产品名称：**URMC-099**
产品别名：**URMC-099**

生物活性：					
Description	URMC-099 is an orally bioavailable and potent mixed lineage kinase type 3 (MLK3) (IC50=14 nM) inhibitor with with excellent blood-brain barrier penetration properties.				
IC_{50} & Target	MLK3	LRRK2	FLT3	FLT1	ABL1 (T315I)
	14 nM (IC_{50})	11 nM (IC_{50})	4 nM (IC_{50})	39 nM (IC_{50})	3 nM (IC_{50})
	ABL1	SGK	SGK1	AurA	AurB
	6.8 nM (IC_{50})	67 nM (IC_{50})	201 nM (IC_{50})	108 nM (IC_{50})	123 nM (IC_{50})
	AurC	IKKβ	IKKα	TNFα	ROCK1
	290 nM (IC_{50})	257 nM (IC_{50})	591 nM (IC_{50})	460 nM (IC_{50})	1030 nM (IC_{50})
	ROCK2	CDK1	CDK2	TRKA	c-MET
	111 nM (IC_{50})	1125 nM (IC_{50})	1180 nM (IC_{50})	85 nM (IC_{50})	177 nM (IC_{50})
	TRKB	IGF1R	LCK	MEKK2	SYK
	217 nM (IC_{50})	307 nM (IC_{50})	333 nM (IC_{50})	661 nM (IC_{50})	731 nM (IC_{50})
	AMPK	JNK1	SRC	ZAP70	ERK2
	1512 nM (IC_{50})	3280 nM (IC_{50})	4330 nM (IC_{50})	5050 nM (IC_{50})	6290 nM (IC_{50})
	P38α	CYP3A4			
	12050 nM (IC_{50})	16.2 μM (IC_{50})			
In Vitro	The effect of URMC-099 (URMC099) on the in vitro growth of the “brain homing” MDA-MB-231 BR cells expressing eGFP (eGFP8.4) and their parental cell line, MDA-MB-231 is tested. The cells are treated with either 200 nM URMC-099 or vehicle alone. Cells treated with URMC-099 grow at a similar rate to those treated with vehicle. Cell viability is >99% in all cases[2].				
In Vivo	URMC-099 has moderate terminal elimination half-life ($t_{1/2}$=1.92 h, 2.14 h and 2.72 h for C57 BL/6 mice (10 mg/kg, oral dosing), C57 BL/6 mice (2.5 mg/kg, iv), C57 BL/6 mice (10 mg/kg, iv))[1]. The effect of URMC-099 (URMC099) on tumor formation in vivo is analyzed using a well characterized mouse xenograft model of breast cancer brain metastasis. For these experiments, eGFP8.4 cells are inoculated into the left ventricle of immunodeficient nu/nu mice; animals are then treated with either URMC-099 (10 mg/kg) or vehicle alone, every 12 hours for 20 days. This dose of URMC-099 is chosen because it has been shown to be sufficient to effectively inhibit MLK3 in mice, with good penetration of the blood-brain barrier and potent inhibition of the phosphorylation of Jun N-terminal kinase (JNK) in brain tissue. On day 21 the mice are sacrificed and number of BM is assessed. Fifteen mice are used for each treatment group. BM are detected in 60% of mice, which is consistent with previous studies using this xenograft model by other investigators. URMC-099 treatment significantly ($p<0.05$, two-tailed t-test) increases the total number of brain metastasis (BM) in mice. For micrometastases, the pattern is similar to that observed for total BM. The number of macrometastases is statistically indistinguishable between mice treated with URMC-099 or vehicle[2].				
	In Vitro: **DMSO : ≥ 33 mg/mL (78.28 mM)** **H_2O : < 0.1 mg/mL** (insoluble)				

Solvent&Solubility

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.3723 mL	11.8613 mL	23.7225 mL
	5 mM	0.4745 mL	2.3723 mL	4.7445 mL
	10 mM	0.2372 mL	1.1861 mL	2.3723 mL

*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

In Vivo:

请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 **In Vitro** 方式配制澄清的储备液，再依次添加助溶剂：

——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶

1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 2.17 mg/mL (5.15 mM); Clear solution

此方案可获得 ≥ 2.17 mg/mL (5.15 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 21.7 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。

2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline)

Solubility: ≥ 2.17 mg/mL (5.15 mM); Clear solution

此方案可获得 ≥ 2.17 mg/mL (5.15 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 21.7 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。

3.请依序添加每种溶剂： 10% DMSO →90% corn oil

Solubility: ≥ 2.17 mg/mL (5.15 mM); Clear solution

此方案可获得 ≥ 2.17 mg/mL (5.15 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。

以 1 mL 工作液为例，取 100 μL 21.7 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。

References

[1]. Goodfellow VS, et al. Discovery, synthesis, and characterization of an orally bioavailable, brain penetrant inhibitor of mixed lineage kinase 3. J Med Chem. 2013 Oct 24;56(20):8032-48.

[2]. Rhoo KH, et al. Pharmacologic inhibition of MLK3 kinase activity blocks the in vitro migratory capacity of breast cancer cells but has no effect on breast cancer brain metastasis in a mouse xenograft model. PLoS One. 2014 Sep 29;9(9):e108487.

实验参考：

Cell Assay	MDA-MB-231, MCF10A, HS578t and MDA-MB-231 EGFP8.4 cells are seeded in a 24 well plate at an initial density of 5.0×10^4 cells/mL in 0.5 mL of media. The cells are treated with either 200 μM of URMC-099 or vehicle (0.002% DMSO). Cell number in each well is measured by trypsinizing the cells and counting them with a hematocytometer. The viability is tested by trypan blue dye

	exclusion. Each condition is tested in triplicate[2].
Animal Administration	Mice[2] 6 to 8 week old female nu/nu mice are injected intraperitoneally with URMC-099 at a dose of 10 mg/kg, or vehicle, twice daily for 20 days. On day 21 mice are sacrificed by CO_2 suffocation. Brains are removed and fixed with 4% formaldehyde in PBS overnight, then transferred to 30% sucrose in PBS. The brains are then quickly frozen by immersing into isopentane cooled on dry ice. The frozen brains are sectioned coronally every 30 micrometers. Eight sections starting at bregma 2.0 and separated by 360 μm are mounted on glass slides for tumor evaluation under the microscope. The number of brain metastasis (BM) is counted by examining eGFP signals under a fluorescence microscope at 20× magnification[2].
References	[1]. Goodfellow VS, et al. Discovery, synthesis, and characterization of an orally bioavailable, brain penetrant inhibitor of mixed lineage kinase 3. J Med Chem. 2013 Oct 24;56(20):8032-48. [2]. Rhoo KH, et al. Pharmacologic inhibition of MLK3 kinase activity blocks the in vitro migratory capacity of breast cancer cells but has no effect on breast cancer brain metastasis in a mouse xenograft model. PLoS One. 2014 Sep 29;9(9):e108487.