CHAPTER 1

GENERAL INTRODUCTION AND SCOPE OF THIS THESIS

Cancer is the second-leading cause of death worldwide, surpassed only by cardiovascular disease [1]. Globally, about 1 in 5 people will develop cancer during their lifetime, with 1 in 9 men and 1 in 12 women dying from the disease [2]. In 2022, the World Health Organization's International Agency for Research on Cancer reported approximately 20 million new cancer cases and 9.7 million deaths worldwide [2]. In 2023, an estimated 18.5 million new cases and 10.4 million deaths occurred globally, excluding non-melanoma skin cancers [1]. These numbers will continue to grow, as the global cancer burden is expected to almost double by 2050, reaching over 35 million new cases and 18.6 million deaths annually, largely driven by population growth, aging, and lifestyle-associated risk factors [1]. Metastatic disease, which accounts for two-thirds – and in some reports up to 90% – of cancer-related deaths in solid tumors [3], remains particularly devastating, with a median survival of only about 10 months for affected patients [4]. Therefore, even though over the past two decades major advances have deepened our understanding of the molecular mechanisms underlying cancer initiation, progression, metastasis, and therapy response, much remains to be done to transform these discoveries into lasting improvements in cancer care.

Part of the enduring challenge in curing cancer stems from its complexity and diversity. No two tumors are truly alike. While tumors of the same type may share hallmark molecular features, they differ markedly between patients, particularly at advanced disease stages. This variability extends beyond genomic alterations to include differences in the tumor microenvironment, immune landscape, and epigenetic regulation, all of which shape tumor behavior and therapeutic response. At the genomic level, it is extremely rare for two metastatic tumors to share an identical fingerprint [5–8]. Additionally, treatment exposure further shapes the tumor genome and contributes to this diversity by imposing an evolutionary bottleneck that selects for therapy-resistant clones. One of the largest pan-cancer analyses of metastatic solid tumors to date showed that 96% of driver mutations within individual metastatic lesions are clonal [7]. Consistently, roughly half of patients with annotated treatment histories (53%) harbor treatment-enriched known or suspected tumor drivers [8], underscoring the strong selective pressure exerted by anticancer therapies during tumor evolution.

The growing understanding of the vast tumor heterogeneity and therapeutic selection pressures has ushered in an era of precision oncology. One-size-fits-all therapeutic approaches are suboptimal and are increasingly being replaced by targeted therapies tailored to the molecular features of each patient's individual tumor [9,10].

Precision oncology: reality or illusion?

The central tenet of precision oncology is to deliver the right drug(s) to the right patient at the right time [11]. Chronic myeloid leukemia (CML) is the poster child for precision oncology. Since its introduction in 2002 as first-line treatment, imatinib, a tyrosine

kinase inhibitor (TKI) targeting the BCR-ABL1 fusion oncoprotein that drives CML, has revolutionized therapy and transformed a once fatal malignancy into a manageable chronic condition with a near-normal life expectancy when treated early [12]. Other successes in precision oncology include trastuzumab for HER2-positive breast cancer [13]; EGFR [14], ALK [15] and RET inhibitors [16] for lung cancers; and NTRK inhibitors for TRK fusion-positive cancers [17]. These targeted drugs have improved clinical outcomes in solid cancers, but with much more modest impact compared to imatinib in CML. Nevertheless, biomarker-guided therapy is the strongest determinant of improved clinical outcomes such as higher response rate (RR) and longer progression-free survival (PFS) and overall survival (OS) [18–21]. Notably, targeted therapies are not intrinsically more effective than cytotoxic agents; rather, their success depends on appropriate biomarker selection to guide their use. When administered without a matching biomarker, their efficacy is minimal and can be even inferior to that achieved with conventional chemotherapy [19,21].

Despite significant promise and a few isolated success stories, the overall impact of targeted therapies on patient outcomes has unfortunately been limited. A meta-analysis of 346 phase I trials involving over 13,000 patients found that biomarker-based selection improved response rates; however, only 30% of patients with refractory cancers responded at all, and median PFS was just 5.7 months compared with 2.95 months in unselected cohorts [21]. Similarly, reviews of cancer drugs approved by the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) have shown that most offered minimal or no gains in OS or quality of life [22,23]. On average, new anticancer agents approved by the EMA and FDA between 2003 and 2013 extended OS by only about 3.4 months relative to previously available treatments [24].

The limited benefits of targeted therapies likely stem from multiple factors. Drug approvals frequently rely on surrogate endpoints that show weak correlation with OS [25]. In addition, the coexistence of distinct genomic alterations across tumor subclones can undermine the effectiveness of single-agent monotherapies, emphasizing the need for rational combination strategies that balance efficacy with tolerable toxicity. Timing also plays a critical role: most targeted agents are tested in heavily pretreated metastatic patients who have exhausted standard-of-care options. Administering these therapies earlier, before extensive clonal evolution and emergence of resistance, may be key to improving outcomes [10,26]. This underscores the importance of incorporating routine molecular profiling at diagnosis to match patients with the most effective therapy from the outset.

The evolution of genome sequencing: from the first human genome to routine molecular profiling

Sequencing of the first full human genome was a landmark scientific achievement that required enormous resources, global collaboration, and more than a decade to complete. The Human Genome Project ultimately cost about $2.7-3 billion USD, took 13 years

(1990-2003), and involved thousands of researchers at more than 20 research centers and universities across the world [27]. This is in stark contrast to the speed and cost efficiency of modern whole genome sequencing (WGS). Today, the cost for WGS is projected to be around $200, with a goal of reaching $100 per genome in the future. Even though WGS for clinical use requires extensive data analysis, interpretation, and use of consumables, which can raise total costs to several thousand dollars per genome [28], the dramatic reduction in sequencing costs has enabled genomic profiling of hundreds of cancer-associated genes as part of routine cancer care in many developed countries. This, in turn, has enabled the identification of hundreds of actionable genomic alterations and spurred the development of new targeted and immunotherapies. However, despite these significant advances, the functional impact of alterations in many oncogenic pathways remain incompletely understood.

From genomic profiling to biological and clinical insight

Translating genomic profiling results into clinical decision-making critically depends on understanding the functional consequences of individual genetic alterations. However, many variants detected in known cancer driver genes remain classified as variants of unknown significance (VUS), limiting their utility for therapy selection. This is particularly consequential when such variants occur in genes that are amenable to targeting by clinically approved therapies. There is therefore a pressing need for systematic and rapid functional characterization of VUS to distinguish biologically relevant driver events from neutral (passenger) variants and thereby support accurate and personalized treatment allocation.

Recent advances in high- and moderate-throughput functional genomics platforms have enabled the systematic interrogation of the oncogenic potential and drug sensitivity of large numbers of cancer-associated variants across clinically actionable genes. These approaches have been particularly instrumental in uncovering the oncogenic activity and underlying mechanisms of rare and low-frequency variants that would be difficult to prioritize based on recurrence alone [29–35]. For example, functional annotation of rare mutations in pancreatic ductal adenocarcinoma (PDAC) identified activating mutations in NADK, uncovering a previously underappreciated role for redox homeostasis in tumorigenesis and highlighting a potential therapeutic vulnerability [32]. Similarly, saturation mutagenesis and large-scale functional screens of kinases such as EGFR [29,33], HER2 [34] and FGFRs [35] have uncovered numerous low-prevalence activating and resistance-conferring mutations, many of which localize to outside the kinase domain yet remain sensitive to approved targeted therapies. Collectively, these studies demonstrate that systematic functional interrogation of rare variants can reveal novel mechanisms of oncogenic activation and expand the population of patients who may benefit from existing drugs.

Despite these successes, current functional annotation efforts remain heavily biased toward single-nucleotide variants and small indels, leaving other classes of VUS, including structural variants and gene fusions, far less systematically explored. Indeed, both experimental screening platforms and *in silico* saturation mutagenesis approaches have predominantly focused on missense alterations [29–31,33–36], whereas the functional and clinical consequences of complex rearrangements, domain truncations and non-canonical splice variants remain poorly characterized. Addressing this imbalance will be essential for achieving a comprehensive functional interpretation of the cancer genome and for fully realizing the promise of precision oncology.

SCOPE OF THIS THESIS

Building on these knowledge gaps, the present thesis aims to advance our understanding of the functional and clinical implications of genomic alterations in the fibroblast growth factor receptor (FGFR) family, as well as to elucidate mechanisms of therapeutic resistance within the PI3K-AKT-mTOR pathway.

In **Chapter 2** [37], we review the biological and clinical relevance of canonical in-frame FGFR fusions and complex non-canonical structural FGFR variants in cancer. We discuss their oncogenic mechanisms, their value as predictive biomarkers of therapeutic response, and the emerging challenges and opportunities for FGFR-targeted therapy. Central to this review is the discovery that exon 18 (E18) truncations in *FGFR2* and *FGFR3* are critical for oncogenic activation, a finding that, as later detailed in **Chapters 3-5** [38–40], has redefined our understanding of FGFR-driven tumorigenesis and informed the design of clinical trials evaluating next-generation FGFR inhibitors. Together, these insights highlight the importance of more precisely defining which *FGFR* genomic alterations are truly oncogenic and therapeutically actionable.

In **Chapter 3** [38], we use a combination of transposon-based screening, mouse tumor modeling and functional assays to identify C-terminal truncation of E18 in *FGFR2* as a potent single-driver oncogenic event. Analysis of human oncogenomic datasets reveals that this truncation can arise through multiple mechanisms (e.g. rearrangements, partial amplifications, and truncating mutations), all converging on the production of E18-truncated *FGFR2* (*FGFR2*$^{\Delta E18}$) oncogenic transcripts. In contrast, the oncogenic competence of full-length *FGFR2* amplification depends on the presence of additional co-driver alterations. Importantly, we show that expression of *FGFR2*$^{\Delta E18}$ is a predictive biomarker of FGFR-targeted therapy response, with direct implications for patient selection and the design of therapeutic strategies.

Building on these findings, in **Chapter 4** [39], we investigate the molecular mechanisms through which loss of the C-terminal tail leads to FGFR2 oncogenic activation. Through a systematic functional analysis of *Fgfr2*-E18 variants, we identify a previously unrecognized phenylalanine-serine (F703-S704) motif within the C-terminal region that mediates autoinhibitory binding of the C-tail to the kinase domain. This **'**kinase domain-binding and suppression**'** (KDBS) motif is essential for restraining FGFR2 kinase activity under normal conditions. Disruption or truncation of this motif, either through E18 loss or targeted mutation, abolishes this autoinhibitory control, resulting in constitutive activation of FGFR2 and aberrant stimulation of the PI3K-AKT-mTOR and STAT3 signaling pathways. We show that permutation of the KDBS motif, together with additional regulatory C-terminal sites, fully phenocopies the oncogenic competence of FGFR2$^{\Delta E18}$, establishing the KDBS motif as a central molecular switch controlling FGFR2 activation. These insights reveal a structural and mechanistic basis for oncogenic FGFR2 activation through C-terminal truncation and uncover potential therapeutic vulnerabilities, as combined inhibition of FGFR2 with PI3K-AKT-mTOR or STAT3 pathway inhibitors may overcome on-target resistance to FGFR-targeted therapies in *FGFR2$^{\Delta E18}$*-positive cancers.

Prompted by our findings in FGFR2, in **Chapter 5** [40], we investigate whether analogous C-terminal tail loss in its closely related FGFR3 paralog similarly drives oncogenicity. Intriguingly, we uncover that FGFR3 exhibits divergent behavior: unlike FGFR2, FGFR3 requires both E18 truncation and fusion to a partner gene retaining the expression of a dimerizing domain to effectively drive oncogenic signaling and tumorigenesis. We also functionally validate the oncogenic potential of previously uncharacterized clinical *FGFR3* gene fusions (FGFR3$^{\Delta E18}$-ADD1, FGFR3$^{\Delta E18}$-TNIP2, and FGFR3$^{\Delta E18}$-JAKMIP1). All these fusions contain a self-dimerizing domain contributed by the fusion partner, confer resistance to HER2/EGFR tyrosine kinase inhibitors, yet remain sensitive to pharmacologic FGFR inhibition *in vitro*.

Whereas **Chapters 2-5** focus on elucidating the oncogenic mechanisms of FGFR signaling and their implications for targeted therapy, **Chapter 6** [41] broadens this scope to explore mechanisms of therapeutic resistance within the PI3K-AKT-mTOR pathway, one of the major signaling axis frequently co-opted by receptor tyrosine kinases such as FGFRs. Despite the clinical promise of mTOR inhibitors (mTORi) in breast cancer, treatment efficacy remains limited by intrinsic and acquired resistance. Through multiomic profiling of treatment-naïve and mTORi-resistant mouse invasive lobular carcinomas, we identify MYC activation as a key driver of resistance. MYC counteracts mTORi-induced translational suppression by sustaining ribosomal protein synthesis and tumor growth. Moreover, mTORi-resistant cells depend on MYC activity, as combined inhibition of mTOR and MYC restores therapeutic sensitivity. Importantly, we show that MYC activation correlates with poor response to the mTORi everolimus in metastatic breast cancer patients, establishing MYC as a

potential driver of clinical mTORi resistance that may stratify breast cancer patients for mTOR-targeted therapies.

Finally, the general discussion in **Chapter 7** reflects on the main findings presented in this thesis, highlights remaining challenges, and outlines directions for future research.

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