Donated Chemical Probe

ERK5 Inhibitor Probe BAY-885

June, 2018

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ERK5 Probe BAY-885

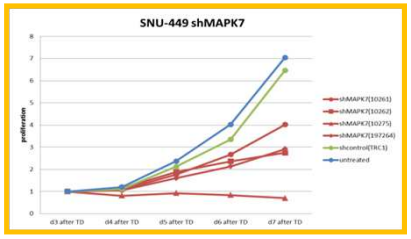
Target rationale: ERK5 amplification

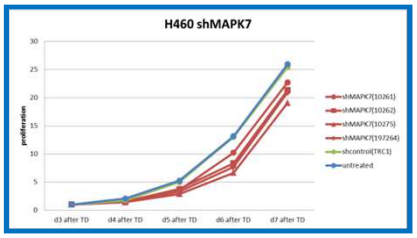
Rationale

- MAPK7/ERK5 is a key integrator of cellular signal transduction
- Several cancer types display genomic ERK5 amplifications, e.g. HCC
- MAPK7/ERK5 inhibition reduces proliferation and survival and induces apoptosis selectively in ERK5 amplified cells

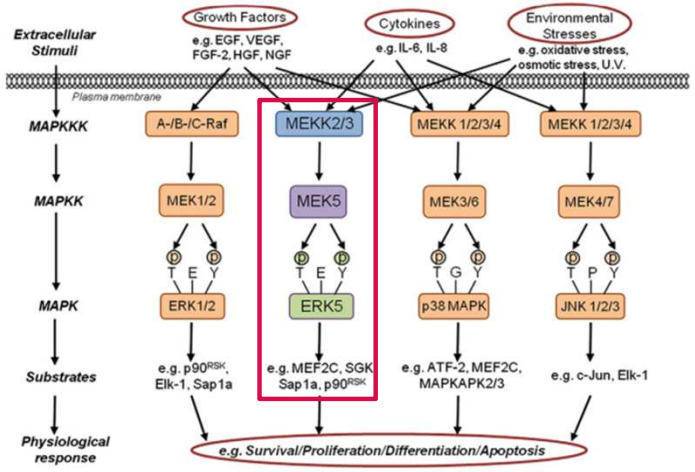
Validation (literature & in house data)

- ERK5 silencing in ERK5 amplified cells induces apoptosis (but not in non-amplified cells)
- ERK5 silencing induces anti-proliferative effects in cell lines with genomic ERK5 amplification versus non-amplified controls Zen et al., 2009 – see backup





Signal Cascade



ERK5 plays a role in various cellular processes such as proliferation, differentiation and cell survival.

Inhibition of ERK5 blocks proliferation and survival of ERK5 amplified tumors

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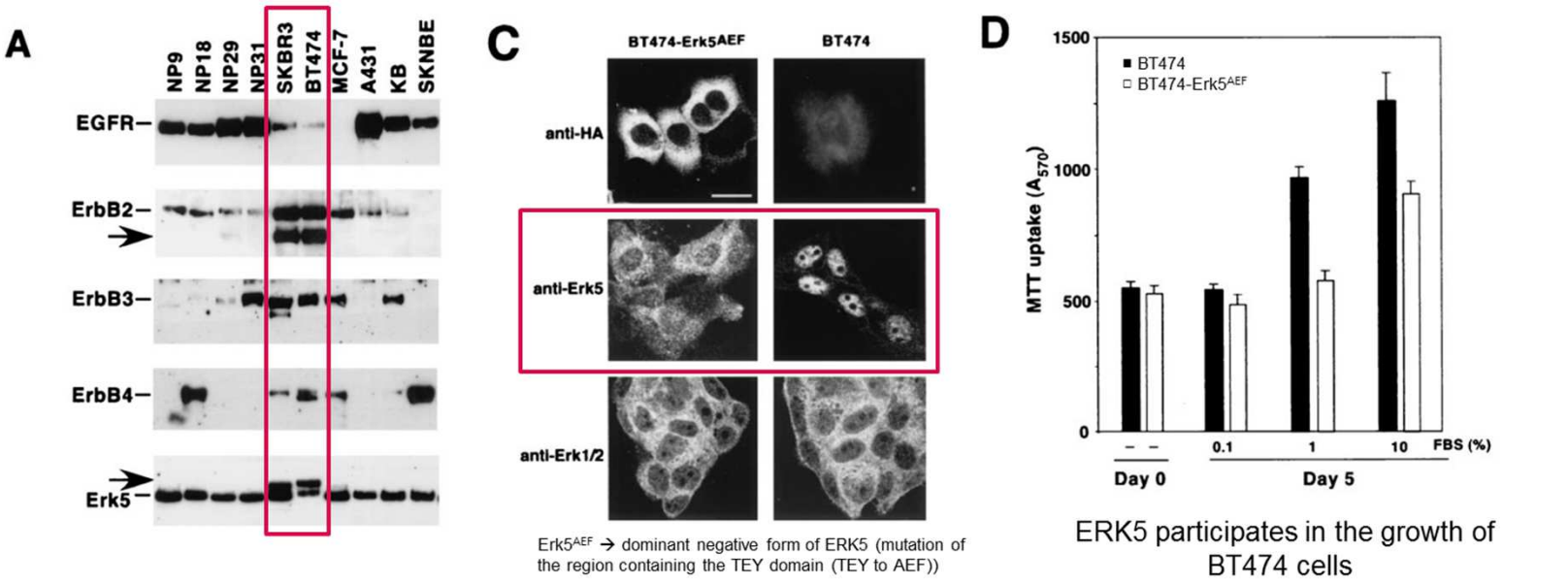
Target rationale: ERK5 activation



Erk5^{AEF} → dominant negative form of ERK5 (mutation of the region containing the TEY domain (TEY to AEF))

Cell lines with ErbB2 overexpression show constitutively active ERK5 (p-ERK5 located in the nucleus)

ERK5 participates in the growth of BT474 cells

Esparis-Ogando, 2002. Molecular and Cellular Biology

Cell lines with constitutively active ERK5 depend on it for proliferation and survival

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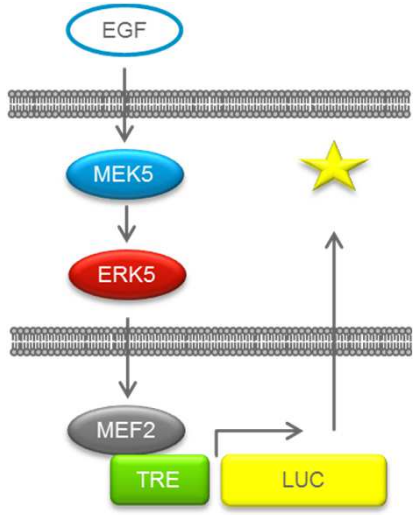
Cellular mechanistic assay: SN12C-MEF2-luc

ERK5 cellular mechanistic assay (SN12C-MEF2-Luciferase reporter cell line (clone 37))

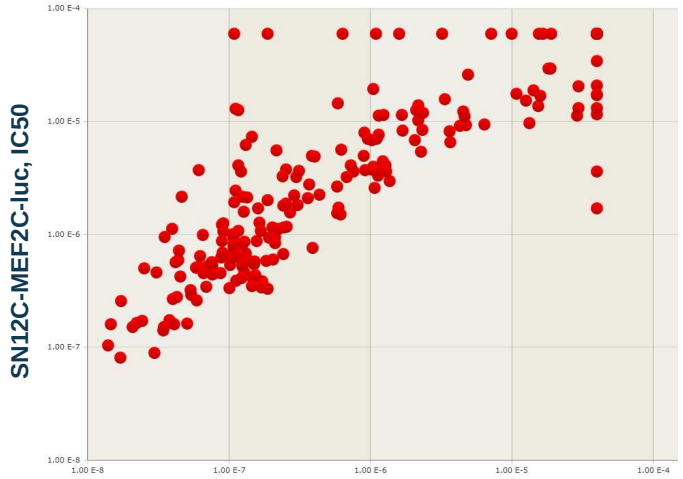


- **MEF2 transcriptional activity** (transcription factor MEF2 is directly activated by ERK5)
- **Stimulation with growth factor (EGF)**

TRE → MEF2 transcription response element

Assay conditions:

- **Nr cells:** 10,000 cells/well
- **Stimulation:** EGF (100 ng/ml; 2h)
- **Drug incubation:** 16h
- **Throughput:** 384-well format
- **S/B:** 7.0 ± 1.9 (n=2)
- **Z-factor:** 0.8 ± 0.1 (n=2)



Good correlation between ERK5 biochemical and cellular mechanistic assays

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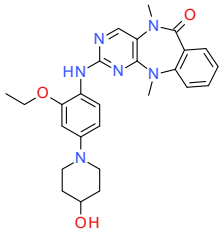
Known ERK5 inhibitors

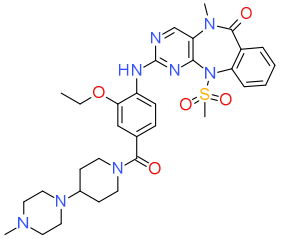
XMD8-92

- Originally described as a potent and selective ERK5 inhibitor with *in vivo* antitumor efficacy (*Yang et al, 2010. Cancer Cell*).
- However, a recent study demonstrated that the biological activity of XMD8-92 derived from an off-target activity on bromodomains (BRD4) (*Lin et al, 2016. PNAS*).
- Literature data:
  - ERK5 IC_{50} (lysate, KiNativ): 190 nM
  - BRD4 K_d (DiscoveRx): 170 nM
- In-house data:
  - ERK5 biochemical IC_{50}: 199 nM
  - ERK5 cellular IC_{50}/IC_{90}: 886 / 6900 nM



AX15836

- ERK5 inhibitor with improved potency and selectivity (vs. BRD4), but with described pharmacokinetic liabilities (*Lin et al, 2016. PNAS*).
- In-house data suggests that this compound might precipitate easily in culture medium (IC_{50} curves in V shape).
- Literature data:
  - ERK5 IC_{50} (lysate, KiNativ): 8 nM
  - BRD4 K_d (DiscoveRx): 3600 nM
- In-house data:
  - ERK5 biochemical IC_{50}: 15 nM
  - ERK5 cellular IC_{50}/IC_{90}: 86 / >30000 nM



Available preclinical ERK5 inhibitors have different liabilities

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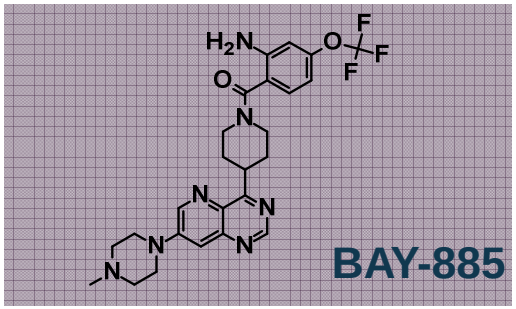
In vitro profile



POTENCY (IC_{50} [nM])	
ERK5 (@ 250µM ATP) IC_{50}	35
Mechan. SN12C-MEF2-luc IC_{50}	115
2D proli SN12C IC_{50}	> 30 000
2D proli SNU-449 IC_{50}	> 30 000
2D proli BT-474 IC_{50}	> 30 000
2D proli SK-BR-3 IC_{50}	> 30 000

Properties & Physchem	
LogD @pH 7.5	2.4
BEI / LLE (ERK5 @250µM ATP)	15.7 / 5.1
Sw pH 6.5 [mg/L]	218
MW corr / TPSA [g*mol / Å²]	477 / 101
Stability (r /h plasma) [%]	nd
Stability pH 1,7,10 (24h) [%]	stable

***in vitro* DMPK Properties**

Caco2 permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	184		107		0.6	
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	liver mics (h)		0.8		38	
	rat hepatocytes		3.7		13	
	human hepatocytes					
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	> 20	> 20	> 20	> 20	> 20	> 20, no hint on TDI
PXR	green					
CYP induction						

Selectivity

In-house kinase panel	highly selective., 1 kinase hit with IC_{50}: 3 µM
Eurofins @ 1 µM (kinase panel)	highly selective

SAFETY

Cytotox	
hERG IC_{50} [µM]	> 10

- Highly potent and selective ERK5 inhibitor
- Good solubility and excellent permeability, low to moderate metabolic stability

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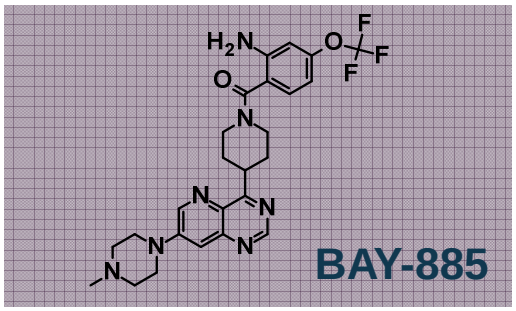
Selectivity profile



POTENCY (IC_{50} [nM])	
ERK5 (@ 250µM ATP) IC_{50}	35
Mechan. SN12C-MEF2-luc IC_{50}	115
2D proli SN12C IC_{50}	> 30 000
2D proli SNU-449 IC_{50}	> 30 000
2D proli BT-474 IC_{50}	> 30 000
2D proli SK-BR-3 IC_{50}	> 30 000

- BAY-885 was profiled in Eurofins kinase panel
- 350 kinases were tested @ 1µM BAY-885
- Residual kinase activity below 50% reported for
  - Fer (h): 38%
  - EphB3 (h): 42%
  - EphA5 (h): 57%
- For other kinases, residual activity reported ≥ 80%

Microsoft Excel Worksheet

- BAY-885 is a highly selective ERK5 inhibitor
- Fer (tyrosine-protein kinase Fer) acts downstream of EGFR to promote activation of NF-kB and cell proliferation

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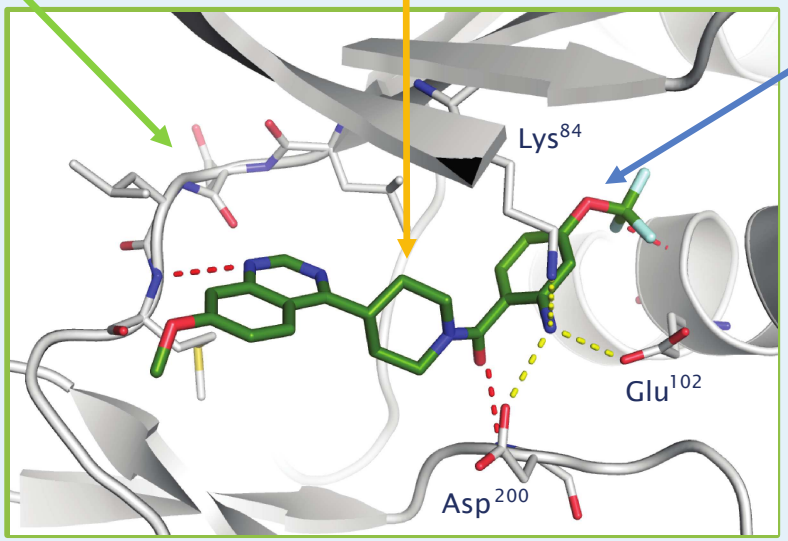
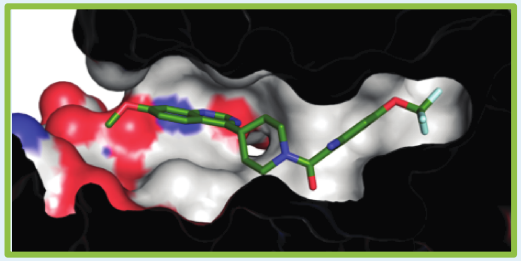
X-ray Structure in Complex with an analog of BAY-885

Hinge region
Single H-bonding interaction

Central piperidine
Conformation allows compound to simultaneously bind at hinge and in back pocket

Hydrophobic back pocket
occupied by OCF_3 group. The pyramidalized NH_2 group makes hydrogen-bonds with Lys^{84}, Asp^{200} and Glu^{102} side-chains.





X-ray of a quinazoline derivative in complex with ERK5

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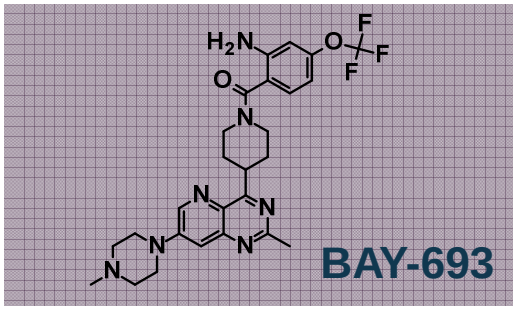
In vitro profile of negative control BAY-693



POTENCY (IC_{50} [nM])	
ERK5 (@ 250µM ATP) IC_{50}	6400
Mechan. SN12C-MEF2-luc IC_{50}	11 000
2D proli SN12C IC_{50}	29 300
2D proli SNU-449 IC_{50}	19 500
2D proli BT-474 IC_{50}	27 600
2D proli SK-BR-3 IC_{50}	22 400

Properties & Physchem	
LogD @pH 7.5	2.6
BEI / LLE (ERK5 @250µM ATP)	10.6 / 2.6
Sw pH 6.5 [mg/L]	-
MW corr / TPSA [g*mol / Å²]	488 / 101
Stability (r /h plasma) [%]	nd
Stability pH 1,7,10 (24h) [%]	stable

in vitro DMPK Properties

Caco2 permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	234		107		0.5	
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	liver mics (h)		0.6		55	
	rat hepatocytes		3.3		22	
	human hepatocytes					
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	> 20	> 20	> 20	> 20	> 20	>20, hint on TDI
PXR						
CYP induction						

Selectivity

In-house kinase panel	33 kinases tested, 1 kinase with IC_{50}: 3 µM
Eurofins @ 1 µM (kinase panel)	nd

SAFETY

Cytotox	
hERG IC_{50} [µM]	

BAY-693 recommended as negative control for the chemical probe

ERK5 Probe BAY-885

Summary / conclusion

Probe criteria	
Inhibitor/agonist potency: goal is < 50 nM (IC_{50}, Kd)	**Surpasses criteria**; high potency in biochemical ERK5 assay with IC_{50} = 35nM
Selectivity within target family: goal is >30-fold	**Surpasses criteria**; highly selective vs all kinases in Eurofins panel
Selectivity outside target family: describe the off-targets (which may include both binding and functional data)	not done
On target cell activity for cell-based targets: goal is < 1 micromolar IC_{50}/EC_{50}	**Surpasses criteria**; active in cellular mechanistic assay (IC50 =115 nM) demonstrating on-target cell activity
On target cell activity for secreted targets: appropriate alternative such as mouse model or other mechanistic biological assay, e.g., explant culture	not applicable
Neg control: in vitro potency – > 100 times less; Cell activity – >100 times less potent than the probe	**Surpasses criteria**; BAY-693 with biochemical activity IC_{50} = 6.4 µM and on target activity IC_{50} = 11 µM)

We recommend ERK5 inhibitor BAY-885 to be accepted as donated chemical probe, with BAY-693 as negative control

ERK5 Probe BAY-885

Acknowledgements

Ulf Bömer
Simon Holton
Clara Lemos
Duy Nguyen
Christian Lechner
Stefan Prechtl
Detlev Sülzle
Franziska Siegel

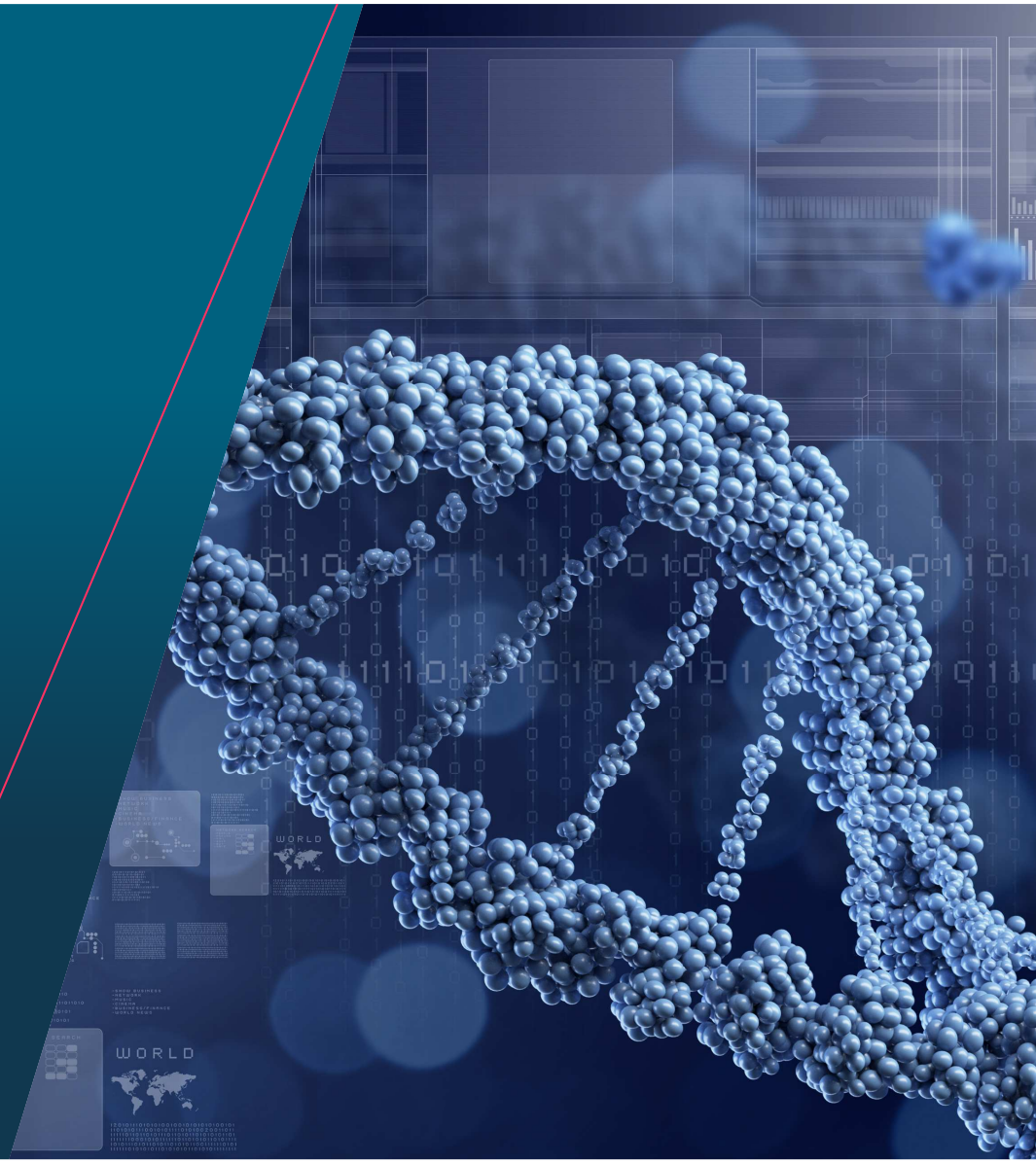
Andrea Hägebarth
Marcus Bauser

Lars Wortmann
Sarah Wagner
Knut Eis
Patrick Steigemann
Atanas Kamburov
Andreas Steffen
Philip Lienau
Sabine Zitzmann-Kolbe
Florian Prinz
Ralf Lesche
Ursula Egner
Uwe Eberspächer
Gregor Fachinger
Peter Spreyer
Cora Scholten
Léa Bouché

Dominik Mumberg
Carl Friedrich Nising
Franz von Nussbaum

Enrico Stasik
Maria Buhl
Steve Baethge
Franziska Scholze

Thank You



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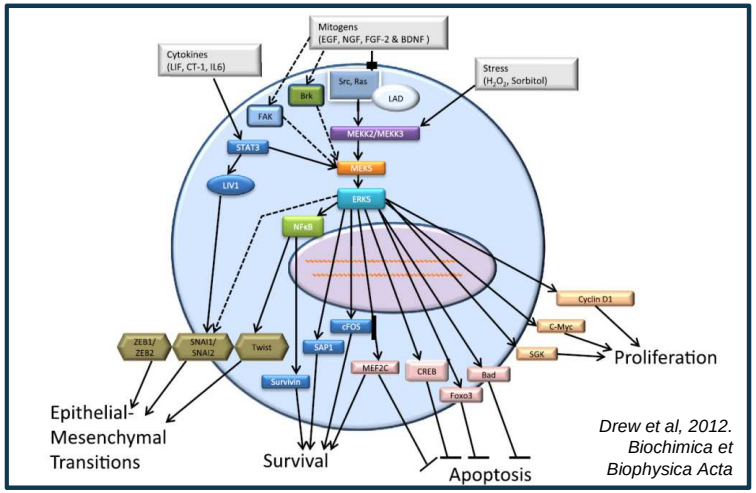
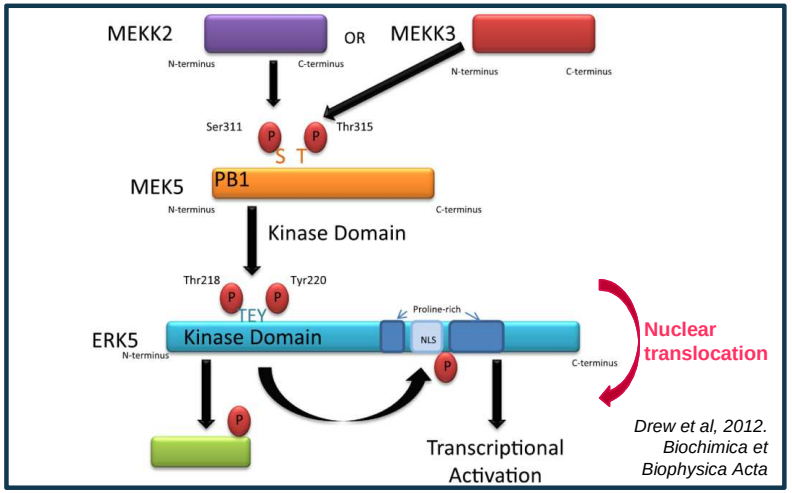
Scientific rationale – ERK5 signaling



Drew et al, 2012. Biochimica et Biophysica Acta



Drew et al, 2012. Biochimica et Biophysica Acta

- The MEK5/ERK5 pathway is activated by cell stressors, mitogens or cytokines
- The upstream activators of ERK5 are MEKK2/3 and MEK5
- Upon activation, ERK5 autophosphorylates and shuttles to the nucleus where it phosphorylates/transcriptionally activates various downstream targets
- ERK5 signaling contributes to proliferation and survival and prevents apoptosis

MAPK7/ERK5 is a key integrator of cellular signal transduction and it plays a role in various cellular processes such as proliferation, differentiation and cell survival.

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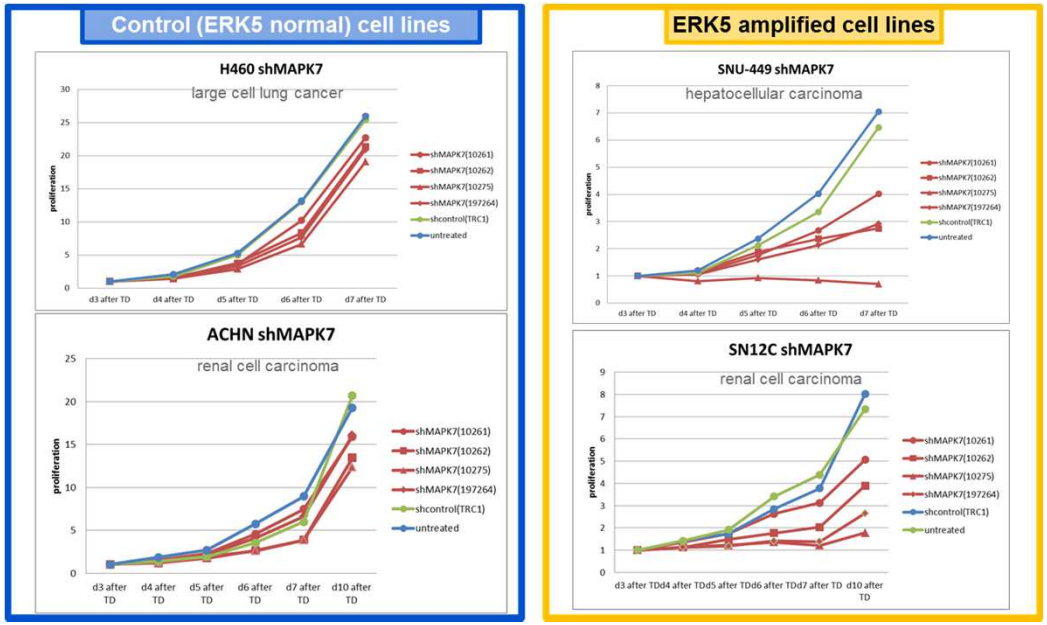
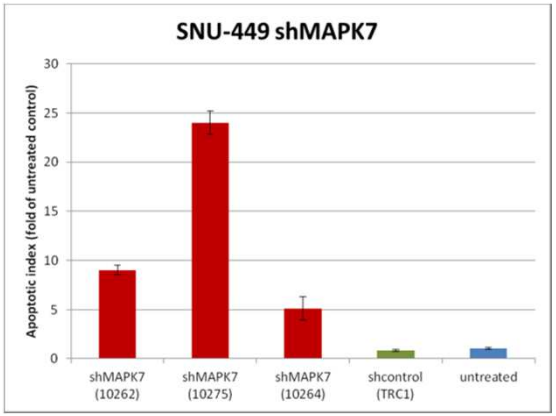
Target rationale: ERK5 amplification



ERK5 silencing induces anti-proliferative effects in cell lines with genomic ERK5 amplification vs. non-amplified controls



ERK5 silencing induces apoptosis in ERK5 amplified cells

ERK5 depletion blocks proliferation and induces apoptosis in ERK5 amplified tumors

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Target Rationale – ERK5 amplification (literature)

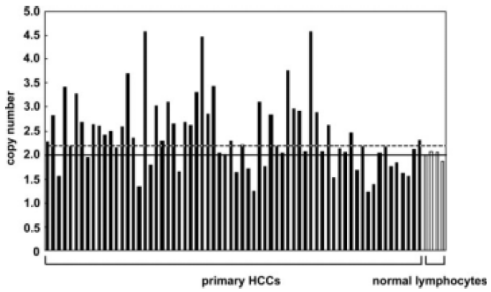
GENES, CHROMOSOMES & CANCER 48:109–120 (2009)

RESEARCH ARTICLES

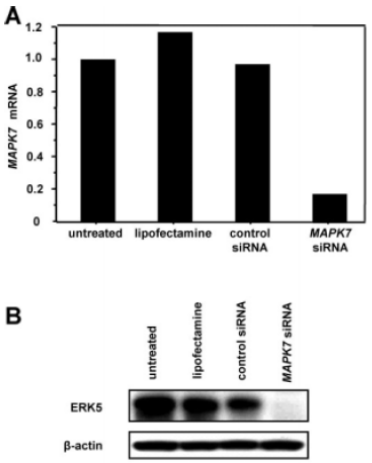
ERK5 is a Target for Gene Amplification at 17p11 and Promotes Cell Growth in Hepatocellular Carcinoma by Regulating Mitotic Entry

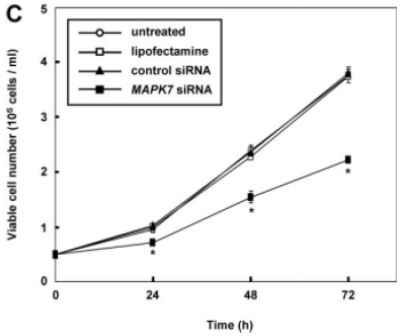
Keika Zen,[1] Kohichiroh Yasui,[1]* Tomoaki Nakajima,[1] Yoh Zen,[2] Kan Zen,[3] Yasuyuki Gen,[1] Hironori Mitsuyoshi,[1] Masahito Minami,[1] Shoji Mitsufuji,[1] Shinji Tanaka,[4] Yoshito Itoh,[1] Yasuni Nakanuma,[2] Masafumi Taniwaki,[5] Shigeki Arii,[4] Takeshi Okanoue,[1] and Toshikazu Yoshikawa[1]

[1]Molecular Gastroenterology and Hepatology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan
[2]Department of Human Pathology, Kanazawa University Graduate School of Medicine, Kanazawa, Japan
[3]Division of Cardiovascular Medicine, Omihachiman Community Medical Center, Omihachiman, Japan
[4]Department of Hepato-Biliary-Pancreatic Surgery, Tokyo Medical and Dental University, Tokyo, Japan
[5]Molecular Hematology and Oncology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan



- An increase in ERK5 copy number was detected in 35 of 66 primary HCC tumors





- siRNA-mediated downregulation of ERK5 suppressed the growth of SNU-449 cells

ERK5 promotes the growth of ERK5-amplified HCC cells

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Target Rationale – relevance of kinase activity

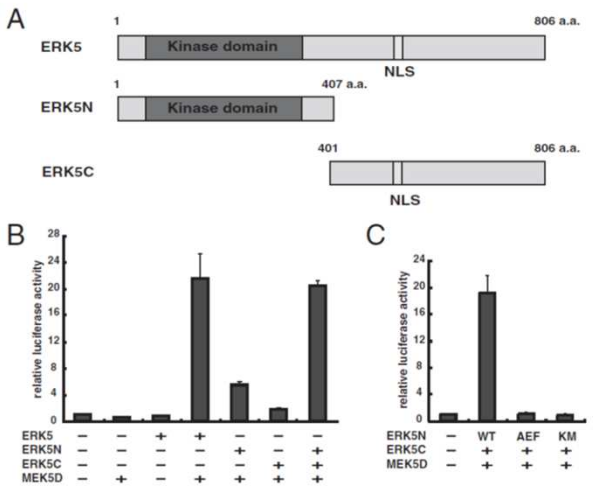
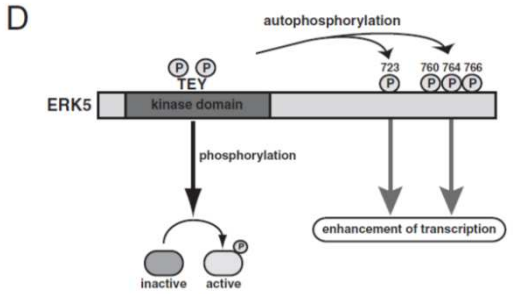


FIGURE 1. **Both the N-terminal- and C-terminal-halves of ERK5 are required for the activation of AP-1 activity.** *A*, mouse ERK5 and ERK5 deletion mutants used in these experiments. *B* and *C*, indicated expression plasmids were transfected with pAP1-Luc, the reporter plasmid, in NIH 3T3 cells. After 24 h, the cells were harvested and then lysates were subjected to the luciferase assay.



Morimoto et al, 2007. J Biol Chem

Supportive literature:

Kasler et al, 2000. Mol Cell Biol
Mulloy et al, 2003. Oncogene
Morimoto et al, 2007. J Biol Chem
Erazo et al, 2013. Mol Cell Biol
+
Nishimoto and Nishida, 2006. EMBO Rep
Drew et al, 2012. Biochim Biophys Acta
Lochhead et al, 2012. Biochem Soc Trans
Simões et al, 2016. Drug Discov Today

- The ERK5 kinase domain is required for the ERK5-induced transcriptional activation (phosphorylation/activation of downstream TF + autophosphorylation that results in activation of the TAD leading to enhancement of transcription) and proliferation
- Supported by in-house data showing that ERK5i are able to abrogate ERK5-induced MEF2 transcriptional activity (SN12C-MEF2-luc reporter cell line)

ERK5 Probe BAY-885

Methods

- High-throughput TR-FRET (time-resolved fluorescence energy transfer) -based kinase inhibition assay was used to identify potential ERK5 inhibitors (Figure 1). MAP2K5 (mitogen-activated protein kinase kinase 5) and ATP-activated full-length ERK5 was incubated with the test compounds, biotinylated substrate peptide, and 250 µM ATP for 120 min. The amount of phosphorylated peptide was quantified with a phosphospecific detection antibody and generic TR-FRET detection reagents.

- A quinazoline cluster was identified as the initial hit by high-throughput screening and was selected as a starting point for optimization guided by X-ray. On-target activity of ERK5 inhibitors was shown by inhibition of transcriptional activity of *MEF2* (myocyte enhancer factor 2, directly activated by ERK5) (Figure 2). SN12C-MEF2-luciferase reporter cells were treated with different concentrations of ERK5 inhibitors for 16 h and stimulated with EGF (epidermal growth factor) to increase the assay window. The luciferase (luminescence) signal was determined with ONE-Glo™.

- The *in vitro* efficacy of the compounds was tested by proliferation assay on cell lines with either genomic *ERK5* amplification (breast cancer: MFM-223, renal cell carcinoma: SN12C, hepatocellular carcinoma: SNU-449) or constitutive activation of *ERK5* (breast cancer: BT-474, SK-BR-3). The NCI-H460 large cell lung cancer cell line was used as a control. Assays were performed in 384-well format with 96 h compound incubation. Cell viability was determined with a CellTiter-Glo® luminescent assay.

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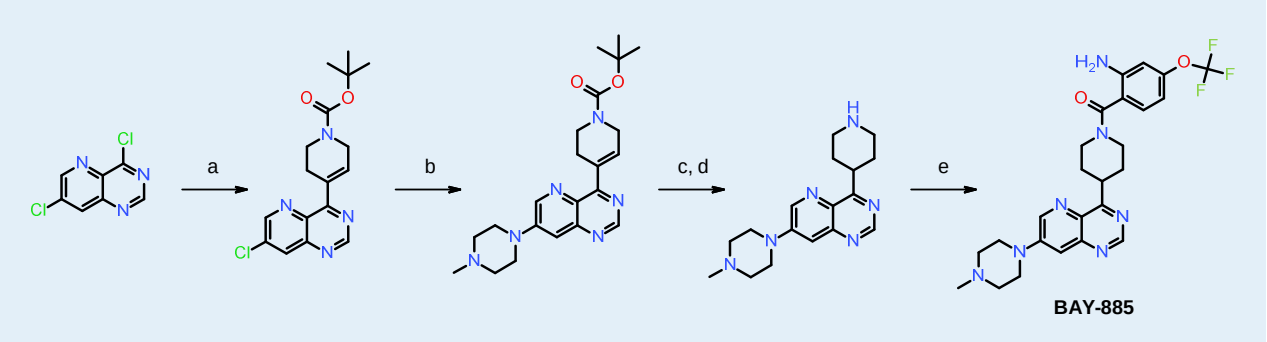
Compound synthesis



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Competitors

Competitor analysis on MAPK7/ERK5 inhibitors

Gregor Fachinger, **January 2017**
Sources: Thomson Cortellis (former IDdb3), MedTrack database, Prous-Integrity database, Company homepages.

- The **multi-kinase inhibitor TG-02** identified by S-Bio and developed by Lee Pharma and Tragara is **still in Phase 1 clinical trials**, no update
- Kesios entertains **two programs on ERK5 at the preclinical level**, which have not progressed into the clinic. Same is true for Northern Institute for Cancer Research.
- The actual **status of XMD-8-92** developed by Scripps and ActivX is inconsistently reported in the databases and **currently unclear**.
- The **patent literature** provides indication of development **activities on ERK5 inhibitors or modulators** by **Kyorin Pharmaceuticals, Merck KG and Astex** (new since last year) as well as academic institutions.

- Given the unselective profile of TG-02, no advanced relevant competitor activities for a selective ERK5/MAPK7 inhibitor have been reported.
- Recommendation to observe the field going forward.