

产品名称: **LGK974**

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生物活性:				
Description	LGK974 (WNT974) is an orally bioavailable and specific Porcupine (PORCN) inhibitor with an IC₅₀ of 0.1 nM.			
IC ₅₀ & Target	Porcupine[1]			
In Vitro	LGK974 effectively displaces [3H]-GNF-1331 with an IC50 of 1 nM in the PORCN radioligand binding assay. LGK974 potently reduces Wnt-dependent AXIN2 mRNA levels in HN30 cells with an IC50 of 0.3 nM[1].			
In Vivo	LGK974, a drug that targets Porcupine, a Wnt-specific acyltransferase. LGK974 potently inhibits Wnt signaling, has strong efficacy in rodent tumor models, and is well-tolerated. Toxicology studies are performed on nontumor bearing rats at 3 and 20 mg/kg. At the efficacious dose of 3 mg/kg per day for 14 d, LGK974 is well-tolerated without abnormal histopathological findings in Wnt-dependent tissues, including the intestine, stomach, and skin. When rats are administrated a very high dose of 20 mg/kg per day for 14 d, loss of intestinal epithelium is observed, consistent with the concept that Wnt is required for intestinal tissue homeostasis[1].			
Solvent&Solubility	In Vitro: DMSO : ≥ 32 mg/mL (80.72 mM) H₂O : < 0.1 mg/mL (insoluble) * "≥" means soluble, but saturation unknown.			
	<div>Preparing Stock Solutions</div>	<div>Solvent Mass Concentration</div>	1 mg	5 mg
		1 mM	2.5224 mL	12.6122 mL
		5 mM	0.5045 mL	2.5224 mL
		10 mM	0.2522 mL	1.2612 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℃，6 months；-20℃，1 month。 -80℃ 储存时，请在 6 个月内使用，-20℃ 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 →90% (20% SBE-β-CD in saline) Solubility: 2.5 mg/mL (6.31 mM); Suspended solution; Need ultrasonic and warming 此方案可获得 2.5 mg/mL (6.31 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水溶液中，混合均匀。			
	2.请依序添加每种溶剂： 10% DMSO →90% corn oil			

	<p>Solubility: ≥ 2.5 mg/mL (6.31 mM); Clear solution</p> <p>此方案可获得 ≥ 2.5 mg/mL (6.31 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。</p> <p>以 1 mL 工作液为例, 取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中, 混合均匀。</p>
References	<p>[1]. Liu J, et al. Targeting Wnt-driven cancer through the inhibition of Porcupine by LGK974. <i>Proc Natl Acad Sci U S A</i>. 2013 Dec 10;110(50):20224-9.</p> <p>[2]. Tammela T, et al. A Wnt-producing niche drives proliferative potential and progression in lung adenocarcinoma. <i>Nature</i>. 2017 May 18;545(7654):355-359.</p>
实验参考:	
Cell Assay	<p>HN30 cells and UMSCC cells are used. For TaqMan assay, 2×10^6 cells per well are plated into six-well cell culture plates and treated with or without LGK974 in amultipoint dose-response. RNA samples are collected after 48 h. For colony formation assays, 2×10^3 cells per well are plated into six-well cell culture plates with or without compound treatment. Cells are stained with crystal violet 1 wk later[1].</p>
Animal Administration	<p>Mice and Rats[1]</p> <p>Nude mice (or nude rats) bearing the mouse mammary tumor virus-Wnt1, HN30, or SNU1076 tumors are randomized according to tumor volume. LGK974 is formulated in 10% (vol/vol) citrate buffer (pH 2.8)/90% (vol/vol) citrate buffer (pH 3.0) or 0.5% MC/0.5% Tween 80 and administered by oral gavage at a dosing volume of 10 μL/g animal body weight. Body weight is monitored daily, and tumor sizes are assessed three times per week after the tumors are palpable. Tumor sizes are determined by using caliper measurements. Tumor volumes are calculated with a formula (length\timeswidth\timesheight)/2. The plasma concentrations and exposures of LGK974 in the tumor-bearing nude mice (n=2 per dosing group) are determined on day 14. Blood samples (50 μL) are collected by serial retroorbital sampling at 1, 3, 7, 16, and 24 h postdose. The blood samples are centrifuged, and plasma is separated and frozen until analysis by liquid chromatography/MS/MS. For tolerability studies, LGK974 is administrated to nontumor-bearing Wistar rats one time per day by oral gavage at 3 or 20 mg/kg per day. Necropsies are performed at the end of the study. Tissues are fixed in 10% (vol/vol) neutralbuffered formalin, sectioned, and subjected to H&E staining.</p>
Kinase Assay	<p>Radioligand binding assay: using the aforementioned membrane preps, filtration binding assays are performed. To reduce nonspecific binding, 96-well filtration plates are precoated as suggested by the manufacturer with 0.1% BSA and then washed four times with 0.1% BSA. Membrane preps (50 μg total protein) are incubated in polypropylene 96-well plates with 6.6 nM 3H-GNF-1331 in the presence or absence of a testing compound in binding buffer (50 mM Tris, pH 7.5, 5 mM $MgCl_2$, 1 mM EDTA, 0.1% BSA) plus EDTA-free protease inhibitor mixture in a final volume of 150 μL for 3 h at room temperature. Binding reaction mixtures are then transferred to the precoated 96-well filtration plates, filtered, and washed using a 96-pin FilterMate Harvester. Radioactive signals are obtained using a Microplate Scintillation Counter TopCount. Curve fitting is performed using Prism[1].</p>
References	<p>[1]. Liu J, et al. Targeting Wnt-driven cancer through the inhibition of Porcupine by LGK974. <i>Proc Natl Acad Sci U S A</i>. 2013 Dec 10;110(50):20224-9.</p> <p>[2]. Tammela T, et al. A Wnt-producing niche drives proliferative potential and progression in lung adenocarcinoma. <i>Nature</i>. 2017 May 18;545(7654):355-359.</p>