

Inhibiting the extracellular signal-regulated kinase 1/2 (ERK1/2) cascade in cancer and the heart: for better or worse, in sickness and health?

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Review

Inhibiting the Extracellular Signal-regulated Kinase 1/2 (ERK1/2) Cascade in Cancer and the Heart: for Better or Worse, in Sickness and Health?

Angela Clerk *, Shona U Amadi, Samuel J Smith, and Peter H Sugden

School of Biological Sciences, University of Reading, Reading RG6 6AS, UK

* Correspondence: a.clerk@reading.ac.uk

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Abstract: The extracellular signal-regulated kinases 1 and 2 (ERK1/2) are the prototypic mitogen-activated protein kinases, first discovered and investigated in the context of cell division and their role in cancer. ERK1/2 are phosphorylated and activated by upstream kinases, MEK1/2 (also known as MKK1/2) that are in turn phosphorylated and activated by RAF kinases (RAF1, BRAF, ARAF), these being activated by small G proteins of the RAS family (HRAS, KRAS, NRAS). The oncogenic nature of the pathway has resulted in the generation of highly specific inhibitors that are successfully used to treat cancer, particularly melanoma. Those in clinical use currently inhibit some isoforms of RAS, RAF kinases and MEK1/2, with additional inhibitors of these kinases in clinical trials. New drugs are now entering the clinic to inhibit ERK1/2 themselves. The ERK1/2 cascade is also important in the heart. It promotes cardiomyocyte hypertrophy and cardioprotection to counter pathophysiological stresses, and plays a significant role in enhancing cardiac fibrosis with detrimental consequences for cardiac function. Here, we summarise the role of ERK1/2 signalling in cancer and the heart, we outline the development of ERK1/2 cascade inhibitors for cancer providing information on those that are approved as cancer treatments and those which are in clinical trials, and we discuss the known and predicted consequences of these ERK1/2 cascade inhibitors for the heart. Integral with this, we consider whether these drugs are necessarily detrimental to the heart or if/when they may be repurposed to prevent or treat heart failure.

Keywords: Cancer; heart failure; cardiac hypertrophy; cardiac fibrosis; extracellular signal-regulated kinase; inhibitor

1. Introduction: Cancer vs Heart Failure in Society

Cancer and heart failure are leading causes of death and disability, having a huge impact on patients, their families and healthcare systems. For both diseases, the problem is increasing worldwide. The numbers of new cases of cancer are predicted to rise from 19.3 million in 2020 to >28 million by 2040, but the drive to understand the molecular basis of cancer over the last 50 years, along with new targeted therapies, means that 10-year survival rates for some cancers are now over 80% [1]. Heart failure cases already exceed 64 million and heart failure is now considered a worldwide pandemic [2]. In contrast to cancer, new therapeutic targets for managing or treating heart failure remain limited. The heterogeneity of cancer is well-recognised by society, and the molecular nature of the individual cancers is taken into account by the scientific/clinical community when considering treatment. For cancer, a precision medicine approach is already taken for many patients (as with, for example, HER2+ breast cancer treated with anti-HER2 therapies, first introduced in 1998 [3]). Heart failure is not as well-recognised, being generally viewed by society as a single disease. It is largely defined according to loss of function of the heart and the worldwide



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consensus from 2021 developed a categorisation according to left ventricular ejection fraction (LVEF): heart failure with reduced ejection fraction (HFrEF; LVEF<40%), heart failure with mildly reduced ejection fraction (HFmrEF; LVEF 41–49%) and heart failure with preserved ejection fraction (HFpEF; LVEF>50%) [4]. This does not provide a clear definition of underlying mechanisms and so cannot facilitate a precision medicine approach.

All drugs developed to treat disease have potential for off-target toxicities, in addition to on-target side effects. Anti-cancer therapies are designed to stop cell proliferation and cell growth, promote cell death directly or via the immune system, destroy cell survival systems and prevent cell migration/invasion, and so have significant potential for on-target toxicities. These drugs can have significant effects on the heart (cardiotoxicities) including prolongation of the QT interval in the cardiac cycle which increases the risk of arrhythmia, effects on LVEF, myocardial ischaemia and hypertension [5,6]. Apart from cardiac toxicities during their therapy, as more patients survive their cancer, there is increased risk of cardiovascular problems developing as a consequence of their cancer treatment [7,8]. As with heart failure in general, the underlying mechanisms for each of these toxicities varies, and the field can benefit from stratification of these different effects. As a first approach, it has been suggested that cardiotoxicities could be classed according to whether the effects are directly on the myocardium affecting the contractile cells themselves, indirect effects on the myocardium (e. g. innervation, perfusion etc.) or due to inflammation (i. e. a form of myocarditis) [5]. Nevertheless, most emphasis on diagnosis of cardiotoxicity is based on LVEF and HFrEF [9]. Because of the increasing incidence of cancer therapy-related cardiovascular risk, there are already many reviews on the topic in general. Here, we focus on the drugs which target the pivotal signalling cascade that drives cell division, the prototypic mitogen-activated protein kinases (MAPKs) now known as the extracellular signal-regulated kinases 1/2 (ERK1/2) [10,11]. As explained below, each component of the cascade is a target for anti-cancer therapies. However, this pathway is also important in the heart and we discuss the known or likely effects of these therapies.

2. ERK1/2 Signalling as a Target for Cancer

2.1. The ERK1/2 Cascade

All MAPK cascades contain a core of 3 protein kinases, each of which is regulated by phosphorylation by upstream kinases (Figure 1). Thus, a MAPK kinase kinase (MAP3K) phosphorylates and activates a MAPK kinase (MKK) which phosphorylates the MAPK. For the ERK1/2 cascade [10,11], the core cascade is initiated by RAF family kinases of which there are 3 isoforms (RAF1, BRAF and ARAF). These kinases phosphorylate and activate MEK1 and MEK2 (also known as MKK1 and MKK2). MEK1 and MEK2 operate as heterodimers [12], and are highly specific dual-specificity kinases whose only substrates appear to be ERK1/2. Thus, MEK1/2 phosphorylate ERK1/2 on both Thr and Tyr residues of a TEY motif to induce full activation. The regulation of each component of the cascade is complex as reviewed in [10,11], with activating and inhibitory phosphorylations affecting protein kinase activities directly, in addition to phosphorylations that promote or disrupt protein-protein interactions. The latter may facilitate or prevent activation, or target the enzymes to different compartments of the cell. ERK1/2 have numerous substrates in the cytoplasm and nucleus that include transcription factors and downstream kinases such as the p90 ribosomal S6 kinases (p90RSKs) that also play an important role in regulating gene expression and promoting cell proliferation.

The ERK1/2 cascade is activated by a wide range of stimuli including peptide growth factors such as epidermal growth factor (EGF) that bind to and activate receptor tyrosine kinases (RTKs). Activated RTKs recruit signalling proteins to the receptor, including adapter proteins such as GRB2 that bring other proteins to the complex like SOS, an exchange factor for RAS small G proteins (HRAS, KRAS and NRAS) [11,13]. RAS acts as a molecular switch for the system: it is inactive when bound to GDP and exchange of GDP for GTP, catalysed by exchange factors such as SOS, results in a conformational change and activation. The innate GTPase activity in RAS terminates its biological activity and is enhanced by RAS-activating proteins (RAS-GAPs), returning it to the inactive state.

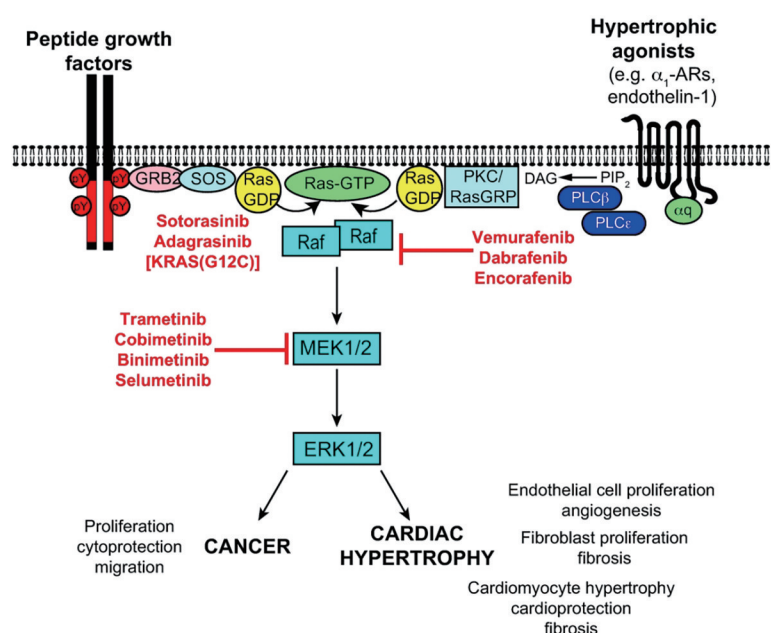


Figure 1. Activation of the ERK1/2 cascade and small molecule inhibitors of the cascade in use for cancer. Peptide growth factors bind to receptor tyrosine kinases causing receptor dimerization and transphosphorylation. The phospho-Tyr recruits the adapter protein GRB2 that binds to SOS, an exchange factor for RAS.GDP. SOS promotes GDP-GTP exchange on RAS and RAS.GTP brings RAF kinases to the membrane where they dimerize and become activated by phosphorylation. RAF kinases phosphorylate and activate MEK1/2 that phosphorylate and activate ERK1/2. Agonists such as endothelin-1 bind to and activate Gq protein-coupled receptors (GqPCRs) to activate the α_q subunit by exchange of GTP for GDP. This activates phospholipase C (PLC) isoforms that hydrolyse phosphatidylinositol 4,5 bisphosphate (PIP₂) to produce diacylglycerol (DAG). DAG activates protein kinase C (PKC) isoforms and the RASGRP family of RAS exchange factors. These promote GDP-GTP exchange on RAS, stimulating the ERK1/2 cascade. ERK1/2 signalling causes cancer by increasing cell proliferation, cytoprotection and cell migration. ERK1/2 are also involved in cardiac hypertrophy acting on cardiomyocytes, fibroblasts and endothelial cells to promote cardiomyocyte hypertrophy, fibroblast proliferation, angiogenesis, cardioprotection and fibrosis. ERK1/2 cascade inhibitors used clinically for cancer target and inhibit RAF kinases (vemurafenib, dabrafenib, encorafenib) and MEK1/2 (trametinib, cobimetinib, binimetinib, selumetinib) with new inhibitors specific for mutant KRAS(G12C) only recently available. These drugs are effective cancer therapies but have potential to affect the heart.

Activated RAS-GTP binds to RAF kinases, bringing them to the membrane for activation by phosphorylation and dimerisation. BRAF and RAF1 can each act as homo- or heterodimers to activate MEK1/2 [14], an important consideration for the development of inhibitors for these kinases. The kinases that phosphorylate RAF kinases are not well defined but RAF1 requires phosphorylation of Ser338 and Tyr341 for activation, potentially by the Ser/Thr kinase PAK1 and Src family tyrosine kinases [15]. BRAF activity is increased by phosphorylation of Ser445 (equivalent to Ser338 in RAF1) but Asp448 substitutes for the phospho-Tyr in RAF1 and the additional negative charge in this position results in high basal activity. RAF kinases form a node for signal integration in the ERK1/2 cascade but are not equivalent. Although the function of BRAF may be solely to phosphorylate MEK1/2, RAF1 inhibits pro-apoptotic kinases including ASK1 and MST2, potentially acting in a kinase-independent manner to sequester them and/or block interaction with downstream kinases [16], whilst ARAF may serve as a scaffold for the other RAF kinases [17].

The ERK1/2 cascade is subject to negative feedback control. The requirement of ERK1/2 for dual phosphorylation of both the Thr and Tyr residues in the TEY motif for full activation means that removal of one or both of these phosphorylations reduces their activity. Early studies showed that one of the immediate early genes upregulated by ERK1/2 signalling is a dual-specificity phosphatase (DUSP) with potential to dephosphorylate and inhibit ERK1/2 [18]. This MAPK phosphatase (MKP1 or DUSP1) was the first of a family of DUSPs to be identified, some of which have clear specificity for ERK1/2 (e.g. DUSP6) [19].

Whereas an immediate early gene such as DUSP1 may be involved in negative feedback control of the activated system, DUSP6 is constitutively expressed and may regulate basal ERK1/2 signalling to manage the threshold for activation. In cardiomyocytes, the Tyr residue of ERK1/2 may be dephosphorylated independently of the phospho-Thr, to produce a monophosphothreonyl form that retains activity, but which may have altered substrate specificity [20]. The identification of this monophospho- form of ERK1/2 raises questions about whether Tyr and Ser/Thr phosphatases other than DUSPs are involved in negative regulation of ERK1/2 activity. Other negative feedback control systems are also important for the ERK1/2 cascade. Thus, SOS1 can be phosphorylated by ERK1/2 and/or p90RSK, inhibiting its ability to promote RAS-GTP loading [21], and feedback inhibitory phosphorylation of RAF1 results in its hyperphosphorylation and desensitization [22].

2.2. Oncogenic Potential of the ERK1/2 Cascade and Development of Anti-cancer Drugs for Inhibition of the Pathway

Activation of ERK1/2 promotes cell division, and any mutations that lead to its activation can be oncogenic [11]. This can include increased activity or expression of receptors that activate the pathway (e.g. increased expression of HER2, a member of the EGF receptor family, accounts for ~15% of breast cancers [23]), mutations in facilitating proteins (e.g. the tyrosine phosphatase SHP2 [24]), or mutations within the pathway itself. Approximately 30% of human cancers are caused by mutations in RAS [11]. The most potent oncogenic driver is HRAS, one of the earliest oncogenes to be identified [25]. However, it accounts for only ~3% of RAS-driven cancers with mutations in KRAS being more prevalent (~85% of cases) [26]. Oncogenic mutations for all isoforms are particularly common in residues 12, 13 and 61 and result in an increase in the rate of GDP-GTP exchange. Although the oncogenic potential of RAS has been known since the early 1980s along with the mutations, it has proved “undruggable” for many years with the first bona fide RAS inhibitors being approved for emergency clinical use over 30 years later in 2022 [27]. These (sotorasib, adagrasib) target the third most commonly mutated form, KRAS(G12C), and are highly specific, utilising the cysteine residue in the GDP-bound (inactive) form for covalent modification, locking the protein in an inactive conformation.

RAF1 was the first member of the RAF kinase family to be identified as a proto-oncogene, but subsequent GWAS experiments identified BRAF as the major oncogenic driver in melanoma with mutation of a single residue (Val600) being responsible for ~90% of BRAF-driven cancers [28]. This resulted in great efforts being made to develop BRAF inhibitors, ideally selective for this common mutation. As a protein kinase with a characteristic ATP-binding site, BRAF is a much more tractable therapeutic target than RAS and the first inhibitors were approved for clinical use for melanoma patients within just 10 years [29]. The three inhibitors so far approved (vemurafenib, dabrafenib, encorafenib) were designed to target mutated BRAF(V600E/K) and are only approved for treatment of patients who have tumours with this mutation, but all have potential to inhibit wild-type BRAF and RAF1, albeit at slightly higher concentrations. Thus, in cell-free assays, the IC₅₀ for vemurafenib inhibition of BRAF(V600E) is 31 nM, whereas IC₅₀ values for inhibition of wild-type RAF1 or BRAF are 48 nM and 100 nM, respectively [30]. Similarly, the IC₅₀ for dabrafenib inhibition of BRAF(V600E) is 0.7 nM with values for wild-type RAF1 and BRAF being 6.3 and 5.2 nM [31], whilst encorafenib has an IC₅₀ of 0.4 nM for BRAF(V600E) that is similar to that for wild-type BRAF and RAF1 [32,33]. This has implications for other tissues including the heart.

Vemurafenib, dabrafenib and encorafenib are all Type 1 or 1.5 inhibitors which bind to a conformation of BRAF that can result in paradoxical activation of ERK1/2 in the presence of activated RAS, the “RAF paradox” [14]. As described above, activated RAS recruits RAF to the membrane for dimerization and activation. With the RAF paradox, RAF remains inactive in the presence of high concentrations of inhibitor; at lower concentrations of inhibitor, only one of the two protomers may be inhibited, with the other retaining signalling potential in a conformation that may be refractory to the inhibitor. Nevertheless, BRAF inhibitors are successful although (as with many cancer treatments) patients can develop resistance and other pathologies (e.g. squamous cell carcinomas [34]). Partly because of this, they are usually used in combination with a MEK inhibitor (vemurafenib with cobimetinib; dabrafenib with trametinib; encorafenib with binimetinib).

Oncogenic mutations in MEK1/2 are less common than in RAS or RAF kinases, and mutations in ERK1/2 themselves are rare [11]. The reasons may relate to the innate amplification potential of a protein

kinase signalling cascade, with mutations at the top of the cascade being potentially more potent in driving oncogenesis. From a therapeutic standpoint, inhibiting the pathway by intervention at any level can be a successful strategy. MEK inhibitors have been developed over many years but, whilst several proved useful for *in vitro* studies of ERK1/2 signalling in cellular responses (e.g. PD98059, U0126, PD184352 [35]) the first of these with clinical application (trametinib) was not approved until 2013 [36], with subsequent approval for use of the MEK inhibitors cobimetinib and binimetinib with vemurafenib and encorafenib (RAF inhibitors), respectively. RAF/MEK inhibitors were originally only approved for treatment of melanoma driven by BRAF(V600E/K) mutations. However, this has been extended with FDA approval for use of dabrafenib/trametinib for non-small cell lung cancer, thyroid cancer, tissue-agnostic cancers with BRAF (V600E) mutations, and paediatric low grade glioma [37–40]. In addition, cobimetinib is approved for use with histiocytic neoplasms [41], vemurafenib is approved for Erdheim-Chester disease [42], encorafenib/binimetinib combination therapy is approved for non-small cell lung cancer [43], whilst another MEK inhibitor, selumetinib has been approved for neurofibromatosis [44]. The importance of these inhibitors in the clinical toolbox for cancer is highlighted by the fast-track designation granted in 2023 for a new BRAF inhibitor, ABM-1310, for glioblastoma (<http://www.abmtx.com/site/newsdetails/88>), in addition to the numerous clinical trials in progress for use of inhibitors that have already been approved for other cancers (Table 1; Supplementary Table S1).

Table 1. Numbers of active clinical trials of ERK1/2 cascade inhibitors with approval for clinical use. The numbers are based on the trials listed in Supplementary Table S1, representing trials from clinicaltrials.gov that are active (recruiting or not-recruiting) or for which recruiting is due to start. * ATP competitive inhibitor; ** Allosteric inhibitor; § other inhibitor type.

Inhibitor	Target	Phase 1	Phase 2	Phase 3	Inhibitor type
Sotorasib	KRAS(G12C)	3	15	4	§ [109]
Adagrasib	KRAS(G12C)	2	8	3	§ [110]
Vemurafenib (without cobimetinib)	RAF	2	18	4	* [25]
Vemurafenib with cobimetinib	RAF/MEK	1	15	3	
Dabrafenib without trametinib	RAF	0	2	0	* [111]
Dabrafenib with trametinib	RAF/MEK	4	17	3	
Encorafenib without binimetinib	RAF	4	8	2	* [112]
Encorafenib with binimetinib	RAF/MEK	1	26	4	
Trametinib (without dabrafenib)	MEK	9	19	0	** [113]
Cobimetinib (without vemurafenib)	MEK	6	19	1	** [114]
Binimetinib (without encorafenib)	MEK	8	19	0	** [115]
Selumetinib	MEK	6	19	5	** [115]

The ERK1/2 cascade inhibitors currently in clinical use are shown in Figure 1, but the importance of the pathway is underscored by continued development of inhibitors for every step in the pathway. There are at least 3 new small molecule inhibitors of KRAS(G12C) progressing through Phase 3 clinical trials with other RAS inhibitors in Phase 1 or 2 (Table 2; Supplementary Table S2). Additionally, a novel RAF/MEK combined inhibitor is in phase 2 trials, with several inhibitors of RAF or MEK at various stages of development, whilst inhibitors of ERK1/2 themselves are in Phase 2 clinical trials (Table 3; Supplementary Table S3). The drugs so far developed to target the ERK1/2 cascade are small molecule inhibitors, many of which target the ATP-binding site of the different protein kinases for inhibition. However, new approaches are being developed to increase specificity, avoid the RAF paradox effect of current RAF inhibitors, and reduce potential toxicity (see, for example, [13]). A key step forward in drug development is the use of PROTACs (proteolysis targeting chimeras) that combine a small molecule inhibitor of the protein of interest with an E3 ubiquitin ligase binder, resulting in ubiquitinylation of the target protein that is then degraded by the proteasome [45]. One advantage is that the target is degraded, which theoretically avoids problems of

incomplete inhibition and, for RAF kinases, potential paradoxical activation of ERK1/2 signalling (although as we discuss below, this is not necessarily the case). Another advantage is that the enzymatic catalytic process means that the drug is reused and targets many molecules instead of just one. Consequently, lower concentrations of drug can be effective, reducing potential side-effects. This also means that a PROTAC does not need to bind its target protein with high affinity and can bind lower affinity sites, expanding potential targeting strategies.

Table 2. Numbers of active clinical trials of RAS inhibitors. The numbers are based on the trials listed in Supplementary Table S2, representing trials from clinicaltrials.gov that are active (recruiting or not-recruiting) or for which recruiting is due to start. X, any amino acid. * Covalent inhibitor; ** Non-covalent inhibitor; § other inhibitor type.

Inhibitor	Target	Phase 1	Phase 2	Phase 3	Inhibitor type
Divarasib	KRAS(G12C)	2	2	0	* [116]
Garsorasib	KRAS(G12C)	1	4	1	* [117]
Opnurasib	KRAS(G12C)	1	2	1	** [118]
MRTX-1133	KRAS(G12D)	0	1	0	§ [119]
RMC-6236	RAS(G12X)	1	0	0	§ [120]
RMC-6291	RAS(G12X)	1	1	0	± [120]

Table 3. Numbers of active clinical trials of inhibitors for RAF, MEK1/2 and ERK1/2. The numbers are based on the trials listed in Supplementary Table S3, representing trials from clinicaltrials.gov that are active (recruiting or not-recruiting) or for which recruiting is due to start. * ATP competitive; ** RAF dimer inhibitor; *** allosteric inhibitor; § other inhibitor type.

Inhibitor	Target	Phase 1	Phase 2	Phase 3	Inhibitor type
ABM-1310	BRAF(V600E)	1	0	0	?
Exarafenib (KIN-2787)	Pan-RAF	1	0	0	** [121]
Naporafenib (LXH-254)	BRAF, RAF1 (ARAF)	4	1	0	** [122]
Lifirafenib (BGB-283)	Pan-RAF, EGFR (and other kinases)	1	0	0	* [123]
Tovorafenib (TAK-580, MLN2480, DAY101)	BRAF, RAF1 (ARAF)	1	3	1	** [122]
Belvarafenib (HM-95573, GDC-5573, RG-6185)	Pan-RAF	2	1	0	** [124]
XP-102 (BI-882370)	Pan-RAF	0	1	0	* [125]
CFT1946	BRAF (V600)	0	1	0	§ [41, 126]
Tinlorafenib (PF-07284890, ARRY-461)	BRAF	2	0	0	* [127]
PF-07799933 (ARRY-460)	BRAF	2	0	0	* [128]
Pimasertib (AS-703026)	MEK1/2	0	2	0	*** [129]
Mirdametinib (PD0325901)	MEK1/2	1	7	0	*** [130]
E6201	MEK1/2	0	1	0	* [131]
Tunlametinib (HL-085)	MEK1/2	1	4	2	? [132]
PF-07799544 (ARRY-134)	MEK1/2	1	0	0	?
Avutometinib (VS-6766, CH5126766 RO5126766)	Dual RAF/MEK inhibitor	1	10	1	*** [133]
Ulixertinib (BVD-523)	ERK1/2	3	4	0	* [134]
LY3214996	ERK1/2	2	2	0	* [135]
ERAS-007 (ASN007)	ERK1/2	0	2	0	* [136]
ASTX029	ERK1/2	0	1	0	*** [137]

PROTACs have been explored for RAS, BRAF and MEK in the preclinical setting. For RAS, the drug LC-2 was based on the small molecule inhibitor adagrasib [specific for KRAS(G12C)] with a binder to recruit von Hippel-Lindau (VHL) E3 ligase [46]. Proof-of-concept studies for BRAF PROTACs used dabrafenib and BI882370 linking either to VHL or cereblon E3 ligases [47]. The best were based on BI882370 and promoted degradation of BRAF (V600E), suppressing proliferation of BRAF(V600E)-driven tumours. Another compound, SJF-0628, is based on vemurafenib coupled to VHL [48]. These PROTACs appear to be more effective at inhibiting tumour growth than the original BRAF binder. However, whilst SJF-0628 has selectivity for oncogenic BRAF (V600E), it binds to and degrades activated wild-type BRAF but retains paradoxical activation of ERK1/2. This is postulated to result from interaction of the vemurafenib-based moiety of SJF-0628 with residual BRAF, resulting in activation of ERK1/2 via the RAF paradox in a similar manner to vemurafenib alone. However, the issue is not fully resolved. Interestingly, the study highlighted the potential to sensitise the system to inhibition by using a MEK inhibitor to prevent negative feedback phosphorylation of SOS1 (see section 2.1), resulting in a greater pool of BRAF in the active conformation that is amenable to inhibitor binding. Although there is limited information about structure and function, at least one PROTAC that targets BRAF (V600E) is already in Phase1/2 Clinical trials [49]. MS432 and MS934 are MEK1/2 PROTACs developed from mirdametinib (PD0325901, a compound in Phase 1 and Phase 2 clinical trials; Table 3 and Supplementary Table S3) coupled to VHL [50]. Other MEK1/2 PROTACs have been developed from refametinib (RDEA119; BAY869766) [51]. Given the efficacy of the MEK inhibitors that are already clinically approved, the advantage of MEK-targeting PROTACs remains to be established. In general, PROTAC research is a rapidly moving field [52]. It remains to be seen how much benefit is gained from the degraders over the small molecule inhibitors.

3. The ERK1/2 Cascade in Cardiac Hypertrophy and Heart Failure: for Better or Worse?

3.1. Cardiomyocyte and Cardiac Hypertrophy

The adult mammalian heart is a robust organ required to manage the blood supply for the body. It accommodates the physiological and pathological events of life, managing an increased workload by increasing in size (i.e. cardiac hypertrophy: growth over and above that which is expected at a specific maturational stage) [53,54]. Physiological stresses such as exercise training and pregnancy increase the workload on the heart, requiring it to increase its output. This is accommodated by cardiac hypertrophy (i.e. enlargement of the heart), largely resulting from an increase in the size of the contractile cardiomyocytes (cardiomyocyte hypertrophy) without any significant accumulation of fibrotic material. Physiological hypertrophy is generally reversible and the heart returns to its normal size on cessation of exercise or delivery of the infant. Pathophysiological stresses also increase the workload on the heart (e.g. hypertension results in pressure-overload on the heart) and cause cardiac hypertrophy, but with different consequences. Initially, cardiomyocytes hypertrophy to increase cardiac output but, in the longer term, other pathological features develop (e.g. increased fibrosis, loss of capillaries). The initial compensatory cardiac hypertrophy becomes decompensated and the heart starts to fail. This may lead to dilated cardiomyopathy and heart failure. Understanding the mechanisms associated with physiological vs pathological hypertrophy and, for the latter, compensated vs decompensated hypertrophy has been a focus for research over many years.

Mammalian cardiomyocytes are terminally-differentiated, largely withdrawing from the cell cycle soon after birth [55,56]. Subsequent growth of the heart results from an increase in the size of cardiomyocytes, coupled with an increase in the supporting structures and the capillary network (maturation growth). Cardiomyocytes are large cells so, although they constitute ~70% of the volume of the adult myocardium, they are only ~30% of the cell number [57]. Other cells are mostly endothelial cells forming the capillary network and fibroblasts that produce the matrix of the heart. Adult cardiomyocytes respond to an increase in workload by increasing in size (hypertrophy) without any significant increase in cell division (hyperplasia) [53,58,59]. This is associated with an increase in myofibrillar apparatus, along with isoform switching and re-expression of genes normally expressed during foetal development. In pathological settings, this compensation is not sustained and, at a cellular level, there is loss of cardiomyocytes, there can be loss of endothelial cells and capillaries resulting in reduced oxygenation of the heart, and there is usually increased fibrosis [60–62].

3.2. The ERK1/2 Cascade in the Heart

ERK1/2 were first shown to be activated in cardiomyocytes and the heart by hypertrophic stimuli over 30 years ago [63]. Since then, many groups worldwide have invested great efforts in understanding how they are regulated and the consequences of their activation [64,65]. In addition to activating MEK1/2 and ERK1/2 themselves, peptide growth factors and hypertrophic stimuli such as endothelin-1 or α 1-adrenergic agonists activate RAS, RAF kinases, and p90RSKs [66–70], all of which are key targets for anti-cancer therapies as described above. Early studies of RAF kinases did not identify BRAF in cardiomyocytes or the heart at the protein level, although BRAF mRNA was detected [70,71]. This is presumably because the antibodies available at the time were not sufficiently sensitive. However, subsequent proteomics analysis of the cardiomyocyte kinome not only demonstrated that BRAF protein is expressed in neonatal and adult rat cardiomyocytes, but also showed the levels of expression are potentially higher than those of RAF1 [72]. Furthermore, BRAF is readily detected by immunoblotting of rodent and human cardiomyocytes and/or hearts using the antibodies that are now commercially-available [68]. BRAF forms pre-existing heterodimers with RAF1 in rat cardiomyocytes and is found in preformed high molecular weight complexes [68]. As in other cells, the basal activity of BRAF is high, but this activity and that of RAF1 is increased by endothelin-1. BRAF plays a physiologically relevant role in cardiomyocytes (as explained below), and activation of BRAF in cardiomyocytes promotes cardiomyocyte hypertrophy whilst cardiomyocyte-specific BRAF knockout compromises cardiac adaptation to hypertension in mice [68,73].

Many studies have used MEK1/2 inhibitors to implicate ERK1/2 in cardiomyocyte responses, along with molecular interventional methods for manipulating the pathway. These studies all indicate that the pathway is important in promoting cardiomyocyte hypertrophy and cytoprotection [64,65,74]. As a driver of cell proliferation ERK1/2 signalling is undoubtedly important in cardiac non-myocytes and can influence endothelial cell and fibroblast accumulation. However, ERK1/2 signalling is also directly implicated in the development of cardiac fibrosis. For example, microRNA-21 activates ERK1/2 signalling in cardiac fibroblasts by downregulation of an endogenous inhibitor of the pathway, Spry1, and promotes fibroblast survival along with interstitial fibrosis in the heart [75]. Moreover, recent studies of interleukin 11 (IL11) highlight the role of ERK1/2 signalling in the interplay between cardiomyocytes and cardiac fibroblasts to increase fibrosis in the heart [76–78].

Preclinical studies of ERK1/2 signalling using genetically-altered mice further implicate the pathway in cardiac pathologies. Gain-of-function studies with cardiomyocyte-restricted expression of MEK1 or knock-in of the V600E mutation in BRAF indicate that pathway activation in isolation promotes cardiomyocyte hypertrophy, along with a compensated form of cardiac hypertrophy [68,79]. In addition, various studies targeting ERK1/2 for inhibition or gene deletion indicate that it is important in cardiomyocytes, promoting hypertrophy and cardioprotection [65]. Of the RAF kinases, RAF1 is particularly important in cardioprotection. Cardiomyocyte-specific deletion of RAF1 in mice leads to cardiac dysfunction/cardiomyopathy at baseline, whilst expression of dominant-negative RAF1 enhances cardiac dysfunction/cardiomyopathy induced by pressure-overload [80,81]. In both situations, there is increased cardiomyocyte apoptosis. This is potentially linked to non-ERK1/2 signalling effects of RAF1, probably via inhibition of ASK1 (apoptosis signal regulating kinase 1), since concomitant deletion of ASK1 in mice with cardiomyocyte-specific deletion of RAF1 prevents cardiomyopathy [81]. As discussed above, there was confusion for many years about whether BRAF is expressed in cardiomyocytes [70]. However, BRAF is expressed in cardiomyocytes and is upregulated in ventricular tissue from human heart failure patients [68]. Furthermore, cardiomyocyte-specific deletion of BRAF inhibits cardiomyocyte hypertrophy and interstitial fibrosis in a mouse model of hypertension [73]. The latter observation is consistent with studies of IL11 that illustrate the importance of cardiomyocyte ERK1/2 signalling in the fibrotic response [76–78].

Apart from its role in pathological hypertrophy and the adult heart, the ERK1/2 cascade is obviously important during early development. Germline mutations in various different components of the RAS-RAF-MEK-ERK1/2 pathway that increase ERK1/2 signalling cause congenital abnormalities, collectively known as the “RASopathies”. These include Noonan Syndrome (NS), Noonan Syndrome with Multiple Lentigines (NSML, previously termed LEOPARD syndrome) and cardiofaciocutaneous syndrome, diseases with overlapping clinical features, varying penetrance and various phenotypes [82]. Perhaps unsurprisingly the

underlying mutations vary both in the affected component and the specific mutation. For example, Noonan Syndrome is caused by mutations in SOS1, KRAS, NRAS, MEK2, in addition to ancillary proteins PTPN11 (SHP2) that facilitates activation of RAS and a scaffolding protein, SHOC2 [83]. NSML is linked to mutations in SHP2 and RAF1 whilst cardiofaciocutaneous syndrome has mutations in KRAS, BRAF, MEK1 and MEK2 [84]. Although individual syndromes are viewed as rare diseases, RASopathies overall affect ~1/1000 live births with cardiac defects in 60-90% of patients. Congenital problems include valve and septal defects, and patients may develop hypertrophic cardiomyopathy [85]. Genetically-altered mice carrying the same mutations recapitulate the same features and the cardiac defects appear to result from developmental abnormalities in cardiomyocytes and endothelial cells [86].

The conclusion of many years of research on ERK1/2 in the heart is that this pathway is equally as important in the heart as in cancer. It undoubtedly plays a vital role in cardiac development requiring strict control of activity. In the adult heart, although it may or may not be important in a healthy physiological setting, it clearly plays a significant role in the adaptive response to pathophysiological stresses. ERK1/2 promote cardiomyocyte hypertrophy and survival but also drive the fibrosis designed to assist in repair but which ultimately compromises the function of the heart. Is ERK1/2 signalling for better or for worse for the heart? Clearly, this depends on the context and we argue that the context determines the effects of anti-cancer drugs designed to inhibit it.

4. ERK1/2 Cascade Inhibitors and the Heart – in Sickness and in Health?

Not unreasonably, the focus of all new anti-cancer therapeutics is success or otherwise in treating cancer, rather than consideration of effects on the heart. This emphasis means that patients may be excluded from early clinical trials if they have a cardiovascular complication (e. g. hypertension). Consequently, although cardiac-related adverse events may develop during clinical trials, cardiovascular complications may not emerge until a particular therapy is used clinically in a wider population over a prolonged period. The corollary is that some drugs could emerge with beneficial effects in a cardiovascular setting. BRAF and MEK inhibitors have been in use clinically for over 10 years and there are some cardiac complications, but there are also indications that these inhibitors may be useful in specific cardiac conditions.

4.1. In Health.

A first consideration is whether inhibitors for the ERK1/2 cascade are detrimental to the heart in patients with no overt cardiovascular complications (i.e. a “healthy” heart). Since cardiotoxicity does not develop in the majority of cancer patients treated with RAF or MEK inhibitors, the evidence suggests that this is not the case. Of the RAF and MEK inhibitors used clinically, vemurafenib (alone or in combination with cobimetinib) is reported to cause prolongation of the QT interval, the time between ventricular depolarization and repolarization [87–89]. QT prolongation is classified as grades 1–4 (450–480 ms, 481–500 ms, >500 ms or >600 ms change, signs of serious arrhythmia [90]). Notably, QT prolongation was not reported as an adverse event in the early trials in which patients with a QT interval >470 ms were excluded [91], although it was reported in studies of the wider population as affecting up to 13.4 % of patients [92,93]. QT prolongation was reported in 4 of 194 patients treated with encorafenib, although there were no reports of QT prolongation in 192 patients treated with encorafenib in combination with binimetinib [94], so the degree to which encorafenib causes QT prolongation appears substantially less than vemurafenib. None of the studies show QT prolongation with dabrafenib and hypertherapeutic dosing with dabrafenib has no effect on QT interval [94,95], so the effect of vemurafenib (and possibly encorafenib) does not appear to be related to inhibition of BRAF *per se*. Nevertheless, effects of these drugs on QT interval remains a problem and 4 of 21 patients treated with ABM-1310 (the recent BRAF inhibitor to be fast-tracked for approval by the FDA) developed a problem with QT prolongation [96]. Other than vemurafenib, dabrafenib appears to be the only RAF inhibitor to have been in extensive trials/treatment as monotherapy and there are no obvious cardiovascular adverse effects [88,89]. One study of encorafenib monotherapy reported 3 patients out of 276 with LV dysfunction [32]. It therefore appears that inhibition of BRAF is not generally detrimental to the heart. This is borne out in preclinical studies of dabrafenib in young healthy male mice and of mice with cardiomyocyte BRAF knockout in which there is no obvious cardiac dysfunction [73,97].

As discussed above, the Type 1/1.5 RAF inhibitors currently used in cancer can promote ERK1/2 signalling via the “RAF paradox” where there is activation of RAS and the inhibitor is not at a saturating concentration [14]. This presumably accounts for the increase in squamous cell carcinomas that can develop with these RAF inhibitors, an effect that can be mitigated by combination therapy with a MEK inhibitor [98]. MEK inhibitors alone or in combination with a RAF inhibitor cause hypertension in some patients and/or reduced ejection fraction [99]. This is seen with different MEK inhibitors (trametinib, selumetinib and cobimetinib) indicating that it is an on-target effect. Hypertension induced by MEK inhibitor therapy affects significant numbers of patients (e. g. ~26% of patients develop hypertension with trametinib [100,101]). Trametinib also causes reduced LVEF in 7–11% of patients [100,101]. However, the majority of patients do not have a significant cardiovascular problem with MEK inhibitor therapy. This is supported by preclinical studies of trametinib in healthy male mice where the drug shows no effect on heart function over 7 d [68] or with more prolonged administration over 28 d (Clerk et al. unpublished data). Others have detected significant cardiotoxicity in mice treated with trametinib beyond 30 days, with ~50% survival at 60 days and reduced ejection fraction, associated with increased oxidative stress and markers of inflammation [102]. The differences between the studies could simply reflect duration of treatment but could be due to experimental conditions. Both studies reported a dosage of 1 mg/kg/d trametinib but we used osmotic minipumps for constant drug delivery whereas Beck et al. [102] provided trametinib in the food with likely variation in intake throughout the day.

The overall conclusion is that inhibiting ERK1/2 signalling in patients does not have a significant effect on a healthy heart at least in the short term. This could simply be because the heart is a robust organ and, unless compromised by disease, cardioprotective mechanisms are sufficient to manage the effects of ERK1/2 inhibition. An alternative explanation is that ERK1/2 signalling is not required in a normally functioning heart, so inhibiting the pathway has no significant consequence in this setting. Having said this, we need to remain vigilant, with established ERK1/2 cascade inhibitors being used more widely for treating more cancers and in more patients, as more patients survive their cancers and live longer and as increasing numbers and types of new drugs enter the market.

4.2. In Disease

Although most patients treated with RAF and MEK inhibitors report no overt cardiovascular events, a significant proportion do, as outlined above, with increased QT interval resulting from vemurafenib (and perhaps encorafenib) treatment in some patients [92–94], and hypertension and cardiac dysfunction being caused by MEK inhibitors in others [99–101]. As more patients treated with ERK1/2 cascade inhibitors for more prolonged periods and more survive their cancer, there has to be consideration that these drugs may affect the heart in later life either because cardioprotective mechanisms become compromised or as cardiovascular diseases begin to manifest themselves. The mechanisms by which trametinib and other MEK inhibitors cause hypertension are unknown, but this affects a substantial number of patients. Similarly, the reason for the fall in ejection fraction with trametinib is largely unexplored, but the reports of cardiac dysfunction in ~11% of patients has to be of major concern [100,101]. In our hands, trametinib inhibits cardiomyocyte hypertrophy in a model in which we activated ERK1/2 signalling [68], and it suppresses cardiac adaptation to hypertension induced by angiotensin II (Clerk et al. unpublished data). This could be beneficial in the short term, but if cardiomyocytes rely on ERK1/2 signalling to maintain survival and/or manage an increased workload, inhibiting ERK1/2 signalling is likely to prove damaging in longer term. Further investigation is clearly warranted.

Of the three RAF inhibitors currently used clinically, each has a different potential effect on the heart. Vemurafenib and encorafenib are Type 1 inhibitors that bind to RAF and lock it in an active conformation, whereas dabrafenib is a Type 1.5 inhibitor that can bind to both active and inactive conformations of the enzyme. These drugs can all induce activation of ERK1/2 via the RAF paradox [14]. Our studies indicate that terminally-differentiated cardiomyocytes do not behave in entirely the same way as proliferating cells with respect to the RAF paradox. Unlike cancer cells in which activation of RAS promotes RAF dimerization, RAF dimers appear to be preformed in cardiomyocytes, with the majority of BRAF forming heterodimers with RAF1 [68]. Dabrafenib failed to induce any significant paradoxical activation of ERK1/2 in these cells

[97]. However, the Type 1 RAF inhibitors SB590885 (not used clinically) and encorafenib each promote paradoxical activation of ERK1/2, stimulate MEK1/2-dependent changes in gene expression and promote hypertrophy in cultured cardiomyocytes [68]. These drugs also promote compensated hypertrophy of mouse hearts *in vivo*, with an increase in cardiomyocyte size in the absence of fibrosis, similar to transgenic mice with cardiomyocyte-specific expression of MEK1 or knock-in of the V600E mutation in BRAF [68,79]. The data suggest that Type 1 RAF inhibitors with RAF paradox-inducing effects could be used to boost cardiomyocyte function. The requirements of such a drug may differ from those required for cancer. For cancer, drugs with a long half-life are likely to be more effective as a therapy but, for effective activation of ERK1/2 signalling in cardiomyocytes via the RAF paradox, a compound with a short half-life would help to ensure submaximal levels of inhibition are achieved. Such drugs would have oncogenic potential so could only be used in the short term, but patients with a failing heart waiting for a transplant require short-term urgent treatment.

The intended target for dabrafenib was BRAF(V600E/K) and there are no reports of cardiotoxicity with the monotherapy treatments originally used [88,89]. Indeed, preclinical studies in a mouse model of hypertension indicate that dabrafenib may be beneficial and reduce cardiomyocyte hypertrophy and cardiac fibrosis [97]. It is not currently certain if this is due to inhibition of ERK1/2 signalling, results from activation of non-ERK1/2 signalling from RAF kinases or is an off-target effect. RAF1 reduces cardiomyocyte death via the pro-apoptotic kinase ASK1, potentially in a kinase-independent manner [16,103,104]. ASK1 is considered a therapeutic target for fibrosis in many diseases, and this is a possible mechanism of action for the effects of dabrafenib on hypertension-associated pathology in mice. The ASK1 inhibitor, selonsertib, has not succeeded in phase II clinical trials for pulmonary arterial hypertension [105] or phase III trials for fibrosis reduction in non-alcoholic steatohepatitis [106]. However, these are diseases with severe and established fibrosis. In our hands, selonsertib is effective in reducing cardiac fibrosis in mice with developing hypertension resulting from angiotensin II [107], so the effect of dabrafenib could be at least partly due to enhanced inhibition of ASK1 by RAF1. Alternatively or additionally, the effect of dabrafenib could result from off-target inhibition of the pro-apoptotic kinase RIPK3 since it is equipotent for inhibition of RIPK3 and RAF kinases [108]. Dabrafenib appears more effective than selonsertib at inhibiting cardiac hypertrophy in mice with developing hypertension [97,107], suggesting it could have multi-target effects. Irrespective of the mechanism involved, dabrafenib may have beneficial effects on the heart to reduce accumulation of fibrotic material and thus maintain cardiac health.

As a final consideration of possible benefits of ERK1/2 cascade inhibitors for cardiac pathologies, it is important to return to the RASopathies (see above). Approximately 10% of these patients have hypertrophic cardiomyopathy and at least some can benefit from MEK inhibitors [85]. Off-label use of trametinib has already proved successful with remission of cardiac hypertrophy in several neonates with Noonan Syndrome and severe cardiac hypertrophy or congestive heart failure [109–111]. It has also been used to treat multifocal atrial tachycardia in a pre-term neonate with Noonan Syndrome [112]. These severe cases clearly merited emergency treatment, and they demonstrate the potential benefits of ERK1/2 cascade inhibitors for the RASopathies as a whole.

5. Conclusions and Future Prospects

The 30 year history of ERK1/2 signalling in the heart has been tremendously exciting with many discoveries, but it has also been enormously challenging to try to understand their functional role. In many ways, the heart field “borrowed” from the cancer field for this work, but the heart is arguably more complex, needing to balance contractile function of terminally-differentiated cardiomyocytes with maintenance and repair systems including proliferation of fibroblasts and fibrosis. The ERK1/2 cascade inhibitors developed for cancer are proving useful experimentally both in cultured cells and in preclinical models to obtain a clearer picture of what this pathway does and how it is regulated. Beyond this, as outlined in section 4 and illustrated in Figure 2A, studies in preclinical models have (probably surprisingly) started to suggest ways in which at least some of these inhibitors may be useful for treating cardiac pathologies.

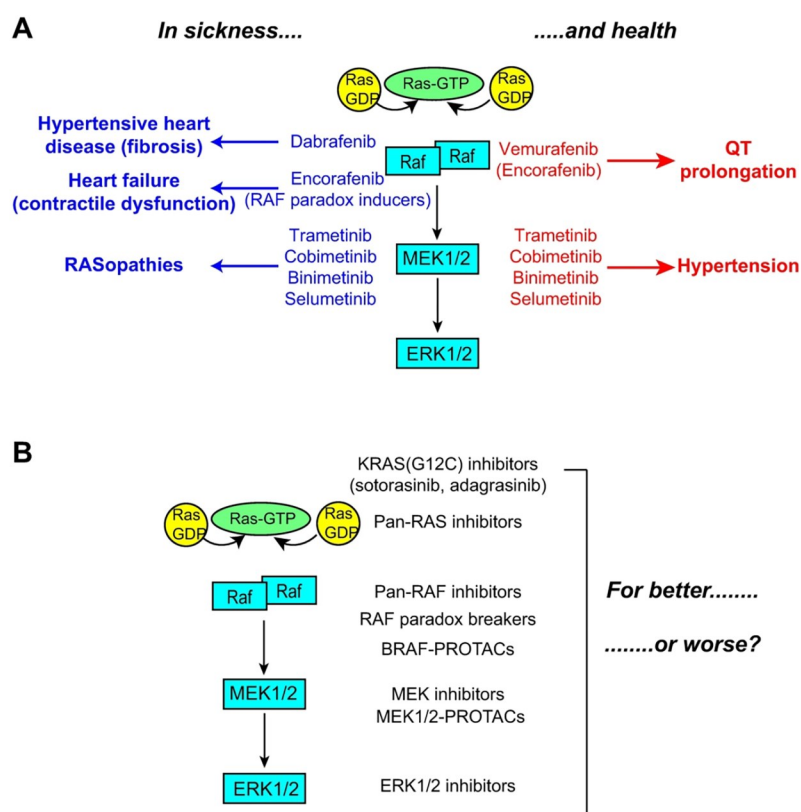


Figure 2. ERK1/2 cascade inhibitors and the heart: in sickness and health, for better or worse? **A**, Inhibitors of the ERK1/2 cascade may cause problems for a healthy heart (red type, right), with vemurafenib (and potentially encorafenib to some degree) causing QT prolongation in ~13% of patients, whilst MEK1/2 inhibitors cause hypertension in up to 26% of patients. It remains to be established if these patients have a pre-existing predisposition. However, ERK1/2 cascade inhibitors could offer benefits in certain cardiac conditions (blue type, left). Inhibition of the pathway with MEK1/2 inhibitors may be beneficial for mutations causing congenital diseases (the RASopathies), dabrafenib may reduce fibrosis in hypertensive heart disease and RAF paradox inducers may provide temporary benefit in promoting cardiomyocyte hypertrophy and function in a terminally failing heart. **B**, The effects on the heart of new classes of inhibitors targeting the ERK1/2 cascade are difficult to predict. Whereas covalent modifiers of specific oncogenic mutations may be benign since heart cells should not carry these mutations [e.g. RAS inhibitors that target KRAS(G12C)], other inhibitors that block the pathway completely (e.g. pan-RAF inhibitors or RAF paradox breakers, new MEK inhibitors and ERK1/2 inhibitors) may be expected to have similar effects as the current MEK1/2 inhibitors. However, new classes of inhibitor such as PROTACs that cause degradation of the target protein via the proteasome may cause additional cellular stresses and the consequences for the heart remain to be established.

The cancer field continues to evolve and, as indicated in section 2 and illustrated in Figure 2B, many more new types of ERK1/2 cascade inhibitor are entering the clinic or in development. The greatest advance is the development of RAS inhibitors (Table 2; Supplementary Table S2). These are largely targeted to mutated forms of RAS, and most are covalent inhibitors targeting the cysteine residue of KRAS (G12C) for modification [27]. As such, they appear very specific. However, of 116 patients treated with adagrasib, one patient died of cardiac failure [113]. This may be unrelated to any inhibition of RAS, particularly since there are no reported cardiac problems with sotorasinib [114], but these drugs are in very early stages of clinical assessment and it will be important to monitor their potential cardiotoxicity. BRAF and MEK inhibitors continue to be developed (Table 3; Supplementary Table S3) with a huge market potential for these drugs to treat an increasing number of different cancers. With the RAF paradox effects of current RAF inhibitors, the approach to inhibiting BRAF has moved to develop “paradox breakers” and allosteric inhibitors that are not prone to activating ERK1/2 signalling in the same way [13]. The effects of these drugs on the heart are difficult to predict, but as the RAF inhibitors become more effective, with development of pan-RAF inhibitors that avoid the RAF paradox, they may be expected to have the same consequences as MEK

inhibitors with respect to inhibiting ERK1/2 signalling. However, the consequences may differ for isoform-selective inhibitors such as PLX8394, a paradox-breaker which prevents homodimerization of BRAF or heterodimerisation of BRAF with RAF1, but which still permits RAF1 homodimer signalling [115]. In addition to RAF and MEK1/2, there are efforts to develop inhibitors for ERK1/2 themselves (Table 3; Supplementary Table S3). The first of these in clinical trials, ulixertinib, carries a low risk of QT prolongation [116], and the current trials all exclude any significant cardiovascular disease. However, ERK1/2 are the only known substrates of MEK1/2, so on-target effects of ERK1/2 inhibitors may be expected to have similar consequences for the heart as on-target effects of MEK inhibitors.

There is considerable excitement about the development of PROTACs for enzyme degradation [45], as discussed above. The likely effects of PROTAC drugs as a whole, irrespective of their protein target, on cardiomyocytes and the heart are impossible to predict. Proteostasis is vital for the heart and disruption of the system is associated with increase oxidative stress and development of cardiomyopathies [117,118]. Whilst they may not have any significant effect on the heart and cardiomyocytes, PROTAC drugs have the potential to overload and stress the system. Our preliminary data suggest this may well be the case, with activation of stress-responsive MAPKs, the c-Jun N-terminal kinases (JNKs; Clerk et al. unpublished data). This stress will be additional to the intended consequences of disrupting the signalling pathway. Furthermore, loss of individual proteins will alter protein stoichiometry, an important factor in intercellular signalling in non-cancerous cells. For example, RAF kinases exist in large, preformed complexes in cardiomyocytes [68], so loss of BRAF is likely to alter the RAF kinase complement in those complexes, potentially altering the signalling profiles in these cells.

As a final comment, just as cancer research evolves to find new methods for treating cancer, the cancers themselves also evolve with resistance to RAS, BRAF and MEK inhibitors, often a result of rewiring of signalling pathways and activation of parallel systems [119]. This leads to consideration of therapeutic combinations of other drugs with ERK1/2 cascade inhibitors such as immune checkpoint inhibitors [120]. The consequences of these combinations for the heart remain to be seen.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.sciltp.com/journals/ijddp/2024/2/375/s1>, Table S1: Active clinical trials of ERK1/2 cascade inhibitors with approval for clinical use; Table S2: Active clinical trials of RAS inhibitors; Table S3: Active clinical trials of inhibitors for RAF, MEK1/2 and ERK1/2.

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