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; dEGFR1, a constitutively active variant of Drosophila Egfr; DFG, Asp-Phe-Gly; DOHaD, Developmental Origins of Health and Disease; dp110GTAAX, a membrane-targeted active Drosophila p110; dRetM955T, 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.

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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\(^5\)\(^6\) (Figure 1).

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.

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 hyper-proliferation 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 ‘super-competitor’, 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.\(^16\)\(^18\) Besides, some of lgl mutant alleles cause proliferation rather than cell death (eg, a cleaned-up allele of lgl\(^i\)) or alleles of lgl\(^{2753}\), lgl\(^{2531}\), lgl\(^{2339}\), or lgl\(^{665\\alpha} \text{YAMAMURA et A l.} \)\(^11\)\(^12\) 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
thyroid cancer (TC). 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<sup>M955T</sup>) mimicking RET<sup>M955T</sup> in MTC patients<sup>33-36</sup> (Table 1). They served to validate the lead chemical ZD6474 to generate vandetanib as the first targeted therapy for MTC.<sup>4</sup> 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<sup>34</sup> (described later).

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

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.<sup>38</sup> In these fly models, the ptc promoter directs transgene expression in developing epithelia including wing, eye, and leg discs and other tissues.<sup>33</sup> 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<sup>38</sup> (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.<sup>41</sup>

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.<sup>42</sup> To understand how such diversities affect CRC development, GEMMs for intestinal tumors have made pivotal contributions.<sup>43</sup> 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.<sup>44-47</sup>

Unfortunately, GEMMs with complex genotypes require enormous efforts to generate and maintain.<sup>48</sup> 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<sup>49</sup> (Figure 3) as well as patient genomic data from TCGA.<sup>50</sup> Active ras<sup>G12V</sup> 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)              | RET<sup>MF18T</sup> | GMR-dRet<sup>MF58T</sup> | 'Rough eye' by cell proliferation | Vandetanib as the first targeted therapy for MTC | Section 2.2.1 36 |
|                                  |                  | ptc>dRet<sup>MF58T</sup> | 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 (AP56-45) 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        | ptc>CCDC6-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 |
|                                  | NCOA4-RET        | ptc>NCOA4-RET         |                       |                       | |
| Colorectal cancer (CRC)          | KRA<sub>G12V</sub>, losses of TP53, PTEN, APC, and/or SMAD4 | byn>ras<sup>G12V</sup>, p53<sup>RNAi</sup>, pten<sup>RNAi</sup>, apc<sup>RNAi</sup>, and/or smad4<sup>RNAi</sup> | Proliferation, EMT and/or distant metastasis of transformed cells in 32 multigenic flies, with ras<sup>G12V</sup>, p53<sup>RNAi</sup>, pten<sup>RNAi</sup>, apc<sup>RNAi</sup> 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 |
|                                  | KRA<sub>G11A</sub>, biallelic losses of APC, TP53, and FBXW7, heterozygous mutations of SMARCA4, FAT4, MAPK14, and CDH1 (a refractory CRC patient) | byn>ras<sup>G12V</sup>, apc<sup>RNAi</sup>, p53<sup>RNAi</sup>, p38a<sup>RNAi</sup>, shg<sup>RNAi</sup> | Expansion of the hindgut | A personalized combination therapy between the MEK inhibitor drug trametinib and the calcium metabolism modifier drug zolendronate being effective in the patient | Section 2.2.2 73 |
| Non-small-cell lung cancer (NSCLC) | KRA<sub>G12V</sub>, loss of PTEN | btl>ras<sup>G12V</sup>, pten<sup>RNAi</sup> | 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<sup>dp110CAAX</sup> | 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.
and/or smad4 recapitulated major CRC pathologies including cell proliferation, EMT and distant metastasis, with \( \text{ras}^{G12V}, \text{p53RNAi}, \text{ptenRNAi}, \text{apcRNAi} \) 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.

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 \( \text{Apc};\text{Kras};\text{Pten} \) 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. Interestingly also in flies, social isolation accelerated progression of their gut tumors within 21 d, 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. As the most commonly mutated oncogene in NSCLC, KRAS renders it resistance against adjuvant chemotherapy and EGFR inhibitors.

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 \textit{breathless} (btl)-GAL4 targeted \textit{Drosophila} \( \text{ras}^{G12V} \) misexpression and pten knockdown to the trachea causing tumor-like growths and lethality in early larval stages. 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 \( \text{KRAS}^{G12S} \) (Table 1).

Drosophila also helped to create novel therapeutic strategies for patients with the \( \text{KIF5B-RET} \) fusion oncogene, the most relevant fusion driver in NSCLC. 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 \( \text{KIF5B-RET} \)-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.

To overcome this situation, Read et al. established flies modeling GBM genotype by expressing activated isoforms of \textit{Drosophila} \textit{Egfr} (\textit{dEGFR}^C) and \textit{p110} (\textit{dp110CAAX}) using glia-specific repo-GAL4. These transgenes induced glial proliferation, infiltration, and loss of cell polarity recapitulating human glioma to cause larval lethality.
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. Notably they are both risk factors established already for all cancer types, thus elucidating their pathogenesis can give clues for cancer prevention.

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. 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\textsuperscript{GLY34},csk\textsuperscript{−/−}). 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.

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 \( \beta \) cells. 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 (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.

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**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-\textsuperscript{dRet\textsuperscript{M955T}} flies, the patched ptc\textsuperscript{−} promoter drives expression of an M955T isoform of Drosophila Ret (dRet\textsuperscript{M955T}) modeling R\textsuperscript{E257E}R\textsuperscript{M955T} mutation in MTC patients. Left panel, a larval wing disc harboring GFP-labeled, dRet\textsuperscript{M955T}\textsuperscript{−}\textsuperscript{−} expression 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 μg/ml 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-\textsuperscript{dRet\textsuperscript{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 (\textsuperscript{-}isoC\textsubscript{6}C\textsubscript{2}F\textsubscript{2}) 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.

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### 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. Studies below demonstrate that Drosophila serves as a whole-animal platform to advance the field.

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#### 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. 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.

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...
shown promise in patients with liver or kidney cancer or MTC as well as in a Drosophila MTC model \( ptc\textgreater d\text{Ret}^{M955T} \) where the \( ptc \) promoter drives expression of \( d\text{Ret}^{M955T} \) (Figure 4A), but sorafenib causes new tumors or even fatal toxicity in patients.\(^6\)因此，我们尝试创造一种新方法来制造新型抗癌药物：通过改进已有的KI药物，我们朝着独特的激酶目标网络方向发展，以降低其毒性通过Drosophila与哺乳动物的互补使用。

Picking MTC and sorafenib as models of cancer type and KI drug, respectively, first we performed chemical genetic screening in \( ptc\textgreater d\text{Ret}^{M955T} \) flies for all of 252 kinases in the entire kinome in the presence of sorafenib. Introducing a heterozygous mutation of each kinase into \( ptc\textgreater d\text{Ret}^{M955T} \) 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 (APSS-16-2) and A6 (AP6-45) 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 xenografts34,71 (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 KRASG12A 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 mo73 (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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