Donated Chemical Probe

TBK1/IKKε Inhibitor Probe BAY-985

June, 2018

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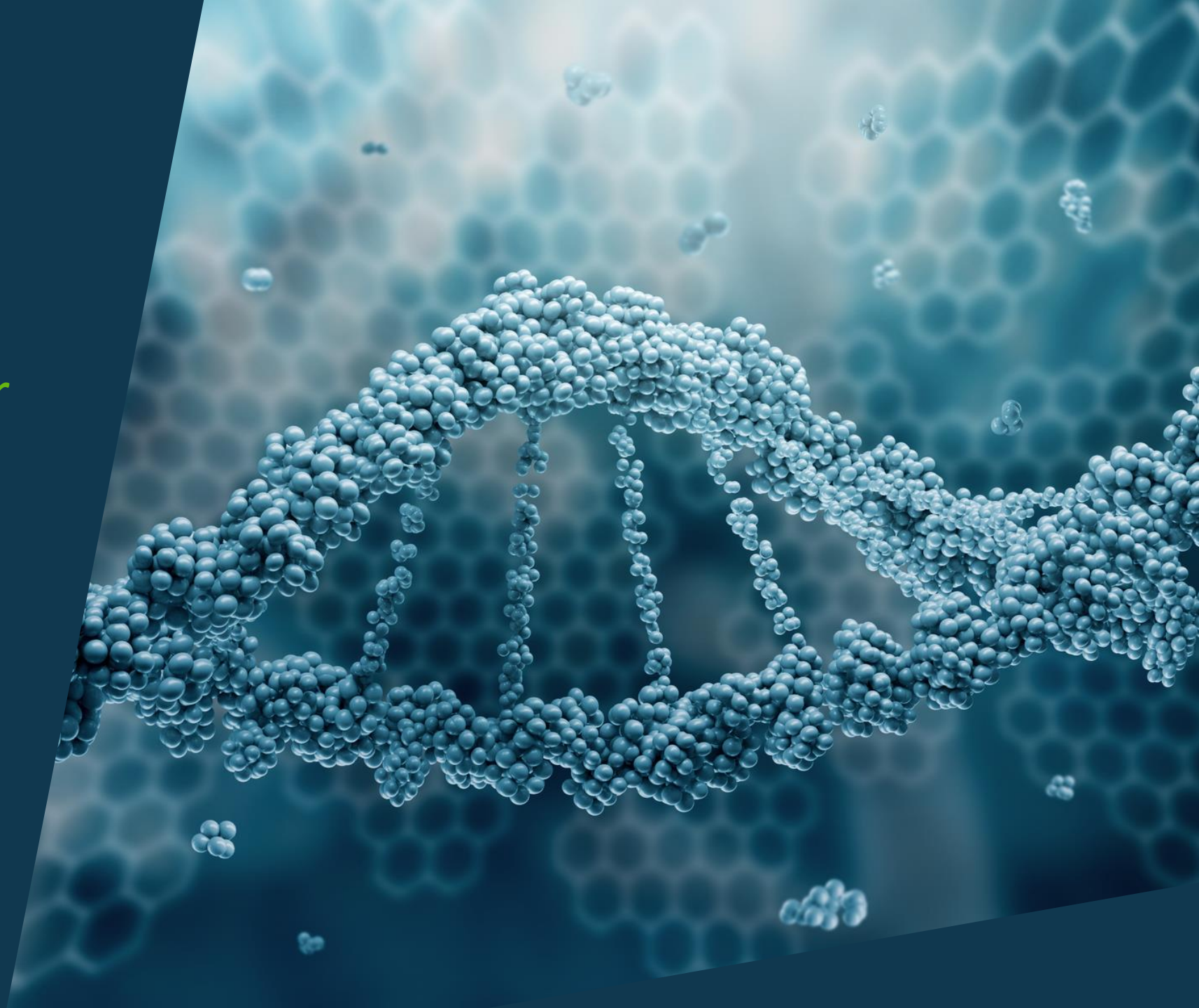
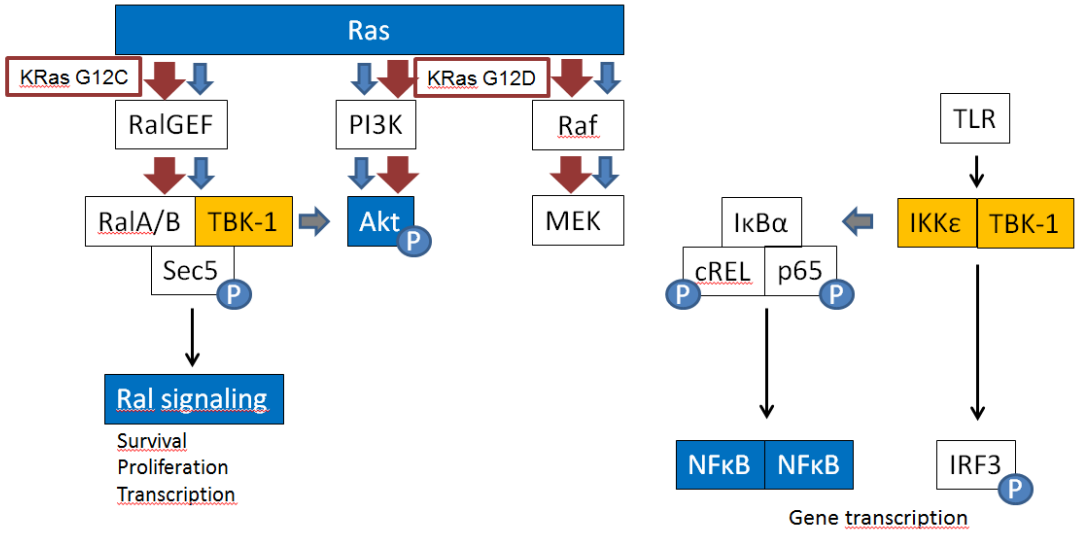


TBK1/IKKε Probe BAY-985

Scientific Rationale

- IKKε and TBK1 are closely related serine/threonine kinases, originally identified being involved in **innate anti-viral immune response**
- TBK1/IKKε are highly expressed in **prostate, colon, breast cancer**
- TBK1 interacts with **Ras-effector RalA/RalB pathway especially in KRAS G12C/D/V mutants** and in cooperation with MEK
- Inhibition of TBK1 in **NRAS mutant melanoma** rescues resistance to MEK
- TBK1/IKKε interact with **PI3K/Akt and NFκB pathway**
- IKKε inhibition prevents **NFκB activation** and blocks **oncogenic transformation in breast cancer**
- TBK1/IKKε promote survival of aggressive KRAS mut and p53 loss driven **lung carcinomas** by activating CCL5 and IL-6 in **cooperation with JAK**

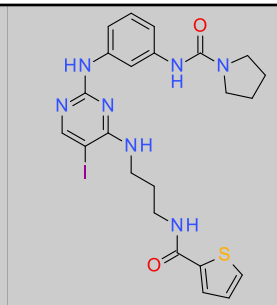
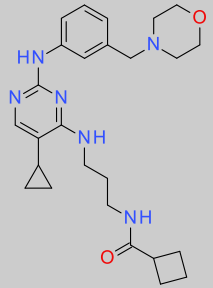


Multiple lines of evidence support TBK1/IKKε as innovative target for cancer therapy.

Our compound was developed as a TBK1/IKKε inhibitor for treatment of cancer.

TBK1/IKKε Probe BAY-985

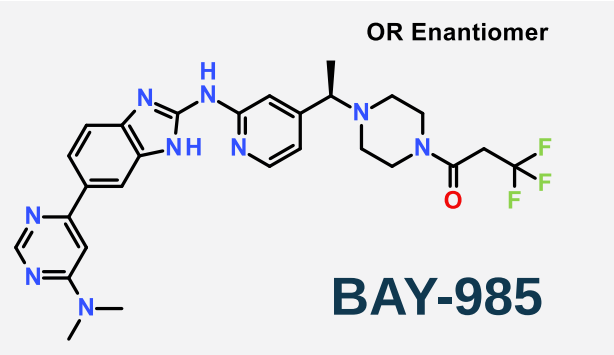
Commercial TBK1/IKKε inhibitors

	BX795	MRT67307
		
	Developed as PDK1i	
Lit. data: TBK1 IC_{50}: IKKε IC_{50}:	 **6 nM** **41 nM**	 **19 nM** **160 nM**
Off targets	PDK1 (111 nM) Aurora B (31 nM) ERK8 (140 nM) MARK1 (55 nM) MARK2 (53 nM) MARK3 (81 nM) MARK4 (19 nM) NUAK1 (5 nM) VEGFR (157 nM) MLK1 (50 nM) MLK2 (46 nM) MLK3 (42 nM)	MARK1 (27 nM) MARK2 (52 nM) MARK3 (36 nM) MARK4 (41 nM) SIK2 (67 nM) Aurora B, JAK2, MLK1, MLK3 (>90% inh. @1µM)

There are two postulated TBK1/IKKε inhibitors commercially known.
However, both compounds show weak selectivity profile inhibiting several kinases beyond TBK1/IKKε with comparable activity.

TBK1/IKKε Probe BAY-985

Overall Profile



Single enantiomer
(absolute stereochemistry under investigation)

Key Data

TBK1 Biochem. low/high ATP [nM]	2 / 28
IKKε low ATP Biochem. [nM]	2
Cellular Mech. pIRF3 [nM]	74
Anti-Proliferation SK-MEL-2 [nM]	900

Molecular Properties

MW [g/mol]	553
MW corr.	512
TPSA [Å^2]	106

PhysChem

Sw DMSO [mg/L]	0.8
logD (pH 7.5)	2.5

In vitro DMPK

		CL_b [L/h/kg]			F_{max} [%]		
LM	h/m/r/d	0.5/2.2/1.7/0.92			62/59/58/56		
Hep	Rat	1.7			58		
CaCo2		A-B [nm/s]			B-A [nm/s]		ratio
		8.9			147		17
CYP inhibition IC_{50} [µM]		1A2	2C8	2C9	2D6	3A4	3A4 preinc.
		>20	0.9	17	>20	>20	>20
PXR assay		Yellow					

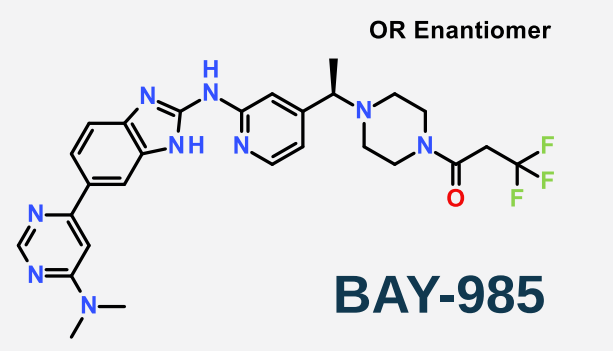
Safety

hERG [µmol/L]	4.7

- BAY-985 is a highly potent TBK1/IKKε inhibitor that shows anti-proliferative activity in tumor cells
- BAY-985 shows moderate stability across species and low permeability (strong efflux)
- BAY-985 shows activity at hERG

TBK1/IKKε Probe BAY-985

Highly selective TBK1/IKKε inhibitor



Key Data

TBK1 Biochem. low/high ATP [nM]	2 / 28
IKKε Biochem. [nM]	2
Cellular Mech. pIRF3 [nM]	74

Bayer internal kinase panel

Selectivity ratio vs TBK1 < 100x for FLT3 only
FLT3 IC_{50} = 123 nM (75x)
MEK5 IC_{50} = 847 nM (518x)

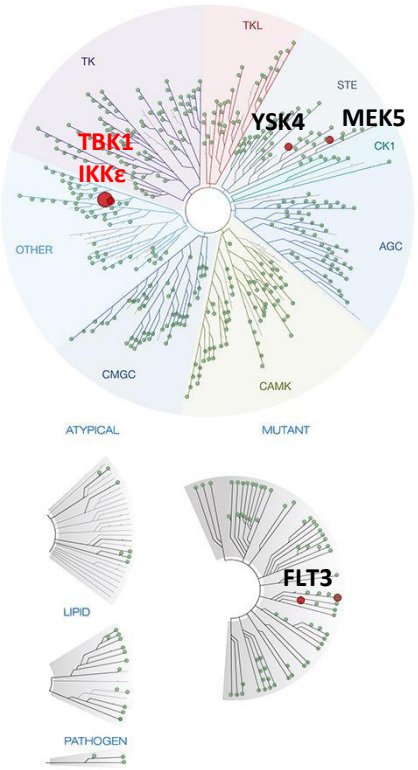
DiscoverX kinase panel

Additional Kds measured
TBK1 : Kd = 1.5 nM
DRAK1 : Kd = 74 nM (49x)
YSK4 : Kd = 9.6 nM (6x)

Eurofins (selected) kinase panel

IC_{50} DRAK1 = 310 nM (105x)

> 70% inhibition @ 100 nM

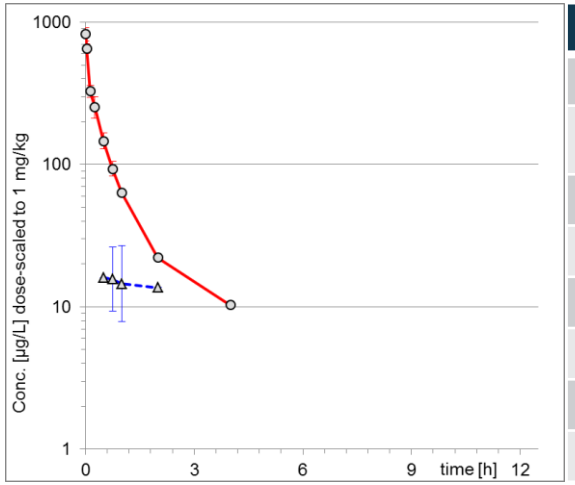


- BAY-985 shows high selectivity in Bayer internal kinase panel (IC_{50} of FLT3 only < 100-fold vs TBK1)
- BAY-985 shows high selectivity in DiscoverX kinase panel, but potent activity on **YSK4** detected
- BAY-985 shows > 100-fold selectivity on TBK1 vs DRAK1 in Eurofins IC50 measurement

TBK1/IKKε Probe BAY-985

In vivo PK

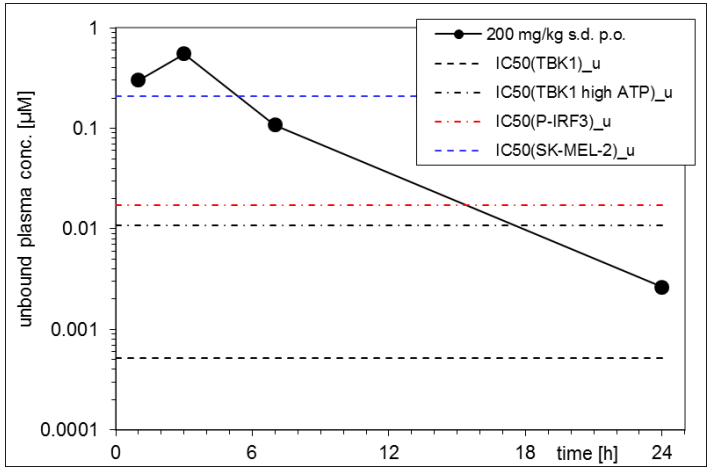
Low dose rat PK



Route		i.v.	p.o.
Dose	mg/kg	0.3	0.3
$AUC(0\text{-}t_{last})_{norm}$	kg*h/L	0.25	0.011
AUC_{norm}	kg*h/L	0.27	0.029
$C_{max,norm}$	kg/L		0.016
CL_b	L/h/kg	4.0	
V_{ss}	L/kg	2.9	
$t_{1/2}$	h	0.79	1.0
F	%		11

- Blood clearance is high (95% HBF), V_{ss} is high, $t_{1/2}$ is short and %F is low (no ΔF)
- In vivo blood clearance is higher than expected from in vitro clearance
- Bioavailability as expected from in vivo blood clearance

Exposure after single dose in mice



AUC(0-tlast)	h*µM	129
$AUC(0\text{-}t_{last})$	mg*h/L	71
$AUC(0\text{-}t_{last})_{norm}$	kg*h/L	0.36
C_{max}	µM	28
$C_{max,norm}$	kg/L	0.076
C (24H) /C_{max}		0.48%

MTD > 200 mg/kg 2QD p.o. for 7 days in nude mice (vehicle: PEG400/ Ethanol/ Water 60:10:30)

Administering 200 mg/kg p.o. single dose in mice:

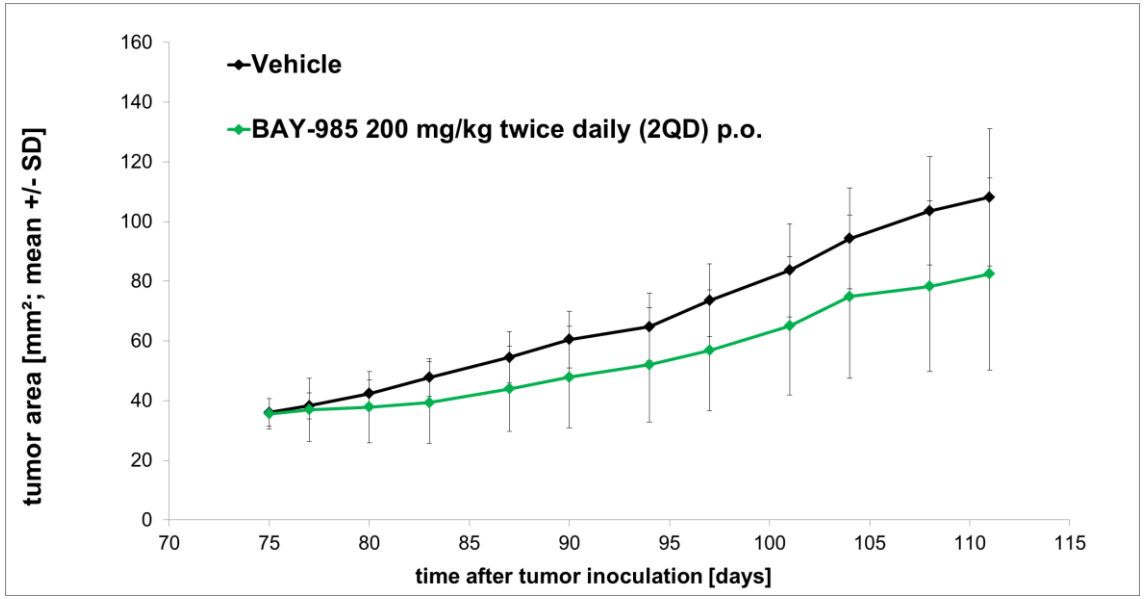
- Exposure is moderate and increases dose proportionally (compared to single dose 100 mg/kg p.o.)
- $IC_{50,u}$ (TBK1/IKKe high ATP) is covered for appr. 17h
- $IC_{50,u}$ (P-IRF3) is covered for appr. 15h
- $IC_{50,u}$ (SK-MEL-2) is covered for appr. 5h

When administering 200 mg/kg p.o. twice daily (2QD), anti-proliferative IC_{50},u (SK-MEL-2) should be covered for appr. 12h in steady-state and IC_{50},u (P-IRF3) should be covered for 24h.

TBK1/IKKε Probe BAY-985

In vivo anti-tumour efficacy

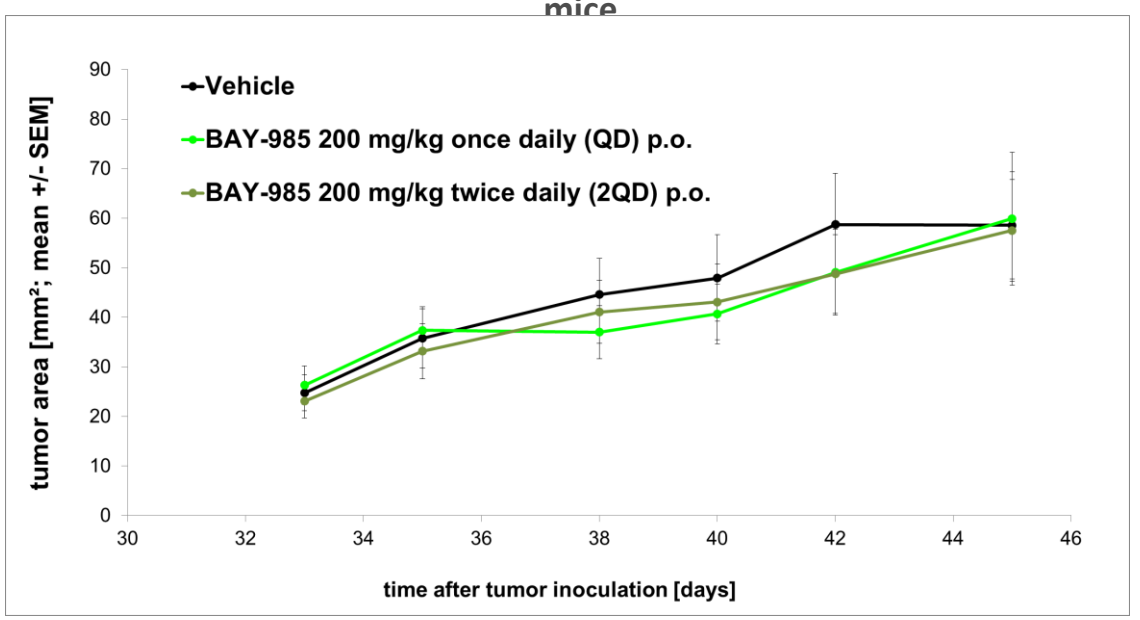
Tumour growth human SK-MEL-2 melanoma xenograft model in nude mice



Treatment vs Control ratio based on final tumour area (T/C_{TA}): vehicle = 1.00; BAY-985 = 0.80
No critical body weight loss (> 10%) or toxicity observed

- Minor anti-tumor activity of TBK1/IKKε inhibitor BAY-985 in SK-MEL-2 xenograft model

Tumour growth human MDA-MB-231 TNBC* xenograft model in nude mice



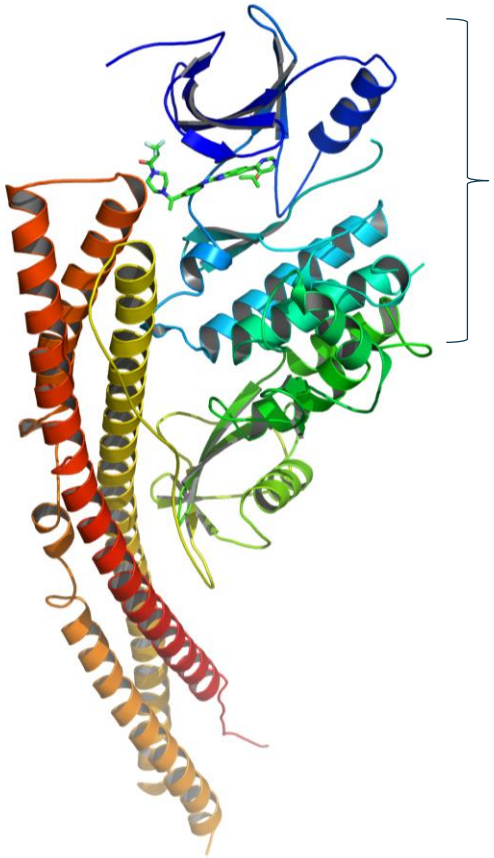
No critical body weight loss (> 10%) or toxicity observed

- No anti-tumor activity of TBK1/IKKε inhibitor BAY-985 in MDA-MB-231 xenograft model

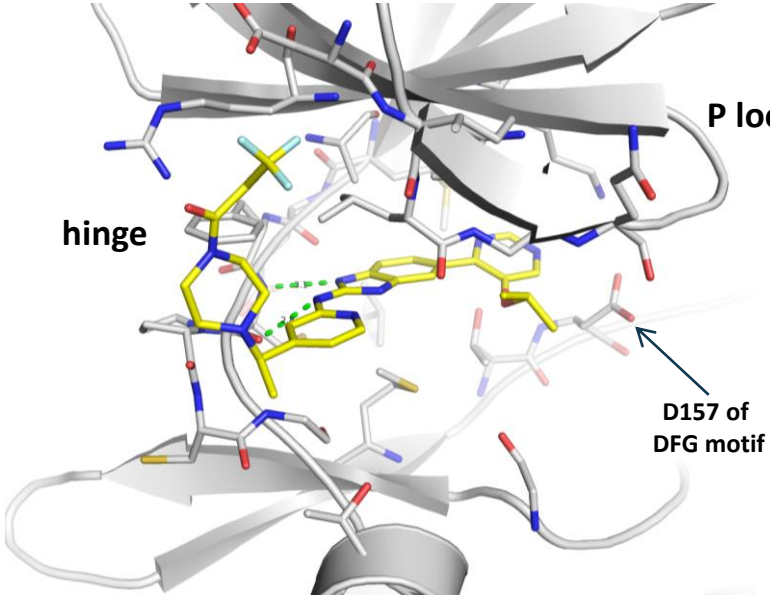
(*) Inflammatory breast cancer (TNBC) is charactereized by cytokine/ chemokine/ growth factor driven infiltration with inflammatory immune cells, promoting aggressiveness of tumor growth; Hypothesis of anti-tumor activity via anti-inflammatory activity of TBK1/IKKε inhibition

TBK1/IKKε Probe BAY-985

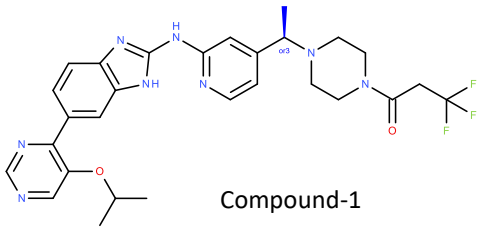
X-ray crystal structure (Prof. Daniel Panne, University of Leicester)



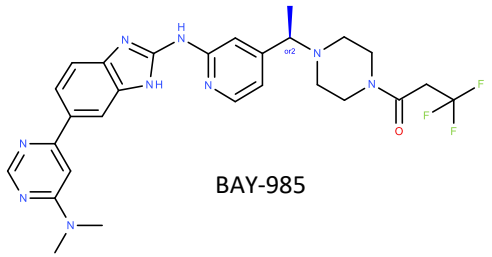
Near full-length TBKs in complex with compound-1, a close derivative of BAY-985 (Daniel Panne, manuscript in preparation)



View into ATP site



Compound-1



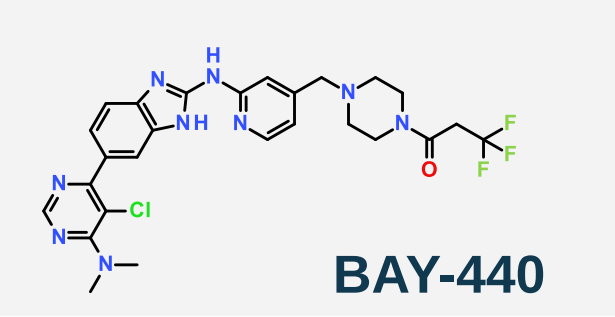
BAY-985

In collaboration with Prof. Daniel Panne, compound-1 was co-crystallized with near-full length TBK1. X-ray structure (3 Å resolution) shows:

- Compound binds into ATP site and forms two H bonds to hinge region.
- Stereo center identified as having (*R*)-configuration
- Pyrimidin inserts into deeper pocket, adjacent to Asp157 from DFG motif

TBK1/IKKε Probe BAY-985

Overall Profile of negative control



Key Data

TBK1 Biochem. low/high ATP [nM]	1140 / >20000
IKKε Biochem. [nM]	1280
Mech. pIRF3 [nM]	3890
Proli. SK-MEL-2 [nM]	6330

In vitro DMPK

		CL_b [L/h/kg]	F_{max} [%]	
LM	Hum.	Ongoing		
Hep	Rat			
CaCo2		A-B [nm/s]	B-A [nm/s]	ratio
		Ongoing		

Molecular Properties

MW [g/mol]	574
MW corr.	516
TPSA [Å²]	106

PhysChem

Sw DMSO [mg/L]	n.d.
logD (pH 7.5)	2.70

- BAY-440 is > 500-fold less active on TBK1 and IKKε (low ATP)
- BAY-440 is inactive in the TBK1 high ATP assay

TBK1/IKKε Probe BAY-985

Summary / Conclusion

Probe criteria	BAY-985
Inhibitory/agonistic biochemical potency: goal < 50 nM (based on IC50, Kd)	**Surpasses criteria** High potency in biochemical assay with IC50 on TBK1 of 2 nM low at ATP and 18 nM at high ATP (on IKKε 2 nM at low ATP)
Selectivity within target family: goal > 30-fold (based on biochemical IC50, Kd)	**Surpasses criteria** High kinase selectivity for TBK1 and IKKε (selectivity ratio < 100-fold, except YSK4 and FLT3)
Selectivity outside target family: describe the off-targets (including binding as well as functional data)	**Surpasses criteria** Clean in Lead Profiling Screen
On target cell activity for cell-based targets: goal < 1 µM (based on cellular effective and mechanistic IC50)	**Surpasses criteria** Potent cellular mechanistic activity, moderate anti-proliferative activity (SK-MEL-2)
On target cell activity for selected targets (appropriate alternative such as mouse model or other mechanistic biological assay, e.g. explant culture)	Minor anti-tumor efficacy in SK-MEL-2 and no activity in MDA-MB-231 xenograft experiments
Negative control: in vitro potency – > 100-fold less than probe; cellular activity – > 100-fold less than probe	**Surpasses criteria** BAY-440 > 500-fold less potent than probe in biochemical assays

We ask for acceptance of TBK1/IKKε inhibitor BAY-985 as chemical probe, accompanied by BAY-440 as negative control.

TBK1/IKKε Probe BAY-985

Acknowledgements

TBK1/IKKε Core Team

Antje Wengner (LOPL)
Julien Lefranc (LOC)
Volker Schulze
Anne Mengel
Florian Prinz
Detlef Stöckigt
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Michael Brüning
Christian Hildebrandt
Howard Williams
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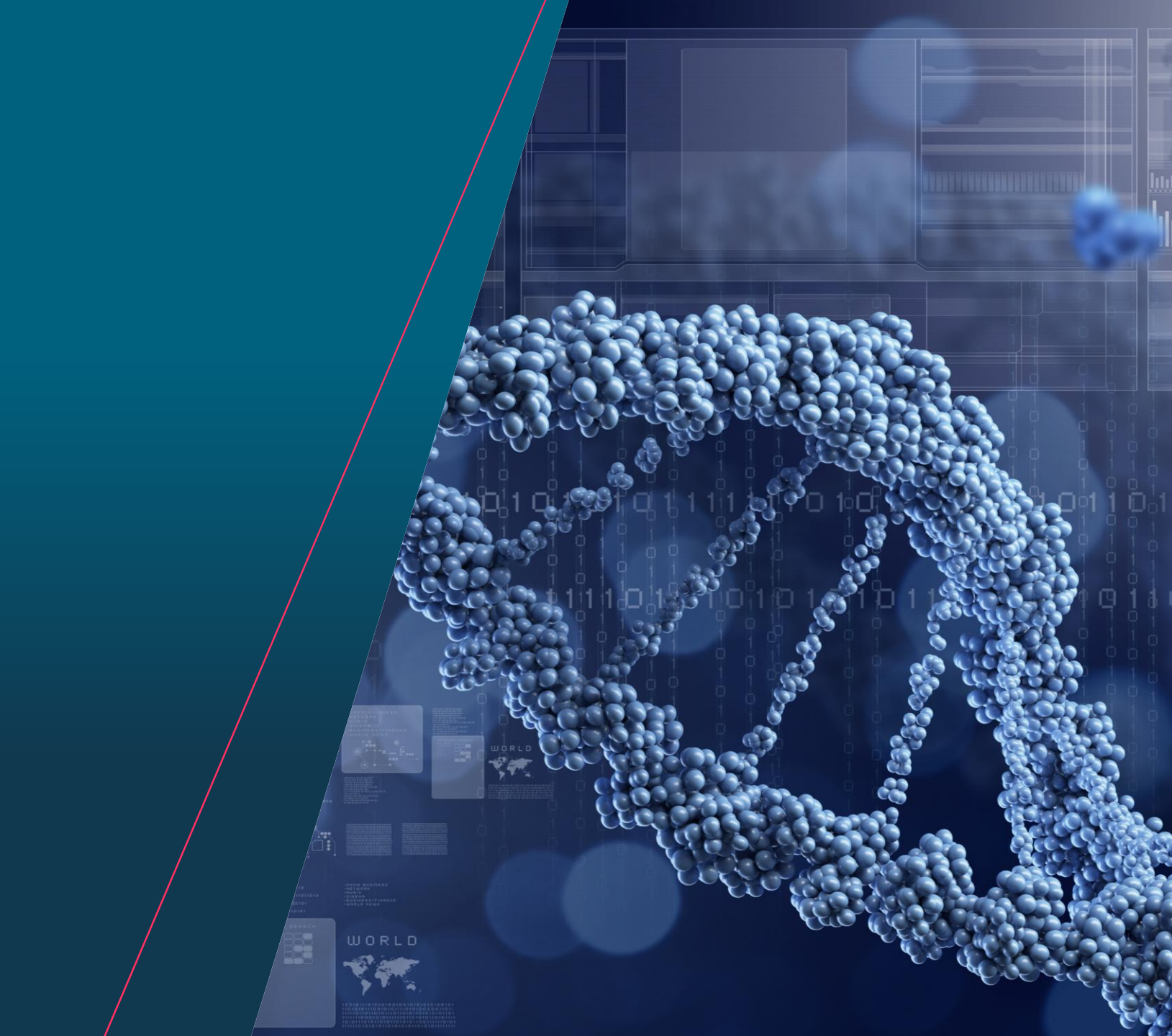
Marion Rudolph
Tunde Lawrence
Sven Golfier

ER Moderators

Andrea Hägebarth
Marcus Bauser

Dominik Mumberg
Carl Friedrich Nising
Franz von Nussbaum

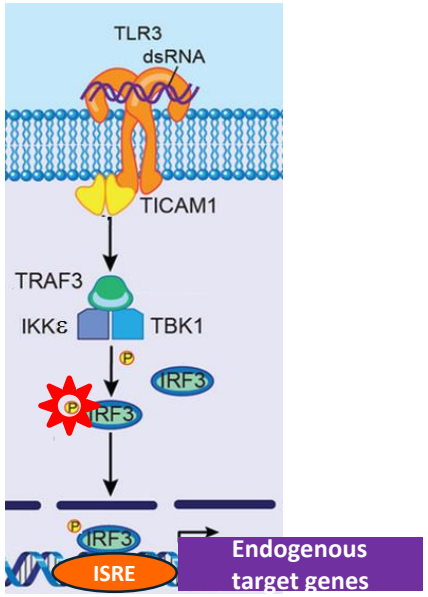
Thank You



TBK1/IKKε Probe BAY-985

Assay description - HTRF

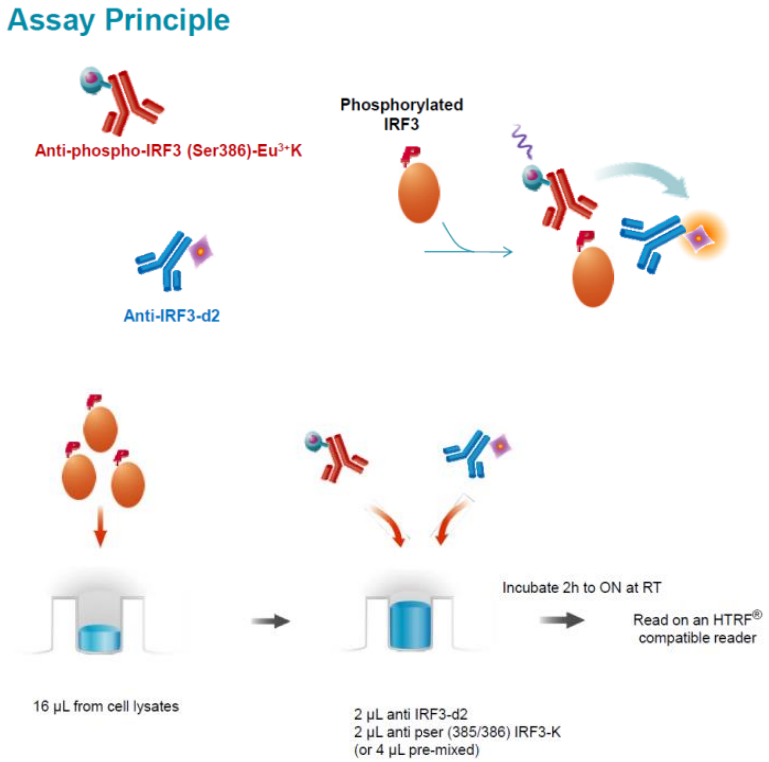
Cell-based pIRF3 mechanistic assay and endogenous gene regulation



IRF3 phosphorylation

1) Reduction of IRF3 target gene expression (IFIT1, IFIT2)

2) pIRF3 HTRF assay
→ *loss of IRF3 phosphorylation*
→ **candidate**



- HTRF assay is simple, fast and robust

TBK1/IKKε Probe BAY-985

Relevance of YSK4 off-target hit

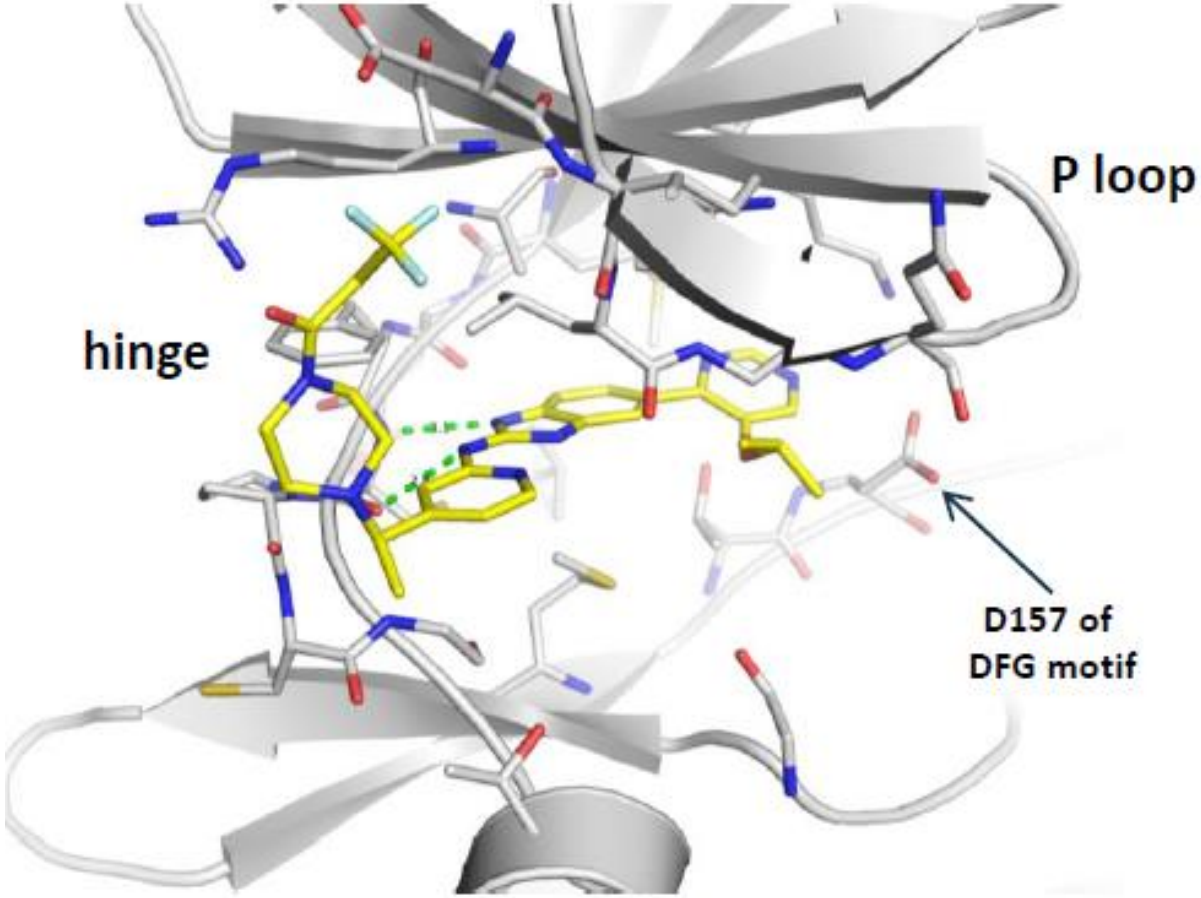
YSK4 is known as MAP3K19 (mitogen-activated kinase kinase kinase 19)
Currently, there are no literature data available demonstrating a relevant role in cancer. Based on genomic/proteomic analyses possible functions were postulated (inferred from biological aspect of ancestors): regulation of apoptosis, activation of MAPKK activity, regulation of mitotic cell cycle, activation of protein kinase activity, role in stress-activated MAPK cascade.

Follow up activities to assess the function and impact of YSK4:

- Evaluation of micronuclei induction (cell cycle effects) by compounds with strong activity on YSK4 did not demonstrate any effects
- Knockdown of YSK4 (Achilles data) did not affect tumor cell line proliferation esp. not in those cell lines where TBK1 inhibitors show activity

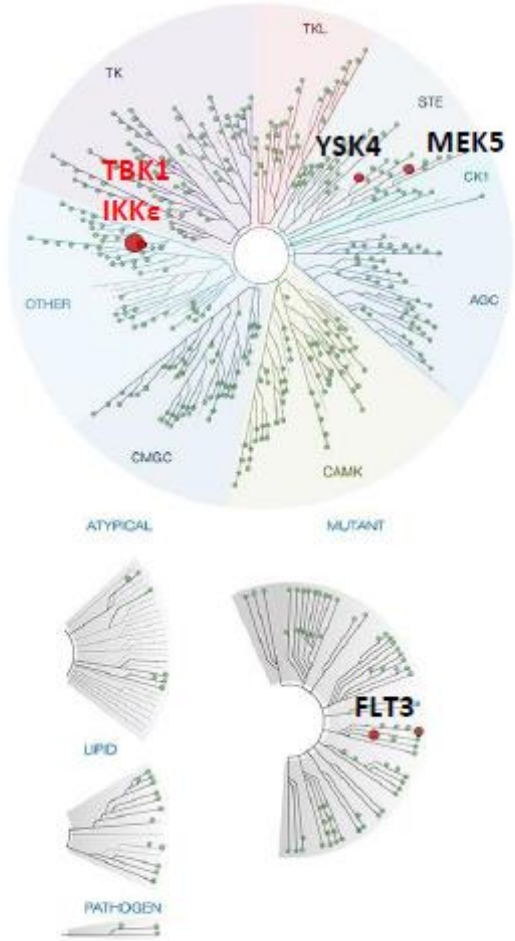
YSK4 was considered as non-relevant off-target in relation to cancer.

TBK1-Ikke Bay donated Probe

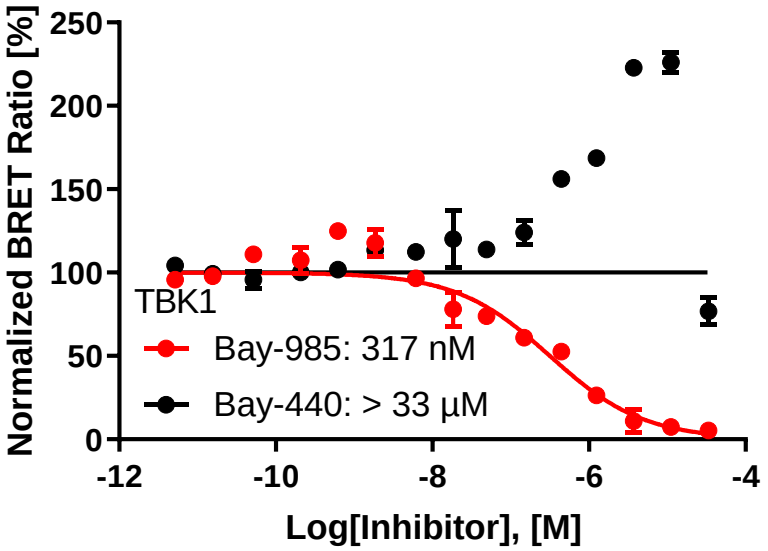


View into ATP site

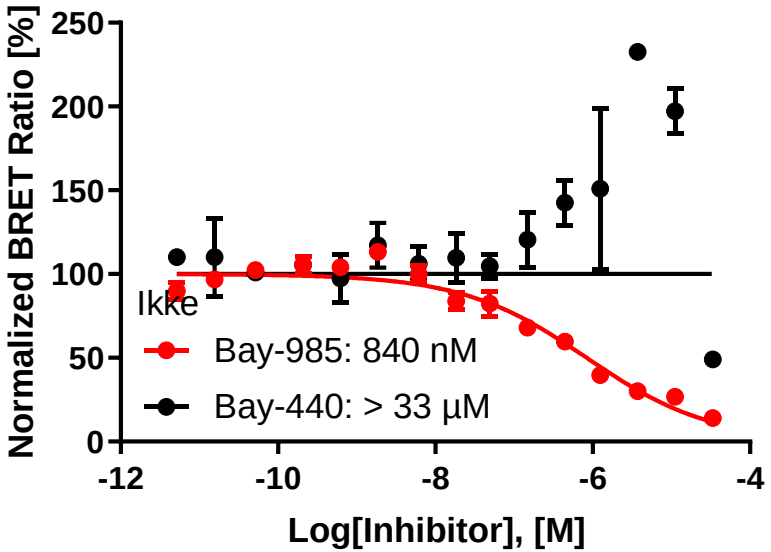
> 70% inhibition @ 100 nM



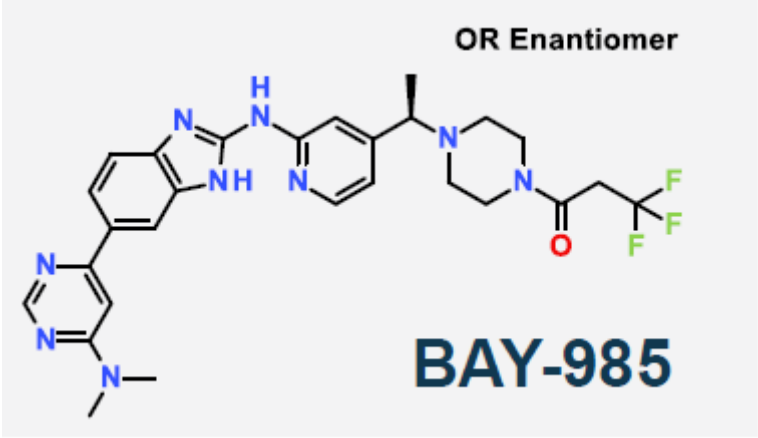
NanoBRET donated probes Bayer TBK1/Ikke

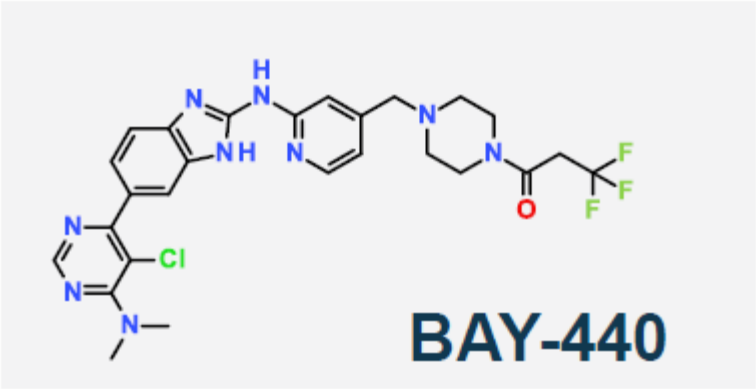


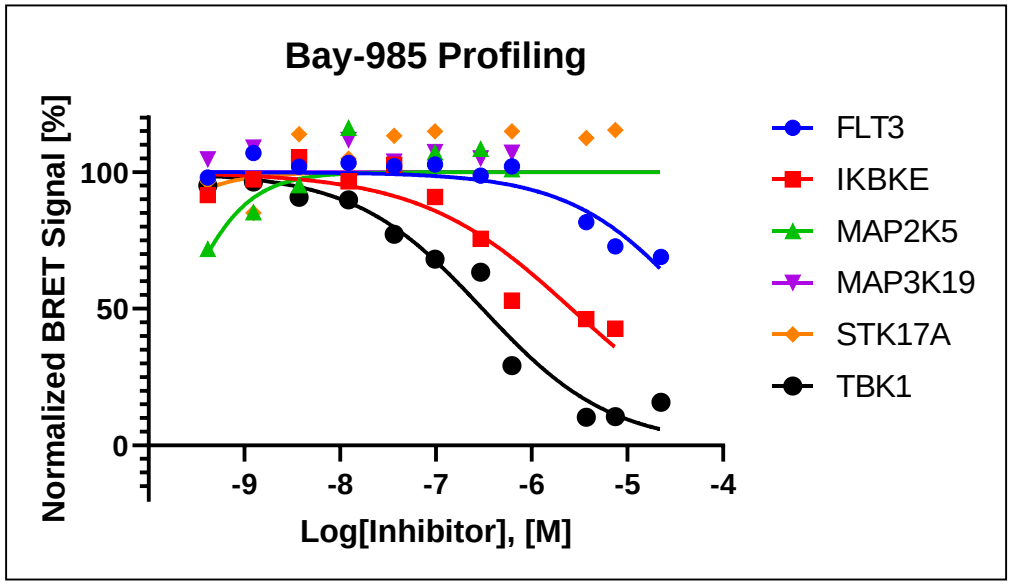
z' = 0.78 @ [tracer5]=$IC_{50,\ tracer5}$=100 nM

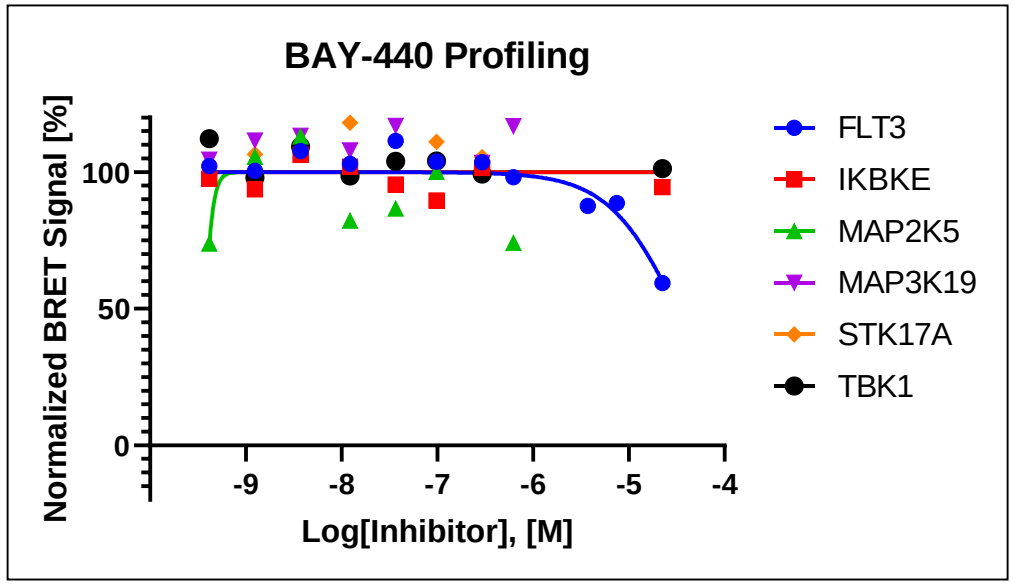


z' = 0.62 @ [tracer5]=$IC_{50,\ tracer5}$= 250 nM









Bay-985, Neg: Bay-440				Bay-985		Bay-440 (NC)	
	BAY-985			Run 1:	Run 2:	Run 1:	Run 2:
Target	in vitro IC50 DiscoverX, [M]	in vitro Bayer, [M]	NanoBRET name	NanoBRET IC50, [M]	NanoBRET IC50, [M]	NanoBRET IC50, [M]	NanoBRET IC50, [M]
TBK1	1,50E-09	2,80E-08	TBK1	3,17E-07	3,07E-07	>33µM	>20µM
Ikke		2,00E-09	IKBKE	8,40E-07	2,61E-06	>33µM	>20µM
YSK4	9,60E-09		MAP3K19		>20µM		>20µM
DRAK1	7,40E-08		STK17A		>20µM		>20µM
MEK5		8,47E-07	MAP2K5		>20µM		>20µM
FLT3		1,23E-07	FLT3		>20µM		>20µM