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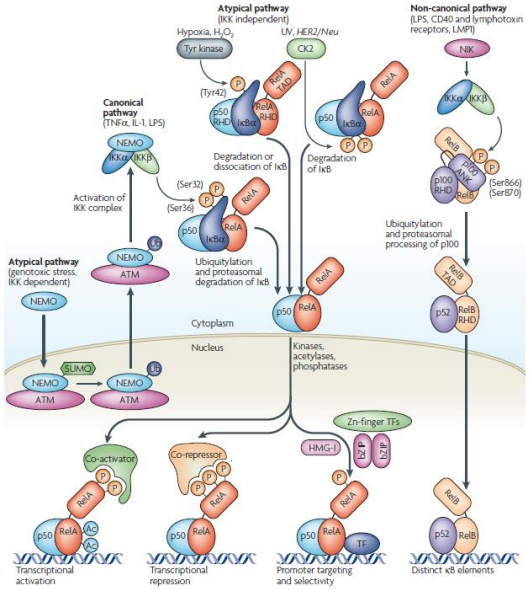

Targeting IKKα in cancer

Professor Simon P. Mackay
Small Molecule Drug Discovery Group
University of Strathclyde

Hayden & Ghosh (2008) ***Cell 132***, 344

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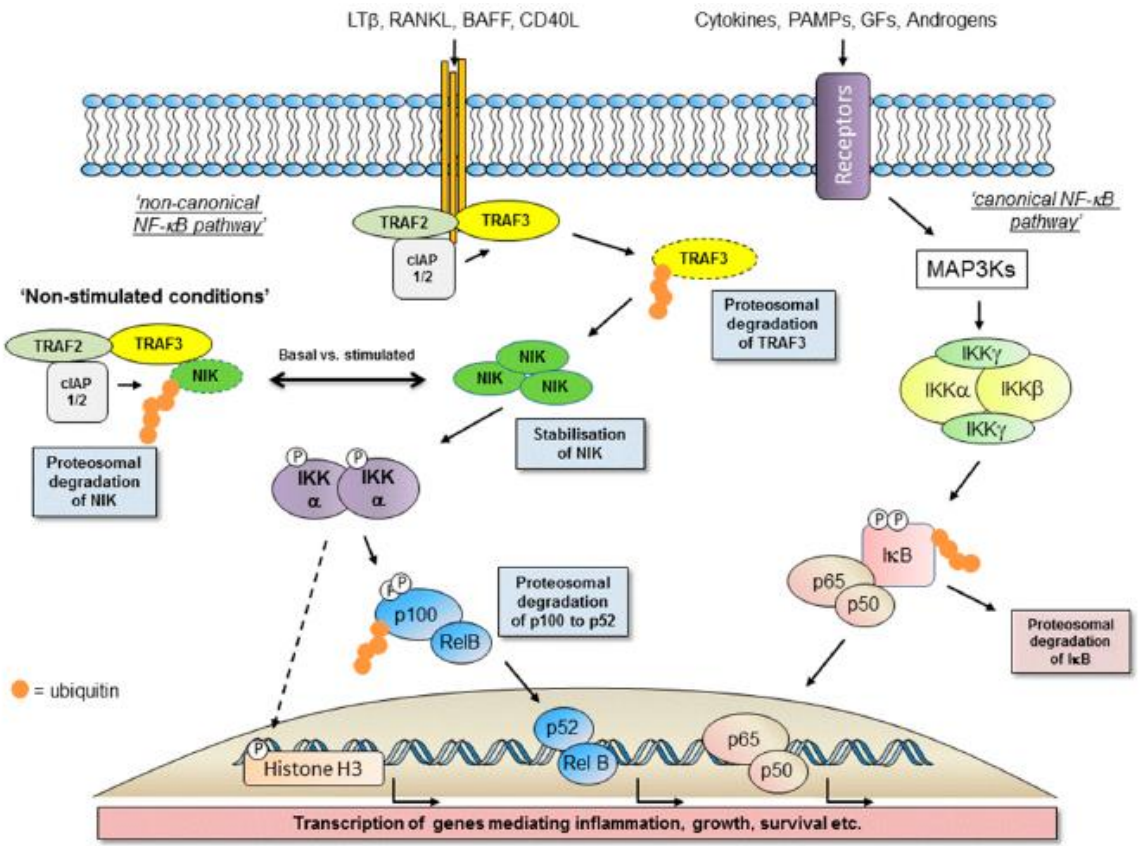
IKKα in cell signalling

Activation of IKKα stimulates responses via the regulation of diverse pathways and gene expression both independent of and through the direct regulation of NF-kB.



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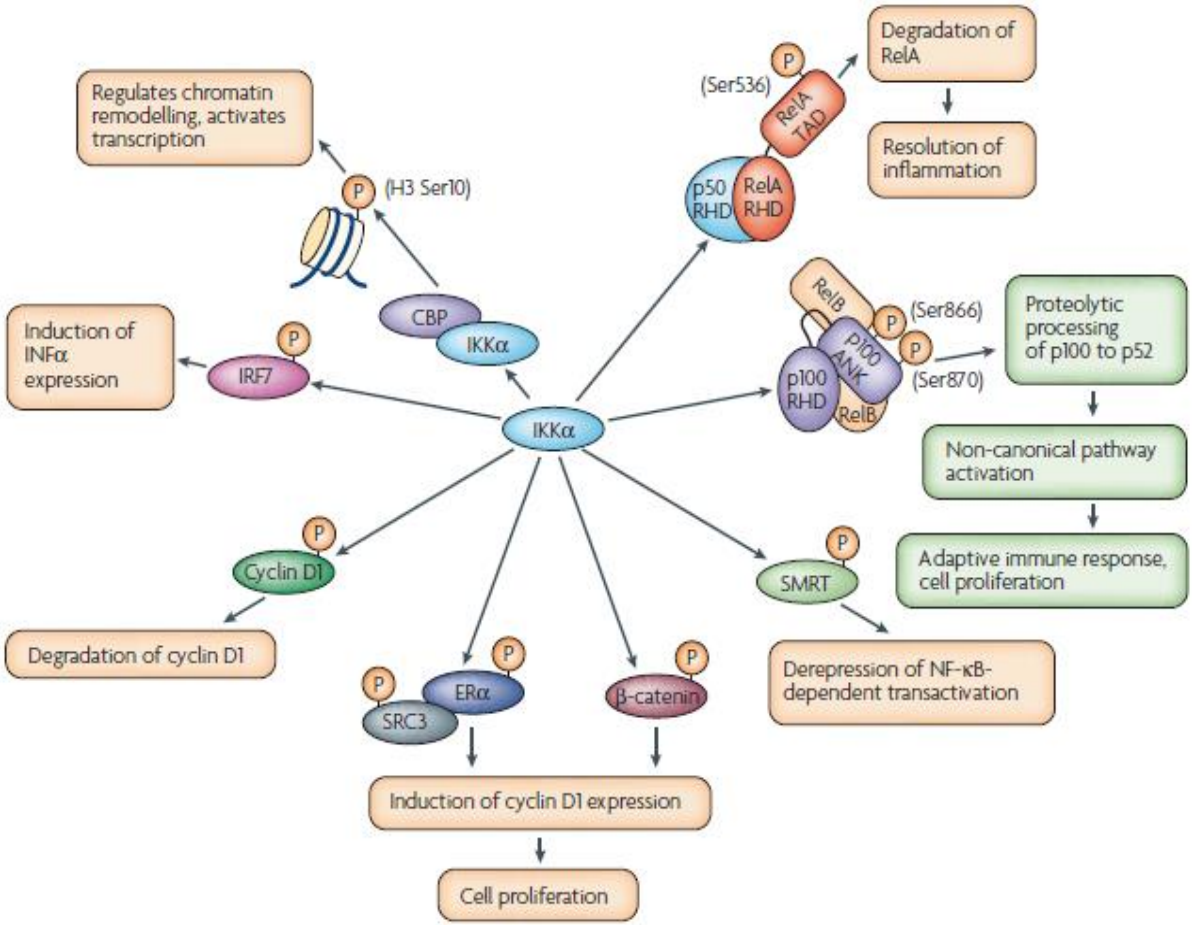


Perkins (2009)

***Hallmarks of cancer**: IKKa-mediated regulation of genes/proteins contributing to acquired capabilities*

Kwak et al., (2011); Park et al., (2005)

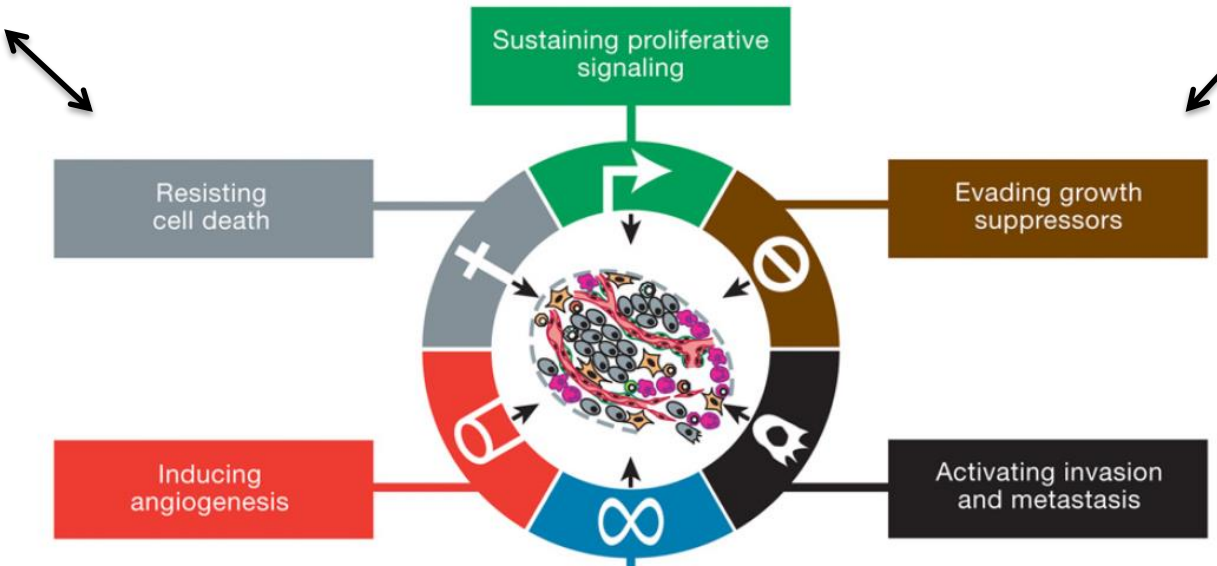
Cyclin D1 expression/phosphorylation
→ Cell cycle progression
AR/ER expression/transactivation
→ Hormone dependent/independent signalling and growth

Schumm et al., (2006)

Modulation of p53, p21 (WAF/CIP1), PUMA, Chk1 expression

Tu et al., (2006)
Ammirante et al., (2013)

Activating E2F1/inactivating Rb
Modulation of p53



Ammirante et al. (2010); Ammirante et al., (2014)

CXCL13, CXCL13 expression
→ Modulation of angiogenesis,
→ stromal support of tumours

Dejardin et al., (2002)

Telomerase reactivation
→ C250T hTERT promoter mutation
→ 30% of GBMs

Li et al., (2015)

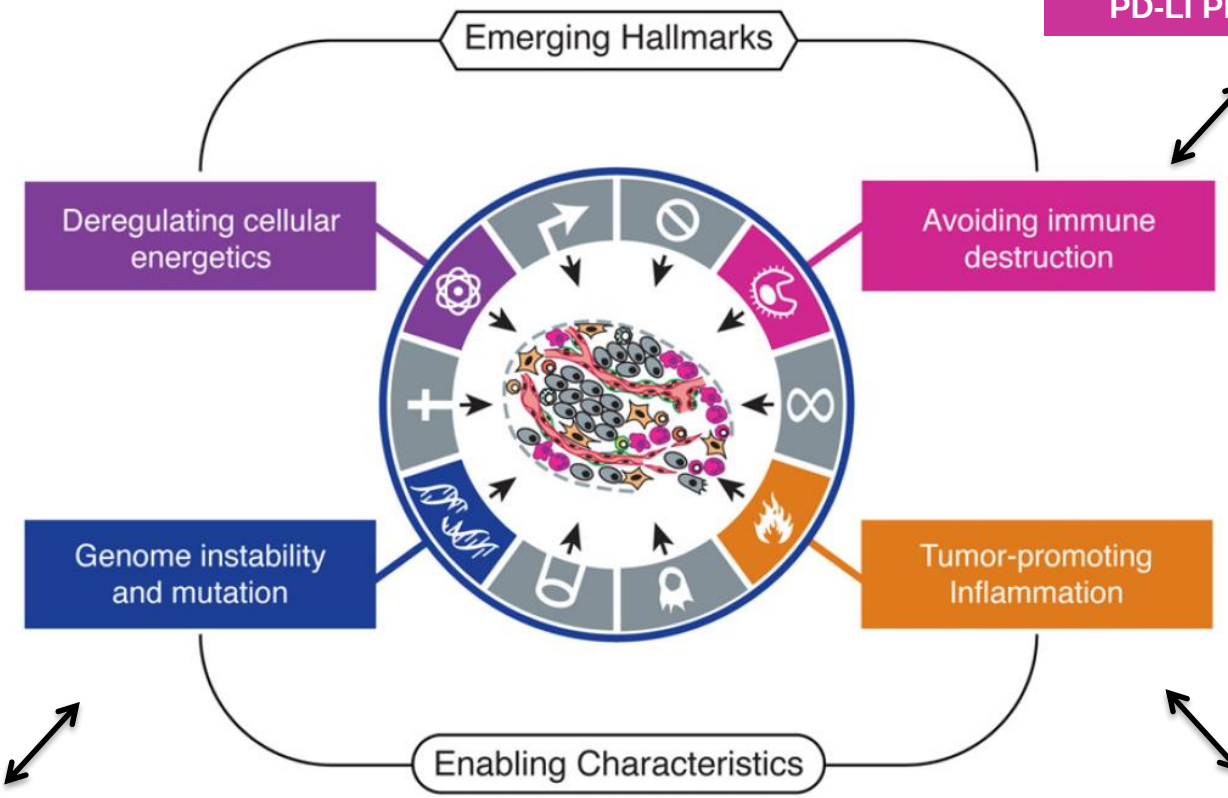
ICAM/VCAM expression
→ Increased cell-cell contact/migration
MMP2/9 induction
→ Increased invasion
Maspin downregulation
→ Decreased suppression of invasion

Hanahan & Weinberg, 2011

***Hallmarks of cancer**: IKKa-mediated regulation of genes/proteins contributing to acquired capabilities*

see Sun et al. (2017)

**Modulation of B/T-lymphocyte, DC function??
PD-LI PD1 expression??**



Dejardin et al., (2002); Ammirante et al. (2014)

Elevated AURK A/B expression/activity

Prajapati et al; (2006); Irelan et al. (2007)

**Cytokine/chemokine induction
→ Il-6, IL-8, CXCL12, CXCL13 expression
→ supports stromal-tumour microenvironment??**

Hanahan & Weinberg, 2011

IKKα: a target in multiple cancer processes

IKKα mediates both p52-RelB NF-κB–dependent and NF-κB–independent transcription

- *Aside of driving a proliferative response, IKKa-p52-RelB signalling regulates genes and proteins that underpin acquired capabilities (or Hallmarks) of cancer*
- *Majority of 'Hallmarks' may be targeted via IKKa with our small molecule inhibitors*
- *Relevant in solid tumour (prostate, pancreatic, breast, colorectal etc.) and haematological settings*
- *Stromal-tumour microenvironment (CXCL12 &13/CXCR axes) and potentially immune-evasion may be targetable*
- *Tumour re-emergence and drug resistant scenarios are targetable in defined settings*
  - AR hormone-independent signalling in CRPC, specific breast cancers (TNBC)
  - hTERT/telomerase complex reactivation in approx. 30% of glioblastomas
  - Rebound IKKα- NF-κB signalling generated in response to classic cytotoxics and agents driving DNA damage responses (DDRs) (combination approaches)

The health need we are primarily seeking to address:

- For castrate reisistant prostate cancer (CRPC), current therapies are severely limited
- Of 1 in 8 men diagnosed with prostate cancer, 25% will die of metastatic disease.
- Despite the approval of four new agents (carbazitaxel, abiraterone, enzalutamide and provenge) that have been shown to prolong life for up to 3-9 months in advanced PC patients, it remains an incurable disease.

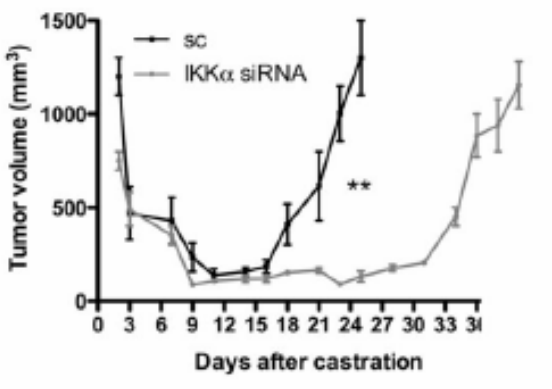
How CRPC develops:

- PC cells develop a series of strategies to survive and grow in response to ADT
- Development of resistance to ADT results in patient relapse and emergence of CRPC
- Molecular processes underlying this transition are not completely understood, although it is now recognised that despite insensitivity to ADT, the tumours remain under the control of androgen-signalling
- Defined mechanisms of resistance include
  - AR gene amplification, mutation, remodelling of AR-regulated genes, deregulation of co-regulators
  - Ligand-independent AR activation in response to GFs and IL-8
  - IL-8 signalling promotes androgen-independent proliferation of PC cells via induction of AR expression and activation
  - PTEN loss induces a selective upregulation of IL-8 signalling that sustains the growth and survival in PTEN-deficient prostate carcinoma

Waugh & Wilson (2008) Clin Cancer Res 14, 6735-6741; Waugh et al (2008) 29, 1148-1156; Waugh et al (2013) European Urology64, 177-188; Vancurova et al (2013) J Immunol, 191, 2837-2846

Links between castrate resistant prostate cancer (CRPC) and IKKα

- A mutation that prevents IKKα activation slows down prostate cancer growth and inhibits metastasis in TRAMP mice
- Suppression of IKKα by siRNA delays the appearance of CRPC in the murine myc-CaP allograft model of PC
- The amount of active nuclear IKKα in mouse and human prostate cancer correlates with metastatic progression
- Nuclear IKKα appears to provide a mechanism for hormone resistance in the development of CRPC
- Deletion of BAG3 which is required for IKKα nuclear translocation delays development of castrate-resistant disease
- Inflammatory responses induced by the dying primary tumour may contribute to the failure of ADT
- Individuals who produce high levels of lymphotoxin are more likely to develop CRPC and should be the main beneficiaries of therapies that reduce its effects.



Ammirante et al. (2007) Nature 446, 690; Lessard L et al. (2007) Cell Signal. 19, 1093; Derwent et al (Arch Dermatol Res (2016) 308, 733-742; *Ammirante et al. (2010) Nature 464, 302; Ammirante et al (2013) Genes Dev. 27; 1435-40; Ammirante et al (2011) Cell Death Dis. 2:e139. doi: 10.1038*

Links between AR-signalling and IKKα

- Nuclear localisation of RelB is associated with higher grade tumours
- The treatment of prostate cancer cells with androgens induces accumulation of p52
- IKKα-activated NF-κB2/p52 promotes:
  - castrate-resistant growth in LNCaP cells by inhibiting cell cycle arrest and apoptotic cell death induced by androgen deprivation
  - resistance of prostate cancer cells to enzalutamide by the activation of AR and its splice variants
  - prostate cancer cell growth *in vitro* and *in vivo* and may play a critical role in the progression of castration-resistant prostate cancer via increased activation of AR
- Overexpression of IKKα in prostate cancer cells increases AR-dependent activity and IKKα catalytic activity is required for increased nuclear AR
- IKKα plays a crucial role in progression to CRPC by enhancing p100 processing and mobilising p52:AR dimers in a hormone-dependent manner
- Silencing of IKKα reduces androgen receptor activity and gene expression

Nadimintiny et al (2008) Prostate 68, 1725-1733; Nadimintiny et al (2013) Mol Cancer Ther 12, 1629-1637; Nadiminty et al (2010) Cancer Res 70, 3309-3319; Marienfeld et al (2012) Neoplasma 14, 178-189

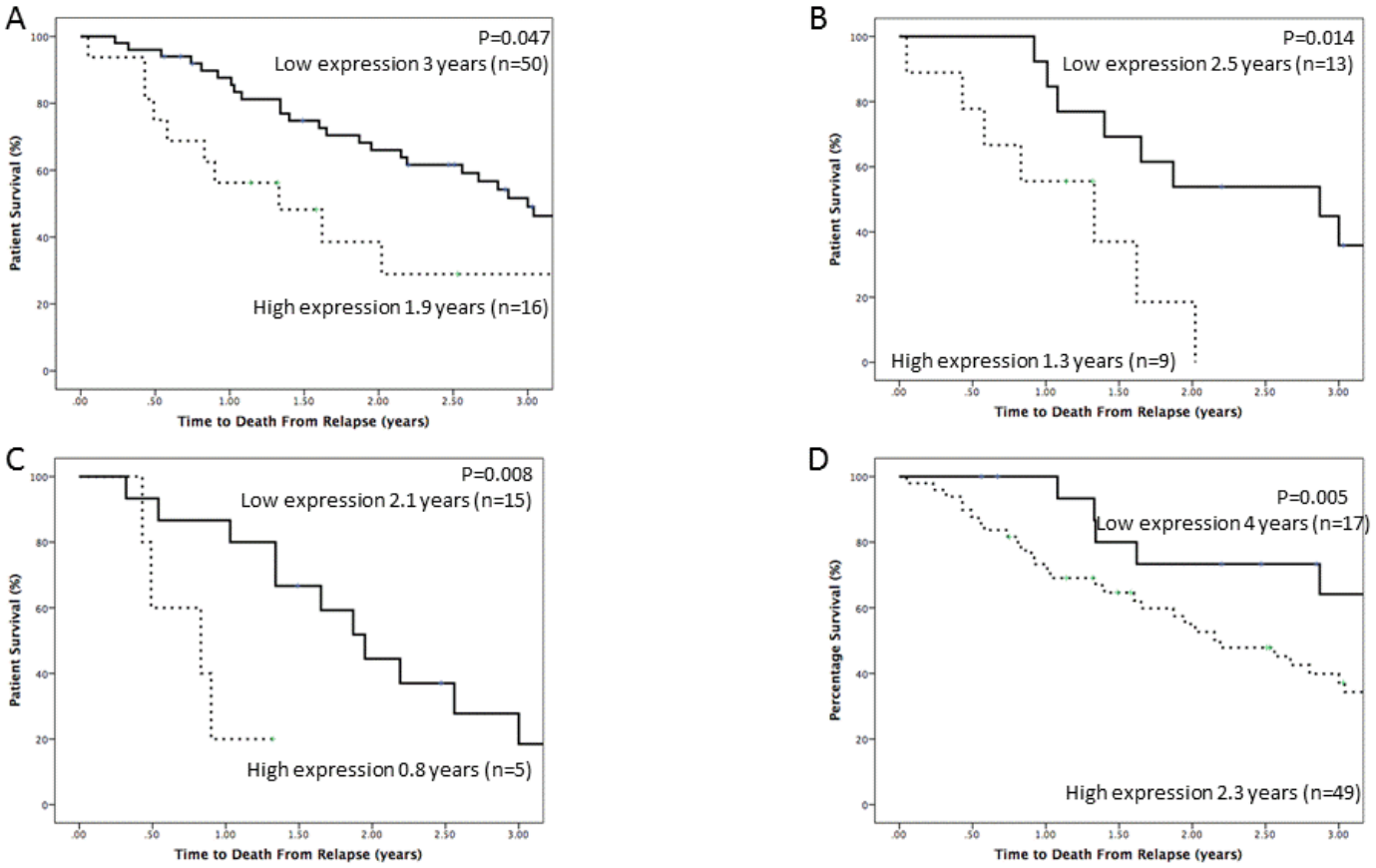
Clinical positioning of IKKα inhibitors in CRPC

- Despite the observed benefit of combining ADT and chemotherapy in pre-castrate patients, many men still exhibit clinical progression to CRPC.
- The development of novel agents that selectively target critical signalling pathways underpinning disease progression and resistance is essential.
- Effective targeting of IKKα may therefore enhance ADT responses by concurrently inhibiting androgen-driven and androgen-independent AR activity.
- IKKα inhibition may also abrogate inflammatory microenvironment signalling and eliminate tumour-promoting stimuli from adjacent stroma and infiltrating monocytes.
- IKKα inhibitors have the potential to alter disease course, restore/prolong sensitivity to AR-targeted therapy and improve survival. Moreover, their use in hormone-sensitive *de novo* metastatic disease may significantly extend the benefit duration of conventional therapies and reduce the overall incidence of CRPC.

Potential clinical trial scenarios:

- Last-line therapy in patients with CRPC that have failed standard-of-care treatment
- Combination therapy with ADT to prevent the emergence of CRPC/prolong sensitivity to ADT
- Combination therapy in CRPC patients to restore sensitivity to ADT/reduce resistance development to chemotherapy
- Single-agent therapy to prevent the emergence of CRPC

Clinical relevance of IKKα in CRPC - will it help stratification?



IKKα immunoreactivity was assessed in 66 castrate resistant prostate cancer specimens.

A: In CRPC specimens, high cytoplasmic IKKα expression (above the upper quartile) was significantly associated with shorter TTDR. **B**: This observation was potentiated in patients with high AR expression, in CRPC specimens with high AR expression, high cytoplasmic IKKα expression was significantly associated with shorter TTDR. **C**: This observation was potentiated in patients with highly proliferating tumours, in CRPC specimens with high Ki67 expression, high cytoplasmic IKKα expression was significantly associated with shorter TTDR. **D:** In CRPC specimens, high nuclear IKKα expression (above the lower quartile) was significantly associated with shorter TTDR.

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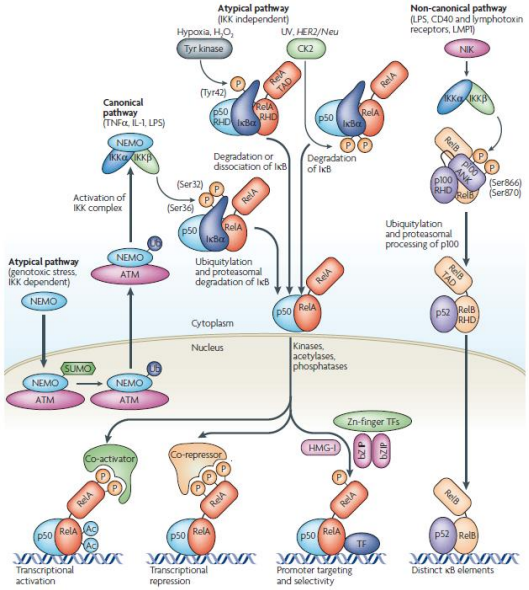

IKK Drug Discovery Programme

Hayden & Ghosh (2008) ***Cell 132***, 344

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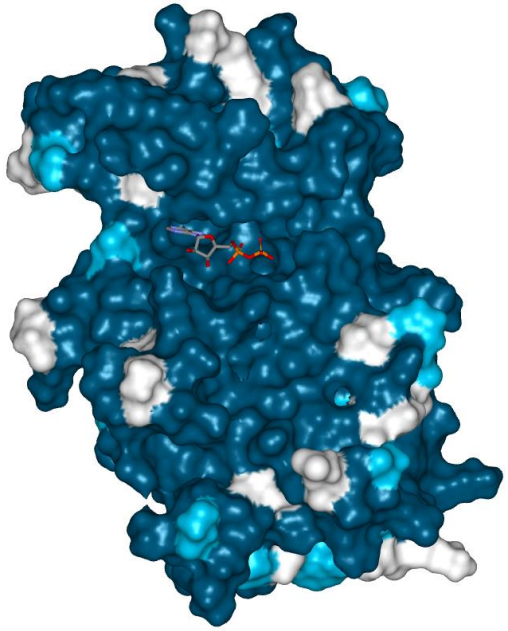
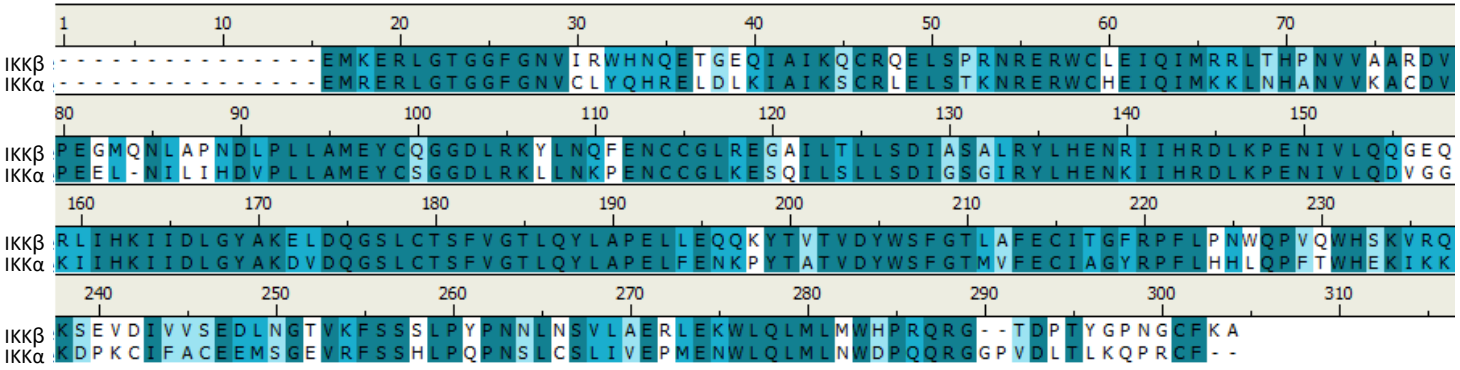
Is the target druggable and are there selectivity challenges?

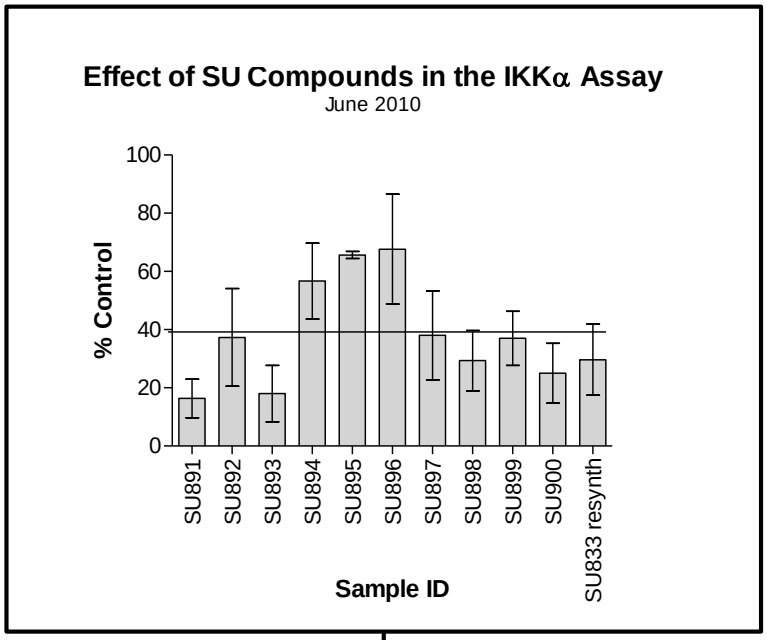


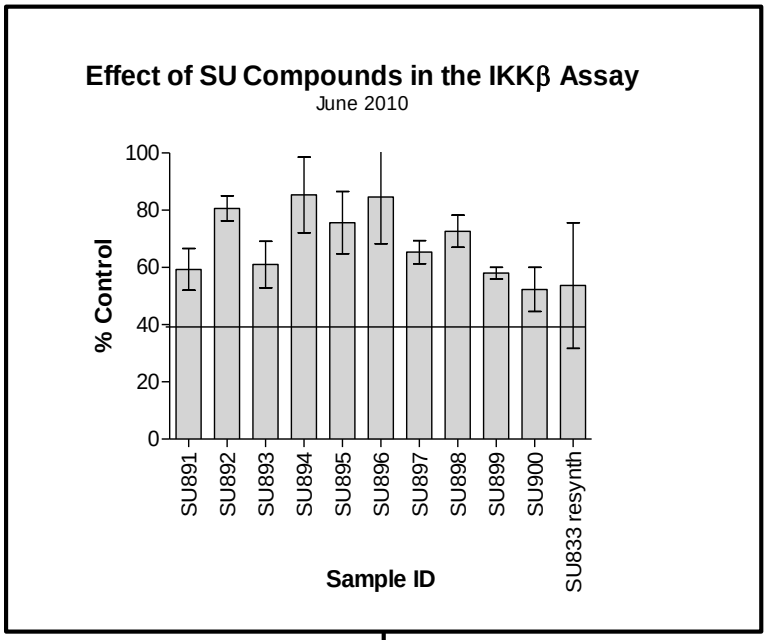
Sequence identity between IKKα and IKKβ:

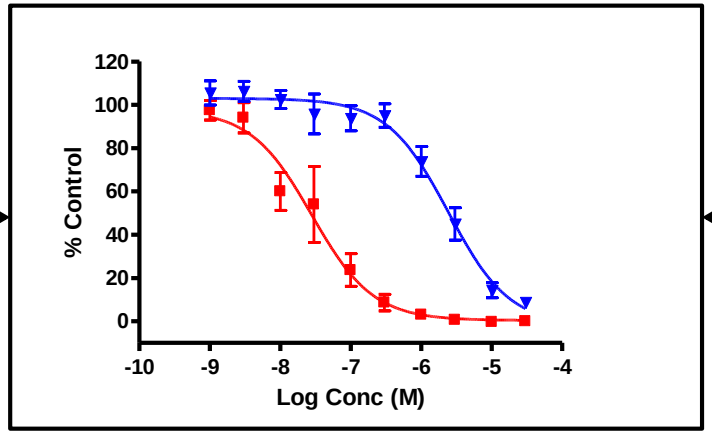
- Identical residues
- Non-identical but similar residues
- Non-identical and dissimilar residues



In-house library was designed and assessed against both kinases before determining IC_{50}/K_i values







IKKα and IKKβ activity is assessed in 96-well plate format using a DELFIA-based assay with antibodies to phosphorylated peptide substrates

Identifying PD readouts for IKKα and IKKβ to assess selective on-target engagement by compounds in cells

Target engagement with IKKα – measured by assessing (Western Blot)

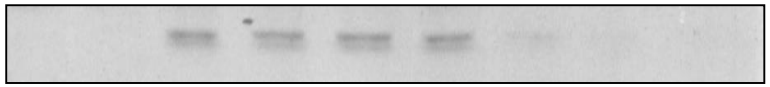
- *The inhibition of p100 phosphorylation by IKKα in cells*
- *The inhibition of p100 processing to p52*
- *The inhibition of RelB translocation to the nucleus*

Target *non*-engagement with IKKβ – measured by assessing (Western Blot)

- *The appearance of IkB-α as a consequence of off-target IKKβ inhibition (IkB-α is no longer being degraded)*
- *The inhibition of p65 phosphorylation by IKKβ*
- *The inhibition of p105 phosphorylation by IKKβ*

SU1433 demonstrates target engagement with IKKα and phenotypic effects in different cancer cell lines

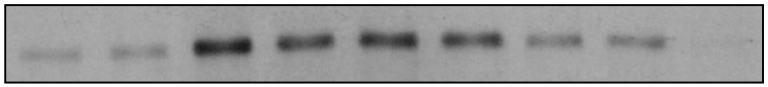
A



← p-p100

IC_{50} = 0.6 μM

---	---	+	+	+	+	+	+	+	LTα1β2 (15ng/ml)
---	---	---	+	---	---	---	---	---	DMSO (0.05%)
---	10	---	---	0.1	0.3	1	3	10	SU1433 (μM)



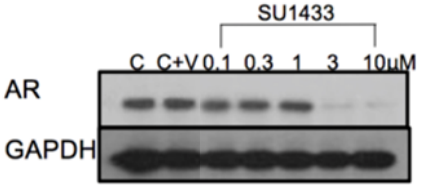
← Nuclear Rel B

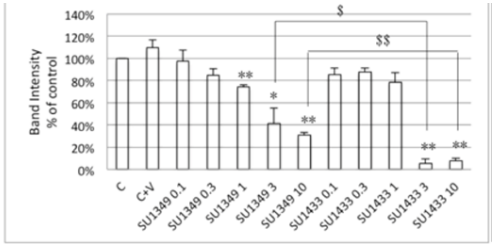
IC_{50} = 0.4μM

---	---	+	+	+	+	+	+	+	LTα1β2 (10ng/ml)
+	---	+	---	---	---	---	---	---	DMSO (0.015%)
---	3	---	0.01	0.03	0.3	0.1	1	3	SU1433 (μM)

A: Effect of SU1433 on primary PD-readouts for IKKα in PC3M cells

B





C

MDA-MB-231:

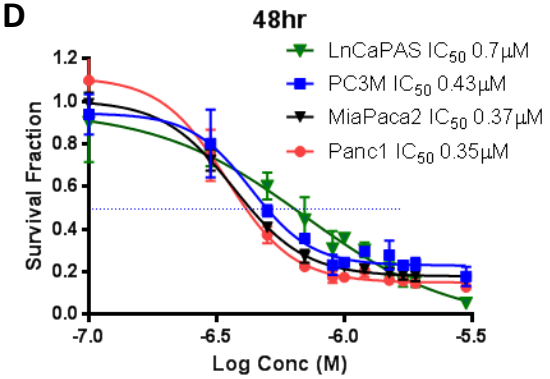
← p-p100

...	10	...	...	0.1	0.3	1	3	10	SU1433 (μM)
...	...	...	+	...	...	...	...	...	DMSO (0.05%)
...	...	+	+	+	+	+	+	+	LTα1β2 (20 ng/ml)

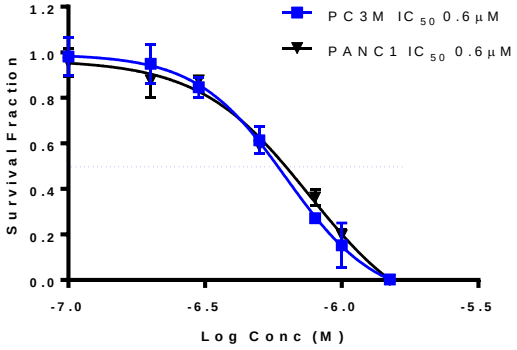
B: SU1433 reduces AR protein expression in LNCaP AI cells

C: SU1433 reduces p100 phosphorylation in a TNBC cell line

D



E

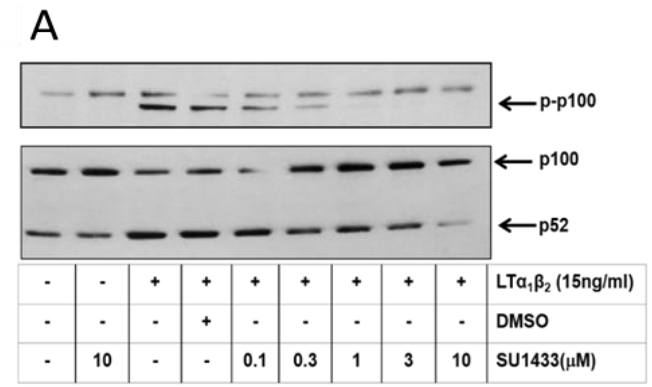


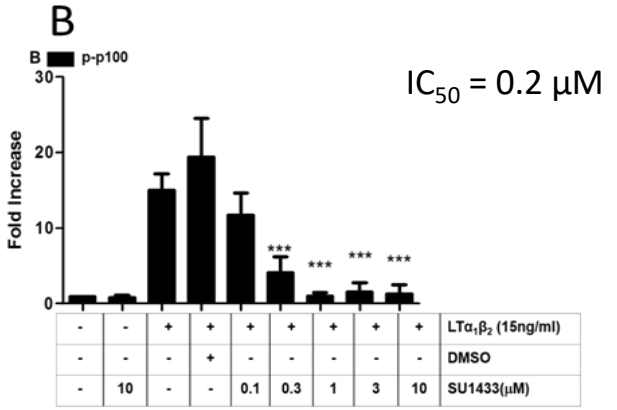
D. Treatment with **SU1433** for 48 hours reduced the viability of PC3M, LNCaP, PANC1 and MiaPaca2 cells.
E. the effect of SU1433 on the clonogenic capacity of PC3M and PANC1 cells following 8 day continuous treatment.

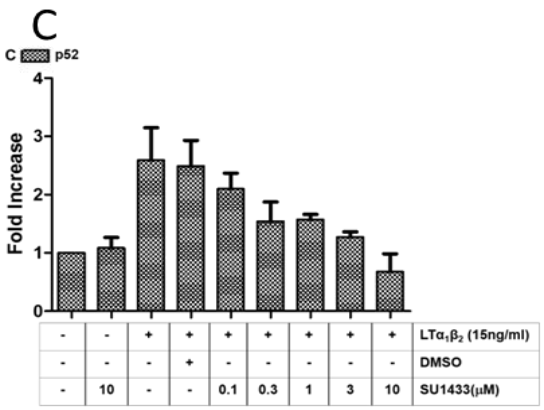
SU1433 inhibits IKKα PD markers in the non-canonical NF-kB pathway in pancreatic cancer cells

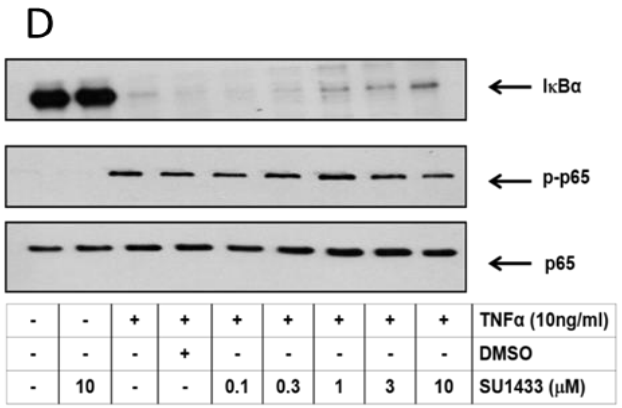
Effect of SU1433 in Panc1 cells.

SU1433 inhibits $LT\alpha_1\beta_2$ induced phosphorylation of p-p100 and had no effect on TNFα-mediated IkBα degradation and phosphorylation of p65 NF-kB in Panc-1 cells



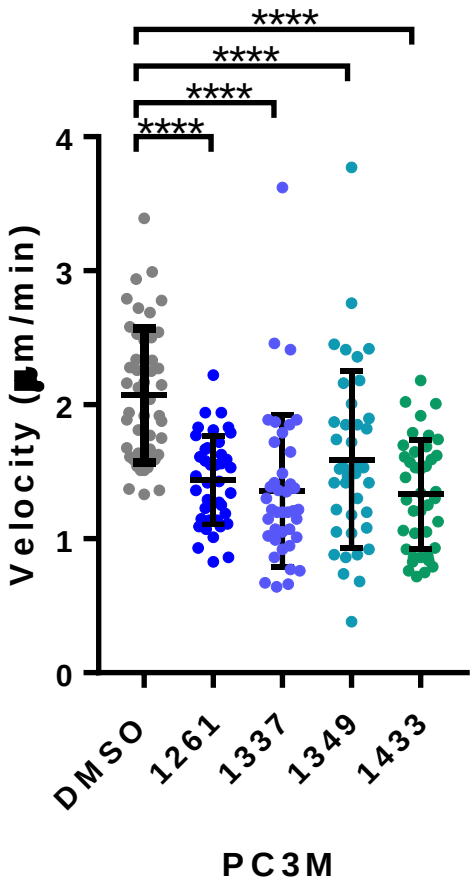


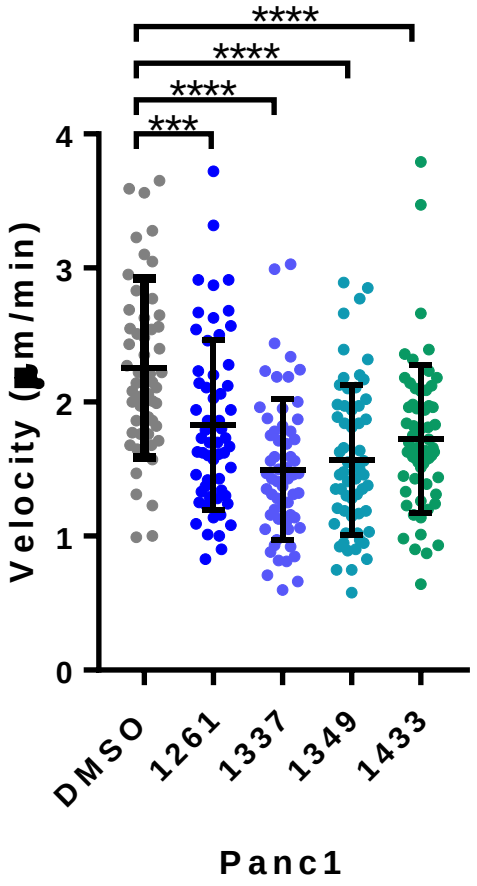




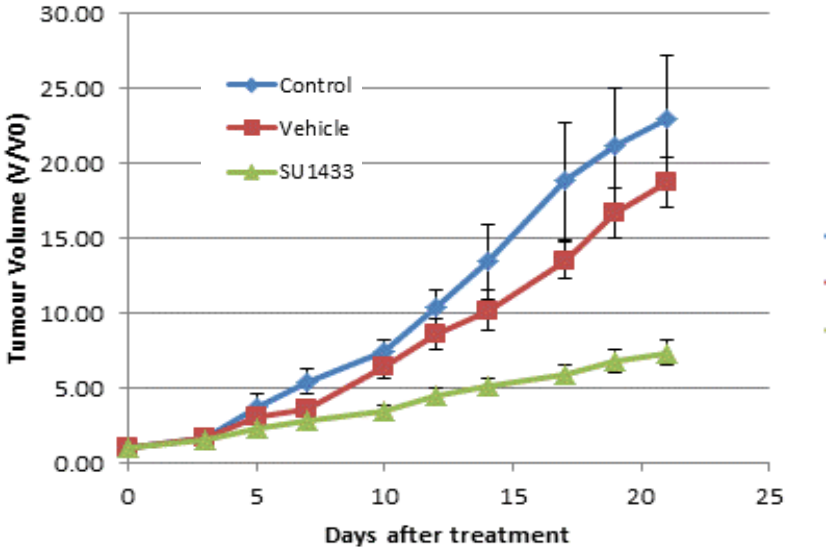
Phenotypic effects of SU1433

SU1433 (and SU1349) inhibits PC3M and Panc1 cell migration at 1 µM as assessed by timelapse microscopy





Studies in nude mice bearing PC3M xenografts show SU1433 inhibits tumour growth by 60%



Dunn's multiple comparisons test	Mean rank diff.	Significance	Adjusted P Value
Control vs. vehicle	1.875	ns	>0.9999
Control vs. treated	12.38	**	0.0014
Vehicle vs. treated	10.5	**	0.0089

Figure 3A: Xenografts were established in nude mice by subcutaneous injection of 5 × 10^6 PC3M-Luc-c6 cells. After 8 days, mice bearing tumours of approximately 60 mm^3 in volume were randomized into 3 treatment groups of 8 mice in each. One group of mice received intraperitoneal injection (i.p.) of 50 mg/kg SU1433 once daily dissolved in a vehicle of DMSO (5%), solutol HS15 (5%) and 15% w/v hydroxypropyl-β-cyclodextrin in water for injection (90%) for a total of 21 days. Another group received once daily i.p injection of the vehicle (100µL) alone whilst the last group received no treatment.

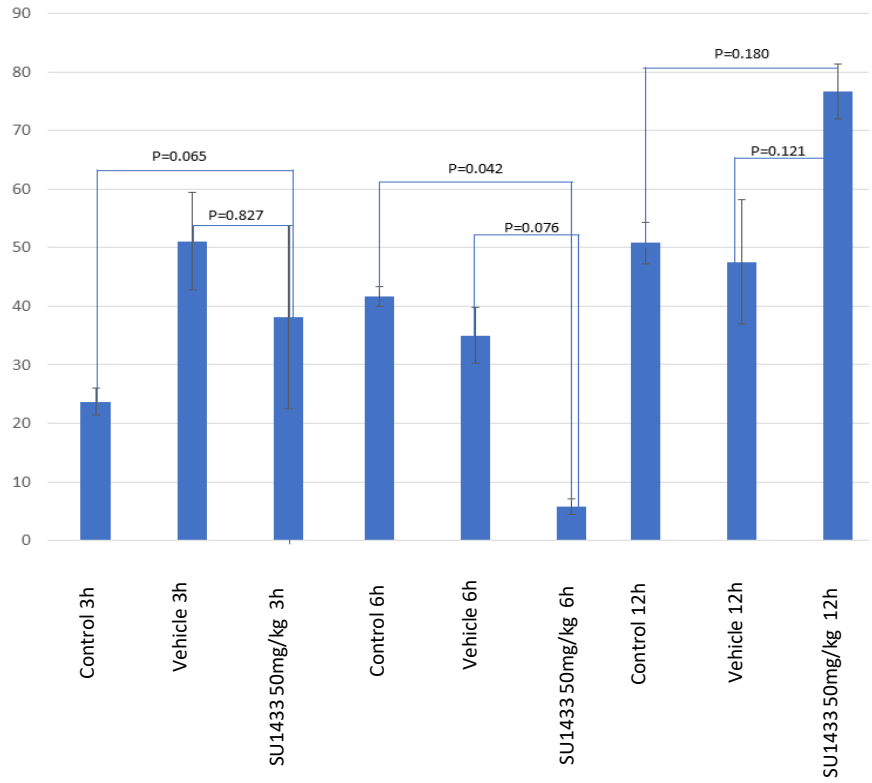


Figure 3B: Staining for proliferative marker Ki67 indicates that in tumour tissue harvested after a single 50mg/kg i.p. dose of SU1433, an antiproliferative effect was exerted, (6h), then dissipated as the compound was eliminated (12h)

DMPK (*in vivo* - mouse)	i.v.	i.p.	p.o.
Clearance (mL/min/kg)	120		
Terminal half-life (h)	0.2	2.1	1.6
V_{ss} (L/kg)	1.3		
Bioavailability (%)		97	17

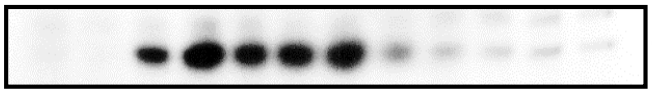
SU1433 has excellent selectivity across the kinome

Kinase	SU1433	Kinase	SU1433	Kinase	SU1433	Kinase	SU1433
Abl	94	CHK2	104	IRAK1	88	Pim-1	109
ACK1	95	CK1δ	74	IRAK4	104	PKBα	100
ALK	107	CK2	57	JAK2	77	PKCα	100
Arg	104	CLK2	-1	JAK3	92	PKCη	93
AMPKα1	99	c-RAF	102	JNK1α1	87	PKCμ	89
ARK5	102	cSRC	97	JNK3	89	PKD2	111
Aurora-A	104	DAPK1	98	KDR	89	Plk1	101
Aurora-B	93	DYRK2(h	22	Lck	101	PRAK	118
Aurora-C	89	EGFR	93	LIMK1	88	PRK2	66
BRK	97	EphA1	41	MAPK1	89	Ret	96
CaMKI	91	ErbB4	103	MEK1	82	ROCK-I	92
CaMKIIβ	99	Fes	103	MARK1	97	ROCK-II	71
CaMKIIγ	90	FGFR1	112	Mer	83	Rsk1	108
CaMKIδ	102	FGFR3	100	Met	109	SAPK3	12
CaMKIIδ	94	Flt1	102	MINK	86	SAPK4	58
CaMKIV	111	Flt3	54	MKK7β	101	SGK	116
CDK1	98	Flt4(h)	71	MSK1	116	Syk	95
CDK2	94	Fms(h)	111	MST2	100	TAK1	104
CDK3	96	GCK(h)	69	mTOR	100	TAO1	39
CDK5	84	GSK3α(h)	84	NEK2	93	TBK1	86
CDK6	100	GSK3β(h)	72	PAK2	100	TrkA	93
CDK7	42	Haspin(h)	54	PAR-1Bα	85	TrkB	107
CDK9	48	HIPK1(h)	66	PDGFRβ	138	ZAP-70	111
CHK1	105	IR(h)	109	PDK1	88	ZIPK	111

Kinome profile for **SU1433**: % residual activity @ 1 μM (at ATP K_m)

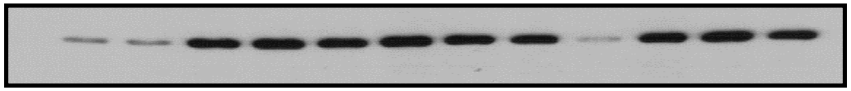
SU1644 is more potent and selective against IKKα than SU1433.

A



p-p100
IC_{50} = 0.05 μM

-	-	-	+	+	+	+	+	+	+	+	+	LTα1β2(15ng/ml)
-	-	+	-	+	-	-	-	-	-	-	-	DMSO (10 μM)
-	10	-	-	-	0.01	0.03	0.1	0.3	1	3	10	SU1644 (μM)



p-p65
IC_{50} > 10 μM

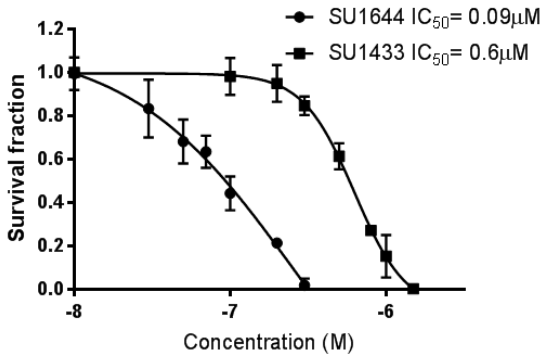
IκBα
IC_{50} > 10 μM

-	-	-	+	+	+	+	+	-	+	+	+	TNFα (10ng/ml)
-	-	+	-	+	-	-	-	+	-	-	-	DMSO (10 mM)
-	10	-	-	-	0.01	0.03	0.1	-	10	3	1	SU1644 (mM)

A. SU1644 is more potent against IKKα-driven markers (e.g. p100 phosphorylation) in PC3M cells (IC_{50} < 0.1 μM) and inactive against IKKβ-driven markers (IC50 > 10 μM)

SU1644 Biochemical IC_{50}s:
IKKα = 15 nM
IKKβ = 1100 nM

B



B. SU1644 is the most potent compound to-date at reducing the clonogenic capacity of PC3M cells following 8 day continuous treatment (IC_{50} = 0.09 μM .

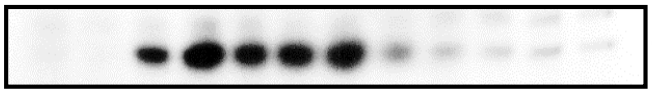
SU1644 *in vitro* DMPK properties

SU1433: Murine S9 fraction C_L=18.5 μl/min/mg; $t_{1/2}$ = 75 min
SU1644: Murine S9 fraction stable
SU1433: Plasma stability (amount remaining after 2 h): 100%
SU1644: Plasma stability (amount remaining after 2 h): 100%

SU1433: Solubility= 65 μM; Caco2 permeability= 10.7×10^{-6} cm s^{-1}; efflux = 2.8
SU1644: Solubility= 2 μM; Caco2 permeability= 2.6×10^{-6} cm s^{-1}; efflux = 1.5

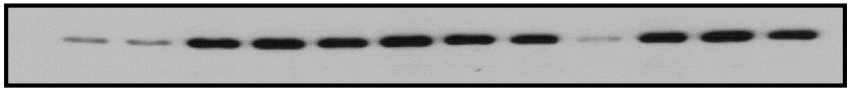
SU1433 and SU1644 enhance the response to enzalutamide/abiraterone

A



p-p100
IC_{50} = 0.05 µM

-	-	-	+	+	+	+	+	+	+	+	+	LTα1β2(15ng/ml)
-	-	+	-	+	-	-	-	-	-	-	-	DMSO (10 µM)
-	10	-	-	-	0.01	0.03	0.1	0.3	1	3	10	SU1644 (µM)



p-p65
IC_{50} > 10 µM

IκBα
IC_{50} > 10 µM

-	-	-	+	+	+	+	+	-	+	+	+	TNFα (10ng/ml)
-	-	+	-	+	-	-	-	+	-	-	-	DMSO (10 mM)
-	10	-	-	-	0.01	0.03	0.1	-	10	3	1	SU1644 (mM)

A. SU1644 is more potent against IKKα-driven markers (e.g. p100 phosphorylation) in PC3M cells (IC_{50} < 0.1 µM) and inactive against IKKβ-driven markers (IC50 > 10 µM)

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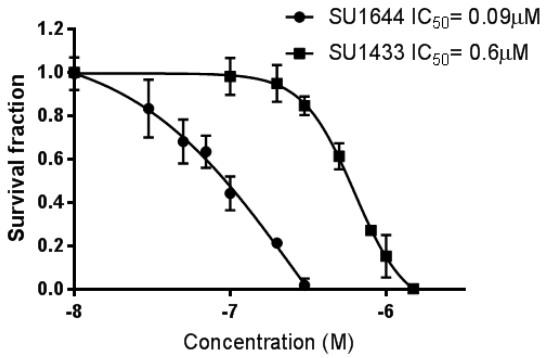
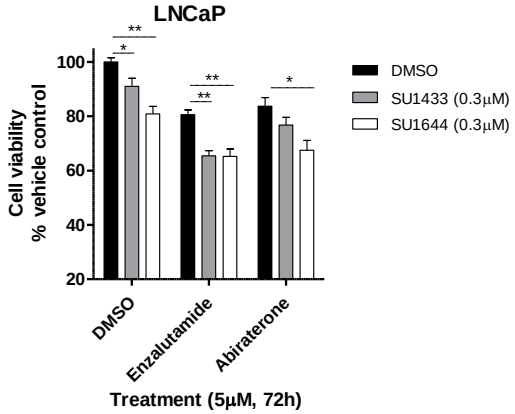
B



B. SU1644 is the most potent compound to-date at reducing the clonogenic capacity of PC3M cells following 8 day continuous treatment (IC_{50} = 0.09 µM .

C



C. Cell viability data demonstrating improved response to enzalutamide (5µM) or abiraterone (5 µM) in LNCaP cells in the presence of SU1433 and SU1644 (0.3µM).

SU1644 *in vitro* DMPK properties

SU1433: Murine S9 fraction C_L=18.5 µl/min/mg; $t_{1/2}$ = 75 min
SU1644: Murine S9 fraction stable
SU1433: Plasma stability (amount remaining after 2 h): 100%
SU1644: Plasma stability (amount remaining after 2 h): 100%

SU1433: Solubility= 65 µM; Caco2 permeability= 10.7x10^{-6} cm s^{-1}; efflux = 2.8
SU1644: Solubility= 2 µM; Caco2 permeability= 2.6x10^{-6} cm s^{-1}; efflux = 1.5

Current position of the programme

- IKKα plays a pivotal role in a number of cancers of unmet need including advanced prostate cancer, multiple myeloma, pancreatic, colorectal and breast cancer.

- We have a first-in-class lead series that demonstrates potent and selective target engagement for IKKα over IKKβ that produce desirable phenotypic outcomes in prostate and pancreatic cancer cells

- SU1433, despite having a short half-life *in vivo*, retards tumour growth of PC3M xenografts in mice.

- We're addressing the metabolic liabilities of SU1433 through the development of SU1644, but solubility needs to be improved

- Compound development is now focused on generating a compound that can be administered orally with steady-state plasma concentrations for sustained pharmacological exposure

Requirements to take the project forward (subject to funding).

- Further positioning of IKKα in prostate cancer using existing tool compounds (SU1433/SU1644)
  - Requires evaluation as an inflammatory mediator of progression and the development of resistance in cell-based models
  - Requires evaluation as an oncogenic mediator in cell-based models

- Development of SU1433/SU1644 into an '*in vivo* ready' preclinical lead compound
  - *In vivo* PK parameters determined
  - PK/PD target engagement studies performed in appropriate mouse models determined
  - Efficacy studies performed in appropriate mouse models

IKKα: further positioning studies in prostate cancer

In collaboration with David Waugh (Queensland University of Technology), aim to assess compounds meeting progression criteria for PD and phenotypic outputs in the following pre-clinical cell-based models.

Primary Screening Collection:

- Human AR-expressing lines reflecting the transition to castrate-resistant disease (LNCaP and castrate-resistant LNCaP C4-2/C4-2b derivatives, plus AR-amplified VCaP cells and AR/ARv7-expressing 22Rv1 cells)
- Human metastatic lines including AR-null PC3 (bone metastasis) and androgen-insensitive DU145 cells (brain metastasis)

Secondary Screening Collection:

- Drug-resistant clones of LNCaP cells
- Modified PC3 and DU145 clones that model reconstitution of the tumour suppressor gene PTEN in PC3 cells or shRNA-mediated repression of PTEN expression in DU145 cells.
- PNT1A/RWPE-1 – quasi-normal prostate epithelial cell lines plus human prostate stromal fibroblasts
- Murine prostate cancer cell line (DVL3) generated from a Pb-Cre-driven homozygous deletion of PTEN in mouse prostate epithelium and other related proprietary in-house lines derived from this line

IKKα: further positioning studies in prostate cancer

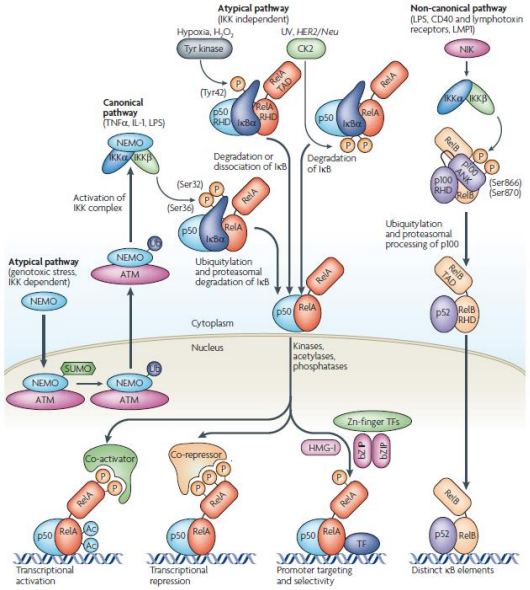
The results of these studies will direct the translation into relevant *in vivo* models below for lead compounds alone and in combination with conventional treatments (e.g. enzalutamide therapy)

- Standard Xenograft Model of CRPC: an androgen-sensitive LNCaP xenograft model and for different models of castrate-resistance, the LNCaP-C4/2-b model and the AR-amplified VCaP model

- Genetically-Annotated Models of Prostate Cancer: tumours may become addicted to the IKKα pathway under specific genetic backgrounds and/or following exposure to stress. A number of genetically-manipulated cell lines (*PTEN, p53, Rb*) have been developed at QUB. These genetic markers associate with transcriptional responses that modulated genes are shown to underpin resistance to hormone therapy.

- Syngeneic Model of Prostate Cancer: Have developed a murine prostate cancer cell line (DVL3) which will allow compound evaluation in immune competent BALB/C mice to assess the wider impacts on the immunological microenvironment and determine how this may assist in generating an effective immune response.

University of Strathclyde Science



Appendix

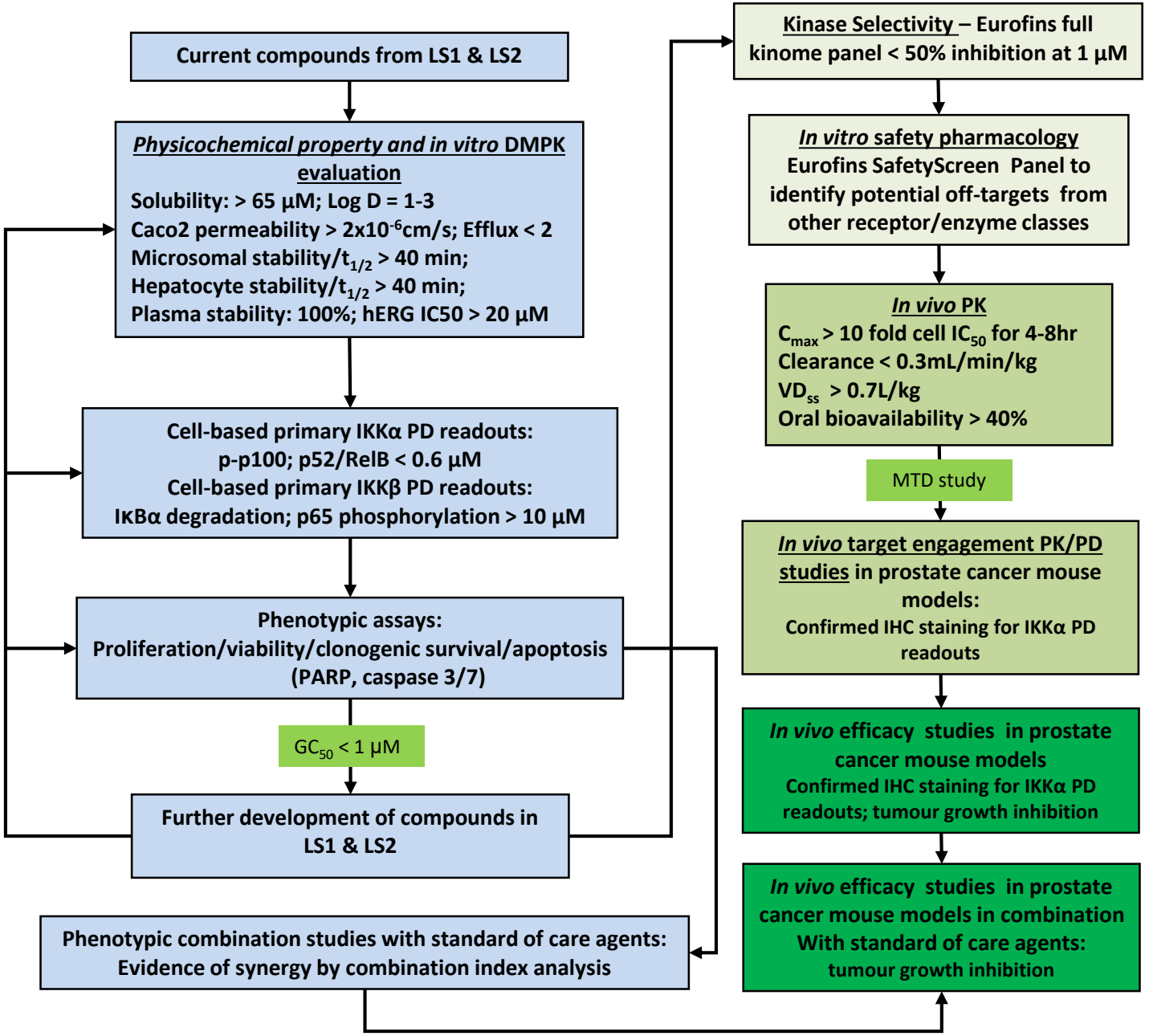
Hayden & Ghosh (2008) ***Cell 132***, 344

CANCER RESEARCH UK

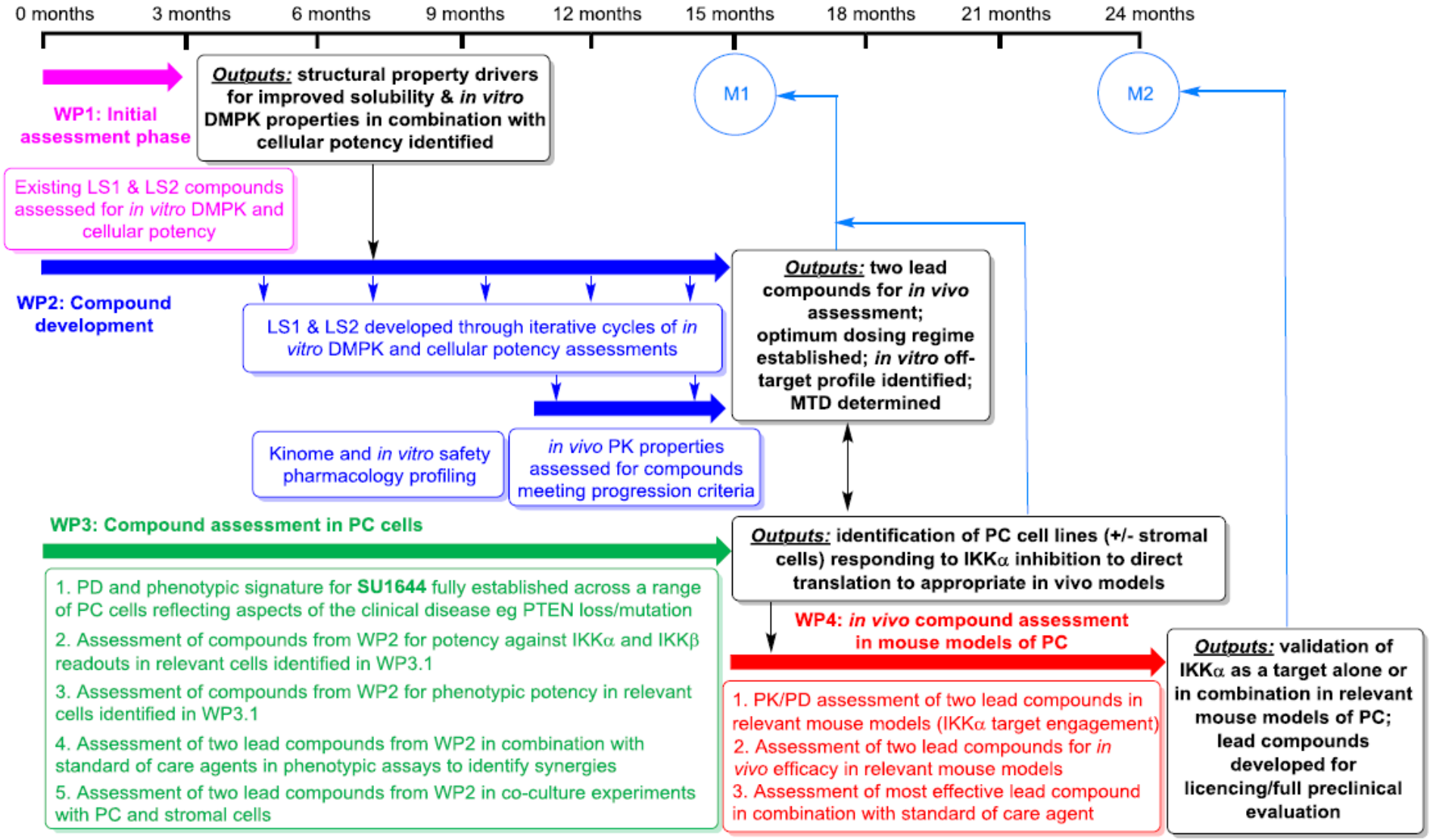
MRC Medical Research Council

PROSTATE CANCER UK

The strategy adopted to deliver a preclinical candidate



Project plan (CRPC)

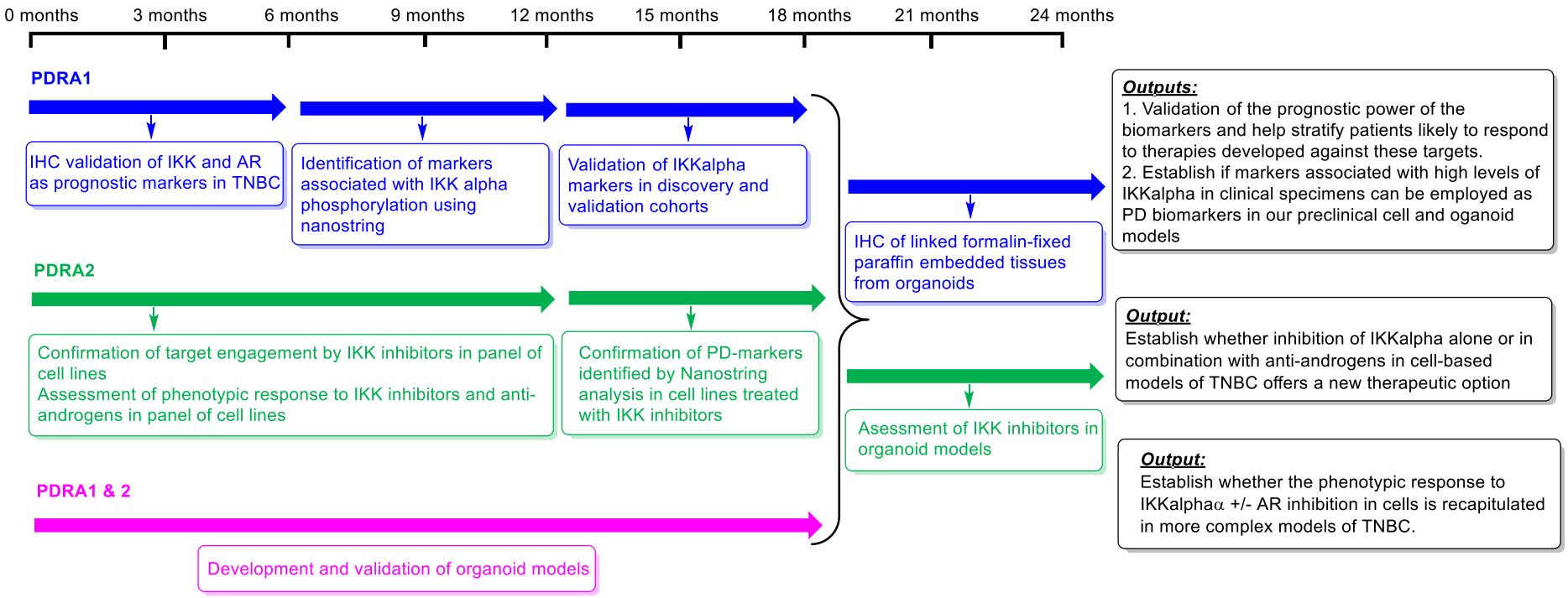


Other potential cancer indications for IKKα related to AR

- TNBC accounts for 10-20% of breast cancers and is a heterogeneous group of tumours defined by their lack of oestrogen receptor (ER), progesterone receptor (PR) and Her2 receptor.
- TNBC currently has the highest mortality rates of all subtypes due to its aggressive nature and the lack of availability of targeted therapies.
- IKKα is associated with overexpression of p100/52 and the development of breast tumours
- In TNBC, knockdown of IKKα results in cell cycle arrest and induction of apoptosis in MDA-MB-231 TNBC cells
- In MDA-MB-231 and MDA-MB-468 TNBC xenografts, knockdown of IKKα results in a reduction in cell migration, invasion and development of metastases, whilst restoration of IKKα expression rescues the invasive ability of both cell lines and the subsequent development of metastases in these models .

Zhang T et al. (2013) Molecules. 18, 845-58. Farhana L et al (2005) Cancer Res. 65:4909; Jayaraman S, Doucet M, et al (2016) Mol Cancer Res. 14, 730-9

Project plan (TNBC)



Other potential cancer indications for IKKα

- Pancreatic cancer
  - The non-canonical NF-kB pathway via p100 is constitutively activated in many patient cases
  - Non-canonical NF-kB pathway components and target genes are reported to be upregulated in pancreatic cancer
  - Inflammatory stimuli promote growth and invasion of pancreatic cancer cells through a non-canonical NF-kB mechanism of PP2A repression
  - Mesothelin-activated NF-kB induces elevated IL-6 expression, which acts as a growth factor to support pancreatic cancer cell survival and proliferation
  - loss of TRAF2 mediated degradation of NIK leads to IKKα activation in pancreatic cancer cells

Wharry et al (2009) Cancer Biol. Ther. 8; 1567-76; Nishina et al (2009) BBRC. 388; 96-101;Yao et al (2011) Carcinogenesis 32; 1013-1024; Doppler et al (2013) PLoS ONE 8; e53676; Baldwin et al (2013) Cancer Discov. 3; 690-703; Tao et al (2016) Cell Cycle 15; 381-393; Edwards et al (2017) Int. J. Cancer 1633-1644

Other potential cancer indications for IKKα

- Leukaemia, lymphoma and myeloma:
  - Around 20% of MM patients reported to have mutations leading to activation of the non-canonical pathway
  - Rearrangements of the IKKα substrate *nfκb2* (p100) gene occur in a range of leukaemias leading to constitutive activity. Constitutive processing still requires IKKα
  - IL-6 induced motility, survival and proliferation in patient-derived CLL samples is reduced by the pan-IKK inhibitor BMS-345541

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Demchenko et al (2010) Blood, 115, 3541-3552; Verhasselt et al (2013) Br J Haematol. 163, 621-30; Margalef et al. (2012) Cell Reports 2, 840-845

IKKα: positioning the target in colorectal cancer

In collaboration with the Beatson Institute (Owen Sansom), we aim to explore the utility of SU1433/SU1644 in cell-based models of colorectal cancer under the recently awarded CRUK Accelerate Award.

- Organoid models from patient derived cell lines developed in the host laboratory will be employed to test the phenotypic response to IKKα inhibition.
- The results of these studies will direct the translation into relevant *in vivo* models *for in vivo* ready lead compounds

IKKα: positioning the target in haematological cancers

Genetic abnormalities leading to constitutive NF-κB activity have been found in approximately 20% of MM patients and 40% of MM cell lines. Most of the genetic abnormalities involve the non-canonical NF-κB pathway

In collaboration with Chris Pepper at Sussex University, we will explore the utility of SU1433/SU1644 in cell-based models of MM

Compound status and projected development

	Lead chemotype being developed *at project start*	Property criteria of lead compound *at project end*	Property criteria of *subsequent clinical candidate*
	SU1433		
Physicochemical Properties			
Measured logD pH 7.4	3.4	<4.0	<4.0
PSA	89 Å^2	<130 Å^2	<130 Å^2
Aqueous solubility pH 7.4	65 µM	>100 µM	>100 µM
Aqueous stability pH 7.4	<1% degradation > 48h	<1% degradation > 48h	<1% degradation > 48h
Molecular structure	No undesirable features	No undesirable features	No undesirable features
Number of HB donors and acceptors (HBD/A)	HBD: 3/HBA: 2	HBD <10/HBA <5	HBD <10/HBA <5
In vitro Biological Activity			
IKKα IC_{50} (biochemical)	11 nM	<50 nM	<50 nM
Selectivity over IKKβ (biochemical)	2300 nM	>20-fold	>20-fold
Inhibition of p100 phosphorylation in two PC cell lines (IKKα PD readout) IC_{50}	0.5 µM (LNcAP; PC3M)	<0.5 µM	<0.5 µM
Inhibition RelB nuclear translocation in two PC cell lines (IKKα PD readout) IC_{50}	0.4 µM (LNcAP; PC3M)	<0.5 µM	<0.5 µM
Inhibition of p65 phosphorylation in two PC cell lines (IKKβ PD readout) IC_{50}	> 10 µM	> 10 µM (>20-fold)	> 10 µM (>20-fold)
Inhibition of IkB degradation in two PC cell lines (IKKβ PD readout) IC_{50}	> 10 µM	> 10 µM (>20-fold)	> 10 µM (>20-fold)
Phenotypic assay: antiproliferative activity IC_{50} in two PC cell lines	0.6 µM	<0.5 µM	<0.5 µM

	Lead chemotype being developed *at project start*	Property criteria of lead compound *at project end*	Property criteria of *subsequent clinical candidate*
	SU1433		
In vitro DMPK and toxicity assays			
Hepatocyte clearance (mouse)	70 µl/min/10^6 cell	<48 µl $min^{-1}10^6$ cell	Equivalent values in human hepatocytes
Hepatocyte half-life (mouse)	20 min	> 30 min	Equivalent values in human hepatocytes
Plasma stability (% remaining after 2h)	100%	100%	100%
Plasma protein binding	97%	<95%	<95%
CYP450 inhibition (1A2; 2C9; 2C19; 2D6; 3A4)	> 25 µM for all isoforms	> 25 µM for all isoforms with no time dependent inhibition	> 25 µM for all isoforms with no time dependent inhibition
hERG inhibition	2 µM (*in vitro*)	> 25 µM (*in vitro*)	> 25 µM (*in vitro*) plus minimal changes in QRS interval and contractibility in rabbit ventricular wedge assay
Receptor/channel selectivity pharmacology panel screening	ND	<10% inhibition of all mammalian receptors/enzymes at 10 µM	<10% inhibition of all mammalian receptors/enzymes at 10 µM
Kinome screening	Only CLK2 (see Table 1; Figures & Tables document)	<10% inhibition of key kinases at 1µM	<10% inhibition of key kinases at 1µM
Genotoxicity	ND	Negative in mini-Ames test	Negative in mini-Ames test
Reactive metabolites	No evidence of reactive metabolites – alkyne stable	No evidence of reactive metabolites	No evidence of reactive metabolites
In vivo DMPK Assays			
Clearance	120 ml/min/kg *[mouse]*	< 30 ml min^{-1} kg *[mouse]*	Equivalent metabolic/total clearance in 2 other species e.g. rat/dog/mini-pig (<0.3x hepatic blood flow)
Plasma terminal half-life	2.1 h *[mouse]*	> 5 h *[mouse]*	See above
V_D	1.3 L/kg *[mouse]*	1-5 L/kg *[mouse]*	V_D determined in 2 other species e.g. rat/dog/mini-pig (1-5 L/kg)
F_{po}	17% *[mouse]*	> 50% *[mouse]*	F_{po} determined in 2 other species e.g. rat/dog/mini-pig >80%
Plasma protein binding	ND	< 95% [mouse]	Characterised across species to understand impact on PD in different species models
Dose proportionality	ND	AUC and C_{max} demonstrate dose proportionality	AUC and C_{max} demonstrate dose proportionality
Repeat dose exposure	ND	No unexpected compound accumulation on repeat dosing	No unexpected compound accumulation on repeat dosing

	Lead chemotype being developed *at project start*	Property criteria of lead compound *at project end*	Property criteria of *subsequent clinical candidate*
	SU1433		
***In vivo* pharmacology**			
PK/PD	*Evidence of antiproliferative effect at 6h by Ki67 staining (Figure 5B; Figures & Tables document)*	*In vivo* evidence of IKKα-targeting, with modulation dependent on compound exposure in 2 or more mouse models of PC	*In vivo* evidence of IKKα-targeting, with modulation dependent on compound exposure in 2 or more mouse models of PC
Dose to produce efficacy [mouse]	50 mg/kg	<20mg/kg	<10mg/kg
Efficacy measure [in two mouse models of prostate cancer]	Reduced tumour growth over 21 day by 60%	> 50% inhibition of tumour growth in 2 or more different mouse models of PC (alone or in combination with standard-of-care agents) at a dose level <3x MTD over 28 days' treatment	> 80% inhibition of tumour growth in 2 or more different mouse models of PC (alone or in combination with standard-of-care agents) at a dose level <3x MTD over 28 days' treatment
Toxicity	No gross toxicity: < 10% total body weight loss during 21 day treatment	No gross toxicity; e.g. < 10% total body weight loss during 28 day treatment	No gross toxicity; e.g. < 5% total body weight loss during 28 day treatment
Chemical tractability			
Complexity and length of synthetic route	API synthesis in 4 steps using starting materials from commercial sources	API synthesis < 8 stages. Starting materials available from commercial sources/multiple suppliers	API synthesis < 12 stages. Starting materials available from commercial sources/multiple suppliers
Scalability of synthetic route		Route able to produce > 5g of lead compound in 98% purity, with no more than 0.5% of a single impurity as determined by HPLC. No steps to involve semi-prep-HPLC purification	Route able to produce > 50g of lead compound in 98% purity, with no more than 0.5% as a single impurity as determined by HPLC. No steps to involve semi-prep-HPLC purification
Solid state properties	Crystalline, non-hygroscopic form can be produced	Potential to generate crystalline, stable, non-hygroscopic form	Crystalline, stable, non-hygroscopic form in single polymorph
Patentability and freedom to operate			
Structural novelty	Freedom to operate around core scaffold	Patentability of core scaffold established	Patentability of core scaffold maintained
Prior art	No issues arising from 3rd Party IP rights or publications	No issues arising from 3rd Party IP rights or publications compromising IP	No issues arising from 3rd Party IP rights or publications compromising IP
Patent applications	Patent application pending around candidate cmpd	Patent application pending including exemplification of candidate cmpd.	Patent application filed which includes exemplification of candidate cmpd

The programme team

University of Strathclyde

Programme Director:	Simon Mackay
Biology Director:	Andrew Paul
Formulation Director:	Gavin Halbert (CRUK Formulation Unit)

Chemistry FTEs

- 4 Postdoctoral chemists
- 2 Graduate Students

Biology FTEs

- 4 Postdoctoral biologists
- 2 Graduate students

- *Programme Partners*

- University of Newcastle
  - Neil Perkins*
  - Herbie Newell
  - Elaine Willmore

University of Glasgow

- Joanne Edwards
- Hing Leung
- Danny Huang
- Andrew Biankin
- David Chang

- Cancer Research Technology
  - Tim Hammonds*
  - Angus Lauder*
  - Martin Swarbrick*

- Queens University Belfast
  - David Waugh
  - Ian Mills

* Expert Advisory Board Members