7

CHAPTER 7

GENERAL DISCUSSION

Precision cancer medicine requires precision data. To deliver the right drug(s) to the right patient at the right time, precision oncology must integrate several layers of information describing both the tumor and the individual patient to guide treatment selection. This includes genomic data (e.g., presence of somatic or germline mutations, copy-number changes, and structural rearrangements), other multiomic tumor profiling information (e.g., transcriptomics, epigenomics, proteomics, metabolomics, immune composition), presence of biomarkers predictive of response or resistance, longitudinal clinical characteristics (e.g., disease stage, histology, comorbidities, prior treatment), and complementary imaging and pathology data. Patient context such as demographics, lifestyle and environmental factors further influence treatment feasibility and effectiveness. Ultimately, the promise of precision oncology is realized when these diverse data streams are systematically integrated over time, enabling evidence-based therapeutic decisions that continuously learn from outcomes and refine care for current and future patients with similar characteristics.

Although precision oncology depends on the integration of multiple complementary data streams, genomic profiling remains the cornerstone of its current clinical implementation. The identification of somatic and germline alterations that drive tumorigenesis and create therapeutic vulnerabilities has enabled patient stratification and the development of targeted therapies that have led to meaningful improvements in clinical outcomes. In some cases, these advances have been transformative. Chronic myeloid leukemia (CML), once a fatal disease, has become a manageable chronic condition following the introduction of the tyrosine kinase inhibitor (TKI) imatinib (which targets the BCR-ABL1 fusion oncoprotein that drives CML) as first-line therapy in 2002. In solid tumors, outcome improvements have generally been more modest but nonetheless clinically significant, particularly among patients harboring actionable alterations in genes such as *HER2*, *EGFR*, *ALK*, *RET* and *NTRK*, who benefit from matched targeted therapies [1–5]. Together, these examples illustrate how linking specific genomic alterations to mechanism-based treatments can fundamentally alter the treatment of cancer. Consistent with this, biomarker-guided therapy is one of the strongest predictors of improved clinical outcomes in oncology [6–9].

Despite these successes, and as discussed in **Chapter 1**, the overall benefit of targeted therapies across cancer types remains limited. Indeed, all new anticancer agents approved by the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) between 2003 and 2013 extended overall survival by an average of only 3.4 months [10]. The reasons underlying these limited gains are multifactorial [11–13]. However, a critical and increasingly evident challenge is the incomplete functional understanding of the numerous genetic alterations identified through routine tumor genomic profiling, many of which remain classified as variants of unknown significance (VUS). Determining whether such variants represent biologically relevant tumor-driving events or neutral alterations

with no impact on tumorigenesis or therapeutic response is essential, particularly in known cancer genes for which targeted therapies already exist. Addressing this knowledge gap is crucial to improving patient stratification and enabling more accurate and personalized treatment allocation.

1. DECODING ONCOGENIC SIGNALING AND THERAPY RESISTANCE: INSIGHTS FROM C-TERMINAL FGFR2/3 TRUNCATIONS AND MYC-DRIVEN MTOR INHIBITOR RESISTANCE

Genomic alterations in the *FGFR* family in cancer

As introduced in **Chapter 2** [14], genomic alterations affecting the FGFR family (FGFR1-FGFR4) are recurrent across many cancer types. In fact, up to 7.1% of all human cancers harbor alterations in the *FGFR* genes [15–19]. As tumor sequencing technologies and real-world genomic datasets expand, new *FGFR* alterations are continuously uncovered across multiple cancer types. However, the precise functional roles and therapeutic implications of many of these alterations remain unknown. It is crucial to define which *FGFR* alterations represent genuine oncogenic drivers with therapeutic relevance and which ones do not. While gene amplifications and hotspot mutations have long been recognized as oncogenic drivers, only recent oncogenomic and functional studies (including those presented in this thesis) have uncovered previously underappreciated *FGFR* rearrangements as a distinct and clinically relevant class of tumor-driving alterations that can dictate therapeutic response to FGFR inhibitors.

C-terminal truncation of *FGFR2*: an oncogenic driver and predictive biomarker

In **Chapter 3** [20], a Sleeping Beauty transposon-based insertional mutagenesis screen identified loss of exon 18 (E18) of *Fgfr2* (*Fgfr2*$^{\Delta E18}$) – which encodes its C-terminal tail – as a driver alteration in mammary tumorigenesis in mice. Prompted by these findings, we systematically interrogated large-scale human oncogenomic datasets from the Hartwig Medical Foundation (HMF; >2,000 whole-genome sequencing (WGS) profiles with a subset of matched RNA-seq data) and Foundation Medicine (FMI; >249,000 targeted sequencing profiles) to determine the prevalence and spectrum of *FGFR2* alterations. Our analyses uncovered that human tumors express diverse *FGFR2*$^{\Delta E18}$ transcripts arising from multiple distinct alternative splicing events and genomic alterations. These include *FGFR2*-E1-E17 partial amplifications, canonical in-frame fusions with 3'-partner genes, non-canonical rearrangements, E18 splice-acceptor-site mutations and E18-truncating mutations. Notably, almost half of all alterations potentially perturbing E18 in the FMI cohort (44.6%, n = 610) were initially classified as VUS, with the remainder consisting of in-frame gene fusions.

We demonstrated that E18 truncation of *Fgfr2* constitutes a bona fide tumor driver alteration that is targetable by FGFR inhibitors by functionally interrogating *Fgfr2*$^{\Delta E18}$ variants using *in vitro* assays (i.e., cell outgrowth, downstream signaling, 3D colony formation, proteomics, phosphoproteomics and FGFR inhibitor sensitivity assays) together with somatic *in vivo* tumor modeling of mammary glands in mice. Importantly, we showed that this oncogenic activity is independent of fusion partners. To assess the clinical relevance of these findings, we re-analyzed the phase II FIGHT-202 clinical trial of the FGFR inhibitor pemigatinib in patients with advanced cholangiocarcinoma, which revealed that objective responses are largely restricted to tumors harboring fusions or rearrangements affecting the *FGFR*-I17/E18 hotspot region, with an objective response rate of 35.5% compared with patients harboring other or no *FGF/FGFR* alterations [21,22]. Further stratification showed that 80-100% of patients with *FGFR2* in-frame fusions or other non-canonical rearrangements achieved objective response or durable stable disease, irrespective of rearrangement type or *FGFR2* amplification status. These data suggest that *FGFR2* fusions and non-canonical rearrangements that involve E18 are predictive biomarkers for response to the FGFR inhibitor pemigatinib and, conceivably, for other FGFR-targeted therapies. Future studies in larger patient cohorts should confirm that alterations involving E18 reliably predict benefit from FGFR inhibitors across different cancer types.

Together, our findings establish C-terminal truncation of *FGFR2* as a recurrent, fusion partner-independent tumor driving alteration that is sensitive to FGFR inhibition. In doing so, we can re-classify many of the *FGFR2*-E18 alterations previously considered VUS as clinically actionable driver events, thereby expanding the population of patients who may benefit from FGFR-targeted therapies. Future clinical trials evaluating FGFR inhibitors should therefore include all patients with E18-truncating alterations, not only those with known hotspot mutations or known oncogenic E18-truncating fusions. More broadly, our work underscores the importance of functional characterization of somatic variants in cancer driver genes to guide patient selection for targeted therapies.

FGFR2 amplification within a co-driver landscape

One unexpected observation from our *in vivo* mammary tumorigenesis studies in **Chapter 3** [20] was that expression of full-length *Fgfr2* (*Fgfr2*^{FL}) – which mirrors *FGFR2* amplification (*FGFR2*^{amp}) in human cancers – showed surprisingly limited oncogenic competence. This is surprising given that *FGFR2* amplification has long been regarded as a tumor-driving event. Our findings revealed that its oncogenic potential depends on additional co-driver alterations that are tissue-specific. We demonstrated that *Fgfr2*^{FL} requires cooperative genetic co-alterations such as *Trp53* or *Pten* loss, or overexpression of *Myc*, *Ccnd1*, or *Fgf3* to promote tumorigenesis in the mammary gland of mice, consistent with the enrichment of these alterations in human breast cancers harboring *FGFR2*^{amp}. Such context dependence might also explain why *FGFR2*-amplified tumors show limited objective responses to FGFR inhibitors in clinical trials [23–25]. If tumor growth is sustained by additional oncogenic

drivers, inhibition of the FGFR signaling axis alone is unlikely to achieve durable control. In this setting, combinatorial therapeutic strategies are likely to yield more effective clinical outcomes. This is particularly relevant in advanced malignancies, which frequently harbor multiple driver events, with an average of 4-5 identifiable alterations in a single tumor [26,27].

In the context of *FGFR2*^{amp} tumors, combination therapy may not only improve response rates but also overcome resistance mechanisms that emerge under selective therapeutic pressure. For instance, it has been shown that *CCND1* amplification confers resistance to FGFR inhibitors [28], and early clinical data indicate that concurrent FGFR and CDK4/6 inhibition yields superior responses compared with monotherapy [29,30]. Moreover, FGFR inhibitors exhibit strong synergy with agents targeting PI3K [31–35] or EGFR/AXL [36]. Accordingly, ongoing clinical trials are evaluating FGFR-targeted therapies in combination with PI3K and CDK4/6 inhibitors, immune checkpoint inhibitors, chemotherapy and DNA damage response agents. Importantly, the potential additive or overlapping toxicities of these combinations must be carefully considered, as increased treatment-related adverse effects may limit dose intensity and long-term tolerability. One potential strategy to mitigate this challenge is the exploration of multiple low-dose combination regimens, which aim to maintain pathway suppression while reducing toxicity burdens [37]. Moving forward, a deeper mechanistic understanding of co-occurring driver alterations, resistance pathways, and treatment-associated toxicities will be essential to rationally design biomarker-guided combination therapies that maximize efficacy while maintaining an acceptable safety profile.

In contrast to *Fgfr2*^{FL}, *Fgfr2*$^{\Delta E18}$ acts as a potent oncogene capable of driving tumorigenesis independently of cooperating alterations. Consistent with this, *FGFR2*$^{\Delta E18}$ alterations in human cancers are largely mutually exclusive with *TP53* hotspot mutations, *PTEN* loss-of-function, and *MYC*, *CCND1*, or *FGF3/4/19* amplifications [20,38,39].

The oncogenicity of *FGFR2*^{amp} may be influenced not only by accompanying co-driver alterations but also by its capacity to generate *FGFR2*$^{\Delta E18}$ transcripts. Alternative splicing of *FGFR2* that generates shorter C-terminal isoforms is well documented and particularly linked to breast and gastric cancers [40–43]. Furthermore, cell lines that express the shorter C-terminal transcripts show more transforming potential [20,40,43].

FGFR3: a different story

FGFR2 and FGFR3 are two members of the FGFR family that share a highly conserved overall architecture and many key residues and functional domains, including the kinase catalytic core and docking sites for adaptor proteins [44]. We identified recurrent *FGFR3* breakpoints in both the FMI and HMF oncogenomic datasets that also result in C-terminal truncation. Given their structural and functional similarities, we reasoned that these *FGFR3* alterations

might mirror the oncogenic mechanism observed for *FGFR2* C-terminal truncations. We therefore hypothesized that loss of the FGFR3 C-terminal tail (encoded by E18) would be sufficient to confer oncogenic activity. However, functional annotation of *FGFR3* alterations in **Chapter 5** [45] revealed a distinctly different biology. In contrast to FGFR2, C-terminal truncation alone is insufficient to drive transformation. Instead, FGFR3 requires both C-terminal truncation and fusion to a partner gene that retains the expression of a functional dimerizing domain to effectively drive oncogenic signaling and tumorigenesis.

Nevertheless, not all *FGFR3* E18-truncating alterations occur in the context of a fusion with a dimerizing partner. In the FMI cohort analyzed in **Chapter 5** [45], E18-truncating mutations accounted for 12.3% (98/799) of all alterations predicted to generate a C-terminally truncated *FGFR3* lacking a fusion partner. Such an elevated percentage raises the question of whether C-terminally truncated *FGFR3* can confer oncogenic activity in combination with specific co-driver alterations. Our findings suggest that this might be the case. Tumors harboring *FGFR3*$^{\Delta E18}$ alterations devoid of a partner gene (i.e., E18-truncating mutations, intergenic or internal rearrangements, or partial amplifications) were enriched for alterations in *KRAS*, *ERBB2*, *MYC*, *PIK3CA* and *PTEN*, compared to tumors containing *FGFR3*$^{\Delta E18}$ fusions with a dimerizing partner. This supports a model in which, in the absence of a dimerizing partner, C-terminally truncated *FGFR3* may require additional tumor co-drivers to achieve full transforming potential.

C-terminal tail-mediated autoinhibition of FGFR2 versus FGFR3

RTK signaling output is tightly controlled by autoinhibition [46]. Cells have evolved multiple regulatory mechanisms to prevent hyperactive FGFR signaling, and several studies have hinted at the importance of the C-terminal region in fine-tuning receptor activity [47,48]. Autoinhibitory functions have also been attributed to the C-terminal segments of other RTKs, highlighting a broader regulatory principle across the RTK superfamily. For example, the EGFR C-terminal tail stabilizes an unphosphorylated kinase domain dimer [49], whereas the MET C-tail limits kinase function by restricting ATP access to the active site [50], thereby suppressing receptor signaling. Consistent with this regulatory role, truncation of the C-terminal regions of EGFR, MET, HER2 and other RTKs promotes pro-tumorigenic activities [51–54].

In the FGFRs, phosphorylation of multiple tyrosine and serine residues within the C-terminal tail by downstream signaling components establishes negative feedback loops that promote receptor endocytosis and attenuate FGFR signaling [55–58]. Moreover, the distal portion of the C-terminal tail of FGFRs interacts with the inhibitory adaptor protein GRB2 [47,59–61], while its proximal part contains the clathrin-mediated endocytosis motif [47,62]. Together, these features position the FGFR C-terminal tail as a key negative regulatory module that restrains excessive receptor activation.

The divergence in the functional consequences of C-terminal truncation of *FGFR2* versus *FGFR3* raises the question of whether the C-terminal tails of these paralogs exert different autoinhibitory effects on their respective kinase domains. Loss of the FGFR2 C-terminal tail represents a critical determinant of FGFR2 oncogenicity (**Chapter 3** [20]) indicating that, under normal conditions, the intact tail functions to suppress excessive signaling. In **Chapter 4** [63], we systematically dissected the FGFR2 C-terminal tail to identify the precise residues required for this suppressive function. Our findings revealed a previously unannotated phenylalanine-serine motif located in the proximal region of E18 to be essential for binding of the C-tail to the kinase domain and for restraining FGFR2 kinase activity. Accordingly, we termed these two residues the 'kinase domain-binding and suppression' (KDBS) motif. Functional *in vitro* and *in vivo* efforts demonstrated that the C-terminal tail suppresses FGFR2 oncogenicity through this KDBS motif and the concurrent regulation of endocytosis and the PI3K/ERK negative-feedback loop. Loss of either the entire C-tail or the KDBS motif activates PI3K-AKT-mTOR and STAT3 signaling and promotes transformation.

Notably, FGFR3 is the only member of the FGFR family that does not fully conserve the KDBS motif. Instead of a phenylalanine-serine, FGFR3 contains a phenylalanine-alanine sequence. This serine-to-alanine change could potentially diminish the capacity of the FGFR3 C-terminal tail to exert autoinhibitory control over the kinase domain. The replacement of serine with alanine removes the polar hydroxyl group that could otherwise stabilize hydrogen bonds with the kinase domain, reduces favorable polar interactions that contribute to binding affinity, and may alter the local conformation or flexibility of the proximal C-terminal segment. In addition, the absence of a serine residue eliminates a potential phosphorylation site, thereby removing an extra layer of post-translational regulation. Collectively, these changes might weaken tail-kinase interactions and attenuate C-terminal tail-mediated suppression of receptor activity in FGFR3. As a result, FGFR3 likely operates under comparatively lower baseline C-terminal-mediated autoinhibition than FGFR2, such that truncation or removal of the FGFR3 C-terminal tail does not produce a substantial increase in signaling output. Without the release of a strong inhibitory constraint, FGFR3$^{\Delta E18}$ alone would be insufficient to drive oncogenic transformation and instead require an additional activating mechanism, such as fusion to a partner that promotes receptor dimerization, or other co-driver alterations (**Chapter 5** [45]).

Collectively, our findings from **Chapters 3-5** [20,45,63] underscore the importance of dissecting the biology of each FGFR paralog individually, rather than extrapolating functional or clinical implications of similar alterations across the receptor family. Our work demonstrates that even subtle structural differences between FGFR2 and FGFR3 can dictate distinct mechanisms of oncogenic activation. In addition, cellular and tissue context may further modulate the functional and therapeutic consequences of a given *FGFR* alteration.

Although C-terminal truncations of *FGFR2* and *FGFR3* occur across multiple cancer types, their distribution is not uniform, with certain tumor types showing relative enrichment. For instance, C-terminal truncating *FGFR2* alterations are more frequent in cholangiocarcinoma, gastroesophageal and breast cancers (**Chapter 3** [20]), whereas *FGFR3* alterations are more prevalent in bladder, glioblastoma and lung cancers (**Chapter 5** [45]). Together, these observations emphasize that the experimental models used to investigate *FGFR*-driven oncogenic mechanisms and therapeutic vulnerabilities should take tumor (sub)type into account, as the tissue of origin could shape the biological implications of *FGFR* alterations. More broadly, it remains to be investigated whether other RTKs beyond the FGFR paralogs contain phenylalanine-serine KDBS motifs and, if so, whether these motifs play a role in the autoinhibitory regulation of their kinase activity.

FGFR3$^{\Delta E18}$ fusions beyond *TACC3*

In **Chapter 5** [45], we observed a strong predominance of *TACC3* as the fusion partner in *FGFR3* E18-truncating (*FGFR3$^{\Delta E18}$*) rearrangements, accounting for 85.3% of the 799 tumors analyzed in the FMI cohort. All other rearrangements were rare, with intergenic space rearrangements comprising the second largest category (3.3% of all cases) and partner genes other than *TACC3* occurring in fewer than 2% of cases. Although *FGFR3$^{\Delta E18}$-TACC3* fusions were first reported as oncogenic drivers in glioblastoma in 2012 [64], to our knowledge we were the first to demonstrate their causal role in mammary tumorigenesis through *in vivo* somatic modeling in both wild-type and *Wap-Cre;Cdh1$^{F/F}$* mice, a model of invasive lobular carcinoma (ILC). Mechanistically, we showed that transformation depends strictly on FGFR3 kinase activity, while the contribution of TACC3 is largely structural: its coiled-coil domains provide the FGFR3$^{\Delta E18}$-TACC3 fusion protein with dimerization capacity necessary to promote constitutive receptor activation, whereas its canonical mitotic functions are dispensable. Consistent with this mechanism, tumors driven by *Fgfr3$^{\Delta E18}$-Tacc3* remain sensitive to pharmacologic FGFR inhibition, with tumor growth significantly suppressed by the ATP-competitive FGFR1-3 inhibitor fexagratinib [65], supporting FGFR-directed therapies as a rational treatment strategy for cancers harboring *FGFR3$^{\Delta E18}$-TACC3* fusions. Importantly, expression of the truncated receptor alone is insufficient to drive tumor formation in the mouse mammary gland or lung, indicating that both C-terminal tail loss and a dimerizing partner are required for oncogenicity.

This behavior contrasts sharply with *FGFR2*, for which C-terminal truncation alone is sufficient to confer oncogenic potential and does not rely on a specific fusion partner (**Chapter 3** [20]). Indeed, more than a hundred distinct *FGFR2$^{\Delta E18}$* fusion partners have been identified across tumor types, consistent with a mode of activation that is independent of fusion partner identity [19,20,66] (**Chapter 2** [14]). The striking predominance of *TACC3* among *FGFR3$^{\Delta E18}$* rearrangements, together with the rarity of alternative partners, raises the possibility that some of these low-frequency fusions may represent passenger events rather than bona fide oncogenic drivers. With the exception of *FGFR3$^{\Delta E18}$-BAIAP2L1*

[67], most of these variants remain functionally uncharacterized and their therapeutic relevance is therefore uncertain. To address this gap, in **Chapter 5** [45] we evaluated three previously untested, low-frequency *FGFR3*$^{\Delta E18}$ fusions identified in the FMI and HMF cohorts, namely *FGFR3*$^{\Delta E18}$*-ADD1* (6 cases), *FGFR3*$^{\Delta E18}$*-TNIP2* (11 cases) and *FGFR3*$^{\Delta E18}$*-JAKMIP1* (4 cases). In all 21 cases, the fusion partners retained predicted self-interacting domains. All three FGFR3 fusions proved oncogenic *in vitro*, activating downstream signaling in a kinase-dependent manner and conferring resistance to HER2/EGFR TKIs, yet remaining sensitive to pharmacologic FGFR blockade. This preserved susceptibility to FGFR inhibition underscores a potential therapeutic opportunity for patients harboring these rare *FGFR3* fusions. *In vivo*, however, their tumorigenic capacity diverged. Whereas *Fgfr3*$^{\Delta E18}$*-Jakmip1* functioned as a robust tumor driver in the mouse mammary gland, *Fgfr3*$^{\Delta E18}$*-Tnip2* produced an intermediate phenotype and *Fgfr3*$^{\Delta E18}$*-Add1* failed to induce tumors. One possible explanation for the differences observed between our *in vitro* versus *in vivo* findings is tissue specificity, as *FGFR3*$^{\Delta E18}$*-ADD1* and *FGFR3*$^{\Delta E18}$*-TNIP2* fusions are mainly observed in urothelial carcinomas, whereas our functional modeling was performed in a breast cancer setting. Future research should investigate the *in vivo* oncogenicity of these fusions in the relevant tissue, i.e., urothelium, to more accurately define their oncogenic potential and their therapeutic relevance.

Taken together, our findings broaden the spectrum of actionable *FGFR3*$^{\Delta E18}$ rearrangements beyond *FGFR3*$^{\Delta E18\text{-}TACC3}$ fusions. Although each of these alternative fusions occurs at very low frequency, grouping them according to shared structural features – namely C-terminal truncation of FGFR3 coupled with retention of a functional dimerization domain in the partner – rather than by the specific identity of the partner gene, substantially enlarges the pool of potentially targetable cases. This stratification suggests that more patients than previously appreciated might benefit from FGFR-targeted therapies (discussed more in detail in **Chapter 2** [14]). In clinical practice, however, the rarity of *FGFR3*$^{\Delta E18}$ gene fusions beyond *TACC3* renders conventional, histology-restricted trials largely impractical. Basket trials, which enroll patients based on the presence of a shared molecular alteration irrespective of tumor type, may provide a more feasible and efficient framework to evaluate therapeutic responses to FGFR-targeted therapies in this subgroup [68]. Such biomarker-driven strategies have already transformed precision oncology, leading to tissue-agnostic approvals of targeted therapies, including inhibitors directed against TRK and RET fusions, BRAF^{V600E}, and pembrolizumab for microsatellite instability-high (MSI-H) or tumor mutation burden-high (TMB-H) malignancies, following evidence of clinical benefit across diverse cancer types [3,69–74].

Overcoming treatment resistance

Resistance to targeted therapies remains one of the main limitations of precision oncology and a major cause of treatment failure and poor patient survival [9,10,75,76] (**Chapter 1**). Because tumors often harbor heterogeneous and complex genomic alterations, particularly

in advanced disease stages, monotherapies directed against a single driver rarely achieve durable disease control, since cancer cell plasticity and clonal diversity foster rapid selection and expansion of resistant clones. Resistance may be intrinsic, already present at diagnosis in the form of drug-resistant subclones, or it may emerge as a consequence of therapy. Acquired resistance is therapy-driven: although tumors may initially respond to treatment, they subsequently adapt under therapeutic pressure. This adaptation commonly involves target reactivation (e.g., on-target mutations or gene amplification), bypass signaling pathway activation, alterations in downstream effectors, or non-genetic mechanisms such as phenotypic switching, epigenetic remodeling, or remodeling of the tumor microenvironment [77]. Intratumoral heterogeneity further complicates therapeutic response, as distinct subclones may harbor different resistance mechanisms.

In the context of reversible ATP-competitive small-molecule FGFR inhibitors, acquired resistance most frequently involves secondary mutations in the gatekeeper or molecular brake residues within the FGFR kinase domain (FGFR1-FGFR3), although pathway reactivation via the upregulation or mutation of alternative RTKs or downstream signaling components is also commonly observed (**Chapter 2** [14]). Consistent with these mechanisms, our *in vivo* drug intervention study using the FGFR inhibitor fexagratinib revealed incomplete eradication of *Fgfr3*$^{\Delta E18}$*-Tacc3*-driven tumors (**Chapter 5** [45]). A subset showed reactivation of FGFR3 downstream signaling during treatment outgrowth, whereas others appeared to develop FGFR3-independent resistance, potentially reflecting tumor cell plasticity or stromal adaptations [78–80].

Beyond FGFR-targeted therapy, alterations in the FGF/FGFR signaling axis have been implicated in resistance to chemotherapy, radiotherapy and other targeted agents, including CDK4/6 inhibitors, endocrine therapy and HER2-directed treatments [28,81–85]. Consistently, we demonstrated that expression of *Fgfr3*$^{\Delta E18}$*-Tacc3* and the rare *Fgfr3*$^{\Delta E18}$*-Add1*, *Fgfr3*$^{\Delta E18}$*-Tnip2* and *Fgfr3*$^{\Delta E18}$*-Jakmip1* fusions conferred marked resistance to the dual HER2/EGFR TKIs afatinib and dacomitinib in human bladder cancer cells, underscoring the capacity of oncogenic *FGFR3* rearrangements not only to act as primary drivers but also to reshape therapeutic vulnerabilities across different treatment modalities. Notably, these fusions retained sensitivity to pharmacologic FGFR inhibition, highlighting a potential opportunity for rational combination therapy. In fact, the use of rational combination strategies represents one of the most established approaches to overcome acquired resistance and improve clinical outcomes in precision oncology. This can be achieved through vertical pathway inhibition by targeting multiple nodes within the same signaling cascade to prevent downstream reactivation, as exemplified by combined BRAF and MEK inhibition in melanoma [86], or through horizontal blockade of compensatory bypass pathways, such as the combination of EGFR and MET inhibition in EGFR-mutant non-small cell lung cancer [87].

A context in which vertical pathway co-targeting may be particularly effective is the simultaneous inhibition of mTOR and MYC in breast cancer. MYC functions as a master regulator of multiple cellular processes, acting predominantly as a transcription factor that either directly or indirectly regulates the expression of thousands of genes [88]. It is deregulated in up to 70% of human cancers, and its activation contributes to the acquisition of most, if not all, hallmarks of cancer [89]. In **Chapter 6** [90], we used a transplantable mouse model of ILC, a breast cancer subtype characterized by frequent activation of the PI3K-AKT-mTOR signaling axis, to study the resistance mechanisms acquired during prolonged *in vivo* treatment with the mTOR inhibitor (mTORi) AZD8055. Multiomic analyses of matched therapy-naïve and AZD8055-resistant ILC tumors revealed that MYC activation represents a central feature of mTORi resistance. MYC coordinated both tumor cell-intrinsic and -extrinsic processes involved in response to mTOR inhibition, including the compensation of suppressed protein translation by mTORi. Orthotopic modeling of mouse ILC tumors and human breast cancer-derived xenografts further demonstrated that MYC is an *in vivo* driver of mTORi resistance. Importantly, while our experimental models established MYC as an acquired resistance mechanism, complementary analyses of human cancer cell lines and patient data showed that *MYC* amplification and elevated MYC expression prior to treatment correlated with poor response to mTORi. These observations indicate that MYC contributes not only to acquired resistance but also to intrinsic resistance. Collectively, this work highlights the potential of MYC not only as a therapeutic target but also as a predictive biomarker for patient stratification before treatment initiation.

From a therapeutic perspective, MYC co-targeting represents a rational strategy to overcome mTORi resistance and, potentially, resistance to other anticancer therapies, given that *MYC* amplification occurs across multiple cancer types and has been associated with reduced therapeutic sensitivity, underscoring its broad relevance in treatment resistance. Consistent with this rationale, in **Chapter 6** [90], pharmacologic MYC inhibition using the preclinical compound KJ-Pyr-9 restored sensitivity of *Myc*-amplified, mTOR inhibitor-resistant KEP tumor-derived cells to AZD8055, providing proof-of-concept for this combinatorial approach.

Despite its compelling rationale as a target, MYC has long been considered 'undruggable' due to its intrinsically disordered structure and lack of an enzymatic binding pocket, which complicate traditional small-molecule development. To date, no direct MYC inhibitors have been approved for clinical use. Nonetheless, several MYC-targeting compounds have entered early-phase clinical evaluation, and an expanding array of direct and indirect strategies is currently under active investigation [91,92]. Overall, there is growing optimism that effective pharmacologic targeting of this 'undruggable' target may be feasible after all [92]. The successful development of clinically effective MYC-directed therapies could have transformative implications across tumor types, including in combination strategies designed to prevent or overcome mTORi resistance.

Notably, rational combination therapy is only one of several approaches aimed at overcoming—or at least delaying—the emergence of therapeutic resistance. For instance, in the setting of resistance to reversible ATP-competitive FGFR inhibitors driven by kinase domain mutations in FGFR2, sequential treatment with covalent FGFR inhibitors has been shown to improve clinical outcomes [93]. These observations highlight the importance of developing more selective next-generation inhibitors as well as alternative FGFR-targeting modalities (**Chapter 2** [14]). In parallel, circulating tumor DNA (ctDNA) analysis offers a non-invasive strategy to detect emerging resistance mutations in real time, enabling dynamic treatment adjustments [94–96]. Furthermore, multiomic analysis of resistant tumors can uncover novel therapeutic vulnerabilities. Translating such discoveries into clinical benefit, however, requires sustained efforts in functional validation and therapeutic development.

Taken together, the findings presented in this thesis reinforce the notion that the central challenge of precision oncology lies not only in identifying actionable driver alterations, but also in anticipating resistance mechanisms that may pre-exist or arise during treatment. A deeper understanding of both intrinsic and acquired resistance is essential to extend the duration of therapeutic responses and, ultimately, to improve patient outcomes. Achieving this will require systematic functional validation in appropriate experimental models to distinguish causal resistance drivers from passenger events and to inform rational therapeutic strategies.

2. ADVANCING PRECISION ONCOLOGY BY INTEGRATING LARGE-SCALE GENOMICS, COMPUTATIONAL MODELING AND FUNCTIONAL ANNOTATION

The power of numbers and WGS

The identification of rare but therapeutically relevant alterations, such as the non-canonical *FGFR* rearrangements described in this thesis, underscores the need for large-scale sequencing efforts to reliably uncover low-frequency yet biologically meaningful variants that might otherwise remain overlooked. Rare driver mutations, resistance mechanisms, and complex structural variants often require extremely large cohorts—in some cases numbering in the hundreds of thousands of tumors—to reveal recurrent patterns indicative of functional relevance and to achieve statistical robustness. Major international initiatives, including The Cancer Genome Atlas (TCGA), the International Cancer Genome Consortium (ICGC), the Pan-Cancer Analysis of the Whole Genomes (PCAWG) and the 100,000 Genomes Project (100kGP), among others, have profoundly reshaped our understanding of cancer as a genetically driven disease [27,97–100]. In particular, the application of WGS within these programs has expanded the focus beyond protein-coding regions toward genome-wide

complexity, including structural variation, non-coding alterations, and mutational processes.

Routine clinical diagnostics continue to rely primarily on targeted gene panels or whole exome sequencing (WES), which efficiently detect single-nucleotide variants and small insertions or deletions within predefined cancer genes and capture most currently actionable biomarkers. However, these approaches provide limited resolution of structural rearrangements, copy-number alterations, mutational signatures, and alterations in non-coding regions that comprise 98% of the genome. In contrast, WGS enables unbiased detection across all variant classes, offering a more comprehensive molecular characterization in one single test [27,97,99–101]. Although its widespread clinical adoption remains limited by cost, analytical complexity and challenges in interpreting VUS, the use of WGS is steadily increasing in specific clinical contexts, particularly pediatric cancers, rare tumors, and cancers of unknown primary [102,103]. Of note, although short-read sequencing remains the predominant technology in clinical practice, it may fail to fully resolve complex structural rearrangements, including many *FGFR* fusions and other C-terminal truncations. The limited read length can hinder accurate reconstruction of breakpoint-spanning events and complex genomic alterations such as gene fusions. In contrast, the integration of long-read sequencing technologies could provide greater resolution of such rearrangements, allowing precise delineation of fusion breakpoints and overall structural architecture, and enabling more accurate variant annotation [104–106].

The added value of integrating genomic and transcriptomic profiling

The combined application of whole-genome and transcriptome sequencing (WGTS) has the potential to even further improve diagnostic precision by complementing unbiased DNA-level variant detection with RNA-based assessment of gene expression. This integrated approach has been shown to improve therapeutic stratification and facilitate identification of new biomarkers [103,107–113]. The added value of transcriptome profiling is particularly evident for gene rearrangements, for which WGS alone may not conclusively resolve reading-frame status or transcript structure. This was exemplified in our own analyses in **Chapters 3** [20] and **5** [45]. In **Chapter 5** [45], integration of matched RNA-seq with WGS data from the HMF cohort revealed that 85.7% (12/14) of cases initially classified as "frame-unknown" *FGFR3* E18-truncating rearrangements based on DNA sequencing alone expressed detectable in-frame rearrangement transcripts, underscoring the importance of integrating DNA- and RNA-based sequencing to reliably identify oncogenic rearrangements.

WGTS has the potential to ultimately replace many conventional molecular diagnostic approaches, although its widespread integration into routine oncology practice will require healthcare systems to address key operational challenges. These include

standardized procedures for sample collection and biobanking, robust analytical pipelines, clear frameworks for data interpretation and reporting, and aligned regulatory and reimbursement landscapes [103]. Ultimately, the implementation of either WGS or WGTS into routine clinical care will depend on demonstrable cost-effectiveness. Although sequencing costs have declined considerably over the past decade, comprehensive genomic and transcriptomic profiling remains expensive in clinical settings [103,109,114–116]. Nevertheless, broader adoption may prove economically justified in the long term if these approaches provide the most comprehensive analysis of tumor alterations resulting in improved diagnostic accuracy, reduced multiple redundant testing and more precise therapeutic stratification.

Knowledge resources and data infrastructure in precision oncology

The vast amounts of genomic data generated by next-generation sequencing (NGS) technologies are of limited value without robust computational and statistical methods that enable the extraction of meaningful biological insights from raw sequencing data. Over the past 25 years, numerous computational tools have been developed to annotate both the human genome in general [117] and cancer genomes in particular [118]. In oncology, large-scale consortia such as the ICGC/TCGA PCAWG project have driven the establishment of standardized bioinformatics pipelines that now serve as reference workflows for tumor genomic analyses [27,119]. In parallel, open-access repositories and visualization platforms, such as TCGA, the Genomic Data Commons (GDC), cBioPortal and the UCSC Genome Browser, have markedly accelerated translational cancer research by facilitating large-scale data sharing and integrative omics analyses [118]. Additonally, these resources underpin the development of expert-curated 'knowledge bases' that aggregate and annotate the clinical significance of somatic variants to support their interpretation in the clinical setting. Prominent examples include ClinVar [120], COSMIC [121], CIViC [122] and OncoKB [123,124].

The ultimate goal is to comprehensively integrate genomic alterations with functional evidence and real-world clinical data within unified frameworks that support outcome prediction. Such integration would not only facilitate more informed, tailored treatment decisions by oncologists but also contribute to a learning healthcare system in which insights derived from past and current patients continuously refine care for future patients [102]. One representative example of progress towards this vision is Project GENIE (Genomics Evidence Neoplasia Information Exchange), an international data-sharing initiative led by the American Association for Cancer Research (AACR) [125,126]. To date, this publicly accessible clinico-genomic registry has linked harmonized NGS data with demographic, diagnostic, treatment, and longitudinal outcome information from more than 220,000 patients treated across 20 leading cancer centers worldwide. GENIE has underscored the critical importance of national and international data sharing, collaborative research infrastructures, and secure, large-scale and unified frameworks that enable cross-border access to genomic and clinical patient data [127].

Artificial intelligence in precision oncology

As molecular and clinical datasets continue to expand in scale and complexity, more powerful analytical tools are required. Optimized therapy will likely depend on rational combination therapies that target tumors from different angles, further increasing the analytical demands on precision oncology. In this context, the incorporation of machine learning and artificial intelligence (AI) into diagnostic and decision-support workflows becomes increasingly necessary [128,129]. AI-based approaches are particularly well suited for handling large, multimodal data and identifying subtle patterns and interactions that may not be spotted by conventional methods or the expert human eye [130–134]. Consequently, these approaches hold considerable promise for generating clinically meaningful outcome predictions.

At the same time, methodological rigor is required to ensure generalizability, interpretability and clinical reliability. Algorithm optimization, external validation, and prospective evaluation remain indispensable prerequisites for clinical implementation [128]. Moreover, the integration of AI into precision oncology must proceed transparently and responsibly, with careful consideration of ethical challenges including data privacy and security, informed consent, management of incidental findings, responsible data sharing, and the prevention of genetic data misuse [118,129].

Functional validation as the gold standard

Despite major advances in computational modeling, machine learning and AI, experimental functional characterization remains the gold standard for establishing the biological and clinical relevance of genomic alterations. *In silico* prediction frameworks can provide important speed, scalability, prioritization, and hypothesis-generating capacity, but they cannot substitute for direct experimental evidence. For instance, Tangermann *et al.* [135], who performed saturation mutational scanning of all 11,520 possible kinase domain point mutations across *FGFR1-4* to systematically identify activating and FGFR inhibitor resistance-mediating variants, demonstrated that widely used *in silico* prediction tools such as REVEL, EVE and AlphaMissense failed to reliably classify activating gain-of-function mutations. Moreover, the algorithms could not distinguish between gain- and loss-of-function effects. Tumor recurrence in COSMIC was likewise insufficient as a surrogate for activation. In another example, Ng *et al.* [136] developed a moderate-throughput functional genomic platform and used it to annotate more than a 1,000 VUS—including gene amplifications, point mutations, indels and gene fusions—potentially doubling the number of driver mutations characterized in clinically actionable genes. When the authors compared their experimental annotations against 21 commonly used algorithms that predict mutation effects, their accuracy was variable (50-76%) and none fully recapitulated the experimental classification. Together, these findings further illustrate that computational predictions cannot substitute for direct functional validation to establish causality.

Some machine learning-based methodologies have been described to outperform selected experimental efforts, such as the *in silico* saturation mutagenesis framework boostDM and its capacity to score potential driver mutations in certain cancer genes such as *TP53* or *EGFR* based on patterns derived from tumor sequencing datasets [137]. However, the performance of boostDM is inherently dependent on the availability of extensive, gene-specific training data that are not available for many targets. For example, the boostDM framework provides coverage for *FGFR3* based on mutational data from bladder cancer, but offers no predictions for *FGFR1*, *FGFR2* or *FGFR4*. Thus, even advanced AI-driven approaches remain constrained by data availability and generalizability, and cannot yet replace systematic experimental interrogation.

In this thesis, computational analyses played an essential role in interrogating diverse large-scale datasets derived from cancer patients, mouse models and *in vitro* experimental systems to prioritize candidate alterations. However, rigorous functional *in vitro* and *in vivo* interrogation was required to conclusively demonstrate that (*i*) C-terminal truncation of *FGFR2* is a strong oncogenic driver in cancer (**Chapter 3** [20]); (*ii*) this oncogenic activity is mechanistically driven by loss of the KDBS motif (**Chapter 4** [63]); (*iii*) the oncogenic potential of FGFR3 requires both C-terminal truncation and fusion to a partner with a conserved dimerizing capacity (**Chapter 5** [45]); and (*iv*) MYC activation drives resistance to mTOR inhibitors in breast cancer (**Chapter 6** [90]). Therefore, while computational approaches are indispensable for navigating molecular complexity at scale, experimental validation remains essential to distinguish between causation and mere correlation, and to define true drivers of oncogenicity and therapy resistance.

3. CONCLUSIONS

The work presented in this thesis underscores that systematic functional characterization is essential for translating genomic findings into clinically actionable biomarkers and enabling more precise patient selection for targeted therapies. By demonstrating the divergent oncogenic behavior of the FGFR2 and FGFR3 paralogs following C-terminal truncation, this work provides a strong rationale for expanding FGFR-targeted treatment strategies to patients harboring E18-truncating *FGFR2* alterations or *FGFR3* fusions involving dimerizing partner genes. In parallel, the identification of MYC as a clinically relevant driver of resistance to mTOR inhibition in breast cancer further refines patient stratification, highlighting high MYC expression as a potential biomarker to identify patients less likely to benefit from mTOR-targeted therapy. Together, these findings illustrate how functional interrogation can refine therapeutic stratification and support more informed therapeutic decisions in precision oncology.

Despite remarkable advances over the past two decades, precision oncology remains a developing field. The fact that driver alterations in approximately 5% of cancers remain unidentified [27] underscores that our understanding of tumor biology is still incomplete and that important discoveries remain ahead. Moving forward, precision oncology must extend beyond genomics alone, since genomic alterations represent only the first layer of tumor complexity. Integrated pan-omic approaches combining genomic, transcriptomic, proteomic, metabolomic and immunogenomic data will be essential to better define the full complexity of individual tumors. At the same time, rational biomarker-guided combination strategies should increasingly replace one-size-fits-all monotherapies, particularly as cancers adapt under therapeutic pressure and develop resistance.

Ultimately, translating molecular insight into sustained clinical benefit will require the coordinated integration of large-scale omics datasets, rigorous functional validation, prospectively collected real-world clinical outcome data, and advanced computational and AI-enabled analytical frameworks. Only through this truly multidimensional and translational ecosystem, coupled with continued innovation in drug discovery and development, can genomic insights be reliably transformed into tangible, durable improvements in patient outcomes.

REFERENCES

1. Piccart-Gebhart, M.J. *et al.* (2005) Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med* 353, 1659–1672
2. Soria, J.-C. *et al.* (2018) Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. *N Engl J Med* 378, 113–125
3. Drilon, A. *et al.* (2018) Efficacy of Larotrectinib in TRK Fusion-Positive Cancers in Adults and Children. *N Engl J Med* 378, 731–739
4. Drilon, A. *et al.* (2020) Efficacy of Selpercatinib in RET Fusion-Positive Non-Small-Cell Lung Cancer. *N Engl J Med* 383, 813–824
5. Shaw, A.T. *et al.* (2020) First-Line Lorlatinib or Crizotinib in Advanced ALK-Positive Lung Cancer. *N Engl J Med* 383, 2018–2029
6. Jardim, D.L. *et al.* (2015) Impact of a Biomarker-Based Strategy on Oncology Drug Development: A Meta-analysis of Clinical Trials Leading to FDA Approval. *J Natl Cancer Inst* 107, djv253
7. Schwaederle, M. *et al.* (2015) Impact of Precision Medicine in Diverse Cancers: A Meta-Analysis of Phase II Clinical Trials. *J Clin Oncol* 33, 3817–3825
8. Schwaederle, M. *et al.* (2016) Precision Oncology: The UC San Diego Moores Cancer Center PREDICT Experience. *Mol Cancer Ther* 15, 743–752
9. Schwaederle, M. *et al.* (2016) Association of Biomarker-Based Treatment Strategies With Response Rates and Progression-Free Survival in Refractory Malignant Neoplasms: A Meta-analysis. *JAMA Oncol* 2, 1452–1459
10. Salas-Vega, S. *et al.* (2017) Assessment of Overall Survival, Quality of Life, and Safety Benefits Associated With New Cancer Medicines. *JAMA Oncol* 3, 382–390
11. Prasad, V. *et al.* (2015) The Strength of Association Between Surrogate End Points and Survival in Oncology: A Systematic Review of Trial-Level Meta-analyses. *JAMA Intern Med* 175, 1389–1398
12. Subbiah, V. and Kurzrock, R. (2018) Challenging Standard-of-Care Paradigms in the Precision Oncology Era. *Trends Cancer* 4, 101–109
13. Wahida, A. *et al.* (2023) The coming decade in precision oncology: six riddles. *Nat Rev Cancer* 23, 43–54
14. Yemelyanenko, J. *et al.* (2026) FGFR rearrangements: oncogenic drivers and therapeutic targets. *Trends in Cancer* DOI: 10.1016/j.trecan.2026.01.010
15. Helsten, T. *et al.* (2016) The FGFR landscape in cancer: Analysis of 4,853 tumors by next-generation sequencing. *Clinical Cancer Research* 22, 259–267
16. Krook, M.A. *et al.* (2020) Pan-cancer analysis of FGFR1-3 genomic alterations to reveal a complex molecular landscape. *JCO* 38, 3620–3620
17. Zuo, W. *et al.* (2020) Landscape of *FGF/FGFR* Alterations in 12,372 Chinese Cancer Patients. *Journal of Cancer* 11, 6695–6699
18. Sun, Y. *et al.* (2020) A comprehensive pan-cancer study of fibroblast growth factor receptor aberrations in Chinese cancer patients. *Annals of Translational Medicine* 8, 1290–1290
19. Murugesan, K. *et al.* (2022) Pan-tumor landscape of fibroblast growth factor receptor 1-4 genomic alterations. *ESMO Open* 7, 100641
20. Zingg, D. *et al.* (2022) Truncated FGFR2 is a clinically actionable oncogene in multiple cancers. *Nature* 608, 609–617
21. Abou-Alfa, G.K. *et al.* (2020) Pemigatinib for previously treated, locally advanced or metastatic cholangiocarcinoma: a multicentre, open-label, phase 2 study. *Lancet Oncol* 21, 671–684
22. Rodón, J. *et al.* (2024) Pemigatinib in previously treated solid tumors with activating FGFR1–FGFR3 alterations: phase 2 FIGHT-207 basket trial. *Nat Med* DOI: 10.1038/s41591-024-02934-7

23. Pearson, A. *et al.* (2016) High-Level Clonal FGFR Amplification and Response to FGFR Inhibition in a Translational Clinical Trial. *Cancer Discov* 6, 838–851
24. Van Cutsem, E. *et al.* (2017) A randomized, open-label study of the efficacy and safety of AZD4547 monotherapy versus paclitaxel for the treatment of advanced gastric adenocarcinoma with FGFR2 polysomy or gene amplification. *Ann Oncol* 28, 1316–1324
25. Chae, Y.K. *et al.* (2020) Phase II Study of AZD4547 in Patients With Tumors Harboring Aberrations in the FGFR Pathway: Results From the NCI-MATCH Trial (EAY131) Subprotocol W. *J Clin Oncol* 38, 2407–2417
26. Martincorena, I. *et al.* (2017) Universal Patterns of Selection in Cancer and Somatic Tissues. *Cell* 171, 1029-1041.e21
27. Aaltonen, L.A. *et al.* (2020) Pan-cancer analysis of whole genomes. *Nature* 578, 82–93
28. Formisano, L. *et al.* (2019) Aberrant FGFR signaling mediates resistance to CDK4/6 inhibitors in ER+ breast cancer. *Nat Commun* 10, 1373
29. Uehara, Y. *et al.* (2022) Targeting the FGF/FGFR axis and its co-alteration allies. *ESMO Open* 7, 100647
30. Liu, K. *et al.* (2025) Significant Response to Palbociclib Plus Lenvatinib as Second-line Treatment for CDKN2A/2B Deletion Intrahepatic Cholangiocarcinoma: A Case Report. *J Clin Transl Hepatol* 13, 169–172
31. Packer, L.M. *et al.* (2017) PI3K Inhibitors Synergize with FGFR Inhibitors to Enhance Antitumor Responses in FGFR2mutant Endometrial Cancers. *Mol Cancer Ther* 16, 637–648
32. Holzhauser, S. *et al.* (2019) In vitro antitumor effects of FGFR and PI3K inhibitors on human papillomavirus positive and negative tonsillar and base of tongue cancer cell lines. *Oncol Lett* 18, 6249–6260
33. Holzhauser, S. *et al.* (2020) Targeting Fibroblast Growth Factor Receptor (FGFR) and Phosphoinositide 3-kinase (PI3K) Signaling Pathways in Medulloblastoma Cell Lines. *Anticancer Research* 40, 53–66
34. Lukoseviciute, M. *et al.* (2024) Combined targeted therapy with PI3K and CDK4/6, or FGFR inhibitors show synergistic effects in a neuroblastoma spheroid culture model. *Biomed Pharmacother* 177, 116993
35. Juric, D. *et al.* (2025) PI3Kα inhibitor and degrader inavolisib can co-opt FGFR2 to enhance responses in patients with PIK3CA-mutated solid tumors and in preclinical models. *Clin Cancer Res* DOI: 10.1158/1078-0432.CCR-25-1459
36. Nakamura, R. *et al.* (2024) Triple combination therapy comprising osimertinib, an AXL inhibitor, and an FGFR inhibitor improves the efficacy of EGFR-mutated non-small cell lung cancer. *Cancer Letters* 598, 217124
37. Fernandes Neto, J.M. *et al.* (2020) Multiple low dose therapy as an effective strategy to treat EGFR inhibitor-resistant NSCLC tumours. *Nat Commun* 11, 3157
38. Silverman, I.M. *et al.* (2021) Clinicogenomic Analysis of FGFR2-Rearranged Cholangiocarcinoma Identifies Correlates of Response and Mechanisms of Resistance to Pemigatinib. *Cancer Discov* 11, 326–339
39. D'Souza, N. *et al.* (2024) Comutational patterns in FGFR3-altered non-muscle invasive bladder cancer influence recurrence and progression. *Urologic Oncology: Seminars and Original Investigations* 42, S55
40. Itoh, H. *et al.* (1994) Preferential alternative splicing in cancer generates a K-sam messenger RNA with higher transforming activity. *Cancer Res* 54, 3237–3241
41. Ueda, T. *et al.* (1999) Deletion of the carboxyl-terminal exons of K-sam/FGFR2 by short homology-mediated recombination, generating preferential expression of specific messenger RNAs. *Cancer Res* 59, 6080–6086

42. Tannheimer, S.L. *et al.* (2000) Characterization of fibroblast growth factor receptor 2 overexpression in the human breast cancer cell line SUM-52PE. *Breast Cancer Res* 2, 311–320
43. Moffa, A.B. *et al.* (2004) Transforming Potential of Alternatively Spliced Variants of Fibroblast Growth Factor Receptor 2 in Human Mammary Epithelial Cells1. *Mol Cancer Res* 2, 643–652
44. Dai, S. *et al.* (2019) Fibroblast Growth Factor Receptors (FGFRs): Structures and Small Molecule Inhibitors. *Cells* 8, 614
45. Yemelyanenko, J. *et al.* (2025) C-terminal Truncation and Fusion Partner Determine Oncogenicity of FGFR3. *Cancer Res* DOI: 10.1158/0008-5472.CAN-24-2648
46. Lemmon, M.A. and Schlessinger, J. (2010) Cell signaling by receptor-tyrosine kinases. *Cell* 141, 1117–1134
47. Szybowska, P. *et al.* (2021) Negative Regulation of FGFR (Fibroblast Growth Factor Receptor) Signaling. *Cells* 10, 1342
48. Lin, C.-C. *et al.* (2023) The combined action of the intracellular regions regulates FGFR2 kinase activity. *Commun Biol* 6, 1–14
49. Zhang, X. *et al.* (2006) An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor. *Cell* 125, 1137–1149
50. Schiering, N. *et al.* (2003) Crystal structure of the tyrosine kinase domain of the hepatocyte growth factor receptor c-Met and its complex with the microbial alkaloid K-252a. *Proc Natl Acad Sci U S A* 100, 12654–12659
51. Khazaie, K. *et al.* (1988) Truncation of the human EGF receptor leads to differential transforming potentials in primary avian fibroblasts and erythroblasts. *The EMBO Journal* 7, 3061–3071
52. Woolford, J. *et al.* (1988) Activation of the feline c-fms proto-oncogene: multiple alterations are required to generate a fully transformed phenotype. *Cell* 55, 965–977
53. Akiyama, T. *et al.* (1991) The transforming potential of the c-erbB-2 protein is regulated by its autophosphorylation at the carboxyl-terminal domain. *Mol Cell Biol* 11, 833–842
54. Bardelli, A. *et al.* (1999) A peptide representing the carboxyl-terminal tail of the met receptor inhibits kinase activity and invasive growth. *J Biol Chem* 274, 29274–29281
55. Lorenzi, M.V. *et al.* (1997) Ligand-independent activation of fibroblast growth factor receptor-2 by carboxyl terminal alterations. *Oncogene* 15, 817–826
56. Hart, K.C. *et al.* (2001) Identification of tyrosine residues in constitutively activated fibroblast growth factor receptor 3 involved in mitogenesis, Stat activation, and phosphatidylinositol 3-kinase activation. *Mol Biol Cell* 12, 931–942
57. Nadratowska-Wesolowska, B. *et al.* (2014) RSK2 regulates endocytosis of FGF receptor 1 by phosphorylation on serine 789. *Oncogene* 33, 4823–4836
58. Szybowska, P. *et al.* (2019) Cancer Mutations in FGFR2 Prevent a Negative Feedback Loop Mediated by the ERK1/2 Pathway. *Cells* 8, 518
59. Ahmed, Z. *et al.* (2010) Direct binding of Grb2 SH3 domain to FGFR2 regulates SHP2 function. *Cell Signal* 22, 23–33
60. Ahmed, Z. *et al.* (2013) Grb2 controls phosphorylation of FGFR2 by inhibiting receptor kinase and Shp2 phosphatase activity. *J Cell Biol* 200, 493–504
61. Lin, C.-C. *et al.* (2012) Inhibition of basal FGF receptor signaling by dimeric Grb2. *Cell* 149, 1514–1524
62. Cha, J.Y. *et al.* (2009) Aberrant receptor internalization and enhanced FRS2-dependent signaling contribute to the transforming activity of the fibroblast growth factor receptor 2 IIIb C3 isoform. *J Biol Chem* 284, 6227–6240
63. Zingg, D. *et al.* (2025) The C-terminal Kinase Domain-Binding and Suppression Motif Prevents Constitutive Activation of FGFR2. *Cancer Research* DOI: 10.1158/0008-5472.CAN-24-3349
64. Singh, D. *et al.* (2012) Transforming fusions of FGFR and TACC genes in human glioblastoma. *Science* 337, 1231–1235

65. Gavine, P.R. *et al.* (2012) AZD4547: an orally bioavailable, potent, and selective inhibitor of the fibroblast growth factor receptor tyrosine kinase family. *Cancer Res* 72, 2045–2056

66. Chen, L. *et al.* (2021) Fibroblast growth factor receptor fusions in cancer: opportunities and challenges. *Journal of Experimental & Clinical Cancer Research* 40, 345

67. Nakanishi, Y. *et al.* (2015) Mechanism of oncogenic signal activation by the novel fusion kinase FGFR3-BAIAP2L1. *Molecular Cancer Therapeutics* 14

68. Tao, J.J. *et al.* (2018) Basket Studies: Redefining Clinical Trials in the Era of Genome-Driven Oncology. *Annu Rev Med* 69, 319–331

69. Le, D.T. *et al.* (2017) Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* 357, 409–413

70. Subbiah, V. *et al.* (2020) Dabrafenib plus trametinib in patients with BRAFV600E-mutated biliary tract cancer (ROAR): a phase 2, open-label, single-arm, multicentre basket trial. *Lancet Oncol* 21, 1234–1243

71. Subbiah, V. *et al.* (2022) Tumour-agnostic efficacy and safety of selpercatinib in patients with RET fusion-positive solid tumours other than lung or thyroid tumours (LIBRETTO-001): a phase 1/2, open-label, basket trial. *Lancet Oncol* 23, 1261–1273

72. Marabelle, A. *et al.* (2020) Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. *Lancet Oncol* 21, 1353–1365

73. Salama, A.K.S. *et al.* (2020) Dabrafenib and Trametinib in Patients With Tumors With BRAFV600E Mutations: Results of the NCI-MATCH Trial Subprotocol H. *J Clin Oncol* 38, 3895–3904

74. Wen, P.Y. *et al.* (2022) Dabrafenib plus trametinib in patients with BRAFV600E-mutant low-grade and high-grade glioma (ROAR): a multicentre, open-label, single-arm, phase 2, basket trial. *Lancet Oncol* 23, 53–64

75. Kim, C. and Prasad, V. (2015) Cancer Drugs Approved on the Basis of a Surrogate End Point and Subsequent Overall Survival: An Analysis of 5 Years of US Food and Drug Administration Approvals. *JAMA Intern Med* 175, 1992–1994

76. Davis, C. *et al.* (2017) Availability of evidence of benefits on overall survival and quality of life of cancer drugs approved by European Medicines Agency: retrospective cohort study of drug approvals 2009-13. *BMJ* 359, j4530

77. Sabnis, A.J. and Bivona, T.G. (2019) Principles of resistance to targeted cancer therapy: lessons from basic and translational cancer biology. *Trends Mol Med* 25, 185–197

78. Kas, S.M. *et al.* (2018) Transcriptomics and transposon mutagenesis identify multiple mechanisms of resistance to the FGFR inhibitor AZD4547. *Cancer Research* 78, 5668–5679

79. Katoh, M. (2019) Fibroblast growth factor receptors as treatment targets in clinical oncology. *Nat Rev Clin Oncol* 16, 105–122

80. Katoh, M. *et al.* (2024) FGFR-targeted therapeutics: clinical activity, mechanisms of resistance and new directions. *Nat Rev Clin Oncol* DOI: 10.1038/s41571-024-00869-z

81. Turner, N. *et al.* (2010) FGFR1 amplification drives endocrine therapy resistance and is a therapeutic target in breast cancer. *Cancer Res* 70, 2085–2094

82. Hanker, A.B. *et al.* (2017) HER2-Overexpressing Breast Cancers Amplify FGFR Signaling upon Acquisition of Resistance to Dual Therapeutic Blockade of HER2. *Clin Cancer Res* 23, 4323–4334

83. Zhou, Y. *et al.* (2020) FGF/FGFR signaling pathway involved resistance in various cancer types. *J Cancer* 11, 2000–2007

84. Mao, P. *et al.* (2020) Acquired FGFR and FGF Alterations Confer Resistance to Estrogen Receptor (ER) Targeted Therapy in ER+ Metastatic Breast Cancer. *Clin Cancer Res* 26, 5974–5989

85. Servetto, A. *et al.* (2021) Nuclear FGFR1 Regulates Gene Transcription and Promotes Antiestrogen Resistance in ER+ Breast Cancer. *Clin Cancer Res* 27, 4379–4396

86. Long, G.V. *et al.* (2014) Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. *N Engl J Med* 371, 1877–1888

87. Lu, S. *et al.* (2026) Savolitinib plus osimertinib versus chemotherapy for advanced, EGFR mutation-positive, MET-amplified non-small-cell lung cancer in China (SACHI): interim analysis of a multicentre, open-label, phase 3 randomised controlled trial. *Lancet* 407, 375–387

88. Dhanasekaran, R. *et al.* (2021) The MYC oncogene — the grand orchestrator of cancer growth and immune evasion. *Nature Reviews Clinical Oncology 2021* DOI: 10.1038/s41571-021-00549-2

89. Hanahan, D. (2026) Hallmarks of cancer—Then and now, and beyond. *Cell* 0

90. Bhin, J. *et al.* (2023) MYC is a clinically significant driver of mTOR inhibitor resistance in breast cancer. *Journal of Experimental Medicine* 220, e20211743

91. Duffy, M.J. *et al.* (2025) MYC as a Target for Cancer Treatment: from Undruggable to Druggable? *Target Oncol* 20, 791–801

92. Whitfield, J.R. and Soucek, L. (2025) MYC in cancer: from undruggable target to clinical trials. *Nat Rev Drug Discov* 24, 445–457

93. Facchinetti, F. *et al.* (2024) Understanding and Overcoming Resistance to Selective FGFR Inhibitors across FGFR2-Driven Malignancies. *Clin Cancer Res* 30, 4943–4956

94. Ma, F. *et al.* (2016) ctDNA dynamics: a novel indicator to track resistance in metastatic breast cancer treated with anti-HER2 therapy. *Oncotarget* 7, 66020–66031

95. Cao, H. *et al.* (2020) Circulating Tumor DNA Is Capable of Monitoring the Therapeutic Response and Resistance in Advanced Colorectal Cancer Patients Undergoing Combined Target and Chemotherapy. *Front. Oncol.* 10

96. Ernst, S.M. *et al.* (2025) Utilizing ctDNA to discover mechanisms of resistance to targeted therapies in patients with metastatic NSCLC: towards more informative trials. *Nat Rev Clin Oncol* 22, 371–378

97. Priestley, P. *et al.* (2019) Pan-cancer whole-genome analyses of metastatic solid tumours. *Nature* 575, 210–216

98. Alexandrov, L.B. *et al.* (2020) The repertoire of mutational signatures in human cancer. *Nature* 578, 94–101

99. Li, Y. *et al.* (2020) Patterns of somatic structural variation in human cancer genomes. *Nature* 578, 112–121

100. Rheinbay, E. *et al.* (2020) Analyses of non-coding somatic drivers in 2,658 cancer whole genomes. *Nature* 578, 102–111

101. Roepman, P. *et al.* (2021) Clinical Validation of Whole Genome Sequencing for Cancer Diagnostics. *J Mol Diagn* 23, 816–833

102. Rosenquist, R. *et al.* (2022) Clinical utility of whole-genome sequencing in precision oncology. *Seminars in Cancer Biology* 84, 32–39

103. Cuppen, E. *et al.* (2022) Implementation of Whole-Genome and Transcriptome Sequencing Into Clinical Cancer Care. *JCO Precis Oncol* 6, e2200245

104. Koboldt, D.C. (2020) Best practices for variant calling in clinical sequencing. *Genome Med* 12, 91

105. Kosugi, S. and Terao, C. (2024) Comparative evaluation of SNVs, indels, and structural variations detected with short- and long-read sequencing data. *Hum Genome Var* 11, 18

106. Chen, X. *et al.* (2025) Long-Read Sequencing Outperforms Short-Read Sequencing in Detecting Most Structural Variations. *Serican J Med* 2, 23671

107. Inaba, H. *et al.* (2017) Integration of Next-Generation Sequencing to Treat Acute Lymphoblastic Leukemia with Targetable Lesions: The St. Jude Children's Research Hospital Approach. *Front Pediatr* 5, 258

108. Rusch, M. *et al.* (2018) Clinical cancer genomic profiling by three-platform sequencing of whole genome, whole exome and transcriptome. *Nat Commun* 9, 3962

109. Frank, M.O. *et al.* (2019) Sequencing and curation strategies for identifying candidate glioblastoma treatments. *BMC Med Genomics* 12, 56

110. Walter, W. *et al.* (2021) Clinical application of whole transcriptome sequencing for the classification of patients with acute lymphoblastic leukemia. *BMC Cancer* 21, 886

111. Horak, P. *et al.* (2021) Comprehensive Genomic and Transcriptomic Analysis for Guiding Therapeutic Decisions in Patients with Rare Cancers. *Cancer Discov* 11, 2780–2795

112. Newman, S. *et al.* (2021) Genomes for Kids: The Scope of Pathogenic Mutations in Pediatric Cancer Revealed by Comprehensive DNA and RNA Sequencing. *Cancer Discov* 11, 3008–3027

113. Möhrmann, L. *et al.* (2022) Comprehensive genomic and epigenomic analysis in cancer of unknown primary guides molecularly-informed therapies despite heterogeneity. *Nat Commun* 13, 4485

114. Schwarze, K. *et al.* (2018) Are whole-exome and whole-genome sequencing approaches cost-effective? A systematic review of the literature. *Genetics in Medicine* 20, 1122–1130

115. Schwarze, K. *et al.* (2020) The complete costs of genome sequencing: a microcosting study in cancer and rare diseases from a single center in the United Kingdom. *Genet Med* 22, 85–94

116. Pasmans, C.T.B. *et al.* (2021) Micro-costing diagnostics in oncology: from single-gene testing to whole- genome sequencing. *Expert Review of Pharmacoeconomics & Outcomes Research* 21, 403–413

117. Ji, H.J. *et al.* (2026) Annotating genomes at increased scale and resolution. *Nat Rev Genet* DOI: 10.1038/s41576-026-00937-3

118. Clark, A.J. and James W. Lillard, J. (2024) A Comprehensive Review of Bioinformatics Tools for Genomic Biomarker Discovery Driving Precision Oncology. *Genes* 15

119. Goldman, M.J. *et al.* (2020) A user guide for the online exploration and visualization of PCAWG data. *Nat Commun* 11, 3400

120. Landrum, M.J. *et al.* (2025) ClinVar: updates to support classifications of both germline and somatic variants. *Nucleic Acids Res* 53, D1313–D1321

121. Sondka, Z. *et al.* (2024) COSMIC: a curated database of somatic variants and clinical data for cancer. *Nucleic Acids Res* 52, D1210–D1217

122. Griffith, M. *et al.* (2017) CIViC is a community knowledgebase for expert crowdsourcing the clinical interpretation of variants in cancer. *Nat Genet* 49, 170–174

123. Chakravarty, D. *et al.* (2017) OncoKB: A Precision Oncology Knowledge Base. *JCO Precis Oncol* 2017, PO.17.00011

124. Suehnholz, S.P. *et al.* (2024) Quantifying the Expanding Landscape of Clinical Actionability for Patients with Cancer. *Cancer Discov* 14, 49–65

125. The AACR Project GENIE Consortium *et al.* (2017) AACR Project GENIE: Powering Precision Medicine through an International Consortium. *Cancer Discovery* 7, 818–831

126. Acebedo, A. *et al.* (2025) Collaborating across sectors in service of open science, precision oncology, and patients: an overview of the AACR Project GENIE (Genomics Evidence Neoplasia Information Exchange) Biopharma Collaborative (BPC). *ESMO Real World Data Digit Oncol* 7, 100097

127. Riba, M. *et al.* (2024) The 1+Million Genomes Minimal Dataset for Cancer. *Nat Genet* 56, 733–736

128. Chang, T.-G. *et al.* (2025) Hallmarks of artificial intelligence contributions to precision oncology. *Nat Cancer* 6, 417–431

129. Reardon, B. *et al.* (2026) Convergence of machine learning and genomics for precision oncology. *Nat Rev Cancer* 26, 217–229

130. Kehl, K.L. *et al.* (2021) Artificial intelligence-aided clinical annotation of a large multi-cancer genomic dataset. *Nat Commun* 12, 7304

131. Tran, T.N. *et al.* (2025) AI cancer driver mutation predictions are valid in real-world data. *Nat Commun* 16, 8509

132. Nitta, N. *et al.* (2026) Clinical-grade autonomous cytopathology through whole-slide edge tomography. *Nature* DOI: 10.1038/s41586-025-10094-y

133. Zhao, W. *et al.* (2026) An agentic system for rare disease diagnosis with traceable reasoning. *Nature* DOI: 10.1038/s41586-025-10097-9

134. Brixi, G. *et al.* (2026) Genome modelling and design across all domains of life with Evo 2. *Nature* DOI: 10.1038/s41586-026-10176-5

135. Tangermann, C. *et al.* (2025) Saturation mutagenesis identifies activating and resistance-inducing FGFR kinase domain mutations. *Nat Genet* DOI: 10.1038/s41588-025-02431-8

136. Ng, P.K.-S. *et al.* (2018) Systematic Functional Annotation of Somatic Mutations in Cancer. *Cancer Cell* 33, 450-462.e10

137. Muiños, F. *et al.* (2021) In silico saturation mutagenesis of cancer genes. *Nature* 596, 428–432