产品名称：RAF265
产品别名：RAF265 (CHIR-265)

生物活性：

Description	RAF265 is a potent RAF/VEGFR2 inhibitor.	
IC_{50} & Target	VEGFR2	RAF
In Vitro	The MTT assay reveals that in HT29 and MDAMB231 cells, RAF265 alone shows significant activity with IC_{20} values of 1 to 3 μM and IC_{50} values of 5 to 10 μM. In A549 and HCT116 cells, IC_{20} values are 1 μM for both, but RAF265 concentrations up to 10 μM do not reach IC_{50} values. However, in the presence of 1 nM RAD001, the IC_{50} for RAF265 is 5 μM in A549 cells and 10 μM in HCT116 cells[1].	
In Vivo	In single-compound efficacy studies, optimal dosing of RAD001 and RAF265 is 5 to 12 mg/kg daily and 30 mg/kg every two days, respectively. However, combination tolerability studies in nontumor-bearing mice defin dose-limiting toxicity as a 10% weight loss with the combination of RAD001 at a dose of 12 mg/kg daily and RAF265 at a dose of 20 mg/kg every two days. Therefore, the combination of RAF265 at a dose of 12 mg/kg qd and RAD001 at a dose of 12 mg/kg qd seems to be the maximal tolerated dose. RAD001 and RAF265 are both given at a dose of 12 mg/kg qd, alone or concurrently, over 6 days. After a 2-day stop, the compounds are given for another 6 days, and the treatment is then stopped. To confirm the potential of the combination of RAF265 and RAD001, the antitumor effect of the combination is tested in HCT116 xenografts (*KRAS* mut, *PIK3CA* mut). In HCT116 xenografts, RAD001 or RAF265 given alone shows 60% to 65% and 71% to 72% TVI%, respectively[1].	

Solvent&Solubility

In Vitro:

DMSO : ≥ 26 mg/mL (50.15 mM)

Ethanol : 10 mg/mL (19.29 mM; Need ultrasonic)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.9290 mL	9.6449 mL	19.2898 mL
	5 mM	0.3858 mL	1.9290 mL	3.8580 mL
	10 mM	0.1929 mL	0.9645 mL	1.9290 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: ≥ 1 mg/mL (1.93 mM); Clear solution

此方案可获得 ≥ 1 mg/mL (1.93 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 10.0 mg/mL 的澄清 EtOH 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: 1 mg/mL (1.93 mM); Suspended solution; Need ultrasonic 此方案可获得 1 mg/mL (1.93 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 10.0 mg/mL 的澄清 EtOH 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: 1 mg/mL (1.93 mM); Clear solution; Need warming 此方案可获得 1 mg/mL (1.93 mM)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 10.0 mg/mL 的澄清 EtOH 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Mordant P, et al. Dependence on phosphoinositide 3-kinase and RAS-RAF pathways drive the activity of RAF265, a novel RAF/VEGFR2 inhibitor, and RAD001 (Everolimus) in combination. Mol Cancer Ther. 2010 Feb;9(2):358-68.

实验参考：

Cell Assay	The MTT assay and Bliss additivism model are used to assess the effect of the combination on cell viability. Human A549 and H460 lung, HT29 and HCT 116 colon, and MDAMB231 breast cancer cell lines are used. In each well of a 96-well plate, 1×10^4 cells are grown in 200 μL of medium. After 24 h, RAD001, RAF265, or the combination is added to achieve a final concentration of 0.1 to 10 nM and 0.1 to 10 μM, respectively. After 48 h of treatment, 20 μL of 5 mg/mL MTT solution in PBS is added to each well. After 4 h, supernatant is removed and formazan crystals are discarded in 200 μL of DMSO. Absorbance is then measured at 595 nm using an absorbance plate reader. Data are expressed as the percentage of viable cells in treated relative to nontreated conditions[1]
Animal Administration	Mice[1] The efficacy of the combination is also tested in vivo. A total of 3×10^6 A549, H460, HCT116, or MDAMB231 cells are injected s.c. into the flank region of 6-wk-old female athymic mice. When tumors reach 50 mm3, the mice are randomized into four groups (n=7/group) for the following treatment: vehicle, RAF265 (12 mg/kg daily), RAD001 (12 mg/kg daily), or both. All drug are administered over 14 d (6 d on, 2 d off, 6 d on), and the drug combination is administered concurrently. Control mice receive the respective vehicles of both drugs. Animal weight and tumor volumes are taken twice weekly and expressed relative to initial tumor volume. Tumors are measured until achieving a relative volume of 10 times the initial volume, and the time to this end point is noted. Drug efficacy is assessed based on the tumor growth curve, growth delay, and tumor volume inhibition percentage. The tumor growth curve is designed to depict the evolution of the relative tumor size over time. The tumor volume inhibition percentage (TVI%) is calculated[1]
References	[1]. Mordant P, et al. Dependence on phosphoinositide 3-kinase and RAS-RAF pathways drive the activity of RAF265, a novel RAF/VEGFR2 inhibitor, and RAD001 (Everolimus) in combination. Mol Cancer Ther. 2010 Feb;9(2):358-68.