Examining the efficacy of a novel small molecule inhibitor of EGFR and ERBB2

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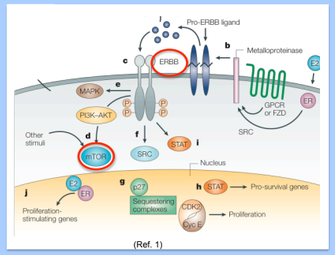
Introduction

Growth factor receptors allow cells to receive growth signals from the extracellular environment, in the form of proteins such as Epidermal Growth Factor (EGF). EGFR and ERBB2 are growth factor receptor tyrosine kinases. In the presence of EGF, EGFR and ERBB2 can heterodimerize to promote cell proliferation and survival. Mutations in the genes encoding EGFR and ERBB2 are found in lung cancer. A subset of these mutations are insertion mutations that have been shown to have oncogenic properties in cultured cells.

The main goal of this project is to test the efficacy of a new drug that targets the ERBB2 pathway. We aim to compare its efficacy to that of two other drugs already on the market and one other drug that is currently in clinical trials.

The EGFR/ERBB2 signal transduction pathway



(Ref. 1)

Small molecule inhibitors that target the ERBB2 pathway have been synthesized, but their clinical efficacy remains elusive. The compound of focus in our studies is DSI-1, a small molecule which is a dual-specificity EGFR and ERBB2 inhibitor. We aim to test how effective this drug is in the laboratory before it undergoes clinical testing. Our studies compare this drug to three other drugs: Erlotinib, Rapamycin, and DSI-2, a compound similar to DSI-1. DSI-2 has been shown to be effective in the laboratory but there are implications that it has not been effective in the clinic.

We are testing the efficacy of DSI-1 and other small molecule inhibitors on hematopoietic Ba/F3 cells expressing several oncogenic ERBB2 mutants. Ba/F3 cells are normally dependent on a cytokine named interleukin-3 (IL-3) for survival and proliferation. However, expression of oncogenes such as the ERBB2 mutants found in lung cancer supports proliferation of the cells in the absence of IL-3. We are exposing these transformed Ba/F3 cells to the inhibitors and assessing the effects on cell survival in the absence of IL-3.

Oncogenic ERBB2 mutations in the kinase domain

Portion of wild-type ERBB2

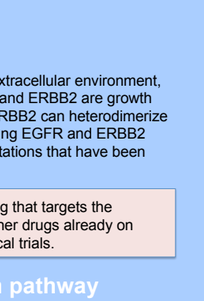
	-1	Ile	Leu	Asp	Glu	Ala	Tyr	Val	Met	Ala	Gly	Val	Gly	Ser	Pro	Tyr	Val	Ser	Arg	Leu	Leu	Gly
2301		CTTAGACGAA	GCATACGTGA	TGGCTGGTGT	GGGCTCCCCA	TATGTCTCCC	GCCTTCTGGG	C
		GAATCTGCTT	CGTATGCACT	ACCGACCACA	CCCGAGGGGT	ATACAGAGGG	CGGAAGACCC	G

Portion of ERBB2 Insertion A775_G776 ins YVMA ("Ins 1")

	-1	Ile	Leu	Asp	Glu	Ala	Tyr	Val	Met	Ala	Tyr	Val	Met	Ala	Gly	Val	Gly	Ser	Pro	Tyr	Val	Ser	Arg	Leu	Leu	Gly
2301		CTTAGACGAA	GCATACGTGA	TGGCATATGT	CATGGCTGGT	GTGGGCTCCC	CATATGTCTC	CGCCTTCTG	GGC
		GAATCTGCTT	CGTATGCACT	ACCGTATACA	GTACCGACCA	CACCCGAGGG	GTATACAGAG	GGCGGAAGAC	CCG

Portion of ERBB2 Insertion A775_G776 ins V,G776C ("Ins 2")

	-1	Ile	Leu	Asp	Glu	Ala	Tyr	Val	Met	Ala	Val	Cys	Val	Gly	Ser	Pro	Tyr	Val	Ser	Arg	Leu	Leu	Gly
2301		CTTAGACGAA	GCATACGTGA	TGGCTGTTTG	TGTGGGCTCC	CCATATGTCT	CCCGCCTTCT	GGGC
		GAATCTGCTT	CGTATGCACT	ACCGACAAAC	ACACCCGAGG	GGTATACAGA	GGGCGGAAGA	CCCG

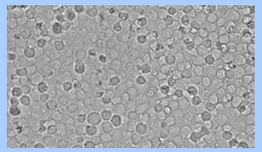


(Ref. 2)

The figure shown above is a structure of the EGFR kinase domain, which is similar to the ERBB2 kinase domain. The pink region is the area in which amino acids are inserted in the ERBB2 mutant proteins.

Materials and Methods

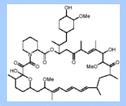
Cell Lines Used



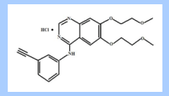
All cell lines used were Ba/F3 hematopoietic cells. Each cell line used overexpresses one of the following ERBB2 constructs:

– ERBB2 Ins 1
– ERBB2 Ins 2
– wild-type ERBB2

Chemical Compounds Used

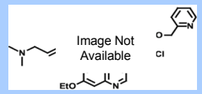


Rapamycin, a molecule that inhibits a downstream signaling protein named mTOR



Erlotinib, an EGFR inhibitor

DSI-1, a dual specificity inhibitor of EGFR and ERBB2



DSI-2, a dual specificity inhibitor of EGFR and ERBB2

Procedural Outline

– Split Ba/F3 cells and grow in IL-3+ media until confluent

http://www.wwmponline.com/cart/images/T/35310-02.jpg

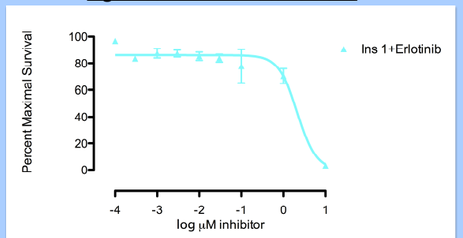
– Incubate cells for 12 hours
– Take cells to Coulter Counter to determine number of viable cells per mL
– Plate appropriate number of cells and grow in media lacking IL-3
– Expose cells to different concentrations of drugs (Rapamycin, Erlotinib, DSI-1, or DSI-2) for 48 hrs

– Perform WST assay by adding reagent, incubating, and reading luminescence in Molecular Devices Spectramax 190
– Analyze WST assay results to determine number of viable cells present under each experimental condition

http://www.bucher.ch/en/products/Molecular-Devices/

Results and Conclusions

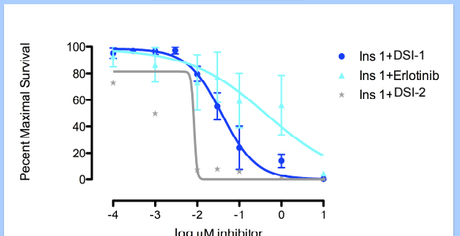
Confirming the Poor Efficacy of Erlotinib Against ERBB2 Mutant Cells



	Ins 1+Erlotinib
IC50	2.143

Conclusion: These data show that our assay is working as expected, with Erlotinib inhibiting cell proliferation only at high concentrations.

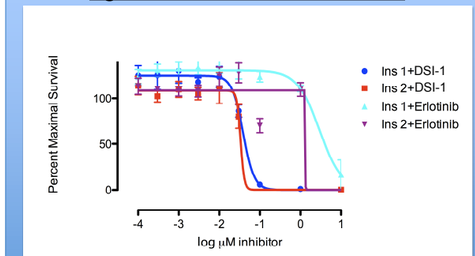
Comparing Efficacies of Erlotinib, DSI-1 and DSI-2



	Ins 1+DSI-1	Ins 1+Erlotinib	Ins 1+DSI-2
IC50	0.03858	0.4340	~ 0.008403

Conclusion: DSI-1 is more effective than Erlotinib, a currently used treatment. DSI-1 may not be quite as effective as DSI-2, a compound currently undergoing clinical testing.

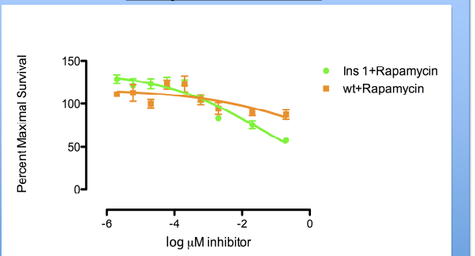
Efficacy of DSI-1 and Erlotinib Against Different ERBB2 Mutants



	Ins 1+DSI-1	Ins 2+DSI-1	Ins 1+Erlotinib	Ins 2+Erlotinib
IC50	0.03861	~ 0.03348	2.962	~ 1.325

Conclusion: DSI-1 is much more effective than Erlotinib in preventing cell proliferation, regardless of the ERBB2 mutant genotype of the cells.

Testing the Efficacy of a Downstream Component Inhibitor



Conclusion: Rapamycin did not seem to be very effective in preventing cell growth. Efficacy of Rapamycin may require higher concentrations or combination with other therapeutics.

References

1. Hynes and Lane. (2005) *ERBB receptors and cancer: the complexity of targeted inhibitors*, Nature Reviews, **5**, 341-353.

2. Stephens et al. (2004) *Intragenic ERBB2 kinase mutations in tumours*, Nature, **431**, 525-526

Acknowledgments

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