Targeted therapy for triple-negative breast cancer

McLaughlin, R.P.

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Author: McLaughlin, R.P.
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Chapter 6

Discussion & Future Perspectives

Breast cancer is the most frequently occurring cancer in women worldwide, and as such represents a substantial clinical burden[1]. It is a profoundly heterogeneous disease which can be sub-classified into multiple histological and molecular subtypes based on anatomical origin and transcriptomic profiling[2–4]. One of the most aggressive forms of breast cancer is the triple-negative subtype (TNBC), which is associated with endemic drug resistance and a significantly poorer survival compared to hormone receptor-positive (HR+) or human epidermal growth factor receptor 2-positive (HER2+) breast cancer[5–9]. Standard clinical management of TNBC is complicated by an acute lack of effective targeted therapies which exploit the unique properties of TNBC cells in order to subvert their aberrant proliferation and subsequent dissemination to distant organs[10]. The principle objective of the studies which constitute this thesis was to identify novel drug targets and/or targeted agents which effectively kill TNBC cells *in vitro*, either as monotherapies or in combination with clinically approved inhibitors. The findings of the chapters presented in this thesis are thus discussed in a broader context whilst relevant points for future investigation are also proposed.

1. The importance of targeting the cell cycle in TNBC

Dysfunctional cell division is one of the key hallmarks of cancer and is linked to abnormalities in the intricate molecular machinery which governs the transition of cells through the strictly controlled transitions that constitute the mammalian cell cycle. Targeting aberrant regulation of this cycle by inhibiting the function of various cyclin-dependent kinases (CDKs) or other kinases involved in cell cycle regulation (e.g. cdc7) is therefore an attractive strategy for targeting TNBC and other cancers. Cancers frequently exhibit altered CDK function resulting in dysregulation of the tightly controlled checkpoints which govern progression into different sections of the

cell cycle[11]. In **Chapter 2** the heterogeneity characteristic of TNBC is evidenced by the differential response of a panel of TNBC cell lines to multiple MEK and AKT inhibitors. Excessive activation of MAPK and/or PI3K pathways is common in TNBC tumours. Deletion of PTEN which negatively regulates Akt activation, or activating mutations in the catalytic subunit of PI3K (PIK3CA) can lead to constitutive Akt activity to which TNBC cells can become addicted[12]. Activating mutations in RAS or RAF, which are located upstream of MEK and ERK, are surprisingly rare in TNBC, however[13,14]. Nonetheless, basal-like tumours are often enriched for an activated RAS transcriptional programme and alterations in negative regulators of RAS/RAF (e.g. DUSP4 and NF1) have been extensively described and linked to metastatic behaviour in TNBC[14–18]. Importantly, in **Chapter 2** we demonstrate that the resistance of cells to dual MEK and Akt inhibition cannot be attributed to crosstalk between MAPK and PI3K pathways since neither MEK nor Akt inhibitors induced up-regulation of the opposing pathway in cell lines resistant to both inhibitors. In order to identify potential vulnerabilities in these double-resistant cell lines, we utilised transcriptomic and proteomic data to identify gene signatures capable of distinguishing double-resistant cell lines from those sensitive to either MEK or Akt inhibition. Cell lines resistant to both inhibitors are enriched for a gene expression signature strongly linked to cell cycle regulation and DNA damage. This enrichment renders double-resistant cell lines relatively more sensitive to CDK inhibitors, which potently induce apoptosis and inhibit proliferation. These results thus highlight the importance of transcriptomic and proteomic profiling in identifying potential novel druggable targets and in the stratification of patients according to drug sensitivity.

In **Chapter 3**, despite high-level EGFR expression in TNBC tumours, we show that TNBC cell lines are overwhelmingly resistant to EGFR-tyrosine kinase inhibitors

(EGFR-TKIs). We confirmed that the resistance of these cells to EGFR-TKIs cannot be attributed to lack of target inhibition since multiple inhibitors effectively reduced EGF-mediated phosphorylation of EGFR in both TKI-resistant and TKI-sensitive TNBC cell lines. By screening a TNBC cell line panel with a kinase inhibitor library in combination with EGFR-TKIs, we demonstrated that simultaneous inhibition of cdc7 and CDK9 function (using kinase inhibitor PHA-767491) sensitised EGFR-TKI-resistant TNBC cells to EGFR inhibition, resulting in cell cycle arrest and eventual apoptosis. Cdc7 is critical for accurate DNA synthesis during S-phase; it phosphorylates mini-chromosome maintenance proteins including MCM2 which subsequently activate DNA helicases which unwind the double-helix and recruit replication factors necessary for DNA synthesis[19,20]. Cdc7 and p53 are both essential for accurate regulation of the G1/S checkpoint; p53 mutations are ubiquitous in TNBC which results in dysfunction or loss of a p53-dependent cdc7-inhibition checkpoint[21]. Since cdc7 inhibition in non-transformed human fibroblasts does not lead to cell cycle arrest due to the presence of intact p53, cdc7 therefore represents a valid, specific anti-cancer target[21]. Moreover, by analysing the association of cdc7 expression with metastasis-free survival (MFS) in a cohort of TNBC patients, we confirmed in **Chapter 3** that cdc7 expression is inversely correlated with MFS in TNBC. Given that PHA-767491 inhibits both cdc7 and CDK9 activity, the evaluation of cdc7-selective inhibitors in TNBC is therefore warranted, particularly in cell lines or tumours lacking functional p53, either as monotherapies or in combination with RTK-targeted agents such as EGFR-TKIs.

2. Exploiting the dysfunctional, CDK-regulated transcriptional machinery in TNBC

During their development, cancers accrue a myriad of damaging genetic and/or epigenetic lesions and aberrations which ultimately disrupt the signal transduction cascades controlling cell proliferation, survival and motility, amongst others[17,22,23]. Despite the fact that the genomic landscape of tumours is littered with innumerable mutations, multiple cancers are addicted to activation of particular signal transduction pathways as a result of activation of certain oncogenes. This renders such cancers sensitive to targeted agents which repress oncogene-driven signal transduction. Whilst targeting RTK-mediated signalling is effective against cancers with clear oncogenic drivers such as oestrogen receptor-positive (ER+) breast cancer, androgen receptor-positive (AR+) prostate cancer or EGFR-mutant non-small cell lung cancer (NSCLC), in TNBC the lack of immediately discernible and pharmacologically amenable driver pathways or mutations severely limits the use of such therapies[24]. Moreover, targeting RTK-mediated signal transduction is often confounded by the functional redundancy that exists within and between multiple families of RTKs, as well as adaptive transcriptional responses in response to oncogenic RTK inhibition which promote up-regulation of alternative RTKs[25,26]. Instead, inhibiting the transcriptional machinery at the root of all RTK-fuelled signal transduction in TNBC represents one method of exploiting so-called “non-oncogene addiction”, a phenomenon whereby despite the absence of mutations in selected genes, malignant cells rely upon their function for their own survival as a result of oncogene-mediated distortion of signal transduction or cellular processes[27]. Amplification of c-MYC in many breast tumours confers a dependency on exceptionally high levels of transcription in order to sustain the MYC-driven

transcriptional programmes which contribute to the maintenance of an oncogenic state[28]. This transcriptional addiction can also be targeted in TNBC and covalent CDK7 inhibitor THZ1 has shown pre-clinical benefit in this context[29].

Consequently, we evaluated the potential of a selection of CDK inhibitors which prevent the function of key transcriptional regulators (e.g. P-TEFb/CDK9 and TFIIH), in addition to CDK1 and CDK2, as possible options for targeted therapy in TNBC in **Chapter 4**. Taking the molecular heterogeneity of TNBC into account, we screened a panel of 20 TNBC cell lines representative of all TNBC molecular subtypes described by Lehmann et al (2016)[4]. Strikingly, inhibitors with potent activity against CDK1, CDK2, CDK9 and CDK7 were extremely effective at preventing the proliferation of TNBC cells at concentrations as low as 100 nM. These anti-proliferative effects were accompanied by induction of apoptosis and G2/M cell cycle arrest. Inhibition of cell migration speed at sub-lethal concentrations also highlighted the potential of these agents to prevent initiation of the metastatic cascade. These effects were comparable to those elicited by clinically-approved pan-CDK inhibitors dinaciclib and flavopiridol, suggesting such compounds could have similar efficacy in a clinical context. Contrastingly, CDK4/6-selective inhibitors such as palbociclib and abemaciclib were generally ineffective, suggesting that targeting CDK4/6-mediated cell cycle progression is futile, except in TNBC cells which express androgen receptor (AR)[30]. This is consistent with other studies which delineate a clear dependency on CDK4/6-mediated regulation of G1/S transition in ER+ or HER2+ breast cancers and in AR+ prostate cancers[31–34]. Androgens and oestrogens promote cell cycle progression by up-regulating Cyclin D1 expression, which can therefore be counteracted by using CDK4/6 inhibitors in combination with anti-hormonal therapy[35]. The tumourigenesis of HER2+ breast cancers is also dependent on concomitant CDK4 activity[36].

Nevertheless, considerable functional redundancy exists between CDKs; CDK12 also phosphorylates the CTD of RNA Polymerase II at Serine 2 to promote transcriptional elongation. The expression of DNA damage response (DDR)-associated genes and NRF2 target genes is particularly sensitive to disruption of CDK12 function, strongly suggesting that CDK12 is involved in the transcriptional regulation of adaptive cellular stress responses[37–39]. Additionally, CDK12 function is required for the termination of transcription; CDK12 depletion prevented the 3' end processing of c-FOS mRNA transcripts induced by EGF stimulation in human embryonic kidney cells, also suggesting that it is required for successful transduction of RTK-mediated signalling via terminal transcription factors[40]. Interestingly, the specificity of CDK12 for particular transcripts has not yet been unequivocally established[38,41]. Considering its aforementioned roles in transcriptional regulation, CDK12 could theoretically compensate for decreased CDK9 activity, and therefore P-TEFb activity, in order to drive productive elongation of mRNA transcripts and overcome growth inhibition by CDK9-specific inhibitors. However, this hypothesis clearly requires experimental validation by assessing and comparing the effects of CDK12 and CDK9 depletion in TNBC cells, either by using RNAi or CRISPR-Cas9 technologies or by developing highly selective and effective inhibitors for these kinases. A CDK12-specific inhibitor THZ531 has been synthesised and exhibited potent activity against T-cell acute lymphoblastic lymphoma (T-ALL) models[42]. Nevertheless, a high degree of structural homology exists between members of the CDK family, particularly in the ATP-binding site, often precluding the development of ATP-competitive inhibitors highly selective for individual CDKs[43,44]. Resistance to single targeted agents invariably develops in the clinic, and for this reason rational combination therapies are increasingly being considered as compulsory in

oncology[25,45]. In several TNBC models and in patient tumours, kinase inhibition induces up-regulation of alternative RTKs which bypass the inhibitory effects of these agents thereby permitting the development of resistance[46,47]. Considering the profound inhibition of proliferation, depletion of BCL-2 family member proteins, and attenuation of transcriptional elongation seen after CDK inhibitor treatment, we therefore combined the CDK inhibitors I-73 and LY3-21 with EGFR-TKIs and bromodomain (BET) inhibitors in **Chapter 5** to determine whether they could sensitise drug-resistant TNBC to such compounds. We confirmed that these agents strongly synergise in *in vitro* models of TNBC, leading to superior inhibition of cell proliferation, cell cycle arrest and induction of apoptosis.

To scrutinise the mechanism of action of these CDK inhibitors and rational combinations further, we performed RNA sequencing to determine the global impact of treatment on gene expression and signal transduction. Transcriptomic profiling identified a select list of CDK inhibitor-sensitive transcription factors significantly enriched in basal-like breast tumours. By virtue of RNA interference-based screening, we confirmed in **Chapter 4** that expression of a number of these transcription factors was essential for the proliferation of multiple TNBC cell lines. SOX9, EN1 and PLAG1 were associated with epithelial-to-mesenchymal transition (EMT), maintenance of basal-like subtype, mitochondria-regulated protection from cell death stimuli, and IGF-mediated signal transduction[48–52]. Moreover, we showed in **Chapter 5** that combined EGFR-TKI and I-73 treatment enhances the impact of I-73 on gene transcription. Additionally, I confirmed that expression of a number of transcription factors differentially and significantly down-regulated after co-treatment with EGFR-TKI and I-73 was strongly linked to poor prognosis in TNBC patients. The use of transcriptional CDK inhibitors in multiple malignancies has permitted the identification

of “super enhancer-associated genes” which are indispensable for cancer cell survival and exquisitely sensitive to CDK7 inhibition[29,53–55]. Considering that SOX9 and EN1 have previously been identified as super enhancer-associated genes in specific TNBC cell lines, expanding the list of such genes should constitute a future priority. Detecting these enhancer regions by virtue of their enrichment for specific epigenetic markers (e.g. acetylation of H3K27 or methylation of H3K4) using Ch-IP sequencing in TNBC cells and xenograft tumours treated with the effective novel CDK inhibitors and rational combinations identified in this thesis could allow identification of novel super enhancer-associated genes in TNBC. This would therefore provide further mechanistic insight whilst illuminating knowledge of the transcriptional programmes which drive heterogeneous tumours like TNBC. Whilst directly targeting transcription factor function remains pharmacologically problematic, an increased understanding of the programmes driving growth and tumour adaptation to therapy could permit the evidence-based identification of synthetic lethal interactions

3. The limitations of *in vitro* compound screening

Notwithstanding the remarkable sensitivity of TNBC cells to these CDK inhibitors *in vitro*, the contribution of the tumour microenvironment to intrinsic or acquired resistance cannot be understated[56–58]. *In vitro* models cannot recapitulate the intricate complexity conferred by the tumour vasculature and surrounding tissues which directly and indirectly control the availability of nutrients and the ability of therapeutics to reach the tumour bulk[59]. Crucially, assessing single CDK inhibitor efficacy, as well as combining EGFR-TKIs (e.g. lapatinib) and I-73, in a PDX-based or xenotransplantation model will be vital in elucidating whether it is possible to replicate the strong synergy observed *in vitro* in **Chapter 5**. Such models would also

provide information on whether this combination is likely to have clinical efficacy in tumours which express high levels of EGFR or which depend on EGFR pathway functionality. More importantly, whether the efficacy or eventual synergy observed is due to inhibition of the aforementioned CDK inhibitor-sensitive or synergy-associated transcription factors indispensable for cell growth *in vitro*, should be investigated. Intriguingly, recent evidence derived from *in vitro* and *in vivo* models of glioblastoma (GBM) revealed a distinct lack of overlap between the transcription elongation factors required for GBM survival in vitro and those necessary for tumour outgrowth in orthotopic patient-derived xenografts (PDX)[60]. Specifically, genes regulating productive transcriptional elongation and pause-release, in particular JMJD6 and BRD4, were essential for enhancer-mediated transcriptional adaptation to the tumour microenvironment[60]. In general, an increased dependency on pause-release and elongation machinery was observed *in vivo.* The tumour microenvironment presents wholly different challenges to tumour proliferation than those encountered by homogenous populations of cancer cell lines cultured on plastic. *In vivo*, tumour cells must adapt to hypoxic conditions and the scarcity of nutrients, whilst also disarming infiltrating immune cells with the power to destroy them, thereby subverting the anti-tumour immune response[61]. Importantly, they must also physically interact, and communicate, with surrounding stromal cells in order to survive and establish a tumour-permissive environment[61–64]. Therefore, an increased dependency on transcriptional programmes which regulate adaptive stress responses is to be expected. In contrast, *in vitro* culture systems are by and large designed to eliminate such stresses. Cultured cell lines receive abundant nutrients and the absence of immunogenic pressure or stromal influence allows these cells to expand rapidly and with ease, facilitating phenotypic drift from the tumour from which they were originally

derived[61,65,66]. It is thus imperative to consider *in vitro* systems such as those utilised throughout this thesis, as mere models and not, therefore, an accurate reflection of the potential of targeted agents in the clinic[65]. Additionally, xenotransplantation of cancer cell lines into mice is associated with its own limitations; unlike PDX-based models, cell line-derived xenografts do not retain the original tumour architecture and do not preserve inter-and intra-tumoural heterogeneity[67,68]. Moving forward, it would therefore be of utmost importance to verify whether any overlap exists between the TNBC-specific transcriptional programmes sensitive to CDK inhibition identified *in vitro* in **Chapters 4 and 5** of this thesis, and those required for TNBC tumour growth *in vivo*. Comparison of RNA seq-derived transcriptomic profiles of PDX-derived tumours treated with single agent I-73 or in combination with EGFR-TKIs, with the profiles of TNBC cell lines cultured *in vitro* or xenotransplanted into mice, may permit further research to focus on the most relevant pathways for *in vivo* tumour growth, instead of those which represent *in vitro* artefacts. However, the fact that the reliance of GBM cells on the function of transcriptional elongation and pause-release factors was enhanced in an *in vivo* context, is reassuring considering how disruption of such molecules so potently eliminates the propensity of TNBC cells to grow *in vitro*.

4. Manipulating the connection between CDK function and the DNA damage response (DDR)

Apart from their impact on CDK9-regulated, P-TEFb-mediated transcriptional elongation, the effective CDK inhibitors tested here also have potent activity against CDK1. The formation of CDK1-Cyclin B1 complexes is an essential component of the mitotic checkpoint during cell division, these complexes being responsible for the reorganisation of nuclear architecture prior to cytokinesis. Although CDK1's primary function is in regulating mitosis, it is also known to influence the stability of proteins

required for precise DNA damage repair, particularly the stability of BRCA1[69]. Depletion of CDK1 can substantially and selectively enhance the efficacy of PARP inhibitors in HR-proficient, BRCA1 wild-type cancer cells, leaving untransformed cells relatively unaffected by combined CDK1 and PARP inhibition[69]. Considering the strong induction of G2/M cell cycle arrest and γ-H2AX induction elicited by I-73 and D11-81 in **Chapter 4**, and the induction of γ-H2AX and PARP1 cleavage by dinaciclib in **Chapter 2**, it is tempting to attribute such effects to inhibition of CDK1 or CDK2. Regardless, dissecting phenotypic responses remains troublesome with pan-CDK inhibitors due to lack of target specificity. However, these DNA damage-related responses could theoretically be exploited by combining the CDK inhibitors described here with currently used chemotherapeutics such as doxorubicin, gemcitabine or cisplatin[70].

Whilst particular attention has been given to inhibitors with potent activity against P-TEFb/CDK9 and other CDKs, namely CDK1, CDK2 and CDK7, the consequences of CDK12 inhibition have not been addressed. CDK12 also phosphorylates the CTD of RNA Polymerase II (POLR2A) and specifically regulates the expression of genes involved in DNA-damage repair[37,71,72]. Recent studies have demonstrated that pan-CDK inhibitor dinaciclib also targets CDK12 function in addition to CDK1, CDK2, CDK5 and CDK9[73]. Treatment of TNBC cells with dinaciclib inhibited the expression of genes linked to homologous recombination (HR) and DDR, effects which were also seen after CDK12 depletion. Dinaciclib-induced CDK12 inhibition or RNAi-mediated CDK12 depletion reduced the capacity of BRCA1/2 wild-type (wt) cells and tumours for HR after exposure to γ-irradiation and were therefore sensitised to PARP inhibitors[73]. Crucially, dinaciclib-mediated CDK12 inhibition abrogated acquired resistance to PARP inhibitors in several PDX models of TNBC[73]. PI3K inhibition in

TNBC cells also induces DNA damage which augments the response of BRCA-wt/HR-proficient cells to PARP1/2 inhibitors, emphasising the links between PI3K signalling and the DNA-damage response (DDR)[74]. Considering that MEK-i/Akt-i double-resistant cells are highly sensitive to pan-CDK inhibitors such as dinaciclib, there is a clear rationale for combining dinaciclib or MEK/AKT inhibition with PARP inhibitors and chemotherapy or radiotherapy in such instances. Investigating whether Akt or MEK inhibitors alter the DNA-damage response themselves, across the differentially sensitive subgroups identified in **Chapter 1**, could therefore also potentially identify DNA-damaging agents as possible combination therapies together with MEK or Akt inhibitors. Additionally, combining the CDK inhibitors evaluated in **Chapter 4** with PARP inhibitors in TNBC cells both proficient and deficient in HR could therefore identify novel rational synergistic combinations for specific groups of TNBC patients. Such combinations may allow the use of lower doses and thereby avoid the off-target toxicity associated with either therapy.

5. Future Perspectives

One of the principle concerns regarding the translation of pre-clinically effective CDK inhibitors to the clinic is the induction of systemic toxicity. First-generation pan-CDK inhibitors flavopiridol and roscovitine showed immense promise in pre-clinical investigations but ultimately failed to deliver in the clinic[75–78]. Similarly, dinaciclib has shown limited potential in patients with solid tumours[75]. A recently completed phase 1 clinical trial of dinaciclib in various multiple solid malignancies revealed that dose-limiting effects included pancytopenia, hyperuricemia and hypotension[79]. Additionally, dinaciclib exhibited potent growth inhibitory effects on normal patient lymphocytes treated *ex vivo*[79]. In breast cancer specifically, dinaciclib has yet to demonstrate superior efficacy over standard-of-care chemotherapy-based treatments such as

capecitabine in patients with advanced disease, with severe neutropenia being reported in around 75% of patients[80]. Pharmacokinetic limitations were also problematic; short half-lives were seen for 2 hour infusions with dinaciclib whilst longer infusions induced hepatotoxicity[79].

In order to maximise the benefit from CDK inhibitors and to combat the high rates of attrition currently associated with the development of such agents, three unsettled issues concerning their use and design must therefore be addressed. Primarily, and as previously mentioned, disentangling the relationships between phenotypic effects and target inhibition is crucial. Without a clear understanding of mechanism of action, clinical translation will be extremely difficult. To this end, functional genomic screening to verify whether phenotypic effects such as apoptosis, cell cycle arrest or autophagy are associated with disruption of particular CDKs in TNBC would be highly appropriate. It is envisaged that pan-CDK inhibitors such as I-73, D11-81 or LY3-21 owe their antineoplastic effects to the combined inhibition of multiple CDKs. Nonetheless, through transcriptomic profiling in **Chapters 4 and 5** we showed that these agents overwhelmingly affect transcription factor function, strongly suggesting that inhibition of transcriptional CDKs is a significant component of their inhibitory effects. Secondly, the identification of patients who will derive maximum benefit from such treatments is absolutely paramount. The clinical trials in which pan-CDK inhibitors have been evaluated, assessed end-point responses such as target inhibition, side-effects, disease progression and long-term survival yet often lacked genomic or transcriptomic profiling of tumours from patients who responded to anti-CDK therapy[79]. Consequently, elucidating whether certain gene signatures or genomic lesions can distinguish CDK inhibitor-sensitive from CDK inhibitor-resistant patients as well as signatures associated with less toxicity, would be invaluable. The

utility of such an approach was emphasised in **Chapter 2**, in which we established a rationale for CDKs as an alternative targeted therapy in TNBC resistant to both MEK and AKT inhibitors, due to the enhanced expression of a cell cycle-related gene signature in these tumours. Considering the mounting pre-clinical evidence that CDK inhibition is synthetic lethal with MYC amplification, stratifying patients based on such a marker would be prudent[81,82]. Disruption of CDK1 function is synthetic lethal with KRAS-mutations in pancreatic ductal adenocarcinoma cell lines and colorectal cancer xenografts and with PARP inhibition in BRCA-mutant and BRCA-wt breast cancers, denoting other possible indications for the use of CDK inhibitors[69,83]. Thirdly, and of particular relevance for their use in combination therapies, absence of a malleable therapeutic window has hampered efforts to successfully combine CDK inhibitors and standard-of-care treatments[75]. The impact of selective CDK9 inhibition on the survival of normal, non-cancerous cells is yet to be determined. The evidence that cancers depend upon transcriptional CDKs such as CDK7 and CDK9 for the execution of oncogenic transcriptional programmes may allow for discrimination between normal and malignant tissue, though disruption of other CDKs such as CDK1 is known to be detrimental for the proliferation and mitosis of normal cells and for maintenance of genomic stability in pluripotent stem cells[84,85]. The clinical balancing act of achieving superior efficacy whilst avoiding the onset of dangerous toxicity is extremely challenging. Thorough toxicological assessment of CDK inhibitors such as I-73 in human tissues which are frequently the sites of adverse reactions is therefore essential. The use of *in vitro* or *ex vivo* screening systems which utilise primary human hepatocytes, renal cells and/or bone marrow-derived haematopoietic stem cells is of particular relevance here. Previously published data showing the substantial selectivity of I-73 for tumour cells over CD34+

haematopoietic cells or human embryonic kidney cells is reassuring in this regard[86,87]. Nonetheless, the dearth of objective responses, as well as the premature termination of clinical trials of pan-CDK inhibitors in a monotherapeutic context, is testament to the necessity for rational combination therapies in solid malignancies.

6. Conclusions

Collectively, the work presented in this thesis stresses the importance of inhibiting CDK function in TNBC as a means to bypass or overcome resistance to already-established molecular-targeted agents and as effective monotherapies. The use of high-throughput screening and compound libraries was essential to the identification of novel molecular targeted therapies effective against *in vitro* models of TNBC, as well as rational synergistic combinations with superior potency. Extensive pre-clinical toxicity testing is, however, required before these results can be translated to a clinical context, as well as further delineation of the relationship between target inhibition and phenotypic response. Additionally, this thesis highlights the importance of omic-based technologies in identifying novel targets and evaluating the transcriptional response of cancer cells to targeted therapies in order to elucidate the signalling networks most sensitive to CDK inhibition.

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