Targeted therapy for triple-negative breast cancer

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Appendix

Summary (English)

Targeted therapy for triple-negative breast cancer

Triple-negative breast cancer (TNBC) is an aggressive form of breast cancer distinguishable from breast cancers dependent upon hormone-regulated growth pathways by the absence of oestrogen and progesterone receptors, as well as the lack of HER2 (human epidermal growth factor receptor 2) expression or gene amplification. Consequently, anti-hormonal therapies (e.g. tamoxifen), which are highly efficacious in HR+ breast cancer, are redundant in the context of TNBC. At present, clinical management of TNBC consists of chemotherapy, radiotherapy and tumour-reductive surgery. The therapeutic arsenal with which TNBC can be treated has failed to expand appreciably since the introduction of chemotherapy, emphasising the desperate need for the development of novel targeted agents to combat this intractable illness. As such, the principle aim of this thesis was to identify novel TNBC-specific dependencies amenable to pharmacological manipulation, with a key emphasis on the identification of novel synergistic combinations comprised of clinically approved agents in conjunction with novel targeted molecules.

Chapter 2 explored the differential sensitivity of TNBC cells to MEK and Akt inhibitors. Kinase inhibitor-based screening of TNBC cell lines showed that they cluster into three separate groups based on their sensitivity to multiple MEK and Akt inhibitors; Group 1 (Akt inhibitor-resistant), Group 2 (MEK inhibitor-resistant) or Group 3 (resistant to both MEK and Akt inhibitors). An increased level of phospho-Akt and mutations in PIK3CA, PIK3R1 and/or PTEN distinguished MEK inhibitor-resistant, Akt inhibitor-sensitive cell lines from those resistant to Akt inhibitors yet sensitive to MEK inhibitors. Co-treatment of double-resistant cell lines with MEK and

Akt inhibitors was not sufficient to overcome their resistance to either agent, nor did it lead to synergistic responses in the other groups. To identify alternative, more appropriate targets for this difficult-to-treat subgroup, transcriptomics- and proteomics-based gene-set enrichment analyses unveiled a significant enrichment of genes linked to cell cycle regulation in MEK/Akt inhibitor double-resistant cell lines. Importantly, cell lines resistant to both MEK and Akt inhibitors showed greater sensitivity to pan-CDK inhibitors dinaciclib and flavopiridol. These agents potently inhibited CDK-mediated signal transduction and induced DNA damage, suggesting that the MEK/AKt inhibitor double-resistant subgroup may benefit from alternative targeted therapy in the form of CDK inhibitors.

Chapter 3 delineates the resistance of TNBC cells to a panel of epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors. Here, I demonstrate that EGFR is expressed at higher levels in TNBC tumours compared to ER+ breast tumours and high expression of EGFR is associated with cell lines conforming to a basal-like subtype. The response of a panel of TNBC cells to EGFR-tyrosine kinase inhibitors (EGFR-TKIs) was therefore evaluated; the majority of TNBC lines being resistant to EGFR-targeted therapy, despite the fact that silencing EGFR markedly attenuated their proliferation. This phenomenon was independent of EGFR pathway functionality since EGF-mediated stimulation of both EGFR-TKI-resistant and EGFR-TKI-sensitive TNBC cell lines activated downstream signalling which was inhibited by treatment with lapatinib, erlotinib and gefitinib. To identify novel compounds which could sensitise EGFR-TKI-resistant TNBC cells to these drugs, I performed a kinase inhibitor library screen in combination with a clinically relevant dose of lapatinib. This screen identified a dual cdc7/CDK9 inhibitor (PHA-767491) as the most promising candidate compound. Combining PHA-767491 with multiple EGFR-TKIs

substantially reduced the proliferation of TNBC cells compared to either monotherapy and induced apoptosis in multiple TNBC cell lines. This co-treatment inhibited signal transduction crucial for cdc7-regulated DNA replication and the initiation of productive mRNA transcription controlled by CDK9, with concomitant G2/M cell cycle arrest. Moreover, increased expression of cdc7 and RNA Polymerase II, the downstream target of CDK9, was associated with poorer metastasis-free survival in TNBC patients. Transcriptomic profiling revealed down-regulation of pathways governing proliferation, transcription and cell survival in TNBC cells treated with combination therapy. Additionally, an elevated expression of several genes involved in the aforementioned signaling pathways, and whose expression is sensitive to the combination, was associated with a worse metastasis-free survival in CMTN patients, suggesting targeting CDK9 in conjunction with EGFR may represent a powerful strategy to combat TNBC.

To scrutinise the potential of targeting aberrant CDK9-mediated transcriptional regulation as a novel target for TNBC, the efficacy of a panel of CDK inhibitors with prominent activity against P-TEFb/CDK9 function was assessed in TNBC cell lines in **Chapter 4.** TNBC cells were sensitive to inhibitors with strong activity against CDK1, CDK2, CDK7 and CDK9, highlighting their exquisite dependence on regulation of the cell cycle and transcription. Treatment with these inhibitors abolished P-TEFb/CDK9-controlled phosphorylation of RNA Polymerase II and rapidly attenuated the expression of multiple anti-apoptotic factors including MCL-1 and BCL-xL. Accordingly, these inhibitors induced dose-dependent apoptosis and DNA damage in TNBC cell lines. CDK9 inhibitor-induced depletion of MCL-1 was found to occur in a proteasome-dependent manner, since proteasome inhibitor MG-132 prevented MCL-1 depletion. Transcriptomic profiling was subsequently performed in order to

elucidate which pathways and transcriptional programmes were most sensitive to disruption by these CDK9 inhibitors. TGF-β, BMP and Wnt/β-catenin signalling, as well as genes involved in cell cycle progression, DNA repair and epithelial-to-mesenchymal transition (EMT), featured prominently amongst the down-regulated genes. The most strongly affected transcripts were enriched for transcription factors; a number of these (SOX9, EN1 and PLAG1) were crucial for the proliferation of multiple TNBC cell lines and were highly expressed in basal-like tumours. Altogether, these findings demonstrate that CDK inhibitors with potent anti-P-TEFb/CDK9 activity are highly efficacious against *in vitro* models of TNBC.

Since resistance to single targeted therapies almost invariably develops in the clinic, the effect of combining the CDK9 inhibitors described in **Chapter 4** with clinically-approved agents or agents under pre-clinical investigation was evaluated in **Chapter 5**. Strong synergy was observed when combining pre-clinical BET inhibitor JQ1 with I-73 or LY3-21, resulting in depletion of MCL-1 and BCL-2 with concomitant activation of the DNA damage response. Combining I-73 and LY3-21 with EGFR-TKIs lapatinib and gefitinib synergistically reversed the insensitivity of TNBC cells to EGFR-tyrosine kinase inhibition and induced G2/M cell cycle arrest accompanied by apoptosis. RNA sequencing-based analysis revealed that co-treatment of TNBC cells with I-73 and lapatinib augmented the general inhibition of transcription induced by I-73. TGF-β signalling, cell cycle progression and stem cell pluripotency were amongst the pathways most strongly down-regulated under co-treatment conditions. A number of transcriptional regulators specifically down-regulated by combining I-73 and lapatinib were associated with a significantly poorer metastasis-free survival in a cohort of TNBC patients. In short, inhibiting P-TEFb/CDK9 function can alleviate the resistance of TNBC cells to EGFR-TKIs and BET inhibitors.

In conclusion, the work presented in this thesis illustrates that CDK inhibitors with potent activity against P-TEFb/CDK9 can be used to target common transcriptional nodes in cancers addicted to receptor tyrosine kinase-mediated signal transduction or which depend on delicately controlled cell cycle progression.