产品名称：**BLZ945**
产品别名：**BLZ945**

生物活性：

Description	BLZ945 is a potent, selective and brain-penetrant CSF-1R inhibitor with an IC_{50} of 1 nM, showing more than 1,000-fold selectivity against its closest receptor tyrosine kinase homologs.
IC_{50} & Target	IC50: 1 nM (CSF-1R), 3.2 μM (c-Kit), 4.8 μM (PDGFRβ), 9.1 μM (Flt3)[1]
In Vitro	Treatment of bone marrow-derived macrophages (BMDMs) with BLZ945 inhibits CSF-1-dependent proliferation (EC_{50}=67 nM), and decreases CSF-1R phosphorylation, similar to CSF-1R antibody blockade. BLZ945 also reduces viability of CRL-2467 microglia, *Ink4a/Arf*$^{-/-}$ BMDMs (PDG genetic background), and NOD/SCID BMDMs. Importantly, BLZ945 treatment in culture does not affect proliferation of any PDG-derived tumor cell lines (all *Csf-1r*-negative), or U-87 MG human glioma cells, and PDG cell tumor sphere formation is unaffected. Thus, BLZ945 has no direct effects on glioma cells, and perturbs macrophage survival through CSF-1R inhibition[1]
In Vivo	Mice are treated with BLZ945 or vehicle, and evaluated for symptom-free survival. Median survival in the vehicle-treated cohort is 5.7 weeks. In striking contrast, BLZ945 significantly improves long-term survival with 64.3% surviving to the 26-week trial endpoint. This endpoint is chosen because *Ink4a/Arf*$^{-/-}$ mice develop spontaneous tumors, including lymphomas and sarcomas, beginning at ~30 weeks. BLZ945 is well-tolerated over long-term treatment, with no visible side-effects, consistent with histopathological studies. Histological grading revealed high-grade, invasive gliomas in all vehicle-treated mice. By contrast, BLZ945-treated animals have significantly less-malignant tumors, and no detectable lesions in 55.6% of asymptomatic mice at the endpoint[1]. Mice receiving BLZ945 shows reduced CSF1R staining in both cervical tumors and the associated stroma, with a significant decrease in CSF1R^{+} stromal macrophages relative to vehicle-treated mice (P<0.05) [2]
Solvent&Solubility	***In Vitro:*** **DMSO : 125 mg/mL** (**313.69 mM;** Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.5095 mL	12.5477 mL	25.0954 mL
	5 mM	0.5019 mL	2.5095 mL	5.0191 mL
	10 mM	0.2510 mL	1.2548 mL	2.5095 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: ≥ 2.08 mg/mL (5.22 mM); Clear solution

此方案可获得 ≥ 2.08 mg/mL (5.22 mM，饱和度未知) 的澄清溶液。

	以 1 mL 工作液为例，取 100 μL 20.8 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: ≥ 2.08 mg/mL (5.22 mM); Clear solution 此方案可获得 ≥ 2.08 mg/mL (5.22 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 20.8 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: ≥ 2.08 mg/mL (5.22 mM); Clear solution 此方案可获得 ≥ 2.08 mg/mL (5.22 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 20.8 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Pyonteck SM, et al. CSF-1R inhibition alters macrophage polarization and blocks glioma progression. Nat Med. 2013 Oct;19(10):1264-72. [2]. Strachan DC, et al. CSF1R inhibition delays cervical and mammary tumor growth in murine models by attenuating the turnover of tumor-associated macrophages and enhancing infiltration by CD8+ T cells. Oncoimmunology. 2013 Dec 1;2(12):e26968.

实验参考：

Cell Assay	Cell growth rate is determined using the MTT cell proliferation kit. Briefly, cells are plated in triplicate in 96-well plates: 1×10^3 cells per well for glioma cell lines, 5×10^3 cells per well for BMDM and CRL-2467, and 2.5×10^3 cells per well for HUVEC and HBMEC cell lines. For all experiments, media is changed every 48 h. Cells are grown in the presence or absence of 6.7-6,700 nM of BLZ945, or 8 μg/mL of CSF-1R neutralizing antibody. To test the sensitivity to PDGFR inhibition, PDGC lines are cultured in the presence of 10,000 nM STI571 or 10,000 nM PTK787 (diluted from 10 mM stock solutions in DMSO). HUVEC and HBMEC cells are supplemented with ECGF supplied by the manufacturer unless otherwise indicated. Reduction of the MTT substrate is detected by colorimetric analysis using a plate reader as per the manufacturer's protocol. 10 μL of MTT labeling reagent is added to each well and then incubated for 4 h at 37°C, followed by the addition of 100 μL MTT solubilization reagent overnight. The mixture is gently resuspended and absorbance is measured at 595 nm and 750 nm on a spectraMax 340pc plate reader[1]
Animal Administration	Mice[2] Tumors are measured using calipers and volumes calculated based on the formula: volume=$(\text{width})^2\times\text{length}/2$. In MMTV-PyMT mouse studies, 56-63 d old female mice are randomized into groups based on tumor volumes and dosed with either 20% Captisol vehicle or 200 mg/kg BLZ945. Dosing is administered by oral gavage once daily and tumor volumes are measured twice weekly. 5A1 rat anti-mouse CSF1 neutralizing antibody or rat IgG control is dosed at 10 mg/kg by intraperitoneal injection every 5 d. To calculate pulmonary metastasis in MMTV-PyMT transgenic mice, formalin-fixed paraffin-embedded lungs are serially sectioned and stained with hematoxylin and eosin. Tumor regions are scored by tumor burden (total tumor area divided by total lung area), size (tumor diameter), and according to the total number of individual metastases counted in a single-blind fashion. These values are averaged across the entire depth of the lung to obtain the

	final value.
References	[1]. Pyonteck SM, et al. CSF-1R inhibition alters macrophage polarization and blocks glioma progression. Nat Med. 2013 Oct;19(10):1264-72. [2]. Strachan DC, et al. CSF1R inhibition delays cervical and mammary tumor growth in murine models by attenuating the turnover of tumor-associated macrophages and enhancing infiltration by CD8+ T cells. Oncoimmunology. 2013 Dec 1;2(12):e26968.