ERK1/2 signalling is frequently de-regulated in human cancer due to mutations in receptor tyrosine kinase (RTKs), RAS (especially KRAS), and BRAF (

pathway inhibition in pancreatic ductal adenocarcinoma (PDAC) (Vaseva et al., 2018) (**Figure 3**). Here we critically review the evidence supporting ERK5 as a mediator of BRAFi/MEKi and ERK1/2i resistance, both in BRAF-mutant melanoma and in RAS-driven tumours. We also review the challenges in targeting ERK5 signalling with small molecules, including the off-target effects of early ERK5 inhibitors (ERK5i) and the paradoxical activation of the transcriptional transactivation domain by ERK5i. Finally, we consider new therapeutic modalities that could be employed to target ERK5.

ERK5 Signalling

Like ERK1/2, ERK5 is the effector kinase of a three-tiered mitogen-activated protein kinase (MAPK) signalling cascade comprising the kinases MEKK2 and MEKK3 that phosphorylate and activate dual specificity kinase MEK5, which in turn phosphorylates the activation-loop T-E-Y motif in the ERK5 kinase domain, thereby activating it (Nishimoto and Nishida, 2006) (**Figure 1**). Whilst, MEK5 and MEK1/2 exhibit high sequence similarity, it is increasingly clear that these pathways are parallel, with few if any shared components. For example, MEK5 does not activate ERK1/2 and MEK1/2 do not activate ERK5. Furthermore, whilst early studies suggested that CRAF might directly activate ERK5 signalling (English et al., 1999), it now seems likely that any effect of RAF on ERK5 signalling is indirect and represents feed forward signalling or pathway cross talk (Lochhead et al., 2016) (see below). Consistent with this, whilst the kinase domains of ERK1/2 and ERK5 exhibit high sequence identity, they tend to have distinct substrates. For example, ERK1/2 phosphorylates members of the FOS family of

sufficient for growth factor-stimulated expression or activation of c-FOS or FRA1 (Gilley et al., 2009). Whilst other ERK1/2 substrates have also been proposed as ERK5 substrates (Sap1a, c-MYC, RSK, and SGK) the best validated substrates of the ERK5

dataset and found that ERK5 expression was variable across tumour type, but patients with high ERK5 expression were associated with worse overall survival time. However, functional studies to define how ERK5 drives this poor prognosis have been plagued by off-target effects of early ERK5 kinase inhibitors (ERK5i), most notably against bromodomain containing proteins (Lin et al., 2016), and confounding paradoxical activation of ERK5 signalling. These matters are descri61i23310TD[J-11t bromo-

ERK1/2 signalling was downregulated with decreased phosphorylation of MEK1/2, ERK1/2, and p90-RSK. In contrast, ERK5 phosphorylation was increased (and in some cell lines ERK5 protein levels were also increased), together with an increase in the mRNA levels of the ERK5 target genes c-MYC and c-JUN. Consistent with Tusa et al. (2018), $BRAF^{V600E}$-mutant melanoma cells were sensitive to ERK5 pathway inhibition using the MEK5 inhibitor, BIX02189 (Benito-Jardón et al., 2019). Furthermore, ERK1/2i-resistant cells were more sensitive to ERK5 pathway inhibition than treatment-naïve cells. These results were confirmed using shRNA to ERK5 or expression of MEK5A a dominant negative form of the ERK5-activating kinase MEK5 in which the activation-loop phosphorylation sites, required to be phosphorylated for MEK5 activity, are mutated to alanine to prevent phosphorylation, creating a non-activatable form of MEK5. These authors also established that insulin-like growth factor receptor 1 (IGF-1R) was upregulated (by stabilization) and ERK1/2i-resistant melanoma cells were dependent on IGF1-R activity (using the IGF1-R inhibitor, linsitinib) for cell proliferation in vitro, spheroids and in xenografts (Fig. 4 and B43 and

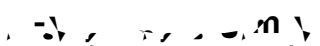
Figu.4(weod)B3vid.n xenograf565(Et,)-roform 857nhory9-n cel88634.1(we84)-524.8(sensiti8e)-432.3(89)-444.5(IGF1-R2

increase in phosphorylated ERK5. In contrast, cells rendered resistant to BRAFi (vemurafenib) plus MEKi (trametinib) or BRAFi (vemurafenib) plus ERK1/2i (SCH772984) exhibited decreased phosphorylated ERK1/2 but increased phosphorylated ERK5 and were sensitive to the MEK5 inhibitor, BIX02189 (Benito-Jardón et al., 2019). One thing that MEKi and the ERK1/2i, SCH772984 have in common is that they both prevent the T-E-Y phosphorylation of ERK1/2. MEKi’

The study by Vaseva provides one example of how ERK5 may contribute to the maintenance of PDAC. However, in contrast to the growing body of evidence in melanoma and other cancers, the rationale for ERK5 inhibition in PDAC is, as yet, less well advanced. Given the high unmet clinical need in PDAC, further work is urgently required here.

for use in the clinic was Imatinib more than 20 years ago. Since then more than 70 protein kinase inhibitors have been approved for clinical use (Cohen et al., 2021). Therefore, it is not surprising

machinery, independent of the therapeutic target, to mediate their effects. These new therapies also face challenges of uptake into the cells and getting to the disease location within the body. Thus, careful consideration should be given to how they will target ERK5 in disease relevant cells.



The last few years have seen an explosion of interest in proteolysis targeting chimeras (PROTACs) as a strategy to drive the degradation of proteins rather than inhibiting certain functions (Chamberlain and Hamann, 2019; Bekes et al., 2022). PROTACs are hetero-bifunctional molecules consisting a ligand for an intracellular target protein-of-interest (POI) and an E3 ubiquitin ligase ligand, joined by a linker which brings the POI and E3 ligase together; this drives polyubiquitylation of the POI and its subsequent degradation by the 26S proteasome. PROTACs offer an alternative approach over classical small molecule inhibitors with some notable advantages. Since the POI is degraded, the PROTAC is recycled to target another copy of the POI. This catalytic mode of action is termed “event-driven pharmacology and sets it aside from the classical one-to-one target-to-inhibitor interaction. Also, by driving destruction of the target POI, PROTACs should provide a more durable effect that will only be reversed by cellular de-ubiquitylase activity or resynthesis of the target POI. Perhaps more importantly, in the context of ERK5, a small molecule inhibitor such as a protein kinase inhibitor typically only targets one function of a protein, whereas the degradation of the protein ablates all functions including catalytic activities, allosteric regulatory sites and scaffolding or protein-protein interaction sites. This may lead to a more pronounced phenotype than targeting just one domain or function; whether this results in too severe a phenotype will ultimately need to be determined empirically, although the phenotype of conditional gene knock-outs in adult mice should inform this approach (Regan et al., 2002).

ERK5 seems like an excellent candidate for a PROTAC-based approach. It has a classical ATP-binding kinase catalytic domain, through which it phosphorylates MEF2 transcription factors. However, like most protein kinases it also has other domains that are sites for further regula. 9 (5 . 8 (u) - 4 (e) 2 b) 2 4 6 . t e

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