Targeting FAK in anticancer combination therapies

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Abstract | Focal adhesion kinase (FAK) is both a non-receptor tyrosine kinase and an adaptor protein that primarily regulates adhesion signalling and cell migration, but FAK can also promote cell survival in response to stress. FAK is commonly overexpressed in cancer and is considered a high-value druggable target, with multiple FAK inhibitors currently in development. Evidence suggests that in the clinical setting, FAK targeting will be most effective in combination with other agents so as to reverse failure of chemotherapies or targeted therapies and enhance efficacy of immune-based treatments of solid tumours. Here, we discuss the recent preclinical evidence that implicates FAK in anticancer therapeutic resistance, leading to the view that FAK inhibitors will have their greatest utility as combination therapies in selected patient populations.

Focal adhesions
Points of cellular plasma membranes that link to extracellular matrix via transmembrane receptors, typically integrin heterodimers.

Focal adhesion targeting (FAT) domain
A protein domain that is involved in the localization of focal adhesion kinase (FAK) to focal adhesions through interactions with other focal adhesion proteins.

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https://doi.org/10.1038/s41568-021-00340-6

Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that classically transduces signalling from cell adhesions to regulate multiple biological cellular functions, including cell survival, migration, and invasion of cancer cells[1,2] (BOX 1). Briefly, engagement of transmembrane integrin receptors with extracellular matrix (ECM) recruits FAK to sites where integrins cluster, termed 'focal adhesions.' FAK does not interact with integrins directly but instead binds to the membrane and to other focal adhesion proteins, such as paxillin and talin, through its carboxy-terminal focal adhesion targeting (FAT) domain[3]. Once recruited to focal adhesions, FAK becomes catalytically active in a multistep process. Initially, inactive FAK forms dimers that interact with phosphatidylinositol 4,5-bisphosphate-rich membranes that dissociate the autoinhibitory interaction between the FERM (4.1 protein, ezrin, radixin and moesin) domain and the kinase domain of FAK and expose the autophosphorylation site tyrosine 397 (Y397) for trans-autophosphorylation[4]. Once phosphorylated, FAK acts as a molecular scaffold and recruits SRC family kinases to phosphorylate FAK on Y576 and Y577 within the activation loop of the kinase domain[4]. Autophosphorylation of FAK on Y397 is a major target of its catalytic activity and is a key step in its priming for activation and subsequent signalling from focal adhesions[2,4,5], thereby making it an attractive target for anticancer therapy. FAK also acts as an adaptor protein at focal adhesions, which can be independent of its kinase activity, to recruit other proteins to link through to the actin cytoskeleton (BOX 1).

FAK is encoded by the *PTK2* gene; *PTK2* mutations are rare in cancer, primarily occurring in tumours of the uterus (9.24% of 530 cases), colon (7.25% of 400 cases) and liver (3.92% of 51 cases), and consisting largely of missense, nonsense or frameshift events according to data from The Cancer Genome Atlas (TCGA) research network[6]. *PTK2* mutations show no preference for location or functional domain. Although *PTK2* gene mutations are rare, gene amplification and increased FAK expression are common in a number of cancers[1,2]. More than 20% of ovarian cancers (including high-grade serious ovarian carcinoma (HGSOC)), lung squamous cell neoplasms, oesophageal cancers and uveal melanomas have an increased copy number of *PTK2*, which is predictive of poor patient outcome according to data from the TCGA research network[7–9].

As first shown by Agochiya et al.[10] in 1999, many cell lines derived from invasive epithelial tumours have increased copies of *PTK2*, located at chromosome band 8q24.3. Amplification of 8q24 is common in tumours[11,12] including increased copies of the *MYC* oncogene[6,13]. The amplification of this chromosomal region is thus suggested to be associated with *MYC*. Yet, in some tumours, such as head and neck squamous cell carcinoma (SCC), *PTK2* gains occur more frequently than *MYC* gains; therefore, it cannot be assumed that it is always, or only, elevated *MYC* expression contributing to cancer phenotypes[7–9].

FAK activation in tumours occurs via well-described mechanisms following engagement of integrin-mediated cell adhesions or, alternatively, by activating mutations in heterotrimeric G proteins in uveal melanoma or mutations of RAS homologue family member A (RHOA) in diffuse gastric cancer, both of which activate RHOA-dependent and FAK-dependent focal adhesion signalling[8,14]. FAK

FERM (4.1 protein, ezrin, radixin and moesin) domain
A protein domain that is involved in localizing proteins to the plasma membrane.

Heterotrimeric G proteins
A GTPase complex made up of three subunits, α, β and γ, that links transmembrane receptors to intracellular signalling pathways.

Ras homologue family member A
(RHOA). A small GTPase primarily associated with regulating the actin cytoskeleton.

also has what are considered non-canonical roles in cancer cells that are experiencing cellular stress, as well as nuclear functions involving regulation of p53 degradation and cytokine expression[15,16].

Proline-rich tyrosine kinase 2 (PYK2; also known as PTK2B) is a closely related paralogue of FAK with ~48% amino acid similarity; it also shares analogous structural domain organization and many protein-binding partners[2,17]. FAK and PYK2 can have redundant roles; for example, PYK2, like FAK, regulates WNT–β-catenin signalling in colorectal cancer models[18]. However, FAK and PYK2 can also perform distinct roles; for example, PYK2 weakly localizes to focal adhesions[19,20] and aberrant PYK2 activation can inhibit cell cycle progression[17,21]. FAK knockout or pharmacological inhibition can increase PYK2 expression or phosphorylation in both normal and cancer cells in vitro and in vivo[22–24]. FAK inhibitors fall into two categories: FAK inhibitors (for example, PF-573,228 (REF.[25]) and IN10018 (also known as BI 853520)[26]) or dual FAK/PYK2 inhibitors (for example, defactinib (also known as VS-6063 or PF-04554878)[27] and PF-562,271 (REF.[28])). Both types of FAK inhibitors are being tested clinically (TABLE 1), and it is not clear whether using FAK inhibitors or dual FAK/PYK2 inhibitors would provide any specific differences in clinical efficacy.

Despite the accumulated evidence that FAK plays important roles in cancer progression[18,29–33], clinical studies of single-agent FAK inhibitors have resulted in limited efficacy[34–37]. Given the genetic, cellular and stromal complexity of advanced solid tumours, it is perhaps not surprising that monotherapies targeting individual signal transduction proteins or pathways are often unsuccessful; single agents targeting molecular drivers of disease are the rare exception, for example BRAF inhibitors in BRAF-mutant melanoma[38]. Melanomas frequently harbour activating mutations (~60%, most commonly V600E) in BRAF, and although mutant BRAF inhibitors such as vemurafenib have performed well clinically, patients often relapse due to several well-documented genetic and non-genetic resistance mechanisms[39,40].

FAK inhibition has recently been identified as a potential strategy to overcome adaptive resistance to chemotherapy[41,42], radiotherapy[30,43–45] or targeted therapies (including BRAF inhibitors in *BRAF*-mutant cancers)[46–48] or therapies that target the immune microenvironment[16,42,49,50]. There are now excellent examples of biological scenarios in which FAK is a key mediator of therapeutic resistance, via tumour cell survival signalling mechanisms or influence over the tumour microenvironment, or both[8,16,41–43,47,48,51–54]. Indeed, it could be argued that FAK inhibitors used as a monotherapy may be likely to supress the more classical adhesion and migration roles of FAK as primary effects, which is perhaps unlikely to have a major impact on the outcomes of patients with advanced cancers; an aspiration of combination therapeutics would therefore be to also specifically target the roles of FAK in buffering therapeutic stress and so trigger cancer cell death or an immune response. Therefore, there is growing interest in how best to use FAK inhibitors as therapeutic combinations in clinical trials. Here we review recent insights that highlight the role of FAK in the regulation of inherent and acquired resistance to antitumour therapies.

Box 1 | **Kinase and adaptor functions of FAK**

Focal adhesion kinase (FAK) was first discovered as a heavily tyrosine phosphorylated protein located at focal adhesions[1,2,137–139]. FAK is also an adaptor protein. Multiple domains, including the so-called FERM domain[5], are involved in the binding of key cellular protein partners and their co-recruitment into larger heteromeric protein complexes. More recently, it has become clear that FAK also acts as a scaffold for transcriptional regulatory complexes in the nucleus[16,50,127], underlining the importance of FAK's adaptor functions in cell biology. These protein–protein interactions have not been directly targeted for therapeutic purposes. However, it is likely that certain scaffolding interactions of FAK are regulated by its kinase activity via protein conformational switches[128].

Nuclear localization of FAK is evident from biochemical fractionation studies and may be a function of cellular response to stress. FAK functions in the nucleus by acting as a scaffold for transcriptional regulators, including p53 in some scenarios[15], and influencing the expression of biologically important target genes, such as those encoding chemokines that influence the tumour microenvironment and antitumour immunity[16,49]. Little is known about why and how FAK translocates to the nucleus or whether the nuclear pools of FAK exist independently with those at peripheral focal adhesions. One hypothesis is that FAK is part of stress-sensing and stress-response machinery at the cell periphery and that upon receipt of specific cues in the cellular environment, FAK moves to the nucleus to perform functions that buffer cells against stress-induced cell death[15]. This may form part of perhaps multiple mechanisms by which elevated FAK expression and activity contribute to tumour cell survival, including under unfavourable conditions such as after chemotherapy[41]. It is equally possible that canonical FAK signalling at cell adhesions, including its kinase and/or scaffolding activity, is an important survival signalling initiating event that can be modulated by FAK translocation to and signalling within the nucleus.

FAK inhibitor-based combinations

The critical role of autophosphorylation in FAK activation has led to the development and clinical testing of a number of therapies that target FAK catalytic activity, including defactinib, IN10018, VS-4718, GSK2256098 and PF-573,228 (REFS[1,2,5]). Among these, defactinib and IN10018 are currently being evaluated as part of combination therapies in phase I or phase II clinical trials (TABLE 1). Defactinib and IN10018 both inhibit FAK at low nanomolar concentrations in vitro (0.4–0.6 nM and 1 nM, respectively)[26,36]. Defactinib was developed as a second-generation FAK inhibitor, and inhibits nine other kinases in vitro with a half-maximal inhibitory concentration (IC_{50}) less than 1 μM (REFS[27,55]) (TABLE 2). By contrast, IN10018 is a FAK inhibitor co-targeting only four other kinases (of 262 tested) with in vitro IC_{50} less than 1 μM (REF.[26]). Inhibition of PYK2 by IN10018 is negligible (IC_{50} = 2–50 μM)[26], while defactinib inhibits PYK2 at low nanomolar concentrations (IC_{50} ranging from 0.6 nM (REF.[36]) to 423.4 nM (REFS[27,55])).

Despite the limited efficacy of FAK inhibitors as monotherapy in cancer treatment[34–37], FAK is clearly an important signalling 'hub' through which cancer cells buffer stress following chemotherapy[41,42], radiotherapy[43–45] or targeted therapies[46–48]. FAK is therefore a rational target for combination therapy, where co-targeting may reveal vulnerabilities that are not evident from use of FAK inhibitors or other therapies alone — especially as FAK inhibitors are orally administered and well tolerated clinically[2,34–36]. However, this approach requires evidence-based combination strategies and clearly

Table 1 | **Active clinical trials with FAK inhibitor combinations**

FAK-targeting compound	Combination agents	Molecular targets	Cancer type	Clinical trials
Defactinib (also known as VS-6063 or PF-04554878)	Pembrolizumab and gemcitabine	PD1	Solid tumours/advanced solid tumours Expansion cohort: pancreatic cancer	NCT02546531 (phase I)[68]
	Pembrolizumab	PD1	Non-small-cell lung cancer, mesothelioma, pancreatic neoplasms	NCT02758587 (phase I/IIA)[67]
	Paclitaxel and carboplatin	–	Ovarian cancer	NCT03287271 (phase I/II) ROCKIF trial[66]
	Pembrolizumab	PD1	Pancreatic ductal adenocarcinoma	NCT03727880 (phase II)[65]
	VS-6766	MEK and RAF	Advanced RAS-mutant solid tumours: non-small-cell lung cancer, low-grade serous ovarian cancer, colorectal cancer and other RAS-mutant solid tumours	NCT03875820 (phase I)[64]
	Pembrolizumab	PD1	Malignant pleural mesothelioma	NCT04201145 (phase Ia-Ib)[63]
	VS-6766	MEK and RAF	Non-small-cell lung cancer with a KRAS activating mutation	NCT04620330 (phase II)[60]
	VS-6766	MEK and RAF	Low-grade serous ovarian cancer	NCT04625270 (phase II)[59]
	Radiotherapy	–	Pancreatic cancer	NCT04331041 (phase II)[61]
	VS-6766	MEK and RAF	Metastatic uveal melanoma,	NCT04720417 (phase II)[100]
IN10018 (also known as BI 853520)	Cobimetinib	MEK	Metastatic uveal melanoma or cutaneous NRAS-mutant melanoma	NCT04109456 (phase Ib)[62]

FAK, focal adhesion kinase; PD1, programmed cell death 1.

defined patient stratification, without which combination therapies are likely to fail. Preliminary data from an early clinical trial in patients with advanced or refractory ovarian cancer demonstrated partial responses to the combination of defactinib and paclitaxel[56], while preliminary results from a trial of GSK2256098 in combination with the MEK inhibitor trametinib in patients with advanced pancreatic cancer showed no activity[57]. In addition, the combination of GSK2256098 and trametinib failed to show any improvement in patients with advanced mesothelioma or other solid tumours and RAF–MEK–ERK pathway activation, with the best response being disease stabilization in 13 patients (38%)[58].

To identify patients most likely to benefit from FAK inhibitor-based combinations, a greater understanding of how FAK signalling interacts with druggable oncogenic drivers and of the role of FAK in adaptive and acquired therapeutic resistance is required. To this end, we describe preclinical studies that highlight an important role for FAK in the survival and growth of particular cancers after therapeutic intervention, providing a rationale for specific combination therapies; in turn, each of these preclinical studies have led to clinical trials evaluating FAK inhibitors (defactinib or IN10018) in combination with RAF and/or MEK inhibitors, anti-programmed cell death 1 (PD1) immunotherapy, and chemotherapy or radiotherapy[59–68] (TABLE 1). We select key exemplars of FAK-mediated control of resistance to therapy that represent exciting co-targeting opportunities.

FAK mediates resistance to therapy

FAK in HGSOC chemotherapy resistance. *PTK2* is amplified in HGSOC, the subtype of ovarian cancer with the highest mortality[69], more than in any other cancer. Importantly, increased FAK expression is concordant with *PTK2* gains in HGSOC, and this is associated with tumour progression and a poor prognosis[2,41]; in contrast, no such association exists for MYC in this setting[70,71]. As we now know that cellular roles for FAK extend beyond promotion of cell adhesion and motility, HGSOC represents a good model system in which to dissect the connections between FAK and its role in chemoresistance.

Standard of care for patients with HGSOC is cytoreductive surgery followed by carboplatin (which induces DNA damage) and paclitaxel (which stabilizes microtubules) chemotherapy to kill residual tumour cells[72,73]. In contrast to pancreatic cancer, which displays high levels of FAK tyrosine phosphorylation in the stromal cells surrounding tumours (as evidenced by immunohistochemical staining)[54,74], HGSOC is characterized by elevated FAK tyrosine phosphorylation within the tumour cells themselves[41]. Although basic research[75] and clinical studies[56,76] have examined the role of FAK in promoting paclitaxel resistance in HGSOC, the role of FAK

Programmed cell death 1 (PD1). A protein expressed on the surface of cells that inhibits the activation of the immune system.

Stromal cells
Connective tissue cells such as fibroblasts that support the other cells of that organ.

Hippo pathway
A signalling pathway that controls organ size by regulating cell proliferation and apoptosis that can be dysregulated in cancer.

in mediating platinum chemotherapy resistance is less well understood. In paired patient tumour samples taken before and after several cycles of carboplatin and paclitaxel chemotherapy, FAK tyrosine phosphorylation was elevated after chemotherapy in non-necrotic residual tumour cells[41]. In HGSOC models in vivo, FAK Y397 phosphorylation — a surrogate for activity — increased upon sublethal cisplatin treatment of platinum-resistant tumours. Interestingly, treatment with cisplatin, but not paclitaxel, led to increased FAK Y397 phosphorylation in anchorage-independent tumourspheres in vitro[41]. Since platinum-induced cell stress can activate FAK, it has been suggested that FAK activation may function to permit acquired platinum tumour resistance[41].

Few ovarian tumour models exist to study the impact of copy number alterations on tumour state. Immortalized ovarian epithelial cells (ID8) from mice form slow-growing tumours[77]; however, isolation and expansion of early ascites cells from these mice generated spontaneously aggressive counterparts that readily form tumours in vivo[78]. These cells contained chromosome gains encompassing several amplicons in common with HGSOC[41]. One region of interest included murine chromosome band 15qA1-D3, which contained the genes encoding MYC, FAK and RECQL4, a region orthologous to human 8q24.3, which is often amplified in epithelial cancers. Exome sequencing did not reveal de novo oncogenic somatic mutations, supporting the notion that gains in this region may be an important driver of tumour malignancy. These aggressive tumour cells were therefore termed 'KMF cells', denoting gains in the genes encoding KRAS, MYC and FAK[41]. KMF cells exhibited elevated FAK Y397 phosphorylation, increased β-catenin transcriptional activity, stem-like properties, including enhanced tumoursphere-forming capability in vitro and augmented detoxifying enzyme (such as aldehyde dehydrogenase) expression, and greater intrinsic resistance to cisplatin-mediated cytotoxicity compared with the parental ID8 cells[41] (FIG. 1). As β-catenin is linked to cisplatin resistance in HGSOC[79], and FAK signalling to β-catenin is an adaptive chemoresistance pathway in *BRAF*-mutated colon cancer[46], this pathway may promote resistance to radiotherapy and other chemotherapies in HGSOC and other types of cancer[18,30,45]. A central role for FAK in promoting stemness-associated characteristics suggests that FAK inhibitors could complement classical chemotherapy by targeting populations of cells that are resistant to treatment[41]. This rationale underlies the ROCKIF trial[66] (TABLE 1), in which FAK inhibitors are being used to resensitize platinum-resistant ovarian cancers by targeting stem-like cells in the tumour compartment.

With use of the KMF model of HGSOC, pharmacological and genetic approaches were used to delineate the role of FAK in both intrinsic and acquired resistance to platinum chemotherapy. Transcriptomic and bioinformatic analyses of FAK-knockout and FAK-reconstituted murine KMF cells, as well as data from a TCGA HGSOC cohort, revealed 135 targets that were upregulated in HGSOC, including genes associated with chemoresistance, stemness and the regulation of cell metabolism. All 135 genes were induced by the expression of an active form of β-catenin within cells lacking FAK[41]. Among these, many were related to DNA repair and DNA replication processes. Kinase-dependent FAK signalling was also linked to increased expression of Hippo pathway components[41]. In uveal melanoma, FAK activates the transcriptional activator YAP via MOB1 phosphorylation, also resulting in Hippo pathway inhibition[8]. Although the activation of WNT–β-catenin signalling by FAK may represent an important adaptive signalling response to stress[80], it is clear that FAK activity is more complex, since β-catenin overexpression and activation was sufficient to induce chemoresistance, yet paradoxically insufficient to rescue FAK-null tumour growth defects in KMF mice[41].

Transcriptomic analysis of patient HGSOC samples showed elevated FAK mRNA levels, which was prognostic for decreased relapse-free survival among patients receiving chemotherapy, and altered expression of a set of 36 genes[41]. Within this set of genes, none was documented as an oncogene or a tumour suppressor in the COSMIC database. Among 25 upregulated transcripts, many were products of genes on chromosome arm 8q that were amplified, like FAK, and six were products of genes on other chromosomes (*ST6GALNAC5*, *SPON1*, *PTGER3*, *KRT14*, *NRP2* and *ATP10A*). Among

Table 2 | **Kinase selectivity of current clinical FAK inhibitors**

Compound	Protein target (gene name)	Assay type and IC_{50} (nM)			
		Kinobeads[27]	Kinase inhibition[36]	Z'-LYTE[26]	DELFIA[26]
Defactinib (also known as VS-6063 or PF-04554878)	*PTK2*[a]	0.5	0.6	–	–
	CDKL5	16.5	–	–	–
	KIAA0195	20.7	–	–	–
	FAM58A, *FAM58BP*	236.6	–	–	–
	MOB1A, *MOB1B*	318.2	–	–	–
	FLT3	339.8	–	–	–
	CABLES1	371.2	–	–	–
	FIBP	404.7	–	–	–
	PYK2	423.4	0.6	–	–
	NUAK2	453.6	–	–	–
	KLHL6	494.2	–	–	–
	AURKA	622.3	–	–	–
	CDK12	730.2	–	–	–
	STK16	892.9	–	–	–
	MELK	897.0	–	–	–
	MAP3K11	902.1	–	–	–
	NTRK1	917.5	–	–	–
IN10018 (also known as BI 853520)	*PTK2*[a]	–	–	38.1	1
	FER	–	–	903	–
	FES	–	–	1,040	–
	PYK2	–	–	2,000	>50,000

The kinobead assay profiles the interaction of small-molecule inhibitors with the endogenous proteome[133]. The Z'-LYTE and DELFIA assays are fluorescence-based readouts of kinase activity, reviewed in Ma et al.[134]. FAK, focal adhesion kinase; IC_{50}, half-maximal inhibitory concentration. [a]The *PTK2* gene encodes FAK.

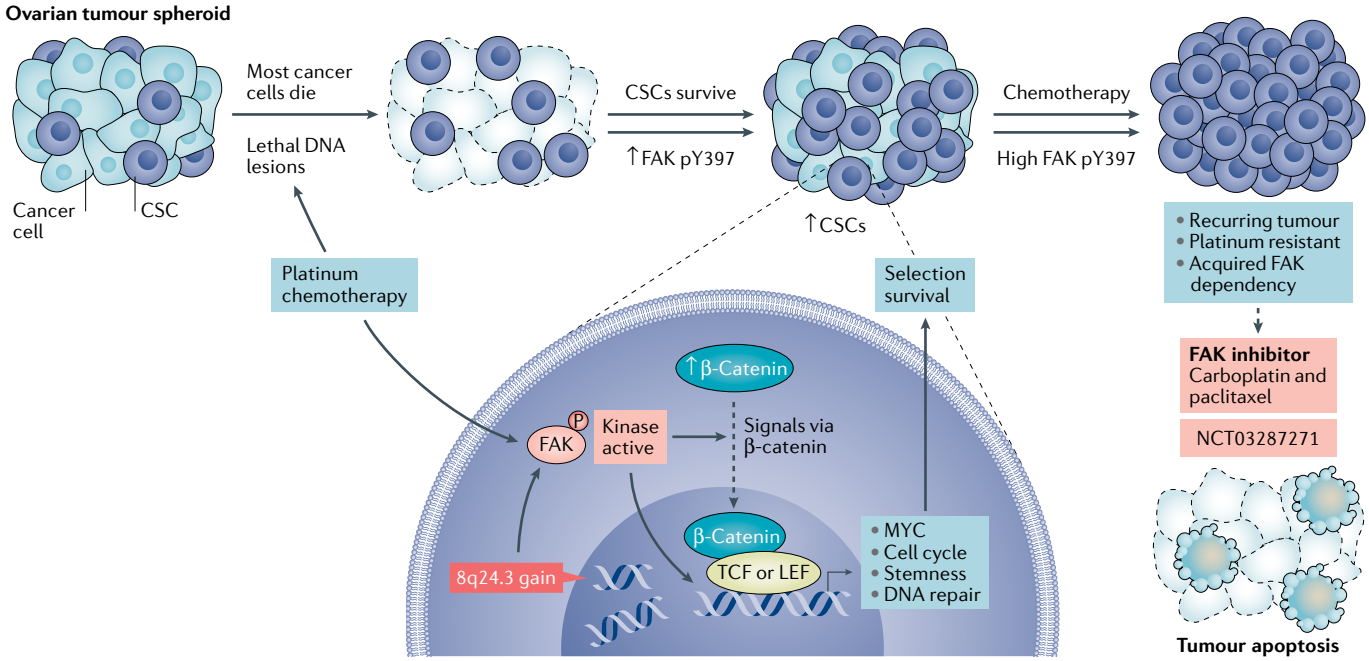

Fig. 1 | **FAK mediates resistance to therapy in high-grade serous ovarian cancer.** The figure illustrates chromosome gain at 8q24.3, a region that includes the FAK-encoding gene *PTK2*, and downstream consequences of focal adhesion kinase (FAK) activation in high-grade serous ovarian cancer (HGSOC). Gene breakage, gains or losses are common drivers of HGSOC phenotypes; more than 70% of patient tumours have gains at both the *PTK2* locus and the *MYC* locus within 8q24. FAK signalling sustains tumoursphere growth and cancer stem cell (CSC) survival and increases platinum resistance. Although the mechanisms of FAK activation in tumourspheres remain to be determined, intrinsic FAK activity is needed for β-catenin-associated increased expression of MYC, as well as genes involved in cell cycle, pluripotency and DNA repair. Spontaneous and induced platinum resistance is associated with high levels of FAK tyrosine phosphorylation and acquired dependence on FAK activity in cell culture. As the combination of a small-molecule FAK inhibitor and cisplatin triggers platinum-resistant tumour cell apoptosis, a clinical trial[66] is now testing the combination of FAK inhibition, carboplatin and paclitaxel for recurrent platinum-resistant HGSOC, a condition for which no approved treatments are available. LEF, lymphoid enhancer-binding factor; pY397, tyrosine 397 phosphorylation; TCF, T cell factor.

the 11 FAK-downregulated genes associated with poor patient outcome was brain-expressed X-linked protein 1 (BEX1)[81], which is thought to function as a tumour suppressor; a potential inverse link between BEX1 and FAK expression remains under investigation[41].

The most enriched signalling pathways at the genomic level, in patients with HGSOC who will receive chemotherapy, are those that modulate lipid metabolism[82]. This may reflect the metastatic tropism of HGSOC cells to lipid-rich secondary sites, such as omentum[83]. However, HGSOC cells are also highly enriched in copies of genes regulating cell adhesion, ECM and FAK signalling pathways. HGSOC tumours with *PTK2* gene amplification frequently have gains in a number of stemness-related genes, including *SOX2*, *SOX9* and *OCT4* (also known as *OCT3* and *POU5F1*)[41]. SOX9 is linked to both WNT signalling and ERK activation[84]. Analysis of the time to recurrence and stemness-associated genes that are gained in HGSOC revealed two, *DUSP1* and *IER5*, whose protein products may cooperate with FAK and whose levels are significantly elevated in patients with decreased overall survival[41]. Importantly, both are linked to chemoresistance; DUSP1 via its modulation of the JNK MAPK signalling pathway[85], and IER5 via its key role in DNA repair[86]. The coincident amplification of the *PTK2*, *DUSP1* and *IER5* genes may therefore prove useful as HGSOC biomarkers for rapid disease recurrence or progression, and they could be useful co-targets in this lethal gynaecologic disease.

FAK in resistance to RAS–RAF–MEK pathway inhibition. Activation of the RAS–RAF–MEK signalling pathway, in particular by mutation of the genes encoding RAS or RAF proteins, is frequent in many common cancers[38,87]. FAK is activated following inhibition of the RAS–RAF–MEK pathway in several preclinical tumour models[46,48,88] (FIG. 2) and patient tumours[89,90]. Conversely, FAK signalling can be negatively regulated by the RAS–RAF–MEK pathway; in mutant RAS-expressing fibroblasts, ERK-mediated phosphorylation of FAK on S910 provides feedback leading to FAK inactivation by dephosphorylation and focal adhesion turnover during migration[91]. Loss of this negative regulation may partially explain the activation of FAK in cancer cells following RAS–RAF–MEK pathway blockade.

In preclinical models of melanoma and colorectal cancer with mutant BRAF, treatment with RAF inhibitors (dabrafenib, GDC-0879 or vemurafenib) or MEK inhibitors (trametinib) induces a rapid activation of FAK within hours[46–48]. This can occur via tumour cell-intrinsic mechanisms or tumour cell-extrinsic mechanisms; for example, treatment of mice bearing melanoma tumours with

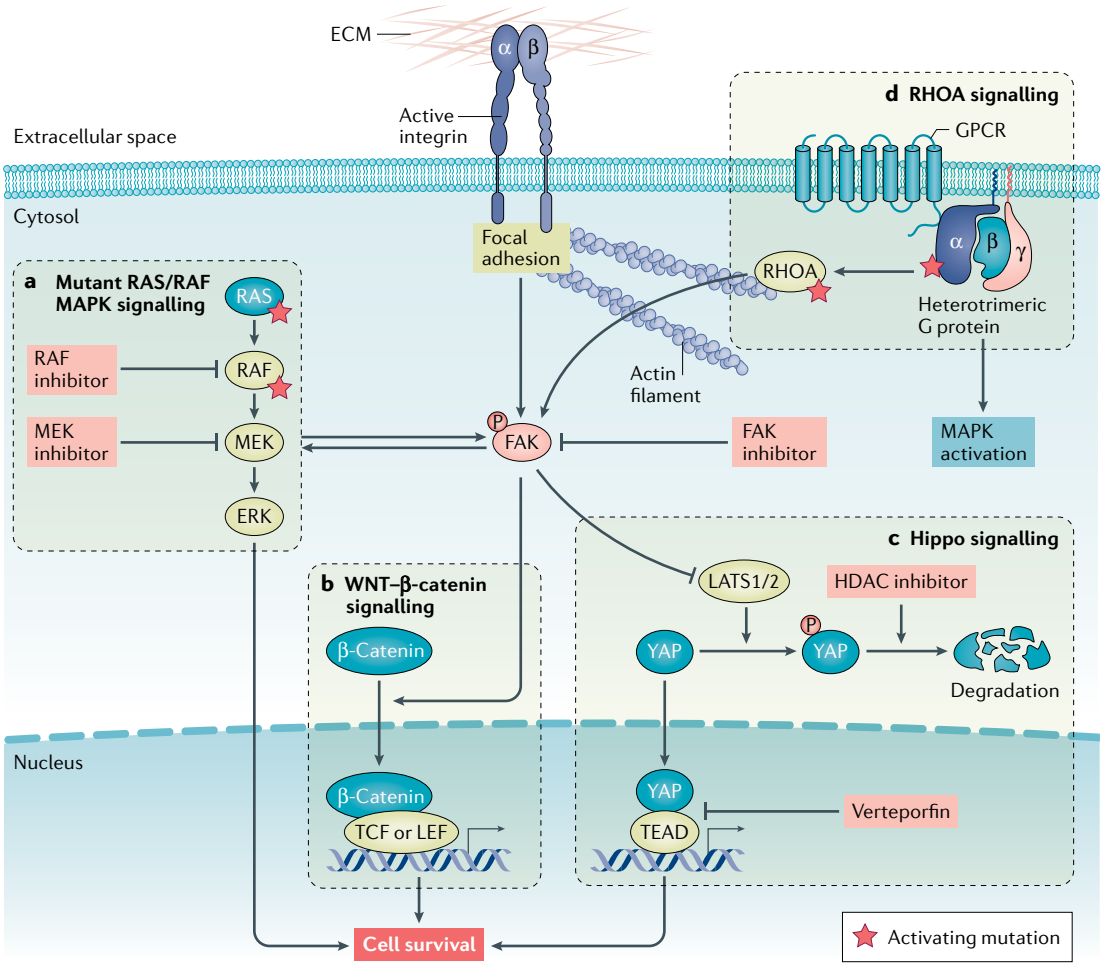

Fig. 2 | **Molecular targets for FAK inhibitor combination therapy.** Focal adhesion kinase (FAK) supports myriad oncogenic processes. **a** | Activation of the RAS–RAF–MEK signalling cascade is a common oncogenic driver in many tumour types and can be activated by mutations in a tumour type-specific manner. In RAS-mutant or RAF-mutant cancer cells, blockade of the RAS–RAF–MEK pathway using either RAF or MEK inhibitors activates FAK and promotes cell survival by reactivation of ERK signalling. **b** | In BRAF-mutant colorectal cancer cells, RAS–RAF–MEK–ERK pathway blockade activates FAK in a β1 integrin-independent and SRC-independent way and promotes WNT–β-catenin signalling and survival. **c** | Activated FAK in diffuse gastric cancer and uveal melanoma alleviates the negative regulation of the transcriptional activator YAP by large tumour suppressor kinases 1 and 2 (LATS1/2). As mutant heterotrimeric G protein $G\alpha_q$ subunit (GNAQ/GNA11) signalling in uveal melanoma also activates the ERK pathway, the combination of FAK (defactinib or IN10018) with RAF and/or MEK (VS-6766 or cobimetinib) inhibition is being tested in clinical trials[62,100]. FAK activity can promote the nuclear translocation of YAP, and combinations of FAK inhibitors with inhibitors of YAP expression (for example, histone deacetylase (HDAC) inhibitors) or transcriptional activity (for example, verteporfin) may be needed to reinforce inhibition of oncogenic YAP signalling. **d** | The small GTPase RAS homologue family member A (RHOA) regulates the actin cytoskeleton. Activation of the RHOA signalling pathway by gain-of-function mutation of *RHOA* together with inactivation of the tumour suppressor cadherin 1 gene (*CDH1*) in diffuse gastric cancer activates FAK and subsequent YAP, PI3K and β-catenin signalling. In an alternative mechanism, activating mutations in $G\alpha_q$ subunits (GNAQ or GNA11) of heterotrimeric G proteins in uveal melanoma activate FAK via the RHOA pathway to support YAP signalling and tumour growth. ECM, extracellular matrix; GPCR, G protein-coupled receptor; LEF, lymphoid enhancer-binding factor; TCF, T cell factor.

vemurafenib leads to activation of melanoma-associated fibroblasts (MAFs), which remodel the ECM into a cancer cell-protective environment[48]. In melanoma cells treated with vemurafenib, FAK is activated as part of a JUN–FAK–SRC pathway that promotes dedifferentiation of cells expressing the low-affinity nerve growth factor receptor, and these melanoma cells are tolerant of BRAF-V600E inhibition. Co-targeting of BRAF (with vemurafenib) and FAK (with the dual FAK/PYK2 inhibitor PF-562,271 or defactinib) impairs the acquisition of this vemurafenib-tolerant state and induces cell death[47]. In BRAF-V600E colorectal cell lines, BRAF inhibitors activate the WNT–β-catenin pathway via activation of FAK, independently of β1 integrin and SRC, and vemurafenib and PF-562,271 together reduce growth of HT-29 colorectal tumours in vivo. Interestingly, 'reinforced' inhibition of RAF–MEK signalling by targeting BRAF-V600E (with vemurafenib) and MEK (with trametinib) in a triple combination with the FAK inhibitor PF-562,271 robustly blocks HT-29 tumour growth in vivo[46].

In preclinical models of non-small-cell lung cancer (NSCLC), cells with mutated KRAS depend on FAK for survival, and pharmacological inhibition with the dual FAK/PYK2 inhibitor PF-562,271 or VS-4718 inhibits tumour growth[44]. However, treatment of patients with KRAS-mutant NSCLC with the FAK/PYK2 inhibitor defactinib showed only modest effects, demonstrating the disparity sometimes found between preclinical models and clinical efficacy[37]. Preliminary results from a phase I clinical trial (NCT03875820) report FAK activation in KRAS-G12V NSCLC tumours from patients treated with the dual RAF/MEK inhibitor VS-6766 (formerly CH5126766 or RO5126766)[89,90]. In addition, this study (NCT03875820) showed preliminary clinical activity of defactinib in combination with VS-6766 in the NSCLC and low-grade serous ovarian cancer cohorts[89,90], which led to initiation of two phase II clinical trials[59,60] (TABLE 1). Therefore, FAK activation in patients with tumours harbouring RAS or BRAF mutations treated with RAF and/or MEK inhibitors may be susceptible to clinical combinations that include a FAK inhibitor.

FAK in YAP-mediated therapeutic resistance. As part of the 'consensus integrin adhesome', FAK sits at the core of an ECM–focal adhesion signalling network[92] (BOX 1) that transduces mechanical cues into transcriptional responses by mechanisms that include the cytoplasmic to nuclear translocation of the transcriptional co-activator YAP and its paralogue TAZ[93,94]. YAP and TAZ activation correlates with grade, state of metastasis and poor outcome in breast, lung, liver, pancreatic and skin cancer[95], and YAP and TAZ signalling plays an important role in both the tumour cell compartment and the stromal compartment[94,96]. Dysregulation of components of the Hippo pathway, and of YAP in particular, is widely implicated in resistance to anticancer therapies; one study in melanoma demonstrated that short hairpin RNA-mediated knockdown of YAP is synthetically lethal with BRAF inhibitors in BRAF-V600E melanoma cells[97]. In addition, diffuse gastric cancer has gain-of-function mutations in *RHOA* (for example, Y42C) that synergize with inactivation of the tumour suppressor cadherin 1 gene (*CDH1*) and drive FAK-dependent activation of YAP signalling in *Cdh1*$^{-/-}$*Rhoa*^{Y42C} mice[14]. Furthermore, uveal melanoma displays amplification of *PTK2* and activating mutations in heterotrimeric G protein $G\alpha_q$ subunits (GNAQ and GNA11) that induce protumorigenic YAP signalling via the TRIO–RHOA–FAK pathway in uveal melanoma cell lines in vitro and in vivo[8] (FIG. 2). In both of these preclinical models, FAK-dependent YAP activation is abolished by the dual FAK/PYK2 inhibitor VS-4718 or PF-573,228, and tumour growth is inhibited[8,14]. Uveal melanoma, unlike cutaneous melanoma, does not display activating mutations in BRAF, but activating mutations in GNAQ or GNA11 proteins stimulate the RAS–RAF–MEK pathway and combined inhibition of the YAP and RAS–RAF–MEK pathways suppresses tumour growth[98]. Using knowledge of the unique genetic landscape of uveal melanoma and an unbiased genetic screen, Paradis et al. reveal that horizontal inhibition of FAK and the adaptive activation of MEK–ERK results in cancer cell death and tumour regression[99]. Targeting FAK (and therefore YAP where YAP activation is FAK dependent) and MEK in metastatic uveal and cutaneous melanoma is the subject of two combination clinical trials evaluating FAK inhibitors (defactinib or IN10018) combined with MEK inhibitors (VS-6766 or cobimetinib)[62,100] (TABLE 1).

In mouse skin, YAP and TAZ are required for homeostasis and wound healing; conditional genetic deletion of *Ptk2* in mouse skin blocks YAP nuclear translocation following chemically induced inflammation[101]. YAP is strongly localized to the nucleus of mouse skin papillomas and SCCs generated by chemically induced carcinogenesis, and pharmacological inhibition of FAK using PF-573,228 inhibits the accumulation of nuclear YAP[101]. Therefore, FAK appears to be crucial for the activation of YAP and TAZ signalling during chemically induced skin tumour development, and this is consistent with conditional deletion of FAK in the same model inhibiting SCC tumour progression[29]. Using SCC cells derived from this model expressing a FAK mutation to mimic a kinase inhibitor, a chemical–genetic phenotypic screen identified FAK-dependent reorganization of the actin cytoskeleton following treatment with histone deacetylase (HDAC) inhibitors, such as vorinostat[51]. Combination of the dual FAK/PYK2 inhibitor VS-4718 with HDAC inhibitors synergistically inhibited the in vivo growth of SCC tumours[51] by cooperatively inhibiting YAP nuclear localization and expression. In vitro, HDAC inhibitors reduce YAP expression in many cancer cell lines[102], and while HDAC inhibitors have failed to live up to their potential as single anticancer agents, in part due to toxicity, they are being investigated in combination with other agents where it may be possible to lower their doses[103]. It remains to be seen whether the clinical use of FAK inhibitors to block nuclear translocation of YAP can be effective in combination with inhibitors of YAP expression (for example, HDAC inhibitors)[51,102] or with inhibitors of YAP transcriptional activity (for example, verteporfin[104] or bromodomain inhibitors[105]) (FIG. 2).

FAK activation by the microenvironment

There is abundant evidence that FAK is regulated by, and regulates, the tumour microenvironment, including the tumour immune cell compartment and antitumour immunity. We discuss a number of contexts in which FAK inhibitors may modulate the tumour microenvironment to influence responses to therapy.

FAK regulates microenvironment-mediated resistance in melanoma. In a mouse model of BRAF-mutant melanoma, tumour cells display a heterogeneous response to treatment with the BRAF inhibitor vemurafenib[48]; Hirata et al. used intravital imaging to show that tumour areas with a low stromal density are sensitive to the BRAF inhibitor, while areas with high stromal density are more resistant. In this case, resistance is mediated by activation of MAFs in response to vemurafenib. Activated MAFs lay down, and remodel, ECM, resulting in a tumour environment with increased rigidity, creating protective microenvironmental 'sanctuaries' where tumour cells are no longer sensitive to the BRAF inhibitor. ECM-driven

Consensus integrin adhesome
The proteins that make up the core cell adhesion machinery of integrin adhesion complexes.

Regulatory T cells
(T_{reg} cells). A subpopulation of T cells that suppress the immune response.

resistance is distinct from other types of resistance where signalling networks are reprogramed to bypass mutant BRAF signalling, leading to reactivation of ERK signalling, for example by switching to other RAF isoforms or (re)activation of receptor tyrosine kinase signalling[39]. The MAF-driven adaptive resistance mechanism is dependent on a β1 integrin–FAK signalling axis in the melanoma cells (FIG. 3). This process is rapid, as cells respond within hours to vemurafenib treatment to begin remodelling the ECM; 'protected' tumour cells can then provide the foundation for eventual relapse and tumour regrowth. MAF-driven resistance is negated by the use of the FAK inhibitors PF-573,228, PF-562,271 and FAKi14; melanoma cells isolated from high stromal environments can be rendered sensitive again, demonstrating the importance of the modified tumour microenvironment[48]. Increased ECM deposition and/or stiffness can also activate YAP signalling[94], highlighting the commonality of FAK signalling in different therapeutic resistance mechanisms of cancer cells. FAK (IN10018) and MEK (cobimetinib) inhibitors are being investigated in a clinical trial involving patients with metastatic melanoma (TABLE 1).

FAK regulates the immune microenvironment in solid tumours. Activation of the antitumour immune response has shown great promise for the treatment of a number of cancers. Tumours evade immune-mediated killing through numerous mechanisms, including downregulation of antigen processing or presentation pathways, expression and secretion of immunoregulatory molecules such as immune checkpoint ligands and suppressive cytokines, and the assembly of a highly immunosuppressive microenvironment[106]. Overcoming these mechanisms of immunosuppression is central to many therapeutic strategies aimed at stimulating, or reinvigorating, a natural antitumour immune response; FAK is emerging as a promising target in this context. In a chemical carcinogenesis mouse model of SCC[29], FAK depletion from malignant cells or treatment of tumours with the small-molecule FAK/PYK2 inhibitor VS-4718 resulted in complete immune-mediated tumour regression and lasting immunological memory[16]. FAK-dependent expression of CC-chemokine ligand 5 (CCL5) and the cytokine transforming growth factor β2 (TGFβ2) in SCC cells was found to increase the intratumoural density of regulatory T cells (T_{reg} cells), thereby shifting the balance of T_{reg} cells to cytotoxic $CD8^+$ T lymphocytes in favour of immune evasion. This was a kinase-dependent function of nuclear FAK that required its association with the multifunctional cytokine interleukin-33, thereby enabling FAK to functionally interact with a network of chromatin modifiers and transcriptional regulators linked to genes encoding proinflammatory chemokines, including CCL5 (REFS[16,50]) (FIG. 4). Nuclear FAK seems to accumulate in response to cellular stress[15], and in the SCC study[16] it was not present in the relevant normal cell counterparts, namely skin keratinocytes, implying that FAK inhibitors may offer improved disease-specific immune modulation when compared with some more direct immune-targeted therapies.

In line with FAK-mediated immune modulation, several studies have now shown that FAK inhibitors

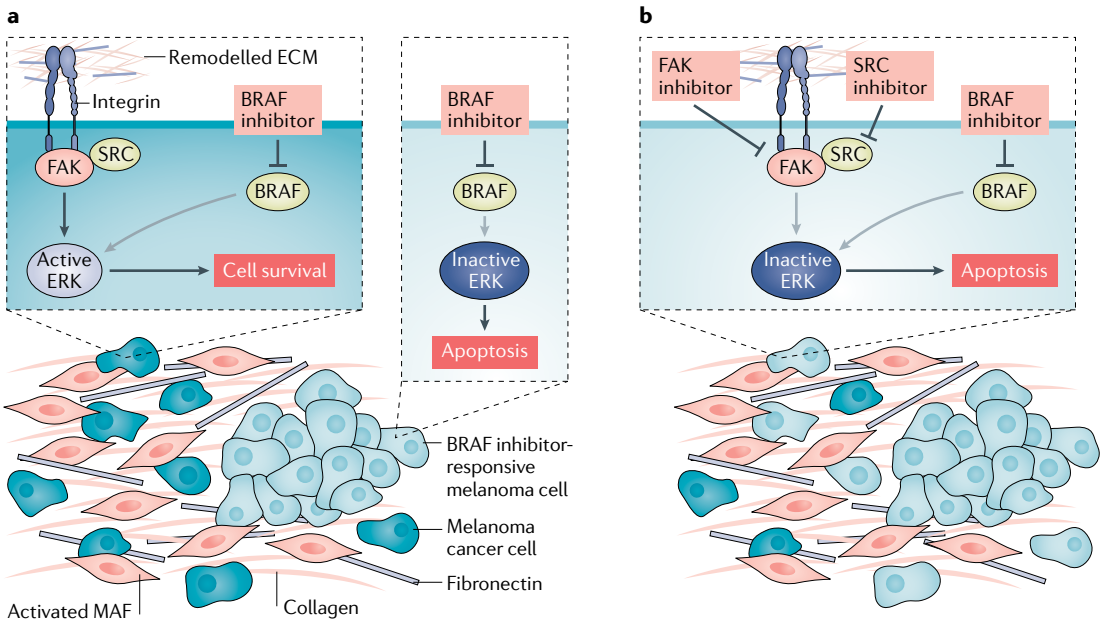

Fig. 3 | **FAK regulates adaptive resistance to targeted therapy in melanoma.** High stromal density is found in BRAF-mutant melanomas containing cancer cells and melanoma-associated fibroblasts (MAFs). **a** | BRAF inhibitors block mutant BRAF signalling and inhibit prosurvival signalling via ERK to induce cancer cell apoptosis. In tumour areas with stromal rich environments, MAFs remodel the extracellular matrix (ECM), leading to activation of β1 integrin receptors. Ligation of β1 integrin activates focal adhesion kinase (FAK) and SRC, which in turn reactivates ERK-dependent prosurvival signalling, bypassing mutant BRAF signalling and rendering melanoma cells rapidly resistant to vemurafenib. **b** | BRAF inhibitor resistance in melanoma cells driven by stromal remodelling of the ECM can be targeted with FAK inhibitors to resensitize tumour cells to BRAF inhibitors. Adapted with permission from REF.[135], Elsevier.

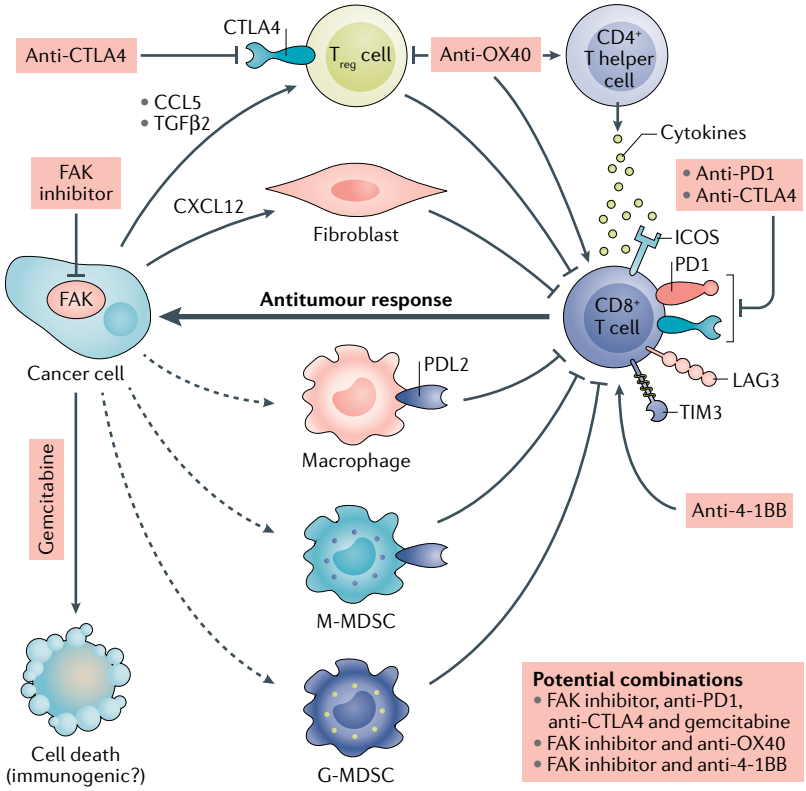

Fig. 4 | **FAK mediates protective effects of the tumour immune microenvironment, creating opportunities for combination therapy.** Focal adhesion kinase (FAK) inhibition can modulate the cellular and molecular composition of the immunosuppressive tumour microenvironment. FAK-dependent expression of CC-chemokine ligand 5 (CCL5) and transforming growth factor-β2 (TGFβ2) has been shown to impact regulatory T (T_{reg}) cell numbers in squamous cell carcinoma tumours[16], while CXC-chemokine ligand 12 (CXCL12) has been linked to promoting pancreatic fibroblast proliferation[42]. In some cases, FAK inhibition has also been shown to result in a decrease in the numbers of macrophages, monocytic myeloid-derived suppressor cells (M-MDSCs) and granulocytic myeloid-derived suppressor cells (G-MDSCs) in tumours[42,136]. These cell types can also act to supress antitumour $CD8^+$ T cell activity. Therefore, a decrease in their abundance is likely to contribute to the enhanced antitumour activity of FAK inhibitors in combination with immunotherapies. The mechanisms through which FAK inhibition can regulate macrophages, M-MDSCs and G-MDSCs remain to be defined (dashed arrows). Preclinical studies support further development of FAK inhibitors in combination with other immunotherapies such as anti-OX40 or anti-4-1BB. There are four clinical trials testing a FAK inhibitor (defactinib) in combination with an anti-PD1 antibody (pembrolizumab) in patients with pancreatic and mesothelioma cancers[63,65,67,68].

can sensitize mouse cancer models to immunotherapies[42,49]. Indeed, treatment of genetically engineered (*Ptf1a*^{Cre}*Kras*^{G12D}*Trp53*$^{flox/+}$) and transplantable mouse models of pancreatic cancer with the dual FAK/PYK2 inhibitor VS-4718 is able to overcome resistance to a combination of immunotherapy (anti-PD1 with or without anti-cytotoxic T lymphocyte antigen 4 (CTLA4)) and chemotherapy (gemcitabine), resulting in increased overall survival[42]. This synergistic activity of the combination is underpinned by targeting of both the tumour-protective fibrotic microenvironment and the immunosuppressive microenvironment by a FAK inhibitor which sensitizes the malignant cells to chemotherapy and immunotherapy[42].

The FAK inhibitor IN10018 can also promote antitumour immunity, resulting in stable disease or tumour regression, against transplantable mouse models of SCC and breast cancer that express CD80 (REF.[49]), which is a ligand for both the immune stimulatory T cell co-receptor CD28 and the inhibitory receptor CTLA4 (REFS[107,108]). IN10018 reduces the intratumoural frequency of $CTLA4^+$ immune cells, promoting an antitumour immune response against SCC tumours that is dependent on both CD80 and CD28 (REF.[49]). In the same study involving mouse models of SCC and pancreatic cancer that lack tumour cell CD80 expression, FAK inhibition combined with activating antibodies targeting the inducible T cell co-stimulatory receptors OX40 (also known as TNF receptor superfamily member 4 (TNFRSF4)) and 4-1BB (also known as TNFRSF9) also resulted in robust antitumour immunity, and in SCC this can induce complete tumour regression. In addition to decreasing the intratumoural frequency of T_{reg} cells in SCC tumours, IN10018 treatment results in broad downregulation of the immune checkpoint ligand PDL2 in the tumour microenvironment and elevated expression of the inducible T cell co-stimulator (ICOS) on effector $CD8^+$ T cells. Elevated expression of ICOS on $CD8^+$ T cells is important for the increased efficacy of IN10018 in combination with anti-OX40 and of IN10018 in combination with anti-4-1BB, while PDL2 regulation may also contribute to the efficacy of IN10018 in combination with anti-OX40 (REF.[49]).

Thus, FAK regulates multifaceted immune evasion programmes in multiple tumour contexts that can impact the efficacy of antitumour T cell responses (FIG. 4). These studies have led to the first clinical trials testing FAK inhibitors in combination with immunotherapies. Defactinib is currently being studied in combination with the PD1 receptor inhibitor pembrolizumab in patients with pancreatic cancer, NSCLC and mesothelioma[63,65,67], and in combination with both pembrolizumab and gemcitabine in patients with advanced pancreatic cancer[68] (TABLE 1).

FAK is at the early stages of investigation as an immuno-oncology target, and many questions remain regarding the mechanisms that underpin the immunomodulatory activity of FAK and dual FAK/PYK2 inhibitors. Most research to date has focused on the role of FAK in tumour cells (BOX 1). However, both FAK and PYK2 are expressed in a range of cell types present within the tumour microenvironment, and dual inhibition of FAK and PYK2 function in these cells may also be important. For example, FAK is expressed and phosphorylated in human T cells[109], where it can be found in complex with the T cell receptor (TCR)[110]. CD4 and CD8 (also known as CD8A) co-receptors recruit the SRC family kinase LCK to the peptide MHC–TCR complex, where it can phosphorylate tyrosine residues in the immunoreceptor activation motifs of CD3, the ζ-chains of the TCR complex and the kinase ZAP70 (REF.[111]). The TCR complex must be sufficiently phosphorylated in order to initiate a cellular response. In a leukaemic T cell line and activated peripheral blood $CD4^+$ T cells from healthy individuals, FAK depletion using microRNAs sensitized T cells to low-dose TCR stimulation, resulting in enhanced TCR signalling, cytokine production and expression of the activation marker CD69 (REFS[110,112]).

Cytotoxic T lymphocyte antigen 4
(CTLA4). A protein expressed by regulatory T cells that functions as an immune check point to inhibit the immune response.

FAK is required for association of carboxy-terminal SRC kinase (CSK) with LCK at the TCR–CD4 complex, resulting in inhibition of LCK activity, and implying that FAK may act as a rheostat to prevent inappropriate T cell activation[110]. These findings support the hypothesis that targeting FAK in T cells may sensitize them to low-affinity tumour antigens, thereby helping to promote antitumour immunity. FAK can also interact with and phosphorylate the adaptor protein linker for the activation of T cells (LAT) on Y171 to promote dissociation of T cells to dendritic cell conjugates and increased T cell motility[113]. Prolonged conjugation to dendritic cells is important for optimal activation of naive T cells[114,115], suggesting that FAK inhibitors could have benefits in this regard also. By contrast though, the dual FAK/PYK2 inhibitor PF-562,271 can impair ZAP70 phosphorylation, $CD4^+$ T cell activation and interaction of T cells with antigen-presenting cells in a mouse model expressing an ovalbumin peptide-specific TCR, OTII[116]. However, genetic depletion of FAK in $CD4^+$ T cells from OTII mice resulted in only modest differences in adhesion to intercellular adhesion molecule 1 (ICAM1) and conjugation to antigen-presenting cells at higher doses of soluble ovalbumin peptide stimulation, suggesting that the action of PF-562,271 could not be explained solely by inhibition of FAK[116]. PYK2 is also expressed by human T cells, and the relative roles of FAK and PYK2 in regulating T cell biology may not be functionally redundant[112], raising the possibility that FAK-specific inhibitors and dual FAK/PYK2 inhibitors may elicit different immunomodulatory activities that would influence outcomes in patients. Further work is required to reach a consensus on the relative functions of FAK and PYK2 in regulating T cell biology that is relevant to the antitumour T cell response since this may have important consequences for how we develop FAK and dual FAK/PYK2 inhibitors as combination immunotherapies moving forward.

The necessary cautionary tale

While the overwhelming body of evidence supports the conclusion that FAK inhibitors may be beneficial in the treatment of cancer, a small number of preclinical studies have found that FAK loss in specific cell types can contribute to enhanced tumour progression and metastasis. For example, FAK deletion in haematopoietic cells can increase the incidence of liver metastasis in the RIP-Tag2 mouse model of pancreatic cancer, and the incidence of liver, lung and bone metastasis following tail vein injection of B16 melanoma cells into mice[117]. FAK depletion in pericytes is reported to promote angiogenesis and tumour growth in mouse models of lung, melanoma and pancreatic cancer[118], while FAK depletion or expression of non-phosphorylatable FAK-Y397F in endothelial cells inhibits metastasis, tumour angiogenesis and growth, and increases sensitivity to chemotherapy[52,119–121].

FAK deletion in cancer-associated fibroblasts can increase cancer cell glycolysis in a way that is linked to tumour growth in mouse models of lung and pancreatic cancer[122]; by contrast, FAK depletion in cancer cells can downregulate glycolysis[123], and so the situation is complex. In tumour samples from patients with pancreatic ductal adenocarcinoma (PDAC), elevated levels of FAK Y397 phosphorylation are observed in cancer-associated fibroblasts[54], and in mouse models of PDAC and breast cancer, targeting of FAK in the stromal compartment by either genetic or pharmacological means prevents metastasis[53,54]. However, prolonged pharmacological inhibition of FAK in PDAC models eventually leads to stromal depletion that is associated with drug resistance[124]. Transient pharmacological inhibition of the RHOA effector RHO-associated protein kinase (ROCK) in pancreatic tumour stroma is sufficient to sensitize tumours to chemotherapy and reduce metastasis[125], and such a treatment strategy could be adopted in concert with FAK inhibitors to prevent the development of drug resistance. Therefore, there are complex preclinical data which imply that the role of FAK in distinct cell types within tumours can contribute in a nuanced way to the overall tumour microenvironment and therapeutic responses. On this basis, it is difficult to predict how the antitumour, and potentially protumour, effects of FAK inhibitors will be integrated in patients to produce particular responses. This requires data from ongoing and future clinical trials, likely involving combination therapies, but it is noteworthy that, as yet, there are no reports that FAK inhibitors are toxic or promote tumour progression in patients[34–36,89,126].

Conclusions

Preclinical and clinical evaluation of molecularly targeted therapies against cancer driver pathways shows that tumours almost always display inherent or acquired resistance. Solid tumours consist of a complex heterogeneous mixture of cancer cells, immune cell populations, and stromal cells, and for this reason, combination therapies are generally required for durable responses. As a key coordinator of cellular responses to environmental cues and a mitigator of cellular stresses including therapeutic interventions, FAK is an attractive common target supporting a myriad of oncogenic processes and resistance mechanisms. FAK is likely to be a more effective target when inhibitors are used in the context of combination therapies, especially if tumour cells rely on anchorage-dependent signalling initiated from the microenvironment. We do not dismiss the potential for future targeting of the protein–protein scaffolding functions of FAK, as these play a vital role in cancer cell biology[127–129], especially if tumour-specific adaptor functions are defined. For example, proteolysis targeting chimera (PROTAC)-mediated degradation of FAK may also be considered as a useful therapeutic strategy that would simultaneously remove FAK's catalytic and adaptor functions[130–132]. Aside from FAK activation itself[9], robust biomarkers are required to identify patients with tumours whose survival and growth are driven by chemoprotective FAK-dependent signalling, whether by intrinsic or extrinsic mechanisms, that could guide the best use of FAK inhibitors in the clinic.

Published online 17 March 2021

RHO-associated protein kinase
(ROCK). A serine/threonine kinase downstream effector of RAS homologue family member A (RHOA) involved in the formation of actin stress fibres.

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Acknowledgements
M.C.F. is supported by a Cancer Research UK Programme Award (C157/A24837). J.C.D. is supported by a Cancer Research UK (C42454/A28596) and BrainTumour Charity (GN-000676) award. D.D.S. and D.G.S. are supported by funding from the US National Institutes of Health (R01 CA247562 and R01 CA254342). A.S. is supported by a Cancer Research UK Career Development Award (C39669/A25919).

Author contributions
All authors made a substantial contribution to discussion of the content and writing the article. M.C.F., J.C.D., A.S., D.G.S. and D.D.S. researched data for the article. M.C.F., J.C.D., D.G.S. and D.D.S. reviewed and edited the manuscript before submission.

Competing interests
A.S. has received research funding from Boehringer Ingelheim to work on IN10018 (then BI 853520) and is on the scientific advisory board of InxMed in relation to the development of IN10018. All other authors declare no competing interests.

Peer review information
Nature Reviews Cancer thanks J.-L. Guan, and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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