design assisted by computers along with cellular or biochemical assays. Compounds identified as hits undergo further evaluation using pre-clinical animal and cellular models whilst analyzing the effect of the target molecule. On the other hand, the phenotypic evaluation comprises identifying compounds against a panel of cancer cell lines and then testing in mice tumor models. The main concern with cancer cell lines is that they fail to recap the molecular heterogeneity or mechanisms of tumor resistance. This is also extended to three-dimensional *in vitro* cultures which recap most of the cellular contact and extracellular matrix properties but still cannot provide the proper physiological environment. *Drosophila* on the other hand provides a highly efficient *in vivo* model for high-throughput screening whilst recapping the surrounding tumor microenvironment (Richardson, Willoughby, & Humbert, 2001)

2. Drosophila Contribution to Anti-Cancer Drug Discovery

Drosophila research granted the field of anti-cancer drug development important findings that can be classified into two major approaches: Firstly, with fundamental research, *Drosophila* contributed to the discovery of new proliferation genes that acted as potential targets in anti-cancer therapy such as Aurora kinases and pathways involving: *Notch, Hippo, Hh* (*Sonic Hh*), *and Wingless* (*Wnt*). Whereby discovery of the gene roles in *Drosophila* preceded the link of the human homologues to cancer (Gonzalez, 2013). The second approach is *Drosophila*'s direct contribution through modeling of human diseases and using these models for drug screening. Although fruit flies have been used for decades as models for human diseases, their use as drug screening models is new. Neurodegenerative disease models in *Drosophila* (S. Chang et al., 2008; Desai et al., 2006; Pandey & Nichols, 2011) were the first to be tested for drug screening and then *Drosophila* cancer models were either used for discovery of new drugs or for re-purposing of FDA approved ones to treat different pathologies. As an example is the study using *Drosophila* larvae Ras-driven tumor model for high-throughput screening which identified the glutamine analogue, acivicin, as a tumor inhibitor in an adapted 96-well plate chemical screening (Willoughby et al., 2013). Another example is the previously described *Drosophila* eye RET-driven cancer model that helped in validating the effects of vandetanib in a whole animal organism; this time the oncogene *Ret* was directed to epithelial cells in *Drosophila* and used as a drug screening platform. AD5 was identified in this model as a drug that can recue *Drosophila* Ret-driven lethality (Dar, Das, Shokat, & Cagan, 2012). Markstein *et al*.

went further to demonstrate the response of *Drosophila* stem cell tumors to drug screening using the fruit fly intestine as a source of stem cells. This study highlighted the fact that drugs acting on *Drosophila* tumor stem cells affect the cells microenvironment and could lead to the hyperproliferation of their wild type counterparts which fuels tumor recurrence; thus shedding the light on the need to combine stem cell targeting drugs with drugs that have other targets for optimal therapy (Markstein et al., 2014). This finding was also elucidated in a mouse breast cancer model whereby chemotherapeutics were targeting cancer cells but at the same time insinuating the production of TNF-α in surrounding cells which fuels cancer cells survival later on (Acharyya et al., 2012). *Drosophila* has been also used as an *in vivo* model to identify radiation sensitizers that can be combined with ionizing radiation to effectively target cancer cells (Gladstone & Su, 2011). Another important aspect of *Drosophila* contribution to anti-cancer drug discovery is a study that utilized fruit flies for selecting personalized treatments. In this study by Bangi *et al.* (Bangi et al., 2019), a *Drosophila* model was built that reflected the complexity of a colorectal cancer patient's genomic data. The model was produced by alteration of *Drosophila* orthologs of nine genes that were identified from the patient's genomic analysis in the fruit fly hindgut using GAL4/UAS system. Screening for drug candidates that can reverse the flies lethality identified trametinib, a Ras pathway inhibitor, and zoledronate (a bisphosphonate) as drug candidates. Treating the patient with this combination resulted in a progression-free interval of three months but a partial response of the target lesions for eight months involving a maximum reduction in target lesions of 45%. This study

showcased a highly genetically complex model that can be generated using fruit flies which can help identifying personalized patients' treatments.

Collectively, the advantages of *Drosophila* as a research organism discussed earlier in genetic screening also apply to drug screening whereby the ease of handling, short life cycle, and the large brood size makes drug screening highly efficient. Moreover, the small size of *Drosophila* larvae and adults allows drug screening in the whole organism in small compartments such as vials and microtiter plates which is cost effective relative to mice husbandry whilst bypassing animal care ethical considerations.

3. Drug Delivery Concerns

The most important question to consider when administering drugs to fruit flies is the targeted developmental stage. Embryonic, larval, and adult stages can all be used in drug screening models with different approaches and degrees of hardships in drug delivery. For embryos, the major limitation is a waxy layer in the egg shell that hinders drug permeability and, therefore, dechorionation is employed to remove this outer layer and then different embryo permeabilization solvents are utilized to enhance access of drugs (Rand, Kearney, Dao, & Clason, 2010). For larvae, since they feed throughout the three larval instar stages, long exposure to the drug can be simply attained by mixing prepared drug solutions with food. Food can be either dried instant media (Willoughby et al., 2013) or containing low-melting-point agarose to allow for drug solubility at 37ºC (Markstein et al., 2014). Shorter drug exposures for larvae can be accomplished by placing the drugs in a dilute solution of yeast paste (Pandey & Nichols, 2011). Injection

is another method for drug delivery to larvae although rarely used for drug screening due to the difficulties accompanying the procedure which hinders efficiency in high-throughput screening and is mainly used in immunity studies for bacterial injections (Dionne, Ghori, & Schneider, 2003). Adult flies comprise the advantage of having a myriad of approaches for drug delivery ranging from mixing the drug with fly food or saturating a filter paper with drug/sucrose (Nichols, Ronesi, Pratt, & Sanders-Bush, 2002), presenting it as a vapor cocaine (McClung & Hirsh, 1998) and ethanol (Moore et al., 1998), injecting it through the adult abdomen for rapid distribution in the fly body (Dzitoyeva, Dimitrijevic, & Manev, 2003) or dropping it on the nerve chord which becomes exposed after decapitation (Torres & Horowitz, 1998). An important concern with oral administration of drugs is drug's taste; flies might find a certain drug unpalatable and refrain from consuming the food mixed with it. Hereby, a rewarding substance such as sucrose, banana or yeast paste can be mixed with the drug to encourage flies to eat. It is noteworthy to mention that there are also feeding assays in flies that could be followed to monitor if a drug's taste is influencing food intake (Ja et al., 2007). Although all of the described drug delivery approaches can serve the purpose of drug screening not all of them coincide with the aims of high-throughput drug screening. As a matter of fact, the most efficient is mixing the drug directly in fly food or agarose/sucrose and aliquoting into wells harboring individual flies (Pandey & Nichols, 2011).

Aside from the drug route of delivery, one should consider the optimal effective concentration that lies within the therapeutic window and the time of exposure.

The most effective approach is screening at high concentrations to observe and look out for any drug toxicity or effect and then going back to testing lower concentrations (Richardson et al., 2001). Drug concentrations that are considered to be physiologically effective can range from 0.01 mM to 100 mM as a final concentration in food with 1 mM to about 10 mM being the most widely used concentrations in studies (Pandey & Nichols, 2011). *In vivo* concentrations of course will be lower than the concentration in food and it might be necessary in some studies to determine the *in vivo* concentration employing high-performance liquid chromatography or mass spectrometry (Kuklinski, Berglund, & Ewing, 2010). Another very crucial point to take care of is the vehicle concentration in food such as dimethyl sulfoxide (DMSO) since most of the drugs are soluble in DMSO as stock solutions. It is most commonly accepted that larvae can tolerate up to 0.1% DMSO in food (Richardson et al., 2001). A study by Cvetković *et al*., identified 0.04% DMSO as the no observed effect concentration and 0.42% as the concentration that leads to 50% larval lethality (LC50) (Cvetković et al., 2015).

Collectively, the main advantage that *Drosophila* can add to the pipeline of anti-cancer drug discovery is shaping a pre-clinical whole animal *in vivo* model that can filter out from a library of drugs real efficacious hits and preclude disguised hits that often fail in subsequent tests due to toxicity or bioavailability issues. One can look at a fly model in drug testing as a model having the advantages of a cell culture screening but within a relevant physiological microenvironment.

4. Limitations of the System

a. Biological limitations

It is not surprising to have major biological differences between flies and humans which could hinder the identification of certain drugs. Taking cell cycle as an example, flies lack controllers of cell cycle such as p15-Ink4 arm in cyclinD-Cdk4 regulation and Arf-Mdm2 arm in p53 regulation. On another note, flies lack adaptive immunity, blood, and lymphatic vessels and exhibit only simple innate immunity which precludes assessment of drug effects on neo-vasculature and immunity. Moreover, certain mammalian organs do not have flies equivalents such as breast, prostate, thyroid gland, kidneys, spleen, pancreas, liver, and lungs; although, there are organs in the flies that execute similar functions. For example Malpighian tubules have similar function to kidneys (Jung, Denholm, Skaer, & Affolter, 2005), the fat body as an equivalent to adipose tissue and liver (A. C. Jung et al., 2005), and the tracheal system as equivalent to lungs (Roeder, Isermann, Kallsen, Uliczka, & Wagner, 2012). Another setback is the fact that for high-throughput screening only orally administered drugs can be assessed which strips drug screens from drugs that necessitate different routes of delivery. Finally, the differences between mammals and *Drosophila* on the level of pharmacodynamics and pharmacokinetics of drugs cuts off the chances of directly translating drugs into clinics (Richardson et al., 2001).

b. Technical limitations

While most of the published *Drosophila* studies on drug screening have been done manually, *Drosophila* drug screens can be adapted to automated systems. In the manual screening, setting up en-masse crosses can do the trick of collecting large numbers of the desired fly developmental stage (embryo, larva, adult). The main challenge remains in the subsequent steps which can be automated using robots (such as Biomek NXP, Beckman Coulter) that dispense a certain number of embryos for example on the desired drug concentration or dispense food to drug vials/wells. Therefore, technical limitations lie in the initial stages for setting up large number of crosses required to produce the embryos/larvae/adults for drug screening process; this implies huge number of virgin females that need to be crossed to males which is labor intensive since it necessitates timely collections and manual identification of males and females (Richardson et al., 2001). However, even this step is amenable to automation by using a virginizer stock whereby males have a cell death gene (*hid*) on *Y* chromosome controlled by the inducible heat-shock-promoter *hsp70*; so when larvae are heat shocked all male flies die leaving females only thereby skipping the virgining step (Mummery-Widmer et al., 2009). Another level of limitation is the identification of the effective drug concentrations and having it accessible for administration to flies. Moreover, at some instances the vehicle that is required to dissolve the drug might be toxic in a fly which precludes the whole drug screening process.

In conclusion, no study to date tested a *Drosophila melanogaster* BCR-ABL1 model for drug screening which can serve as an efficient addition to the field of CML to

help speed up the drug discovery process; which is a necessity especially regarding the emerging resistant mutations and the fact that TKIs are non-curative lifelong treatments. This kind of models can also help answer untackled questions regarding wild type and mutant BCR-ABL1 pathophysiological interactions and might provide new therapeutic targets to consider. Hence, developing this model and wisely using its advantages along with combining it to *in vitro* and *in vivo* mouse models might be the key to reach important future milestones in CML studies.

CHAPTER II

SPECIFIC AIMS

Throughout the history of CML treatment, TKIs revolutionized the therapeutic field however, their success was outshined by the emergence of resistant mutations, their inability to eradicate LSCs and therefore inability to provide a cure. Moreover, despite the fact that CML represents a malignancy with a very well-studied cytogenetic hallmark, the *BCR-ABL1* fusion gene, the mechanisms lying behind the transformative potential still partially require further investigations. Therefore, CML is still a pathology that requires a more vigilant assessment for curative therapeutic interventions and pathophysiologic interactions of its causative aberrant fusion gene (*BCR-ABL1*) especially for its arising mutations that confer resistance to standard treatments. One such simple, multicellular, and genetically tractable animal model that is exploited in recent years for modeling human diseases, including cancer, is *Drosophila melanogaster*. **Therefore, the experimental work described in this dissertation aims in overall to establish an efficient *Drosophila melanogaster* model of human BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ to serve as a credible platform for treatment and genetic screening thereby allowing for deciphering potential therapeutic targets and functional interactors**.

1. Specific aim 1: Characterize the phenotypes associated with the expression of human BCR-ABL1^{p210} and BCR-ABL1 $^{p210/T315I}$ in *Drosophila melanogaster*.

Drosophila melanogaster provides an *in vivo* animal model with a myriad of tissues that can be exploited for expressing human oncogenes and establishing an

"efficient" phenotype. The term "efficient" can hold several characteristics such as scorable, rapidly developed or easily visualized all of which make from a phenotype a suitable one for future drug/genetic screening. Therefore, we started by targeting the expression of BCR-ABL1^{p210} and BCR-ABL1 $^{p210/T315I}$ to various *Drosophila* tissues by employing the binary UAS/GAL4 system for tissue specific and temporal expression of the human transgenes, which allowed using at some instances different promoters/temperatures for expression in the same tissue in the aim of picking the suitable model for drug/genetic screening.

Objectives of specific aim 1:

a. **Generation** of transgenic *Drosophila melanogaster* lines expressing human BCR-ABL1^{p210} and BCR-ABL1 $^{p210/T315I}$.
b. **Expression** of human BCR-ABL1^{p210} and BCR-ABL1 $^{p210/T315I}$ in different *Drosophila* tissues/organs: compound eyes, wings, hemocytes, and ubiquitously.
c. **Quantification** and **assessment** of the severity of the obtained phenotypes upon expression of human BCR-ABL1^{p210} and BCR-ABL1 $^{p210/T315I}$ in the selected *Drosophila* tissues.

2. Specific aim 2: Test the sensitivity of the characterized phenotypes to treatment screening with clinically approved TKIs.

After establishment of the different phenotypes obtained upon human BCR-ABL1^{p210} and BCR-ABL1 $^{p210/T315I}$ expression; the phenotypes were tested for their sensitivity to treatment screening using approved TKIs for CML treatment (imatinib,

nilotinib, dasatinib, and ponatinib). This aim is based on the notion that if the model is to be used for deciphering new potential therapeutics it should show sensitivity to approved TKIs. Once we started, there were no studies modeling CML in *Drosophila* or administering CML approved TKIs to flies; this implied optimization of the drug screening procedure for optimal efficacy and credibility of the model was the first step to embark on.

Objectives of specific aim 2:

a. **Identification** of the optimal dose and toxicity for each TKI to be used. Since TKIs are solubilized in DMSO as stock solutions, we had to make sure DMSO is not toxic to flies. As a matter of fact we found two studies in the literature that studied the effect of DMSO on flies (Cvetković et al., 2015; Nazir, Mukhopadhyay, Saxena, & Chowdhuri, 2003) which aided in the choice of the maximal percentage of DMSO to be tried for each drug.

b. **Discernment** of the appropriate temperature and promoter to be used for drug screening. Since UAS/GAL4 system is used for specific tissue expression of the transgenes, this meant we had control over the severity of the phenotype produced in each tissue. For optimal drug sensitivity we investigated which promoter and which temperature would be optimal for our study.

3. Specific aim 3: Perform genetic epistasis experiments to identify potential genes whose knockdown is associated with phenotypic changes.

After establishing the drug screening model, we aimed to test if this model is amenable to genetic screening as well which could broaden the applications of the model and help decipher potential CML therapeutic targets by knocking down certain genes that might play a role in aggravating or attenuating the obtained phenotypes; especially with the advent of genetic tools in *Drosophila*. We employed RNA interference (RNAi) as a gene silencing method and the UAS/GAL4 system to knock down the gene of interest specifically in a BCR-ABL1 expressing tissue.

Objectives of specific aim 3:

a. **Generation** of a viable and fertile screening line of *Drosophil*a that expresses human BCR-ABL1^{p210} or BCR-ABL1 $^{p210/T315I}$ in the desired tissue driven by the specific GAL4 promoter to be crossed to fly lines carrying RNAi constructs targeting the gene of interest.

b. **Assessment** of the aggravating or attenuating effect of the knockdown of gene of interest on the phenotypes studied.

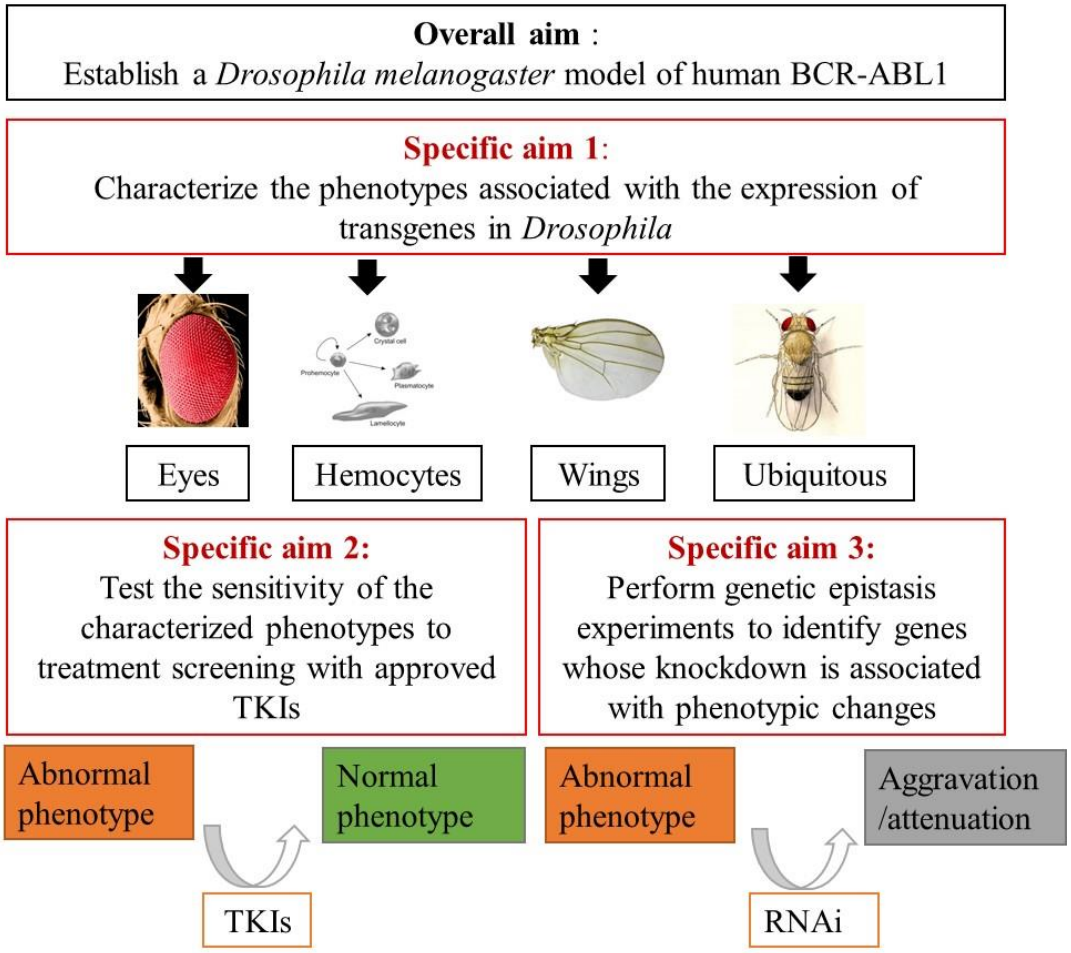


Figure 9. Schematic diagram summarizing the aims of the dissertation. The overall aim of this dissertation is establishing a *Drosophila melanogaster* model of human BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ to serve as a platform for drug and genetic screening.

CHAPTER III

MATERIALS AND METHODS

Fly work was done following institutional guide for the care and use of laboratory animals whereby there is no need for Institutional Animal Care and Use Committee (IACUC) review/approval for research work that does not involve vertebrate animals.

A. *Drosophila* Culture Media

1. Standard Agar-Based Culture Medium

Fly stocks were maintained in a 25°C incubator with 12-hour (hr) light-12-hr dark cycle and 60-70% humidity on standard agar medium that was prepared by mixing 45 g of polenta, 5 g of mashed potato, 11 g of yeast, and 3.3 g of agarose with 45 mL of molasses in 600 mL of distilled water, heated in a microwave three times for three minutes each and then allowed to cool before adding 4 mL of propionic acid as an antimicrobial under a chemical fume hood. Food is then poured into polystyrene *Drosophila* vials or bottles, covered with a mesh, and allowed to solidify at room temperature overnight.

2. Culture Media for Treatment Groups

Treatment groups involve two different phenotypes based on the promoter used to drive the expression of BCR-ABL1. The first phenotype is the eye phenotype and the

second is the lethality phenotype. For experiments involving the addition of drug to food, flies were kept at 18°C incubator (eye phenotype analysis) or at 25°C (lethality phenotype analysis) with 12-hr light-12-hr dark cycle and 60-70% humidity on a special instant *Drosophila* medium (Carolina Biological) that is blue in color to allow visualization of food inside flies and larvae abdomen indicating food intake. This type of food does not require heating which eliminates the possibility of drug decomposition. The drug is dissolved in a water solution and then an equal volume of dry instant food is placed inside a vial. The drug solution is then poured gradually to allow reconstitution of the powder inside the vial. For eye phenotype analysis females were allowed to lay eggs after mating directly on the surface of the prepared food vial mixed with drug whereas for lethality phenotype analysis, eggs laid after mating were collected first on grape juice plates and then a known number of eggs was transferred to the surface of food vial containing the drug.

a. Grape juice plates for embryo harvesting

The medium is set up by preparing a 3% agar solution in grape juice and mixing very well before boiling the mixture in a microwave until obtaining a clear grape juice solution where all agar is dissolved then after slight cooling, the mixture is poured into small plates, allowed to solidify at room temperature and then stored for later use at 4°C.

B. Setting Up *Drosophila* Mating Crosses

For all crosses mentioned in this dissertation, virgin female flies were collected according to certain criteria including: emptying a bottle of the desired genotype the evening before the intended day for virgin flies collection, flies were then subsequently collected early the next morning and every 2-3 hours during the day and virgins were identified based on the presence of a dark spot in their distended abdomens which is the last meal the larvae had before formation of pupae. Virgin females are bigger than non-virgin females and have lighter body pigmentation as well. Virgin females from a desired genotype were mated to males from another desired genotype by placing them in a food vial with a pinch of dry yeast and allowed to mate on the desired temperature until the observation of egg laying on the surface of food, then parents are discarded or flipped to new vials and F1 progeny were allowed to develop and timely collected.

C. Generation of Transgenic *Drosophila* Strains

Transgenic flies, harboring the human BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315l}$ were generated using the Phi C31 integrase system, a site-specific integration system (Groth, Fish, Nusse, & Calos, 2004), and were inserted on the third chromosome for GAL4-UAS expression. Myc tag was added at N-terminus to identify expression of the BCR-ABL1 protein. BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ were inserted into pUAST-attB *Drosophila* expression vector (custom DNA cloning). The sequence of the pUASTattB vector can be found in the GenBank data base under the accession number EF362409. pUAST-attB-myc BCR-ABL1 ^{p210} and pUAST-attB-myc BCR-ABL1

$^{p210/T315l}$ were injected into y1 w67c23; P(CaryP) ABLattP2 (8622 BDSC) embryos in order to generate transgenic flies (BestGene Inc, Chino Hills, CA).

D. Induction of Cell and Tissue-Specific Transgenes Expression: GAL4-UAS System

For the targeted expression of BCR-ABL1 in *Drosophila* cells, the binary GAL4-UAS system was used. Flies carrying the UAS-BCR-ABL1 constructs (Table 2) were crossed to flies carrying GAL4 drivers (Table 2). The GAL4-UAS binary system utilizes the yeast protein (GAL4) which acts as a transcription activator and is very well characterized (Ptashne, 1988). GAL4 binds to upstream activating sequences (UAS) to drive the ectopic expression of a transgene in a spatial and temporal manner. UAS is a weak promoter that is incapable of driving the expression of the transgene in the absence of GAL4. The GAL4 and UAS sequences will initially be present in two separate transgenic lines. In the GAL4 transgenic line, we can find the activator protein (GAL4) next to the tissue /cell promoter, however, there is no transgene to be activated (Driver strain). In the UAS line (Responder strain), the transgene to be activated is present fused to UAS (GAL4 binding sites) but in a silent state in the absence of the activator (GAL4). Upon crossing the two lines, the promoter will target the expression of GAL4 in the progeny in a tissue/cell specific manner then GAL4 will bind to UAS and drive the expression of the transgene in the specified tissue (Phelps & Brand, 1998) (Figure 10).

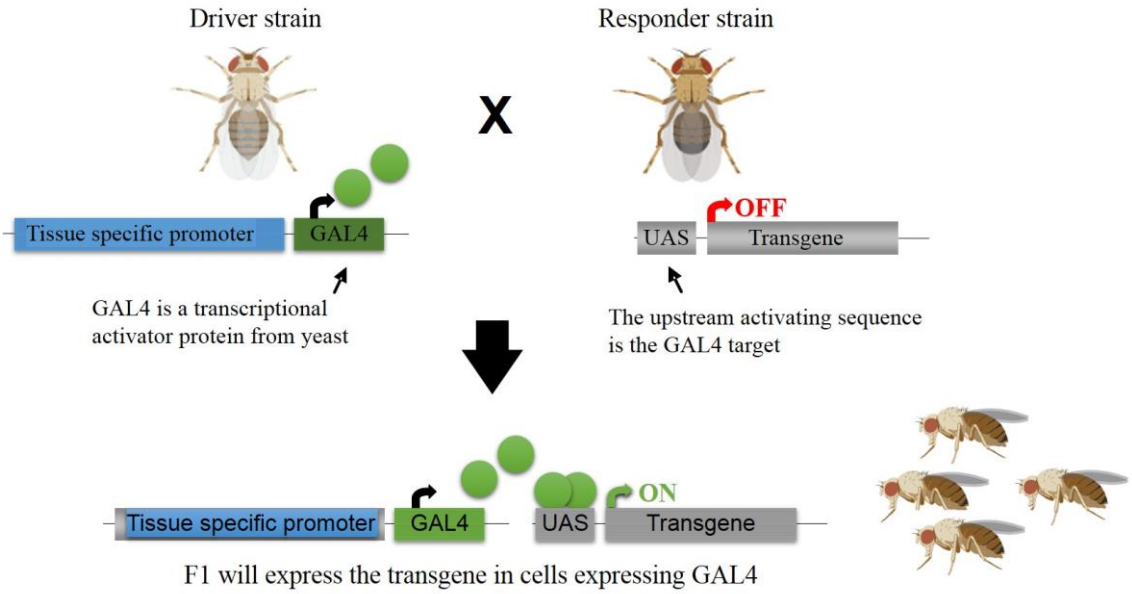


Figure 10. The fly GAL4-UAS binary transgenic expression system. The GAL4 and UAS sequences will initially be present in two separate transgenic lines. Upon crossing the two lines, the promoter will target the expression of GAL4 in the progeny in a tissue/cell specific manner then GAL4 will bind to UAS and drive the expression of the transgene in the specified tissue (Flies cartoon from Biorender.com).

E. RNAi-Mediated Knockdown of Genes:

Knockdown of potential genes in the desired BCR-ABL1 expressing tissue was done through combining the RNAi gene knockdown with the transgenic GAL4-UAS system in *Drosophila*. This method has the advantage of spatial and temporal control of the gene knockdown (Perrimon, Ni, & Perkins, 2010) (Figure 11). For this, we have prepared a viable and fertile *Drosophila* line expressing BCR-ABL1^{p210} or BCR-ABL1$^{p210/T315I}$ in *Drosophila* eyes under the control of the eye specific promoter GMR-GAL4. Virgin females from this line were then crossed to males carrying UAS-RNAi (of gene of interest) and F1 progeny were examined for lethality or modification of the eye

phenotype. Control crosses consisted of mating flies from BCR-ABL1 screening line with control RNAi line.

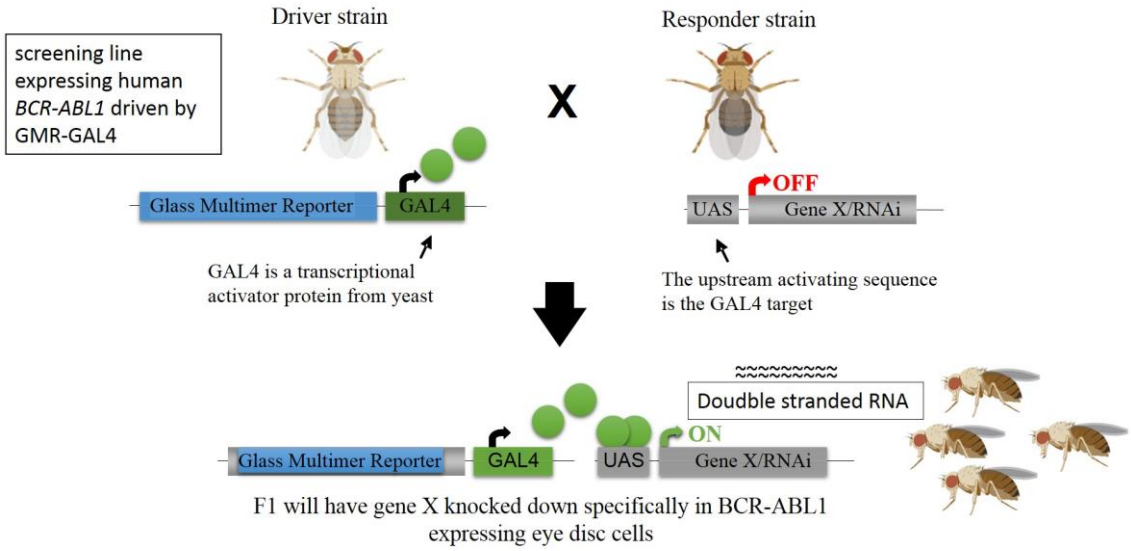


Figure 11. Schematic representation of the spatio-temporal control of RNAi-mediated knockdown of genes in *Drosophila*. Combining the RNAi gene knockdown with the transgenic GAL4-UAS system in *Drosophila***.** A viable and fertile *Drosophila* line expressing BCR-ABL1^{p210} or BCR-ABL1$^{p210/T315I}$ in *Drosophila* eyes under the control of the eye specific promoter GMR-GAL4 will be crossed to males carrying UAS-RNAi of gene of interest. (Flies cartoon from Biorender.com).

F. Fly Strains

Table 2 shows the different fly strains used throughout this study. Virgin females from GAL4 strain were crossed to males with UAS constructs. In all mating schemes the control cross was w^{1118} flies crossed to the specified GAL4 driver strain. Fly strains were obtained from Bloomington Drosophila Stock Center (BDSC).

Fly strain	Genotype	Description (Thurmond et al., 2018) FB2019_02
w^{1118} (*white*)	w^{1118}*;+;+*	Reference strain carrying a recessive mutation in the X chromosome that causes the absence of eye pigmentation causing white colored eyes
GMR-GAL4 (BDSC 1104)	*w*;GMR-GAL4;+*	Driver construct inducing the expression of the Gal4 protein under the control of the of *glass multimer reporter* (*gmr*) gene promoter to induce ectopic expression of target genes during larval development in the developing eyes and specifically in cells that lie behind the morphogenetic furrow
Sevenless (sev)-Gal4 (BDSC 5793)	w^{1118}*;sev-GAL4;+*	Driver construct inducing the expression of the Gal4 protein under the control of the *sevenless* (*sev*) gene promoter to induce ectopic expression of target genes during larval development in photoreceptors R3, R4, R7, and the cone cells and weakly in R1 and R6 in the eye imaginal disc
MS1096-Gal4 (BDSC 8860)	w^{1118}*MS1096-GAL4;+;+*	Driver construct inducing the expression of the Gal4 protein under the control of the of *MS1096* gene promoter to induce early ectopic expression of target genes in the dorsal wing pouch
Hemolectin Δ -GAL4 (BDSC 30140)	w^{1118}*; Hml-GAL4,UAS-EGFP*	Driver construct inducing the expression of the Gal4 protein under the control of the of hemolectin (*hml*) gene promoter to induce ectopic expression of target genes in the majority of circulating

		hemocytes, hemocytes precursors and lymph glands.
Actin-5C-Gal4 (BDSC 4414)	*y1 w^{1118}; Act5CGAL4/CyO*	Driver construct inducing the expression of the Gal4 protein under the control of the of *actin-5C* gene promoter to induce ectopic expression of target genes ubiquitously in *Drosophila.*
Engrailed-Gal4	*y1 w* ; en-GAL4;+*	Driver construct inducing the expression of the Gal4 protein under the control of the of *engrailed* gene promoter to induce ectopic expression of target genes in the posterior compartment of embryonic segments.
UAS-BCR-ABL (BDSC 9571)	*w*; UAS-P210/CyO*	Expresses exons 1-3 of the human Breakpoint cluster region (*bcr*) gene fused to the fly abelson (*abl*) gene under UAS control.
UAS-BCR-ABL1^{p210}(Al Outa et al., 2019)	*+; +;UAS-P210*	Expresses the human Breakpoint cluster region (*bcr*) gene fused to human abelson (*abl*) gene under UAS control.
UAS-BCR-ABL1$^{p210/T315I}$ (Al Outa et al., 2019)	*+; +;UAS-P210^{T315I}*	Expresses the human Breakpoint cluster region (*BCR*) gene fused to human abelson (*ABL1*) gene harboring the T315I mutation in the kinase domain under UAS control.

Table 2. Fly strains. Fly strains that are used throughout this study are shown in this table along with their genotypes and a brief description of their expression patterns.

G. Tyrosine Kinase Inhibitors Studies

Imatinib (I-5577), nilotinib (N-8207), dasatinib (D-3307), and ponatinib (P-7022) were obtained from LC laboratories, MA, USA. Stock solutions were dissolved in DMSO and stored at -20°C and the required dilution of TKI was prepared in distilled water and added to instant *Drosophila* medium. Since DMSO is known to be toxic to

Drosophila (Cvetković et al., 2015), 0.03% DMSO was used for low TKI concentrations and 0.3% for high concentrations (eye phenotype analysis). For lethality phenotype analysis, 0.1% DMSO was the highest concentration to be used which is known to be the maximum DMSO concentration that can be tried when the end point to be assessed is lethality (Levine & Cagan, 2016).

H. Scanning Electron Microscopy (SEM)

Adult flies were fixed with 2% gluteraldehyde and 2% formaldehyde in phosphate buffered saline (PBS) (1x) overnight at 4ºC and then washed three times for 10 minutes each with PBS (1x), dehydrated with a series of increasing ethanol concentrations (30%, 50%, 70%, 80%, 90% and twice in 100%), dried with a critical point dryer (k850, Quorum Technologies), mounted on standard aluminum head covered with standard carbon adhesive tabs and coated with 20 nm layer of gold. SEM analysis was performed using a Tescan, Mira III LMU. FEG (SEM) Field Emission Gun, secondary electron detector was used for capturing the electron microscopy images. F1 progeny of each given genotype displayed essentially identical phenotypes and randomly selected representative images are shown. SEM images were processed using Adobe Photoshop CS6.

I. Scoring of Eye phenotypes and Measurement of Eye Defect Area

A grading score, that was modified from the score previously published (Margret Shirinian et al., 2015), was used for quantification of the severity of the rough

eye phenotype and involved ten different levels of increasing severity labelled from 1 to 10. The score is based on the number of ommatidial fusions, the extent of bristle organization and ommatidial loss (Table 3). For measurement of the posterior eye defect area (area with total loss of ommatidial facets), Image J (Schneider, Rasband, & Eliceiri, 2012) was used. SEM images were coded by one researcher and scoring of the rough eye phenotype or measurement of posterior eye defect area was performed by another researcher who was blinded to the images. The average scores or areas measurements were used for statistical comparison. For rough eye phenotype n=20 adult flies from each genotype at each temperature was scored and the experiment was done in triplicate. For measurement of area of eye defect in posterior end of the eye an average of n=20-30 flies from each group was quantified and the experiment was done at least two times.

Score	Criteria
0	Regular ommatidial facets and bristle organization
1	- Scattered areas of non-polarized bristle alignments - And **less than 4** scattered areas displaying fusions of ommatidial facets
2	- Scattered areas of non-polarized bristle alignments - And **10-20** fusions of ommatidial facets that are scattered or in the same area - with/without duplicated bristles

	- with/without few lens defects manifested as holes in the ommatidial facets
3	- Scattered areas of non-polarized bristle alignments - And **20-40** fusions of ommatidial facets that are scattered or in the same area - with/without duplicated bristles - with/without few lens defects manifested as holes in the ommatidial facets
4	**One large surface area** of non-polar bristle alignments and fusions of ommatidial facets of the same large area - with/without duplicated bristles - with/without few lens defects manifested as holes in the ommatidial facets
5	- Multiple non-polar bristle alignments - And one large surface area of fusions of ommatidial facets and/or duplicated bristles - with/without few lens defects manifested as holes in the ommatidial facets
6	- Multiple non-polar bristle alignments - And scattered areas of incompletely developed ommatidial facets and/or duplicated bristles - with/without lens defects manifested as holes - with/without a characteristic groove of lost ommatidial facets on the lower end of the eye
7	- Multiple non-polar bristle alignments - And one large surface area of incompletely developed ommatidial facets and/or duplicated bristles -With/without lens defects manifested as holes in the residual ommatidial facets - With/ without a characteristic groove of lost ommatidial facets on the lower end of the eye
8	- Multiple non-polar bristle alignments - And/or duplicated bristles - with total loss of ommatidial facets

	- And with/without 1 area of missing bristles
9	- Multiple non-polar bristle alignments - And/or duplicated bristles - With total loss of ommatidial facets - With more than 1 area of missing bristles
10	Few dispersed bristles across the eye with total loss of ommatidial facets

Table 3. Grading score for quantification of eye roughness. Scoring was based on the extent of ommatidia and mechanosensory bristles organization and loss.

J. Western Blot Analysis

To determine transgene expression levels, adult fly heads were homogenized in 2x Laemmli sample buffer (catalog #161-0737, Bio-Rad Laboratories). Samples were loaded on 8% SDS-polyacrylamide gel, subjected to electrophoresis and transferred to a 0.2 µm pore–size nitrocellulose membrane (catalog# #1620112, Bio-Rad laboratories). After blocking the membrane with 5% bovine serum albumin (BSA) in tris (hydroxymethyl)- aminomethane,-buffered saline with 0.05% tween-20, the blots were incubated with specific antibodies against c-ABL (SC-23, 1:1000, Santa Cruz Biotechnology, Santa Cruz, CA) and phospho-c-ABL (#2868, 1:500, Cell Signaling Technology). Proteins of interest were detected with HRP-conjugated anti-mouse (SC-2318, 1:5000, Santa Cruz Biotechnology, Santa Cruz, CA) and anti-rabbit (NA934, 1:5000 ,GE Healthcare) IgG antibody and visualized with Clarity Western ECL substrate (#1705061, Bio-Rad laboratories) detection reagent to visualize the immunoreactive bands.

K. Immunofluorescence Staining

Third instar larval eye discs were dissected in PBS (1x) with 0.3% Triton X-100 and were fixed in 4% formaldehyde for 20 minutes at room temperature. Discs were washed in PBS-Triton X-100 three times for 20 minutes each and placed in blocking solution of 5% normal goat serum (NGS) in PBS (1x) overnight at 4°C. Samples were then incubated in primary antibody diluted in blocking solution overnight at 4°C. The following antibodies were used: mouse anti-ELAV (Developmental Studies Hybridoma bank, 1:30), rabbit anti- c-Myc tag (GenScript, 10 μg/mL). The eye discs were then washed in PBS-Triton X-100 three times for 20 minutes each and incubated with fluorochrome-conjugated secondary antibodies AlexaFluor-488 anti-rabbit or AlexaFluor-594 anti-mouse for 2 hours (Abcam, 1:500). Eye discs were incubated in DAPI solution (10^{-3}mg/mL, Molecular Probes) for 5 minutes and washed in PBS-Triton X-100 three times for 20 minutes each. Finally, samples were mounted onto microscope slides with gold anti-fade solution (Invitrogen) for subsequent confocal analysis (Zeiss LSM 710). All images were acquired and analyzed using the Zeiss ZEN 9 imaging software.

L. Lethality Phenotype Analysis

Engrailed-GAL4 was used to drive the expression of BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ in posterior compartments of imaginal discs. Virgin females from engrailed-GAL4 line were crossed to males from BCR-ABL1 lines. Flies from engrailed-GAL4> *w* ^{11118} were used as control. Parent flies were allowed to mate and

females laid eggs on the surface of grape juice plate to which a thin layer of yeast solution was added on the surface. Then parents were discarded and a known number of embryos was transferred to a vial containing either 0.1% DMSO only or the desired concentration of drug mixed with food. Embryos were monitored for survival into the desired stage (Figure 12). Images of pupae or adults were taken using Olympus SZX10 stereomicroscope.

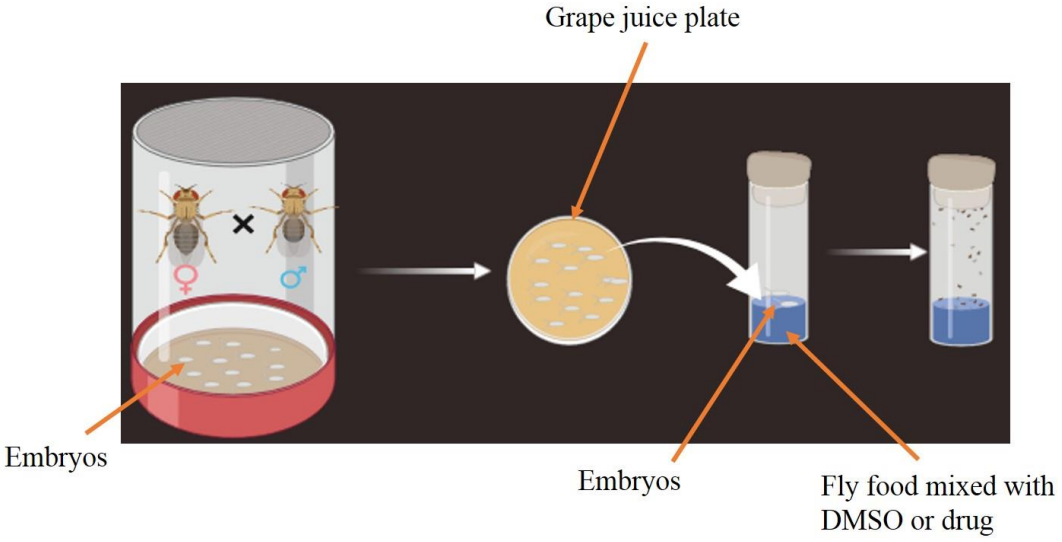


Figure 12. Procedure followed for lethality assay. Embryos expressing BCR-ABL1^{p210} or BCR-ABL1$^{p210/T315I}$ under the control of engrailed-GAL4 are transferred from grape juice plates to the surface of food mixed with either DMSO or drug and then allowed to reach the desired developmental stage. (Created with Biorender.com)

M. Hemocytes Count

To determine the hemocytes count for each genotype, wandering third instar larvae were collected by a soft brush from the walls of every genotype vial and each larva was bled using fine forceps by opening the outer cuticle gently and allowing hemolymph which contains circulating hemocytes to disperse in PBS (1x). The bleed was then transferred to a hemocytometer and cells were counted under the microscope and calculated as number of cells per 1 mL of bleed. Hemocytes numbers from 30 independent larvae from each genotype were used for analysis (Figure 13).

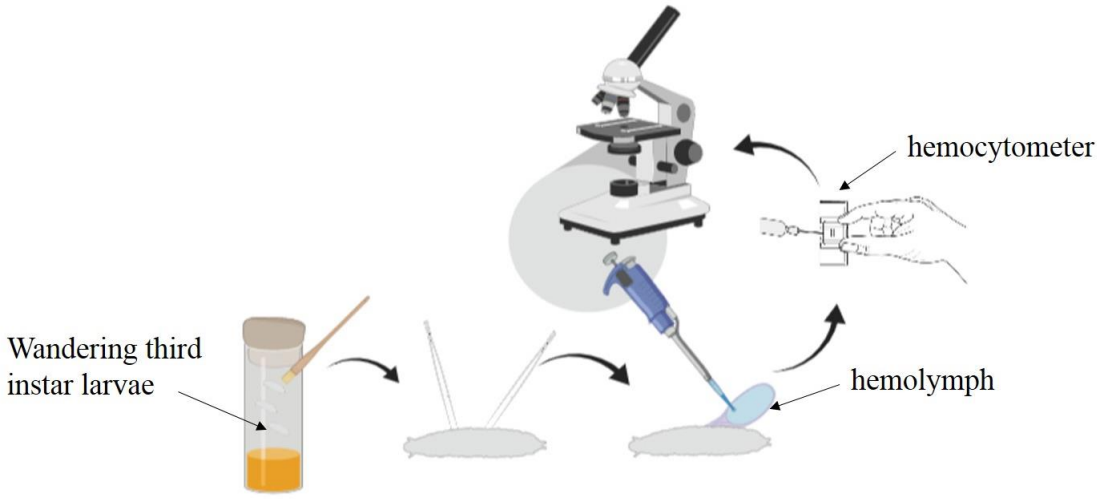


Figure 13. Procedure for counting circulating hemocytes from *Drosophila* larvae. Wandering third instar larvae are picked gently using a brush and then the outer cuticle is opened under the microscope using fine forceps then hemolymph is collected and the number of circulating hemocytes is counted using a hemocytometer. (Created with Biorender.com)

N. Statistical Analysis

The statistical significance of difference between the average scores of rough eye phenotype and average scores of posterior eye defect area was evaluated using two-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. One–way ANOVA was used when comparing averages of posterior eye defect area for dose response and hemocytes count and was followed by Tukey's multiple comparisons test. Associations with $p < 0.05$ were considered significant. Statistical tests were done using GraphPad Prism 6.0 software.

Chapter IV

RESULTS

A. Expression of Chimeric Human/Fly BCR-ABL1 in *Drosophila* Eyes Induces Transformation

Previously, Fogerty *et al*. (Fogerty et al., 1999), generated *BCR-ABL1* transgenic fly lines of human/fly chimeric BCR-ABL1 proteins encoded by 5' human P210 BCR-ABL1 sequences fused to 3' fly *Abl* sequence. They included *Drosophila Abl* carboxy-terminal amino acids because it is required for proper localization of Abl protein to the axon bundles (Figure 14).

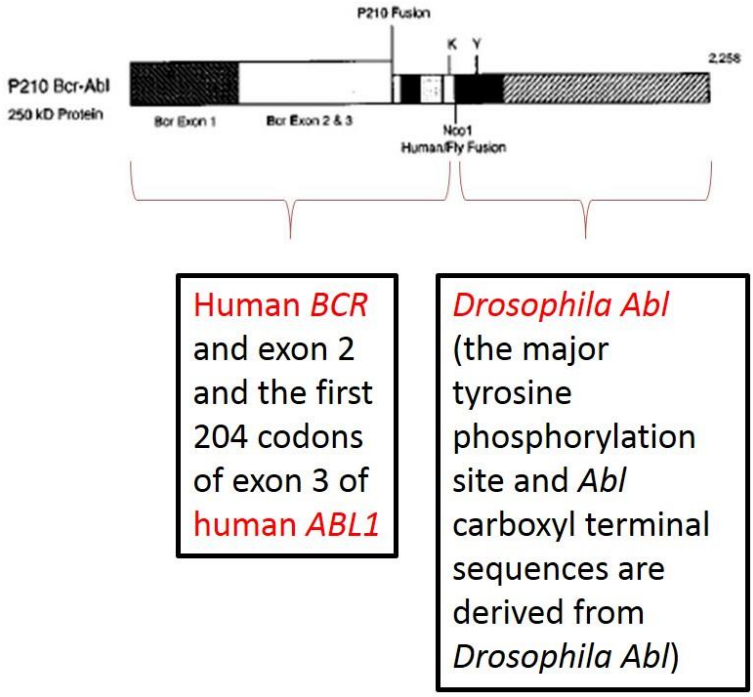


Figure 14. **Human/*Drosophila* chimeric *BCR-ABL1*^{p210}.** The human/fly chimeric P210 is shown. *BCR* exons 1 – 3 are fused to human *ABL1* exon 2. The first 204 codons of human *ABL1* exon 3 are fused to *Drosophila abl* sequence. Modified from (Fogerty et al., 1999).

1. Targeted-Expression of Chimeric BCR-ABL1 Using Sev-Gal4 and GMR-GAL4 Results in Severe Rough Eye Phenotype

We started with replicating Fogerty *et al*. results with sev-GAL4 promoter and in addition we tried the GMR-GAL4 promoter which produces more severe phenotypes. GMR-GAL4 and sev-GAL4 drive the expression of BCR-ABL1 in different sites in the eye and hence produce phenotypes with differing severities. GMR-GAL4 drives the expression in all differentiating photoreceptor cells posterior to the morphogenetic furrow (Freeman, 1996) and sev-GAL4 drives the expression in a subset of photoreceptors mainly R7 (Ray & Lakhotia, 2015). *GMR-GAL4>w^{1118}* or sev-GAL4>*w^{1118}* flies were used as a control. GAL4-UAS system is temperature sensitive which allowed us to control BCR-ABL1 expression levels (Duffy, 2002). Therefore, crosses were performed at 18ºC, 25ºC, and 29ºC allowing for a reciprocal increase in transgene expression upon increased temperatures. Eclosed flies were imaged using light microscopy and SEM. *Drosophila* eyes expressing chimeric human/fly BCR-ABL under the control of sevenless-GAL4 promoter at 25°C resulted in severe transformation of the eye tissue similar to what Fogerty *et al*. described and was characterized by almost complete loss of ommatidial facets, misplaced mechanosensory bristles which are duplicated at some instances, ommatidial fusions and holes in ommatidial facets which indicate lens defects (Figure 15). The severity of the phenotype increased at 29°C to include loss of more ommatidial facets and mechanosensory bristles. Expression under the control of GMR-GAL4 at 25°C and 29°C resulted in a more severe phenotype than sev-GAL4 and was characterized by loss of eye tissue completely leaving a scar.

This prompted us to try a lower temperature (18°C) with GMR-GAL4 which did show restoration of the eye tissue but with major disruption of its development. The eye showed complete loss of ommatidial facets and misplaced /duplicated mechanosensory bristles (Figure 16).

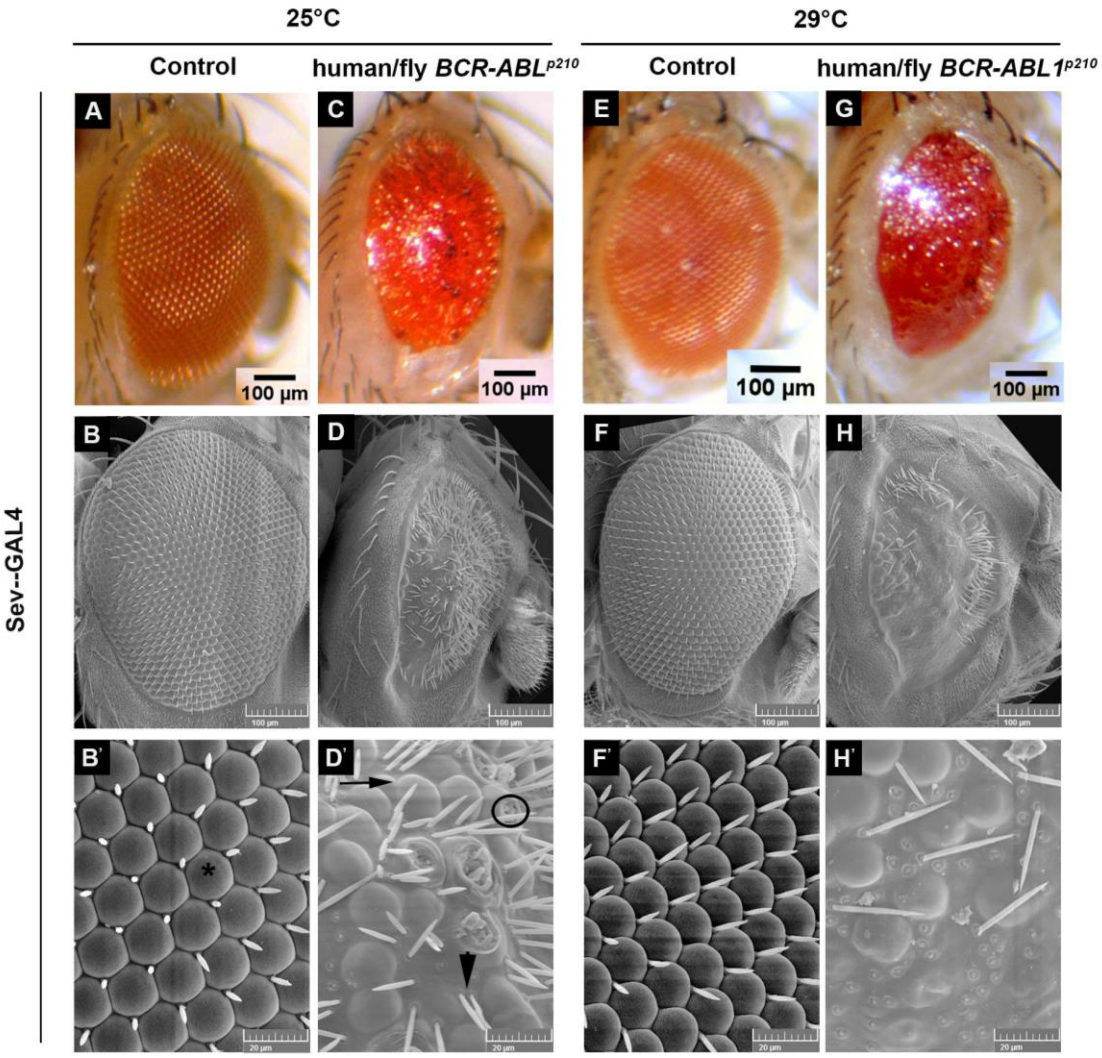
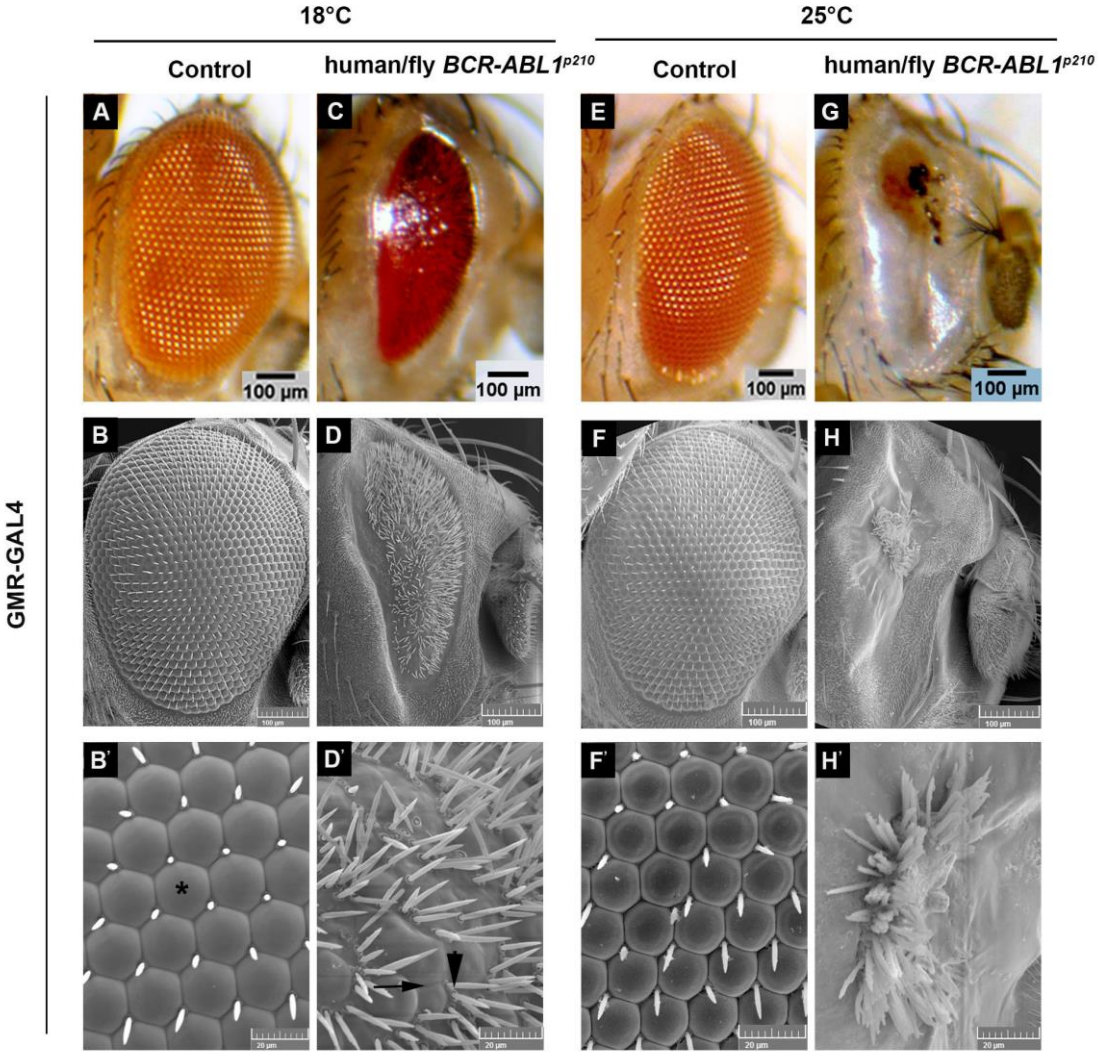


Figure 15. **Rough eye phenotype induced by overexpression of chimeric human/fly BCR-ABL1^{p210} using sev-GAL4**. Light (A, C, E, G,) and scanning electron (B-B', D-D', F-F', H-H') micrographs of adult *Drosophila melanogaster* compound eyes from control or chimeric human/fly BCR-ABL1^{p210} expressing eyes. Flies were raised on 25ºC (A-B-B', C-D-D') or 29ºC (E-F-F', G-H-H'). B', D', F', and H' are high magnification of the centermost region in B, D, F, and H, respectively (1,370 x). Ommatidial facets are depicted in (B') by (*), misplaced mechanosensory bristles depicted by arrowheads, lens defects by circle and ommatidial fusions are shown by arrow in (D'). Sev-GAL4>w^{1118} flies were used as a control. Posterior is to the left.


18°C
25°C
Control
human/fly BCR-ABL1p210
Control
human/fly BCR-ABL1p210
GMR-GAL4
A
C
E
G
B
D
F
H
B'
D'
F'
H'
100 µm
100 µm
100 µm
100 µm
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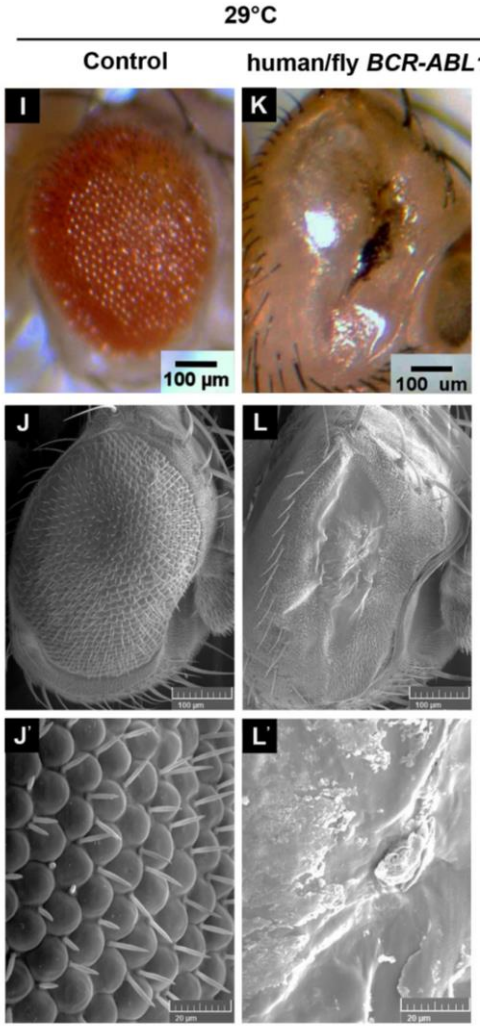


Figure 16. **Rough eye phenotype induced by overexpression of chimeric human/fly BCR-ABL1^{p210} using GMR-GAL4**. Light (A, C, E, G, I, K) and scanning electron (B-B', D-D', F-F', H-H', J-J', L-L') micrographs of adult *Drosophila melanogaster* compound eyes from control and chimeric human/fly BCR-ABL1^{p210} expressing eyes. Flies were raised on 18ºC (A-B-B', C-D-D'), 25ºC (E-F-F', G-H-H') or 29ºC (I-J-J', K-L-L'). B', D', F', H', J' and L' are high magnification of the centermost region in B, D, F, H, J, and L, respectively (1,370 x). Ommatidial facets are depicted in (B') by (*), misplaced mechanosensory bristles depicted by arrowheads, and ommatidial fusions are shown by arrow in (D'). *GMR-GAL4>w^{1118}* flies were used as a control. Posterior is to the left.

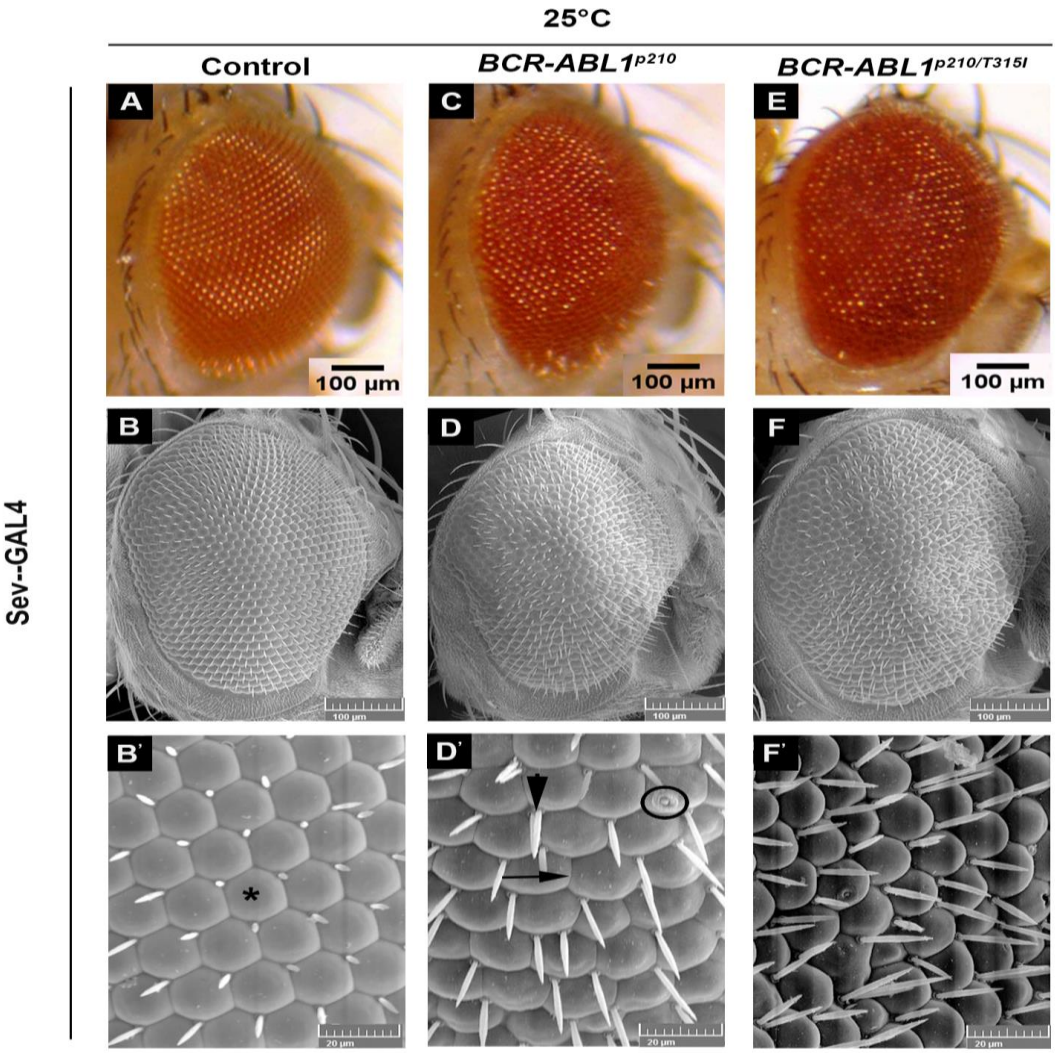
B. Expression of Full Human BCR-ABL1 in *Drosophila* Eyes Induces Transformation

To assess the transformative potential of human BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ in *Drosophila*, we started by expressing the transgenes in the fly compound eye using different eye drivers and evaluations of phenotypes were performed using a grading score (Table 2) which categorized the severity of the phenotype based on the extent of mechano-sensory bristles alignment, misplacement, and duplication as well as the extent of ommatidial facets loss indicating disruption in cellular proliferation and differentiation collectively defining interrupted normal development (Xin, Weng, Xu, & Du, 2002).

1. Targeted-Expression of Human BCR-ABL1 Using Sev-Gal4 Results in Mild Rough Eye Phenotype

The expression of human BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ in *Drosophila* eyes using sev-Gal4 resulted in mild transformation of the eye tissue. The phenotype was characterized with mild ommatidial fusions and few duplicated/misplaced mechanosensory bristles along with few ommatidial holes indicating lens defects (Figure 17) at both temperatures 25°C and 29ºC. Scoring the results reflected the described phenotype whereby no significant difference was shown between the two temperatures for BCR-ABL1^{p210} (average roughness: 2.6 at 25°C and 2.8 at 29°C) and BCR-ABL1$^{p210/T315I}$ (average roughness: 2.8 at 25°C and 29°C) as well as there was no difference between BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ at each

temperature (Figure 18). This prompted us to proceed with GMR-GAL4 since the phenotype with sev-GAL4 would be too mild to show any difference if to be used as the drug screening platform.



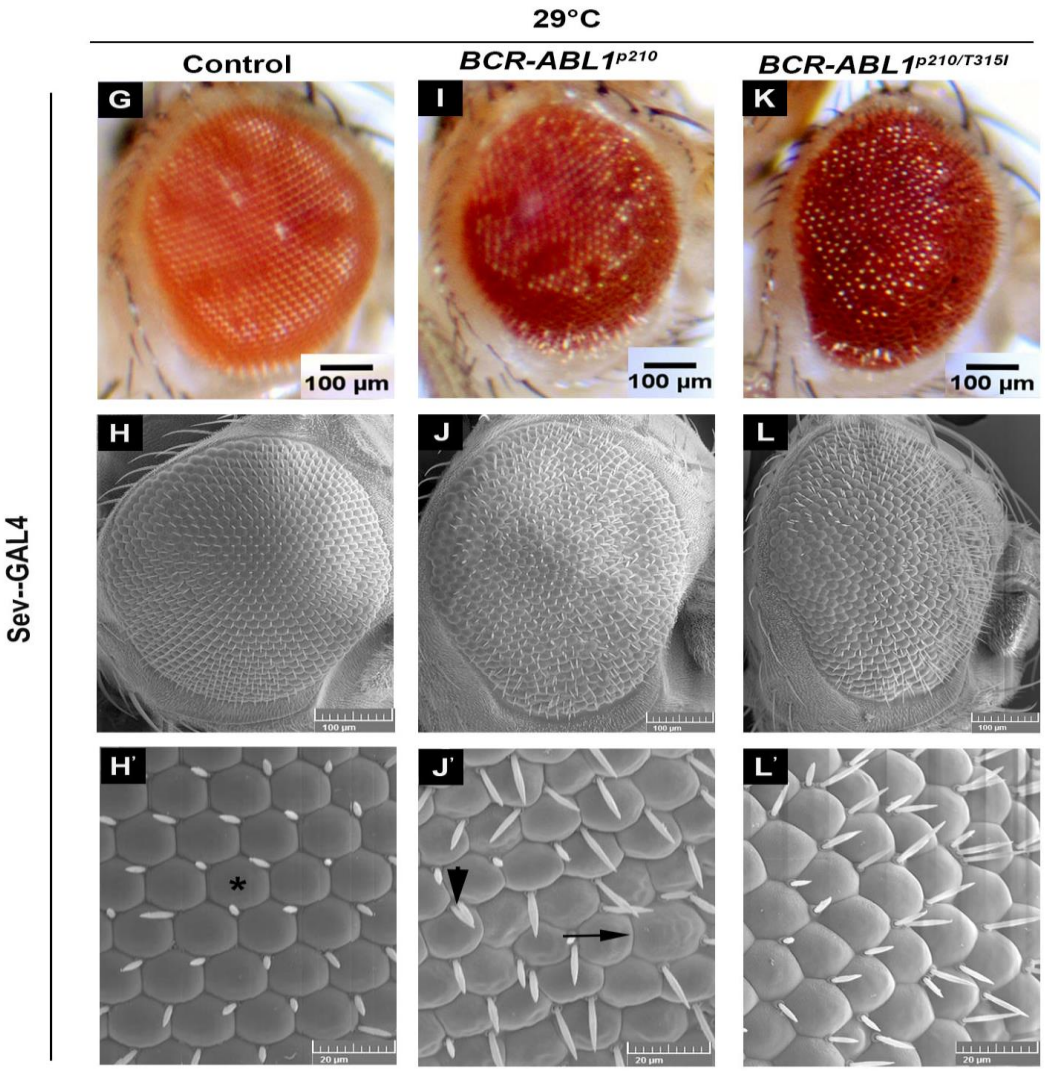


Figure 17. Rough eye phenotype induced by overexpression of human BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ using sev-GAL4. Light (A, C, E, G, I, K) and scanning electron (B-B', D-D', F-F', H-H', J-J', L-L') micrographs of adult *Drosophila melanogaster* compound eyes from control, human BCR-ABL1^{p210} or BCR-ABL1$^{p210/T315I}$ expressing flies. Flies were raised on 25ºC (A-B-B', C-D-D', E-F-F') or 29ºC (E-F-F', G-H-H', I-J-J', K-L-L'). B', D', F', H', J', and L' are high magnification of the centermost region in B, D, F, H, J, and L, respectively (1,370 x). Ommatidial facets are depicted in (B') by (*), misplaced mechanosensory bristles depicted by arrowheads, lens defect by circle and ommatidial fusions are shown by arrow in (D'). *Sev-GAL4>w^{1118}* flies were used as a control. Posterior is to the left.

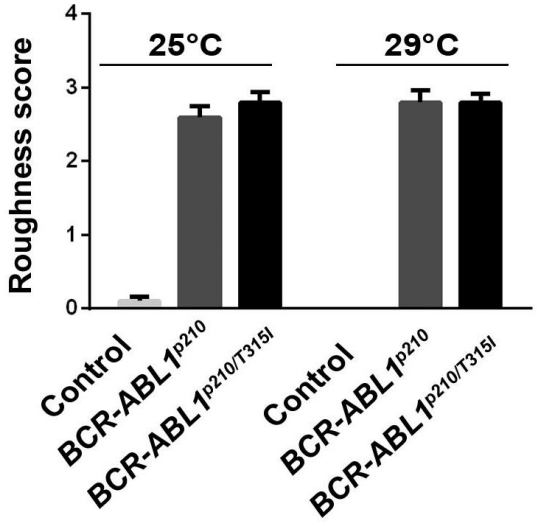


Figure 18. Roughness score of human BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ expressing eyes using sev-GAL4. Grading score was used for quantification of the eye roughness. Data represent mean ± SEM.

2. Targeted-expression of Human BCR-ABL1 Using GMR-Gal4 Results in Severe Rough Eye Phenotype

We opted to test the expression of human BCR-ABL1 under the control of GMR-GAL4 since sev-GAL4 resulted in a very mild phenotype. At 18ºC, BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ flies exhibited a rough eye phenotype characterized by ommatidial fusions and areas of lost ommatidial facets, particularly at the posterior end of the eye, as well as multiple ectopic mechano-sensory bristles which are duplicated at some instances. At 25ºC, a more severe rough eye was observed in both BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ with loss of the majority of ommatidial facets. At 29ºC, the severity increased to involve loss of the majority of mechano-sensory

bristles in addition to the total loss of ommatidial facets in both BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ expressing flies (Figure 19, Figure 20). The average roughness of BCR-ABL1^{p210} significantly increased from 6.2 at 18ºC to 8.2 ($P < 0.0001$) at 25ºC and to 9.5 ($P < 0.0001$) at 29ºC (Figure 19). As for BCR-ABL1$^{p210/T315I}$, the average roughness significantly increased from 6.6 at 18ºC to 8.9 ($P < 0.0001$) at 25ºC and to 10 ($P < 0.0001$) at 29ºC (Figure 20). Western blot analysis confirmed the expression and phosphorylation of BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ in *Drosophila* eyes (Figure 21).

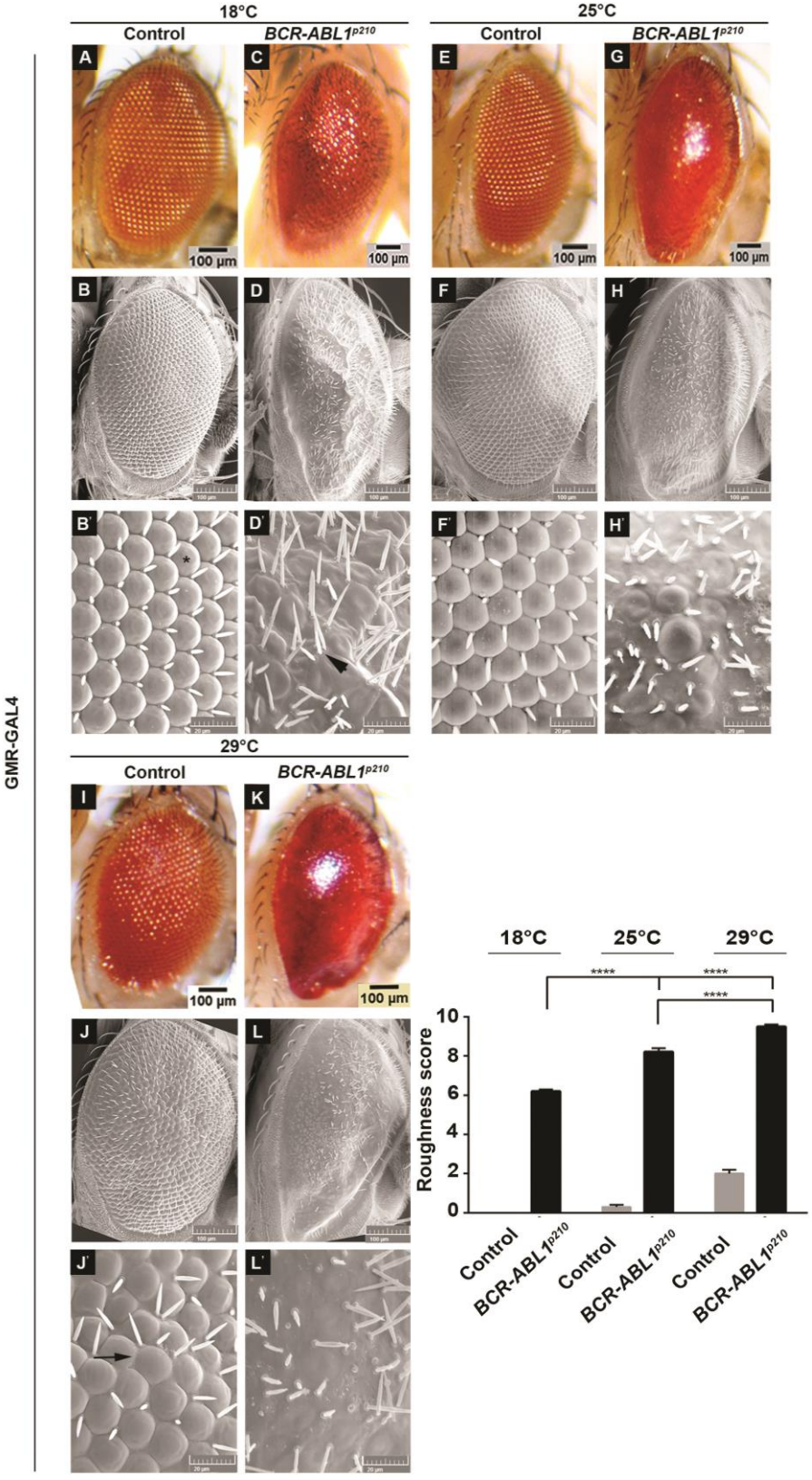

GMR-GAL4
18°C
Control
BCR-ABL1p210
25°C
Control
BCR-ABL1p210
29°C
Control
BCR-ABL1p210
A
C
E
G
B
D
F
H
B'
D'
F'
H'
I
K
J
L
J'
L'
100 µm
18°C
25°C
29°C
****
****
****
Roughness score
10
8
6
4
2
0
Control
BCR-ABL1p210
Control
BCR-ABL1p210
Control
BCR-ABL1p210

Figure 19. Rough eye phenotype induced by overexpression of human BCR-ABL1^{p210} using GMR-GAL4. Light (**A, C, E, G, I, K**) and scanning electron (**B-B', D-D', F-F', H-H', J-J', L-L'**) micrographs of adult *Drosophila* compound eyes expressing BCR-ABL1^{p210}. Flies were raised on 18ºC (**A-B-B', C-D-D'**), 25ºC (**E-F-F', G-H-H'**) or 29ºC (**I-J-J', K-L-L'**). **B', D', F', H', J'** and **L'** are high magnification of the centermost region in **B, D, F, H, J,** and **L,** respectively (1,370 x). Ommatidial facets are depicted in (B') by (*), misplaced mechanosensory bristles in (D') depicted by arrowheads and ommatidial fusions in (J') are shown by arrow. Posterior is to the left. Lower right panel represents quantification of severity of roughness of the adult fly eye expressing BCR-ABL1^{p210} and cultured at 18 ºC, 25 ºC or 29 ºC using the grading scale. Data represent mean ± SEM. ****, *P < 0.0001*.

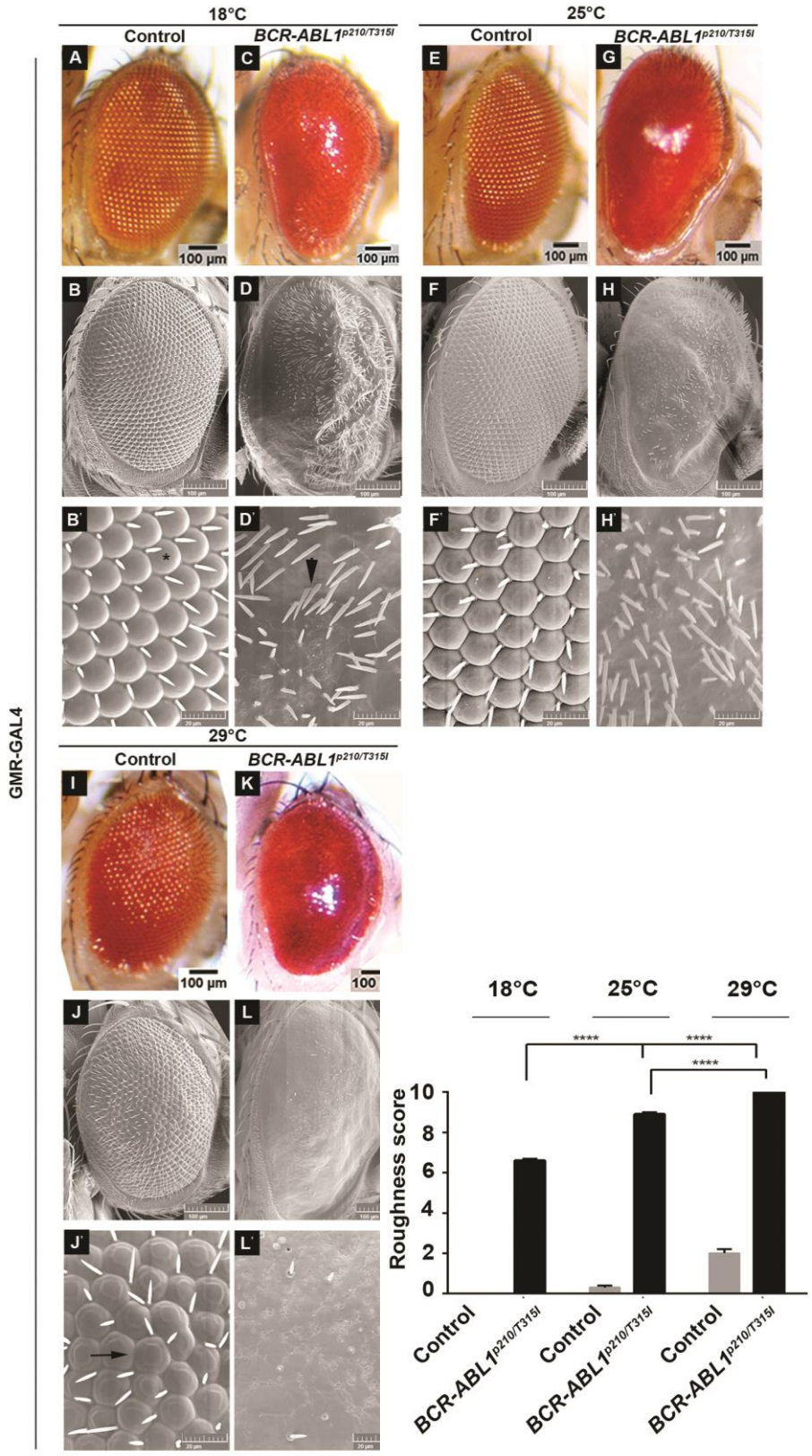

18°C
25°C
29°C
Control
BCR-ABL1p210/T315I
GMR-GAL4
A
B
B'
C
D
D'
E
F
F'
G
H
H'
I
J
J'
K
L
L'
100 µm
18°C
25°C
29°C
****
****
****
Roughness score
0
2
4
6
8
10
Control
BCR-ABL1p210/T315I
Control
BCR-ABL1p210/T315I
Control
BCR-ABL1p210/T315I

Figure 20. Rough eye phenotype induced by overexpression of human BCR-ABL1$^{p210/T315I}$ using GMR-GAL4. Light (A, C, E, G, I, K) and scanning electron (B-B', D-D', F-F', H-H', J-J', L-L') micrographs of adult *Drosophila melanogaste*r compound eyes expressing BCR-ABL1$^{P210/T315I}$. Flies were raised on 18ºC (A-B-B', C-D-D'), 25ºC (E-F-F', G-H-H') or 29ºC (I-J-J', K-L-L'). B', D', F', H', J' and L' are high magnification of the centermost region in B, D, F, H, J, and L, respectively (1,370 x). Ommatidial facets are depicted in (B') by (*), misplaced mechanosensory bristles in (D') depicted by arrowheads and ommatidial fusions in (J') are shown by arrow. Posterior is to the left. Lower right panel represents quantification of severity of roughness of the adult fly eye expressing BCR-ABL1 $^{p210/T315I}$ and cultured at 18ºC, 25ºC or 29ºC using the grading scale. Data represent mean ± SEM. ****, *P < 0.0001*.

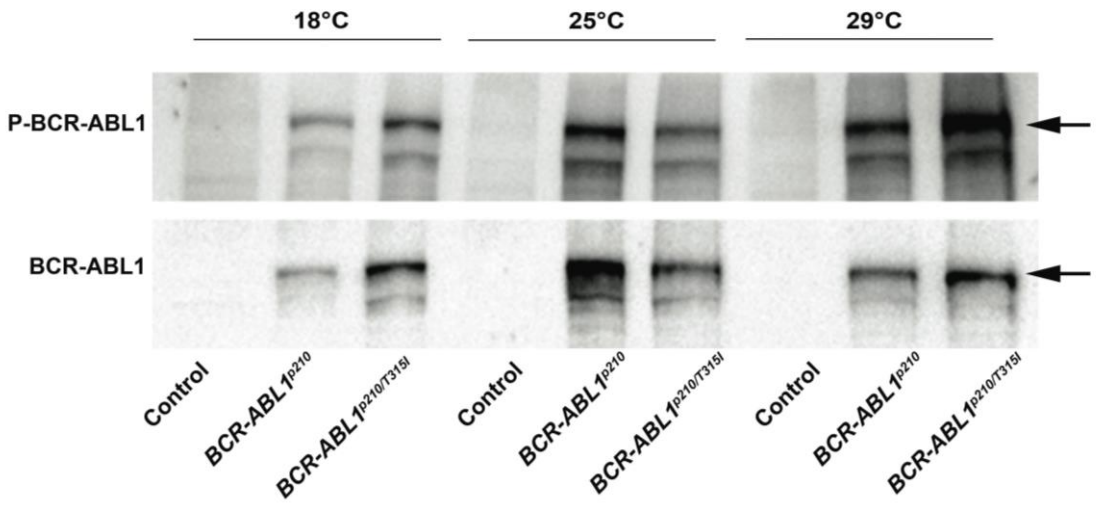


Figure 21. Protein expression of BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ in *Drosophila* eyes. Representative Western blot of the expression of BCR-ABL1 and phosphorylated levels in transgenic adult fly heads expressing BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ at different temperatures (18ºC, 25ºC, and 29ºC). Genotypes indicated are under the control of eye specific promoter GMR-GAL4. GMR-GAL4>w^{1118} were used as control.

C. Expression of BCR-ABL1 ^{p210} and BCR-ABL1$^{p210/T315I}$ in Eye Imaginal Discs Results in Photoreceptors Disorganization

GMR-GAL4 drives the expression of BCR-ABL1 in all cells posterior to the morphogenetic furrow which is a wave of cell differentiation that takes place during larvae life to establish the eye field. Therefore, we wanted to detect the expression of BCR-ABL1 in these eye discs which will eventually develop into the adult eye by staining for Myc tag antibody. We also stained them with ELAV which is a pan neural marker that marks photoreceptor neurons in the eye disc and shows their neural fate (Campos, And, & White, 1985). Flies were raised at 18ºC. Confocal images show the

highly ordered, tightly arrayed, and properly aligned ommatidial clusters from control (GMR-GAL4>w^{1118}) eye discs along with the boundary of the morphogenetic furrow sweeping the eye disc (marked with a white arrow). However, eye discs expressing BCR-ABL1^{p210} or BCR-ABL1$^{p210/T315I}$ exhibited a disturbed arrangement of the photoreceptors with loss of the tight arrays and proper alignment with gaps between ommatidial clusters (Figure 22).

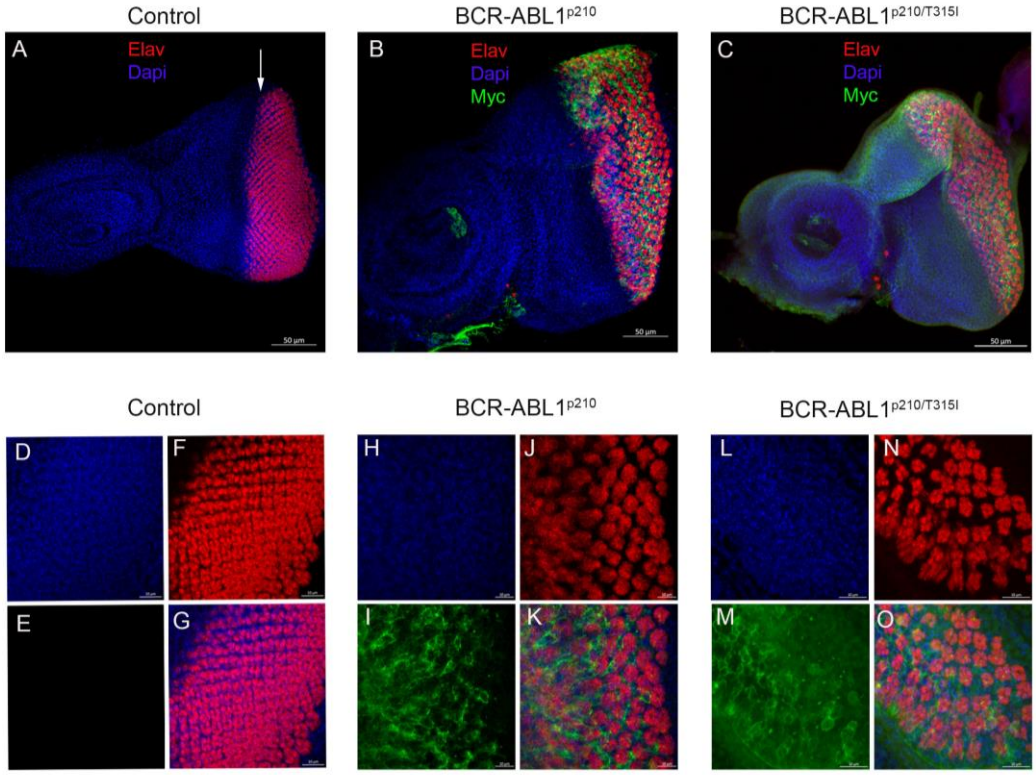


Figure 22. Expression of BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ in *Drosophila* eye discs results in photoreceptors disorganization. Maximum intensity projection at low magnification showing ELAV staining photoreceptors in control eye discs **(A)**, Myc expression in ELAV positive photoreceptor cells indicating expression of BCR-ABL1^{p210} **(B)** and BCR-ABL1$^{p210/T315I}$ **(C)**. Scale bar is 50 µm. Morphogenetic furrow is marked by an arrow in (A). **(D-O)** are maximum intensity projection at higher

magnification of the posterior end of the eye disc. Scale bar is 10 µm. Posterior is to the right.

D. Human BCR-ABL1 Induces Proliferation of Hemocytes in *Drosophila* Larvae

To express BCR-ABL1^{p210} and BCR-ABL1 $^{p210/T315I}$ in *Drosophila* blood cells, we used *hml$^{\Delta}$-GAL4, UAS-GFP* which drives the expression of the transgenes during late embryonic life and all larval stages in the majority of hemocytes and hemocyte precursors (Sinenko et al., 2010). BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ resulted in a significantly increased proliferation of circulating hemocytes raising their numbers four times and five times their wild-type control levels, respectively ($P< 0.0001$). BCR-ABL1$^{p210/T315I}$ showed a significant increase in the number of circulating hemocytes as compared to BCR-ABL1^{p210} ($P< 0.0001$) (Figure 23).

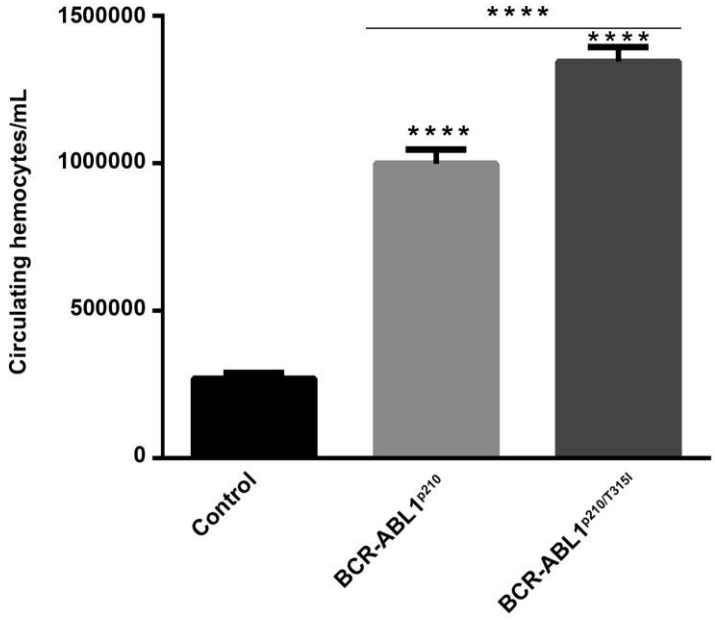


Figure 23. BCR-ABL1^{p210} and BCR-ABLI $^{p210/T315I}$ induce increased proliferation of circulating hemocytes in *Drosophila* larvae. Hemocytes from third instar larvae were collected and counted for each genotype. *Hml$^{\Delta}$-GAL4, UAS-GFP> w ^{1118}* were used as control. Data represent mean ± SEM (****, *P < 0.0001*).

E. Expression of Human BCR-ABL1 in *Drosophila* Wing Discs Results in Wing Deformation

Another *Drosophila* tissue that is employed in oncogene expression studies is the wing disc. In a manner similar to the fruit fly's eye development, the wing disc is derived from the wing imaginal disc. During embryogenesis the wing cells are derived from the ectoderm through the formation of an invagination at the intersection of a dorsal/ventral and anterior-posterior stripe controlled by Wingless and Decapentaplegic, respectively (Figure 24) (Brody, 1999). By means of the GAL4-UAS system we were able to express BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ in *Drosophila* wings using MS1096-Gal4 (Table 2) which drives the early expression of the transgenes in the dorsal wing pouch. Female flies were allowed to lay eggs after mating and parents were discarded then F1 progeny was allowed to develop until the third instar larvae stage. Then, a known number of larvae was transferred with a fine brush to a new vial and allowed to develop further into adult stage. Each individual fly was examined and grouped into either having normal wings or abnormal folding of the wing tissue which appears wrinkled indicating disruption in development or a more severe phenotype characterized by abnormal eclosure whereby the wings remain attached to the pupal case as the adult fly tries to eclose or the most severe phenotype characterized by pupal death whereby the adult fly dies inside the pupal case before eclosing. Around 60 total flies were examined from each genotype and the results were displayed as percentage of flies (%) having a certain phenotype. Control crosses showed normal eclosure with normal wings in 100% of the flies. BCR-ABL1^{p210} expressing flies displayed mainly

flies eclosing with abnormal wings (75%) which indicates disruption to the normal development of the wing disc, flies with eclosure defect (15%), and flies with pupal death (10%). On the other hand, BCR-ABL1$^{p210/T315I}$ showed mainly flies with the most severe phenotype which is pupal death (66%) followed by flies with eclosure defect (30%) and flies with abnormal wings (4%) (Figure 25).

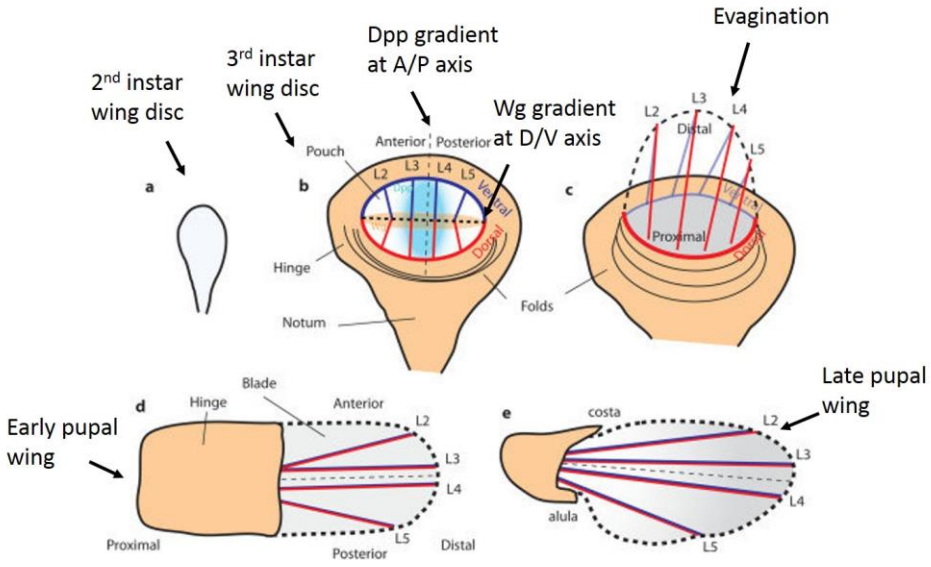


Figure 24. Overview of *Drosophila* wing development**. (**Modified from Brody,1999)

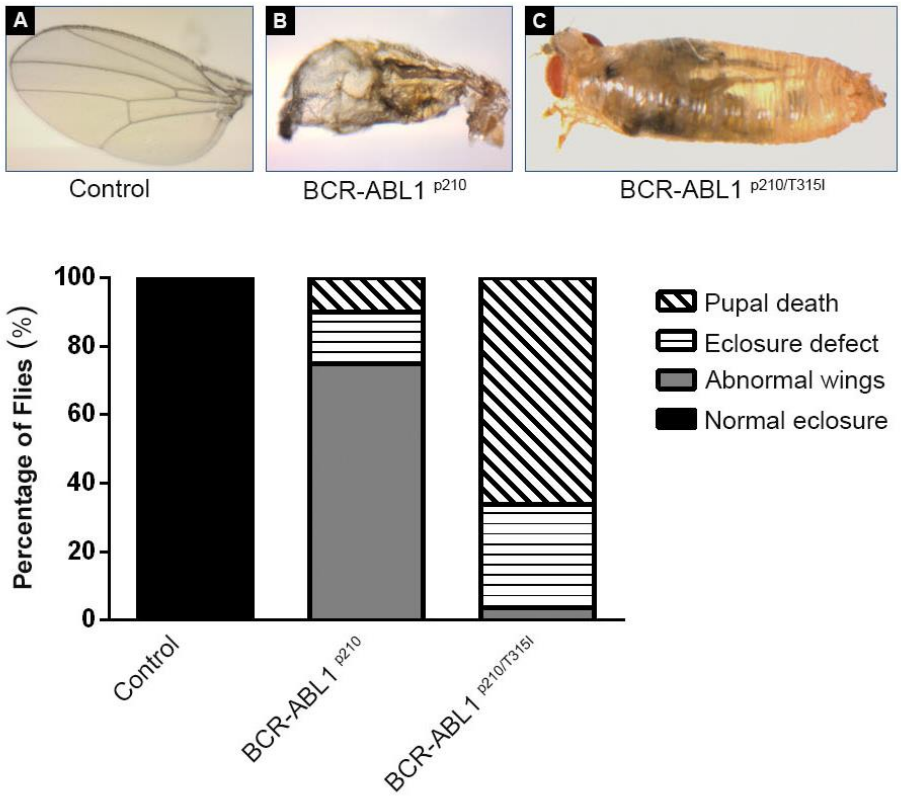


Figure 25. **Wing deformation phenotype induced by BCR-ABL1 expression.** Representative light microscopy images of (A) Control wings (B) abnormal wings as main phenotype of BCR-ABL1 ^{p210} expressing flies (C) eclosure defect as main phenotype of BCR-ABL1 $^{p210/T315I}$ expressing flies. Lower graph is the quantification of percentage of flies (%) with normal eclosure, abnormal wings, eclosure defect and pupal death. The genotypes indicated are under the control of the wing disc driver MS-1096 GAL4. Flies were kept at 25°C.

F. Expression of Human BCR-ABL1 Results in Transformation of *Drosophila* Tissues with Differential Phenotypes Between BCR-ABL1 ^{p210} and BCR-ABL1$^{p210/T315I}$

We used different GAL-4 drivers to be able to characterize distinct BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ induced phenotypes for later drug and/or genetic screening. The below table summarizes the different phenotypes obtained at two temperatures (25°C and 29°C). In most of the cases BCR-ABL1$^{p210/T315I}$ resulted in a more severe phenotype than wild type BCR-ABL1^{p210}.

Promoter	**Lineage**	**Phenotype (25°C)**		**Phenotype (29°C)**	
		p210	*p210^{T315I}*	*p210*	*p210^{T315I}*
Actin-5C-GAL4	Ubiquitous	Lethal	Lethal	Lethal	Lethal
Engrailed-GAL4	Imaginal discs	Pupal lethal	Embryonic/larval lethal	Lethal	Lethal
MS1096-GAL4	Wing disc	Wing defect	Eclosure defect	Not tested	Not tested
GMR-GAL4	Eye discs	Severe rough eye	Severe rough eye	Severe rough eye	Severe rough eye
Sev-GAL4	Eye discs	Mild rough eye	Mild rough eye	Mild rough eye	Mild rough eye
Hml-gal4	Hemocytes	Not tested	Not tested	Increase in hemocytes	Increase in hemocytes

Table 4. Summary of phenotypes induced by human BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ under the control of different GAL4 driver lines at two temperatures (25°C and 29°C)

G. Tyrosine Kinase Inhibitors Studies

For establishing a drug screening model, we picked two tissues for testing their sensitivity for phenotype reversal when feeding flies TKIs. The first model used the adult fly compound eye expressing BCR-ABL1 under the control of GMR-GAL4 at 18ºC. This promoter produces a visible and easily scorable phenotype which is milder in severity than expression at higher temperatures and at the same time shows more transformative potential of BCR-ABL1 than expression with sev-GAL4.

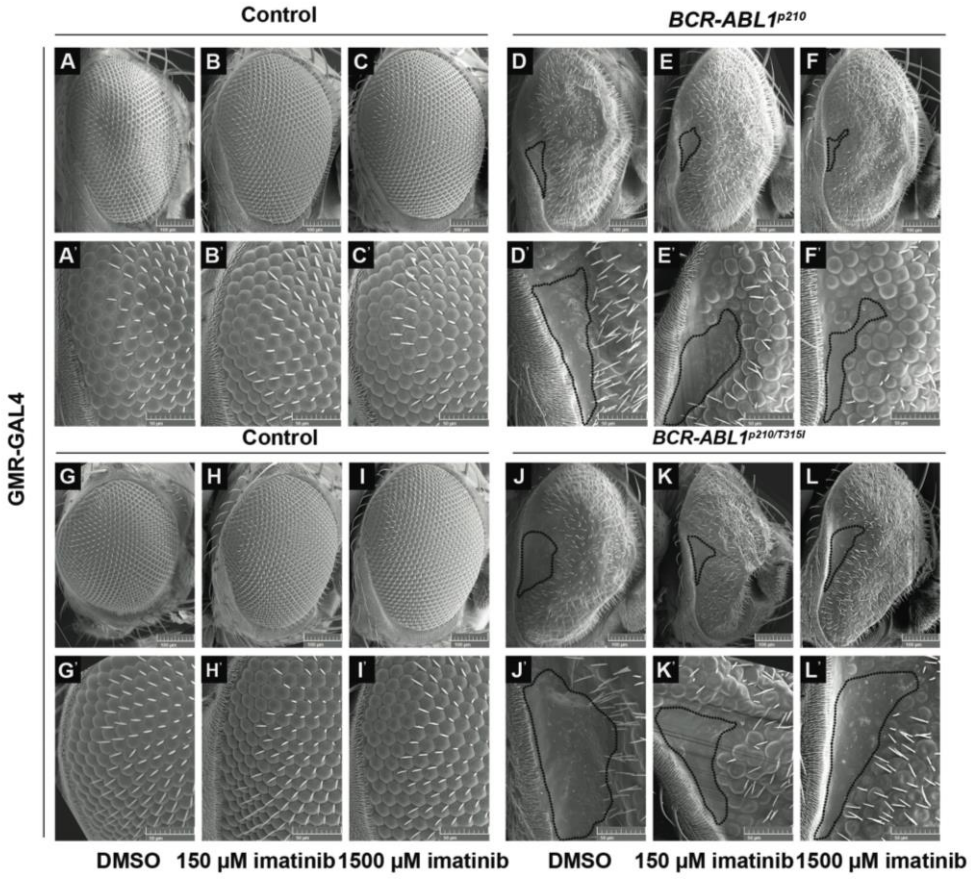
The second model used the lethality phenotype upon expression of BCR-ABL1 using engrailed-GAL4 at 25ºC. Four TKIs were tested which included imatinib, nilotinib, dasatinib, and ponatinib.

1. Tyrosine Kinase Inhibitors Screening Using Adult Drosophila Compound Eyes

a. Imatinib and Nilotinib Show Limited Efficacy in Rescuing Human BCR-ABL1^{p210} Mediated Defects in *Drosophila*

BCR-ABL1^{p210} flies were crossed to GMR-Gal4 flies and progeny were fed on multiple concentrations of the TKIs (treated) or on DMSO alone (untreated). Untreated BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ flies showed the same defects described previously at 18ºC, focusing particularly on the posterior end of the eye with a characteristic defective area characterized by loss of ommatidial facets. The posterior eye defect area in untreated BCR-ABL1^{p210} flies showed an average of 4580 μm^2 and

4044 μm^2 on 0.03% DMSO and 0.3% DMSO, respectively. On the other hand, untreated BCR-ABL1$^{p210/T315I}$ expressing flies showed a wider area of defect at the posterior end with an average significant increase in defect area to 11148 μm^2 ($P < 0.0001$) and 8728 μm^2 ($P < 0.0001$) on 0.03% DMSO and 0.3% DMSO, respectively, as compared to untreated BCR-ABL1^{p210} expressing flies. Feeding 150 µM or 1500 µM imatinib to BCR-ABL1^{p210} expressing flies did not eliminate the posterior eye defect. However, the average posterior eye defect area showed a tendency to decrease with 1500 µM imatinib (3047 μm^2) as compared to that of 150 µM imatinib (4142 μm^2) and untreated flies (4044 μm^2) (Figure 26). Interestingly, the percentage of flies with total rescue (total disappearance of the posterior eye defect) with 150 µM and 1500 µM imatinib was 4% and 21%, respectively. Similarly, feeding 28 µM or 280 µM nilotinib to BCR-ABL1^{p210} expressing flies did not eliminate the posterior eye defect. However, the average posterior eye defect area showed a tendency to decrease with 280 µM nilotinib (2480 μm^2) as compared to that of 28 µM nilotinib (3871 μm^2) and untreated flies (4044 μm^2) (Figure 27). The percentage of flies with total rescue with 28 µM and 280 µM nilotinib was 7% and 13%, respectively.


Control
BCR-ABL1p210
GMR-GAL4
A
B
C
D
E
F
A'
B'
C'
D'
E'
F'
Control
BCR-ABL1p210/T315I
G
H
I
J
K
L
G'
H'
I'
J'
K'
L'
DMSO
150 µM imatinib
1500 µM imatinib
DMSO
150 µM imatinib
1500 µM imatinib

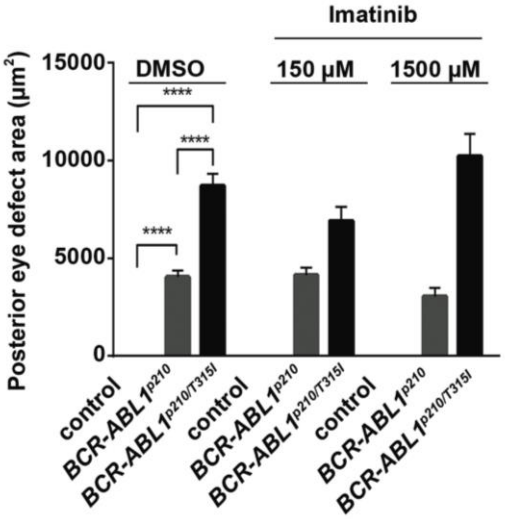
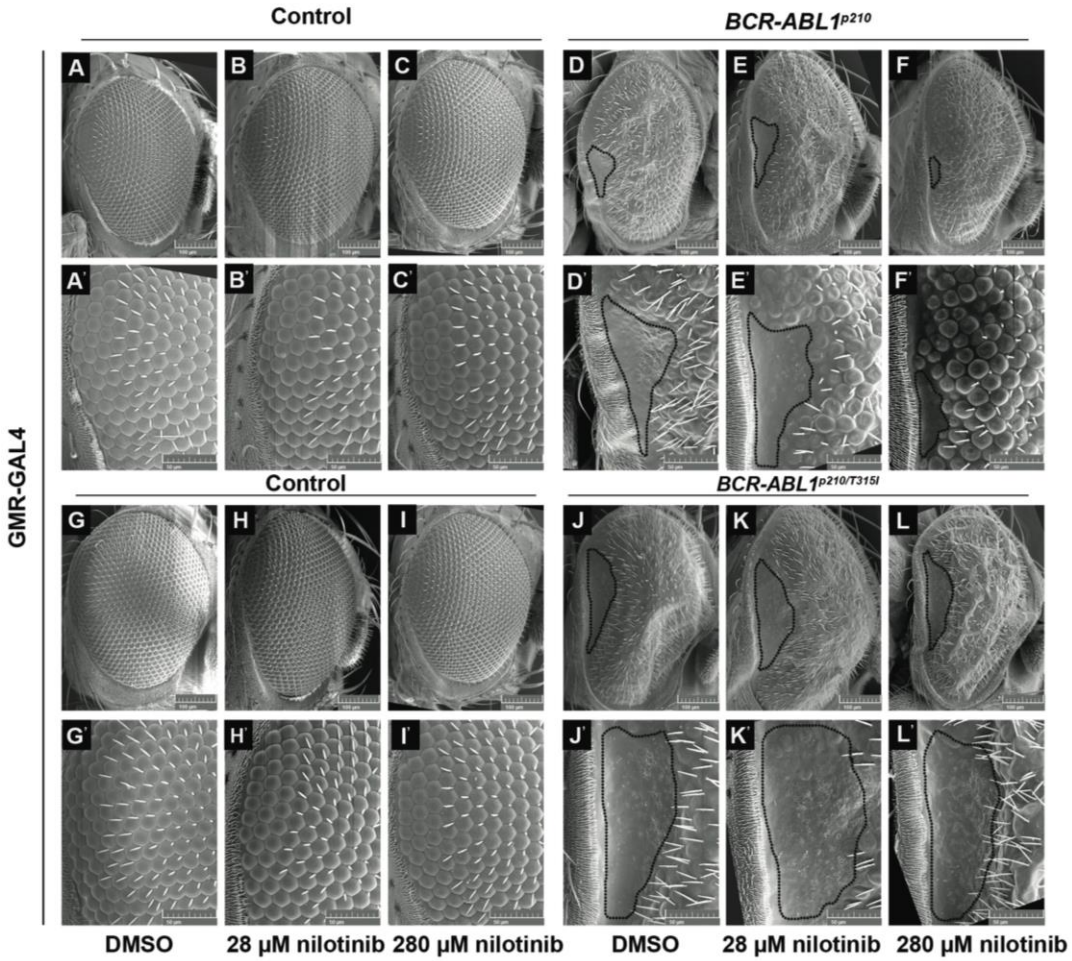

Imatinib
DMSO
150 µM
1500 µM
Posterior eye defect area (µm2)
15000
10000
5000
0
****
****
****
control
BCR-ABL1p210
BCR-ABL1p210/T315I
control
BCR-ABL1p210
BCR-ABL1p210/T315I
control
BCR-ABL1p210
BCR-ABL1p210/T315I

Figure 26. Imatinib shows a tendency to decrease *BCR-ABL1^{p210}* mediated eye defect. Scanning electron micrographs (**A-A', L- L'**) of adult *Drosophila* compound eyes from flies fed on 0.3% DMSO only (**A-A', D-D', G-G', J-J'**) or imatinib (**B-B', C-C', E-E', F-F', H-H', I-I', K-K', L-L'**). Posterior is to the left. GMR-GAL4>*w^{1118}* were used as control. **A'-L'** are high magnification of the posterior end of the eye in **A-L** respectively (692 x). Normal development in control flies fed on DMSO (**A-A', G-G'**) or imatinib (**B-B', C-C', H-H', I-I'**) is observed. *BCR-ABL1^{p210}* (**D-D'**) and *BCR-ABL1$^{p210/T315I}$* (**J-J'**) expressing flies fed on DMSO show characteristic defective area with loss of ommatidial facets. Area is marked with a representative dashed line. Feeding low or high dose imatinib to *BCR-ABL1^{p210}* (**E-E', F-F'**) and *BCR-ABL1$^{p210/T315I}$* (**K-K', L- L'**) retained the defective area in the posterior end of the eye marked with a dashed line. Compare to **D-D'** and **J-J',** respectively. Lower panel represents measurement of the posterior eye defect area (μm^2). Data represents mean ± SEM. ****, *P < 0.0001*.


Control
BCR-ABL1p210
GMR-GAL4
A
B
C
D
E
F
A'
B'
C'
D'
E'
F'
Control
BCR-ABL1p210/T315I
G
H
I
J
K
L
G'
H'
I'
J'
K'
L'
DMSO
28 μM nilotinib
280 μM nilotinib
DMSO
28 μM nilotinib
280 μM nilotinib

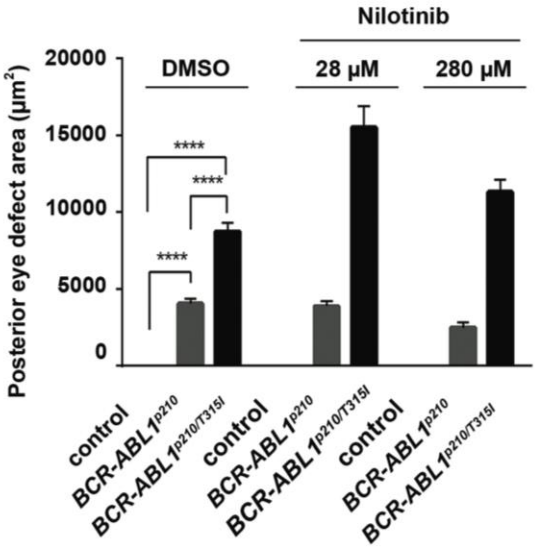

Nilotinib
DMSO
28 μM
280 μM
Posterior eye defect area (μm2)
20000
15000
10000
5000
0
****
****
****
control
BCR-ABL1p210
BCR-ABL1p210/T315I
control
BCR-ABL1p210
BCR-ABL1p210/T315I
control
BCR-ABL1p210
BCR-ABL1p210/T315I

Figure 27. Nilotinib shows a tendency to decrease *BCR-ABL1^{p210}* mediated eye defect. Scanning electron micrographs (**A-A', L-L'**) of adult *Drosophila* compound eyes from flies fed on DMSO only (**A-A', D-D', G-G', J-J'**) or nilotinib (**B-B'-C-C', E-E'-F-F', H-H'-I-I', K-K'-L-L'**).Posterior is to the left. GMR-GAL4>*w^{1118}* were used as control. **A'-L'** are high magnification of the posterior end of the eye in **A-L** respectively (692 x). Normal development in control flies fed on DMSO (**A, A'-G, G'**) or nilotinib (**B-B'-C-C', H-H'-I- I'**) is observed. *BCR-ABL1^{p210}* (**D-D'**) and *BCR-ABL1$^{p210/T315I}$* (**J-J'**) expressing flies fed on DMSO show characteristic defective area with loss of ommatidial facets. Area is marked with a representative dashed line. Feeding low or high dose nilotinib to *BCR-ABL1^{p210}* (**E-E'-F-F'**) and *BCR-ABL1$^{p210/T315I}$* (**K-K'-L-L'**) retained the defective area in the posterior end of the eye marked with a dashed line. Compare to **D-D'** and **J-J',** respectively. Lower panel represents measurement of the posterior eye defect area (μm^2). Data represents mean ± SEM. ****, *P < 0.0001*.

b. Dasatinib and Ponatinib Rescue Human BCR-ABL1p210 Mediated Defects in *Drosophila*

Testing the potent TKIs (dasatinib and ponatinib) showed more efficient rescue. Feeding 20 µM dasatinib or 280 µM ponatinib to BCR-ABL1^{p210} expressing flies improves the overall eye ommatidial arrangement and more specifically eliminates the characteristic posterior eye defect by restoring its normal ommatidial development (Figure 28). The average posterior eye defect area significantly decreased from 4580 μm^2 (in untreated flies) to 0 μm^2 (*P < 0.0001*) with 20 µM dasatinib and from 4044 μm^2 (in untreated flies) (Figure 29) to 267 μm^2 (*P < 0.0001)* with 280 µM ponatinib (Figure 30, Figure 31). The percentage of flies with total rescue was 100% with dasatinib and 86% with ponatinib. A dose-response analysis for BCR-ABL1^{p210} expressing flies

treated with dasatinib showed a significant decrease in the average posterior eye defect area from 4580 μm^2 in untreated flies to 2372 μm^2 ($P < 0.0001$) with 1 µM dasatinib, to 131 μm^2 ($P < 0.0001$) with 10 µM, and to 0 μm^2 ($P < 0.0001$) with 20 µM dasatinib, (Figure 32, Figure 33). The percentage of flies with total rescue increased from 25% to 92% and to 100% with 1µM, 10µm and 20µM dasatinib respectively. Similarly, ponatinib also showed a dose-response whereby the average posterior eye defect area decreased significantly from 4044 μm^2 in untreated flies to 1684 μm^2 ($P < 0.0001$) with 28 µM and to 267 μm^2 ($P < 0.0001$) with 280 µM ponatinib (Figure 30). The percentage of flies with total rescue increased from 48% to 86% with 28 µM and 280 µM ponatinib, respectively.

BCR-ABL1$^{p210/T315I}$ mutation is known to exhibit resistance to imatinib, nilotinib, and dasatinib in CML patients and this was confirmed in our model whereby the characteristic posterior eye defect did not show ommatidial rescue when feeding BCR-ABL1$^{p210/T315I}$ expressing flies imatinib, dasatinib or nilotinib (Figures 26-29). However, feeding ponatinib to BCR-ABL1$^{p210/T315I}$ expressing flies did not show the expected rescue of the posterior eye defect (Figure 30, Figure 31). Western blot analysis confirmed the expression and phosphorylation of BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ in *Drosophila* eyes from untreated or treated flies (Figure 29, Figure 31).

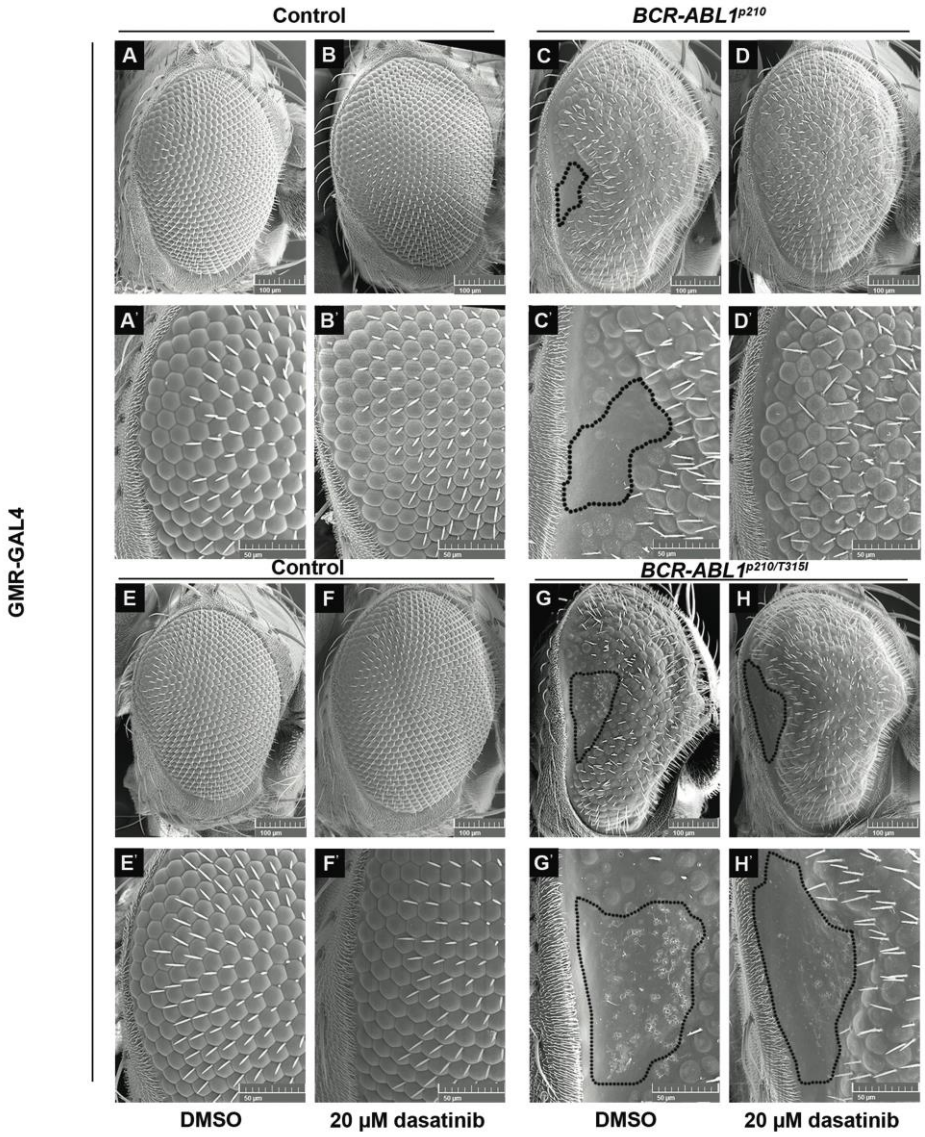

Control
BCR-ABL1p210
GMR-GAL4
A
B
C
D
A'
B'
C'
D'
Control
BCR-ABL1p210/T315I
E
F
G
H
E'
F'
G'
H'
DMSO
20 µM dasatinib
DMSO
20 µM dasatinib

Figure 28. Dasatinib rescues *BCR-ABL1^{p210}* mediated eye defect and shows target specificity *in vivo*. Scanning electron micrographs of adult *Drosophila* compound eyes from flies fed on 0.03% DMSO only (**A-A', C-C', E-E', G-G'**) or dasatinib (**B-B', D-D', F-F', H-H'**). Posterior is to the left. GMR-GAL4>*w^{1118}* were used as control. **A'-H'** are high magnification of the posterior end of the eye of **A-H,** respectively (692 x). Normal development in control flies fed on DMSO (**A-A', E-E'**) or dasatinib (**B-B', F-F'**) is observed. *BCR-ABL1^{p210}* (**C-C'**) and *BCR-ABL1$^{p210/T315I}$* (**G-G'**) expressing flies fed on DMSO show characteristic defective area with loss of ommatidial facets. Area is marked with a representative dashed line. Ommatidial development in this area was restored with *BCR-ABL1^{p210}* flies fed on 20 µM dasatinib (**D-D'**).Compare to **(C-C')**. BCR-ABL$^{p210/T315I}$ flies showed no restoration of ommatidial development (**H-H').** Compare to **(G-G').**

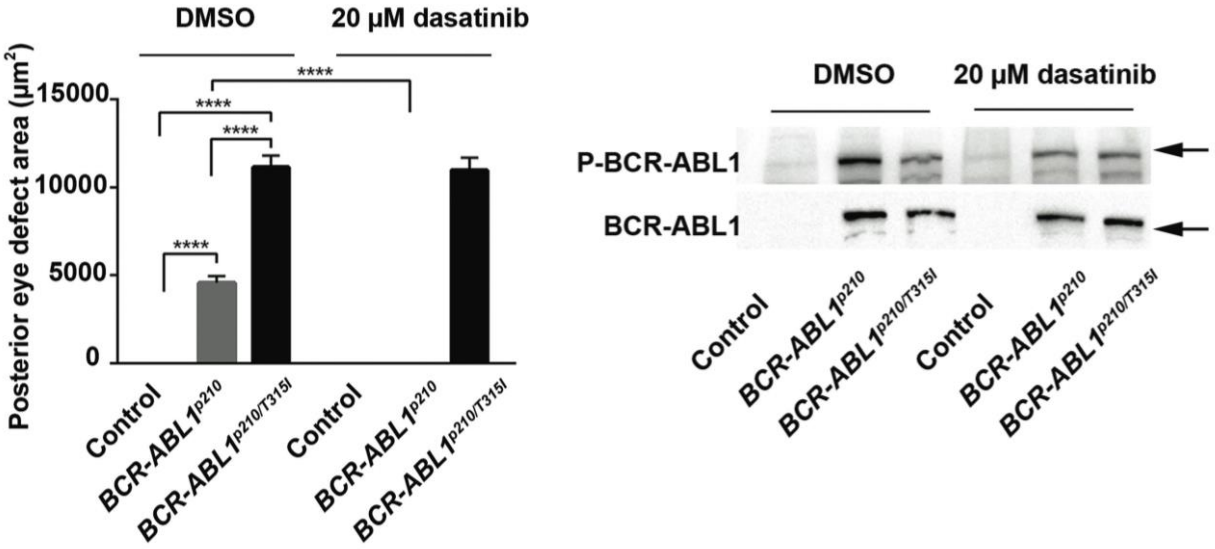


Figure 29. Measurement of the posterior eye defect area (μm^2) of dasatinib treated flies. Data represents mean ± SEM. ****, *P < 0.0001*. B. Representative Western blot of the expression of BCR-ABL1 and phosphorylated levels in transgenic untreated and treated adult fly heads. Genotypes indicated are under the control of eye specific promoter GMR-GAL4.

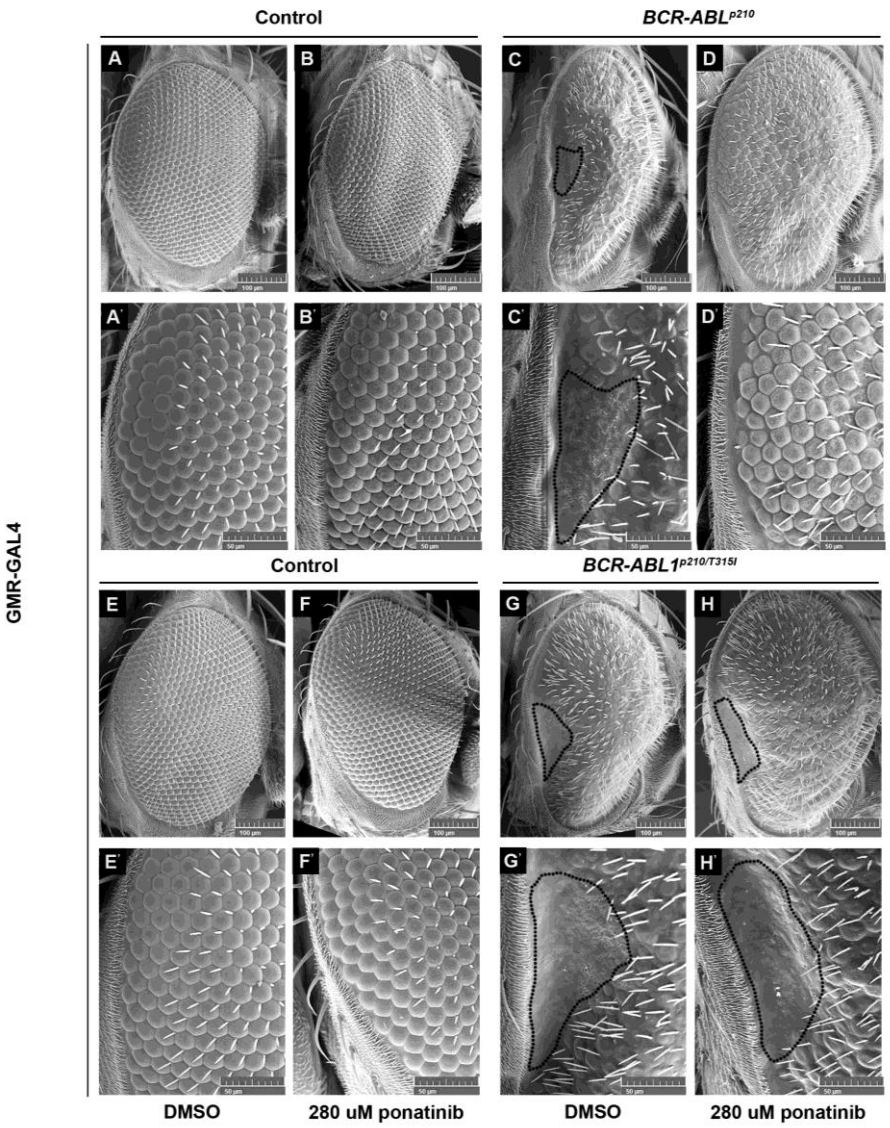


Figure 30. Ponatinib rescues *BCR-ABL1*^{p210} mediated eye defect. Scanning electron micrographs of adult *Drosophila* compound eyes from flies fed on 0.3% DMSO only **(A-A', C-C', E-E', G-G')** or ponatinib **(B-B', D-D', F-F', H-H')**. Posterior is to the left. GMR-GAL4>w^{1118} were used as control. **A'-H'** are high magnification of the posterior end of the eye of **A-H** respectively (692 x). Normal development in control flies fed on DMSO **(A-A', E-E')** or ponatinib (**B-B', F-F'**) is observed. BCR-ABL1^{p210} **(C-C')** and BCR-ABL1$^{p210/T315I}$ **(G-G')** expressing flies fed on DMSO show characteristic defective area with loss of ommatidial facets. Area is marked with a

representative dashed line. Ommatidial development in this area was restored with BCR-ABL1^{p210} flies fed on ponatinib (**D-D'**). Compare to (**C-C'**). BCR-ABL$^{p210/T315I}$ flies showed no restoration of ommatidial development (**H, H'**). Compare to **(G, G').**

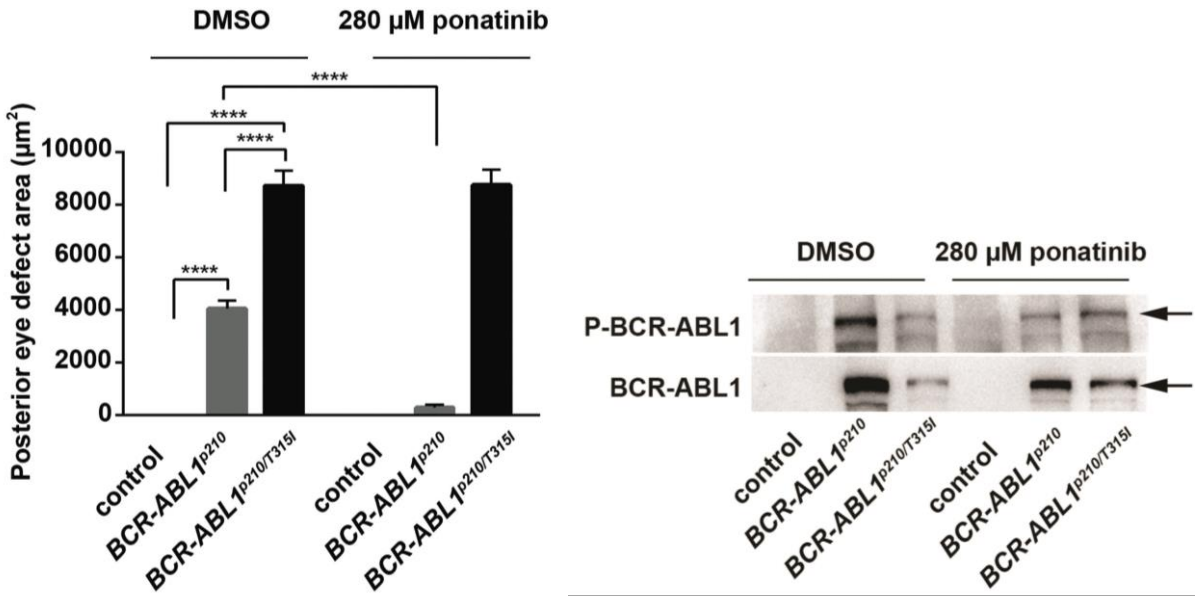


Figure 31. **Measurement of the posterior eye defect area (μm^2) of ponatinib treated flies**. Data represents mean ± SEM. ****, *P < 0.000.* B. representative Western blot of the expression of BCR-ABL1 and phosphorylated levels in transgenic untreated and treated adult fly heads. Genotypes indicated are under the control of eye specific promoter GMR-GAL4.

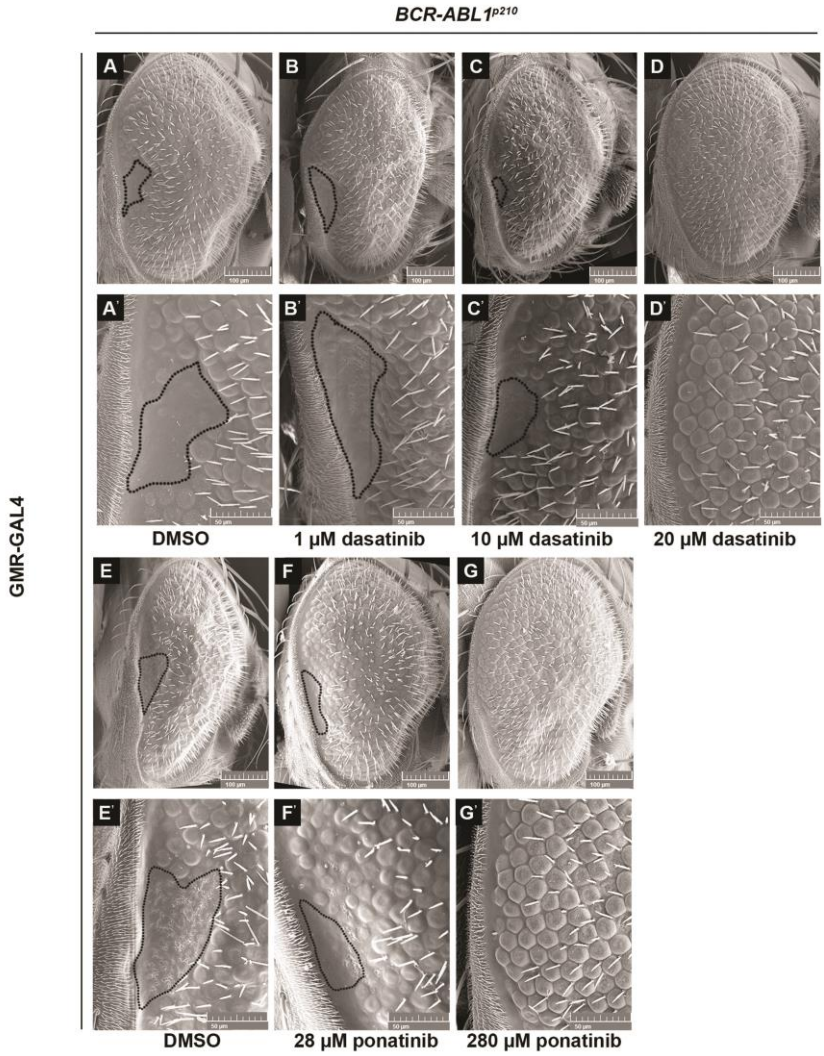


Figure 32. Dasatinib and ponatinib rescue *BCR-ABL1^{p210}* mediated eye defect in a dose dependent manner. Scanning electron micrographs of adult *Drosophila* compound eyes from flies expressing BCR-ABL1^{p210} and fed on 0.03% DMSO (**A-A'**), 1 µM (**B-B'),** 10 µM **(C-C')** or 20 µM (**D-D'**) dasatinib and flies fed on 0.3% DMSO (**E-E'),** 28 µm ponatinib **(F-F')** or 280 µM ponatinib **(G-G').** Posterior is to the left. **A'-G'** are high magnification of the posterior end of the eye of **A-G** respectively (692 x). Posterior eye defect area is marked with a representative dashed line.

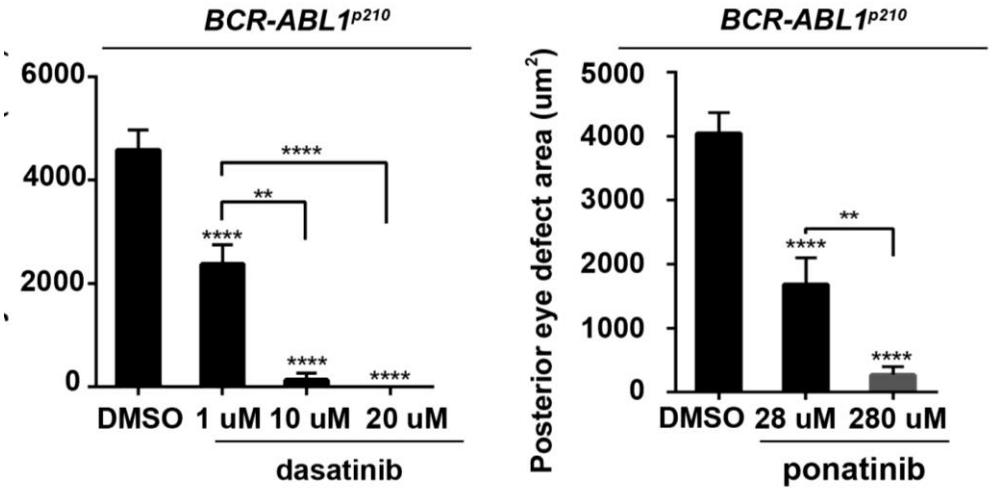


Figure 33. **Dose reponse measurements of the posterior eye defect area (μm^2) for dasatinib and ponatinib treated flies**. Data represent mean ± SEM. ** *P< 0.01;* **** *P < 0.0001.*

2. ***Tyrosine Kinase inhibitors Screening Using Lethality Phenotype***

Upon expression of BCR-ABL1^{p210} in *Drosophila* imaginal discs using engrailed-GAL4, F1 progeny showed mainly pupal lethality while BCR-ABL1$^{p210/T315I}$ revealed earlier lethality of embryonic/larval stages at 25°C indicating a more severe phenotype. We tested whether dasatinib (20 μM) and ponatinib (100 μM) which showed reversal of rough eye phenotype could reverse pupal lethality and lead to eclosure of adult flies expressing BCR-ABL1^{p210}. We also tested whether ponatinib (100 μM) could reverse larval lethality in BCR-ABL1$^{p210/T315I}$ expressing flies.

a. Dasatinib and Ponatinib Reverse BCR-ABL1^{p210} Mediated Pupal Lethality

For quantification of the rescuing effects of dasatinib and ponatinib in BCR-ABL1^{p210} expressing flies under the control of engrailed-GAL4, embryos expressing BCR-ABL1^{p210} were transferred to food mixed with DMSO (0.1%), dasatinib (20 μM) or ponatinib (100 μM) then the percentage of eclosing adults or pharate adults was reported and normalized to percentage of surviving adult control flies (*engrailed-Gal4> w ^{1118}*). Control flies showed a survival of 35% of eclosed adults while BCR-ABL1^{p210} expressing flies did not show any eclosing or even pharate adults on DMSO. On the other hand, treatment of BCR-ABL1^{p210} flies with 20 μM dasatinib resulted in a major developmental shift towards the adult stage whereby 32% of the flies eclosed normally out of their pupal cases as well as 21% formed pharate adults which are adults formed inside the pupal case but failed to eclose. Treatment with 100 μM ponatinib resulted in 17% eclosing adults and 25% paharate adults (Figure 34).

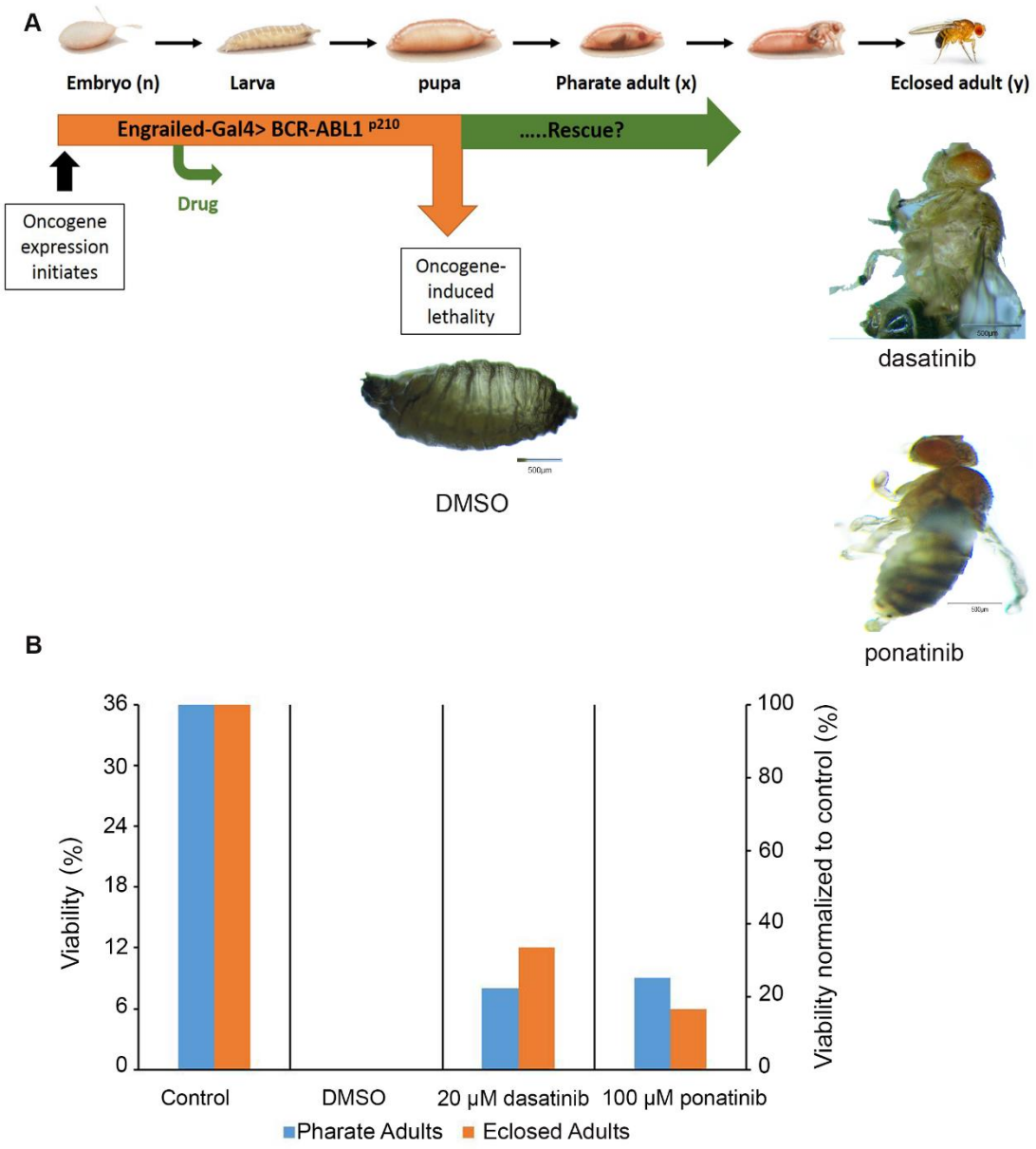


Figure 34. **Rescue of BCR-ABL1^{p210} mediated developmental block at pupal stage by dasatinib and ponatinib. (A)** Quantification of rescue was based on the number of embryos (n) that survived as pharate adults (x) and eclosed adults (y). **(B)** Left axis shows percent viability of control pharate and eclosed adult flies and of pharate (x/n) or eclosed (y/n) untreated (DMSO) or treated (20 µM dasatinib and 100 µM ponatinib) BCR-ABL1^{p210} expressing flies. Right axis shows percent viability normalized to percent of control adults. Total n of 200 embryos per condition.

b. Ponatinib Reverses BCR-ABL1 $^{p210/T315I}$ Mediated Larval Lethality

Embryos expressing BCR-ABL1$^{p210/T315I}$ under the control of engrailed-GAL4 were transferred to food mixed with 0.1% DMSO or 100 μM ponatinib and the percentage of pupae forming was determined for each condition and normalized to percentage of pupae in control. No formation of pupae was detected in untreated flies however the developmental block at larval stage was partially reversed upon treatment with 100 μM ponatinib (Figure 35).

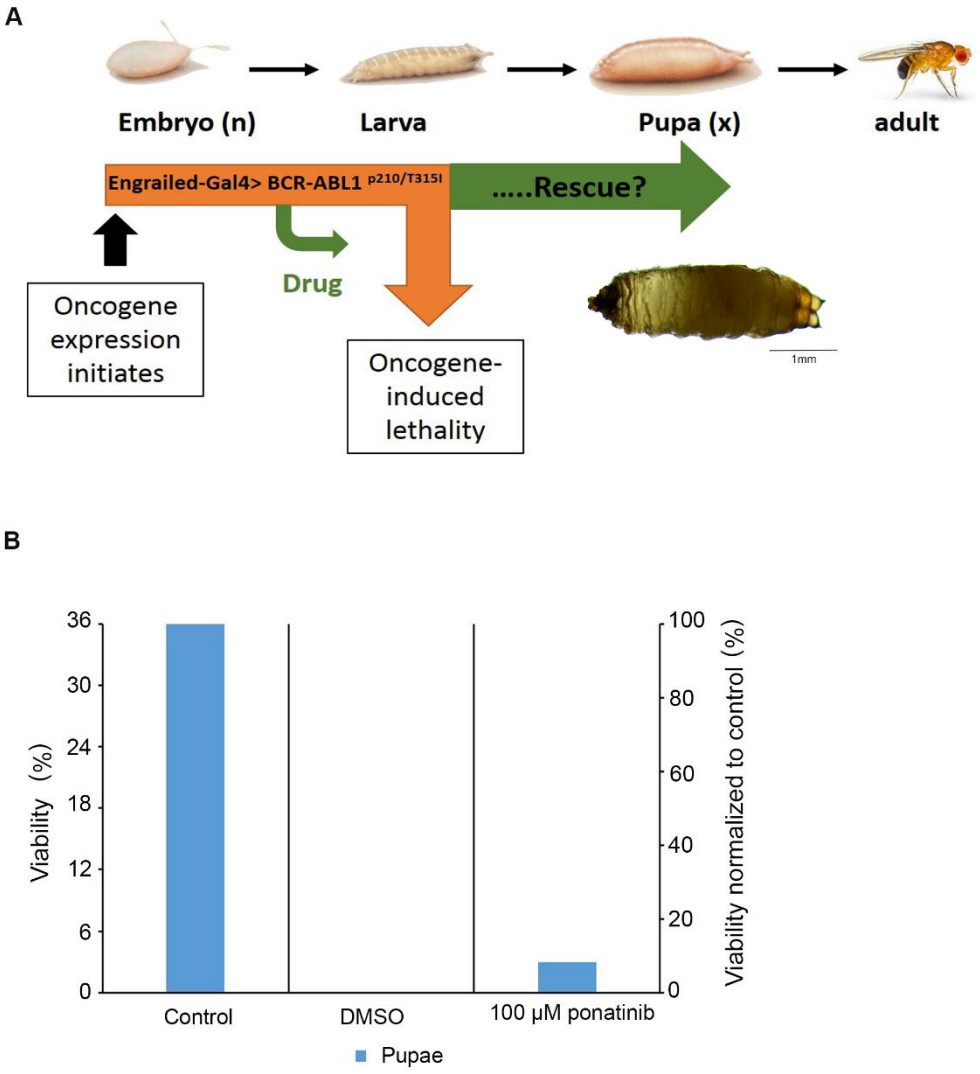


Figure 35. **Partial reversal of BCR-ABL1$^{p210/T315I}$ mediated developmental block at larval stage by ponatinib. (A)** Quantification of the suppression was based on the number of embryos (n) that survived as pupae (x). **(B)** Left axis shows percent viability of control pupae formed and of untreated (DMSO) or treated (100 µM ponatinib) BCR-ABL1$^{p210/T315I}$ expressing flies. Right axis shows percent viability normalized to percent of control pupae. Total n of 200 embryos per condition.

H. Knockdown of Genes in a Cell and Tissue-specific Manner (RNAi)

For RNAi experiments, we prepared a small scale screen that involves the knockdown of certain genes that belong to well defined pathways in *Drosophila*; many of which play crucial roles in proliferation and differentiation of eye disc cells and at the same time are implicated in CML pathogenesis (Figure 34). Two viable and fertile *Drosophila* lines were prepared which express either BCR-ABL1^{p210} or BCR-ABL1$^{p210/T315I}$ in the compound eyes and virgin females from these lines were crossed to males from different RNAi lines. Experiments were performed at 29°C and are also planned to be tried at 25°C. We have just started with the screen and the tested lines are shown along with their resultant phenotype in Table 5. Mainly the knockdown of genes in Toll pathway (Toll, Myd88, and Dif) resulted in lethality (Table 5).

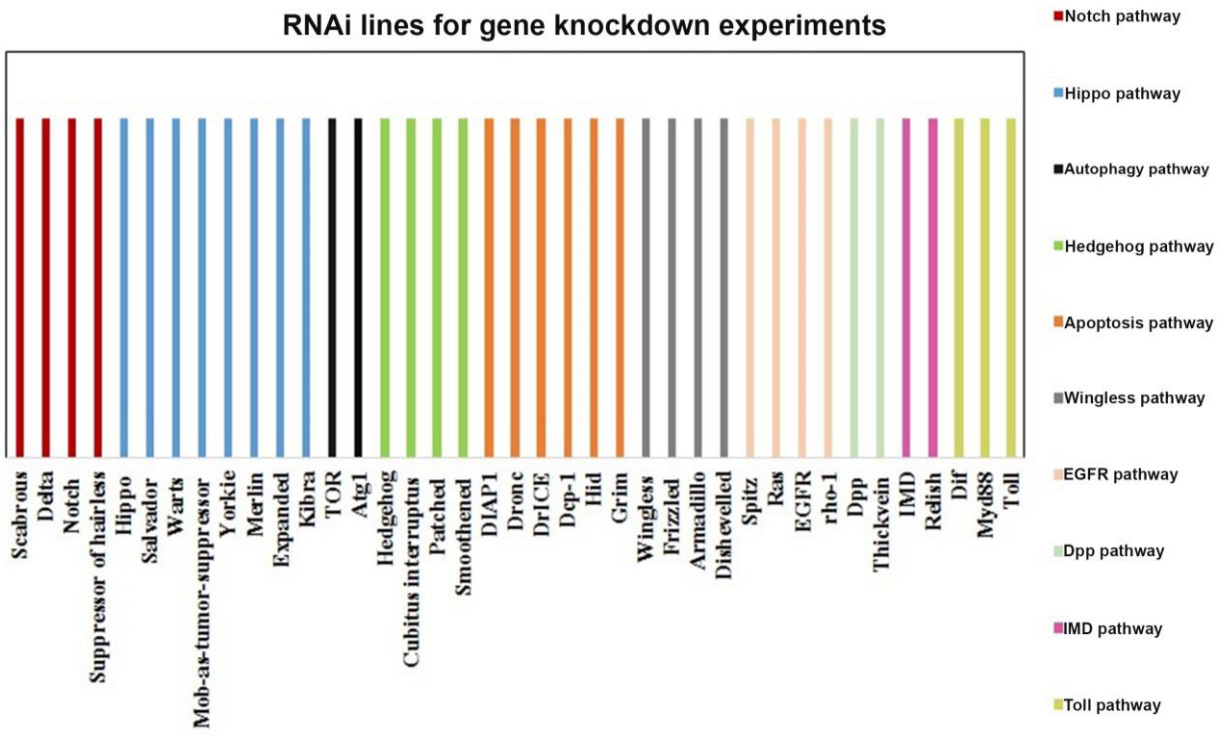


Figure 36. Multicolored chart showing genes to be knocked down in BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ expressing *Drosophila* eyes. Genes are clustered into different colors based on the pathway they belong to.

Gene	VDRC ID	BCR-ABL1^{p210} Phenotype	BCR-ABL1$^{p210/T315I}$ Phenotype
Dorsal related immunity factor (Dif)	100537	Not tested	Lethality
Dorsal related immunity factor (Dif)	100532	Lethality/minor aggravation of eye phenoype	Not tested
Relish	49413	No change in eye phenotype	No change in eye phenotype
Myd88	25399	Lethality	Lethality
Toll	100078	Not tested	Lethality
IMD	101834	Not tested	No change in eye phenotype

TABLE 5. **Phenotypes induced by RNAi-mediated knockdown of genes in BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ expressing *Drosophila* eyes.**

Chapter V

DISCUSSION

Eleven years ago was the last time a publication discussed *Drosophila* as a potential model for studying the biological functions of BCR-ABL1 (Traci L. Stevens et al., 2008). This study came out nine years after Fogerty *et al.* (Fogerty et al., 1999*)* who were the first to propose using *Drosophila* for studying BCR-ABL1 pathogenesis. However, both of these studies used chimeric human/fly BCR-ABL1. We were to be the first to report *Drosophila* as a potential CML model using the full human BCR-ABL1 oncogene, but during our writing up for the manuscript Bernardoni *et al.* (Bernardoni et al., 2019) published the eye phenotypes induced by human BCR-ABL1. We however, validated the BCR-ABL1 model for drug screening.

A. *Phenotypes Induced by BCR-ABL1*

Previously, it was demonstrated that chimeric human/fly BCR-ABL1^{p210} expression in CNS and eye imaginal discs results in disruption of normal differentiation (Fogerty et al., 1999). We replicated their findings in *Drosophila* eyes but we also tested GMR-GAL4 which is known to be expressed in all photoreceptor cells. We did indeed notice the increase in severity with GMR-GAL4 whereby the eye tissue totally disappeared at 25ºC and 29ºC and only few flies could eclose. We then generated the full human BCR-ABL1^{p210} transgenic flies and tested the transformative potential of the transgene in the fly eye disc using sev-GAL4 and GMR-GAL4. Sev-GAL4 did produce

a milder phenotype than GMR-GAL4 and both phenotypes were milder than those of the chimeric human/fly. This proved that human BCR-ABL1 could interfere with *Drosophila* signaling pathways in the eye and disrupt normal development of its photoreceptors. The notion that eye phenotypes were milder than those produced by the chimeric BCR-ABL1 can be explained by the presence of fly Abl carboxy-terminal amino acids in the chimeric construct which gives it the advantage of proper localization to axon bundles (Henkemeyer, West, Gertler, & Hoffmann, 1990). We were then interested in exploring the phenotype induced by the most elusive and famous mutation human BCR-ABL1$^{p210/T315I}$. Using the same eye specific GAL4 drivers, T315I mutation resulted in disruption of normal eye development and in some instances, particularly with GMR-GAL4 at 18°C upon the measurement of the posterior eye defect, it showed a greater area of disruption as compared to BCR-ABL1^{p210}. The observed eye phenotypes upon expression of BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ could be due to cellular proliferation failure or altered cellular fate determination. BCR-ABL1 might be disrupting the differentiation of photoreceptor cells and hence producing degeneration. Staining of the eye discs with anti-ELAV which is a neuronal marker of photoreceptor cells committed to a neuronal fate revealed a disturbed arrangement of ELAV-positive ommatidial clusters with gaps between the clusters indicating that BCR-ABL1 could mess up with the differentiation of photoreceptor cells.

In addition to *Drosophila* eyes, we expressed human BCR-ABL1 in other tissues to have a better characterization of the model and which could later serve as

potential phenotypes for drug or genetic screening. Dealing with a blood cancer necessitated the expression of BCR-ABL1 in hematopoietic tissues to explore the transformative potential on this level. Using Hml Δ-GAL4 which drives the expression in *Drosophila* hemocytes and hemocytes precursors, we detected a significant increase in the number of circulating hemocytes in BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ expressing larvae as compared to the control. Again T315I mutation revealed tendency towards a more severe phenotype. While this approach indicates that there is an increase in a population of hemocytes, it cannot further delineate what type of cells are particularly involved in this phenotype and whether precursor cells are among the population of cells. For this, we can employ hemocytes immunostaining using antibodies directed against plasmatocytes (P1, anti-Nimrod), Peroxidasin (Pxn), lamellocytes (L1) and crystal cells (PPO, prophenoloxidase) which all serve as differentiation markers in hemocytes. The detection of cells that do not express any of these differentiation markers could indicate the presence of precursor cells and hence better characterize the phenotype. On the other hand, wingless signaling (Wg) is required for maintenance of precursor stem cell-like population of hemocytes in *Drosophila* lymph gland (Sinenko, Mandal, Martinez-Agosto, & Banerjee, 2009); those cells express low *hemolectin* (*hml*) levels as well (Sinenko et al., 2010). Therefore, detection of such cells can further validate whether precursor cells contribute to the observed phenotype. Moreover, we have detected melanotic tumors (around 32%) in BCR-ABL1$^{p210/T315I}$ expressing flies under the control of Hml Δ-GAL4 (data not shown, unpublished data). Melanotic tumors often signal an immune response involving

cellular aggregation, phagocytosis, encapsulation, and melanization leading to the formation of black spots that can be easily seen through the larval cuticle (Minakhina & Steward, 2006). Therefore melanotic tumors formation requirs the assistance from plasmatocytes, lamellocytes, and crystal cells for clearing what is sensed as a threat. Going back to the characterization of the type of cells involved in the proliferation phenotype of hemocytes, we can speculate whether an increased precursor hemocytes burden is responsible for melanotic tumor formation. The fact that BCR-ABL1^{p210} did not show melanotic tumors coupled to the lower hemocytes burden as compared to BCR-ABL1$^{p210/T315I}$ further necessitates the identification of the type of hemocytes involved in the proliferation phenotypes. Furthermore, dissection of melanotic masses and staining for Hemese, filamentous actin, and DNA can help us identify if hemocytes are actually present and the nature of these masses (Minakhina & Steward, 2006).

On another level, the hematopoietic model can be further pushed for genetic screening whereby we can identify genes aggravating or attenuating the observed phenotypes by employing RNAi-mediated knockdown of candidate genes coupled to UAS-GAL4 system. Genetic screening might lead us to important insights on unique genetic interactions of BCR-ABL1$^{p210/T315I}$. Furthermore, the genetic screening that we have already started using the eye phenotype could give us hints on genes that could be further validated in *Drosophila* lines expressing BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ in hematopoietic tissues. We have prepared for this purpose screening lines expressing BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ in *Drosophila* hemocytes under the control of Hml Δ-GAL4. Candidate genes in Toll and IMD pathways might

be of particular interest especially that these pathways have been implicated in melanotic tumor formation (Minakhina & Steward, 2006).

We are as well trying to characterize the effect of BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ expression in hematopoietic system on the flies' innate immunity as well through studying the levels of expression of immune inducible antibacterial peptides such as diptericin and drosomycin.

Collectively, we are trying to establish an *in vivo Drosophila* CML model where BCR-ABL1 is expressed in hematopoietic compartments and that could complement *in vivo* studies done on mice. On another note, the model might also serve in the future as a validating drug screening model for drug hits determined by the fly eye or lethality phenotypes. Work using the hematopoietic system is still undergoing.

Expression of BCR-ABL1 in *Drosophila* wings resulted in the disruption of normal wing development again with a more disruptive phenotype associated with T315I mutation. Ubiquitous expression using actin-5C-GAL4 resulted in lethality with both BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$. Finally, using engrailed-GAL4 which drives the expression in the posterior compartments of *Drosophila* imaginal discs resulted in pupal lethality mainly with BCR-ABL1^{p210} and earlier embryonic/larval lethality with BCR-ABL1$^{p210/T315I}$. Whether T315I mutation confers a fitness advantage for BCR-ABL is not well understood or confirmed. Studies show conflicting results and used different approaches for answering the question which makes analysis complicated. Studies suggested a similar (Miething et al., 2006) or reduced (Corbin et al., 2002; Griswold et al., 2006) kinase activity relative to wild type BCR-ABL1 with

one study reporting an increase in the autophosphorylation as well as phosphorylation of other proteins (Yamamoto, Kurosu, Kakihana, Mizuchi, & Miura, 2004); however, they analyzed a truncated form of BCR-ABL. On the other hand, an interesting study (Skaggs et al., 2006) discussed the fact that cell clones carrying T315I mutation was detected in CML patients prior to treatment and this argues that the mutation confers a fitness advantage over the cell clones carrying the wild type BCR-ABL. In this study, they detected an increase in oncogenicity of T315I despite a decrease in kinase activity. To resolve the paradox, they employed quantitative global phosphoproteome profiling to discover that T315I mutation exhibits a unique phosphopeptide signature translated as alterations in P-loop phosphorylation which might affect the signaling interactions normally followed by wild type BCR-ABL1. While this might be a plausible explanation for the increase in severity of phenotypes detected with T315I mutation in *Drosophila* tissues, further development of the model as a genetic screening platform could tackle the question.

B. Drug Screening Using TKIs

1. Drug Screening Using Eye Phenotype

The *Drosophila* eye, with its highly organized reiterative ommatidial structure, constitutes an efficient and relatively easy read out capable of amplifying subtle changes caused by disturbance to normal development. Therefore, we chose this epithelial monolayer as a target tissue for expressing human BCR-ABL1^{p210} and human BCR-ABL1$^{p210/T315I}$. Bernardoni *et al.* (Bernardoni et al., 2019) recently showed that

expression of human BCR-ABL1^{p210} in *Drosophila* eyes was destructive to the normal eye development and resulted in a "glazy" eye phenotype as demonstrated by light microscopy images. We went further to investigate the effect of increased temperature on transgene expression as well as used SEM analysis in addition to light microscopy to show the subtle details of the eye phenotypes. Moreover, we opted to investigate whether one of the most elusive BCR-ABL1 mutations (T315I) behaves similarly or differently to the wild type variant. We found that, with increased temperature, the rough eye phenotype was more prominent in T315I mutant BCR-ABL1. To validate our model for treatment screening, we focused on a specific area in the posterior end of the eye which was evident to be defective in both BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ expressing flies. BCR-ABL1$^{p210/T315I}$ expressing flies showed more severe phenotype characterized by a wider defective area of lost ommatidial facets as compared to flies expressing the wild type variant BCR-ABL1^{p210} indicating that the transformation capacity of T315I is much higher than the wild type BCR-ABL1^{p210}. We further validated the model by assessing the capability of the conventional treatments used in clinics for CML patients in improving the eye defects observed in the adult eyes of BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ flies. These TKIs include imatinib as first generation TKI, nilotinib and dasatinib as second and ponatinib as third generation TKI. Dasatinib and ponatinib resulted in the full rescue of BCR-ABL1^{p210} eye defect in 100% and 86% of flies, respectively. Imatinib and nilotinib exhibited a lower percentage of rescue, 21% and 13%, respectively; this might be attributed to the difference in drug potencies among imatinib and other TKIs whereby compared to imatinib, dasatinib

exhibits 325-fold higher potency for BCR-ABL1 inhibition *in vitro* whereas nilotinib is only 20 folds more potent (T. O'Hare et al., 2005). Another possible explanation for the limited rescuing efficacy of imatinib and nilotinib could be due to activation of dAbl by BCR-ABL1 expression shown previously by Bernardoni *et al.*, (Bernardoni et al., 2019) where it was demonstrated that human BCR-ABL1 expression interferes with dAbl signaling pathway and increases Ena phosphorylation, a dAbl target. On the other hand, using *Drosophila* wing epithelium as an *in vivo* model, Singh *et al*. (Singh, Aaronson, & Mlodzik, 2010) demonstrated that activated dAbl exerts a positive feedback loop on *Drosophila* Src members leading to an increase in their activity and hence signal amplification. It is well known that both dAbl (Xiong & Rebay, 2011) and *Drosophila* Src (Takahashi, Endo, Kojima, & Saigo, 1996) play important roles in *Drosophila* eye development; therefore it is possible that upon human BCR-ABL1 expression in *Drosophila* eyes, the dAbl signaling pathway is activated which in its turn activates *Drosophila* Src members and amplifies BCR-ABL1 mediated effects. Interestingly, Src is one of the kinases inhibited by dasatinib and ponatinib but not imatinib and nilotinib, therefore, this might possibly explain the more robust rescuing effect seen by dasatinib and ponatinib. Dasatinib demonstrated target specificity *in vivo* whereby BCR-ABL1$^{p210/T315I}$ flies fed on dasatinib showed the expected resistance to treatment. BCR-ABL1$^{p210/T315I}$ resistance to imatinib and nilotinib was also confirmed as there was no rescue of ommatidial development. In contrary to what was expected, ponatinib was not successful in rescuing progeny expressing BCR-ABL1$^{p210/T315I}$. While it is hard to explain this phenomenon we would like to focus on the fact that the eye defect area was

significantly larger upon BCR-ABL1$^{p210/T315I}$ expression as compared to that of BCR-ABL1^{p210}. Noting this significant increase in the average posterior eye defect area, we hypothesize that the phenotype was still very severe to allow for any drug reversal. Moreover, noting that the choice of the dose was limited by DMSO toxicity, ponatinib dose used may not have been high enough to reverse the defect.

Collectively, we propose an *in vivo* model for BCR-ABL1 driven transformation where we show the efficacy of the current potent treatments in reversing a very subtle phenotype in a specific location in the posterior end of the adult compound eye. This system could be stretched to assess the efficacy of novel compounds by performing high-throughput library testing *in vivo*. We believe that a *Drosophila* CML model to screen for potential compounds is required in this field especially that TKIs which are currently used do not target CML stem cells and hence are not curative.

2. Drug Screening Using Lethality Phenotype

We have recently started testing the lethality phenotype as a more efficient model for drug screening whereby drug reversal is detected through suppression of lethality of a developmental stage in *Drosophila* life cycle. As discussed above, we have mainly detected pupal lethality upon BCR-ABL1^{p210} expression and earlier embryonic/larval lethality with BCR-ABL1$^{p210/T315I}$. Preliminary results showed that dasatinib and ponatinib suppress pupal lethality in BCR-ABL1^{p210} expressing flies, relieved the developmental block, and allowed the formation of pharate adults and even eclosing adults. Flies eclosing showed wing defects and that is because the wing disc is

one of the discs targeted by engrailed-GAL4. However, drugs did not show reversal of the wing defect but could reverse the developmental block. This might be attributed to the fact that correction of the wing defect might require higher doses of the drugs. We were mainly interested in testing whether ponatinib could reverse T315I lethality although this is challenging because maybe most of the lethality is taking place at the embryonic stage with no chance to feed on the drug since only larvae can feed. Nevertheless we were able to detect larvae capable of reaching the pupal stage which means a one step further in development upon treatment. These pupae are the embryos which could make it through the larval stage and had access to the drug and hence the reversal in lethality. While ponatinib did not show reversal of eye phenotype, here it has the potential to reverse a lethality phenotype which might indicate a tissue-dependent effect of the drug. On the other hand, possible approaches for by passing the embryonic lethality and allowing more larvae to access the drug could be done through the temperature sensitive GAL80ts fly line which can negatively bind GAL4 and inhibit transgene expression at a specific temperature then by shifting to a different temperature the transgene can be expressed at the desired developmental stage. At the same time, the method employed to test for reversal of lethality also should be properly optimized for ensuring optimal conditions for reversal.

C. Genetic Screening

Besides the use of the established eye model for drug screening, it can also be exploited for genetic screening. Although CML has a well-known driving oncogene (BCR-ABL1) and several signaling pathways are well identified; it is still crucial to

investigate the pathophysiologic interactions of BCR-ABL1, especially when mutated. Genetic screening could give us insights on what additional targets might be activated with mutations and hence constitute potential therapeutic targets. We started with a small scale genetic screen using RNAi to knockdown genes of interest using the eye phenotype which could give a visible phenotype easily detected and assessed. We started by chosing few candidates for a mini-screen of genes in the Hh, Notch, Dpp, Wg, EGFR, apoptosis, autophagy, immunity (Toll and IMD), and Hippo pathways. Hh, Notch, Wg, EGFR, and Dpp are known to be crucial pathways in *Drosophila* eye development, acting cooperatively as switches that determine cell fate (Ross Cagan, 2009). Hh initiates the wave of neuronal differentiation (morphogenetic furrow) and induces the expression of Dpp for maintaining a preproneural state in cells ahead of the furrow (C. Thomas & Ingham, 2003). On the other hand, Wg signaling is a crucial determinant of the anterior-posterior and dorsal-ventral patterning of the eye disc and contributes to Notch activation leading to growth of the eye disc (Legent & Treisman, 2008). Notch also orchestrates cell fate, adhesion, and assembly in *Drosophila* eye (Ross Cagan, 2009). EGFR signaling is thought to be involved in proliferation, growth control and survival, of eye disc cells (Domínguez, Wasserman, & Freeman, 1998). Meanwhile, the Hippo pathway was shown to be involved in the regulation of retinal differentiation and cellular growth in eye discs and aids in the progression of the morphogenetic furrow (Wittkorn, Sarkar, Garcia, Kango-Singh, & Singh, 2015) . For instance, the sculpting of *Drosophila* eye with its highly arranged ommatidial structures requires an interplay between cell growth and cell death; hereby the *Drosophila*

apoptotic pathway comes into the picture. Cell death is integral for the precise removal of excess unwanted cells. Failure of apoptosis results in roughened eyes due to variation in the number of pigment cells surrounding ommatidial units (Brachmann & Cagan, 2003). Autophagy as well represents a housekeeping mechanism for controlling retinal homeostasis, pigment granule synthesis, and eye development (Lorincz, Takats, Karpati, & Juhasz, 2016). In addition to the above mentioned pathways, we were interested as well to test the effect of Knockdown of immune pathways genes such as Toll and IMD which are known in *Drosophila* to play part in innate immune response. This is part of the hematopoietic system study whereby we are trying to figure out the effect of BCR-ABL1 expression in *Drosophila* hematopoietic system on innate immunity pathways and the role of these pathways in BCR-ABL1 induced hematopoietic phenotypes as mentioned above.

At the same time if we link these genes to the roles of their mammalian homologues in human CML we can find that studies implicated them in several aspects of CML disease ranging from increased cellular proliferation, resistance, disease development as well as potential roles in maintenance of LSCs; For example Hh signaling is implicated in the disease progression and resistance and constitutes a pertinent therapeutic target (Zeng et al., 2015). Notch signaling is thought to be involved in inhibition of growth of human CML cells (Yin et al., 2009). Deregulation of Hippo signaling was recently shown to play a role in CML progression and pathogenesis (Marsola et al., 2018). Modulation of the bone marrow microenvironment

by CML cells was demonstrated to be mediated through EGFR signaling (Corrado et al., 2016).

Collectively, it seems evident that the study of the role of these genes in BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ expressing *Drosophila* tissues, particularly in the eye, will give us better insights on BCR-ABL1 genetic interactions in this model, help us better understand the genetic players behind BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ induced eye phenotypes as well as might decipher unique genetic interactions for the T315I mutant. Moreover, candidate genes that show profound reversal or aggravation of the eye phenotype might give us hints on potential drugs to be tested in this model as well as will be validated in a *Drosophila* line expressing BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ in hematopoietic system using Hml Δ-GAL4. Preliminary results in RNAi screen have shown lethality upon the knockdown of Dif and Myd88 in BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ expressing eyes. The phenotype might indicate that both Dif and Myd88 were reducing the toxic effects of BCR-ABL1^{p210} and BCR-ABL1$^{p210/T315I}$ expression in the eye discs; however, it is worth noting that testing at a lower temperature (25 ºC) might allow for better resolution of the knockdown-mediated phenotype.

D. Limitations of the Study

The use of *Drosophila* as a drug screening model is relatively new and this leaves us with technical as well as biological limitations. The major concern when feeding larvae a drug is how much of the drug is entering the larva system and do all

larvae eat equally? While this might not be our main concern in this study since we are testing already used drugs in CML, this is a major factor to consider when establishing a dose response for a new drug using this model. Another technical concern is the type of food to use when mixing it with drug, while the type of food we used served the purpose, better fly food mixtures tailored for drug screening could facilitate the process. Moreover, the severe eye phenotype resulting from T315I expression precluded ponatinib reversal and higher doses necessitated higher DMSO concentrations which is toxic to flies. This highlights the issue of DMSO toxicity in flies that limits the concentration of drugs to be tested. At the same time, although there are a lot of conserved biological processes between the fly and human, one should still vigilantly assess and know the differences that exist at some levels for example BCR-ABL1 signaling pathway in *Drosophila* eyes might not mimic all aspects of BCR-ABL1 oncogenic potential.

E. Future Perspectives

This study established the basis of drug screening in a fruit fly CML model and this could be later exploited for high-throughput drug screening whereby drug libraries could be tested for identification of drug hits that can be later validated in *in vitro* CML cell lines and *in vivo* CML mouse models. This could open up for discovery of potentially new therapeutics in the field of CML, especially for emerging resistant mutations.

REFERENCES

Abelson, H. T., & Rabstein, L. S. (1970). Lymphosarcoma: Virus-induced Thymic-independent Disease in Mice. *Cancer Research, 30*(8), 2213-2222.

Acharyya, S., Oskarsson, T., Vanharanta, S., Malladi, S., Kim, J., Morris, P. G., . . . Massague, J. (2012). A CXCL1 paracrine network links cancer chemoresistance and metastasis. *Cell, 150*(1), 165-178. doi: 10.1016/j.cell.2012.04.042

Agaisse, H., Petersen, U.-M., Boutros, M., Mathey-Prevot, B., & Perrimon, N. (2003). Signaling Role of Hemocytes in Drosophila JAK/STAT-Dependent Response to Septic Injury. *Developmental Cell, 5*(3), 441-450. doi: 10.1016/S1534-5807(03)00244-2

Agaisse, H., Petersen, U. M., Boutros, M., Mathey-Prevot, B., & Perrimon, N. (2003). Signaling role of hemocytes in Drosophila JAK/STAT-dependent response to septic injury. *Dev Cell, 5*(3), 441-450.

Al Outa, A., Abubaker, D., Bazarbachi, A., El Sabban, M., Shirinian, M., & Nasr, R. (2019). Validation of a Drosophila model of wild type and T315I mutated BCR-ABL1 in chronic myeloid leukemia: An effective platform for treatment screening. *Haematologica*. doi: 10.3324/haematol.2019.219394

Allan, N., Shepherd, P., & Richards, S. (1995). UK Medical Research Council randomised, multicentre trial of interferon-αn1 for chronic myeloid leukaemia: improved survival irrespective of cytogenetic response. *The Lancet, 345*(8962), 1392-1397.

Amoyel, M., Anderson, A. M., & Bach, E. A. (2014). JAK/STAT pathway dysregulation in tumors: a Drosophila perspective. *Semin Cell Dev Biol, 28*, 96-103. doi: 10.1016/j.semcdb.2014.03.023

Anderson, A. M., Bailetti, A. A., Rodkin, E., De, A., & Bach, E. A. (2017). A Genetic Screen Reveals an Unexpected Role for Yorkie Signaling in JAK/STAT-Dependent Hematopoietic Malignancies in Drosophila melanogaster. *G3: Genes|Genomes|Genetics, 7*(8), 2427-2438. doi: 10.1534/g3.117.044172

Andersson, A., Eden, P., Lindgren, D., Nilsson, J., Lassen, C., Heldrup, J., . . . Fioretos, T. (2005). Gene expression profiling of leukemic cell lines reveals conserved molecular signatures among subtypes with specific genetic aberrations. *Leukemia, 19*(6), 1042-1050. doi: 10.1038/sj.leu.2403749

Ashburner, M. (1989). *Drosophila. A laboratory handbook*: Cold spring harbor laboratory press.

Ayyaz, A., Li, H., & Jasper, H. (2015). Haemocytes control stem cell activity in the Drosophila intestine. *Nat Cell Biol, 17*(6), 736-748. doi: 10.1038/ncb3174

Baccarani, M., Deininger, M. W., Rosti, G., Hochhaus, A., Soverini, S., Apperley, J. F., . . . Hehlmann, R. (2013). European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. *Blood, 122*(6), 872-884. doi: 10.1182/blood-2013-05-501569

Bangi, E., Ang, C., Smibert, P., Uzilov, A. V., Teague, A. G., Antipin, Y., . . . Cagan, R. L. (2019). A personalized platform identifies trametinib plus zoledronate for a patient with KRAS-mutant metastatic colorectal cancer. *Science advances, 5*(5), eaav6528-eaav6528. doi: 10.1126/sciadv.aav6528

Baril, C., Gavory, G., Bidla, G., Knaevelsrud, H., Sauvageau, G., & Therrien, M. (2017). Human NUP98-HOXA9 promotes hyperplastic growth of hematopoietic tissues in Drosophila. *Dev Biol, 421*(1), 16-26. doi: 10.1016/j.ydbio.2016.11.003

Bazzoni, G., Carlesso, N., Griffin, J. D., & Hemler, M. E. (1996). Bcr/Abl expression stimulates integrin function in hematopoietic cell lines. *J Clin Invest, 98*(2), 521-528. doi: 10.1172/jci118820

Ben-Neriah, Y., Daley, G. Q., Mes-Masson, A. M., Witte, O. N., & Baltimore, D. (1986). The chronic myelogenous leukemia-specific P210 protein is the product of the bcr/abl hybrid gene. *Science, 233*(4760), 212-214.

Bennett, D., Lyulcheva, E., & Cobbe, N. (2015). Drosophila as a Potential Model for Ocular Tumors. *Ocul Oncol Pathol, 1*(3), 190-199. doi: 10.1159/000370155

Bernardoni, R., Giordani, G., Signorino, E., Monticelli, S., Messa, F., Pradotto, M., . . . Cilloni, D. (2019). A new BCR-ABL1 Drosophila model as a powerful tool to elucidate the pathogenesis and progression of chronic myeloid leukemia. *Haematologica, 104*(4), 717-728. doi: 10.3324/haematol.2018.198267

Bhatia, R., Munthe, H. A., & Verfaillie, C. M. (1999). Role of abnormal integrin-cytoskeletal interactions in impaired beta1 integrin function in chronic myelogenous leukemia hematopoietic progenitors. *Exp Hematol, 27*(9), 1384-1396.

Biron, C. A. (2001). Interferons α and β as immune regulators—a new look. *Immunity, 14*(6), 661-664.

Bolin, R. W., Robinson, W. A., Sutherland, J., & Hamman, R. F. (1982). Busulfan versus hydroxyurea in long-term therapy of chronic myelogenous leukemia. *Cancer, 50*(9), 1683-1686.

Botham, C. M., Wandler, A. M., & Guillemin, K. (2008). A transgenic Drosophila model demonstrates that the Helicobacter pylori CagA protein functions as a eukaryotic Gab adaptor. *PLoS pathogens, 4*(5), e1000064.

Boulet, M., Miller, M., Vandel, L., & Waltzer, L. (2018). From Drosophila Blood Cells to Human Leukemia. In M. Yamaguchi (Ed.), *Drosophila Models for Human Diseases* (pp. 195-214). Singapore: Springer Singapore.

Bourbon, H.-M., Gonzy-Treboul, G., Peronnet, F., Alin, M.-F., Ardourel, C., Benassayag, C., . . . Vincent, A. (2002). A P-insertion screen identifying novel X-linked essential genes in Drosophila. *Mechanisms of Development, 110*(1), 71-83. doi: https://doi.org/10.1016/S0925-4773(01)00566-4

Brachmann, C. B., & Cagan, R. L. (2003). Patterning the fly eye: the role of apoptosis. *Trends Genet, 19*(2), 91-96. doi: 10.1016/s0168-9525(02)00041-0

Branford, S., & Hughes, T. (2006). Detection of BCR-ABL mutations and resistance to imatinib mesylate. *Methods Mol Med, 125*, 93-106.

Branford, S., Rudzki, Z., Walsh, S., Grigg, A., Arthur, C., Taylor, K., . . . Hughes, T. P. (2002). High frequency of point mutations clustered within the adenosine

triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. *Blood, 99*(9), 3472-3475.

Branson, K., Robie, A. A., Bender, J., Perona, P., & Dickinson, M. H. (2009). High-throughput ethomics in large groups of Drosophila. *Nat Methods, 6*(6), 451-457. doi: 10.1038/nmeth.1328

Bras, S., Martin-Lanneree, S., Gobert, V., Auge, B., Breig, O., Sanial, M., . . . Waltzer, L. (2012). Myeloid leukemia factor is a conserved regulator of RUNX transcription factor activity involved in hematopoiesis. *Proc Natl Acad Sci U S A, 109*(13), 4986-4991. doi: 10.1073/pnas.1117317109

Breig, O., Bras, S., Martinez Soria, N., Osman, D., Heidenreich, O., Haenlin, M., & Waltzer, L. (2014). Pontin is a critical regulator for AML1-ETO-induced leukemia. *Leukemia, 28*(6), 1271-1279. doi: 10.1038/leu.2013.376

Brody, T. (1999). The Interactive Fly: gene networks, development and the Internet. *Trends Genet, 15*(8), 333-334.

Buchdunger, E., Zimmermann, J., Mett, H., Meyer, T., Müller, M., Druker, B. J., & Lydon, N. B. (1996). Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative. *Cancer Research, 56*(1), 100-104.

Buchdunger, E., Zimmermann, J., Mett, H., Meyer, T., Müller, M., Regenass, U., & Lydon, N. B. (1995). Selective inhibition of the platelet-derived growth factor signal transduction pathway by a protein-tyrosine kinase inhibitor of the 2-phenylaminopyrimidine class. *Proceedings of the National Academy of Sciences, 92*(7), 2558-2562.

Cagan, R. (2009). Principles of Drosophila eye differentiation. *Curr Top Dev Biol, 89*, 115-135. doi: 10.1016/s0070-2153(09)89005-4

Cagan, R. (2009). Principles of Drosophila eye differentiation. *Current topics in developmental biology, 89*, 115-135. doi: 10.1016/S0070-2153(09)89005-4

Campos, A. R., And, D. G., & White, K. (1985). Mutant alleles at the locus elav in Drosophila melanogaster lead to nervous system defects. A developmental-genetic analysis. *Journal of neurogenetics, 2*(3), 197-218.

Carroll, M., Ohno-Jones, S., Tamura, S., Buchdunger, E., Zimmermann, J., Lydon, N. B., . . . Druker, B. J. (1997). CGP 57148, a tyrosine kinase inhibitor, inhibits the growth of cells expressing BCR-ABL, TEL-ABL, and TEL-PDGFR fusion proteins. *Blood, 90*(12), 4947-4952.

Chan, L., Karhi, K., Rayter, S., Heisterkamp, N., Eridani, S., Powle, R., . . . Greaves, M. (1987). A novel abl protein expressed in Philadelphia chromosome positive acute lymphoblastic leukaemia positive acute lymphoblastic leukaemia. *Nature, 325*(6105), 635.

Chand, D., Yamazaki, Y., Ruuth, K., Schönherr, C., Martinsson, T., Kogner, P., . . . Marra, M. A. (2013). Cell culture and Drosophila model systems define three classes of anaplastic lymphoma kinase mutations in neuroblastoma. *Disease models & mechanisms, 6*(2), 373-382.

Chang, S., Bray, S. M., Li, Z., Zarnescu, D. C., He, C., Jin, P., & Warren, S. T. (2008). Identification of small molecules rescuing fragile X syndrome phenotypes in Drosophila. *Nat Chem Biol, 4*(4), 256-263. doi: 10.1038/nchembio.78

Chang, T., Mazotta, J., Dumstrei, K., Dumitrescu, A., & Hartenstein, V. (2001). Dpp and Hh signaling in the Drosophila embryonic eye field. *Development, 128*(23), 4691-4704.

Chang, T., Shy, D., & Hartenstein, V. (2003). Antagonistic relationship between Dpp and EGFR signaling in Drosophila head patterning. *Dev Biol, 263*(1), 103-113.

Chawla-Sarkar, M., Lindner, D., Liu, Y.-F., Williams, B., Sen, G., Silverman, R., & Borden, E. (2003). Apoptosis and interferons: role of interferon-stimulated genes as mediators of apoptosis. *Apoptosis, 8*(3), 237-249.

Chen, R., Ma, B., Yang, K., Tian, J., Liu, Y., & Zhao, L. (2011). Interferon alfa versus interferon alfa plus cytarabine combination therapy for chronic myeloid leukemia: a meta-analysis of randomized controlled trials. *Current Therapeutic Research, 72*(4), 150-163.

Cilloni, D., & Saglio, G. (2012). Molecular pathways: BCR-ABL. *Clin Cancer Res, 18*(4), 930-937. doi: 10.1158/1078-0432.ccr-10-1613

Clarke, C. J., & Holyoake, T. L. (2017). Preclinical approaches in chronic myeloid leukemia: from cells to systems. *Exp Hematol, 47*, 13-23. doi: 10.1016/j.exphem.2016.11.005

Claudio, B. (1958). Melanotic tumors in DROSOPHILA. *Journal of Cellular and Comparative Physiology, 52*(S1), 371-381. doi: doi:10.1002/jcp.1030520417

Cohen, G. B., Ren, R., & Baltimore, D. (1995). Modular binding domains in signal transduction proteins. *Cell, 80*(2), 237-248.

Corbin, A. S., Agarwal, A., Loriaux, M., Cortes, J., Deininger, M. W., & Druker, B. J. (2011). Human chronic myeloid leukemia stem cells are insensitive to imatinib despite inhibition of BCR-ABL activity. *J Clin Invest, 121*(1), 396-409. doi: 10.1172/jci35721

Corbin, A. S., Buchdunger, E., Pascal, F., & Druker, B. J. (2002). Analysis of the structural basis of specificity of inhibition of the Abl kinase by STI571. *J Biol Chem, 277*(35), 32214-32219. doi: 10.1074/jbc.M111525200

Corbin, A. S., La Rosee, P., Stoffregen, E. P., Druker, B. J., & Deininger, M. W. (2003). Several Bcr-Abl kinase domain mutants associated with imatinib mesylate resistance remain sensitive to imatinib. *Blood, 101*(11), 4611-4614. doi: 10.1182/blood-2002-12-3659

Cornelison, A. M., Kantarjian, H., Cortes, J., & Jabbour, E. (2011). Outcome of treatment of chronic myeloid leukemia with second-generation tyrosine kinase inhibitors after imatinib failure. *Clinical Lymphoma Myeloma and Leukemia, 11*, S101-S110.

Corrado, C., Saieva, L., Raimondo, S., Santoro, A., De Leo, G., & Alessandro, R. (2016). Chronic myelogenous leukaemia exosomes modulate bone marrow microenvironment through activation of epidermal growth factor receptor. *Journal of cellular and molecular medicine, 20*(10), 1829-1839. doi: 10.1111/jcmm.12873

Correa, S., Binato, R., Du Rocher, B., Castelo-Branco, M. T., Pizzatti, L., & Abdelhay, E. (2012). Wnt/beta-catenin pathway regulates ABCB1 transcription in chronic myeloid leukemia. *BMC Cancer, 12*, 303. doi: 10.1186/1471-2407-12-303

Cortes, J., Rousselot, P., Kim, D. W., Ritchie, E., Hamerschlak, N., Coutre, S., . . . Baccarani, M. (2007). Dasatinib induces complete hematologic and cytogenetic responses in patients with imatinib-resistant or -intolerant chronic myeloid leukemia in blast crisis. *Blood, 109*(8), 3207-3213. doi: 10.1182/blood-2006-09-046888

Cortes, J. E., Kantarjian, H., Shah, N. P., Bixby, D., Mauro, M. J., Flinn, I., . . . Talpaz, M. (2012). Ponatinib in refractory Philadelphia chromosome-positive leukemias. *N Engl J Med, 367*(22), 2075-2088. doi: 10.1056/NEJMoa1205127

Cortes, J. E., Kim, D. W., Pinilla-Ibarz, J., le Coutre, P., Paquette, R., Chuah, C., . . . Kantarjian, H. (2013). A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. *N Engl J Med, 369*(19), 1783-1796. doi: 10.1056/NEJMoa1306494

Corwin, H. O., & Hanratty, W. P. (1976). Characterization of a unique lethal tumorous mutation in Drosophila. *Mol Gen Genet, 144*(3), 345-347.

Crozatier, M., & Meister, M. (2007). Drosophila haematopoiesis. *Cell Microbiol, 9*(5), 1117-1126. doi: 10.1111/j.1462-5822.2007.00930.x

Crozatier, M., Ubeda, J.-M., Vincent, A., & Meister, M. (2004). Cellular Immune Response to Parasitization in Drosophila Requires the EBF Orthologue Collier. *PLOS Biology, 2*(8), e196. doi: 10.1371/journal.pbio.0020196

Crozatier, M., & Vincent, A. (2011). Drosophila: a model for studying genetic and molecular aspects of haematopoiesis and associated leukaemias. *Dis Model Mech, 4*(4), 439-445. doi: 10.1242/dmm.007351

Cvetković, V. J., Mitrović, T. L., Jovanović, B., Stamenković, S. S., Todorović, M., Đorđević, M., & Radulović, N. (2015). Toxicity of dimethyl sulfoxide against Drosophila melanogaster. *Biologica Nyssana, 6*(2).

Daley, G. Q., Van Etten, R. A., & Baltimore, D. (1990). Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science, 247*(4944), 824-830.

Dar, A. C., Das, T. K., Shokat, K. M., & Cagan, R. L. (2012). Chemical genetic discovery of targets and anti-targets for cancer polypharmacology. *Nature, 486*(7401), 80-84. doi: 10.1038/nature11127

Das, J., Chen, P., Norris, D., Padmanabha, R., Lin, J., Moquin, R. V., . . . Barrish, J. C. (2006). 2-aminothiazole as a novel kinase inhibitor template. Structure-activity relationship studies toward the discovery of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1- piperazinyl)]-2-methyl-4-pyrimidinyl]amino)]-1,3-thiazole-5-carboxamide (dasatinib, BMS-354825) as a potent pan-Src kinase inhibitor. *J Med Chem, 49*(23), 6819-6832. doi: 10.1021/jm060727j

Dash, S., Siddam, A. D., Barnum, C. E., Janga, S. C., & Lachke, S. A. (2016). RNA-binding proteins in eye development and disease: implication of conserved RNA granule components. *Wiley Interdiscip Rev RNA, 7*(4), 527-557. doi: 10.1002/wrna.1355

Dazzi, F., Capelli, D., Hasserjian, R., Cotter, F., Corbo, M., Poletti, A., . . . Gordon, M. Y. (1998). The kinetics and extent of engraftment of chronic myelogenous leukemia cells in non-obese diabetic/severe combined immunodeficiency mice reflect the phase of the donor's disease: an in vivo model of chronic myelogenous leukemia biology. *Blood, 92*(4), 1390-1396.

de Almeida, M. H., Fogliatto, L., & Couto, D. (2014). Importance of adherence to BCR-ABL tyrosine-kinase inhibitors in the treatment of chronic myeloid leukemia. *Rev Bras Hematol Hemoter, 36*(1), 54-59. doi: 10.5581/1516-8484.20140014

De Gregorio, E., Han, S. J., Lee, W. J., Baek, M. J., Osaki, T., Kawabata, S., . . . Brey, P. T. (2002). An immune-responsive Serpin regulates the melanization cascade in Drosophila. *Dev Cell, 3*(4), 581-592.

Deininger, M. W., Goldman, J. M., Lydon, N., & Melo, J. V. (1997). The tyrosine kinase inhibitor CGP57148B selectively inhibits the growth of BCR-ABL-positive cells. *Blood, 90*(9), 3691-3698.

Deininger, M. W. N., Goldman, J. M., & Melo, J. V. (2000). The molecular biology of chronic myeloid leukemia. *Blood, 96*(10), 3343-3356.

Denhardt, D. T. (1996). Signal-transducing protein phosphorylation cascades mediated by Ras/Rho proteins in the mammalian cell: the potential for multiplex signalling. *The Biochemical journal, 318 (Pt 3)*(Pt 3), 729-747.

Desai, U. A., Pallos, J., Ma, A. A., Stockwell, B. R., Thompson, L. M., Marsh, J. L., & Diamond, M. I. (2006). Biologically active molecules that reduce polyglutamine aggregation and toxicity. *Hum Mol Genet, 15*(13), 2114-2124. doi: 10.1093/hmg/ddl135

Dick, J. E. (2008). Stem cell concepts renew cancer research. *Blood, 112*(13), 4793-4807. doi: 10.1182/blood-2008-08-077941

Dimarcq, J. L., Imler, J. L., Lanot, R., Ezekowitz, R. A., Hoffmann, J. A., Janeway, C. A., & Lagueux, M. (1997). Treatment of l(2)mbn Drosophila tumorous blood cells with the steroid hormone ecdysone amplifies the inducibility of antimicrobial peptide gene expression. *Insect Biochem Mol Biol, 27*(10), 877-886.

Dionne, M. S., Ghori, N., & Schneider, D. S. (2003). Drosophila melanogaster Is a Genetically Tractable Model Host for Mycobacterium marinum. *Infection and Immunity, 71*(6), 3540-3550. doi: 10.1128/iai.71.6.3540-3550.2003

Domínguez, M., Wasserman, J. D., & Freeman, M. (1998). Multiple functions of the EGF receptor in Drosophila eye development. *Current Biology, 8*(19), 1039-1048. doi: https://doi.org/10.1016/S0960-9822(98)70441-5

Druker, B. J., Guilhot, F., O'Brien, S. G., Gathmann, I., Kantarjian, H., Gattermann, N., . . . Larson, R. A. (2006). Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N Engl J Med, 355*(23), 2408-2417. doi: 10.1056/NEJMoa062867

Druker, B. J., & Lydon, N. B. (2000). Lessons learned from the development of an abl tyrosine kinase inhibitor for chronic myelogenous leukemia. *The Journal of clinical investigation, 105*(1), 3-7.

Druker, B. J., Sawyers, C. L., Kantarjian, H., Resta, D. J., Reese, S. F., Ford, J. M., . . . Talpaz, M. (2001). Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *New England Journal of Medicine, 344*(14), 1038-1042.

Druker, B. J., Talpaz, M., Resta, D. J., Peng, B., Buchdunger, E., Ford, J. M., . . . Ohno-Jones, S. (2001). Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *New England Journal of Medicine, 344*(14), 1031-1037.

Druker, B. J., Tamura, S., Buchdunger, E., Ohno, S., Segal, G. M., Fanning, S., . . . 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), 561-566.

Duffy, J. B. (2002). GAL4 system in Drosophila: a fly geneticist's Swiss army knife. *Genesis, 34*(1-2), 1-15. doi: 10.1002/gene.10150

Dzitoyeva, S., Dimitrijevic, N., & Manev, H. (2003). Gamma-aminobutyric acid B receptor 1 mediates behavior-impairing actions of alcohol in Drosophila: adult RNA interference and pharmacological evidence. *Proc Natl Acad Sci U S A, 100*(9), 5485-5490. doi: 10.1073/pnas.0830111100

Elrod-Erickson, M., Mishra, S., & Schneider, D. (2000). Interactions between the cellular and humoral immune responses in Drosophila. *Curr Biol, 10*(13), 781-784.

Evans, C. J., Hartenstein, V., & Banerjee, U. (2003). Thicker than blood: conserved mechanisms in Drosophila and vertebrate hematopoiesis. *Dev Cell, 5*(5), 673-690.

Evans, C. J., Liu, T., & Banerjee, U. (2014). Drosophila hematopoiesis: Markers and methods for molecular genetic analysis. *Methods, 68*(1), 242-251. doi: 10.1016/j.ymeth.2014.02.038

Fang, Y., Soares, L., Teng, X., Geary, M., & Bonini, N. M. (2012). A novel Drosophila model of nerve injury reveals an essential role of Nmnat in maintaining axonal integrity. *Curr Biol, 22*(7), 590-595. doi: 10.1016/j.cub.2012.01.065

Feller, S. M., Knudsen, B., & Hanafusa, H. (1994). c-Abl kinase regulates the protein binding activity of c-Crk. *Embo j, 13*(10), 2341-2351.

Fogerty, F. J., Juang, J. L., Petersen, J., Clark, M. J., Hoffmann, F. M., & Mosher, D. F. (1999). Dominant effects of the bcr-abl oncogene on Drosophila morphogenesis. *Oncogene, 18*(1), 219-232. doi: 10.1038/sj.onc.1202239

Fortini, M. E., Simon, M. A., & Rubin, G. M. (1992). Signalling by the sevenless protein tyrosine kinase is mimicked by Rasl activation. *Nature, 355*(6360), 559.

Fossgreen, A., Bruckner, B., Czech, C., Masters, C. L., Beyreuther, K., & Paro, R. (1998). Transgenic Drosophila expressing human amyloid precursor protein show gamma-secretase activity and a blistered-wing phenotype. *Proc Natl Acad Sci U S A, 95*(23), 13703-13708. doi: 10.1073/pnas.95.23.13703

Franke, T. F., Kaplan, D. R., & Cantley, L. C. (1997). PI3K: downstream AKTion blocks apoptosis. *Cell, 88*(4), 435-437.

Freeman, M. (1996). Reiterative use of the EGF receptor triggers differentiation of all cell types in the Drosophila eye. *Cell, 87*(4), 651-660.
Gainor, J. F., & Chabner, B. A. (2015). Ponatinib: Accelerated Disapproval. *Oncologist, 20*(8), 847-848. doi: 10.1634/theoncologist.2015-0253
Galloway, J. L., & Zon, L. I. (2003). 3 Ontogeny of hematopoiesis: Examining the emergence of hematopoietic cells in the vertebrate embryo *Current Topics in Developmental Biology* (Vol. 53, pp. 139-158): Academic Press.
Gateff, E. (1978). Malignant neoplasms of genetic origin in Drosophila melanogaster. *Science, 200*(4349), 1448-1459.
Gateff, E. (1994). Tumor-suppressor genes, hematopoietic malignancies and other hematopoietic disorders of Drosophila melanogaster. *Ann N Y Acad Sci, 712*, 260-279.
Ghosh, S., Singh, A., Mandal, S., & Mandal, L. (2015). Active hematopoietic hubs in Drosophila adults generate hemocytes and contribute to immune response. *Dev Cell, 33*(4), 478-488. doi: 10.1016/j.devcel.2015.03.014
Gillet, J. P., Varma, S., & Gottesman, M. M. (2013). The clinical relevance of cancer cell lines. *J Natl Cancer Inst, 105*(7), 452-458. doi: 10.1093/jnci/djt007
Gladstone, M., & Su, T. T. (2011). Screening for radiation sensitizers of Drosophila checkpoint mutants. *Methods Mol Biol, 782*, 105-117. doi: 10.1007/978-1-61779-273-1_9
Godin, I., & Cumano, A. (2002). The hare and the tortoise: an embryonic haematopoietic race. *Nat Rev Immunol, 2*(8), 593-604. doi: 10.1038/nri857
Gold, K. S., & Brückner, K. (2014). Drosophila as a model for the two myeloid blood cell systems in vertebrates. *Exp Hematol, 42*(8), 717-727. doi: 10.1016/j.exphem.2014.06.002
Goldman, J. M. (2010). Chronic myeloid leukemia: a historical perspective. *Semin Hematol, 47*(4), 302-311. doi: 10.1053/j.seminhematol.2010.07.001
Gonzalez, C. (2013). Drosophila melanogaster: a model and a tool to investigate malignancy and identify new therapeutics. *Nat Rev Cancer, 13*(3), 172-183. doi: 10.1038/nrc3461
Gordon, M. Y., Dowding, C. R., Riley, G. P., Goldman, J. M., & Greaves, M. F. (1987). Altered adhesive interactions with marrow stroma of haematopoietic progenitor cells in chronic myeloid leukaemia. *Nature, 328*(6128), 342-344. doi: 10.1038/328342a0
Gorre, M. E., Mohammed, M., Ellwood, K., Hsu, N., Paquette, R., Rao, P. N., & Sawyers, C. L. (2001). Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science, 293*(5531), 876-880. doi: 10.1126/science.1062538
Goto, A., Kadowaki, T., & Kitagawa, Y. (2003). Drosophila hemolectin gene is expressed in embryonic and larval hemocytes and its knock down causes bleeding defects11Two hml-GAL4 lines (w1118; P{w + mc = GAL4-Hml} 5-6 and w1118; P{w + mc = GAL4-Hml}6-4) and one homozygote of d-hml-GAL4, UAS-gfpnlacZ, UAS-gfp[S65T]/d-hml-GAL4, UAS-gfpnlacZ, UAS-gfp[S65T] (w1118; P{w + mc = GAL4-Hml} 6-4 P{w + mc = UAS-GFP::lacZ.nls} 15.1)

transgenic lines are available from the Bloomington Stock Center with the stock numbers of 6395, 6396, and 6397, respectively. *Dev Biol, 264*(2), 582-591. doi: https://doi.org/10.1016/j.ydbio.2003.06.001

Goto, A., Kumagai, T., Kumagai, C., Hirose, J., Narita, H., Mori, H., . . . Kitagawa, Y. (2001). A Drosophila haemocyte-specific protein, hemolectin, similar to human von Willebrand factor. *Biochemical Journal, 359*(Pt 1), 99-108.

Griswold, I. J., MacPartlin, M., Bumm, T., Goss, V. L., O'Hare, T., Lee, K. A., . . . Deininger, M. W. (2006). Kinase domain mutants of Bcr-Abl exhibit altered transformation potency, kinase activity, and substrate utilization, irrespective of sensitivity to imatinib. *Mol Cell Biol, 26*(16), 6082-6093. doi: 10.1128/mcb.02202-05

Groth, A. C., Fish, M., Nusse, R., & Calos, M. P. (2004). Construction of transgenic Drosophila by using the site-specific integrase from phage phiC31. *Genetics, 166*(4), 1775-1782.

Grzeschik, N. A., Parsons, L. M., Allott, M. L., Harvey, K. F., & Richardson, H. E. (2010). Lgl, aPKC, and Crumbs regulate the Salvador/Warts/Hippo pathway through two distinct mechanisms. *Curr Biol, 20*(7), 573-581. doi: 10.1016/j.cub.2010.01.055

Hales, K. G., Korey, C. A., Larracuente, A. M., & Roberts, D. M. (2015). Genetics on the Fly: A Primer on the Drosophila Model System. *Genetics, 201*(3), 815-842. doi: 10.1534/genetics.115.183392

Hanahan, D., & Weinberg, R. A. (2000). The hallmarks of cancer. *Cell, 100*(1), 57-70.

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell, 144*(5), 646-674. doi: 10.1016/j.cell.2011.02.013

Hanratty, W. P., & Ryerse, J. S. (1981). A genetic melanotic neoplasm of Drosophila melanogaster. *Dev Biol, 83*(2), 238-249.

Hao, S. X., & Ren, R. (2000). Expression of interferon consensus sequence binding protein (ICSBP) is downregulated in Bcr-Abl-induced murine chronic myelogenous leukemia-like disease, and forced coexpression of ICSBP inhibits Bcr-Abl-induced myeloproliferative disorder. *Mol Cell Biol, 20*(4), 1149-1161.

Henkemeyer, M., West, S. R., Gertler, F. B., & Hoffmann, F. M. (1990). A novel tyrosine kinase-independent function of Drosophila abl correlates with proper subcellular localization. *Cell, 63*(5), 949-960.

Hochhaus, A., Kreil, S., Corbin, A. S., La Rosee, P., Muller, M. C., Lahaye, T., . . . Hehlmann, R. (2002). Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. *Leukemia, 16*(11), 2190-2196. doi: 10.1038/sj.leu.2402741

Hochhaus, A., O'Brien, S. G., Guilhot, F., Druker, B. J., Branford, S., Foroni, L., . . . Larson, R. A. (2009). Six-year follow-up of patients receiving imatinib for the first-line treatment of chronic myeloid leukemia. *Leukemia, 23*(6), 1054-1061. doi: 10.1038/leu.2009.38

Holz, A., Bossinger, B., Strasser, T., Janning, W., & Klapper, R. (2003). The two origins of hemocytes in Drosophila. *Development, 130*(20), 4955-4962. doi: 10.1242/dev.00702

Honti, V., Csordas, G., Kurucz, E., Markus, R., & Ando, I. (2014). The cell-mediated immunity of Drosophila melanogaster: hemocyte lineages, immune compartments, microanatomy and regulation. *Dev Comp Immunol, 42*(1), 47-56. doi: 10.1016/j.dci.2013.06.005

Honti, V., Csordás, G., Márkus, R., Kurucz, É., Jankovics, F., & Andó, I. (2010). Cell lineage tracing reveals the plasticity of the hemocyte lineages and of the hematopoietic compartments in Drosophila melanogaster. *Molecular Immunology, 47*(11), 1997-2004. doi: https://doi.org/10.1016/j.molimm.2010.04.017

Huang, F., Reeves, K., Han, X., Fairchild, C., Platero, S., Wong, T. W., . . . Clark, E. (2007). Identification of candidate molecular markers predicting sensitivity in solid tumors to dasatinib: rationale for patient selection. *Cancer Res, 67*(5), 2226-2238. doi: 10.1158/0008-5472.can-06-3633

Ilaria, R. L., Jr., & Van Etten, R. A. (1996). P210 and P190(BCR/ABL) induce the tyrosine phosphorylation and DNA binding activity of multiple specific STAT family members. *J Biol Chem, 271*(49), 31704-31710.

Inagaki, H. K., Kamikouchi, A., & Ito, K. (2010). Protocol for quantifying sound-sensing ability of Drosophila melanogaster. *Nat Protoc, 5*(1), 26-30. doi: 10.1038/nprot.2009.206

Iqbal, N., & Iqbal, N. (2014). Imatinib: a breakthrough of targeted therapy in cancer. *Chemotherapy research and practice, 2014*, 357027-357027. doi: 10.1155/2014/357027

Irving, P., Ubeda, J. M., Doucet, D., Troxler, L., Lagueux, M., Zachary, D., . . . Meister, M. (2005). New insights into Drosophila larval haemocyte functions through genome-wide analysis. *Cell Microbiol, 7*(3), 335-350. doi: 10.1111/j.1462-5822.2004.00462.x

Ito, M., Hiramatsu, H., Kobayashi, K., Suzue, K., Kawahata, M., Hioki, K., . . . Nakahata, T. (2002). NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. *Blood, 100*(9), 3175-3182. doi: 10.1182/blood-2001-12-0207

Ja, W. W., Carvalho, G. B., Mak, E. M., de la Rosa, N. N., Fang, A. Y., Liong, J. C., . . . Benzer, S. (2007). Prandiology of Drosophila and the CAFE assay. *Proc Natl Acad Sci U S A, 104*(20), 8253-8256. doi: 10.1073/pnas.0702726104

Jabbour, E., Kantarjian, H., Jones, D., Talpaz, M., Bekele, N., O'Brien, S., . . . Cortes, J. (2006). Frequency and clinical significance of BCR-ABL mutations in patients with chronic myeloid leukemia treated with imatinib mesylate. *Leukemia, 20*(10), 1767-1773. doi: 10.1038/sj.leu.2404318

Jennings, B. H. (2011). Drosophila – a versatile model in biology & medicine. *Materials Today, 14*(5), 190-195. doi: https://doi.org/10.1016/S1369-7021(11)70113-4

Jung, A. C., Denholm, B., Skaer, H., & Affolter, M. (2005). Renal Tubule Development in Drosophila: A Closer Look at the Cellular Level. *Journal of the American Society of Nephrology, 16*(2), 322-328. doi: 10.1681/asn.2004090729

Jung, S.-H., Evans, C. J., Uemura, C., & Banerjee, U. (2005). The Drosophila lymph gland as a developmental model of hematopoiesis. *Development, 132*(11), 2521. doi: 10.1242/dev.01837

Jung, S. H., Evans, C. J., Uemura, C., & Banerjee, U. (2005). The Drosophila lymph gland as a developmental model of hematopoiesis. *Development, 132*(11), 2521-2533. doi: 10.1242/dev.01837

Kantarjian, H., Shah, N. P., Hochhaus, A., Cortes, J., Shah, S., Ayala, M., . . . Baccarani, M. (2010). Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med, 362*(24), 2260-2270. doi: 10.1056/NEJMoa1002315

Kantarjian, H. M., Keating, M. J., Talpaz, M., Walters, R. S., Smith, T. L., Cork, A., . . . Freireich, E. J. (1987). Chronic myelogenous leukemia in blast crisis: analysis of 242 patients. *The American journal of medicine, 83*(3), 445-454.

Karim, F. D., Chang, H. C., Therrien, M., Wassarman, D. A., Laverty, T., & Rubin, G. M. (1996). A screen for genes that function downstream of Ras1 during Drosophila eye development. *Genetics, 143*(1), 315-329.

Karim, F. D., & Rubin, G. M. (1998). Ectopic expression of activated Ras1 induces hyperplastic growth and increased cell death in Drosophila imaginal tissues. *Development, 125*(1), 1-9.

Kelliher, M. A., McLaughlin, J., Witte, O. N., & Rosenberg, N. (1990). Induction of a chronic myelogenous leukemia-like syndrome in mice with v-abl and BCR/ABL. *Proc Natl Acad Sci U S A, 87*(17), 6649-6653.

Kenyon, K. L., Ranade, S. S., Curtiss, J., Mlodzik, M., & Pignoni, F. (2003). Coordinating proliferation and tissue specification to promote regional identity in the Drosophila head. *Dev Cell, 5*(3), 403-414.

Kipreos, E. T., & Wang, J. Y. (1990). Differential phosphorylation of c-Abl in cell cycle determined by cdc2 kinase and phosphatase activity. *Science, 248*(4952), 217-220.

Kipreos, E. T., & Wang, J. Y. (1992). Cell cycle-regulated binding of c-Abl tyrosine kinase to DNA. *Science, 256*(5055), 382-385.

Kocks, C., Cho, J. H., Nehme, N., Ulvila, J., Pearson, A. M., Meister, M., . . . Ezekowitz, R. A. B. (2005). Eater, a Transmembrane Protein Mediating Phagocytosis of Bacterial Pathogens in Drosophila. *Cell, 123*(2), 335-346. doi: 10.1016/j.cell.2005.08.034

Koschmieder, S., Gottgens, B., Zhang, P., Iwasaki-Arai, J., Akashi, K., Kutok, J. L., . . . Huettner, C. S. (2005). Inducible chronic phase of myeloid leukemia with expansion of hematopoietic stem cells in a transgenic model of BCR-ABL leukemogenesis. *Blood, 105*(1), 324-334. doi: 10.1182/blood-2003-12-4369

Krzemień, J., Dubois, L., Makki, R., Meister, M., Vincent, A., & Crozatier, M. (2007). Control of blood cell homeostasis in Drosophila larvae by the posterior signalling centre. *Nature, 446*, 325. doi: 10.1038/nature05650

https://www.nature.com/articles/nature05650#supplementary-information

Kuklinski, N. J., Berglund, E. C., & Ewing, A. G. (2010). Micellar capillary electrophoresis--electrochemical detection of neurochemicals from Drosophila. *J Sep Sci, 33*(3), 388-393. doi: 10.1002/jssc.200900634

Kumar, J. P., & Moses, K. (2001). EGF receptor and Notch signaling act upstream of Eyeless/Pax6 to control eye specification. *Cell, 104*(5), 687-697.

Kurucz, E., Vaczi, B., Markus, R., Laurinyecz, B., Vilmos, P., Zsamboki, J., . . . Ando, I. (2007). Definition of Drosophila hemocyte subsets by cell-type specific antigens. *Acta Biol Hung, 58 Suppl*, 95-111. doi: 10.1556/ABiol.58.2007.Suppl.8

Lahaye, T., Riehm, B., Berger, U., Paschka, P., Muller, M. C., Kreil, S., . . . Hochhaus, A. (2005). Response and resistance in 300 patients with BCR-ABL-positive leukemias treated with imatinib in a single center: a 4.5-year follow-up. *Cancer, 103*(8), 1659-1669. doi: 10.1002/cncr.20922

Laneuville, P. (1995). Abl tyrosine protein kinase. *Semin Immunol, 7*(4), 255-266. doi: 10.1006/smim.1995.0030

Lanot, R., Zachary, D., Holder, F., & Meister, M. (2001). Postembryonic hematopoiesis in Drosophila. *Dev Biol, 230*(2), 243-257. doi: 10.1006/dbio.2000.0123

le Coutre, P., Mologni, L., Cleris, L., Marchesi, E., Buchdunger, E., Giardini, R., . . . Gambacorti-Passerini, C. (1999). In vivo eradication of human BCR/ABL-positive leukemia cells with an ABL kinase inhibitor. *J Natl Cancer Inst, 91*(2), 163-168.

Lee, T. S., Potts, S. J., Kantarjian, H., Cortes, J., Giles, F., & Albitar, M. (2008). Molecular basis explanation for imatinib resistance of BCR-ABL due to T315I and P-loop mutations from molecular dynamics simulations. *Cancer, 112*(8), 1744-1753. doi: 10.1002/cncr.23355

Legent, K., & Treisman, J. E. (2008). Wingless signaling in Drosophila eye development. *Methods Mol Biol, 469*, 141-161. doi: 10.1007/978-1-60327-469-2_12

Lemaitre, B., Meister, M., Govind, S., Georgel, P., Steward, R., Reichhart, J. M., & Hoffmann, J. A. (1995). Functional analysis and regulation of nuclear import of dorsal during the immune response in Drosophila. *Embo j, 14*(3), 536-545.

Levine, B. D., & Cagan, R. L. (2016). Drosophila Lung Cancer Models Identify Trametinib plus Statin as Candidate Therapeutic. *Cell Rep, 14*(6), 1477-1487. doi: 10.1016/j.celrep.2015.12.105

Levinson, N. M., Kuchment, O., Shen, K., Young, M. A., Koldobskiy, M., Karplus, M., . . . Kuriyan, J. (2006). A Src-like inactive conformation in the abl tyrosine kinase domain. *PLoS Biol, 4*(5), e144. doi: 10.1371/journal.pbio.0040144

Lewis, J. M., & Schwartz, M. A. (1998). Integrins regulate the association and phosphorylation of paxillin by c-Abl. *J Biol Chem, 273*(23), 14225-14230.

Li, Y., Clough, N., Sun, X., Yu, W., Abbott, B. L., Hogan, C. J., & Dai, Z. (2007). Bcr-Abl induces abnormal cytoskeleton remodeling, beta1 integrin clustering and increased cell adhesion to fibronectin through the Abl interactor 1 pathway. *J Cell Sci, 120*(Pt 8), 1436-1446. doi: 10.1242/jcs.03430

Linford, N. J., Bilgir, C., Ro, J., & Pletcher, S. D. (2013). Measurement of lifespan in Drosophila melanogaster. *Journal of visualized experiments : JoVE*(71), 50068. doi: 10.3791/50068

Lombardo, L. J., Lee, F. Y., Chen, P., Norris, D., Barrish, J. C., Behnia, K., . . . Borzilleri, R. M. (2004). Discovery of N-(2-chloro-6-methyl- phenyl)-2-(6-(4-(2-hydroxyethyl)- piperazin-1-yl)-2-methylpyrimidin-4- ylamino)thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. *J Med Chem, 47*(27), 6658-6661. doi: 10.1021/jm049486a

Lorincz, P., Takats, S., Karpati, M., & Juhasz, G. (2016). iFly: The eye of the fruit fly as a model to study autophagy and related trafficking pathways. *Exp Eye Res, 144*, 90-98. doi: 10.1016/j.exer.2015.06.013

Lowenberg, B. (2003). Minimal residual disease in chronic myeloid leukemia. *N Engl J Med, 349*(15), 1399-1401. doi: 10.1056/NEJMp038130

Lugo, T. G., Pendergast, A. M., Muller, A. J., & Witte, O. N. (1990). Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. *Science, 247*(4946), 1079-1082.

Luo, H., Hanratty, W. P., & Dearolf, C. R. (1995). An amino acid substitution in the Drosophila hopTum-l Jak kinase causes leukemia-like hematopoietic defects. *Embo j, 14*(7), 1412-1420.

Ma, L., Roderick, J., Kelliher, M. A., & Green, M. R. (2016). High-Throughput Screening of Tyrosine Kinase Inhibitor Resistant Genes in CML. *Methods Mol Biol, 1465*, 159-173. doi: 10.1007/978-1-4939-4011-0_14

Makhijani, K., Alexander, B., Tanaka, T., Rulifson, E., & Bruckner, K. (2011). The peripheral nervous system supports blood cell homing and survival in the Drosophila larva. *Development, 138*(24), 5379-5391. doi: 10.1242/dev.067322

Mandal, L., Banerjee, U., & Hartenstein, V. (2004). Evidence for a fruit fly hemangioblast and similarities between lymph-gland hematopoiesis in fruit fly and mammal aorta-gonadal-mesonephros mesoderm. *Nature Genetics, 36*, 1019. doi: 10.1038/ng1404

Mandal, L., Banerjee, U., & Hartenstein, V. (2004). Evidence for a fruit fly hemangioblast and similarities between lymph-gland hematopoiesis in fruit fly and mammal aorta-gonadal-mesonephros mesoderm. *Nat Genet, 36*(9), 1019-1023. doi: 10.1038/ng1404

Mandal, L., Martinez-Agosto, J. A., Evans, C. J., Hartenstein, V., & Banerjee, U. (2007). A Hedgehog- and Antennapedia-dependent niche maintains Drosophila haematopoietic precursors. *Nature, 446*, 320. doi: 10.1038/nature05585

Markstein, M., Dettorre, S., Cho, J., Neumüller, R. A., Craig-Müller, S., & Perrimon, N. (2014). Systematic screen of chemotherapeutics in Drosophila stem cell tumors. *Proc Natl Acad Sci U S A, 111*(12), 4530-4535. doi: 10.1073/pnas.1401160111

Márkus, R., Laurinyecz, B., Kurucz, É., Honti, V., Bajusz, I., Sipos, B., . . . Andó, I. (2009). Sessile hemocytes as a hematopoietic compartment in Drosophila melanogaster. *Proceedings of the National Academy of Sciences, 106*(12), 4805-4809. doi: 10.1073/pnas.0801766106

Marsola, A., Simoes, B. P., Palma, L. C., Berzoti-Coelho, M. G., Burin, S. M., & de Castro, F. A. (2018). Expression of Hippo signaling pathway and Aurora kinase genes in chronic myeloid leukemia. *Med Oncol, 35*(3), 26. doi: 10.1007/s12032-018-1079-6

Martinsson, T., Eriksson, T., Abrahamsson, J., Caren, H., Hansson, M., Kogner, P., . . . Hallberg, B. (2011). Appearance of the novel activating F1174S ALK mutation in neuroblastoma correlates with aggressive tumor progression and unresponsiveness to therapy. *Cancer Res, 71*(1), 98-105. doi: 10.1158/0008-5472.can-10-2366

Maru, Y., & Witte, O. N. (1991). The BCR gene encodes a novel serine/threonine kinase activity within a single exon. *Cell, 67*(3), 459-468.

McClung, C., & Hirsh, J. (1998). Stereotypic behavioral responses to free-base cocaine and the development of behavioral sensitization in Drosophila. *Curr Biol, 8*(2), 109-112.

McGuire, S. E., Deshazer, M., & Davis, R. L. (2005). Thirty years of olfactory learning and memory research in Drosophila melanogaster. *Prog Neurobiol, 76*(5), 328-347. doi: 10.1016/j.pneurobio.2005.09.003

McWhirter, J. R., Galasso, D. L., & Wang, J. Y. (1993). A coiled-coil oligomerization domain of Bcr is essential for the transforming function of Bcr-Abl oncoproteins. *Mol Cell Biol, 13*(12), 7587-7595.

McWhirter, J. R., & Wang, J. Y. (1993). An actin-binding function contributes to transformation by the Bcr-Abl oncoprotein of Philadelphia chromosome-positive human leukemias. *Embo j, 12*(4), 1533-1546.

Melo, J. V. (1996). The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. *Blood, 88*(7), 2375-2384.

Miething, C., Feihl, S., Mugler, C., Grundler, R., von Bubnoff, N., Lordick, F., . . . Duyster, J. (2006). The Bcr-Abl mutations T315I and Y253H do not confer a growth advantage in the absence of imatinib. *Leukemia, 20*(4), 650-657. doi: 10.1038/sj.leu.2404151

Minakhina, S., & Steward, R. (2006). Melanotic mutants in Drosophila: pathways and phenotypes. *Genetics, 174*(1), 253-263. doi: 10.1534/genetics.106.061978

Moore, M. S., DeZazzo, J., Luk, A. Y., Tully, T., Singh, C. M., & Heberlein, U. (1998). Ethanol intoxication in Drosophila: Genetic and pharmacological evidence for regulation by the cAMP signaling pathway. *Cell, 93*(6), 997-1007.

Mummery-Widmer, J. L., Yamazaki, M., Stoeger, T., Novatchkova, M., Bhalerao, S., Chen, D., . . . Knoblich, J. A. (2009). Genome-wide analysis of Notch signalling in Drosophila by transgenic RNAi. *Nature, 458*(7241), 987-992. doi: 10.1038/nature07936

Muyrers-Chen, I., Rozovskaia, T., Lee, N., Kersey, J. H., Nakamura, T., Canaani, E., & Paro, R. (2004). Expression of leukemic MLL fusion proteins in Drosophila affects cell cycle control and chromosome morphology. *Oncogene, 23*(53), 8639-8648. doi: 10.1038/sj.onc.1207904

Nazir, A., Mukhopadhyay, I., Saxena, D. K., & Chowdhuri, D. K. (2003). Evaluation of the No Observed Adverse Effect Level of Solvent Dimethyl Sulfoxide in

Drosophila melanogaster. *Toxicology Mechanisms and Methods, 13*(2), 147-152. doi: 10.1080/15376510309846

Nelson, R. E., Fessler, L. I., Takagi, Y., Blumberg, B., Keene, D. R., Olson, P. F., . . . Fessler, J. H. (1994). Peroxidasin: a novel enzyme-matrix protein of Drosophila development. *The EMBO journal, 13*(15), 3438-3447.

Nichols, C. D., Ronesi, J., Pratt, W., & Sanders-Bush, E. (2002). Hallucinogens and Drosophila: linking serotonin receptor activation to behavior. *Neuroscience, 115*(3), 979-984.

Noens, L., van Lierde, M. A., De Bock, R., Verhoef, G., Zachee, P., Berneman, Z., . . . Abraham, I. (2009). Prevalence, determinants, and outcomes of nonadherence to imatinib therapy in patients with chronic myeloid leukemia: the ADAGIO study. *Blood, 113*(22), 5401-5411. doi: 10.1182/blood-2008-12-196543

O'Brien, S., Guilhot, F., & Goldman, J. al., e.(2008). International randomized study of interferon versus STI571 (IRIS) 7-year follow-up: sustained survival, low rate of transformation and increased rate of major molecular response (MMR) in patients (pts) with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP) treated with Imatinib (IM). *Blood, 112*, 76.

O'Brien, S. G., Guilhot, F., Larson, R. A., Gathmann, I., Baccarani, M., Cervantes, F., . . . Druker, B. J. (2003). Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med, 348*(11), 994-1004. doi: 10.1056/NEJMoa022457

O'Hare, T., Deininger, M. W. N., Eide, C. A., Clackson, T., & Druker, B. J. (2011). Targeting the BCR-ABL Signaling Pathway in Therapy-Resistant Philadelphia Chromosome-Positive Leukemia. *Clinical Cancer Research, 17*(2), 212-221. doi: 10.1158/1078-0432.ccr-09-3314

O'Hare, T., Shakespeare, W. C., Zhu, X., Eide, C. A., Rivera, V. M., Wang, F., . . . Clackson, T. (2009). AP24534, a pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. *Cancer Cell, 16*(5), 401-412. doi: 10.1016/j.ccr.2009.09.028

O'Hare, T., Walters, D. K., Stoffregen, E. P., Jia, T., Manley, P. W., Mestan, J., . . . Druker, B. J. (2005). In vitro activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. *Cancer Res, 65*(11), 4500-4505. doi: 10.1158/0008-5472.can-05-0259

Oftedal, P. (1953). [The histogenesis of a new tumor in Drosophila melanogaster, and a comparison with tumors of five other stocks]. *Z Indukt Abstamm Vererbungsl, 85*(3), 408-422.

Oh, I. H., & Humphries, R. K. (2012). Concise review: Multidimensional regulation of the hematopoietic stem cell state. *Stem Cells, 30*(1), 82-88. doi: 10.1002/stem.776

Osman, D., Gobert, V., Ponthan, F., Heidenreich, O., Haenlin, M., & Waltzer, L. (2009). A Drosophila model identifies calpains as modulators of the human leukemogenic fusion protein AML1-ETO. *Proc Natl Acad Sci U S A, 106*(29), 12043-12048. doi: 10.1073/pnas.0902449106

Palis, J., & Yoder, M. C. (2001). Yolk-sac hematopoiesis: The first blood cells of mouse and man. *Experimental Hematology, 29*(8), 927-936. doi: https://doi.org/10.1016/S0301-472X(01)00669-5

Pandey, U. B., & Nichols, C. D. (2011). Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery. *Pharmacol Rev, 63*(2), 411-436. doi: 10.1124/pr.110.003293

Pane, F., Frigeri, F., Sindona, M., Luciano, L., Ferrara, F., Cimino, R., . . . Rotoli, B. (1996). Neutrophilic-chronic myeloid leukemia: a distinct disease with a specific molecular marker (BCR/ABL with C3/A2 junction). *Blood, 88*(7), 2410-2414.

Pendergast, A. M., Muller, A. J., Havlik, M. H., Maru, Y., & Witte, O. N. (1991). BCR sequences essential for transformation by the BCR-ABL oncogene bind to the ABL SH2 regulatory domain in a non-phosphotyrosine-dependent manner. *Cell, 66*(1), 161-171.

Pendergast, A. M., Quilliam, L. A., Cripe, L. D., Bassing, C. H., Dai, Z., Li, N., . . . et al. (1993). BCR-ABL-induced oncogenesis is mediated by direct interaction with the SH2 domain of the GRB-2 adaptor protein. *Cell, 75*(1), 175-185.

Peng, C., & Li, S. (2016). Chronic Myeloid Leukemia (CML) Mouse Model in Translational Research. *Methods Mol Biol, 1438*, 225-243. doi: 10.1007/978-1-4939-3661-8_13

Perrimon, N., Ni, J.-Q., & Perkins, L. (2010). In vivo RNAi: today and tomorrow. *Cold Spring Harbor perspectives in biology, 2*(8), a003640.

Phelps, C. B., & Brand, A. H. (1998). Ectopic Gene Expression inDrosophilaUsing GAL4 System. *Methods, 14*(4), 367-379.

Poon, C. L., Lin, J. I., Zhang, X., & Harvey, K. F. (2011). The sterile 20-like kinase Tao-1 controls tissue growth by regulating the Salvador-Warts-Hippo pathway. *Dev Cell, 21*(5), 896-906. doi: 10.1016/j.devcel.2011.09.012

Pricl, S., Fermeglia, M., Ferrone, M., & Tamborini, E. (2005). T315I-mutated Bcr-Abl in chronic myeloid leukemia and imatinib: insights from a computational study. *Mol Cancer Ther, 4*(8), 1167-1174. doi: 10.1158/1535-7163.mct-05-0101

Ptashne, M. (1988). How eukaryotic transcriptional activators work. *Nature, 335*(6192), 683-689.

Puil, L., Liu, J., Gish, G., Mbamalu, G., Bowtell, D., Pelicci, P. G., . . . Pawson, T. (1994). Bcr-Abl oncoproteins bind directly to activators of the Ras signalling pathway. *Embo j, 13*(4), 764-773.

Qiu, P., Pan, P. C., & Govind, S. (1998). A role for the Drosophila Toll/Cactus pathway in larval hematopoiesis. *Development, 125*(10), 1909-1920.

Quintás-Cardama, A., Kantarjian, H. M., & Cortes, J. E. (2009). Mechanisms of Primary and Secondary Resistance to Imatinib in Chronic Myeloid Leukemia. *Cancer Control, 16*(2), 122-131. doi: 10.1177/107327480901600204

Rajak, P., Dutta, M., & Roy, S. (2015). Altered differential hemocyte count in 3rd instar larvae of Drosophila melanogaster as a response to chronic exposure of Acephate. *Interdisciplinary toxicology, 8*(2), 84-88. doi: 10.1515/intox-2015-0013

Ramet, M., Lanot, R., Zachary, D., & Manfruelli, P. (2002). JNK signaling pathway is required for efficient wound healing in Drosophila. *Dev Biol, 241*(1), 145-156. doi: 10.1006/dbio.2001.0502

Rand, M. D., Kearney, A. L., Dao, J., & Clason, T. (2010). Permeabilization of Drosophila embryos for introduction of small molecules. *Insect Biochem Mol Biol, 40*(11), 792-804. doi: 10.1016/j.ibmb.2010.07.007

Rao, P. R., Makhijani, K., & Shashidhara, L. S. (2008). Human APC sequesters beta-catenin even in the absence of GSK-3beta in a Drosophila model. *Oncogene, 27*(17), 2488-2493. doi: 10.1038/sj.onc.1210890

Ray, M., & Lakhotia, S. C. (2015). The commonly used eye-specific sev-GAL4 and GMR-GAL4 drivers in Drosophila melanogaster are expressed in tissues other than eyes also. *J Genet, 94*(3), 407-416.

Read, R. D., Bach, E. A., & Cagan, R. L. (2004). Drosophila C-terminal Src kinase negatively regulates organ growth and cell proliferation through inhibition of the Src, Jun N-terminal kinase, and STAT pathways. *Mol Cell Biol, 24*(15), 6676-6689.

Read, R. D., Goodfellow, P. J., Mardis, E. R., Novak, N., Armstrong, J. R., & Cagan, R. L. (2005). A Drosophila model of multiple endocrine neoplasia type 2. *Genetics, 171*(3), 1057-1081.

Ready, D. F., Hanson, T. E., & Benzer, S. (1976). Development of the Drosophila retina, a neurocrystalline lattice. *Dev Biol, 53*(2), 217-240.

Redaelli, S., Piazza, R., Rostagno, R., Magistroni, V., Perini, P., Marega, M., . . . Boschelli, F. (2009). Activity of bosutinib, dasatinib, and nilotinib against 18 imatinib-resistant BCR/ABL mutants. *J Clin Oncol, 27*(3), 469-471. doi: 10.1200/jco.2008.19.8853

Reimels, T. A., & Pfleger, C. M. (2016). Methods to Examine the Lymph Gland and Hemocytes in Drosophila Larvae. *Journal of visualized experiments : JoVE*(117), 54544. doi: 10.3791/54544

Reiter, L. T., Potocki, L., Chien, S., Gribskov, M., & Bier, E. (2001a). A systematic analysis of human disease-associated gene sequences in Drosophila melanogaster. *Genome research, 11*(6), 1114-1125. doi: 10.1101/gr.169101

Reiter, L. T., Potocki, L., Chien, S., Gribskov, M., & Bier, E. (2001b). A systematic analysis of human disease-associated gene sequences in Drosophila melanogaster. *Genome Res, 11*(6), 1114-1125. doi: 10.1101/gr.169101

Reuther, G., Fu, H., Cripe, L., Collier, R., & Pendergast, A. (1994). Association of the protein kinases c-Bcr and Bcr-Abl with proteins of the 14-3-3 family. *Science, 266*(5182), 129-133. doi: 10.1126/science.7939633

Richardson, H. E., Willoughby, L., & Humbert, P. O. (2001). Screening for Anti-cancer Drugs in Drosophila. *eLS*, 1-14.

Rix, U., Hantschel, O., Durnberger, G., Remsing Rix, L. L., Planyavsky, M., Fernbach, N. V., . . . Superti-Furga, G. (2007). Chemical proteomic profiles of the BCR-ABL inhibitors imatinib, nilotinib, and dasatinib reveal novel kinase and nonkinase targets. *Blood, 110*(12), 4055-4063. doi: 10.1182/blood-2007-07-102061

Rizki, M. T. (1960). Melanotic tumor ormation in Drosophila. *J Morphol, 106*, 147-157. doi: 10.1002/jmor.1051060203
Rizki, M. T., & Rizki, R. M. (1959). Functional significance of the crystal cells in the larva of Drosophila melanogaster. *J Biophys Biochem Cytol, 5*(2), 235-240.
Rizki, R. M., & Rizki, T. M. (1979). Cell interactions in the differentiation of a melanotic tumor in Drosophila. *Differentiation, 12*(3), 167-178.
Rizki, T. M., & Rizki, R. M. (1984). The Cellular Defense System of Drosophila melanogaster. In R. C. King & H. Akai (Eds.), *Insect Ultrastructure: Volume 2* (pp. 579-604). Boston, MA: Springer US.
Rizki, T. M., & Rizki, R. M. (1992). Lamellocyte differentiation in Drosophila larvae parasitized by Leptopilina. *Dev Comp Immunol, 16*(2-3), 103-110.
Roeder, T., Isermann, K., Kallsen, K., Uliczka, K., & Wagner, C. (2012). A Drosophila asthma model - what the fly tells us about inflammatory diseases of the lung. *Adv Exp Med Biol, 710*, 37-47. doi: 10.1007/978-1-4419-5638-5_5
Rotstein, B., & Paululat, A. (2016). On the Morphology of the Drosophila Heart. *J Cardiovasc Dev Dis, 3*(2). doi: 10.3390/jcdd3020015
Rowley, J. D. (1973). A New Consistent Chromosomal Abnormality in Chronic Myelogenous Leukaemia identified by Quinacrine Fluorescence and Giemsa Staining. *Nature, 243*(5405), 290-293. doi: 10.1038/243290a0
Rugendorff, A., Younossi-Hartenstein, A., & Hartenstein, V. (1994). Embryonic origin and differentiation of the Drosophila heart. *Roux Arch Dev Biol, 203*(5), 266-280. doi: 10.1007/bf00360522
Rugendorff, A., Younossi-Hartenstein, A., & Hartenstein, V. (1994). Embryonic origin and differentiation of the Drosophila heart. *Roux's archives of developmental biology, 203*(5), 266-280.
Sang, J. H., & Burnet, B. (1963). Physiological Genetics of Melanotic Tumors in Drosophila Melanogaster. I. the Effects of Nutrient Balance on Tumor Penetrance in the Tu Strain. *Genetics, 48*(2), 235-253.
Sattler, M., Mohi, M. G., Pride, Y. B., Quinnan, L. R., Malouf, N. A., Podar, K., . . . Neel, B. G. (2002). Critical role for Gab2 in transformation by BCR/ABL. *Cancer Cell, 1*(5), 479-492.
Savage, D. G., Szydlo, R. M., & Goldman, J. M. (1997). Clinical features at diagnosis in 430 patients with chronic myeloid leukaemia seen at a referral centre over a 16-year period. *Br J Haematol, 96*(1), 111-116.
Sawyers, C. L., McLaughlin, J., Goga, A., Havlik, M., & Witte, O. (1994). The nuclear tyrosine kinase c-Abl negatively regulates cell growth. *Cell, 77*(1), 121-131.
Scherfer, C., Karlsson, C., Loseva, O., Bidla, G., Goto, A., Havemann, J., . . . Theopold, U. (2004). Isolation and Characterization of Hemolymph Clotting Factors in Drosophila melanogaster by a Pullout Method. *Current Biology, 14*(7), 625-629. doi: 10.1016/j.cub.2004.03.030
Schindler, T., Bornmann, W., Pellicena, P., Miller, W. T., Clarkson, B., & Kuriyan, J. (2000). Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science, 289*(5486), 1938-1942.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature methods, 9*(7), 671.

Shah, N. P., Nicoll, J. M., Nagar, B., Gorre, M. E., Paquette, R. L., Kuriyan, J., & Sawyers, C. L. (2002). Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell, 2*(2), 117-125.

Shah, N. P., Skaggs, B. J., Branford, S., Hughes, T. P., Nicoll, J. M., Paquette, R. L., & Sawyers, C. L. (2007). Sequential ABL kinase inhibitor therapy selects for compound drug-resistant BCR-ABL mutations with altered oncogenic potency. *J Clin Invest, 117*(9), 2562-2569. doi: 10.1172/jci30890

Shah, N. P., Tran, C., Lee, F. Y., Chen, P., Norris, D., & Sawyers, C. L. (2004). Overriding imatinib resistance with a novel ABL kinase inhibitor. *Science, 305*(5682), 399-401. doi: 10.1126/science.1099480

Shandala, T., Woodcock, J. M., Ng, Y., Biggs, L., Skoulakis, E. M., Brooks, D. A., & Lopez, A. F. (2011). Drosophila 14-3-3epsilon has a crucial role in anti-microbial peptide secretion and innate immunity. *J Cell Sci, 124*(Pt 13), 2165-2174. doi: 10.1242/jcs.080598

Shia, A. K., Glittenberg, M., Thompson, G., Weber, A. N., Reichhart, J. M., & Ligoxygakis, P. (2009). Toll-dependent antimicrobial responses in Drosophila larval fat body require Spatzle secreted by haemocytes. *J Cell Sci, 122*(Pt 24), 4505-4515. doi: 10.1242/jcs.049155

Shirinian, M., Kambris, Z., Hamadeh, L., Grabbe, C., Journo, C., Mahieux, R., & Bazarbachi, A. (2015). A Transgenic Drosophila melanogaster Model To Study Human T-Lymphotropic Virus Oncoprotein Tax-1-Driven Transformation In Vivo. *J Virol, 89*(15), 8092-8095. doi: 10.1128/jvi.00918-15

Shirinian, M., Kambris, Z., Hamadeh, L., Journo, C., Grabbe, C., Mahieux, R., & Bazarbachi, A. (2015). A transgenic Drosophila melanogaster model to study HTLV-I oncoprotein Tax-driven leukemogenesis in vivo. *Retrovirology, 12*(S1), O40.

SHRESTHA, R., & GATEFF, E. (1982). Ultrastructure and Cytochemistry of the Cell Types in the Larval Hematopoietic Organs and Hemolymph of Drosophila Melanogaster. *Development, Growth & Differentiation, 24*(1), 65-82. doi: 10.1111/j.1440-169X.1982.00065.x

Shultz, L. D., Lyons, B. L., Burzenski, L. M., Gott, B., Chen, X., Chaleff, S., . . . Handgretinger, R. (2005). Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. *J Immunol, 174*(10), 6477-6489.

Sinenko, S. A., Hung, T., Moroz, T., Tran, Q. M., Sidhu, S., Cheney, M. D., . . . Banerjee, U. (2010). Genetic manipulation of AML1-ETO-induced expansion of hematopoietic precursors in a Drosophila model. *Blood, 116*(22), 4612-4620. doi: 10.1182/blood-2010-03-276998

Sinenko, S. A., Mandal, L., Martinez-Agosto, J. A., & Banerjee, U. (2009). Dual role of wingless signaling in stem-like hematopoietic precursor maintenance in Drosophila. *Dev Cell, 16*(5), 756-763. doi: 10.1016/j.devcel.2009.03.003

Singh, J., Aaronson, S. A., & Mlodzik, M. (2010). Drosophila Abelson kinase mediates cell invasion and proliferation through two distinct MAPK pathways. *Oncogene, 29*(28), 4033-4045. doi: 10.1038/onc.2010.155

Skaggs, B. J., Gorre, M. E., Ryvkin, A., Burgess, M. R., Xie, Y., Han, Y., . . . Graeber, T. G. (2006). Phosphorylation of the ATP-binding loop directs oncogenicity of drug-resistant BCR-ABL mutants. *Proc Natl Acad Sci U S A, 103*(51), 19466-19471. doi: 10.1073/pnas.0609239103

Sorrentino, R. P., Carton, Y., & Govind, S. (2002). Cellular immune response to parasite infection in the Drosophila lymph gland is developmentally regulated. *Dev Biol, 243*(1), 65-80. doi: 10.1006/dbio.2001.0542

Sparrow, J. C. (1974). The genetics of some second chromosome melanotic tumour mutants of Drosophila melanogaster. *Genet Res, 23*(1), 13-21.

Spiers, A. (1977). The clinical features of chronic granulocytic leukaemia. *Clinics in haematology, 6*(1), 77-95.

Stevens, T. L., Rogers, E. M., Koontz, L. M., Fox, D. T., Homem, C. C., Nowotarski, S. H., . . . Peifer, M. (2008). Using Bcr-Abl to examine mechanisms by which abl kinase regulates morphogenesis in Drosophila. *Mol Biol Cell, 19*(1), 378-393. doi: 10.1091/mbc.e07-01-0008

Stevens, T. L., Rogers, E. M., Koontz, L. M., Fox, D. T., Homem, C. C. F., Nowotarski, S. H., . . . Peifer, M. (2008). Using Bcr-Abl to examine mechanisms by which abl kinase regulates morphogenesis in Drosophila. *Molecular biology of the cell, 19*(1), 378-393. doi: 10.1091/mbc.e07-01-0008

Stofanko, M., Kwon, S. Y., & Badenhorst, P. (2010). Lineage Tracing of Lamellocytes Demonstrates Drosophila Macrophage Plasticity. *PLOS ONE, 5*(11), e14051. doi: 10.1371/journal.pone.0014051

Suryanarayan, K., Hunger, S. P., Kohler, S., Carroll, A. J., Crist, W., Link, M. P., & Cleary, M. L. (1991). Consistent involvement of the bcr gene by 9;22 breakpoints in pediatric acute leukemias. *Blood, 77*(2), 324-330.

Takahashi, F., Endo, S., Kojima, T., & Saigo, K. (1996). Regulation of cell-cell contacts in developing Drosophila eyes by Dsrc41, a new, close relative of vertebrate c-src. *Genes Dev, 10*(13), 1645-1656. doi: 10.1101/gad.10.13.1645

Talpaz, M., Shah, N. P., Kantarjian, H., Donato, N., Nicoll, J., Paquette, R., . . . Bleickardt, E. (2006). Dasatinib in imatinib-resistant Philadelphia chromosome–positive leukemias. *New England Journal of Medicine, 354*(24), 2531-2541.

Tang, L. (2019). Investigating heterogeneity in HeLa cells. *Nat Methods, 16*(4), 281. doi: 10.1038/s41592-019-0375-1

Tepass, U., Fessler, L. I., Aziz, A., & Hartenstein, V. (1994). Embryonic origin of hemocytes and their relationship to cell death in Drosophila. *Development, 120*(7), 1829-1837.

Terriente-Felix, A., Perez, L., Bray, S. J., Nebreda, A. R., & Milan, M. (2017). A Drosophila model of myeloproliferative neoplasm reveals a feed-forward loop in

the JAK pathway mediated by p38 MAPK signalling. *Dis Model Mech, 10*(4), 399-407. doi: 10.1242/dmm.028118

Thaker, H. M., & Kankel, D. R. (1992). Mosaic analysis gives an estimate of the extent of genomic involvement in the development of the visual system in Drosophila melanogaster. *Genetics, 131*(4), 883-894.

Thomas, B. J., & Wassarman, D. A. (1999). A fly's eye view of biology. *Trends Genet, 15*(5), 184-190.

Thomas, C., & Ingham, P. W. (2003). Hedgehog signaling in the Drosophila eye and head: an analysis of the effects of different patched trans-heterozygotes. *Genetics, 165*(4), 1915-1928.

Thurmond, J., Goodman, J. L., Strelets, V. B., Attrill, H., Gramates, L S., Marygold, S. J., . . . Consortium, t. F. (2018). FlyBase 2.0: the next generation. *Nucleic Acids Research, 47*(D1), D759-D765. doi: 10.1093/nar/gky1003

Tokarski, J. S., Newitt, J. A., Chang, C. Y., Cheng, J. D., Wittekind, M., Kiefer, S. E., . . . Klei, H. E. (2006). The structure of Dasatinib (BMS-354825) bound to activated ABL kinase domain elucidates its inhibitory activity against imatinib-resistant ABL mutants. *Cancer Res, 66*(11), 5790-5797. doi: 10.1158/0008-5472.can-05-4187

Torres, G., & Horowitz, J. M. (1998). Activating properties of cocaine and cocaethylene in a behavioral preparation of Drosophila melanogaster. *Synapse, 29*(2), 148-161. doi: 10.1002/(sici)1098-2396(199806)29:2<148::aid-syn6>3.0.co;2-7

Valent, P. (2008). Emerging stem cell concepts for imatinib-resistant chronic myeloid leukaemia: implications for the biology, management, and therapy of the disease. *Br J Haematol, 142*(3), 361-378. doi: 10.1111/j.1365-2141.2008.07197.x

Van Etten, R. A., Jackson, P., & Baltimore, D. (1989). The mouse type IV c-abl gene product is a nuclear protein, and activation of transforming ability is associated with cytoplasmic localization. *Cell, 58*(4), 669-678.

Vidal, M., Wells, S., Ryan, A., & Cagan, R. (2005). ZD6474 suppresses oncogenic RET isoforms in a Drosophila model for type 2 multiple endocrine neoplasia syndromes and papillary thyroid carcinoma. *Cancer Research, 65*(9), 3538-3541.

von Bubnoff, N., Manley, P. W., Mestan, J., Sanger, J., Peschel, C., & Duyster, J. (2006). Bcr-Abl resistance screening predicts a limited spectrum of point mutations to be associated with clinical resistance to the Abl kinase inhibitor nilotinib (AMN107). *Blood, 108*(4), 1328-1333. doi: 10.1182/blood-2005-12-010132

Wang, J. C., Lapidot, T., Cashman, J. D., Doedens, M., Addy, L., Sutherland, D. R., . . . Dick, J. E. (1998). High level engraftment of NOD/SCID mice by primitive normal and leukemic hematopoietic cells from patients with chronic myeloid leukemia in chronic phase. *Blood, 91*(7), 2406-2414.

Wang, L. D., & Wagers, A. J. (2011). Dynamic niches in the origination and differentiation of haematopoietic stem cells. *Nat Rev Mol Cell Biol, 12*(10), 643-655. doi: 10.1038/nrm3184

Warr, C. G., Shaw, K. H., Azim, A., Piper, M. D. W., & Parsons, L. M. (2018). Using Mouse and Drosophila Models to Investigate the Mechanistic Links between Diet, Obesity, Type II Diabetes, and Cancer. *Int J Mol Sci, 19*(12). doi: 10.3390/ijms19124110

Watson, K. L., Justice, R. W., & Bryant, P. J. (1994). Drosophila in cancer research: the first fifty tumor suppressor genes. *J Cell Sci Suppl, 18*, 19-33.

Weisberg, E., Manley, P., Mestan, J., Cowan-Jacob, S., Ray, A., & Griffin, J. D. (2006). AMN107 (nilotinib): a novel and selective inhibitor of BCR-ABL. *Br J Cancer, 94*(12), 1765-1769. doi: 10.1038/sj.bjc.6603170

Weisberg, E., Manley, P. W., Breitenstein, W., Bruggen, J., Cowan-Jacob, S. W., Ray, A., . . . Griffin, J. D. (2005). Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. *Cancer Cell, 7*(2), 129-141. doi: 10.1016/j.ccr.2005.01.007

Widmann, A., Eichler, K., Selcho, M., Thum, A. S., & Pauls, D. (2018). Odor-taste learning in Drosophila larvae. *J Insect Physiol, 106*(Pt 1), 47-54. doi: 10.1016/j.jinsphys.2017.08.004

Wildonger, J., & Mann, R. S. (2005). The t(8;21) translocation converts AML1 into a constitutive transcriptional repressor. *Development, 132*(10), 2263-2272. doi: 10.1242/dev.01824

Willoughby, L. F., Schlosser, T., Manning, S. A., Parisot, J. P., Street, I. P., Richardson, H. E., . . . Brumby, A. M. (2013). An in vivo large-scale chemical screening platform using Drosophila for anti-cancer drug discovery. *Dis Model Mech, 6*(2), 521-529. doi: 10.1242/dmm.009985

Wittkorn, E., Sarkar, A., Garcia, K., Kango-Singh, M., & Singh, A. (2015). The Hippo pathway effector Yki downregulates Wg signaling to promote retinal differentiation in the Drosophila eye. *Development, 142*(11), 2002-2013. doi: 10.1242/dev.117358

Wolff, T., & Ready, D. (1993). In Bate, M. and Martinez Arias, A.(eds.), The Development of Drosophila melanogaster: Cold Spring Harbor Laboratory Press.

Woodcock, K. J., Kierdorf, K., Pouchelon, C. A., Vivancos, V., Dionne, M. S., & Geissmann, F. (2015). Macrophage-derived upd3 cytokine causes impaired glucose homeostasis and reduced lifespan in Drosophila fed a lipid-rich diet. *Immunity, 42*(1), 133-144. doi: 10.1016/j.immuni.2014.12.023

Xin, S., Weng, L., Xu, J., & Du, W. (2002). The role of RBF in developmentally regulated cell proliferation in the eye disc and in Cyclin D/Cdk4 induced cellular growth. *Development, 129*(6), 1345-1356.

Xiong, W., & Rebay, I. (2011). Abelson tyrosine kinase is required for Drosophila photoreceptor morphogenesis and retinal epithelial patterning. *Dev Dyn, 240*(7), 1745-1755. doi: 10.1002/dvdy.22674

Yamamoto, M., Kurosu, T., Kakihana, K., Mizuchi, D., & Miura, O. (2004). The two major imatinib resistance mutations E255K and T315I enhance the activity of BCR/ABL fusion kinase. *Biochem Biophys Res Commun, 319*(4), 1272-1275. doi: 10.1016/j.bbrc.2004.05.113

Yang, H., & Hultmark, D. (2016). Tissue communication in a systemic immune response of Drosophila. *Fly (Austin), 10*(3), 115-122. doi: 10.1080/19336934.2016.1182269

Yin, D.-D., Fan, F.-Y., Hu, X.-B., Hou, L.-H., Zhang, X.-P., Liu, L., . . . Han, H. (2009). Notch signaling inhibits the growth of the human chronic myeloid leukemia cell line K562. *Leukemia Research, 33*(1), 109-114. doi: https://doi.org/10.1016/j.leukres.2008.06.023

Yuan, Z. M., Shioya, H., Ishiko, T., Sun, X., Gu, J., Huang, Y. Y., . . . Kufe, D. (1999). p73 is regulated by tyrosine kinase c-Abl in the apoptotic response to DNA damage. *Nature, 399*(6738), 814-817. doi: 10.1038/21704

Zeng, X., Zhao, H., Li, Y., Fan, J., Sun, Y., Wang, S., . . . Ju, D. (2015). Targeting Hedgehog signaling pathway and autophagy overcomes drug resistance of BCR-ABL-positive chronic myeloid leukemia. *Autophagy, 11*(2), 355-372. doi: 10.4161/15548627.2014.994368

Zettervall, C. J., Anderl, I., Williams, M. J., Palmer, R., Kurucz, E., Ando, I., & Hultmark, D. (2004). A directed screen for genes involved in Drosophila blood cell activation. *Proc Natl Acad Sci U S A, 101*(39), 14192-14197. doi: 10.1073/pnas.0403789101

Zhang, H., & Li, S. (2016). Induction of Chronic Myeloid Leukemia in Mice. *Methods Mol Biol, 1465*, 17-25. doi: 10.1007/978-1-4939-4011-0_2