Novel therapeutic molecular targets in lung cancer: non-V600 mutant *BRAF* and mutant *HER3*

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SUMMARY

In three sequential studies, we pre-clinically investigated several previously unexplored lung cancer-derived *BRAF* mutations as well as a *HER3* mutation and their response to clinically available targeted therapeutics. During the FIELT I clinical study at UZ Brussel, in which 229 non-small-cell lung carcinoma patients were prospectively investigated at the genomic level, twelve patients (5.2%) were identified to harbour a *BRAF* mutation in their tumour and one patient found to harbour a novel *HER3* mutation. As opposed to melanoma, 75% of these non-small-cell lung carcinoma-derived *BRAF* mutations were non-V600. RAF inhibitors have only been clinically developed against *BRAF* V600 mutations because of concerns of paradoxical effect in non-V600 mutant cancers. The status of non-V600 mutations with regards to BRAF inhibition effects was unknown. We functionally analysed thirteen of such tumour-derived *BRAF* non-V600 mutations and demonstrated that all types of *BRAF* mutations cause pathway activation and are sensitive to clinically relevant doses of a combination of type I RAF-inhibitor (dabrafenib) and that paradoxical pathway activation is abrogated by MEK-inhibition (trametinib). This entails that dual inhibition of non-V600 mutations is effective and safe. Further, we investigated the comparative efficacy of two modes of RAF inhibition (type I *vs* type II) in suppressing mutant BRAF-induced ERK signalling. Our preclinical findings in non-V600 BRAF expressing cellular models suggest that the type II RAF-inhibition (AZ628) has more potential than the type I RAF-inhibition (dabrafenib), both as single agent and combined with MEK inhibition in suppressing the ERK pathway independent of the *BRAF* mutation type. We also explored a novel somatic lung cancer-derived V855 *HER3* mutation. Our study provided evidence for oncogenicity of V855 *HER3* in a HER2 and ligand-dependent manner, in murine and human cell lines. Further, we showed that the given V855A *HER3* mutation predicts sensitivity to the clinically available HER-targeting therapeutic afatinib. Our findings support the clinical investigation of non-V600 *BRAF* mutated lung or other cancers with dual RAF and MEK inhibition and *HER3* mutant cancers with afatinib.

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INTRODUCTION

In contrast to classical chemo-based therapies, targeted therapies have shown to be safer and more efficient in some cancers.[1-3] The BCR-ABL tyrosine kinase inhibitors in chronic myeloid leukaemia, HER targeting therapeutics in *HER* mutant lung cancer and RAF-inhibitors in *BRAF* mutant melanoma are some relatively successful stories of such therapies.[2,4-7] In some cancers, such as V600E *BRAF* mutant melanoma, combined targeting (RAF and MEK) has been shown to lead to increased efficacy while reducing the risk of resistance.[5,7]

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During my PhD studies, I investigated previously unexplored lung cancer-derived *BRAF* mutations and a *HER3* mutation in lung cancer and their response to clinically available targeted therapeutics.

FINDINGS

STUDY 1: NON-V600 *BRAF* MUTATIONS RECURRENTLY FOUND IN LUNG CANCER PREDICT SENSITIVITY TO THE COMBINATION OF TRAMETINIB AND DABRAFENIB[8]

One of the most promising targeted therapy regiments since a few years ago has been the combination of a type I RAF-inhibitor (dabrafenib) and a MEK-inhibitor (trametinib) for the treatment of ~50% of patients with advanced melanoma who harbour a specific *BRAF* mutation (V600E).[5,6] In contrast, sensitivity of non-V600 *BRAF* mutations to targeted therapeutics had not been studied in melanoma and other types of cancer. *BRAF* mutations depending on their ability to phosphorylate the MEK substrate in acellular *in vitro* kinase assays, are categorised as high or impaired-kinase.[9] Interestingly, impaired-kinase mutations can still activate the ERK pathway, by forming heterodimers with another RAF kinase family member, CRAF. ERK pathway inhibition has been shown to have growth inhibitory effects in cells harbouring either type of *BRAF* mutations.[9-11]

In a clinical study at the UZ Brussel (FIELT study), 229 non-small-cell lung carcinoma (NSCLC) patients were prospectively investigated at the genomic level for mutations in the *EGFR*, *KRAS*, *NRAS*, *HRAS* and *BRAF* genes.[12] Twelve patients (5.2%) were identified to harbour a *BRAF* mutation in their tumour.[8] Interestingly, 75% of these NSCLC-derived *BRAF* mutations were non-V600. We aimed to functionally characterise several NSCLC-derived *BRAF* mutations and investigate the actionability of non-V600 *BRAF* mutations with clinically available ERK pathway inhibitors. We demonstrated that all types of *BRAF* mutations are sensitive to clinically relevant doses of a combination of type I RAF-inhibitor (dabrafenib) and a MEK-inhibitor (trametinib).[8] We investigated the expression of *BRAF* mutant proteins in HEK293T cells and in the lung epithelial cellular model (BEAS-2B) but also in human cancer cell lines harbouring non-V600 *BRAF* mutations.[8] We observed that dabrafenib as a single agent has only weak effects and it may even induce paradoxical ERK activation in cells also overexpressing CRAF. We showed that the combined targeting of RAF and MEK leads to more prolonged ERK inhibition and has both anti-proliferative and pro-apoptotic effects in cells harbouring either type of non-V600 *BRAF* mutations.[8] Our study provides a basis for the clinical exploration of non-V600 *BRAF* mutant lung cancers upon treatment with trametinib and dabrafenib.

STUDY 2: TYPE II RAF-INHIBITION PREDICTS SUPERIOR ERK PATHWAY SUPPRESSION COMPARED TO TYPE I RAF-INHIBITION IN CELLS EXPRESSING DIFFERENT *BRAF* MUTANT TYPES RECURRENTLY FOUND IN LUNG CANCER[1]

As we showed in the previous study, non-V600 *BRAF* mutations predict for sensitivity to the combination of trametinib and a type I RAF-inhibitor dabrafenib.[8] It came therefore as no surprise that due to the lack of clinical studies with RAF and MEK-inhibitors in non-V600 *BRAF* mutant cancers the clinical activity of inhibitors and mechanisms of insensitivity and resistance to these inhibitors remain largely unrevealed. Dimer signalling leads to major challenges with type I RAF-inhibitors.[13,14] 'Early adaptive sensitivity' and 'paradoxical ERK activation' are the two well-described challenges of RAF targeting upon type I RAF-inhibition in V600 mutant cancers. In contrast to type I inhibitors, type II RAF-inhibitors are thought to catalytically inhibit both of the RAF-dimer partners (in case of dimer formation).[15,16] We postulated that with type II RAF-inhibition, stronger ERK pathway inhibition can be achieved in cells expressing *BRAF* mutants, irrespective of mutation type. We addressed the question regarding the comparative efficacy of type I versus type II RAF-inhibition in suppressing mutant *BRAF*-induced ERK signalling. We compared the effects of dabrafenib and a type II RAF-inhibitor (AZ628) on ERK activity, in HEK293T cells expressing several tumour-derived *BRAF* mutants and a non-V600 and impaired-kinase *BRAF* mutant lung cancer cell line (H1666). In contrast to dabrafenib, AZ628 does not induce paradoxical ERK activation in CRAF expressing cells. Increased CRAF expression desensitises *BRAF*-mutant expressing cells to dabrafenib but not to AZ628. Notably, AZ628 has superior ERK-inhibitory effect in HEK293T cells co-expressing several different *BRAF* mutants with CRAF and in H1666 cells. The combination of AZ628 and trametinib has superior MEK-inhibitory and pro-apoptotic effects in H1666 cells compared to combined trametinib/dabrafenib. Moreover, prolonged treatment of H1666 cells with the combination of AZ628 and trametinib results in a superior cell growth inhibitory effect compared to dabrafenib/trametinib. We concluded that the type II RAF-inhibitor AZ628 has more potential than type I RAF-inhibitor dabrafenib, both as single agent and combined with trametinib for the treatment of non-V600 *BRAF* mutant lung cancer.

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STUDY 3: IDENTIFICATION OF A NOVEL *HER3* ACTIVATING MUTATION HOMOLOGOUS TO EGFR-L858R IN LUNG CANCER[17]

In the FIELT 2 study, our laboratory identified one NSCLC patient with a novel *HER3V855A* somatic mutation in his tumour, which is at a position homologous to the frequently occurring L858R *EGFR* mutation.[17] At the time, no *HER3* mutation had been functionally characterised in cancer. Interest was presumably low as HER3 is considered as a pseudokinase with a very weak kinase activity, although, this receptor has a kinase domain that is very similar to the tyrosine kinase domain of the other HER receptors.[18,19] HER3 has known ligands but it can induce downstream signalling only through forming dimers with other HER receptor molecules (preferably HER2).[19,20] We sought to functionally characterise the novel V855 *HER3* mutation and determine its possible role as a driver oncogenic mutation. Moreover, we investigated whether V855 HER3 predicts sensitivity to candidate targeted therapeutics. Our study provided evidence for oncogenicity of V855 *HER3* in a HER2 and ligand-dependent manner, in murine and human cell lines.[17] Further, we showed that the given V855A *HER3* mutation predicts sensitivity to the clinically available HER-targeting therapeutics. We proposed that patients harbouring an oncogenic *HER3* mutation in their tumours could therapeutically benefit from the tyrosine kinase inhibitor afatinib and the monoclonal HER2-antibody pertuzumab.[17] One year after the publication of our results, our claim has been shown to be relevant in *HER3* mutated urothelial cancer.[21] Our HER3 study has laid the foundation for inclusion of *HER3* mutations in any cancer type in a precision multi-cohort basket trial with afatinib in *HER1*, *HER2* or *HER3* mutant cancers that is being launched in all Belgian university medical oncology departments.

CONCLUSION

Taken all together, we conclude that *BRAF* mutations are not rare in lung cancer and the majority of *BRAF* mutations in lung cancer are non-V600E. Our findings suggest that non-V600E mutations predict sensitivity to the combination of dabrafenib and trametinib. Furthermore, we demonstrate that type II RAF-inhibition is more likely to provide efficient ERK pathway suppression compared to type I RAF-inhibition in *BRAF* mutant cells independent of *BRAF* mutation type. Moreover, V855A *HER3*, a rare NSCLC-derived mutation homologous to L858R *EGFR*, is oncogenic and confers sensitivity to available HER targeting therapeutics.

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