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产品名称：Navoximod
产品别名：GDC-0919; NLG-919

生物活性：

Description	Navoximod (GDC-0919; NLG-919) is a potent IDO (indoleamine-(2,3)-dioxygenase) pathway inhibitor with K_i/EC_{50} of 7 nM/75 nM.	
IC_{50} & Target	IDO	IDO
	7 nM (Ki)	75 nM (EC_{50})
In Vitro	Using IDO-expressing human monocyte-derived dendritic cells (DCs) in allogeneic mixed lymphocyte reaction (MLR) reactions, Navoximod (NLG919) potently blocks IDO-induced T cell suppression and restores robust T cell responses with an ED_{50}=80 nM. Similarly, using IDO-expressing mouse DCs from tumor-draining lymph nodes, Navoximod abrogates IDO-induced suppression of antigen-specific T cells (OT-I) in vitro, with ED_{50}=120 nM[1]. Navoximod inhibits the IDO activity in a concentration-dependent manner with an EC_{50} of 0.95 μM. PEG2k-Fmoc-NLG(L) is less active (EC_{50} of 3.4 μM) in inhibiting IDO compared with free Navoximod while PEG2k-Fmoc-NLG(S) is least active (EC_{50}>10 μM). Coculture of IDO+tumor cells with splenocytes isolated from BALB/c mice leads to significant inhibition of T-cell proliferation. This inhibition is significantly attenuated when the mixed cells are treated with Navoximod. PEG2k-Fmoc-NLG(L) is also active in reversing the inhibitory effect of tumour cells although slightly less potent than Navoximod [3].	
In Vivo	VNavoximod (NLG919) is orally bioavailable (F>70%); and has a favorable pharmacokinetic and toxicity profile. In mice, a single oral administration of Navoximod reduces the concentration of plasma and tissue Kyn by ~50%. In vivo, in mice bearing large established B16F10 tumors, administration of Navoximod markedly enhances the anti-tumor responses of naïve, resting pmel-1 cells to vaccination with cognate hgp100 peptide plus CpG-1826 in IFA. In this stringent established-tumor model, Navoximod plus pmel-1/vaccine produce a dramatic collapse of tumor size within 4 days of vaccination (~95% reduction in tumor volume compare to control animals receiving pmel-1/vaccine alone without Navoximod)[1]. When combined with Temozolomide (TMZ)+radiation therapy (RT), both Navoximod and 1-methyl-D-tryptophan (D-1MT, indoximod) enhance survival relative to mice treated with TMZ+RT alone[2].	

In Vitro:

DMSO : 100 mg/mL (**316.09 mM**; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	**1 mg**	**5 mg**	**10 mg**
	1 mM	3.1609 mL	15.8043 mL	31.6086 mL
	5 mM	0.6322 mL	3.1609 mL	6.3217 mL
	10 mM	0.3161 mL	1.5804 mL	3.1609 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

Solvent&Solubility	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: ≥ 3 mg/mL (9.48 mM); Clear solution 此方案可获得 ≥ 3 mg/mL (9.48 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 30.0 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: ≥ 3 mg/mL (9.48 mM); Clear solution 此方案可获得 ≥ 3 mg/mL (9.48 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 30.0 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: ≥ 3 mg/mL (9.48 mM); Clear solution 此方案可获得 ≥ 3 mg/mL (9.48 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 30.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Mario R. Mautino, et al. Abstract 491: NLG919, a novel indoleamine-2,3-dioxygenase (IDO)-pathway inhibitor drug candidate for cancer therapy. AACR 104th Annual Meeting 2013; Apr 6-10, 2013. [2]. Li M, et al. The indoleamine 2,3-dioxygenase pathway controls complement-dependent enhancement of chemo-radiation therapy against murine glioblastoma. J Immunother Cancer. 2014 Jul 7;2:21. [3]. Chen Y, et al. An immunostimulatory dual-functional nanocarrier that improves cancer immunochemotherapy. Nat Commun. 2016 Nov 7;7:13443.

实验参考：

Cell Assay	The IDO inhibitory effect of PEG2k-Fmoc-NLG is tested by an in vitro IDO assay. Briefly, HeLa cells are seeded in a 96-well plate at a cell density of 5000 cells per well and allowed to grow overnight. Recombinant human IFN-γ is then added to each well with a final concentration of 50 ng/mL. At the same time, various concentrations of PEG2k-Fmoc-NLG(L), PEG2k-Fmoc-NLG(S) or Navoximod (NLG919) (50 nM-20 μM) are added to the cells. After 48 h of incubation, 150 μL of the supernatants per well is transferred to a new 96-well plate. Seventy-five μL of 30% trichloroacetic acid is added into each well and the mixture is incubated at 50°C for 30 min to hydrolyse N-formylkynurenine to kynurenine. For colorimetric assay, supernatants are transferred to a new 96-well plate, mixed with equal volume of Ehrlich reagent (2% p-dimethylamino-benzaldehyde w/v in glacial acetic acid), and incubated for 10 min at RT. Reaction product is measured at 490 nm by a plate reader[3].
	Mice[2] Mice are anesthetized with 4% isoflurane, and the surgical plane of anesthesia is maintained with

Animal Administration	2% isoflurane in oxygen. Mice are immobilized in a stereotactic frame for tumor implantation. Briefly, the skull is shaved and exposed with a 0.5 cm skin incision. With antiseptic technique, 10^5 GL261 cells (suspended in 3 μL RPMI-1640) are injected at the following coordinates with respect to the bregma on the right side (antero-posterior, -2 mm; medio-lateral, 2 mm; dorso-ventral, 3 mm). This placement reproducibly yielded tumor growth in a paracortical area of the posterolateral right frontal lobe. Tumor-bearing mice are treated with combinations of oral DL-1MT (2 mg/mL D-1MT mixed with 2 mg/mL L-1MT) in drinking water, D-1MT (4 mg/mL) in drinking water, Navoximod (6 mg/mL) in drinking water, intraperitoneal cyclophosphamide, intraperitoneal temozolomide, and/or total-body radiation (500 cGy from a ^{137}Cs source), as detailed in figure legends. Mice are observed daily, and sacrificed when they became ill or moribund[2].
References	[1]. Mario R. Mautino, et al. Abstract 491: NLG919, a novel indoleamine-2,3-dioxygenase (IDO)-pathway inhibitor drug candidate for cancer therapy. AACR 104th Annual Meeting 2013; Apr 6-10, 2013. [2]. Li M, et al. The indoleamine 2,3-dioxygenase pathway controls complement-dependent enhancement of chemo-radiation therapy against murine glioblastoma. J Immunother Cancer. 2014 Jul 7;2:21. [3]. Chen Y, et al. An immunostimulatory dual-functional nanocarrier that improves cancer immunochemotherapy. Nat Commun. 2016 Nov 7;7:13443.