LEXTRO
BIO SOLUTIONS

CASE STUDY
LB 002

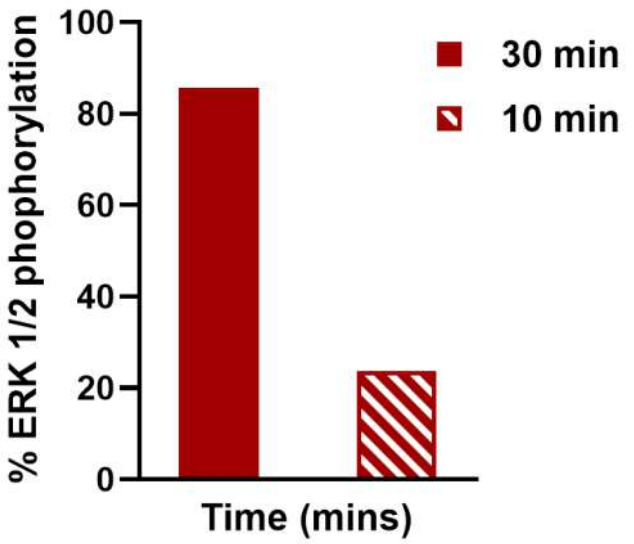
Assessment of EGFR agonist potency by quantitative phospho flow assay of ERK 1/2

Authors: Narasimha Simma, Shalini Kolarkar, Tej Madineni, Murali Addepalli

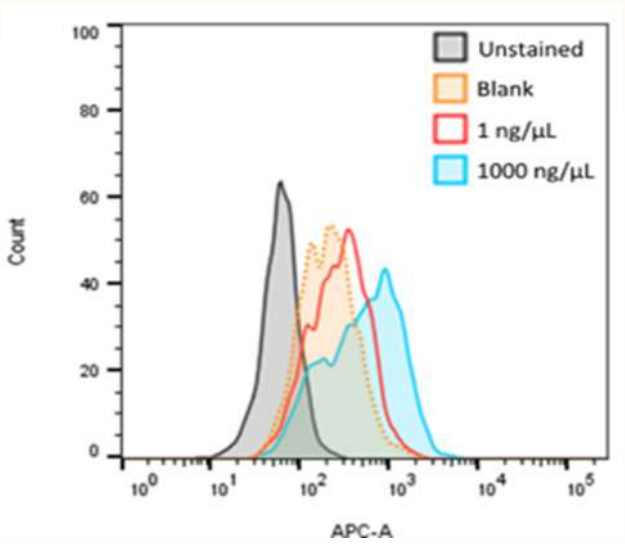
INTRODUCTION

EGFR activation leads to persistent induction of the classical MAPK pathway identified as Extracellular signal-regulated kinase-1 (ERK1) and ERK 2. ERK plays a fundamental role in the EGFR-driven control of epidermal proliferation and inflammation. Aberrant expression of EGFR in epidermal cells lead to hyper sensitivity & inflammation while in cancers induces proliferation. Thus, assessment of EGFR agonists and antagonists has significant role in therapeutics development.

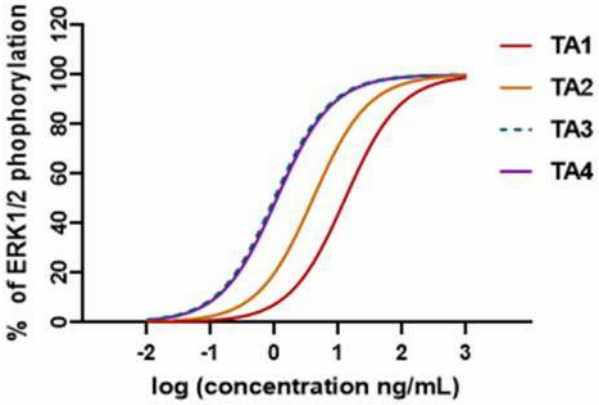
Comparative potency determination for Test Articles, TA1, TA2, TA3 & TA4 in phosphorylation of ERK 1 & 2 in A431 cells:



Serum-starved A431 cells (epidermoid carcinoma cells having constitutive expression of EGFR) were incubated for 30 and 10 min in serum free medium at 37°C, measured the basal levels of pERK1/2 (Thr 202/ Tyr 204) by flow cytometry. Observed 85% of basal pERK1/2 in 30 min as compared to 10 min.



Representative flow cytometry histogram with an overlay of dose-dependent phosphorylation of ERK in TA 1 treated A431 cells.



Dose-response curves measuring pERK in A431 cells treated with TA1-4.

Test Article	TA1	TA2	TA3	TA4
EC_{50*}	12.72	3.99	0.99	1.08

*Determined by GraphPad prism ver. 9.5.0.

CONCLUSION

Application of flow cytometric analysis has identified TA3 is the most potent (EC50=0.9861) while TA1 is the least potent (EC50= 12.72) in phosphorylating ERK.

WHY LEXTRO

Scientists at Lextro Bio Solutions have extensive experience in working with kinase agonists and antagonists where the potencies are determined as EC50 and IC50 respectively in cell-based assays. Our cell-based kinase profile assays include both 2D as well as 3D spheroids (MCTS), Organoids, Tissue samples etc., using cell lines or patient-derived primary tumours / skin tissues.

Tools employed for kinase profiling include-

- Flow cytometry
- ELISA
- Western Blot
- Antibody arrays

Lextro Bio Solutions Pvt. Ltd.
#189, Synergy Square 1 Building, 2nd Floor, Genome Valley, Hyderabad-500 078, India
www.lextrobio.com | contact@lextrobio.com