opnMe by Boehringer Ingelheim

4th generation EGFR^{mut} inhibitor

BI-4732

Boehringer Ingelheim

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Summary

BI-4732 is potent inhibitor against EGFR-activating mutations and on-target resistance mutations, with efficient activity in the central nervous system. It displays remarkable antitumor efficacy *in vitro* and *in vivo* as a single agent in various patient derived models with $EGFR^{C797S}$-mediated osimertinib resistance.

Chemical Structure

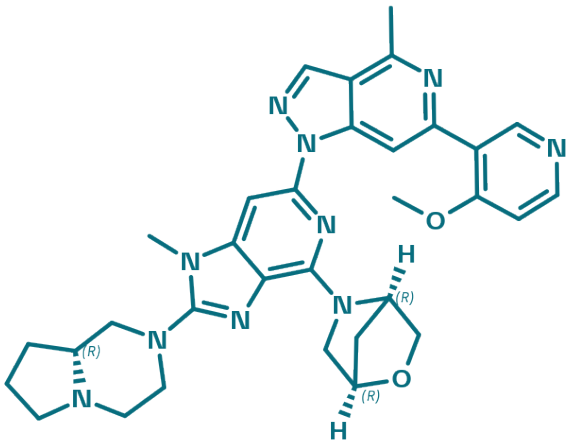

Figure 1 : 2D structure of BI-4732, a selective inhibitor of the oncogenic EGFR variants del19 and L858R in the presence or absence of the TKI resistance mutations T790M and C797S.

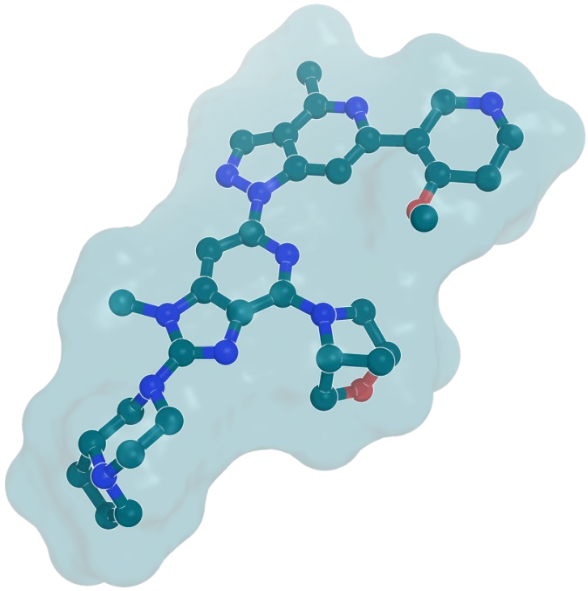

Figure 2: BI-4732, 3D conformation as observed in a model of the complex with EGFR, based on the structure of a complex of EGFR with an inhibitor that is structurally related to BI-4732.

Highlights

BI-4732 is a potent novel fourth-generation epidermal growth factor (EGFR) inhibitor, suitable for *in vitro* and *in vivo* studies. It exhibits potent antitumor effects against on-target resistant EGFR mutations, including C797S, as well as EGFR-activating mutations (E19del and L858R). BI-4732 also displays efficient blood–brain barrier penetration capabilities and robust antitumor activity within the CNS. It is closely related to BI-8128, also available on opnMe.

Target information

EGFR is a transmembrane tyrosine kinase receptor that plays a pivotal role in cellular processes such as proliferation, differentiation, and survival. Upon ligand binding it undergoes dimerization and autophosphorylation, activating downstream signalling pathways including the RAS-RAF-MEK-ERK and PI3K-AKT cascades. It is widely expressed across multiple human tissues, including skin, brain, colon, liver, and lung, with highest expression levels observed in the placenta. EGFR has also been implicated as a component of the cytokine storm which contributes to severe form SARS-CoV-2 infections. Its role in human disease and in particular cancer has been well documented. Often it is associated with specific mutations that are prognostic and sometimes also causative to treatment failure. The most common activating mutations are deletions in exon 19 (del19 or E19del) and a point mutation called L858R in exon 21[1].

These mutations are critical targets for EGFR tyrosine kinase inhibitors (TKIs), which have significantly improved clinical outcomes for patients with EGFR mutant-driven cancers. More than 50% of acquired resistance to the first-generation EGFR-TKIs is caused by the T790M mutation, a threonine to methionine substitution at amino acid position 790 in exon 20 of the EGFR gene[2-3]. This mutation hampers the binding of first-generation reversible EGFR-TKIs to the kinase ATP-binding site of EGFR, leading to drug resistance. To overcome the secondary EGFR T790M mutation, third-generation EGFR-TKIs were developed. Osimertinib is an oral, highly potent, covalent third-generation EGFR-TKI that selectively targets EGFR-activating mutations as well as T790M resistance mutation[4]. Predictably however, resistance to covalent inhibitors has also emerged, in the form of the C797S mutation, which substitutes the reactive cysteine to a serine at amino acid position 797.

BI-4732 is a recently published[5], reversible fourth-generation EGFR-TKI, which is highly potent on the primary activating EGFR variants del19 and L858R and inhibits both variants also in the presence of the acquired EGFR resistance mutations T790M and/or C797S, while sparing wild-type EGFR. The compound is suitable for *in vitro* and *in vivo* studies, being orally bioavailable and demonstrating excellent CNS penetration properties[5]. Compared to the related inhibitor BI-8128, which is also available on opnMe, BI-4732 displays slightly higher potency *in vitro*, and a longer residence time after iv dose *in vivo*.

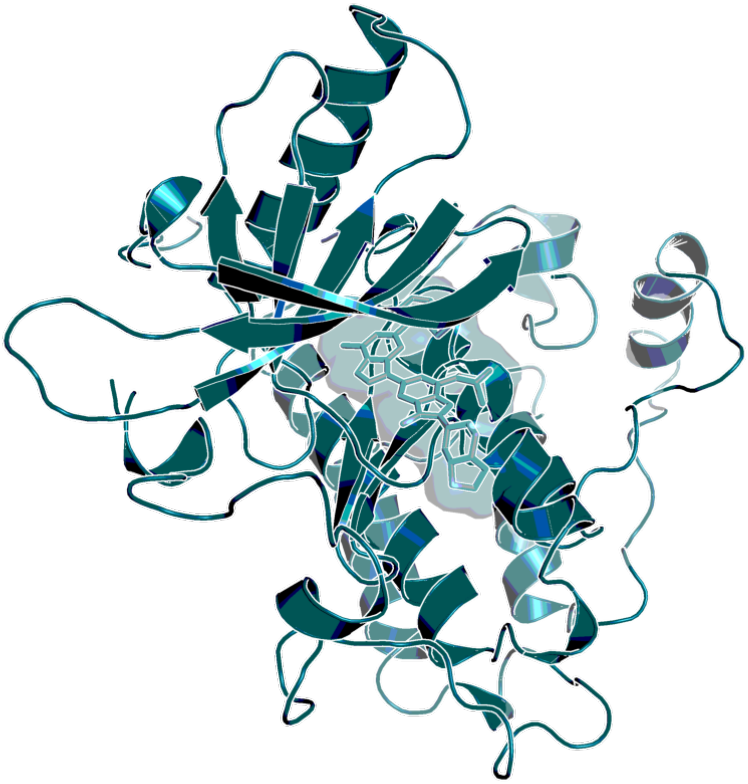

Figure 3: Model of the 3D structure of EGFR in complex with BI-4732, based on an in-house X-ray structure with a highly related inhibitor.

In vitro activity

BI-4732 was profiled for potency and selectivity in engineered Ba/F3 and solid cancer cell lines *in vitro* (see table below for data). BI-4732 demonstrated potent, low nanomolar anti-proliferative activity in Ba/F3 cells that are dependent on the activity of human EGFR variants del19, del19 T790M C797S, L858R, and L858R T790M C797S. An EGFR-independent Ba/F3 cell line and a Ba/F3 clone dependent on EGFR wild-type signaling was only inhibited at much higher concentrations, indicating low off-target cytotoxicity and sparing of EGFR wild-type activity. A parental human PC-9 NSCLC cell line (native EGFR del19 genotype) and a double resistance mutation containing engineered PC-9 T790M C797S variant (EGFR del19 T790M C797S) showed potent growth inhibition by BI-4732 in the single or low double digit nanomolar range, respectively. In line with the proliferation data, BI-4732 reduced the pharmacodynamic phospho-EGFR biomarker in the low nanomolar range in PC-9 variants with and without the resistance mutations T790M and C797S. BI-4732 inhibited A431 cell whose proliferation depends on EGFR wild-type signaling with markedly reduced potency compared to cell models harboring oncogenic EGFR variants demonstrating EGFR wild-sparing activity of the compound. The third-generation EGFR inhibitor and reference compound osimertinib

showed severely impaired or no inhibitory activity in cell models harboring the EGFR C797S mutation, while potently inhibiting EGFR del19 and EGFR L858R cell models.

It should be noted that in a combination treatment with osimertinib, BI-4732 exhibited a synergistic effect in inhibiting PC-9 C797S (EGFR del19 C797S) cell proliferation, compared to monotherapy[5].

PROBE NAME / REFERENCE COMPOUND	BI-4732	BI-8128	OSIMERTINIB
MW [Da][a]	592.7	610.7	499.6
Ba/F3 parental + IL-3 (EGFR-independent) (IC_{50}) [nM][b]	1,367	3,558	1,008
Ba/F3 EGFR wild-type + EGF ligand, proliferation, (IC_{50}) [nM][b]	356	318	56
Ba/F3 EGFR del19, proliferation, (IC_{50}) [nM][b]	1.1	1.7	0.7
Ba/F3 EGFR del19 T790M C797S, proliferation, (IC_{50}) [nM][c]	2.6	3.3	635
Ba/F3 EGFR L858R, proliferation, (IC_{50}) [nM][c]	4.6	9.6	1.6
Ba/F3 EGFR L858R T790M C797S, proliferation, (IC_{50}) [nM][c]	7.8	16	588
A549 (IC_{50}) [nM][c]	1,064	1,615	>1,000

A431 (EGFR wild-type amplification), proliferation, (IC_{50}) [nM][c]	730	492	112
PC-9 parental (EGFR del19 genotype), proliferation, (IC_{50}) [nM][c]	9	16	n.d.
PC-9 T790M C797S (EGFR del19 T790M C797S genotype), proliferation, (IC_{50}) [nM][c]	12	33	>1,000
PC-9 parental (EGFR del19 genotype), phospho-EGFR, (IC_{50}) [nM][d]	6.5	9.6	3.3
PC-9 T790M C797S (EGFR del19 T790M C797S genotype), phospho-EGFR, (IC_{50}) [nM][d]	1.2	1.4	>1,000

[a] The molecule is supplied in salt form. For the molecular weight of the salt form, please refer to the vial label
[b] Ba/F3 cell engineering and cellular proliferation assays were conducted according to the experimental protocol described in Reference 6.
[c] Solid cancer cell line engineering and cellular proliferation assays (A549, PC-9 variants and A431) were conducted according to the experimental protocol described in Reference 6.
[d] Phospho-EGFR inhibition assays were performed by treating the indicated cell lines with a concentration range of BI-4732, BI-8128 or osimertinib for 6 hours followed by cell lysis and phospho-EGFR quantification by semi-quantitative capillary western blotting.

In vitro DMPK and CMC parameters

BI-4732 displays good overall DMPK properties. Good solubility was identified at different pHs. *In vitro* hepatocyte stability data is in accordance with blood clearance data in animal models. Acceptable inhibition properties on a set of CYP-isoforms were also identified. Please note that BI-4732 may be less soluble compared to the related molecule BI-8128.

PROBE NAME	BI-4732	BI-8128
logD @ pH 11	3.6	3.4
Solubility @ pH 7 [µg/mL]	<1	101
MDCK permeability P_{appAB} @ 1 µM [10^{-6} cm/s]	9.1	Low recovery
MDCK efflux ratio	3.3	n.a.
Microsomal stability (human/mouse/rat) [% Q_H]	60 / 34 / 34	48 / <24.2 / 39
Hepatocyte stability (human/mouse/rat) [% Q_H]	27 / 45 / 40	56 / 31 / 22
Plasma Protein Binding (human/mouse/rat) [%]	98.4 / 99.3 / 98.5	97.6 / 98.1 / 96.0
hERG [inh. % @ 1 µM]	4.1	9.5
hERG (IC_{50}) [µM]	14.3	5.6
CYP 3A4 (IC_{50}) [µM]	2.7	6.1
CYP 2C8 (IC_{50}) [µM]	40	>50
CYP 2C9 (IC_{50}) [µM]	13	>50
CYP 2C19 (IC_{50}) [µM]	16	30.6
CYP 2D6 (IC_{50}) [µM]	>50	>50

In vivo DMPK parameters

For *in vivo* studies (oral administration) BI-4732 was formulated in 0.5% natrosol (acidified with HCl). For intravenous studies, the formulation included 25% HP-β-CD as well. BI-4732 shows low to moderate clearance, a medium volume of distribution and high bioavailability in animal models such as mouse and rat. Blood-brain barrier efflux assessed in rats revealed results superior brain permeability for BI-4732 ($K_{p,uu\ brain\ homogenate}$ 0.7) compared to osimertinib ($K_{p,uu\ brain\ homogenate}$ 0.21)[5].

	BI-4732		BI-8128	BI-8128
	Mouse[a]	Rat[b]	Mouse[a]	Rat[b]
Plasma Clearance [% Q_H]	20.3	47.3	29	52
Mean residence time after *i.v.* dose [h]	10.3	8.5	2.5	3.8
t_{max} [h]	3	4	2	4
C_{max} [nM]	747	252	1,200	437
F [%]	69	58	100	57
V_{ss} [L/kg]	11.3	16.5	3.9	8.4

[a] i.v. dose: 1mg/kg; *p.o.* dose: 10 mg/kg
[b] i.v. dose: 1mg/kg; *p.o.* dose: 10 mg/kg

In vivo pharmacology

The *in vivo* efficacy of BI-4732 was investigated using PC-9 cells subcutaneously implanted as xenograft models in mice. Oral administration of BI-4732 at a dose of 10 mg/kg bid and 25 mg/kg bi-daily (6 hours dosing interval between two daily doses) resulted in deep tumor regressions in a parental PC-9 xenograft model (EGFR del19 genotype) and in an engineered PC-9 T790M C797S xenograft model (EGFR del19 T790M C797S genotype) (Figure 4).

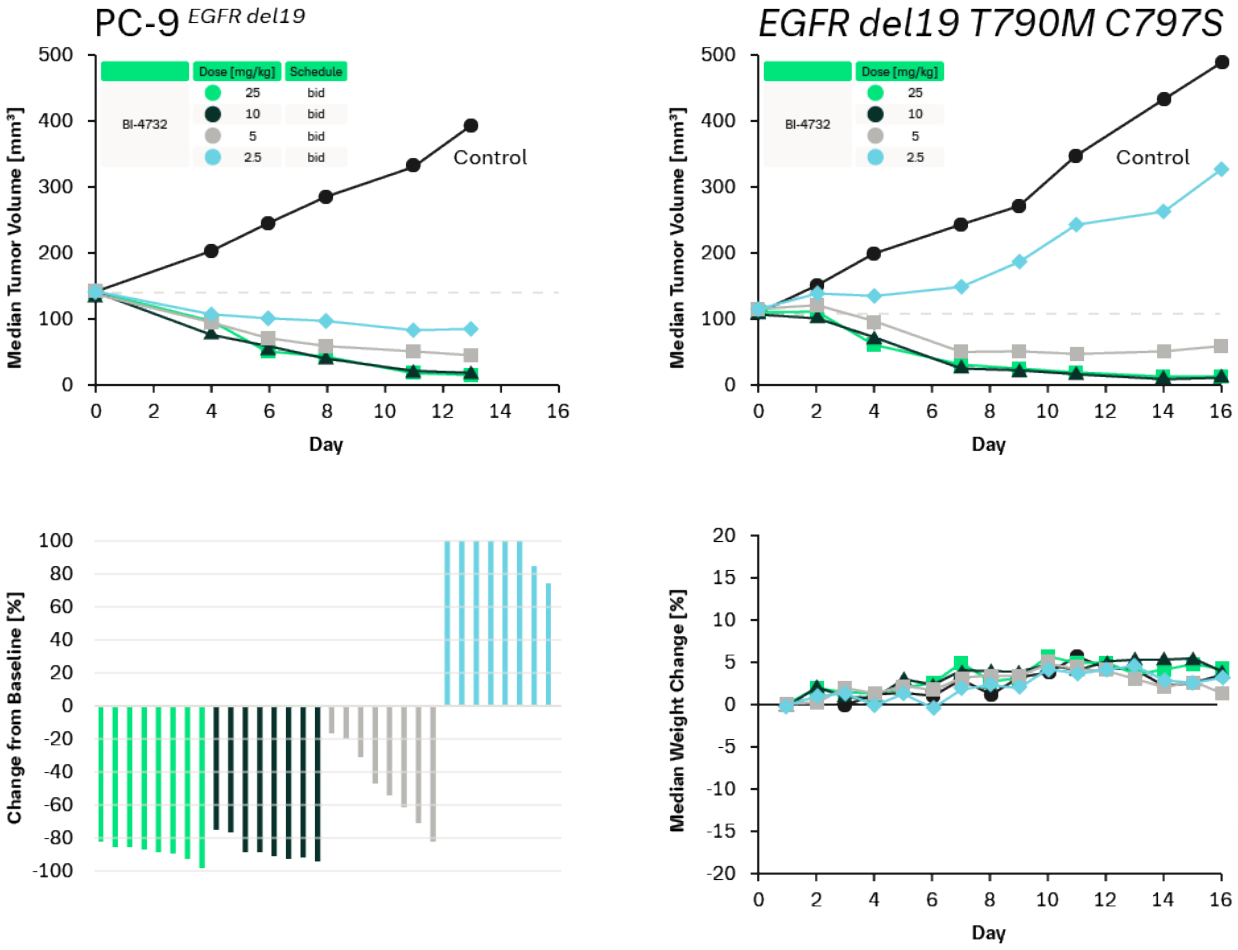

Figure 4: Oral administration of BI-4732 at a dose of 25 mg/kg bid induces tumor regressions in an isogenic set of PC-9 xenograft models with and without the resistance mutations T790M and C797S. Top panel: Median tumor volume post BI-4732 in subcutaneously implanted xenograft experiments employing an isogenic series of PC-9 NSCLC models with the indicated EGFR genotypes. Bottom panel: Waterfall plot representing the percentage of tumor volume change (left) and median body weight change (right) in mice with PC9 (EGFR del19 T790M C797S) xenografts following treatment with BI-4732.

Negative control

There is no negative control available

Selectivity

The selectivity for EGFR was assessed in the SafetyScreen44™, where BI-4732 hit 7 out of 44 kinases more than 50% at the high concentration of 10 µM. BI-4732 was further tested in a kinase panel (number of kinases: 442) and showed selectivity for 340 targets (⩽ 75% inhibition at a concentration of 1 µM). For comparison BI-8128 was tested on 205 targets in a selectivity panel and showed selectivity for 196 targets (⩽ 50% inhibition @ 10 µM). In 13 assays (PDE4D2, M1/H, ALPHA1AH, M4/H, LCK_CE, HERG, M3/H, ADENOSINETRANSPORTER, PKCALPHA(H)@CE) BI-8128 showed inhibition between 51-100% @ 10µM.

SELECTIVITY DATA AVAILABLE	BI-4732	BI-8128
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	Yes	Yes
DiscoverX®	No	No

Reference molecule(s)

The third generation EGFR inhibitor osimertinib is commercially available.

Supplementary data

2D structure files can be downloaded free of charge from opnMe.

References

1. Mok T. S., Wu Y., Thongprasert S., Yang C., Chu D., Saijo N., Sunpaweravong P., Han B., Margono B., Ichinose Y., Nishiwaki Y., Ohe Y., Yang J., Chewaskulyong B., Jiang H., Duffield E. L., Watkins C. L., Armour A. A., Fukuoka M. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med*. **2009**, 361(10):947-57. DOI: 10.1056/NEJMoa0810699, PubMed.
2. Pao W., Miller V. A., Politi K. A., Riely G. J., Somwar R., Zakowski M. F., Kris M. G., Varmus H. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med*. **2005**, 2(3):e73. DOI: 10.1371/journal.pmed.0020073, PubMed.
3. Shih J., Gow C., Yang P. EGFR mutation conferring primary resistance to gefitinib in non-small-cell lung cancer. *N Engl J Med*. **2005**, 353(2):207-8. DOI: 10.1056/NEJM200507143530217, PubMed.
4. Cross D. A. E., Ashton S. E., Ghiorghiu S., Eberlein C., Nebhan C. A., Spitzler P. J., Orme J. P., Finlay M. R. V., Ward R. A., Mellor M. J., Hughes G., Rahi A., Jacobs V. N., Red Brewer M., Ichihara E., Sun J., Jin H., Ballard P., Al-Kadhimi K., Rowlinson R., Klinowska T., Richmond G. H. P., Cantarini M., Kim D., Ranson M. R., Pao W. AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer. *Cancer Discov*. **2014**, 4(9):1046-61. DOI: 10.1158/2159-8290.CD-14-0337, PubMed.
5. Lee E. J., Oh S. Y., Lee Y. W., Kim J. Y., Kim M-J., Kim T. H., Lee J. B., Hong M. H., Lim S. M., Baum A., Woelflingseder L., Engelhardt H., Petronczki M., Solca F., Yun M. R., Cho B. C. Discovery of a Novel Potent EGFR Inhibitor Against EGFR Activating Mutations and On-Target Resistance in NSCLC *Clin Cancer Res*. **2024**, 30(8):1582-1594. DOI: 10.1158/1078-0432.CCR-23-2951, PubMed.
6. Engelhardt H., Böse D., Petronczki M., Scharn D., Bader G., Baum A., Bergner A., Chong E., Döbel S., Egger G., Engelhardt C., Ettmayer P., Fuchs J. E., Gerstberger T., Gonnella N., Grimm A., Grondal E., Haddad N., Hopfgartner B., Kousek R., Krawiec M., Kriz M., Lamarre L., Leung J., Mayer M., Patel N. D., Simov B. P., Reeves J. T., Schnitzer R., Schrenk A., Sharps B., Solca F., Stadtmüller H., Tan Z., Wunberg T., Zoephel A., McConnell D. B. Start Selective and Rigidify: The Discovery Path toward a Next Generation of EGFR Tyrosine Kinase Inhibitors. *J Med Chem*. **2019**, 62(22):10272-10293. DOI: 10.1021/acs.jmedchem.9b01169, PubMed.