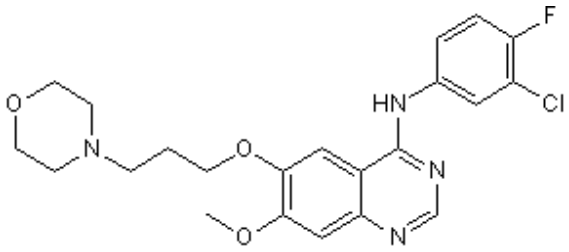
Product Name: Iressa **Cat. No. 3000**

Information on the use of Iressa (Gefitinib) for nonclinical Studies

Introduction

This information has been compiled to assist you in conducting your non-clinical programme of work using Gefitinib. Described in this booklet is a brief overview of some of the physico-chemical properties of Gefitinib together with some observations made in respect to its *in vitro* activity. This booklet also includes recommendations on how to formulate and use the compound in *in vitro e*xperiments.

Laboratory code:	Gefitinib
Physical form:	A white to yellow coloured powder
Chemical name:	4-(3-chloro-4-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy)quinazoline
Structure:	
Molecular formula:	$C_{22}H_{24}ClFN_4O_3$
Relative molecular mass:	446.9
Solubility:	Gefitinib is sparingly soluble in aqueous media but readily soluble in organic solvents e.g. DMSO
Storage	Store below 30°C.

Formulation for use *in vitro*

Gefitinib has been provided to you in the form of a powder.

For use *in vitro*:

Prepare a stock solution in dimethylsulfoxide (DMSO) at 10mM. Dilute as required in cell culture/assay medium. The stock solution may be aliquoted and stored frozen until ready for use. Repeated freeze/thawing's are not recommended.

It is strongly recommended that in order to examine selective effects on EGF/EGFR driven growth *in vitro,* investigations are conducted in the concentration of 0 to 1.0µM. At concentrations above 1.0µM, observations on the effects of Gefitinib on cell behaviour are unlikely to be related solely to the effects on the EGFR signalling pathway.

In vitro activity of Gefitinib Enzyme Inhibition

Gefitinib is a potent sub-micromolar inhibitor (IC_{50} = 0.033 µM) of EGFR TK *in vitro* (Wakeling *et al*, 2002). Activity against the related HER-family member erbB2 was 100-fold less (IC_{50} = >3.7 µM) than that against EGFR TK and, against the receptors for vascular endothelial cell growth factor, KDR (IC_{50} >3.7 µM) and c-flt (IC_{50} > 100 µM), Gefitinib had little or no activity. Gefitinib does not inhibit the activity of the serine/threonine kinases, raf, MEK-1 (>10µM) and ERK-2 (MAPK >100µM).

Cell Growth Inhibition Profile

Gefitinib is a potent and selective inhibitor of EGF-stimulated KB tumor cell growth *in vitro*. Selectivity was demonstrated by the greater than 100-fold difference in IC_{50} for cells grown in the presence (IC_{50} = 0.054 µM) or absence (IC_{50} = 8.8 µM) of EGF. Cytotoxicity was not observed at Gefitinib concentrations of < 25µM. Similarly, Gefitinib selectively inhibited EGF-stimulated growth of HUVEC cells (IC_{50} 0.03 to 0.1 µM) compared with FGF- or VEGF-stimulated growth (IC_{50} 1 to 3mM).

Gefitinib inhibits the proliferation of many cell types including ovarian, breast, colon, prostate, head & neck and lung cancer cells *in vitro*. Enhanced antitumour activity when combined with certain single agent cytotoxics, radiation, and anti-hormonal agents has been observed (Ciardiello *et al*, 2000; Huang *et al*, 2002, Williams *et al*, 2002), as well as many targeted agents.

References

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3. Huang S.M., Li, J., Armstrong, E.A. and Harari, P.M. Modulation of radiation response and tumorinduced angiogenesis after epidermal growth factor receptor inhibition by GEFITINIB (Gefitinib). Cancer Research 62(15) 4300 4306 (2002).
4. Wakeling, A.E., Guy, S.P., Woodburn, J.R., Ashton, S.E., Curry, B.J., Barker, A.J. and Gibson, K.H. GEFITINIB (Gefitinib): An Orally Active Inhibitor of Epidermal Growth Factor Signaling with Potential for Cancer Therapy Cancer Research 62: 5749-5754 (2002).
5. Williams K.J., Telfer, B.A., Stratford, I.J. and Wedge SR. GEFITINIB ('Gefitinib'), a specific oral epidermal growth factor receptor-tyrosine kinase inhibitor, potentiates radiotherapy in a human colorectal cancer xenograft model. British Journal of Cancer 86(7) 1157 - 1161 (2002).

Global info@bio-techne.com bio-techne.com/find-us/distributors TEL +1 612 379 2956
North America TEL 800 343 7475 **Europe** | **Middle East** | **Africa** TEL +44 (0)1235 529449
China info.cn@bio-techne.com TEL +86 (21) 52380373