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REVIEW ARTICLE

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Tiny *Drosophila* makes giant strides in cancer research

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Abstract

Cancer burden has been increasing worldwide, making cancer the second leading cause of death in the world. Over the past decades, various experimental models have provided important insights into the nature of cancer. Among them, the fruit fly *Drosophila* as a whole-animal toolkit has made a decisive contribution to our understanding of fundamental mechanisms of cancer development including loss of cell polarity. In recent years, scalable *Drosophila* platforms have proven useful also in developing anti-cancer regimens that are effective not only in mammalian models but also in patients. Here, we review studies using *Drosophila* as a tool to advance cancer study by complementing other traditional research systems.

KEYWORDS

anti-cancer drug, cancer, *Drosophila*, genetics, whole-body platform

Abbreviations: A5, a sorafenib analog APS5-16-2; A6, a sorafenib analog APS6-45; *btl*, *breathless*; CRC, colorectal cancer; $dEGFR^{\lambda}$, a constitutively active variant of *Drosophila* Egfr; DFG, Asp-Phe-Gly; DOHaD, Developmental Origins of Health and Disease; $dp110^{CAAX}$, a membrane-targeted active *Drosophila* p110; $dRet^{M955T}$, an active M955T isoform of *Drosophila* Ret; EMT, epithelial-mesenchymal transition; GBM, glioblastoma multiforme; GEMM, genetically engineered mouse model; *GMR*, *Glass multiple reporter*; HDS, high dietary sugar; KI, kinase inhibitor; *lgl*, *lethal giant larvae*; *M*, *Minute*; MTC, medullary thyroid cancer; NSCLC, non-small cell lung cancer; PTC, papillary thyroid cancer; *ptc*, *patched*; RNAi, RNA interference; RTK, receptor tyrosine kinase; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TC, thyroid cancer; TCGA, The Cancer Genome Atlas; UAS, Upstream Activation Sequence.

Ryodai Yamamura and Takako Ooshio authors contributed equally to this work.

1 | INTRODUCTION

Thus far, experimental models including cancer cells and genetically engineered mouse models (GEMMs) have made significant contributions to advance cancer research.[1,2] Recently, in addition to these 'traditional' platforms, novel animal models have attracted much attention for their capacity to promote the field, such as zebrafish, the nematode *Caenorhabditis elegans*, and the fruit fly *Drosophila*.

We are interested in introducing particularly *Drosophila* in this review because they offer several advantages complementary to other model organisms in cancer research. Firstly, flies have conserved genes and signaling pathways with humans. Specifically, more than 70% of human genes whose abnormalities cause diseases have a functional ortholog in flies.[3] Secondly, *Drosophila* offers a powerful genetic toolkit including gene-knockout and transgenic stocks. As we describe later, we can also generate flies with complex genotypes due to its advanced reverse genetics. Thirdly, *Drosophila* matures quickly and is highly reproductive without a need for massive lab equipment. For instance, their generation time is only 11-12 d at 25°C, and an adult female can lay 400-500 embryos within 10 d in laboratory vials.[4] These and other valuable features allow us to study fundamental interaction between genes, as well as distinct cells or tissues in not only developmental but also medical biology such as cancer modeling and drug discovery through complimentary use of *Drosophila* with mammals as we introduce below[4,5] (Figure 1).

2 | STUDYING CANCER BIOLOGY WITH FLIES

Cancer originates as a localized disease but it can affect the whole body also, therefore we need whole-body models to understand the mechanisms of its pathogenesis and to develop effective drugs with satisfactory therapeutic index. As one of such models, *Drosophila* has shown its value as genetic and pharmacological toolkits. Namely, their forward genetics allows phenotyping within or between tissues upon naturally occurring mutations while their reverse genetics enables modeling genetic alterations found in patients, which allows animal-level drug explorations for specific genotypes. In this section, we describe cancer biology and candidate therapeutics that *Drosophila* studies have revealed.

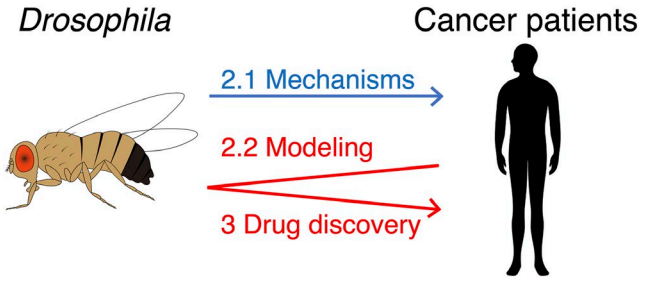


FIGURE 1 Elucidating tumorigenic mechanisms and developing new cancer treatments using *Drosophila*. Forward genetics revealed key tumorigenic mechanisms in *Drosophila* (blue arrow; Section 2.1). Conversely, reverse genetics generated various fly models mirroring patient genotypes (red arrow; Section 2.2). Following drug screening/derivatization in these models identified new therapeutic candidates (red arrow; Section 3)

2.1 | Elucidating cancer mechanisms with flies

Early studies in the 1930s identified mutant *Drosophila* for the gene *lethal giant larvae* (*lgl*) to manifest gross disorganization and hyperproliferation of larval tissues including the brain and imaginal discs.[6] Upon transplantation into wild-type hosts, *lgl* mutant cells invaded their surroundings to colonize.[6] Following genetic analyses in *Drosophila* pinpointed *dlg* and *scrib* to interact with *lgl* to regulate cell polarity whose loss occurs in ~80% of human cancers.[7] Convincingly, expression levels of their human orthologs are lower in several types of cancer compared with those in their normal counterparts.[4,8] These results collectively suggest functional conservation of *lgl*, *dlg*, and *scrib* as tumor suppressors across species.

Additionally, *Drosophila* studies uncovered a process 'cell competition' to eliminate cells with distinct characteristics. The first example came from flies carrying *Minute* (*M*), a mutant allele for a ribosomal gene. When genetic manipulation induced clones with *M* heterozygosity within wild-type wing discs consisting of epithelial monolayers, apoptosis eliminated these clones keeping wing size and shape normal.[9,10] Curiously, cancer-related genes also have key roles in cell competition. As a 'supercompetitor,' a cell overexpressing *Myc* kills surrounding wild-type cells in developing wings.[11,12] Similarly, supercompetition occurs due to a variety of genetic abnormalities in Hippo, WNT/Wg, and JAK-STAT pathways, suggesting a role for supercompetitor as a tumor seed.[13-15]

Conversely, cell competition has an anti-tumor role in different contexts. Namely, wild-type cells eliminate a small population of oncogenic cells lacking *lgl*, *dlg*, or *scrib*, or those harboring SRC activation.[16-20] Besides, some of *lgl* mutant alleles cause proliferation rather than cell death (eg, a cleaned-up allele of lgl^{4} or alleles of lgl^{27S3}, lgl^{E2S31}, lgl^{23S9}, or lgl^{E6S}),[21,22] indicating that *lgl* alleles cause distinct phenotypes. In addition to such genetic alterations, environmental factors also affect cell competition. For example, systemic hyperinsulinemia disturbs elimination of *scrib* mutant cells and promotes tumorigenesis in *Drosophila*.[23]

Like *Drosophila*, mammals also execute cell competition. For instance, a non-transformed epithelial monolayer in culture excludes apically a small population of cells expressing oncogenic *RAS* or *SRC*.[24,25] Also in mice, normal tissues eliminate cells with decreased *Myc* expression, or mutations in either a ribosomal protein, a cell polarity regulator, or Hippo pathway.[26-29] These pieces of evidence raise a fascinating possibility that cell competition works as an intrinsic mechanism preventing carcinogenesis.

2.2 | Fly models for specific cancer types

Reverse genetics has allowed establishing *Drosophila* modeling cancer genotypes. One of the oldest and simplest methods to induce

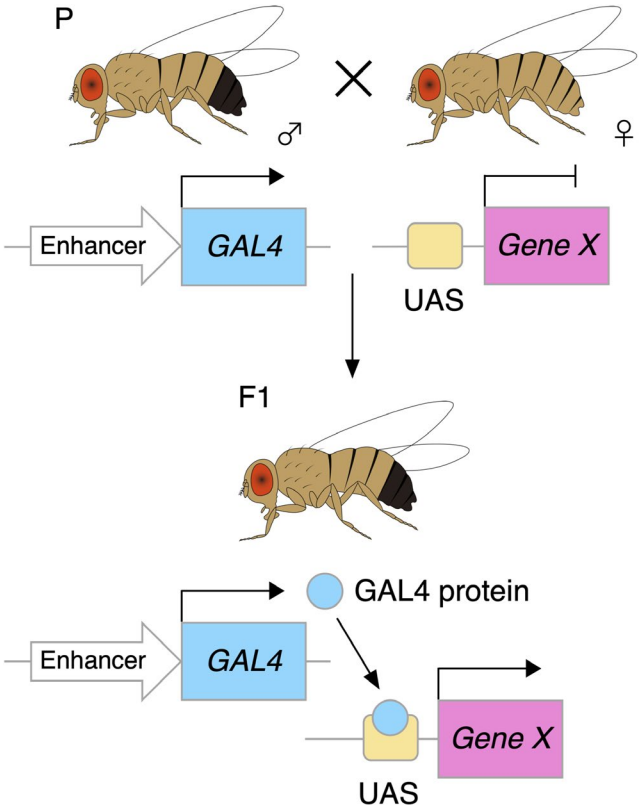


FIGURE 2 The GAL4/UAS system for regulating transgene in *Drosophila*. This system consists of 2 parts: the yeast transcription factor GAL4, driven by cell type-specific or tissue-specific enhancer/promoter, and its target *UAS*. Crossing parent flies carrying either *Enhancer-GAL4* or *UAS-X* produces F1 offspring with *X* induction specifically in cells or tissues of interest

transgene artificially is to utilize a heat shock promoter by putting transgenic flies in a warm incubator.[30] However, heat shock induces transgene throughout the body, which can cause developmental abnormality. Also, there exists leak of transgene expression even without heat shock.[31]

Complementing this tool, the GAL4/UAS system proved useful[31] (Figure 2). In principle, this method employs the yeast transcription factor GAL4 driven by cell type- or tissue-specific enhancer/promoter and its target *UAS* integrated in the fly genome to allow spatial and/or temporal transgene regulation.[31] These and other powerful genetic tools make *Drosophila* an accessible tool to accelerate cancer research as we introduce in this section and Table 1.

2.2.1 | Thyroid cancer models

The case number of TC is increasing dramatically worldwide. In the United States, for example, there is a prediction that TC becomes the fourth most common type of cancer by 2030 replacing colorectal cancer (CRC), making it one of the most pressing health challenges.[32] TC subtypes include papillary TC (PTC) and relatively rare medullary TC (MTC). An active form of cell surface RTK RET is responsible for 90% < of MTC cases, but drug discovery for MTC treatment has been slow largely due to the lack of an efficient research platform.

To tackle this issue, we generated transgenic *Drosophila* models for MTC by inducing in epithelial tissues including eyes and wing discs an active M955T isoform of *Drosophila* Ret (dRet^{M955T}) mimicking RET^{M918T} in MTC patients[33-36] (Table 1). They served to validate the lead chemical ZD6474 to generate vandetanib as the first targeted therapy for MTC.[4] Furthermore, they made intensive chemical genetic screening possible, successfully generating novel lead compounds with much improved efficacy over sorafenib, the Food and Drug Administration (FDA)-approved multikinase inhibitor drug[34] (described later).

In contrast to MTC, PTC accounts for ~85% of all TC cases.[37] PTC has subtypes with different genetic profiles for effectors in the RTK-MAPK pathway including oncogenic *RET* fusion genes in 30% of PTC patients.[38] Although RET inhibitors show efficacy in this population, they also cause severe toxicity.[39] Among *CCDC6-RET* and *NCOA4-RET* fusions identified, the latter causes in patients more severe pathogenesis with undetermined mechanisms and therapeutics.[40]

As with MTC, *Drosophila* became a powerful tool to tackle this cancer. Namely, flies expressing *CCDC6-RET* or *NCOA4-RET* driven by the *patched* (*ptc*) promoter displayed enhanced migration, delamination, and EMT of transformed cells.[38] In these fly models, the *ptc* promoter directs transgene expression in developing epithelia including wing, eye, and leg discs and other tissues.[33] Full kinome screening indicated that NCOA4-RET signaled through kinases including WEE1 distinct from CCDC6-RET. Targeting this NCOA4-RET–WEE1 network by combining sorafenib with the WEE1 inhibitor AZD1775 suppressed above phenotypes, raising a novel candidate therapy for *NCOA4-RET*–positive PTC[38] (Table 1).

2.2.2 | Colorectal cancer models

CRC has the third highest incidence of cancer in both genders globally, with ~1.8 million new cases and 880,000 deaths in 2018, making it as the cancer type with the second highest mortality rate.[41]

CRC harbors combinations of genetic abnormalities in *RAS* oncogenes (*KRAS*/*NRAS*/*HRAS*) and/or in tumor suppressor genes such as *APC*, *TP53*, *SMAD4*, and *LLGL1*.[42] To understand how such diversities affect CRC development, GEMMs for intestinal tumors have made pivotal contributions.[43] For example, we discovered CRC mechanisms including tumor-promoting PGE2-EP2 and NOTCH-ABL-TRIO-RHO pathways, as well as the invasion/metastasis-suppressing *Aes* gene to inhibit NOTCH signaling.[44-47]

Unfortunately, GEMMs with complex genotypes require enormous efforts to generate and maintain.[48] Here, *Drosophila* CRC models proved to be complementary to mammals in scrutinizing quickly the CRC complexity regarding disease mechanisms and drug responses. To model CRC genotypes in flies, Bangi et al employed *byn-GAL4* active in the hindgut corresponding to the human colon[49] (Figure 3) as well as patient genomic data from TCGA.[50] Active *ras*^{G12V} combined with RNA interference (RNAi) knockdown of tumor suppressors *p53*, *pten*, *apc*

TABLE 1 Models and findings in cancer studies using *Drosophila*

Cancer type		Patient genotype	*Drosophila* genotype	*Drosophila* phenotype	Therapeutic candidate	Description (Ref.)
Thyroid cancer (TC)	Medullary TC (MTC)	RET^{M918T}	$GMR\text{-}dRet^{M955T}$	'Rough eye' by cell proliferation	Vandetanib as the first targeted therapy for MTC	Section 2.2.1 [36]
			$ptc>dRet^{M955T}$	Proliferation and migration of transformed cells, and fly lethality	A novel kinase inhibitor (KI) drug lead AD80	Section 2.2.1 [33]
					A novel KI drug lead A6 (APS6-45) with improved therapeutic index in mouse xenografts of human MTC compared with its parent multikinase inhibitor drug sorafenib or cabozantinib, another FDA-approved KI drug for MTC	Section 3.1 [34]
	Papillary TC (PTC)	*CCDC6-RET* *NCOA4-RET*	*ptc>CCDC6-RET* *ptc>NCOA4-RET*	Cell migration, delamination and EMT inducing fly lethality, with *NCOA4-RET* causing more severe phenotypes than *CCDC6-RET*	A combination of sorafenib and the WEE1 inhibitor AZD1775	Section 2.2.1 [38]
Colorectal cancer (CRC)		$KRAS^{G12V}$, losses of *TP53*, *PTEN*, *APC*, and/or *SMAD4*	$byn>ras^{G12V},p53^{RNAi},pten^{RNAi},apc^{RNAi}$, *and/or* $smad4^{RNAi}$	Proliferation, EMT and/or distant metastasis of transformed cells in 32 multigenic flies, with $ras^{G12V},p53^{RNAi},pten^{RNAi},apc^{RNAi}$ causing the most severe phenotypes	A two-step therapy with the proteasome inhibitor bortezomib followed by the PI3K/mTOR inhibitor BEZ235	Section 2.2.2 [50]
		$KRAS^{G13A}$, biallelic losses of *APC*, *TP53*, and *FBXW7*, heterozygous mutations of *SMARCA4*, *FAT4*, *MAPK14*, and *CDH1* (a refractory CRC patient)	$byn>ras^{G12V},apc^{RNAi},p53^{RNAi},ago^{RNAi},put^{RNAi},brm^{RNAi},ft^{RNAi},p38a^{RNAi},shg^{RNAi}$	Expansion of the hindgut	A personalized combination therapy between the MEK inhibitor drug trametinib and the calcium metabolism modifier drug zoledronate being effective in the patient	Section 3.2 [73]
Non-small-cell lung cancer (NSCLC)		$KRAS^{G12V}$, loss of *PTEN*	$btl>ras^{G12V},pten^{RNAi}$	Tracheal tissue proliferation and fly lethality	A combination of trametinib and the HMG-CoA reductase inhibitor drug fluvastatin	Section 2.2.3 [55]
		KIF5B-RET	*ptc>KIF5B-RET*	Proliferation, invasion and EMT of transformed cells harboring invadopodium-like processes	A combination of sorafenib and the EGFR inhibitor drug erlotinib or the microtubule inhibitor drug paclitaxel	Section 2.2.3 [56]
Glioblastoma multiforme (GBM)		Activation mutation or amplification of *EGFR*, activation mutation of *PIK3CA*	$repo>dEGFR^{\lambda},dp110^{CAAX}$	Neoplastic, transplantable glial cells	mTOR, MYC, CCNG1-CDKs and RB-E2F pathways as candidate therapeutic targets	Section 2.2.4 [58]

Note: Genetic engineering has generated *Drosophila* stocks modeling human cancer genotypes, which accelerated identification of disease mechanisms and therapeutic candidates.

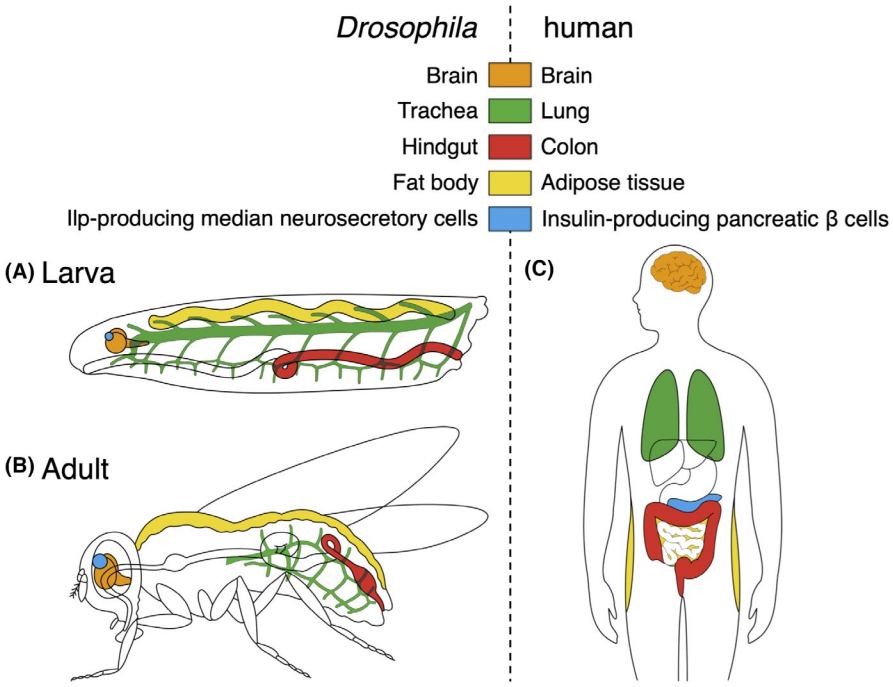


FIGURE 3 Tissue/organ similarity between *Drosophila* and human. Each color indicates functionally similar tissues/organs among *Drosophila* larva (A) and adult (B), and human (C). These similarities allow construction of fly models for human diseases including cancer affecting tissue/organ functions

and/or *smad4* recapitulated major CRC pathologies including cell proliferation, EMT and distant metastasis, with ras^{G12V},$p53^{RNAi}$,$pten^{RNAi}$,apc^{RNAi} causing the most severe phenotypes. Furthermore, each fly line showed distinct responses to anti-cancer reagents, highlighting the importance of personalized medicine based on patient genotypes.[50]

Also, the authors identified *ras* activation and *pten* loss to inhibit mTORC1 as a mechanism for CRC resistance to PI3K/mTOR inhibitors such as BEZ235. Based on this finding, they found significant suppression of cancer traits in cultured human CRC cells, their xenografts, and *Apc*;*Kras*;*Pten* allograft from a CRC GEMM by treating them first with the mTORC1 activator SC79 or bortezomib followed by BEZ235 (Table 1). This study provided a rapid, large-scale platform by combining flies with patient databases and mammalian models (also see Section 3.2).

In addition to exploring cancer complexity, *Drosophila* also serves as a quick platform to test hypotheses from epidemiological studies. Recently, a study demonstrated an association of social isolation with increased risk of cancer death, and indeed rats developed mammary tumors upon lifelong (≤ 18 mo) isolation.[51] Interestingly also in flies, social isolation accelerated progression of their gut tumors within 21 d,[52] highlighting their usefulness in studies on risk factors requiring long-term observation when using mammals.

2.2.3 | Lung cancer models

Globally, lung cancer has long had the highest mortality rate among all cancer types, with NSCLC accounting for 85% of all lung cancer diagnosis.[53] As the most commonly mutated oncogene in NSCLC, *KRAS* renders it resistance against adjuvant chemotherapy and EGFR inhibitors.[54]

In raising therapeutic candidates for this *KRAS*-positive NSCLC, *Drosophila* provided as a test tissue its tracheal system which develops similarly to the vertebrate lung (Figure 3). The *breathless* (*btl*)-*GAL4* targeted *Drosophila* ras^{G12V} misexpression and *pten* knockdown to the trachea causing tumor-like growths and lethality in early larval stages.[55] Following chemical screening for a library of 1192 FDA-approved drugs identified the MEK inhibitor drug trametinib and the HMG-CoA reductase inhibitor drug fluvastatin as candidates to generate a therapeutic cocktail. Indeed, they synergistically inhibited growth of A549 human NSCLC cells carrying active $KRAS^{G12S55}$ (Table 1).

Drosophila also helped to create novel therapeutic strategies for patients with the *KIF5B-RET* fusion oncogene, the most relevant fusion driver in NSCLC.[56] Namely, its product KIF5B-RET activated multiple RTKs including EGFR to offer vulnerabilities to target by combinations of sorafenib with erlotinib or paclitaxel as candidate therapies for *KIF5B-RET*-positive NSCLC, which awaits validation in patients (Table 1).

2.2.4 | Brain tumor models

Gliomas are the most common intracerebral tumors, with GBM as the most aggressive tumor with few effective therapies hence median patient survival being only 15 mo. Studies using GEMMs for GBM have revealed the mechanisms of its development and therapeutic resistance including EGFR-PI3K signaling, but generating novel therapeutic strategies have remained extremely difficult for decades.[57]

To overcome this situation, Read et al. established flies modeling GBM genotype by expressing activated isoforms of *Drosophila* Egfr (dEGFR$^{\lambda}$) and p110 (dp110^{CAAX}) using glia-specific *repo-GAL4*.[58] These transgenes induced glial proliferation, infiltration, and loss of cell polarity recapitulating human glioma to cause larval lethality.[58]

These phenotypes were dependent on TOR, MYC, CCNG1-CDKs and RB-E2F pathways, suggesting them as novel targets for GBM therapy (Table 1). Therefore, flies offer a feasible platform to clarify signaling networks in cancer development.

2.2.5 | Impact of metabolic disorders on cancers

Obesity and type 2 diabetes mellitus (T2DM) are representative metabolic disorders whose prevalence continues to rise worldwide.[59] Notably they are both risk factors established already for all cancer types, thus elucidating their pathogenesis can give clues for cancer prevention.[59]

To this end, mouse, rat, and *Drosophila* models for obesity and T2DM have played substantial roles. Especially, feeding flies with HDS can rapidly generate a dietary-induced obese/T2DM model.[60] Taking this advantage, Hirabayashi et al. unveiled that HDS enhanced tumor growth leading to emergent metastases in flies with increased RAS and SRC activity (ras^{G12V},$csk^{-/-}$).[61] Curiously, *WNT* ortholog *Wg* upregulated insulin receptors to avoid insulin resistance, boosting tumor growth. Therapeutically, the authors successfully delineated anti-tumor efficacy in these flies of a combination between 3 drugs; the T2DM drug acarbose, the WNT signaling inhibitor pyrvinium, and the RAS/SRC/mTOR signaling inhibitor AD81 of their own.[61,62]

In addition to the T2DM studies described in this section, *Drosophila* can help studies also on type 1 diabetes mellitus (T1DM) due to depletion of insulin-producing pancreatic β cells.[63] In flies, complete or partial ablation of insulin-like peptides causes elevated sugar concentrations in larval hemolymph to induce T1DM-like phenotypes, causing developmental delay[64] (Figure 3). To the best of our knowledge, no papers have examined association between T1DM and cancer using these flies. However, *Drosophila* can implement a valuable strategy to reveal causal relationships between cancers and these 2 DMs and even DOHaD, which all develop through complex interplay between genetic and acquired factors.[65]

3 | DRUG DISCOVERY WITH FLIES

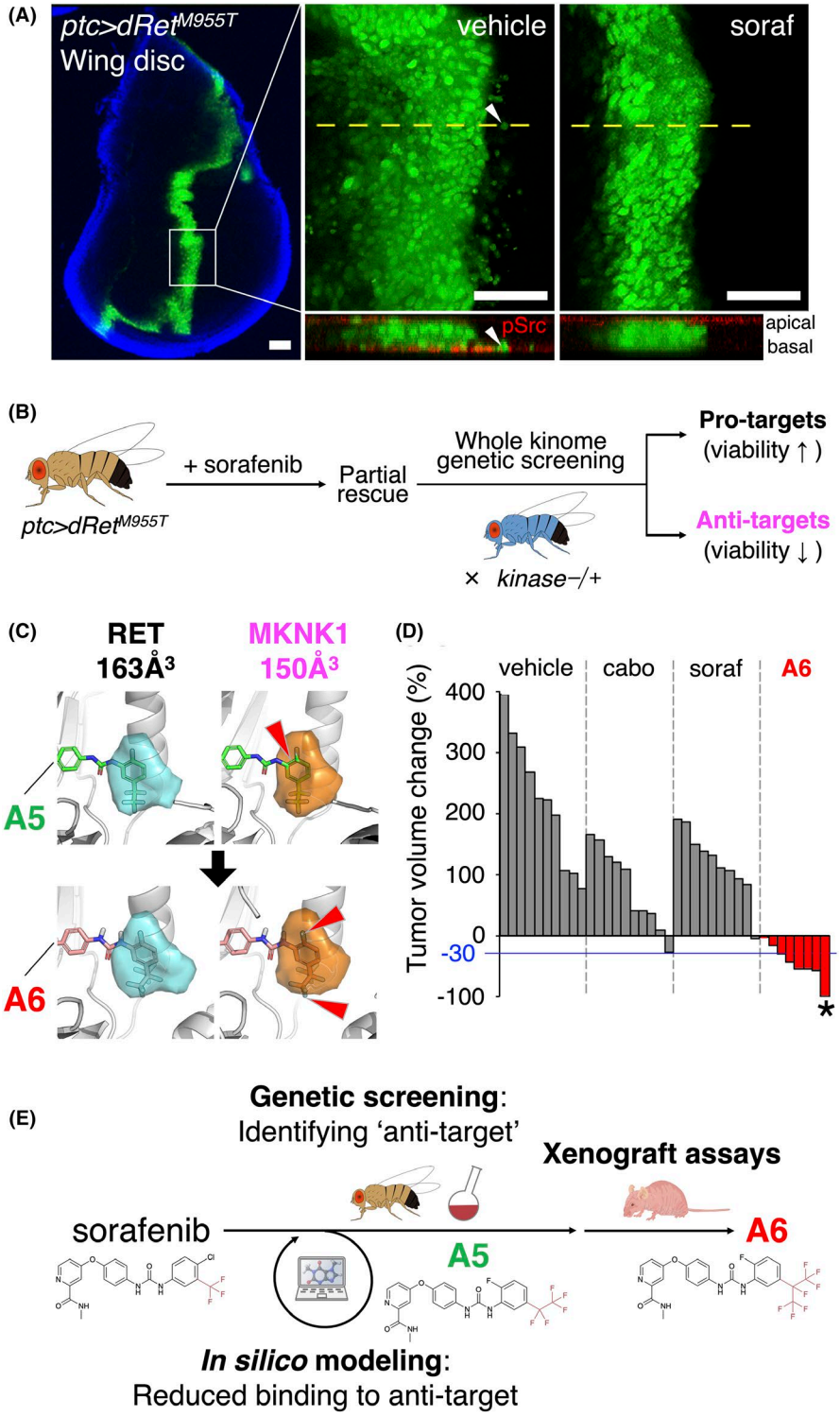
In this section, we introduce 2 studies using *Drosophila* as a platform for chemical biology combined with genetics and as a 'patient avatar' to develop novel therapeutics. Thus far, mammalian models have discovered cancer mechanisms as partly described above. However, in drug discovery, cultured cells cannot fully recapitulate all aspects of cancer development and therapy such as inter-cellular/organ interactions and pharmacokinetics. Also, GEMMs require enormous effort such as time and cost to evaluate novel therapeutic candidates. As a result, the success rate of clinical trials for cancer drug candidates has remained below 4%, demanding a paradigm shift in drug development pipeline.[66] Studies below demonstrate that *Drosophila* serves as a whole-animal platform to advance the field.

3.1 | Creating new drug leads through rational balancing of polypharmacology

To treat cancer, chemotherapy has been one of the primary options for decades. However, chemotherapy elicits significant toxicity such as bone marrow suppression in patients, which often hampers therapeutic protocols.

To overcome this issue, the idea of targeted therapy emerged 3 decades ago aiming to target molecules present primarily in cancer tissues to reduce systemic side effects.[67] Among such molecules, kinases turned out to be fascinating targets, because cancer genomes frequently carry their alterations that cause their deregulation hence cell transformation. Due to these and other reasons, generating KI drugs has been one of the most active areas in drug development.[68] Indeed, the US FDA has approved so far 70 < KIs including ABL inhibitor imatinib with tremendous effects in patients with ABL-positive chronic myeloid leukemia. Unfortunately, however, even approved KI drugs frequently cause unacceptable adverse effects in patients. For example, the multikinase inhibitor drug sorafenib has

FIGURE 4 A whole-animal platform to create novel drug leads through rational balancing of polypharmacology. A, In vivo cell migration assay using a *Drosophila* model for MTC. In these *ptc>dRet*^{M955T} flies, the *patched* (*ptc*) promoter drives expression of an M955T isoform of *Drosophila Ret* (*dRet*^{M955T}) modeling *RET*^{M918T} mutation in MTC patients. Left panel, a larval wing disc harboring GFP-labeled, *dRet*^{M955T}-expressing transformed cells among wild-type cells (black). Blue, DAPI staining outlining the disc margin. Middle top and right top, basal images in DMSO vehicle (middle top; inset box in left panel) or the KI drug sorafenib (soraf)-treated flies (right top; 400 μmol/L in fly food), respectively. Bottom, confocal z-stack images derived from the plane indicated by dotted lines in top panels. Arrowhead, example of basally migrating cell expressing phospho(p)-Src (red). Scale bars, 50 μm. B, Chemical genetic screening identified 'anti-target' kinases whose inhibition by sorafenib accounted for its toxicity. We introduced a heterozygous mutation of each kinase into *ptc>dRet*^{M955T} flies and fed sorafenib to their offspring. As a result, heterozygosity of 9 genes including a sorafenib target MKNK1 reduced significantly the efficacy of sorafenib decreasing fly viability. Besides, we identified 'pro-target' kinases whose heterozygous mutation enhanced the efficacy of sorafenib increasing fly viability. C, In silico prediction of a sorafenib derivative A6 (APS6-45) as a less potent binder compared with another derivative A5 (APS5-16-2) to MKNK1, an anti-target of sorafenib. Compared with sorafenib (not shown) and A5 (top), A6 with a larger modification group (-*iso*C_3F_7) has reduced binding capacity to the DFG pocket in MKNK1 due to steric clashes (arrowhead), although it can still bind to RET with larger pocket size compared with MKNK1. D, A6 suppresses human MTC xenografts. A 4-wk oral administration of A6 reduced the volume of existing tumors more effectively than its seed sorafenib (soraf) or cabozantinib (cabo), another standard of care for MTC treatment. Each bar represents tumor volume change in 1 mouse. Asterisk, complete remission. E, Stepwise evolution of sorafenib. First, we performed genetic screening in *Drosophila* for all of 252 kinases in the entire kinome in the presence of sorafenib to pinpoint MKNK1 as an anti-target of sorafenib. A sorafenib derivative A5 with predictable reduced binding capacity to MKNK1 showed higher efficacy than sorafenib in *ptc>dRet*^{M955T} flies. We further inflated A5 to generate A6, achieving reduced toxicity hence improved therapeutic index in human MTC xenografts



shown promise in patients with liver or kidney cancer or MTC as well as in a *Drosophila* MTC model *ptc>dRet*^{M955T} where the *ptc* promoter drives expression of *dRet*^{M955T} (Figure 4A), but sorafenib causes new tumors or even fatal toxicity in patients.[69]

Therefore, we intended to create an alternative approach to generate novel anti-cancer drugs: to improve an existing KI drug, we evolved it toward a unique network of kinase targets to lower its toxicity through complementary use of *Drosophila* with mammals. Picking MTC and sorafenib as models of cancer type and KI drug, respectively, first we performed chemical genetic screening in *ptc>dRet*^{M955T} flies for all of 252 kinases in the entire kinome in the presence of sorafenib. Introducing a heterozygous mutation of each kinase into *ptc>dRet*^{M955T} flies and feeding sorafenib to them discovered that heterozygosity of 9 genes significantly reduced the efficacy of sorafenib decreasing fly viability. Therefore, we defined these genes as 'anti-targets' of sorafenib whose inhibition by

sorafenib accounted for sorafenib toxicity (Figure 4B). Among these anti-targets, we especially focused on a sorafenib target MKNK1, as its inhibition diminished sorafenib efficacy completely and we successfully modeled in silico the interaction of its allosteric DFG pocket with sorafenib based on 'DFGmodel' platform that we had reported.[70]

In comparison of sorafenib binding to MKNK1 or RET, in silico modeling uncovered ~10% smaller size of the MKNK1 allosteric pocket compared with that in RET, indicating that 'inflated' sorafenib would cause steric clash with MKNK1. Convincingly, sorafenib derivatives A5 (APS5-16-2) and A6 (APS6-45) with enlarged warhead showed tremendously reduced toxicity hence improved efficacy as compared to parent drug sorafenib and cabozantinib, another KI drug for MTC, in human MTC xenografts[34,71] (Table 1, Figure 4C-E).

Collectively this work demonstrates a rational path for balancing polypharmacology in a drug, which enables a sophisticated attack on cellular networks, complementing targeted therapy to open up a new avenue for deriving novel cancer therapeutics. By leveraging this powerful multidisciplinary approach between *Drosophila*, computation, medicinal chemistry and mammals, we are now tackling cancer types for which drug discovery has been problematic, such as pancreatic cancer.

3.1.1 | Personalizing CRC therapy with flies

Cancer patients can benefit from a standard of care early in treatment, but cancers often acquire drug resistance, one of the biggest long-standing issues in treatment. Such resistance can establish through systemic changes including alterations in drug metabolism, genomic sequence of therapeutic targets and/or cancer traits affecting multiple organs, with CRC as an example to have a 5-y survival rate of only 13.3% despite intensive treatment.[72]

To solve this issue, Bangi et al generated a personalized *Drosophila* model for a CRC patient with chemotherapy-resistant liver and lung metastases.[73] Analyzing primary tumors and blood as control in the patient revealed 132 somatic and 965 rare germline variants. Of these, the authors focused on 9 gene alterations due to their potential relevance, including an oncogenic $KRAS^{G13A}$ mutation, biallelic losses of *APC*, *TP53* and *FBXW7*, and heterozygous mutations in *TGFBR2*, *SMARCA4*, *FAT4*, *MAPK14* and *CDH1*. Targeting these alterations to the hindgut epithelium by *byn-GAL4* caused its expansion similarly to their previous CRC model flies.[50]

Subsequent screening for 121 FDA-approved anti-cancer drugs in this model with fly lethality as a readout revealed efficacy of a combination between trametinib and the bisphosphonate class drug zoledronate. Notably, this combination significantly decreased volume of patient tumors by ~45% and controlled their growth for 11 mo[73] (Table 1). These results strongly suggested that this *Drosophila* approach provides a personalized treatment option for patients with refractory cancer.

4 | CONCLUSIONS AND PERSPECTIVES

We have described examples of *Drosophila* contributions to cancer research made thus far. Flies are particularly useful not only as a 'hypothesis-testing' tool but also as a quick and inexpensive 'hypothesis-building' tool by offering genetic and pharmacologic toolkits established during more than 100-y history.[4] In the past decades, there have been marked advances in technologies for cancer research including next-generation sequencing, in vivo imaging, CRISPR-Cas editing and omics analyses. Also, new ideas for treatment are emerging such as immune checkpoint inhibitor drug, sophisticated drug delivery systems, and gut microbiome which affects human diseases including various cancer types.[74,75] Combining these and other powerful modalities with *Drosophila* will accelerate further cancer research by offering a comprehensive framework for exploring the disease complexity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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