UNIVERSITY of HOUSTON
BIOLOGY AND BIOCHEMISTRY

"To examine the combinatorial therapeutic effects of EGFR inhibitor and mTORC2 inhibitor for treatment of pancreatic cancer"

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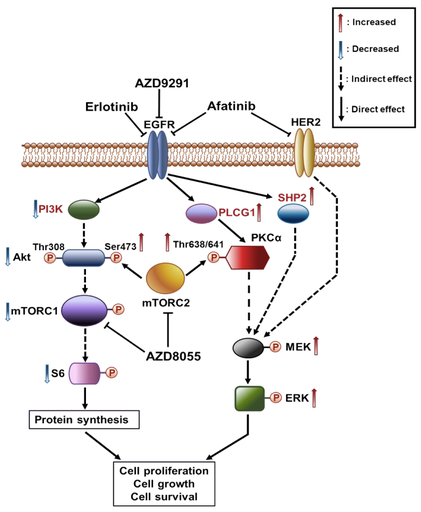
Background

Statistics for pancreatic cancer deaths

Estimated Deaths in 2019	45,750
% of All Cancer Deaths	7.5%



Problem

- Pancreatic cancer is one of the most lethal cancer types that is associated with low survival rate and late diagnosis.
- Despite the highly progressive and aggressive nature of the disease, limited treatment options available for pancreatic cancer.
- Treating pancreatic cancer with a more molecularly targeted, precise approach based on the relevant signaling pathway and protein activities holds great potential to be clinically beneficial to the patients.

Objectives

- Treat cancer cells with a combination of mTORC2 and EGFR inhibitors.
- Observe the cell proliferation using MTT assay and confirm the results with western blot analysis.
- Determine the synergistic effect of drug combination for therapeutic purposes .

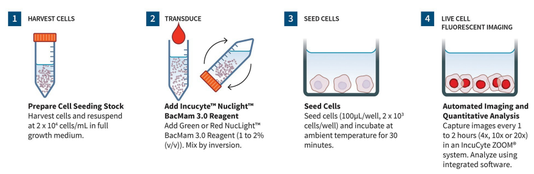
Materials and Methods

Cell Culture and addition of inhibitor

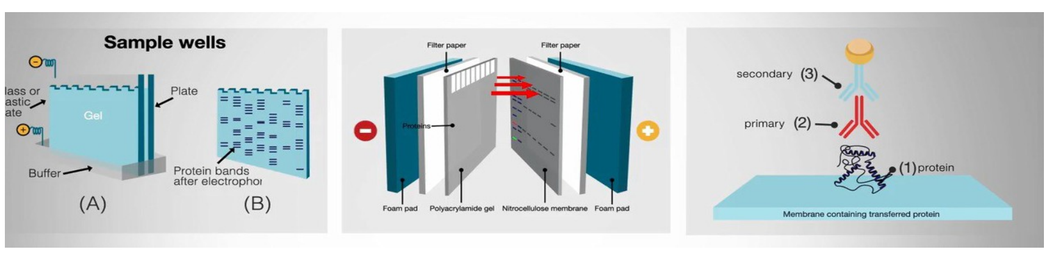
- BxPC-3 cells were seeded into a 96-well culture plate (8,000 cells in 100 µL of media per well) and allowed to grow overnight.
- The next day, the media was removed and fresh media containing the inhibitor of interest was added to each well at defined concentrations.
- DMSO and media without inhibitor (no drug) were used as the controls.

MTT assay

- After 48 hours of incubation, MTT agent (Thiazolyl Blue Tetrazolium Bromide) was added and incubated for 3.5 hours
- Absorbance was measured spectrophotometrically at 570 nm and 620 nM wavelengths using the Molecular Devices SpectraMax Plus 384 Microplate Reader.



Western Blot

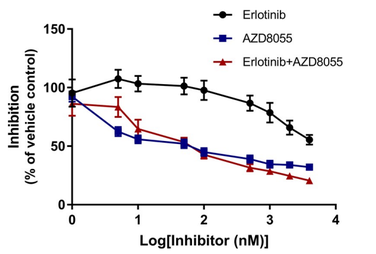


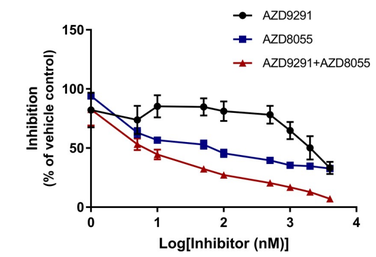
1. Separate proteins by gel electrophoresis
2. Transfer proteins from the gel to a solid support
3. Detect Where we use antibodies specific to the target protein to visualize the protein of interest.

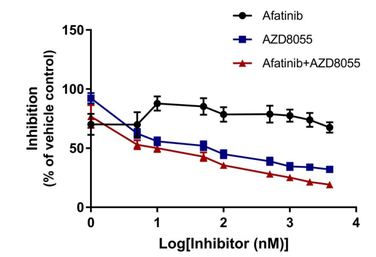
We used the conventional western blot to validate the endogenous protein expression in different EGFRi treatments. Such validation methods are specific and reliable but suffer from being low-throughput and time consuming.

Our future studies therefore aim to improve our peptide library design by utilizing other high-throughput proteomic technology such as mass spectrometry to experimentally validate all those known peptide-protein interactions of interest.

Results







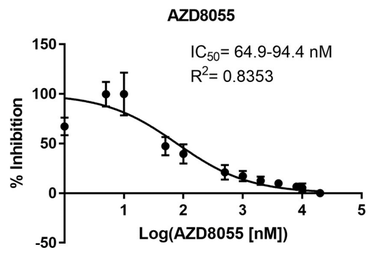


Figure. The data shown above is obtained from MTT assay. It is presented here as normalized %inhibition versus log (concentration [nM]).

IC50 values and SDs were acquired by fitting the data to a sigmoidal curve. GraphPad Prism v.7 software was used to obtain the data curve.

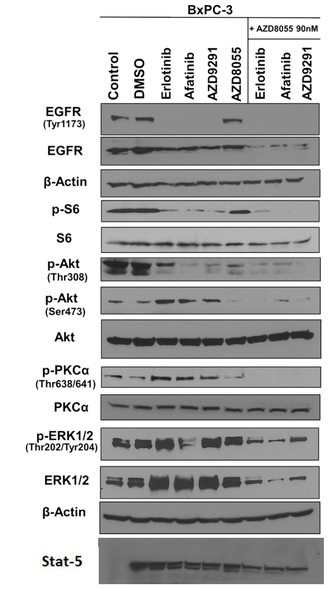


Figure. Western Blot data collection for expression of various proteins. Suppression of EGFR/Akt/mTORC1 and mTORC2/PKCα/ERK pathways can be observed with various

Range of combination index	Description
<0.1	Very strong synergism
0.1-0.3	Strong synergism
0.3-0.7	Synergism
0.7-0.85	Moderate synergism
0.85-0.9	Slight synergism
0.9-1.1	Nearly additive
1.1-1.2	Slight antagonism
1.2-1.45	Moderate antagonism
1.45-3.3	Antagonism
3.3-10	Strong antagonism
>10	Very strong antagonism

Total Dose [AZD9291+AZD8055]	Effect	CI Value
5.0	0.579	0.07522
10.0	0.603	0.07375
50.0	0.740	0.02036
100.0	0.755	0.03347
500.0	0.826	0.06678
1000.0	0.850	0.09366
2000.0	0.885	0.10187
4000.0	0.934	0.06077
8000.0	0.979	0.01140
10000.0	0.975	0.02033

Total Dose [Afatinib + AZD8055] nM	Effect	CI Value
5.0	0.581	0.06316
10.0	0.554	0.30448
50.0	0.656	0.04944
100.0	0.681	0.04011
500.0	0.761	0.00821
1000.0	0.775	0.00875
2000.0	0.807	0.00372
4000.0	0.823	0.00318
8000.0	0.848	0.00148
10000.0	0.855	0.00119

Total Dose [Erlotinib+AZD8055]	Fa	CI Value
5.0	0.338	185.990
10.0	0.421	22.0276
50.0	0.570	0.90654
100.0	0.616	0.39534
500.0	0.730	0.03159
1000.0	0.745	0.03462
2000.0	0.779	0.01672
4000.0	0.810	0.00873
8000.0	0.840	0.00485
10000.0	0.852	0.00372

Figure. Drug synergy was determined by calculating the combination index (CI) and fraction affected (Fa) at different concentrations using the Chou and Talalay method and CompuSyn software.

Conclusion

MTT cell proliferation data represented with the Q curve shows a significant decrease in cell viability when treated with the combination of EGFR and mTORC1/mTORC2 inhibitors. The EGFR inhibitors cause a reduced downstream regulation of PI3K/Akt/mTOR signaling by inhibiting the EGFR phosphorylation. Afatinib is shows inhibitions of EGFR and HER2. Erlotinib and AZD9291 only inhibit EGFR. AZD8055 (mTORC1/mTORC2 inhibitor) that prevents the growth pancreatic cancer cells in combination with EGFR inhibitors (Erlotinib, Afatinib, AZD9291). Combinatorial treatment with EGFRi (Erlotinib, Afatinib, or AZD9291) and mTORi (AZD8055) improved anti-proliferative effects on BxPC-3 cell as compared to EGFRi treatment alone. This combination is shown to be synergetic by Chou and Talalay method, showing high synergetic drug combination at and above IC50.

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