产品名称：CEP-32496
产品别名：Agerafenib

生物活性：

Description

Agerafenib (CEP-32496; RXDX-105) is a highly potent and orally efficacious inhibitor of **BRAF**^{V600E} with a K_d of 14 nM.

IC_{50} & Target

Braf	CRAF	BRaf^{V600E}	c-Kit	Ret	LCK
36 nM (Kd)	39 nM (Kd)	14 nM (Kd)	2 nM (Kd)	2 nM (Kd)	2 nM (Kd)
Abl-1	VEGFR-2	CSF-1R	EPHA2	EGFR	c-Met
3 nM (Kd)	8 nM (Kd)	9 nM (Kd)	14 nM (Kd)	22 nM (Kd)	513 nM (Kd)
JAK-2	MEK-1	MEK-2			
4700 nM (Kd)	7100 nM (Kd)	8300 nM (Kd)			

In Vitro

Agerafenib (CEP-32496) exhibits high potency against several BRAFV600E-dependent cell lines and selective cytotoxicity for tumor cell lines expressing mutant BRAFV600E versus those containing wild-type BRAF. Agerafenib exhibits potent binding (BRAFV600E Kd=14 nM) and cellular activity (pMEK IC50=82 nM and A375 proliferation IC50=78 nM), with activity in the proliferation assay. Agerafenib also exhibits a favorable CYP450 inhibition profile, with measured IC50 values greater than 10 μM versus the CYP1A2, CYP2C9, CYP2D6, and CYP3A4 isoforms and an IC50=3.4 μM versus CYP2C19[1].

In Vivo

Oral administration of Agerafenib (CEP-32496) to Colo-205 tumor xenograft-bearing mice results in significant inhibition of pMEK in tumor cell lysates. For instance, a single 30 mg/kg (po) dose of Agerafenib leads to a 50 and 75% inhibition of normalized pMEK in tumor lysates at the 2 and 6 h postdose time point, respectively (p<0.03), while a 55 mg/kg (po) dose resulted in a 75% to 57% (p<0.03) inhibition of pMEK at 2 through 10 h post administration, with normalization to baseline by 24 h. Agerafenib exhibits an exceptional PK profile in mouse, dog, and cynomolgus monkey. Administration of Agerafenib to beagle dogs (single dose of 1 mg/kg iv and 10 mg/kg po) results in low clearance (CL=5.0 (mL/min)/kg) and excellent bioavailability (%F=100). Similarly, in cynomolgus monkey, the administration of Agerafenib (single dose of 1 mg/kg iv and 10 mg/kg po) leads to high oral exposure due to low clearance (CL=6.7 mL/min/kg) and excellent bioavailability (%F=100)[1].

In Vitro:

DMSO : 50 mg/mL (96.63 mM; Need ultrasonic)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	1.9325 mL	9.6626 mL	19.3252 mL
5 mM	0.3865 mL	1.9325 mL	3.8650 mL
10 mM	0.1933 mL	0.9663 mL	1.9325 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：

Solvent&Solubility	——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: ≥ 2.5 mg/mL (4.83 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.83 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 25.0 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: ≥ 2.5 mg/mL (4.83 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.83 mM，饱和度未知) 的澄清溶液。 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: ≥ 2.5 mg/mL (4.83 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.83 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 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Rowbottom MW, et al. Identification of 1-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl)-3-(5-(1,1,1-trifluoro-2-methylpropan-2-yl)isoxazol-3-yl)urea hydrochloride (CEP-32496), a highly potent and orally efficacious inhibitor of V-RAF murine sarcoma viral oncogene homologue B1 (BRAF) V600E.

实验参考：

Cell Assay	A375 cells are seeded at 10,000 cells per well in DMEM with 10% fetal calf serum and allowed to attach. The cells are washed with PBS and switched to DMEM with 0.5% of serum and incubated overnight. The test compounds (e.g., Agerafenib; 10 μM) are then added at various concentrations with a final DMSO concentration of 0.5% and incubated for 72 h. At the end of incubation, a Cell Titer Blue is added per instructions, and incubation is continued for 3 h. Remaining viable cells are quantified by measuring the strength of the fluorescence signal using SoftMax Pro (excitation at 560 nm and emission at 590 nm). IC_{50} values are derived using a 9-point curve and are presented as mean values from experiments performed in duplicate[1].
Animal Administration	Mice[1] Six to eight week old athymic nu/nu nude mice (20-25 g) are inoculated subcutaneously with Colo-205 tumor cells (1×10^6/mouse) in the right flank. Upon reaching an average tumor volume of 150-200 mm^3 (10-12 days post implantation), animals are randomized into treatment groups (n=10 mice/group). Each group is dosed orally for 14 days with either vehicle only (22% HPβCD) or with Agerafenib at 10, 30, or 100 mg/kg twice daily (BID), and each dose of drug is given in a volume of 0.1 mL per 20 g of body weight, adjusted for the body weight of the animal. Tumor volumes are measured three times weekly using vernier calipers, and volumes are calculated [1].
References	[1]. Rowbottom MW, et al. Identification of 1-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl)-3-(5-(1,1,1-trifluoro-2-methylpropan-2-yl)isoxazol-3-y

	l)urea hydrochloride (CEP-32496), a highly potent and orally efficacious inhibitor of V-RAF murine sarcoma viral oncogene homologue B1 (BRAF) V600E.