Structured Approach to the Development of New Cancer Drugs Using Drosophila

Amin Foroughi Nezhad[1]

[1]Corresponding Author: Faculty of Basic Sciences, Department of Biological Science, Semnan University

November 10, 2022

Abstract

In the past few years, Drosophila has gained prominence as a tool for drug development due to its capacity to screen tiny compounds against intricate disease phenotypes in the setting of a complete animal. Gene-compound interaction studies combine compound feeding with complex genetic modifications in order to better investigate the compound mechanisms of response and resistance. These studies allow for a more in-depth exploration of the compound response and resistance mechanisms. In this section, I will discuss how the chemical screening and testing procedures performed on Drosophila may be applied to the process of generating novel cancer medicines in the present day. A framework for a Drosophila-based cancer drug discovery strategy is what I propose in order to assist the Drosophila research community in making the most of the benefits that Drosophila offers in terms of locating possible treatments and progressing our discoveries into the clinical setting.

Review

Structured Approach to the Development of New Cancer Drugs Using Drosophila

Amin Foroughi Nezhad*

* Corresponding Author: Faculty of Basic Sciences, Department of Biological Science, Semnan University, Semnan, Iran; Email: aminforoughy.77@gmail.com

Abstract: In the past few years, Drosophila has gained prominence as a tool for drug development due to its capacity to screen tiny compounds against intricate disease phenotypes in the setting of a complete animal. Gene-compound interaction studies combine compound feeding with complex genetic modifications in order to better investigate the compound mechanisms of response and resistance. These studies allow for a more in-depth exploration of the compound response and resistance mechanisms. In this section, I will discuss how the chemical screening and testing procedures performed on Drosophila may be applied to the process of generating novel cancer medicines in the present day. A framework for a Drosophila-based cancer drug discovery strategy is what I propose in order to assist the Drosophila research community in making the most of the benefits that Drosophila offers in terms of locating possible treatments and progressing our discoveries into the clinical setting.

Keywords: Drosophila · Cancer drug discovery · Compound screening

1. Introduction

The fruit fly Drosophila melanogaster has been put to extensive use as a model organism in developmental biology and genetics research due to the accessibility of cutting-edge genetic methods and the numerous practical benefits it offers. Research conducted on Drosophila, including genetic screens and studies on epistasis, has resulted in the discovery of novel genes and signaling networks that are involved in key developmental and molecular pathways. This, in turn, has helped to facilitate our understanding of fundamental aspects of development, cell biology, and signal transduction. Drosophila has also demonstrated its use as a disease model, with a number of complex disease states having been successfully replicated in this insect [1-4]. This demonstrates Drosophila's versatility. Drosophila has been the subject of a number of ground-breaking studies over the course of the past two decades [5-12]. These studies have paved the way for future research that will use the genetic power of the fruit fly to identify new therapeutic candidates and investigate the mechanisms of action and resistance of existing drugs. These preliminary studies demonstrated, in a nutshell, the following: 1) compounds can be introduced into flies by feeding or culturing dissected tissues in the presence of compounds; 2) developmental phenotypes and pathway-specific target gene expression can be used as read-outs to monitor the activity of compounds; and 3) compounds can be tested for their ability to modify disease phenotypes generated by genetic manipulations of disease-relevant genes. The findings of these studies indicate, when taken as a whole, that a significant amount of chemical action is conserved in Drosophila, as many of the same compounds that were found to affect the activity of their

targets in experiments conducted on mammalian subjects also had this effect on the activity of their targets when tested on Drosophila. These studies have been discussed in depth elsewhere [1, 13–18], so we won't go over them again here. Instead, we will go on to something different. In this paper, I will discuss how the Drosophila drug-screening and testing methodologies that have been published may be utilized within the framework of the existing cancer drug development pipeline. Specifically, I will focus on how this may be accomplished. A Drosophila-based drug discovery paradigm is proposed in order to address the genetic complexity of cancer. This paradigm makes use of the fly since it offers a one-of-a-kind mix of sophisticated genetic tools and practical benefits.

2. Examination of Multiple Factors

Target-based drug discovery became the industry standard in the treatment of cancer [19-22] around twenty years ago. The idea that pharmacologically affecting the activity of a target that is essential to the formation and/or maintenance of a tumor phenotype would result in a discernible improvement in patient outcomes is the driving force behind this approach. This "target-first" technique begins by locating a genetic vulnerability, which may be done either by functional investigations or data mining of large "omics" datasets. The next step is to employ high throughput compound screening, in silico techniques, and rational design in order to locate compounds that influence the activity of the target. Imatinib (GLEEVEC) [23] and gefitinib (IRESSA) [24] are two examples of medications that have utilized this method with significant levels of success. However, the success rate of target-based drug development strategies in clinical trials has been dismal for the majority of solid tumors [19, 25, 26]. The complex and changeable composition of tumor genomic landscapes is at least partially responsible for the difficulty of the problem. It is difficult to pinpoint a single target whose pharmacological alteration results in a clinically relevant impact due to the presence of highly redundant signaling networks and a large number of feedback mechanisms that compensate for one another. The identification of drugs based on phenotype is a good technique that may be used in conjunction with the discovery of drugs based on targets [19, 26]. This target-agnostic, function-first strategy tries to discover a chemical entity that may either correct a tumor phenotype or destroy cells expressing such phenotypes. The read-outs for the compound screens are cancer-specific phenotypes, and the compound screens use these phenotypes as read-outs. Because the majority of tumor phenotypes are the consequence of emergent interactions between several genomic alterations in complicated and different genetic backgrounds, our technique has a great deal of potential in discovering candidate therapies that may be able to handle the complexity of the illness. Because it allows for the screening of compounds in a complete animal scenario, where both the efficacy and toxicity of the compounds can be evaluated concurrently, Drosophila is an excellent model system for phenotype-based cancer drug development methodologies. This is one of the reasons why Drosophila is an excellent model system. Screening for genetic modifiers is a tried-and-true method that has shown to be one of Drosophila's greatest advantages in the fields of developmental biology and cell signaling [12-32]. Several different organizations have employed this method. Because it has been demonstrated to be an effective read-out in both genetic screens and high throughput compound screens, the rescue from lethality assay is currently the most popular choice for screening compounds. This popularity is based on the fact that it has become the most common assay for screening compounds. The phenotype-based drug development technique has historically suffered from a significant flaw in the form of a shortage of diverse

phenotypic read-outs that may be used for the screening of compounds. Chemicals that are cytotoxic or cytostatic are often found to be suitable for use in therapeutics using high throughput screening methods that rely on cell survival or proliferation as the primary read-out. Drosophila cancer models have been able to successfully capture many of the hallmarks of cancer, including proliferation, apoptosis, senescence, epithelial-mesenchymal transition, migration, and metastasis [2, 33–36]. This has made it possible to develop high throughput screening assays to find compounds that modify these complex and disease relevant phenotypes. There have been previous descriptions [27, 28] of the process of screening drugs in Drosophila using imaging or luciferase-based readouts. It is necessary to perform compound screens that focus on hallmarks of cancer as key assays in order to widen the portfolio of candidate therapies that are currently in clinical development. Proliferation and survival as key assays are not the only hallmarks of cancer that may be tested. Drosophila may be used as a drug discovery platform for cancer, which has a number of advantages. One of these advantages is the ability to construct complicated cancer models that are indicative of the richness and variety of genuine tumor landscapes. Large-scale research into tumor sequencing have led in the development of precision medicine approaches as well as sophisticated designs for biomarker-based clinical trial designs [38–41]. This technique has showed promise [42], but it has not always been enough to predict treatment response [43-48]. Additionally, focusing on individual genomic changes runs the danger of oversimplifying the genetic diversity and complexity of tumor genome landscapes. Because of the genetic complexity of cancer models in Drosophila, it is possible to construct large collections that can be put to use for chemical screening in a very short length of time and at a relatively cheap cost. We were able to show [33] that genetically intricate models of colorectal cancer are resistant to the majority of medications discovered using target-based drug discovery approaches by using a panel of colorectal cancer models produced from sequenced colon tumors. It is possible that the use of such models in compound screens will result in a new generation of candidate therapies that are able to address the complexity of the disease and help pave the way for precision medicine approaches that make use of broader genomic landscapes rather than individual cancer driver alterations for patient stratification.

3. Investigating Multiple Factors of Mechanisms of Action (MoA)

It may be challenging to discover the molecular mechanisms of action (MoAs) of the hits that are identified by phenotype-based compound screens since these screens are not designed to differentiate between targets and mechanisms. Phenotypic screening can be used to discover MoAs that exhibit non-autonomous effects on wild-type cells and tissues that are located in close proximity. Multiple studies conducted on the fruit fly Drosophila have demonstrated that epistasis and gene-compound interaction research may be applied to locate a substance's molecular mechanism of action (MoA). A Drosophila lung cancer model that was produced by targeting oncogenic ras and pten deletion to the tracheal system was used in a chemical screen that led to the discovery of the drug combination known as trametinib/fluvastatin [29]. This is an illustration of how flies may be utilized to explore MoA. Because of the effect that fluvastatin has on the RAS signaling pathway, the combination has a mode of action that includes the capacity of fluvastatin to reduce the systemic toxicity caused by trametinib. The addition of fluvastatin boosted the utility of trametinib by making it feasible to utilize a dosage of trametinib that would otherwise be dangerous. In other words, the inclusion of fluvastatin raised the usefulness of trametinib.

In a study using a stem cell derived intestinal tumor model that was established by targeting oncogenic raf to stem and progenitor cells of the adult intestine [28], a group of chemotherapy agents were found to promote the proliferation of wild-type stem cells while inhibiting the growth of stem cell tumors. This was discovered in a study using a stem cell derived intestinal tumor model. It was discovered that this was the consequence of a non-autonomous influence that was mediated by the production of JAK-STAT ligands. These ligands affected the local tissue microenvironment and encouraged the proliferation of wild-type stem cells that were located in the surrounding area. Bortezomib, an inhibitor of the proteasome, and BEZ235, an inhibitor of the PI3K pathway, were shown to be an effective medication combination with a novel and unique mechanism of action for suppressing the spread of tumor cells into the abdominal cavity when using a genetically complex '4-hit' model of colorectal cancer [33]. This combination was shown to be effective in inhibiting the spread of tumor cells into the abdominal cavity. Bortezomib changed the output of the target signaling pathway, which led to an increased dependency on BEZ235, as was discovered in a research in which flies were used. In order for the combination treatment to be effective, the drugs needed to be given in a certain order and in a specified order of alternating frequency. This was necessary since the unique mechanism that was in action required it. These findings demonstrate how the standard methods utilized in a Drosophila laboratory to investigate fundamental scientific topics can be modified to investigate the mechanism of action of a compound in a whole animal setting. This is an essential but challenging step in the process of developing new medications. In-depth mechanistic investigations in Drosophila have demonstrated that lead compounds discovered by target-based approaches can also acquire clarity on their mechanisms of action [8, 49]. Target-based drug development places a premium on finding selective and highly specific lead compounds, on the theory that these molecules would have more clinical utility [50, 51]. This theory is supported by the fact that this approach places an emphasis on finding selective and highly specific lead compounds. Investigations employing in vitro target profiling, on the other hand, have shown that the great majority of compounds found using this technique really have additional direct targets [52]. In Drosophila, genetic modifier screens and gene-compound interaction assays have been used to explore direct targets that have been discovered by target profiling investigations for the purpose of determining the functional importance of these direct targets [8, 49]. ZD6474, commonly known as vandetanib, is a selective inhibitor of the receptor tyrosine kinase VEGFR2. It was discovered that vandetanib also inhibits the activity of other receptor tyrosine kinases (EGFR, PDGFR, and RET), albeit to a lesser extent [53–55]. Studies on the interaction of ZD6474 with the RET gene in Drosophila have indicated that it is more likely for RET to be carcinogenic in vivo [8]. ZD6474 was finally given approval by the FDA as a medication that can be used to treat metastatic medullary thyroid carcinoma (MTC), a particularly rare kind of thyroid cancer that is caused by oncogenic RET [56].

4. Investigating Multiple Factors of Mechanisms of Resistance

Oncology has one of the worst success rates for clinical trials compared to any other illness sector [57]. The majority of potential lead medications are never tested in human clinical trials, despite the fact that they have fascinating targets, mechanisms of action, and a plethora of information from preclinical studies. Drosophila models have been effectively exploited to examine resistance mechanisms for various medicines [30–33]. This has been accomplished through the discovery of genetic modifiers of drug resistance. In preclinical

mammalian cancer models, several of the plausible medicine combinations that were discovered as a result of these findings have been shown to be effective. This technique provides a fresh alternative for repurposing lead compounds that were failed in clinical trials, either as single therapies targeting the genomic landscapes that are most likely to react, or as part of pharmaceutical combinations that are meant to overcome resistance. It is usual practice to place responsibility for adverse outcomes in clinical research on the insufficient selectivity and specificity of lead molecules. On the other hand, even when a particular target or signaling node is blocked by pharmacological techniques, this is not always sufficient to generate a clinical response [43-48]. Because the actionable genes targeted in these studies are nearly always discovered in the presence of additional altered genes that might modify treatment responsiveness in ways that are unexpected, the lack of a response may be an emergent aspect of complex interactions within the illness network. This is due to the fact that these studies target genes that are almost always discovered in the presence of additional altered genes. Drosophila has evolved as a viable platform for linking response and resistance with wider genomic landscapes, and this has allowed it to be used in the evaluation of lead compounds against huge panels of genetically complex and diverse animals. We have used this technique in order to discover different genomic landscapes that correlate with responsiveness and resistance to these medications [33]. This is because PI3K pathway inhibitors have a relatively poor efficiency as single agents against most solid tumors [43, 58]. These findings clear the way for more sophisticated biomarker-based therapeutic trials, in which patients will be stratified based on genetic landscapes that are more detailed.

5. Experiments in Rational Synthetic Tailoring and Structure-Activity Relationship (SAR)

Investigations into the structure-activity relationship, also known as SAR, are an essential component of the target-based drug development pipeline. During these investigations, a library of compounds that are structurally analogous to one another is developed and tested in order to establish which chemical classes are accountable for the observed level of biological activity. After gaining this knowledge, it will be possible to chemically synthesize lead compounds that have improved specificity, selectivity, and pharmacokinetic and pharmacodynamic (PK/PD) features [59]. The use of a phenotype-based SAR approach in the development of cancer drugs, in which compounds in a SAR series are tested for their ability to modify a complex disease phenotype rather than to specifically and selectively inhibit a single target [49, 60], is perhaps the most novel application of Drosophila in this field. Phenotype-based SAR approaches are used in drug development for a variety of diseases, including Alzheimer's disease, Parkinson's disease, multiple sclerosis An excellent therapeutic index, which is the ratio of efficacy to toxicity, can be achieved by carefully optimizing the pro-target and anti-target profiles of a molecule. This method of rational synthetic tailoring involves the use of chemical synthesis, functional research in Drosophila, and in vitro target profiling in order to identify and remove undesired activities (anti-targets) from a chemical structure while simultaneously keeping favorable activities (pro-targets). This strategy is based on the hypothesis that using compounds with multiple pharmacological actions — those chosen for their optimal pro-target/anti-target profiles rather than their single target specificity — will be more effective in treating human tumors with complex genetic makeups and less likely to develop resistance over time. This hypothesis underpins the overall design of this approach. For the purpose of lead selection and optimization in a variety of tumor types or genetic situations, structurally similar

medicines that have been obtained for SAR research and have had their direct target profiles well defined are a useful resource. It is anticipated that different tumors will have different responses to pro-target and anti-target therapies since different tumors have different genetic landscapes. In certain genomic landscapes, a target that serves as a significant vulnerability also may play an essential protective role in other genomic landscapes. Studies on the genetic makeup of Drosophila can be utilized to calculate the precise pro-target/anti-target profile that is required to target the genomic landscape of a tumor. Then, in the event that a new tumor type or genomic landscape has to be targeted, it is possible to repurpose an existing SAR series in order to locate lead compounds that have the appropriate profile.

6. The Utilization of Drosophila in the Research and Development of Personalized Cancer Drugs

An individualized "fly-to-bedside" clinical research project is now under progress at the Icahn School of Medicine at Mount Sinai to study the usefulness of Drosophila as a therapeutically relevant platform for the development of cancer therapy. This research generates a one-of-a-kind fly model for each cancer patient, which is then put through its paces in the context of an experiment testing a collection of legal drugs. The sequencing of tumor DNA as well as normal (germline) DNA and study of copy number are the cornerstones of this research. The genetic make-up of each individual patient's tumor is subsequently re-created in a Drosophila model that has been tailor-made for that patient. This model is used in iterative screens of drug libraries that have been authorized by the FDA, with rescue-from-lethality serving as the read-out. The goal of these screenings is to determine the medicine combination that works best for each particular patient. A tumor board consisting of doctors, pharmacists, and scientists with expertise in Drosophila genetics, cancer genomics, and clinical trial design reviews the case of each patient, and based on their findings, a personalized treatment approach is devised. Initial discoveries from this project that look promising include the following: A KRAS mutant patient with metastatic colorectal cancer demonstrated a powerful partial response to the 2-drug combination that we designed, followed by several months of stable disease. This patient had previously progressed on multiple FDA-approved therapies (in press). If I could correctly cite it, it would be of great assistance. It will be necessary to collect outcome data from other patients in order to demonstrate that the method is applicable as a specialized treatment option for cancer and to investigate the viability of incorporating it into clinical practice.

7. How to Bring Discoveries to the Bedside without Getting Lost in Translation

The clinical development of prospective medicines is a process that can take a long time and is fraught with difficulty since there are so many moving variables. Because clinical trials are expensive and may require funding from the private sector or foundations, intellectual property (IP) protection is essential to generate interest in the clinical development of lead medications [61]. Validation studies employing mammalian preclinical cancer models are required to be carried out in order to evaluate the mechanism of action and efficacy of hits derived from Drosophila screens. It is necessary to conduct more study into the pharmacokinetic and pharmacodynamic characteristics of hits before determining whether or not they are suitable for clinical development. Because it entails a number of processes that are above the capabilities of a typical Drosophila laboratory, the idea of moving potentially curative treatments that were created using Drosophila into the clinic may be

intimidating to some. I will now explain some of the most important aspects of a Drosophila-based framework for the development of cancer drugs. This framework takes into account the contributions of chemists, physicians, pharmacologists, and other scientists who have experience working with mammalian cancer models and also encourages collaborations with these individuals (Fig. 14.1).

7.1. Selection of a Cancer Model

It is possible that the most important component is selecting a model for cancer drug discovery in which a clinical need for innovative treatments is being addressed. Even while the treatment of cancer continues to be a major priority, there is a possibility that investors may be less enthused about tumor types that already have numerous treatments that have been authorized by the FDA and/or lead compounds that have solid clinical proof. The outcomes achieved by applying best practices in healthcare settings are of similar significance. For example, several combination therapies for multiple myeloma, which have been authorized by the FDA, have been shown to enhance overall longevity by up to ten years [62]. It is possible that pharmaceutical companies may be reticent to sponsor the discovery of new medications that perform better than the requirements that are already in place in this scenario because of the high expense of such studies and the extended time for which they are conducted. When choosing a tumor type, it is important to give careful consideration to the availability of FDA-approved medicines as well as the performance of lead compounds that are currently undergoing clinical development. This will make it easier to form partnerships for the clinical development of lead compounds found by Drosophila screens. If you are conducting research on carcinogenesis, you may find yourself debating whether it would be more beneficial to develop a Drosophila model of a specific tumor type, zero in on a genetic landscape that is shared by several different tumor types, or zero in on a characteristic of tumorigenesis such as invasion. However, the latter two strategies have the potential to provide commercially viable lead therapies with broader potential relevance for the treatment of cancer. The most direct way to address a clinical need is through the use of Drosophila models of a specific tumor type. In some circumstances, identifying the patient population necessary for clinical development and the mammalian preclinical models necessary for validation studies may be challenging. This is something that has to be taken into consideration before screening. Consider how precisely the Drosophila model mimics the genetic landscape of the tumor type of interest. This is an additional essential factor to take into consideration. Employing a panel of genetically sophisticated models that show the whole genomic landscape of a tumor type is one of the most effective ways to make advantage of Drosophila's capabilities as a model system and improve the model's relevance to therapeutic research. This method is also more time efficient. It is feasible to investigate the compound response patterns of various genomic landscapes by screening multiple models simultaneously or by comparing lead compounds discovered from one screen against additional models. The capability of linking chemical response with tumor genotype is a significant tool that can be utilized both in the process of selecting which preclinical model is most suitable for validation studies and in the process of establishing a target patient group for the clinical development of potential medicines. Despite the fact that the majority of cancer-related genes and signaling pathways are highly conserved, not all tumor types or recurrent genomic abnormalities can be recreated in flies. This is the case even though most of these genes are involved. It is difficult to recreate hormone-dependent diseases like breast and prostate cancer in flies because, for example, flies do not have clear

estrogen or androgen orthologs. This makes it possible for researchers to study breast cancer but not prostate cancer. It will be necessary to screen for tumors with highly conserved cancer driver genes and show that the model captures crucial elements of carcinogenesis in order to demonstrate that the Drosophila model has therapeutic relevance. This will be done in order to prove the therapeutic relevance of the Drosophila model. It is difficult to determine the likelihood of success when using high throughput screens due to the imprecise nature of these tests. Therefore, these concerns need to be taken into consideration before screening even begins in order to ensure that there is a feasible path forward for the clinical development of lead compounds if they are identified. This is the case regardless of whether one is designing a new Drosophila cancer model or evaluating the potential of an existing model for cancer drug discovery.

7.2. Selection an Examination Assay

The most common read-out for chemical screening in Drosophila is the rescue of a phenotype that would otherwise be deadly as a result of inducing genetic alterations during development. This is because genetic changes during development can lead to lethal phenotypes. Imaging and luciferase-based Drosophila compound screens are two examples of more disease-specific readouts that may be utilized in screening (described in [27, 28]). It is possible to use assays for other cancer hallmarks, such as invasion, apoptosis, senescence, or tumor metabolism, as primary screening read-outs; however, it is recommended to do thorough pilot studies to assess cost, time, and effort in order to guarantee that a reasonable throughput can be obtained. This is because using these assays as primary screening read-outs could lead to inaccurate results. It is also important to determine the relative weights given to the many genetic alterations that make up a multigenic cancer model's contribution to the screening result. Hits that can address genetic complexity are more likely to be identified if they are based on a phenotype that develops as an emergent property of the specific multigenic combination that is being exploited. This increases the probability that hits will be found. In Drosophila chemical genetic research, substances are frequently administered orally by combining the molecules in question with the food being consumed. It is essential to select a screening assay that takes into account the particular developmental stage of the species, as larval animals only have access to food for a brief length of time. It is preferred to employ compounds that can act as positive controls in order to confirm and calibrate the screening assay. This may be accomplished by using positive controls. If it isn't an option, selecting phenotypes that are lethal to the larval stage is the next best thing. If at all feasible, it is preferable to avoid the development of embryonic lethality as well as other potentially fatal characteristics that might result from genetic modifications made during the embryogenesis process. Read-outs of pupal lethality have been used successfully in compound research and can also serve as helpful read-outs, particularly if the lethality is the consequence of faults in the development of the larva. Nevertheless, determining this with concrete facts can prove to be difficult. If pupal lethal read-outs are employed, the findings of the screening might be biased in favor of compounds that are extraordinarily stable in Drosophila and survive until the development of the pupal stage if these read-outs are used.

7.3. Selection a Multiple Factors Library

Compound libraries for use in de novo drug development can sometimes come from commercial libraries or compound collections compiled in collaboration with individual chemists or pharmaceutical companies. These are only two examples of potential sources for

compound libraries. During the course of the library selection process, it is possible, with the assistance of scientists and experts in intellectual property protection, to locate a library of compounds that fall inside the patentable chemical space and have features similar to those of drugs. Companies should get in touch with the Intellectual Property (IP), Technology Transfer, and Commercialization (TT&C) departments located at most universities if they want to make the most of the resources at their disposal. Before beginning screening, it is essential to ascertain who is the owner of the intellectual property for any discoveries that are produced utilizing chemicals that are gained through collaborations with third parties. Because the majority of academic laboratory research is conducted by postdocs and graduate students, and because the opportunity to publish in a timely manner is a significant factor for them, it is essential to discuss publishing rights and timetables in advance when working with the business sector. Despite their historical value as a source for the identification of new drugs, there has been a significant drop in the number of drug development initiatives that are based on natural products in recent years [63, 64]. This is due to a number of factors, including concerns around intellectual property as well as an inability to simply get sufficient material for clinical trials. When it comes to the repeatability of findings, natural products could provide a challenge due to the high variability that exists from batch to batch, as well as the fluctuations in content and composition that occur due to seasonal and environmental factors. Because of these factors, bringing first hits into the clinic using natural product libraries for compound screening will require identifying active components responsible for the required biological activity and proving that economically viable leads can be created through chemical synthesis. In addition, bringing first hits into the clinic will require a significant amount of time. In de novo drug development, lead optimization is a process that requires a lot of time but is extremely significant. The benefits of this stage include greater efficacy as well as the production of leads with desirable pharmacokinetic and pharmacodynamic (PK/PD) properties. The process of drug repurposing, which entails the examination of medicine libraries for applications that have not been licensed, is an interesting alternative technique. The availability of compounds that have previously been created for human consumption and the existence of extensive preclinical and clinical safety data [65, 66] make it possible to speed up the process of developing these drugs for use in clinical trials for new indications. Despite this, there are still significant regulatory and logistical hurdles that stand in the way of pharmaceutical repurposing [67]. First, there is the possibility that the cost of clinical development will increase if the mechanism of action for the new indication is distinct from the mechanism of action for the original indication or if the new indication contains a target or activity that was not previously discovered. Second, prohibiting the use of generics in ways that are not authorized by the manufacturer is difficult in practice, which reduces the financial potential of repositioning a drug for a different purpose. If a company's drug patents are about to expire or have already expired, the financial incentive for the company to discover new applications for the medication may decrease. In theory, one can get "regulatory data exclusivity" for a new indication, which would prevent others from utilizing clinical data developed for the new indication in regulatory applications for generic counterparts [67]. This would be advantageous for the company developing the new indication. However, it is difficult to monitor this in practice due to the fact that the choice for the least priced medication, the generic, is often given or delivered regardless of the indication. When considering a pharmaceutical repurposing strategy, it is essential to thoroughly investigate whether or not there is a feasible path forward for the clinical development of potential hits. Increasing the weight given to results that are either in the early stages of clinical testing or that have just been licensed by the FDA

with extended protection from generic competition is one option. Another option is to give more weight to results that are in the later stages of clinical testing. Reusing medications in clinical trials that were terminated due to a lack of positive results is yet another approach that might be taken. If these compounds have been through clinical development in the past, finding a new application for them either as single agents or in medicine combinations may help them recoup some of the money they spent on the process. Importantly, compounds whose development was halted because of significant toxicity or unfavorable PK/PD properties would not be ideal candidates for such an approach unless chemical expertise to optimize PK/PD profiles through SAR studies is available. This is because such compounds would not be able to meet the requirements of the approach.

7.4. Studies of Hit Selection and Initial Mechanisms of Action in Drosophila

Following the confirmation of results from the first screen, early follow-up research should be done in Drosophila in order to obtain insight into their putative mechanisms of action (MoAs) and choose suitable preclinical models for mammalian validation. Methods that are commonly used include testing effectiveness in a variety of genetic contexts in order to identify sensitive and resistant genomic profiles, conducting dose response studies in order to determine the optimal dose for future experiments in Drosophila, and using a panel of secondary assays in order to determine which hallmarks of tumorigenesis are targeted by the hit. The existence of confirmed hits in mammalian models makes it possible to conduct additional, more in-depth mechanistic investigations. It's possible that the Drosophila hits with the highest levels of efficiency or biological activity aren't the ones with the most promise for commercial development. When selecting hits for lead optimization, it is essential to have discussions with chemists, pharmacologists, and IP specialists on patentability, PK/PD properties, and the feasibility of altering the chemical structure in order to conduct SAR analyses. It is feasible to increase the likelihood of locating a lead that is suitable for use in commercial enterprises by pursuing several hits in subsequent animal validation tests.

7.5. Verification in Mammals

Drosophila has a high degree of conservation of a number of the genes and signaling pathways that are most frequently associated with cancer. As a consequence, hits obtained from a screening platform based on Drosophila typically display maintained biological activity. However, it is possible that the complexity of the tumor microenvironment and the interactions between the tumor and the stroma are lost in Drosophila models due to variables such as the absence of adaptive immunity and the absence of a substantial stroma. Mammalian validation studies, in which a variety of preclinical models should be utilized if they are accessible, should be included as an essential and early component of the lead selection process. Conventional cell-based drug discovery pipelines have a chance of missing drug candidates with sophisticated processes. However, drug candidates with these processes can be uncovered by employing genetically complex Drosophila cancer models and in vivo screening read-outs. The same quality that makes Drosophila such an appealing platform for drug screening may also make it difficult to find an acceptable preclinical model for mammalian validation when conducting mechanistic investigations that need for complex genetic changes. As a result, it is beneficial to begin early in the process to think about relevant mammalian models for validation studies and to seek collaborations to construct new ones if necessary. In addition, it is important to think about suitable

mammalian models for validation research. After the molecular mechanisms of action (MoAs) of the hits have been uncovered, it is possible that more mammalian study will be required to confirm the hits' efficiency across species. When performed in Drosophila, large-scale exploratory investigations and experiments that require sophisticated genetic modifications can be carried out to unearth more precise hypotheses that can then be tested in mammalian models. This helps to reduce the amount of time, money, and effort that is required for such studies. For instance, in vitro target profiling is an effective way for discovering prospective targets for candidate leads. Drosophila is an excellent tool for evaluating the in vivo relevance of numerous targets that were found in these experiments as well as the relative contributions of each target. Through the use of unbiased genetic screens that aim to uncover modifiers of drug response, it is possible to gain insight into the mechanisms of action and generate more direct ideas that may be tested in mammalian models. This is made possible by the fact that it is possible to generate ideas that may be tested in mammalian models. Exploring the pharmacokinetic and pharmacodynamic properties of potential leads is just as crucial as determining whether or not a biological function has been conserved, particularly in the context of de novo drug development. PK/PD modeling and simulation investigations allow for an early prioritization of hits that have potentially beneficial predicted PK/PD profiles [68, 69]. However, in the end, it is necessary to do experimental research utilizing animal models in order to determine the efficacy, safety, metabolism, and PK/PD profiles of the drug [70, 71]. These assessments may be carried out in collaboration with other parties or outsourced to contract research organizations that are experts in the conduct of studies of this kind.

7.6. Benchmark Quality

Benchmark Quality or Lead optimization refers to research projects with the goals of increasing a prospective lead's efficiency, improving its PK/PD characteristics, and reducing its level of toxicity. This method is both time- and money-consuming, as it typically requires rounds of chemical synthesis, in vitro target profiling, and mammalian validation studies. Additionally, in vitro target profiling can be challenging. The search for potential collaborations with the biotech industry as soon as a lead with preserved biological activity, a favorable projected PK/PD profile, and IP protection that has been secured is one way to cut down on the amount of time and money needed for lead optimization. Another method is to seek out potential collaborations with the pharmaceutical industry. Big Pharma collaborations sometimes need extensive preclinical research and may be more suited for leads that are farther along in the development process [71]. As a result, concentrating on smaller biotech enterprises may be a more prudent strategy at this point in time.

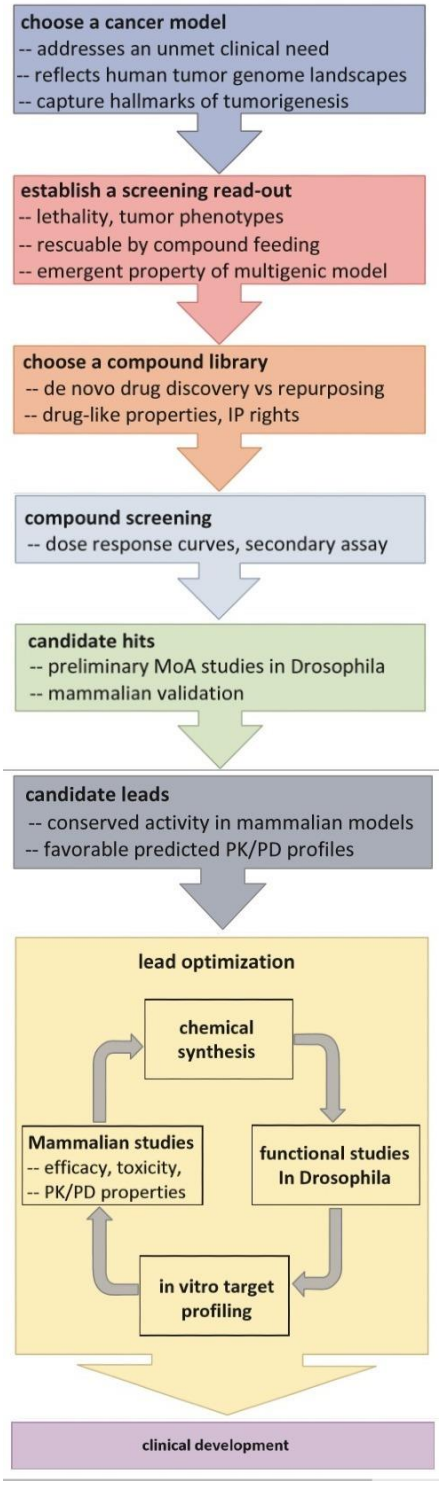

Figure 1: Cancer drugs development through using fruit fly as a model

8. Conclusion

Drosophila has been shown to be useful in a growing body of published research for the identification of new candidate therapies, the exploration of mechanisms of action and resistance for medications in development, and the identification of novel vulnerabilities that may be targeted in future investigations. These three applications are all examples of areas in which Drosophila has been useful. Because the entire cancer research community is becoming aware of our work and realizing the relevance of Drosophila as a platform for drug discovery, the collaborative effort that is essential to move our discoveries into the clinic is now achievable. This is an exciting development. As Drosophila researchers, we have a unique perspective on the development of cancer drugs owing to our familiarity with genetics and our expertise doing in vivo research; nonetheless, this is another another asset that we sometimes fail to acknowledge or undervalue. This, in conjunction with state-of-the-art genetic approaches and the practical benefits of Drosophila, has the potential to pave the way for a new class of candidate medicines that may address the complexity of disease and contribute to the notion of personalized medicine.

Acknowledgments: Figures created with BioRender.com

Funding: N/A

References

1. Pandey UB, Nichols CD (2011) Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery. Pharmacol Rev 63:411–436

2. Sonoshita M, Cagan RL (2017) Modeling human cancers in Drosophila. Curr Top Dev Biol 121:287–309

3. Graham P, Pick L (2017) Drosophila as a model for diabetes and diseases of insulin resistance. Curr Top Dev Biol 121:397–419

4. McGurk L, Berson A, Bonini NM (2015) Drosophila as an in vivo model for human neurodegenerative disease. Genetics 201:377–402

5. Bhandari P, Shashidhara LS (2001) Studies on human colon cancer gene APC by targeted expression in Drosophila. Oncogene 20:6871–6880

6. Radimerski T, Montagne J, Hemmings-Mieszczak M, Thomas G (2002) Lethality of Drosophila lacking TSC tumor suppressor function rescued by reducing dS6K signaling. Genes Dev 16:2627–2632

7. Micchelli CA et al (2003) γ-Secretase/presenilin inhibitors for Alzheimer's disease phenocopy Notch mutations in Drosophila. FASEB J 17:79–81

8. 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 Res 65:3538–3541

9. Desai UA et al (2006) Biologically active molecules that reduce polyglutamine aggregation and toxicity. Hum Mol Genet 15:2114–2124

10. Chang S et al (2008) Identification of small molecules rescuing fragile X syndrome phenotypes in Drosophila. Nat Chem Biol 4:256–263

11. Bangi E, Garza D, Hild M (2011) In vivo analysis of compound activity and mechanism of action using epistasis in Drosophila. J Chem Biol 4:55–68

12. Jaklevic B et al (2006) Contribution of growth and cell cycle checkpoints to radiation survival in Drosophila. Genetics 174:1963–1972

13. Yadav AK, Srikrishna S, Gupta SC (2016) Cancer drug development using Drosophila as an in vivo tool: from bedside to bench and Back. Trends Pharmacol Sci 37:789–806

14. Strange K (2016) Drug discovery in fish, flies, and worms. ILAR J 57:133–143

15. Gladstone M, Su TT (2011) Chemical genetics and drug screening in Drosophila cancer models. J Genet Genomics 38:497–504

16. Markstein M (2013) Modeling colorectal cancer as a 3-dimensional disease in a dish: the case for drug screening using organoids, zebrafish, and fruit flies. Drug Discov Today Technol 10:e73–e81

17. Das T, Cagan R (2010) Drosophila as a novel therapeutic discovery tool for thyroid cancer. Thyroid 20:689–695

18. Das TK, Cagan RL (2013) A Drosophila approach to thyroid cancer therapeutics. Drug Discov Today Technol 10:e65–e71

19. Swinney DC (2013) Phenotypic vs. target-based drug discovery for first-in-class medicines. Clin Pharmacol Ther 93:299–301

20. Hoelder S, Clarke PA, Workman P (2012) Discovery of small molecule cancer drugs: successes, challenges and opportunities. Mol Oncol 6:155–176

21. Sams-Dodd F (2005) Target-based drug discovery: is something wrong? Drug Discov Today 10:139–147

22. Overington JP, Al-Lazikani B, Hopkins AL (2006) How many drug targets are there? Nat Rev Drug Discov 5:993–996

23. Capdeville R, Buchdunger E, Zimmermann J, Matter A (2002) Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. Nat Rev Drug Discov 1:493–502

24. Barker AJ et al (2001) Studies leading to the identification of ZD1839 (IRESSA): an orally active, selective epidermal growth factor receptor tyrosine kinase inhibitor targeted to the treatment of cancer. Bioorg Med Chem Lett 11:1911–1914

25. Swinney DC, Anthony J (2011) How were new medicines discovered? Nat Rev Drug Discov 10:507–519

26. Moffat JG, Rudolph J, Bailey D (2014) Phenotypic screening in cancer drug discovery – past, present and future. Nat Rev Drug Discov 13:588–602

27. Willoughby LF et al (2012) An in vivo large-scale chemical screening platform using Drosophila for anticancer drug discovery. Dis Model Mech 6:521–529

28. Markstein M et al (2014) Systematic screen of chemotherapeutics in Drosophila stem cell tumors. Proc Natl Acad Sci U S A 111:4530–4535

29. Levine BD, Cagan RL (2016) Drosophila lung cancer models identify trametinib plus statin as candidate therapeutic. Cell Rep 14:1477–1487

30. Levinson S, Cagan RL (2016) Drosophila cancer models identify functional differences between ret fusions. Cell Rep 16:3052–3061

31. Das TK, Esernio J, Cagan RL (2018) Restraining network response to targeted cancer therapies improves efficacy and reduces cellular resistance. Cancer Res 78:4344–4359

32. Das TK, Cagan RL (2017) KIF5B-RET oncoprotein signals through a multi-kinase signaling hub. Cell Rep 20:2368–2383

33. Bangi E, Murgia C, Teague AGS, Sansom OJ, Cagan RL (2016) Functional exploration of colorectal cancer genomes using Drosophila. Nat Commun 7:13615

34. Enomoto M, Siow C, Igaki T (2018) Drosophila as a cancer model. Adv Exp Med Biol 1076:173–194

35. Herranz H, Eichenlaub T, Cohen SM (2016) Cancer in Drosophila: imaginal discs as a model for epithelial tumor formation. Curr Top Dev Biol 116:181–199

36. Hou SX, Singh SR (2017) Stem-cell-based tumorigenesis in adult Drosophila. Curr Top Dev Biol 121:311–337

37. Garraway LA, Lander ES (2013) Lessons from the cancer genome. Cell 153:17–37

38. Biankin AV, Piantadosi S, Hollingsworth SJ (2015) Patient-centric trials for therapeutic development in precision oncology. Nature 526:361–370

39. Mendelsohn J (2013) Personalizing oncology: perspectives and prospects. J Clin Oncol 31:1904–1911

40. Simon R, Roychowdhury S (2013) Implementing personalized cancer genomics in clinical trials. Nat Rev Drug Discov 12:358–369

41. Nass SJ et al (2018) Accelerating anticancer drug development — opportunities and trade-offs. Nat Rev Clin Oncol 15:777–786

42. Wong CH (2017) Estimation of clinical trial success rates and related parameters

43. Rodon J, Dienstmann R, Serra V, Tabernero J (2013) Development of PI3K inhibitors: lessons learned from early clinical trials. Nat Rev Clin Oncol 10:143–153

44. Casaluce F et al (2017) Selumetinib for the treatment of non-small cell lung cancer. Expert Opin Investig Drugs 26:973–984

45. Infante JR et al (2012) Safety, pharmacokinetic, pharmacodynamic, and efficacy data for the oral MEK inhibitor trametinib: a phase 1 dose-escalation trial. Lancet Oncol 13:773–781

46. Borthakur G et al (2016) Activity of the oral mitogen- activated protein kinase kinase inhibitor trametinib in RAS-mutant relapsed or refractory myeloid malignancies. Cancer 122:1871–1879

47. Jänne PA et al (2013) Selumetinib plus docetaxel for KRAS-mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study. Lancet Oncol 14:38–47

48. Blumenschein GR Jr et al (2015) A randomized phase II study of the MEK1/MEK2 inhibitor trametinib (GSK1120212) compared with docetaxel in KRAS-mutant advanced non-small-cell lung cancer (NSCLC)†. Ann Oncol 26:894–901

49. Sonoshita M et al (2018) A whole-animal platform to advance a clinical kinase inhibitor into new disease space. Nat Chem Biol 14:291–298

50. Gleeson MP, Hersey A, Montanari D, Overington J (2011) Probing the links between in vitro potency, ADMET and physicochemical parameters. Nat Rev Drug Discov 10:197–208

51. Huggins DJ, Sherman W, Tidor B (2012) Rational approaches to improving selectivity in drug design. J Med Chem 55:1424–1444

52. Davis MI et al (2011) Comprehensive analysis of kinase inhibitor selectivity. Nat Biotechnol 29:1046–1051

53. Ciardiello F et al (2004) Antitumor activity of ZD6474, a vascular endothelial growth factor receptor tyrosine kinase inhibitor, in human cancer cells with acquired resistance to antiepidermal growth factor receptor therapy. Clin Cancer Res 10:784–793

54. Wedge SR et al (2002) ZD6474 inhibits vascular endothelial growth factor signaling, angiogenesis, and tumor growth following oral administration. Cancer Res 62:4645–4655

55. McCarty MF et al (2004) ZD6474, a vascular endothelial growth factor receptor tyrosine kinase inhibitor with additional activity against epidermal growth factor receptor tyrosine kinase, inhibits orthotopic growth and angiogenesis of gastric cancer. Mol Cancer Ther 3:1041–1048

56. Wells SA et al (2012) Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial. J Clin Oncol 30:134–141

57. Wong CH, Siah KW, Lo AW (2018) Estimation of clinical trial success rates and related parameters. Biostatistics 20(2):273–286. https://doi.org/10.1093/ biostatistics/kxx069

58. Massacesi C et al (2016) PI3K inhibitors as new cancer therapeutics: implications for clinical trial design. Onco Targets Ther 9:203–210

59. Guha R (2013) On exploring structure–activity relationships. Methods Mol Biol 993:81–94

60. Dar AC, Das TK, Shokat KM, Cagan RL (2012) Chemical genetic discovery of targets and anti-targets for cancer polypharmacology. Nature 486:80–84

61. Cagan R (2016) Drug screening using model systems: some basics. Dis Model Mech 9:1241–1244

62. Lonial S, Anderson KC (2014) Association of response endpoints with survival outcomes in multiple myeloma. Leukemia 28:258–268

63. Harvey AL, Edrada-Ebel R, Quinn RJ (2015) The re- emergence of natural products for drug discovery in the genomics era. Nat Rev Drug Discov 14:111–129

64. Li JW-H, Vederas JC (2009) Drug discovery and natural products: end of an era or an endless frontier? Science 325:161–165

65. Cha Y et al (2018) Drug repurposing from the perspective of pharmaceutical companies. Br J Pharmacol 175:168–180

66. Pushpakom S et al (2018) Drug repurposing: progress, challenges and recommendations. Nat Rev Drug Discov. https://doi.org/10.1038/nrd.2018.168

67. Breckenridge A, Jacob R (2019) Overcoming the legal and regulatory barriers to drug repurposing. Nat Rev Drug Discov 18:1–2

68. Garralda E, Dienstmann R, Tabernero J (2017) Pharmacokinetic/Pharmacodynamic modeling for drug development in oncology. Am Soc Clin Oncol Educ Book 37:210–215

69. Lavé T, Caruso A, Parrott N, Walz A (2016) Translational PK/PD modeling to increase probability of success in drug discovery and early development. Drug Discov Today Technol 21–22:27–34

70. Stricker-Krongrad A, Shoemake CR, Bouchard GF (2016) The miniature swine as a model in experimental and translational medicine. Toxicol Pathol 44:612–623

71. Lipton SA, Nordstedt C (2016) Partnering with big pharma—what academics need to know. Cell 165:512–515