

# Introducing the *Drosophila Melanogaster* Model for Cancer Research

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**Abstract:** Cancer is the cumulative symptomatology of a cluster of illnesses that affect several systems and are connected to one another. For the better part of the last few decades, the fruit fly *Drosophila melanogaster* has served as a model for researchers looking at human cancers, and they have had a great deal of success doing so. *Drosophila* is advantageous over other model systems in that it is genetically straightforward and provides researchers with access to a wide variety of genetic analysis tools. As a result, it provides a one-of-a-kind opportunity to address concerns about the beginning and progression of cancer, which would be extremely challenging to do using other model systems. In this chapter, we provide a historical overview of *Drosophila* as a model organism for cancer research, summarize the wide variety of genetic tools available, and compare various model organisms and cell culture platforms used in cancer studies. In addition, we briefly discuss some of the most cutting-edge models and concepts in recent *Drosophila* cancer research.

**Keywords:** Cancer · Tumorigenesis · *Drosophila* · Animal models · Genetic tools · Cell competition · Apoptosis induced proliferation · Cachexia · Tumor hotspots · Drug discovery

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## 1. Introduction

After illnesses related to the cardiovascular system, cancer is the second leading cause of mortality in developed nations [1]. There are forecasts that point to cancer becoming the leading cause of death in the United States in the not-too-distant future [2], surpassing cardiovascular disease in that ranking. Cancer is a complex disease that can exhibit a diverse array of clinical manifestations, rates of disease development, and therapeutic responses. Decades of research using a diverse range of methodologies and model systems have made it significantly easier to gain insight into the clinical and molecular mechanisms that are involved in the genesis and progression of the illness [3, 4]. These programs are important in the ongoing quest for novel methods to diagnosing, treating, and preventing this dreadful illness. Because of the powerful genetic tools it employs, *Drosophila melanogaster*, which will be referred to as *Drosophila* from this point forward, is one of the most popular model organisms used to study cancer. This has allowed researchers to gain a better understanding of the molecular and cellular mechanisms that are responsible for the initiation, progression, and invasion of cancer [5-7]. Since the early 1900s, scientists have been doing genetic research on *Drosophila* [8-11]. Our understanding of the genetics underpinning development, innate immunity, the circadian rhythm, and many other biological processes is significantly advanced as a direct result of its contribution. In addition, *Drosophila* has proved extremely useful in the discovery and analysis of signal transduction pathways, many of which have been connected to human diseases such as cancer [6, 9, 12, 13]. There is between 60 and 70

percent conserved sequence homology between the human genome and that of the fruit fly genome [14, 15]. More than seventy-five percent of human disease-causing genes have homologs in *Drosophila* [16, 17], but only forty-eight percent of *Drosophila* genes have been shown to have human homologs. [Citation needed] Because the genome of a *Drosophila* has less genetic redundancy than the genome of a mammal, it is much simpler to completely understand the role that a particular protein plays in the processes of interest within the cell. In addition, the growth time for *Drosophila* at 25 degrees Celsius is ten days, which enables a rapid synthesis of different strains and genotypic combinations. Due to the fact that a single female fruit fly has the capacity to produce up to 500 eggs over the course of her lifetime, these insects may be housed effectively in restricted places at a cheap cost of upkeep and with a high potential for progeny generation. Because the fruit fly *Drosophila* only has two sets of chromosomes, the insertion of balancer chromosomes, which eliminate the possibility of genetic recombination, enables complex genotypes to be maintained indefinitely without the need for regular selection. This eliminates the need for complex genotypes to be carefully selected for. In order to serve the flourishing research community, stock centers maintain the viability of a significant number of mutant and transgenic fly lineages. FlyBase (<http://flybase.org/>) is a dedicated global online database that contains a wealth of information on *Drosophila* genes. It also provides links to supplementary data from the stock centers, validated gene-specific antibody resources, reference articles on PubMed, and related ties to other global databases like the NCBI DNA database and the UniProtKB protein database, providing the scientific community with free, open access to information that is routinely updated. FlyBase can be accessed online. Because of the availability of potent genetic tools, well-established experimental methodologies, and a scientific community that is both collaborative and involved in their work, *Drosophila* has been utilized for decades as a model organism in the study of biology.

## **2. *Drosophila*, a Genetic Model with Access to a Wide Range of Tools**

This model organism's genetic toolkit has been extremely helpful in the discovery of novel mechanisms such as cell competition and compensatory proliferation [6, 18–21], as well as in the development of a number of cancer models that recapitulate aspects of the disease and facilitate in-depth research into the mechanisms that are at the disease's root [5-7, 13, 22–24]. Several excellent reviews [5, 7, 13, 23, 25, 26] highlight how research in the *Drosophila* model system has contributed to our current understanding of the complex nature of this illness, from its development to the use of that information in the therapeutic targeting of the disease in people. These reviews focus on how research in the *Drosophila* model system has contributed to our current understanding of the nature of this illness. The fruit fly *Drosophila* has played a significant role in the development of mutagenesis technology and the application of that technology to the investigation of complex biological systems. In the late 1960s, [27] researchers discovered that mutations might be caused when *Drosophila* were subjected to ethylmethane sulphonate (EMS). In the late 1920s, the early success of X-ray induced mutagenesis [28] triggered the first rush in mutant screening, which led to the initial functional annotation of a large number of genes [29]. These approaches were also responsible for the initial spike in mutant screening. In the 1980s, a large number of genes that are involved in developmental regulation were found, and during this time period, hundreds of new mutant alleles that were created by P-element derived transposable elements were published [10, 30]. Recently, the Berkeley Gene

Disruption Project was successful in carrying out a wide variety of transgenic insertions by making use of a variety of transposable elements. These insertions are currently made available to researchers all around the world by means of stock center repositories [31–33]. The Gal4/UAS system, which was initially established in budding yeast *S. cerevisiae* [34, 35], is the basis for a large number of the modern genetic approaches used to control gene expression in *Drosophila*. These techniques include: This instrument was developed for use in *Drosophila* and is responsible for the endogenous synthesis of the transcriptional activator Gal4 in cells that contain the driver gene. The promoter region of a gene is responsible for this production. The expression of Gal4 causes it to attach to the UAS sequences, which in turn causes the synthesis of any transgenic element that comes after [36]. By subordinating the Gal4 transcription factor to the DNA regulatory regions of the target gene, it is possible to regulate the expression of a transgene using the Gal4/UAS system in a way that is analogous to the regulation of the expression of an endogenous gene. [37] The TARGET (Temporal And Regional Gene Expression Targeting) system was created as a direct result of the success of this strategy. The temperature-sensitive Gal4-inactivating protein Gal80 (Gal80ts) is responsible for repressing Gal4 transcriptional activity in this system when temperatures are within acceptable ranges. In recent years, new techniques for conducting genetic research using the fruit fly model, including as RNA interference (RNAi) and CRISPR-Cas9 based gene editing, have been included into the Gal4/UAS binary expression system [38, 39]. Recently, a method that exploits the ribosomal skipping mechanism of the viral T2A peptide to co-express Gal4 with the endogenous gene of interest [40] was developed. This method is advantageous for genes whose regulatory regions are not known explicitly. [Citation needed] It is not necessary to have any further information besides the open reading frame of the endogenous gene of interest in order to use the T2A-Gal4 method [40]. The recent availability of T2A-Gal4 libraries has resulted in a large rise in the amount of transgenic expression seen in some cell types [41]. Two other binary expression systems that are utilized in conjunction with the Gal4/UAS system are referred to as the LexA-lexAop and QF-QUAS systems [42]. The mosaic analysis is another successful approach that has been developed in *Drosophila* and is perfectly suited for study into the early stages of cancer. This method was developed in *Drosophila*. Through the use of mosaic analysis, it is possible to generate homozygous mutant cells (/) in a background that is heterozygous (+/). This is an appropriate model for research of the beginning of cancer because it avoids the potential lethality associated with numerous mutations [43]. Since cancer often begins from a mosaic scenario, in which a small number of cells within a homotypic tissue system acquire oncogenic mutations, this is an appropriate model for research of how cancer begins. Tumors develop when cancer cells in their microenvironment flourish and overrun their neighbors for oxygen, food, and space [44–46]. This causes the cancer cells to take up more room than their neighbors. In spite of the fact that genes in *Drosophila* associated with cancer were found rather early on, the study of carcinogenesis didn't really get off the ground until the mosaic analysis tool was repurposed. In 1993, the first reports of stable transgenic insertions in the fly genome and a site-specific recombination system using FLP recombinase (FLPase) and its target FLPase Recombination Target (FRT) to catalyze mitotic recombination between homologous chromosomes were published. These advancements provided a significant boost to cancer research in

*Drosophila* [47–49]. The Mosaic method has been adapted for use in mammalian systems, which has made it possible for us to learn about the independence of genes found inside cells as well as the transfer of signals from one clone to another [50–53]. Researchers have used procedures using trans-chromosomal recombination to investigate recessive mutations, which are generally lethal during the larval or embryonic stages of development, in order to investigate their autonomous activities. The FLP/FRT system and the Gal4/UAS system, along with Gal80-mediated repression of transgenic expression in other cells, are the components that make up the Mosaic Analysis using a Repressible Cell Marker (MARCM) technique [54]. This method can be used to study genetic epistasis in *Drosophila* cancer models by driving gene expression or knockdown in mutant clones. Another strategy for producing mosaic tissues is the expression of transgenes in isolated cell populations (clones) inside otherwise wild-type fly populations. Approaches based on cis-chromosomal recombination have been utilized in genome-wide mosaic analysis and screens [49, 55]. One example of this is the FLP-out system, which combines the FLP/FRT and Gal4/UAS systems. FLPase may be used to regulate the proximity of cis-DNA sequences by deleting flanking FRT sites. FLPase is typically generated by a heat-shock promoter. This technique enables a promoter that is located upstream of FRT-STOP-FRT sequences to drive the creation of Gal4 in a promoter > STOP>Gal4 cassette (where > signifies FRT sites), which in turn enables FRT to manufacture Gal4 downstream of it. The FLP-out system has had a significant impact on important research, including the discovery of cell-cell cooperation and rivalry in cancer as well as non-autonomous signaling [18–59]. It is now possible to create a reliable ratio of mutant to non-mutant cells by making changes to the FLP-out method, such as the strategy known as CoinFLP. Additionally, research involving traceable cell lineage may be carried out with the use of the G-TRACE technology [60, 61]. Cre/loxP and CRISPR-Cas9 were initially identified in other systems and then optimized for use in mammals [62–65]. *Drosophila* has profited from the application of these methods, which were initially discovered in other systems. To investigate tumor migration and tumor-host interaction, another technique that was evolved from xenografting methods that were pioneered in mammalian research [66, 67] is called allografting [68]. This technique involves injecting tumors into healthy fly hosts. Single-cell transcriptome studies [71–73], which have recently become popular tools to verify and detect complex concepts in human cancers such as cellular collaboration [74] and discovering tumor heterogeneity [75, 76], should be addressed in the future. [71–73] These studies should be addressed because they have recently become popular tools. Because of the substantial quantity of information that has been accumulated throughout the course of its history, *Drosophila* is an excellent choice to serve as a model organism for the research of this sort.

### **3. Investigation on Cancer-Related Genes and Pathways Utilizing *Drosophila* as a Model System**

Because there are so many genetic resources available for *Drosophila*, it serves as an excellent model for genetic screens that aim to identify the genes and pathways that are involved in a wide variety of biological processes. A significant number of genes that were first discovered in fruit flies have now been revealed to be homologs of human oncogenes or tumor suppressor genes [16, 17]. The fruit fly *Drosophila* was utilized as a model for research

purposes, which led to the discovery of a number of genes and signaling pathways that are connected to cancer. For example, in 1967, Gateff and Schneiderman [77] carried out a genetic screen and found a recessive mutant that had a malignant tumor phenotype. This mutant was revealed to be recessive. Lethal giant larvae (LGL) flies, which have a mutation in just one copy of the gene, develop neoplastic overproliferation of internal tissues and perish as larvae. These flies are referred to as "lethal giant larvae." This discovery was made a long time before it was realized that mutations in retinalblastoma (Rb) may be responsible for recessive oncogenesis [78] or that the term "tumor suppressor genes" was used for the first time in the research on somatic cell hybrids carried out by Harris [79]. Another mutant, discs big (dlg), was obtained from a similar genetic screen with phenotypic similarities to lgl loss-of-function (LOF) imaginal discs almost immediately after the discovery of lgl, the first instance of a tumor suppressor gene ever found. [80] This mutant had phenotypic similarities to lgl loss-of-function (LOF) imaginal discs. Following its discovery and subsequent role in maintaining apicobasal epithelial polarity in the same genetic pathway as lgl and dlg, the gene scribble (scrib) was classified as a neoplastic tumor suppressor gene (nTSG). Additionally, it has been used to create numerous single-gene models of tumorigenesis [12, 81–83]. *Drosophila* served as the model organism for the investigation of a large number of signaling pathways, many of which have now been revealed to have significant roles in the development of human cancer [5, 6, 12]. Notch, Hippo, Dpp, Hedgehog, and Wnt are some of the pathways that fall within this category. Studies conducted on tumor models derived from *Drosophila* have shown that scrib mutations result in significant neoplastic growth in the eye imaginal disc. This is because of oncogenic cooperation between signaling pathways like as Notch and Ras [84]. Both the activation of Notch and the presence of oncogenic Ras have a role in the fusion and invasion of scrib mutant tumors into the ventral nerve cord and the back of the brain [85]. Genetic mosaic screens in *Drosophila* led to the discovery of the Hippo pathway, which is also known as the Salvador/Warts/Hippo (SWH) route [86–93]. It has been shown that human cancers exhibit a kind of dysregulation of the Hippo pathway known as loss of function (LOF) of Hippo pathway genes [82–86, 89, 91]. The loss of function of genes involved in the Hippo pathway results in the expansion of vast tissue areas and a reduction in cell death.

#### **4. *Drosophila* employed as a "Whole Animal" Model for Human Cancer Research**

Patient biopsies and immortalized cell lines that were derived from surgically excised tumor tissues have been significant in developing a solid foundation for therapeutically relevant cancer research [94, 95]. When researching cancer biology and the efficacy of chemotherapeutic medicines, researchers often use HeLa cells and other human cancer cell lines as foundational models [94, 96]. Despite the fact that these resources are essential, a sample of single cancer cell lines only gives a restricted view into a dynamic tumor at a late stage. As a result, *in vivo* "whole animal" model organisms such as Genetically Engineered Mouse Models (GEMMs) and *Drosophila* model systems were utilized in order to investigate the genetic and epigenetic mechanism underlying the beginning and progression of cancer. While GEMMs and *Drosophila* have both helped to our understanding of human cancers, each model has its own set of benefits that make it superior to the other. A number of different tumor models have been generated in *Drosophila* by employing genetically straightforward combinations of oncogenic overexpression and tumor suppressor knockdowns [23, 25, 26, 97]. [23, 25] [26, 97] Because it is a complex "whole animal" system with several interconnected organs and tissue systems that work in harmony to maintain

homeostasis, *Drosophila* can provide phenotypic "readouts" of cancer progression. This is in contrast to cell culture models, which can only provide genotypic "readouts." Studies including comparisons have demonstrated that the fruit fly, often known as *Drosophila*, is a viable alternative to the mouse. Because of its vast array of genetic tools, transgenic constructs, and relative ease of use [98-101], *Drosophila* has been exploited as a model for tumor-promoting genetic cooperations in tumor cell migration and metastasis. Overexpression of the oncogenic isoform dRas1G12V (or simply, RasV12) in the imaginal disc epithelia has been shown to cause tumor transformation in *Drosophila* [102], therefore imitating tissue invasion and metastasis in vivo. [Note: dRas1G12V is also known as RasV12]. This oncogenic isoform has been used in a great number of early-stage genetic screens in an effort to uncover second-loci mutations that may combine with RasV12 to promote oncogenic growth. *Drosophila* is a desirable model because it can be used to create rapid and unbiased genetic screens to find tumor-promoting genetic combinations. This is important because more than thirty percent of human cancers are caused by oncogenic mutations in one of the three Ras orthologs in humans [103], and *Drosophila* can be used to do so. There is evidence that oncogenic Src64B, which is a c-Src homolog, contributes to the metastatic capacity of *Drosophila* imaginal epithelial cell clones [104]. *Drosophila* is a useful platform for researching Ras/Src-driven tumor formation at the whole-animal level [23]. This is because *Drosophila* has been utilized to produce various human cancer models that contain oncogenic activation of Ras and Src. [Citation needed] The malignant brain tumor known as glioblastoma multiforme (GBM) has been effectively modeled in *Drosophila* [24]. GBM is characterized by poor patient outcomes due to insufficient medication absorption, limited treatment efficiency, and rapid drug resistance. These factors all contribute to the disease. In various types of cancer, the epidermal growth factor receptor (EGFR) and phosphatidylinositol-3 kinase (PI3K) pathways are always active, which encourages the formation of tumors [105]. The stimulation of the EGFR and PI3K signaling pathways during embryonic development results in an excessive proliferation of glial cells [106]. This results in a larger brain in the larval stage as well as an overexpression of an oncogenic genetic network that does not rely on the genes that are intended to be targets for the EGFR and PI3K signaling pathways. Therefore, new therapeutic targets have been discovered as a result of research conducted using *Drosophila*, which is a kind of fruit fly. The tumor microenvironment, tissue morphology, angiogenesis, adaptive responses to drugs, tissue invasion, and metastasis are all factors that can be studied using 3D cell culture models like cancer spheroids, which have recently emerged as powerful tissue systems to study cancer biology and drug efficacy [107]. Cancer spheroids are one type of 3D cell culture model. [Note: Despite this, the *Drosophila* model of cancer cachexia [108] indicates the potential utility of whole animal models over spheroid systems. [Cachexia] is a condition in which a patient loses significant amounts of weight. Cachexia is a complex condition that causes muscle loss and has an influence on mortality associated to advanced stages of cancer [105, 109]. It is the result of a remote interaction between a tumor and its host. Systemic inflammation and metabolic dysfunction are assumed to be the root causes of this condition, which has been associated to cancer as well as other diseases such as sepsis [105, 109]. Because cachexia is a wasting phenotype that can manifest in organs and systems other than the underlying tumor, spheroids are not an appropriate model for researching it. In *Drosophila* investigations [108], the insulin signaling antagonist ImpL2 was found to be a strong facilitator of the wasting phenotype. ImpL2 is secreted by malignant tumors, and these research identified it as a significant facilitator of the wasting phenotype. The elimination of ImpL2 leads to an improvement in the wasting phenotype, which suggests

new therapeutic targets for cancer treatment. Therefore, spheroids may be used to further validate comparable molecular fingerprints in mammalian systems by applying a combinatorial approach of *Drosophila* for the discovery of such a factor. This may be done by using spheroids to further validate comparable molecular fingerprints.

## **5. Novel Ideas in Cancer Research Derived from *Drosophila* Investigations**

Our research in *Drosophila* has helped us make significant strides in our knowledge of the underlying processes that maintain tissue homeostasis and how a disruption in those processes might lead to the development of cancer. Competition between adjacent cells in a tissue system is a biological monitoring process that promotes homeostasis by evaluating cellular fitness among neighboring cells. This mechanism was first seen in the wing imaginal discs of *Drosophila* [19, 110-113]. In a competitive approach, neighboring cells with a higher fitness level might cause cells with a lower fitness level to undergo apoptosis [114]. Context has a significant role in determining the relative fitness unit, which is also mechanistically exclusive. This monitoring method was first demonstrated in *Drosophila* [115-117], and depending on the conditions, cancer cells can utilize it to outcompete adjacent wild-type cells and begin the development of neoplastic growth. The approach was first demonstrated in *Drosophila*. Researchers have been able to uncover various genes and factors that play a part in controlling cell competition and fitness levels by using this model system [57]. This model system was used because it allowed the researchers to more easily identify the genes and variables in question. There are a number of variables that have been implicated in cell-on-cell competition, two of which are the proto-oncogene *dmyc* and the Hippo pathway. Activating mutations in *dmyc* and the Hippo pathway have been shown to cause supercompetition [118, 120], which occurs when mutant cells and neighboring wild-type cells are forced to compete with one another. Given the association between Myc family genes and human malignancies [121] and the association between Hippo pathway dysregulation and human lung, colorectal, ovarian, and liver cancers [89–91, 120], supercompetition has been proposed as a cancer-initiation mechanism [122]. This is because of the association between Myc family genes and human malignancies [121]. *Drosophila* was the organism that initially led to the discovery of significant cellular phenomena such as the compensatory proliferation of cells in response to the death of a neighboring cell [123, 124]. Enhanced apoptosis has been linked to accelerating cancer development by signaling proliferation to surrounding cells and triggering an inflammatory response [4, 20, 123]. Despite the fact that increasing programmed cell death in cancer cells has been a common treatment strategy, increasing apoptosis has been linked to accelerating cancer development. Accumulation of reactive oxygen species (ROS) was found to signal macrophages, in a *Drosophila* model of apoptosis-induced proliferation in the eye imaginal disc, to increase the activation of the c-Jun N-terminal kinase (JNK) pathway and initiate cell proliferation [20]. This discovery was made using a *Drosophila* model of apoptosis-induced proliferation in the eye imaginal disc. Cancer cells may develop resistance to drugs through a process known as compensatory proliferation. Enhanced proliferation may lead to an increase in the accumulation of mutations that confer resistance [125], and cancer cells may acquire drug resistance through this process. It is essential to investigate the complex interactions that take place as a result of apoptosis, such as compensatory proliferation, in order to find more efficient methods for treating cancer. The "whole animal" model system that is seen in *Drosophila* is appropriate for this type of research. In addition, the concept of "tumor hotspots" within tissues has been created with the assistance of current research in

*Drosophila* [59, 127]. According to the "seed and soil" theory, which was initially proposed by Dr. Stephen Paget in 1889 [126], metastatic tumor cells, often known as "seeds," may only grow in the microenvironment of a particular organ. Recent research conducted in *Drosophila* has demonstrated that the tissue-intrinsic microenvironment plays a significant role in the establishment of the first tumor [59, 130]. Because they include tissue-intrinsic properties such as favorable cytoarchitecture and endogenous growth-promoting signaling, certain "tumor hotspots" are more sensitive to oncogenic signals or mutations [59, 127]. This makes them more likely to become cancerous. For instance, JAK-STAT signaling acts as an oncogenic driver, causing excessive neoplastic development in the hinge region of the wing imaginal disc [59]. In animals, hotspots for the development of tumors are seen at the intersections of two different epithelial cell types [128, 129]. Recent research conducted in *Drosophila* has showed that the tissue milieu for oncogenic Notch-driven carcinogenesis is promoted by JAK-STAT and JNK signaling towards the posterior margin of the larval salivary gland imaginal ring [130].

## **6. *Drosophila* cancer research with an emphasis on translational aspects**

Over the course of many years, the *Drosophila* model system has contributed, either directly or indirectly, to the development of potential anticancer therapies. In point of fact, *Drosophila* was the first model organism to exhibit synthetic lethality [131, 132]. This provided the theoretical framework for the discovery of PARP inhibitors, which were used to kill BRCA1 and BRCA2 related tumor cells [133]. In more recent years, the *Drosophila* model system has also been used directly for drug screening. This practice began in the 1980s. High Throughput Screening (HTS) and *in silico* virtual screening are two different approaches to the process of target-based drug development, which might be considered independent methodologies. HTS can be used to cause a desirable physiological response in cultured cell lines, which is necessary for the identification of lead compounds that elicit a pharmacological action. However, the development of a medicine and its safe application in clinical settings has traditionally required the use of animal models for drug testing. The inability to reproduce the desired effect of a test molecule obtained using HTS in animal models or the failure to recapitulate in humans the efficacy of a medicine that has been evaluated on animal models is a common bottleneck that occurs during this process [14, 70, 134]. This is one of the reasons why this process takes so long to complete. The use of organ-on-a-chip [135] or organoid models [95, 107], which is merely a costly alternative to animal models, in comparison to the recommended use of *Drosophila* as a parallel drug testing platform [69, 70], has enabled the recent entry of several medications into clinical trials without the use of data from animal models. This was accomplished by employing the organ-on-a-chip [135] or organoid models [95, 107]. Fruit flies, which can be genetically modified in *Drosophila* using a variety of techniques, have been used to mimic multigenic origins of human colon cancer. These fruit flies depict human malignancies more accurately than earlier models did because of this simulation. In one such study, using patient data from The Cancer Genome Atlas, as many as 32 multigenic models of human colorectal cancer were developed for the purpose of further examining medication resistance in a variety of genetic backgrounds [103]. This was done in order to better understand why some people are more likely to develop the disease than others. Cancer models for colorectal and lung cancer have been used to promote combinatorial medication cocktails for a variety of reasons, including overcoming drug resistance and synergizing the effects of the individual drugs [103, 136]. The absence of genetic redundancy in *Drosophila* has made it possible for

large pharmaceutical companies like Novartis and AstraZeneca to test for the medication specificity [134, 136–138]. This has led to *Drosophila* being chosen as a model organism in certain instances rather than the models of vertebrates. Even though the use of invertebrate models for target-based drug screening might not be able to completely circumvent the "lead to drug bottleneck," these models are still useful because they offer an alternative to the animal models that are traditionally utilized in the process of drug discovery and development. Research on medication development in *Drosophila* has the potential to aid in the discovery of targets and pathways that would otherwise be missed by conventional methods. These studies also have the potential to assist in the definition of drug dosage regimens in some instances [103, 136].

## 7. Conclusions

The 'Hallmarks of Cancer,' describing six traits that could best describe the illness, was published in 2000 by Douglas Hanahan and Robert Weinberg [3]. They hypothesized that in order for a normal cell to develop into a malignant one, it must acquire certain hallmark characteristics, such as the ability to proliferate autonomously without external growth signals, resistance to anti-growth signals, avoidance of programmed cell death, the acquisition of unlimited replicative potential through telomere maintenance, sustained angiogenesis for nutrients and oxygen, and tissue invasion via metastasis. In 2011, the list was revised to include additional hallmarks, such as genomic instability in cancer cells and inflammation in the tumor microenvironment as enabling factors that promote cancer progression [4]. Other changes included the deregulation and misappropriation of metabolic pathways to competitively feed cancer cells and the evasion of the immune system. These diagnostic features and enabling qualities point to the disease's complexity and necessitate a broad examination using several modeling approaches. *Drosophila* can genetically mimic most of the characteristics of human cancer [139]. From a genetic standpoint, it has been questioned why *Drosophila* does not show signs of human biology in places like the telomere and telomeric maintenance methods, the adaptive immune system, the process of angiogenesis, and the development of the mammary glands. However, it has been demonstrated that hypoxia response in tumors induces HIF1/Sima-dependent activation of signaling pathways that drive both angiogenesis in humans and tracheogenesis in *Drosophila*, with the same consequence of getting more access to oxygen [140, 141]. Similar signaling responses in the form of JNK and TOLL/NFB pathways are part of the genetic network that constructs the innate immune response to cancer in humans [13, 20]. *Drosophila* is not suitable as a stand-alone model system for testing the efficacy of drugs that are ultimately meant for human trials, nor as a replacement for mammalian testing platforms, due to obvious differences in physiology, oversimplification of signaling networks, and key differences in a drug's ADME (absorption, digestion, metabolism, and excretion) properties. It has, however, been proposed as a low-cost screening platform complementary to other systems and as a "whole animal" cancer screening model with phenotypic readouts to evaluate polypharmacological techniques [69, 134, 137]. *Drosophila* has been used for decades to decipher complicated illnesses by employing advanced genetic techniques created for the model. It has served as both a low-cost hypothesis-building tool for uncovering previously unknown processes of

tumor initiation and progression, and a unique genetic screening platform for the discovery of many genes and pathways involved in cancer. With a positive track record and a strong scientific community, the study of cancer in *Drosophila* will continue to shed light on fundamental topics in cancer biology, leading to the development of new and improved methods for combating the illness.

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