Product Data Sheet

Product Name: Afatinib(BIBW2992)
Cat. No.: GC13296

Chemical Properties

Cas. No.	439081-18-2		
Chemical Name	(E)-N-[4-(3-chloro-4-fluoroanilino)-7-[(3S)-oxolan-3-yl]oxyquinazolin-6-yl]-4-(dimethylamino)but-2-enamide		
SMILES	CN(C)CC=CC(=O)NC1=C(C=C2C(=C1)C(=NC=N2)NC3=CC(=C(C=C3)F)Cl)OC4CCOC4		
Formula	$C_{24}H_{25}ClFN_5O_3$	M.Wt	485.94
Solubility	≥ 24.3mg/mL in DMSO, ≥ 42.1 mg/mL in EtOH with ultrasonic	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 ℃ and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20℃ for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure	Afatinib(BIBW2992)		

Protocol

Cell experiment [1]:

Cell lines	Human pancreatic cancer BxPC-3 cells
Preparation Method	Cells were washed once with 5 ml of RPMI/0.5% FBS and incubated in 5ml of RPMI/0.5% FBS containing no inhibitor, Afatinib (400nM) for 24h at 37℃.
Reaction Conditions	400nM; 24h

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Applications	Afatinib inhibited more potently the proliferation of pancreatic cancer cells in vitro and inhibited the EGF-induced phosphorylation of MAPK (ERK 1/2) and Akt in BxPc-3 cells.

Animal experiment [2]:

Animal models	Mice with *Egfr* exon 19 deletion mutation
Preparation Method	Eleven-week-old transgenic mice of lung cancer models with *Egfr* exon 19 deletion mutation were treated with oral Afatinib (5mg/kg/day), gefitinib (5mg/kg/day), or vehicle alone from 11 to 15 weeks of age.
Dosage form	5mg/kg/day; oral administration; once daily for 4 weeks
Applications	Afatinib-treated mice showed significantly less tumor burden. Afatinib is more effective than gefitinib for treatment of lung cancer induced by the *Egfr* mutation in exon 19.

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References:
[1] Ioannou N, Dalgleish A G, Seddon A M, et al. Anti-tumour activity of afatinib, an irreversible ErbB family blocker, in human pancreatic tumour cells. Br J Cancer. 2011 Nov 8;105(10):1554-62.
[2] Ninomiya T, Takigawa N, Ichihara E, et al. Afatinib prolongs survival compared with gefitinib in an epidermal growth factor receptor-driven lung cancer model. Mol Cancer Ther. 2013. 12(5):589–597.

Background

Afatinib(BIBW2992) is an oral, selective inhibitor of the receptor tyrosine kinases (RTK) of the ErbB family with IC_{50} values of 0.0.5nM for EGFR kinase, 14nM for HER2 kinase, and 1nM for HER4 kinase. The substance irreversibly binds to cysteine 797 of the EGF receptor and the corresponding cysteines 805 and 803 in HER2 and HER4, respectively[1][2].

In vitro, T cells were exposed to 1μM Afatinib for 3 days and then analyzed by FCM for CD62L expression and ROS content. Compared with the negative control, Afatinib significantly increased the expression levels of CD62L and decreased the concentration of ROS. CAR-T cells were exposed to 1μM Afatinib for 3 days and then subjected to analysis or in vivo experiments. Afatinib could modulate CAR-T metabolism and differentiation, which further inhibit exhaustion and enhance persistence, and significantly improve the antitumor performance[3]. Afatinib treatment (400nM) for 24h completely blocked EGF-induced phosphorylation of EGFR, MAPK (ERK1/2) and Akt in human pancreatic cancer BxPC-3 cells. Afatinib also increased proportion of cells in subG1 phase of the cell cycle and reduced the percentage of cells in G0/G1 phase[4]. FaDu human squamous-cell carcinoma model was incubated with Afatinib (3, 30 and 300nM) for up to 9 days. Afatinib revealed a significant and dose-dependent inhibitory effect on tumor cell proliferation and increase of the G0/G1 fraction[5]

In vivo, Afatinib (20 mg/kg/day) was orally administrated as a single agent or in combination with rapamycin (2mg/kg, i.p.) in H1781 non-small cell lung cancer (NSCLC) xenograft mice. The Afatinib/rapamycin combination treatment of mice carrying large (~200mm^3) H1781 tumors resulted in significant tumor regressions in all treated animals whereas single agents delayed or

stopped tumor growth[6]. *Egfr* exon 19 mutation induced lung cancer models were treated with oral Afatinib (5 mg/kg/day),gefitinib (5 mg/kg/day), or vehicle alone from 11 to 15 weeks of age. Afatinib-treated mice showed significantly less tumor burden. Afatinib is more effective than gefitinib for treatment of lung cancer induced by the *Egfr* mutation in exon 19 because it suppresses not only pEGFR but also pHER2 and induces apoptosis over a longer period than gefitinib[7].

References:

[1] Wecker H, Waller C F. Afatinib. Recent Results Cancer Res. 2018:211:199-215.
[2] Regales L, Gong Y, Shen R, de Stanchina E, Vivanco I, Goel A et al (2009) Dual targeting ofEGFR can overcome a major drug resistance mutation in mouse models of EGFR mutant lung cancer. J Clin Invest
[3] Dai Y Y, Liu Y, An L N, et al. Afatinib boosts CAR-T cell antitumor therapeutic efficacy via metabolism and fate reprogramming. J Immunother Cancer. 2024 Nov 17;12(11):e009949.
[4] Ioannou N, Dalgleish A G, Seddon A M, et al. Anti-tumour activity of afatinib, an irreversible ErbB family blocker, in human pancreatic tumour cells. Br J Cancer. 2011 Nov 8;105(10):1554-62.
[5] Schütze C, Dörfler A, Eicheler W,et al. Combination of EGFR/HER2 tyrosine kinase inhibition by BIBW 2992 and BIBW 2669 with irradiation in FaDu human squamous cell carcinoma. Strahlenther Onkol. 2007 May;183(5):256-64.
[6] Perera S A, Li D, Shimamura T,et al. HER2YVMA drives rapid development of adenosquamous lung tumors in mice that are sensitive to BIBW2992 and rapamycin combination therapy. Proc Natl Acad Sci U S A. 2009 Jan 13;106(2):474-9.
[7] Ninomiya T, Takigawa N, Ichihara E, et al. Afatinib prolongs survival compared with gefitinib in an epidermal growth factor receptor-driven lung cancer model. Mol Cancer Ther. 2013. 12(5):589–597.

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