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RESEARCH ARTICLE

Discovery and Characterisation of Highly Cooperative FAK-Degrading PROTACs

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Abstract:
Focal adhesion kinase (FAK) is a key mediator of tumour progression and metastasis. To date, clinical trials of FAK inhibitors have reported disappointing efficacy for oncology indications. We report the design and characterisation of GSK215, a potent, selective, FAK-degrading Proteolysis Targeting Chimera (PROTAC) based on a binder for the VHL E3 ligase and the known FAK inhibitor VS-4718. X-ray crystallography revealed the molecular basis of the highly cooperative FAK-GSK215-VHL ternary complex, and GSK215 showed differentiated *in vitro* pharmacology compared to VS-4718. In mice, a single dose of GSK215 induced rapid and prolonged FAK degradation, giving a long-lasting effect on FAK levels (~96 h) and a marked PK/PD disconnect. This tool PROTAC molecule is expected to be useful for the study of FAK degradation biology *in vivo*, and our results indicate that FAK degradation may be a differentiated clinical strategy versus FAK inhibition for the treatment of cancer.

Introduction

Focal Adhesion Kinase (FAK) is a non-receptor protein tyrosine kinase that transduces signaling from integrins and growth factor receptors to intracellular signaling pathways associated with cell migration, invasion, survival and proliferation.[1] Consistent with the importance of FAK in regulating such events, high levels of FAK protein and mRNA have been reported in several solid tumour types, and are associated with poor clinical outcomes.[1-2] FAK drives cancer cell growth and invasion through kinase-dependent phosphorylation of Y397 and by acting as a kinase-independent scaffold for several signaling proteins.[1, 3] FAK is localised to focal adhesions via binding of the FAT domain to LD repeat motif-containing proteins,[4] while recruitment of integrins and tyrosine kinases through the FAK FERM domain leads to kinase activation via autophosphorylation of Y397.[5] In the nucleus, the FAK FERM domain forms scaffolding interactions with p53 and MDM2, degrading p53 to promote tumour cell survival.[6] Nuclear FAK activity also promotes cancer cell immune evasion by increasing levels of immunoprotective T-regs and decreasing levels of cytotoxic CD8+ T-cells, thereby inducing an immunosuppressive tumour microenvironment.[7]

To date, several ATP-competitive inhibitors targeting FAK kinase activity have been developed. Phase I trials have been conducted with PF-00562271,[8] GSK2256098,[9] defactinib (VS-6063)[10] and BI853520[11] in several solid tumour types. Despite good clinical safety, tolerability and pharmacokinetic profiles, the best responses in these single-agent trials were limited to stable disease, with some agents showing no effect on progression-free survival compared to placebo.[12] These results have prompted the evaluation of FAK inhibitors in combination settings. FAK inhibitors have been found to be synergistic with checkpoint immunotherapy agents in mouse models of pancreatic cancer,[13] and the FAK inhibitor VS-6063 is currently under clinical evaluation in combination with the anti-PD-1 antibody pembrolizumab.[14]

In addition to the signal transduction activity of the FAK kinase domain, several reports indicate that FAK scaffolding functions also have profound effects on cancer cell survival and progression. FAK knock-in mice with P878/881A mutations were shown to exhibit reduced A2 endophilin binding to FAK, leading to a reduction in tumour growth and metastasis.[15] Additionally, disruption of VEGFR-3 interactions with the FAK FAT domain led to apoptosis in breast cancer cells,[16] whereas EGF and PDGF driven-cell motility was reduced by FAK knock-down but not kinase inhibition.[17] FAK can also be phosphorylated by other receptor tyrosine kinases, a potential resistance mechanism of tumour cells against inhibition of Y397 autophosphorylation.[18] The weight of evidence suggests that inhibition of FAK kinase activity may not be sufficient to completely block FAK signaling, and that targeting FAK scaffolding roles could prove to be a more efficacious approach to treating solid tumours. However, known inhibitors of FAK scaffolding functions show only weak potency,[19] and retain the inherent limitations of occupancy-driven inhibitor

10.1002/anie.202109237

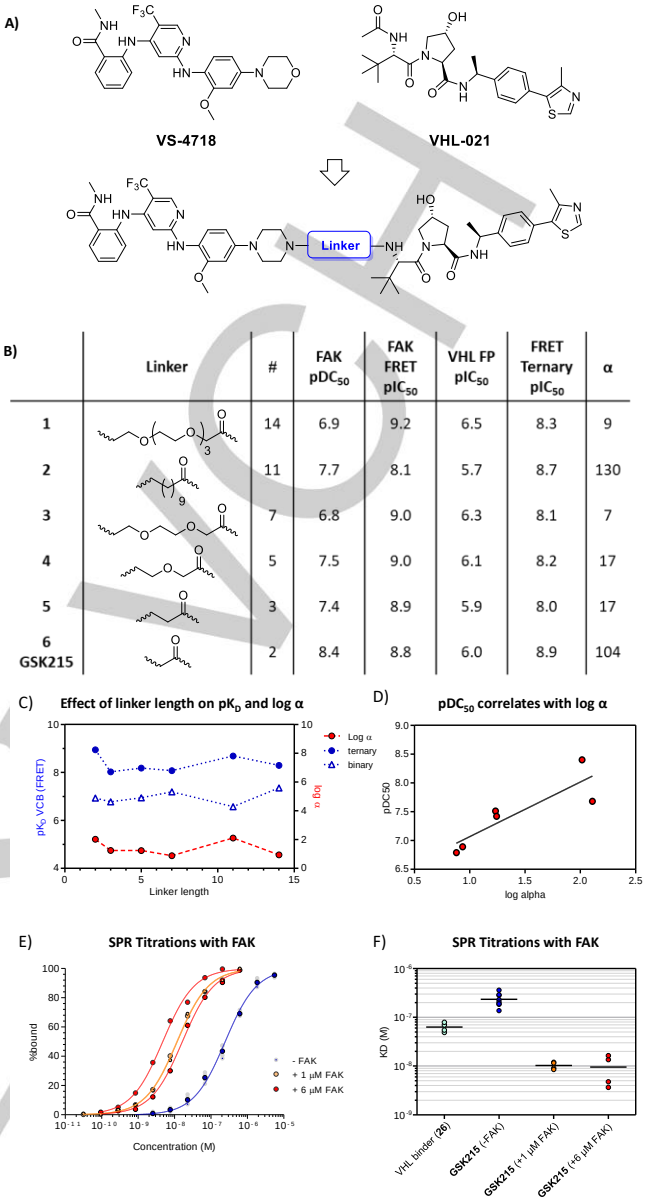
pharmacology. Therefore, addressing both the kinase and non-kinase functions of FAK remains an intriguing prospect for anti-cancer therapy.

Proteolysis Targeting Chimeras (PROTACs) are an emerging class of molecules that offer the possibility to modulate target pharmacology that is inaccessible to molecules that possess only inhibitory activity. PROTACs are heterobifunctional molecules that redirect the ubiquitin proteasome system to degrade selected proteins of interest (POI) by simultaneously recruiting both a POI and an E3 ubiquitin ligase to promote ubiquitination and subsequent degradation of the POI.[20] Due to the formation of stabilising or destabilising protein-protein interactions, the binding affinity of the PROTAC for the E3 ligase or POI may change once bound to the other protein, an effect termed cooperativity. In contrast to a target inhibition strategy, protein degradation has the ability to modulate all functions of a particular POI, which is of particular relevance to targets such as FAK which are known to have important scaffolding functions. In addition, PROTACs can induce a PD/PK disconnect that is dependent on the protein synthesis rate,[21] and in principle they can target "undruggable" proteins where only weak binders are known. Here we present the discovery and characterization of a potent and selective FAK PROTAC molecule, as well as the X-ray crystal structure of the highly cooperative FAK-PROTAC-VHL ternary complex. We show that the PROTAC has differentiated *in vitro* effects on cancer cell growth, motility, and collagen I deposition in human fibroblasts. We further demonstrate the rapid and prolonged degradation of FAK *in vivo* after a single subcutaneous dose of the PROTAC in mice. Collectively, this work highlights the ability of FAK-degrading PROTACs to modulate non-enzymatic and cancer-relevant functions of FAK, and describes FAK PROTAC tool compounds for the further study of FAK *in vitro* and *in vivo* biology.

Results and Discussion

PROTAC design and optimisation

FAK degradation using PROTACs was first reported using promiscuous kinase binders.[22] Recently, more selective FAK PROTACs were published separately by the Crews laboratory (PROTAC-3),[23] Boehringer Ingelheim/University of Dundee (BI-3663, BI-0319)[24] and Rao *et al* (FC-11)[25] (Scheme S1). Although the reported molecules are potent degraders, their effects on cell viability and detailed characterisation of the requisite ternary complexes were not reported. Additionally, *in vivo* FAK degradation has only been described using high doses of PROTAC (50 mg/kg, or 20 mg/kg BID).[26] Here we report the design of FAK PROTACs suitable for use as low-dose *in vivo* tool molecules, and we reveal the structural determinants of the high cooperativity (α) observed in the ternary complexes formed with these compounds.



	Linker	#	FAK pDC$_{50}$	FAK FRET pIC$_{50}$	VHL FP pIC$_{50}$	FRET Ternary pIC$_{50}$	α
1		14	6.9	9.2	6.5	8.3	9
2		11	7.7	8.1	5.7	8.7	130
3		7	6.8	9.0	6.3	8.1	7
4		5	7.5	9.0	6.1	8.2	17
5		3	7.4	8.9	5.9	8.0	17
6 GSK215		2	8.4	8.8	6.0	8.9	104

Figure 1. Investigating the effect of PROTAC linker length. a) Conceptual design of an FAK-VHL PROTAC based on **VS-4718**; b) Linker identity, linker length (number of atoms; #), FAK degradation pDC$_{50}$, individual binary complex and ternary complex binding of PROTACs to FAK and the VCB complex, and ternary complex cooperativity (α). For **3** and **4**: pDC$_{50}$ data generated using formic acid salt and free base respectively, VHL and ternary data generated using TFA salts; c) Comparison of linker length to VCB pK$_D$ (left axis, blue) and cooperativity (right axis, red); d) correlation of cooperativity and degradation; e) binding of **GSK215** to VCB as measuring by SPR, showing increased affinity on addition of FAK protein; f) K$_D$ values for binding of VHL binder (**26**) and PROTAC **GSK215** (± FAK Protein) to VCB, as measured by SPR.

We designed our PROTACs based on the clinical FAK inhibitor **VS-4718**/PND-1186 (FAK pIC$_{50}$ 8.3)[27] and the von-Hippel Lindau (VHL) E3 ligase ligand **VHL-021**.[28] We connected these binders using a variety of linker lengths and chemotypes (See SI for synthesis), and tested FAK degradation using an ELISA protein quantification assay in A549 cells (Figure 1b). Recent reports have suggested ternary complex kinetics and positively cooperative binding can have significant effects on degradation,[29] though increased cooperativity does not always result in increased degradation.[30] To analyse ternary complex formation, we established an FAK-VCB (VHL-ElonginC-ElonginB)

10.1002/anie.202109237

FRET assay capable of determining ternary K_D, and calculated the ternary cooperativity α (α = binary K_D / ternary K_D).

Encouragingly, we observed FAK degradation with a variety of linkers, with all compounds showing strong positive cooperativity (α > 1). Consistent with the complex SAR commonly seen with PROTAC systems, there was no simple correlation between linker length and VCB K_D or cooperativity (Figure 1c). For example, compounds **2** and **6** exhibited amongst the highest ternary cooperativities reported to date despite having linkers with very different lengths. However, the DC_{50} (half-maximal degradation) values did correlate with α (Figure 1d), supporting the importance of positive cooperativity in achieving potent degradation. Interestingly, the most potent degradation was obtained with the unusually short acetamide linker of **6** (hereafter referred to as **GSK215**), and we selected this compound for further investigation.

SPR analysis confirmed the ternary complex formation and high cooperativity of **GSK215** (Figure 1e-f), and a ~24-fold increase in binding of this compound to VCB was measured in the presence of saturating concentrations of FAK protein.

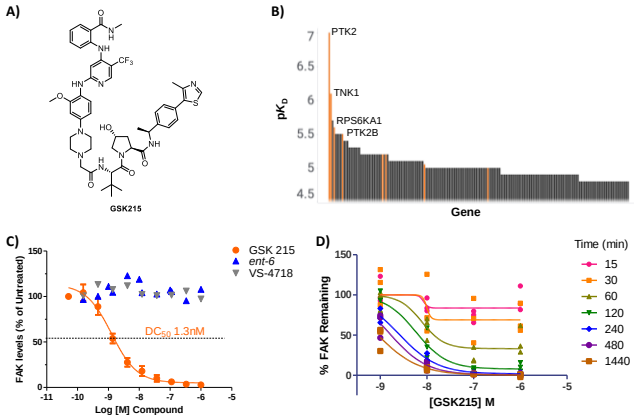


Figure 2. Structure and degradation profiling of FAK-VHL PROTAC **GSK215**. a) Structure of **GSK215**; b) kinome binding selectivity of **GSK215** as determined by KinoBead profiling. Proteins with binding below the lower limit of quantification are shown in grey; c) FAK levels in A549 cells as determined by Western blot, after treatment with **GSK215**, enantiomeric control **ent-6**, and **VS-4718**; d) time-course study of FAK degradation in A549 cells.

Profiling of **GSK215** using a cell lysate kinobead assay showed that the PROTAC maintained high selectivity for binding to the FAK kinase domain. It should be noted that although the FAK binding affinity of **GSK215** is significantly reduced compared to **VS-4718** (pIC_{50} 7.8 vs 8.3, Table S1), the catalytic mechanism of the PROTAC allows it to induce potent FAK degradation. We profiled FAK degradation in A549 non-small-cell lung cancer cells using Western blot and determined a DC_{50} of 1.3 nM (Figure 2c) and maximal degradation (D_{max}) near the limit of the assay quantification (D_{max} 99%). To confirm degradation was PROTAC-mediated, we synthesised the enantiomeric control **ent-6** which is unable to bind to VHL. Neither this compound nor the parent inhibitor **VS-4718** caused any decrease in FAK protein levels (Figure 2c), indicating the critical role of E3 ligase recruitment for degradation. We also observed a strong time-dependence effect on degradation (Figure 2d), with a reduction in FAK levels detectable after 30 min treatment with 0.1 μM **GSK215**, which increased to >90% degradation after 2 hours.

Confirming proteasomal degradation

To confirm that the mechanism of FAK degradation was dependent upon E3 ligase complex recruitment, we demonstrated reduced degradation of FAK upon separate addition of the FAK inhibitor **VS-4718** and the VHL ligand **VHL-021** (Figure 3a), indicating competitive displacement of PROTAC **GSK215** from the ternary complex. Epoxomicin treatment also hindered the ability of **GSK215** to induce FAK degradation, as did separate treatment with MLN4924, a NEDD8 inhibitor that blocks cullin scaffolding support for the E2 ubiquitin-conjugating enzyme (Figure 3c). These experiments provided additional evidence for proteasomal and neddylation dependent PROTAC-induced substrate degradation.

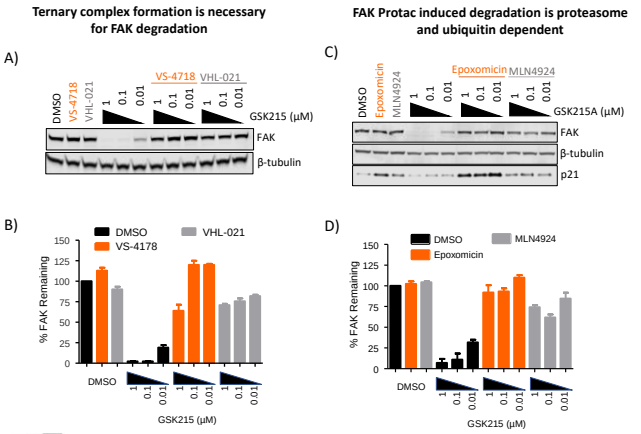


Figure 3. Mechanistic experiments. a,b) Addition of competitive inhibitors **VS-4718** (1 μM) or **VHL-021** (50 μM) reduces FAK degradation; c,d) degradation is rescued by blocking proteasome activity (1 μM epoxomicin) or neddylation (1 μM MLN4924).

Structure of PROTAC ternary complex

To better understand the structural determinants of the high cooperativity observed in engagement of FAK and VHL-ElonginC-ElonginB (VCB) by **GSK215**, a 2.2 Å structure of FAK-**GSK215**-VCB was successfully elucidated by X-ray crystallography (Figure 4a, PDB ID: 7PI4). Crystallizing ternary complexes of PROTACs bound to target proteins and E3 ligase complexes has proven to be challenging, but several examples have been reported. The largest number of VHL ternary complex structures have been reported from bromodomain-containing proteins, such as BRD4[29b] and SMARCA4,[31] with an additional structure of a non-bromodomain protein also having been recently disclosed.[32]

The high ternary complex cooperativity induced by **GSK215** can be explained by many neo-interactions between FAK and VHL that occur at both ends of the PROTAC molecule (Figure 4b). As expected, the FAK binding moiety of the PROTAC is completely enclosed by the FAK protein. However, beyond the FAK ligand binding site, the VHL and FAK proteins make many neo-non-cognate direct protein-protein interactions (PPIs; Figure 4c). These contacts include Van-der-Waals interactions, as well as direct H-bonds, such as that between the sidechain of N67 (VHL) and the backbone carbonyl of C427 (FAK), and a salt bridge between R69 (VHL) and E430 (FAK). At the VHL binding end of the PROTAC ligand, the C-terminal helix of FAK 506-516 lies on top of the VHL ligand, which now sits in an enclosed pocket rather than in an exposed site normally seen with VHL binders.

10.1002/anie.202109237

The total buried surface upon ternary complex formation is 2661 Å^2, with the largest VHL:target protein neo-PPI interface reported to date (721 Å^2, Table S4), which is consistent with the high measured cooperativity.

We were unable to obtain a X-ray crystal structure of **2**, which also demonstrated high cooperativity, bound to the FAK-VCB ternary complex. Given the sharp turn created by the acetamide of **GSK215**, and the close proximity of the FAK- and VHL-binding portions, it is likely that longer linkers would produce different ternary complex structures with alternative positioning of the two proteins. Longer linkers can produce flexible ternary complexes which adopt multiple conformations;[30, 33] we hypothesise that while **2** can induce strongly cooperative ternary binding, the rigid **GSK215** structure is a key factor in its more effective degradation of FAK. A recently reported FGFR-VHL PROTAC includes a similar acetylpiperazine linker,[34] suggesting commonalities in their binding mode and the possibility of transferrable SAR between kinase PROTACs. This work reinforces the importance of thoroughly surveying linker length and rigidity in PROTAC design, and the effect small modifications can have on cooperativity and DC_{50}.

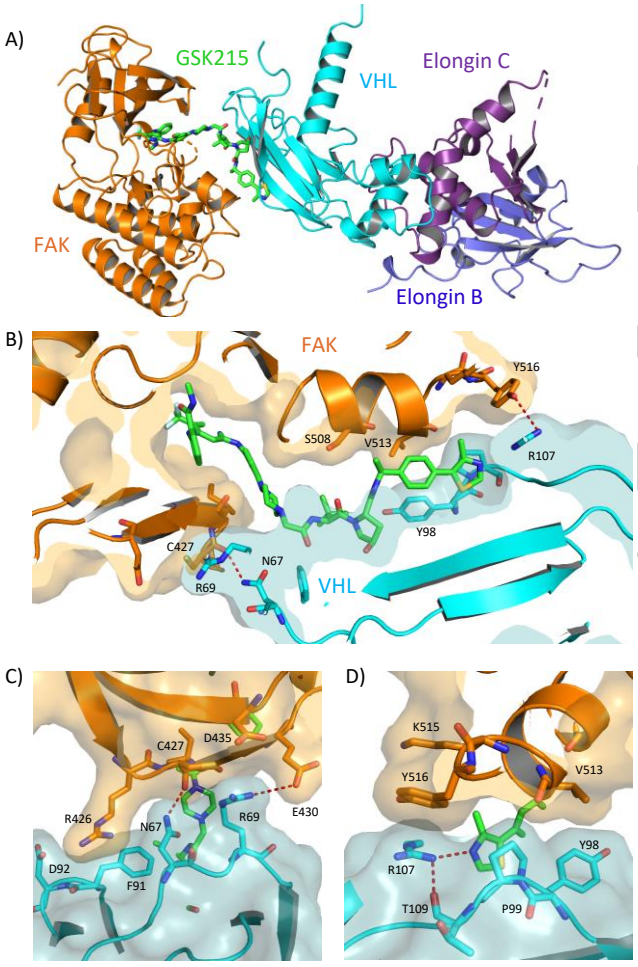


Figure 4. 2.2Å X-ray crystal structure of the FAK-**GSK215**-VCB ternary complex (PDB ID: 7PI4).

GSK215 reveals differential effects of FAK degradation over FAK inhibition

To further profile the PROTAC and discover potential targets of induced degradation, we profiled **GSK215** by multiplexed proteome dynamics profiling (mPDP). mPDP is a mass-spectrometry-based approach, combining dynamic-SILAC labelling with isobaric mass tagging for multiplexed analysis of protein degradation and synthesis.[35] In mature proteins, **GSK215** showed dose dependent degradation of FAK, with selective degradation observed up to 10 nM **GSK215** after 6 h (Figure 6a). At concentrations above 100 nM levels of additional mature proteins were reduced, primarily kinases CDK7, RPS6KA3, MET and GAK. Interestingly, all members of the CAK complex (CDK7, MNAT1 and CCNH) were degraded. These results are in contrast to previously reported FAK degraders. **PROTAC-3** primarily showed reduction of the androgen receptor, p-Akt and p-SRc,[23] while **FC-11** did not degrade CDK7,[26c] highlighting the potential for degraders of differing structure to produce distinct phenotypic effects. **GSK215** showed binding to RPS6KA3 in the KinoBead assay (Table S2), but binding to MET and GAK was below the lower limit of quantification.

In addition, levels of nascent proteins were also reduced, such as FAK and the CAK complex, presumably driven by PROTAC-induced degradation of both mature and nascent protein. For other proteins such as FOSL1, reduction was only seen on the nascent level, indicative of a downstream regulatory effect (Figure 6b). After 24 h treatment (Figure 6d) the selectivity of degradation for **GSK215** was maintained up to 10 nM while at higher concentrations additional targets of induced degradation were observed, with pronounced downstream regulatory effects on nascent protein levels at 100 nM.

Since FAK and the CAK complex subsequently influence cell-cycle progression, we explored non-catalytic FAK biological roles. To simultaneously discover potential differentiating effects of FAK degradation versus kinase inhibition, we also tested **GSK215** alongside **VS-4718** and the corresponding non-degrading enantiomeric control compound **ent-6** in phenotypic assays. **GSK215** repressed 2D cell proliferation in A549 and MCF-7 cells in a concentration dependent manner, but not in BT474 cells (Figure 5a, Figure S5).

Interestingly, we obtained a different result in an anchorage independent cell growth assay, in which inhibition of 3D proliferation upon treatment with **GSK215** was not observed in A549 or MCF-7 cells (Figure S5), but was clearly seen in BT474 cells (Figure 5b). No inhibition of 3D proliferation was seen in any of these cells upon treatment with **ent-6** or **VS-4718**. These results support previous findings that indicate diverse roles for FAK in different growth formats, as well as significant variability between cell lines.

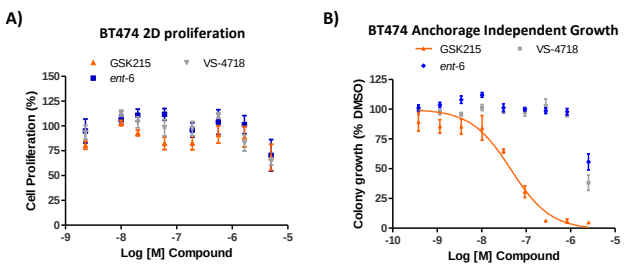


Figure 5. Effect of **GSK215**, enantiomeric control **ent-6** and **VS-4718** on a) 2D proliferation and b) anchorage independent growth in 3D culture media in BT474 cells.

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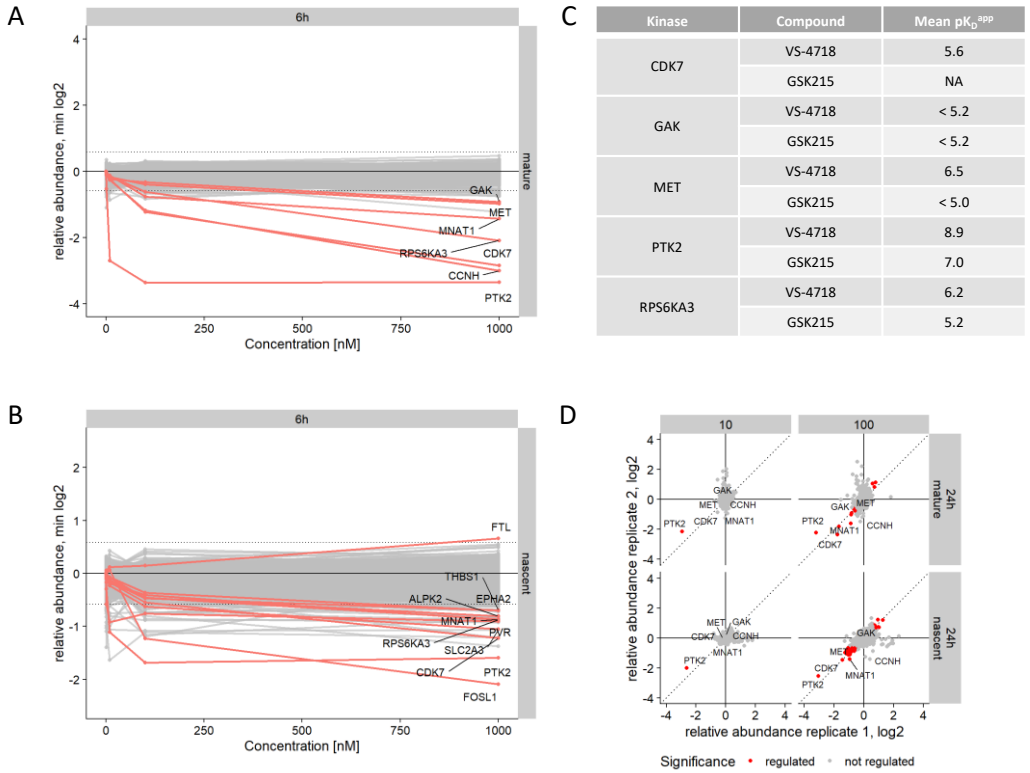

Kinase	Compound	Mean pK_D^{app}
CDK7	VS-4718	5.6
	GSK215	NA
GAK	VS-4718	< 5.2
	GSK215	< 5.2
MET	VS-4718	6.5
	GSK215	< 5.0
PTK2	VS-4718	8.9
	GSK215	7.0
RPS6KA3	VS-4718	6.2
	GSK215	5.2

Figure 6. Proteomics profiling of **GSK215**. a,b) Line chart of mature (a) and nascent (b) protein abundance changes with increasing concentration of **GSK215** after 6 h of treatment. Red lines indicate significantly regulated proteins; c) Kinobeads affinity of **GSK215** and **VS-4718** against kinases degraded as mature protein after 6 h; d) mPDP Scatterplot after 24 h treatment with 10 and 100 nM of **GSK215** for mature and nascent proteins.

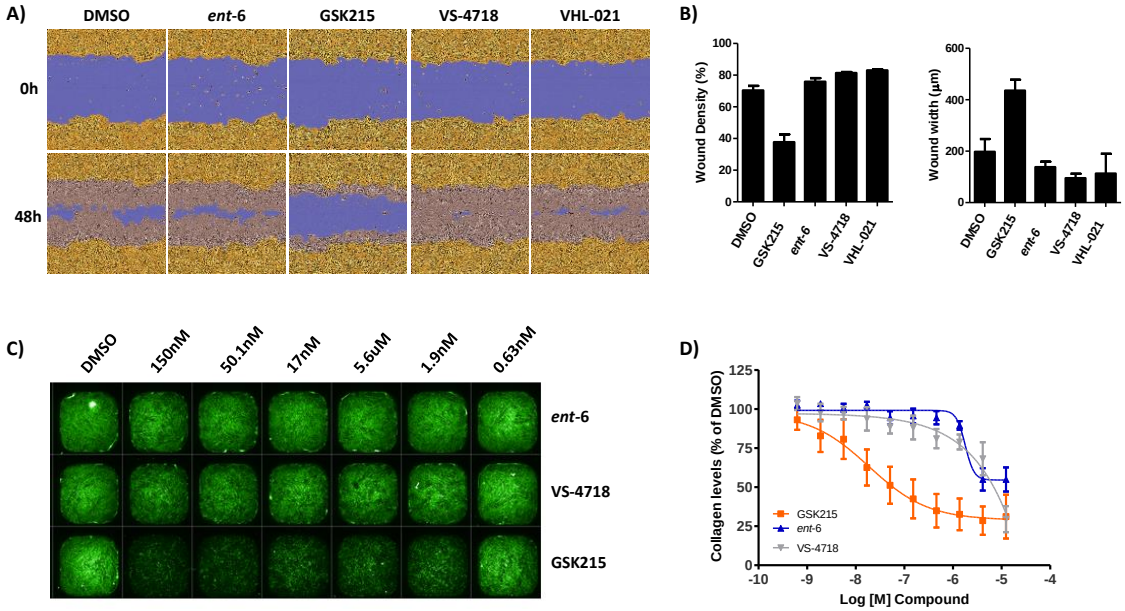

Figure 7. GSK215 inhibits migration, invasion and collagen deposition. a) Representative imaging of wound closure in A549 cell culture following compound treatment (100 nM); b) Bar chart of wound closure assay results with **GSK215** or inhibitor controls (100 nM, n=3); c) Representative imaging of fibroblast collagen deposition following compound treatment; d) effect of **GSK215**, enantiomeric control **ent-6** and **VS-4718** on fibroblast collagen deposition.

To further probe the biological effects of FAK degradation versus FAK inhibition, we studied A549 cell motility in a wound scratch model system upon treatment with **GSK215**. We observed that **GSK215** treatment was better able to suppress cell motility when compared to treatment with FAK kinase inhibitors. Indeed, only treatment with **GSK215** was able to repress simulated wound closure, whereas treatment with **ent-6** or **VS-4718** gave results similar to those seen with untreated cells (Figure 7a,b). Furthermore, potent inhibition of collagen I deposition occurred following treatment of fibroblasts with **GSK215**, with FAK inhibitor controls showing significantly lower efficacy (Figure 7c,d). This effect was independent of cell viability (Figure S6), supporting the role of FAK in collagen I formation, and providing further evidence for the differentiated biology of FAK degradation compared to FAK inhibition.

***In vivo* profile of FAK PROTAC GSK215**

The pharmacokinetic and pharmacodynamic profile of **GSK215** was determined in male CD1 mice following a single subcutaneous 8 mg/kg injection (Figure 8). A composite study design, consisting of serial and terminal blood sampling was used to determine the concentration-time profile of **GSK215** and facilitate measurement of FAK degradation in liver up to 96 h post dose. Endogenous FAK levels in liver tissues were measured and compared to untreated control animals to assess the amount of FAK degradation over time. Figure 8 shows a simulated **GSK215** concentration profile (see Supplementary Information). Following SC injection, **GSK215** was rapidly absorbed from the site of administration with a measured mean C_{max} of 526 ng/mL in blood (T_{max} 0.33 h), with **GSK215** concentrations falling to below the lower level of quantification (LLQ: 1 ng/mL) after 48 h. **GSK215** caused a rapid and profound degradation of FAK in liver over time, with a maximal degradation of ~85% being achieved within 18 h. Endogenous FAK was found to still be reduced by ~60% at 96 h post-dose, at which time the **GSK215** exposure was below the lower limit of quantification. This result highlights the potential for protein degradation therapies to have extended PK/PD effects, which may be due to the slow synthesis rate of the protein.[21]

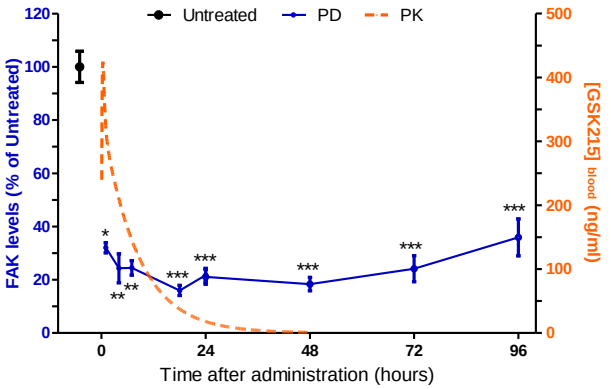

Figure 8. PK/PD of **GSK215** (HCl salt) in naive CD1 mice following a single 8 mg/kg subcutaneous dose.

Conclusion

In summary, we have developed **GSK215** as a potent and selective *in vitro* and *in vivo* FAK degrader tool molecule which shows a differentiated phenotypic response compared to FAK inhibition in cancer cells. The high degradation potency of **GSK215** is derived from an unusually short and rigid linker, which generates a highly cooperative ternary complex which has been characterized by SPR and X-ray crystallography. **GSK215** was found to degrade FAK at low doses *in vivo* in mouse liver, and produced a PK/PD disconnect, with >50% reduction in FAK levels still observed up to 96 hours post-dose, long after the compound has been cleared from blood. We anticipate that **GSK215** will find further use as an *in vitro* and *in vivo* tool compound to probe the effects of FAK degradation, and to explore the potential therapeutic utility of FAK degraders.

Acknowledgements

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Keywords: cancer • drug design • medicinal chemistry • protein degradation • proteolysis-targeting chimeras (PROTACs)

References

[1] F. J. Sulzmaier, C. Jean, D. D. Schlaepfer, *Nat. Rev. Cancer* **2014**, *14*, 598-610.

[2] V. M. Golubovskaya, F. A. Kweh, W. G. Cance, *Histol. Histopathol.* **2009**, *24*, 503-510.

[3] a) W. G. Cance, E. Kurenova, T. Marlowe, V. Golubovskaya, *Sci, Signaling* **2013**, *6*, pe10; b) M. Roy-Luzarraga, K. Hodivala-Dilke, *Clin. Cancer Res.* **2016**, *22*, 3718-3724.

[4] M. D. Schaller, *J. Cell Sci.* **2010**, *123*, 1007-1013.

[5] M. C. Frame, H. Patel, B. Serrels, D. Lietha, M. J. Eck, *Nat. Rev. Mol. Cell Biol.* **2010**, *11*, 802-814.

[6] S. T. Lim, X. L. Chen, Y. Lim, D. A. Hanson, T. T. Vo, K. Howerton, N. Larocque, S. J. Fisher, D. D. Schlaepfer, D. Ilic, *Mol. Cell* **2008**, *29*, 9-22.

[7] A. Serrels, T. Lund, B. Serrels, A. Byron, R. C. McPherson, A. von Kriegsheim, L. Gomez-Cuadrado, M. Canel, M. Muir, J. E. Ring, E. Maniati, A. H. Sims, J. A. Pachter, V. G. Brunton, N. Gilbert, S. M. Anderton, R. J. Nibbs, M. C. Frame, *Cell* **2015**, *163*, 160-173.

[8] J. R. Infante, D. R. Camidge, L. R. Mileshkin, E. X. Chen, R. J. Hicks, D. Rischin, H. Fingert, K. J. Pierce, H. Xu, W. G. Roberts, S. M. Shreeve, H. A. Burris, L. L. Siu, *J. Clin. Oncol.* **2012**, *30*, 1527-1533.

[9] J. C. Soria, H. K. Gan, S. P. Blagden, R. Plummer, H. T. Arkenau, M. Ranson, T. R. Evans, G. Zalcman, R. Bahleda, A. Hollebecque, C. Lemech, E. Dean, J. Brown, D. Gibson, V. Peddareddigari, S. Murray, N. Nebot, J. Mazumdar, L. Swartz, K. R. Auger, R. A. Fleming, R. Singh, M. Millward, *Ann. Oncol.* **2016**, *27*, 2268-2274.

[10] S. F. Jones, L. L. Siu, J. C. Bendell, J. M. Cleary, A. R. Razak, J. R. Infante, S. S. Pandya, P. L. Bedard, K. J. Pierce, B. Houk, W. G. Roberts, S. M. Shreeve, G. I. Shapiro, *Invest. New Drugs* **2015**, *33*, 1100-1107.

[11] M. J. A. de Jonge, N. Steeghs, M. P. Lolkema, S. J. Hotte, H. W. Hirte, D. A. J. van der Biessen, A. R. Abdul Razak, F. De Vos, R. B. Verheijen, D. Schnell, L. C. Pronk, M. Jansen, L. L. Siu, *Targeted Oncology* **2019**, *14*, 43-55.

[12] a) A. Mohanty, R. R. Pharaon, A. Nam, S. Salgia, P. Kulkarni, E. Massarelli, *Expert Opin. Invest. Drugs* **2020**, *29*, 399-409; b) G. Mak, J. C. Soria, S. P. Blagden, R. Plummer, R. A. Fleming, N. Nebot, J. Zhang, J. Mazumdar, D. Rogan, A. Gazzah, I. Rizzuto, A. Greystoke, L. Yan, J. Tolson, K. R. Auger, H. T. Arkenau, *Br. J. Cancer* **2019**, *120*, 975-981.

[13] H. Jiang, S. Hegde, B. L. Knolhoff, Y. Zhu, J. M. Herndon, M. A. Meyer, T. M. Nywening, W. G. Hawkins, I. M. Shapiro, D. T. Weaver, J. A. Pachter, A. Wang-Gillam, D. G. DeNardo, *Nat. Med.* **2016**, *22*, 851-860.

[14] ClinicalTrials.gov, "NCT02758587, Study of FAK (Defactinib) and PD-1 (Pembrolizumab) Inhibition in Advanced Solid Malignancies (FAK-PD1)", can be found under https://clinicaltrials.gov/ct2/show/NCT02758587, **2020**.

[15] H. Fan, X. Zhao, S. Sun, M. Luo, J. L. Guan, *J. Biol. Chem.* **2013**, *288*, 3322-3333.

[16] C. A. Garces, E. V. Kurenova, V. M. Golubovskaya, W. G. Cance, *Cancer Res.* **2006**, *66*, 1446-1454.

[17] D. J. Sieg, C. R. Hauck, D. Ilic, C. K. Klingbeil, E. Schaefer, C. H. Damsky, D. D. Schlaepfer, *Nat. Cell Biol.* **2000**, *2*, 249-256.

[18] T. A. Marlowe, F. L. Lenzo, S. A. Figel, A. T. Grapes, W. G. Cance, *Mol. Cancer Ther.* **2016**, *15*, 3028-3039.

[19] E. V. Kurenova, D. L. Hunt, D. He, A. T. Magis, D. A. Ostrov, W. G. Cance, *J. Med. Chem.* **2009**, *52*, 4716-4724.

[20] a) G. M. Burslem, C. M. Crews, *Cell* **2020**, *181*, 102-114; b) M. Pettersson, C. M. Crews, *Drug Discovery Today: Technol.* **2019**, *31*, 15-27; c) D. P. Bondeson, A. Mares, I. E. Smith, E. Ko, S. Campos, A. H. Miah, K. E. Mulholland, N. Routly, D. L. Buckley, J. L. Gustafson, N. Zinn, P. Grandi, S. Shimamura, G. Bergamini, M. Faelth-Savitski, M. Bantscheff, C. Cox, D. A. Gordon, R. R. Willard, J. J. Flanagan, L. N. Casillas, B. J. Votta, W. den Besten, K. Famm, L. Kruidenier, P. S. Carter, J. D. Harling, I. Churcher, C. M. Crews, *Nat. Chem. Biol.* **2015**, *11*, 611-617.

[21] A. Mares, A. H. Miah, I. E. D. Smith, M. Rackham, A. R. Thawani, J. Cryan, P. A. Haile, B. J. Votta, A. M. Beal, C. Capriotti, M. A. Reilly, D. T. Fisher, N. Zinn, M. Bantscheff, T. T. MacDonald, A. Vossenkamper, P. Dace, I. Churcher, A. B. Benowitz, G. Watt, J. Denyer, P. Scott-Stevens, J. D. Harling, *Commun. Biol.* **2020**, *3*, 140.

[22] H. T. Huang, D. Dobrovolsky, J. Paulk, G. Yang, E. L. Weisberg, Z. M. Doctor, D. L. Buckley, J. H. Cho, E. Ko, J. Jang, K. Shi, H. G. Choi, J. D. Griffin, Y. Li, S. P. Treon, E. S. Fischer, J. E. Bradner, L. Tan, N. S. Gray, *Cell Chem. Biol.* **2018**, *25*, 88-99 e86.

[23] P. M. Cromm, K. T. G. Samarasinghe, J. Hines, C. M. Crews, *J. Am. Chem. Soc.* **2018**, *140*, 17019-17026.

[24] J. Popow, H. Arnhof, G. Bader, H. Berger, A. Ciulli, D. Covini, C. Dank, T. Gmaschitz, P. Greb, J. Karolyi-Ozguer, M. Koegl, D. B. McConnell, M. Pearson, M. Rieger, J. Rinnenthal, V. Roessler, A. Schrenk, M. Spina, S. Steurer, N. Trainor, E. Traxler, C. Wieshofer, A. Zoephel, P. Ettmayer, *J. Med. Chem.* **2019**, *62*, 2508-2520.

[25] H. Gao, Y. Wu, Y. Sun, Y. Yang, G. Zhou, Y. Rao, *ACS Med. Chem. Lett.* **2020**, *11*, 1855-1862.

[26] a) C. M. Crews, P. M. Cromm, A. P. Crew (Yale University, Arvinas Operations Inc.), WO/2020/023851, **2020**; b) N. S. Gray, B. Jiang, B. Nabet, T. Zhang, M. Hao (Dana-Farber Cancer Institute, Inc), WO/2020/069117, **2020**; c) H. Gao, C. Zheng, J. Du, Y. Wu, Y. Sun, C. Han, K, Kee, Y. Rao, *Protein Cell* **2020**, *11*, 534–539.

[27] I. Tanjoni, C. Walsh, S. Uryu, A. Tomar, J. O. Nam, A. Mielgo, S. T. Lim, C. Liang, M. Koenig, C. Sun, N. Patel, C. Kwok, G. McMahon, D. G. Stupack, D. D. Schlaepfer, *Cancer Biol. Ther.* **2010**, *9*, 764-777.

[28] K. Raina, J. Lu, Y. Qian, M. Altieri, D. Gordon, A. M. Rossi, J. Wang, X. Chen, H. Dong, K. Siu, J. D. Winkler, A. P. Crew, C. M. Crews, K. G. Coleman, *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, 7124-7129.

[29] a) M. J. Roy, S. Winkler, S. J. Hughes, C. Whitworth, M. Galant, W. Farnaby, K. Rumpel, A. Ciulli, *ACS Chem. Biol.* **2019**, *14*, 361-368; b) M. S. Gadd, A. Testa, X. Lucas, K. H. Chan, W. Chen, D. J. Lamont, M. Zengerle, A. Ciulli, *Nat. Chem. Biol.* **2017**, *13*, 514-521.

[30] J. Schiemer, R. Horst, Y. Meng, J. I. Montgomery, Y. Xu, X. Feng, K. Borzilleri, D. P. Uccello, C. Leverett, S. Brown, Y. Che, M. F. Brown, M. M. Hayward, A. M. Gilbert, M. C. Noe, M. F. Calabrese, *Nat. Chem. Biol.* **2021**, *17*, 152-160.

[31] W. Farnaby, M. Koegl, M. J. Roy, C. Whitworth, E. Diers, N. Trainor, D. Zollman, S. Steurer, J. Karolyi-Oezguer, C. Riedmueller, T. Gmaschitz, J. Wachter, C. Dank, M. Galant, B. Sharps, K. Rumpel, E. Traxler, T. Gerstberger, R. Schnitzer, O. Petermann, P. Greb, H. Weinstabl, G. Bader, A. Zoephel, A. Weiss-Puxbaum, K. Ehrenhofer-Wolfer, S. Wohrle, G. Boehmelt, J. Rinnenthal, H. Arnhof, N. Wiechens, M. Y. Wu, T. Owen-Hughes, P. Ettmayer, M. Pearson, D. B. McConnell, A. Ciulli, *Nat. Chem. Biol.* **2019**, *15*, 672-680.

[32] C. W. Chung, H. Dai, E. Fernandez, C. P. Tinworth, I. Churcher, J. Cryan, J. Denyer, J. D. Harling, A. Konopacka, M. A. Queisser, C. J. Tame, G. Watt, F. Jiang, D. Qian, A. B. Benowitz, *ACS Chem. Biol.* **2020**, *15*, 2316-2323.

[33] R. P. Nowak, S. L. DeAngelo, D. Buckley, Z. He, K. A. Donovan, J. An, N. Safaee, M. P. Jedrychowski, C. M. Ponthier, M. Ishoey, T. Zhang, J. D. Mancias, N. S. Gray, J. E. Bradner, E. S. Fischer, *Nat. Chem. Biol.* **2018**, *14*, 706-714.

[34] G. Du, J. Jiang, Q. Wu, N. J. Henning, K. A. Donovan, H. Yue, J. Che, W. Lu, E. S. Fischer, N. Bardeesy, T. Zhang, N. S. Gray, *Angew. Chem. Int. Ed. Engl.* **2021**, *60*, 15905-15911

[35] M. M. Savitski, N. Zinn, M. Faelth-Savitski, D. Poeckel, S. Gade, I. Becher, M. Muelbaier, A. J. Wagner, K. Strohmer, T. Werner, S. Melchert, M. Petretich, A. Rutkowska, J. Vappiani, H. Franken, M. Steidel, G. M. Sweetman, O. Gilan, E. Y. N. Lam, M. A. Dawson, R. K. Prinjha, P. Grandi, G. Bergamini, M. Bantscheff, *Cell* **2018**, *173*, 260-274, e25.

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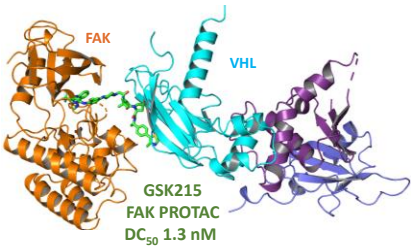

A PROTAC with an unusually short linker potently degrades FAK. SPR and X-ray crystallography revealed a highly cooperative FAK-PROTAC-VCB ternary complex, and FAK degradation showed enhanced effects on 3D cell growth compared to FAK inhibitors. In mice, GSK215 induced rapid and sustained degradation of FAK with a profound PK/PD disconnect.