In Vitro Model to Test Efficacy of Anti-Cancer Drug Candidates and their Synergistic Effects

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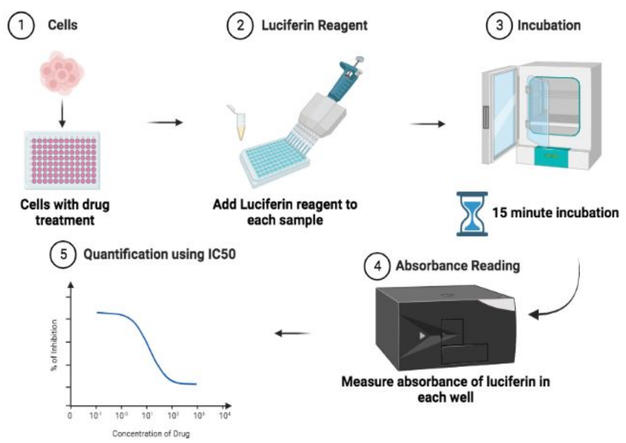
Abstract

- Anti-Cancer Drugs require many steps of testing before they are introduced to the general public including *in vitro*, then *in vivo* testing, and then finally in clinical trials before being approved. In this work, we use an *in vitro* CellTiter-Glo assay to **determine the effectiveness of the anti-cancer drugs** by finding their half-maximal inhibitory concentration.
- In addition to be used as therapeutic agents, drugs are also useful tools to manipulate the function of certain genes and study their biology. The basic hypothesis is if two drugs have synergistic effects on certain phenotype, they are likely to work in a convergent pathway. Here, we test the synergy of proteasome inhibitor and several drugs that target regulators of transcription to **find which part of transcription proteasomes might participate in**.

CellTiter-Glo Luminescent Cell Viability Assay

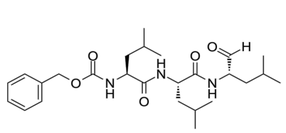


CellTiter-Glo (CTG) Viability Assay:

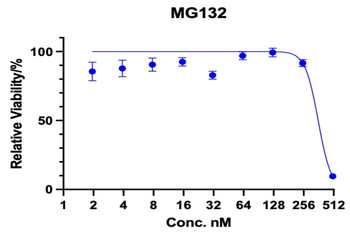
- CTG emits luminescence upon reaction with ATP present in cells
- Luminescence is linearly correlated with the number of cells
- Luninescence is quantified by Tecan Infinite 200 plate reader and half-maximal inhibitory concentration (IC50) is defined by fitting to a Hill function curve

Testing Anti-Cancer Efficacy of Drugs

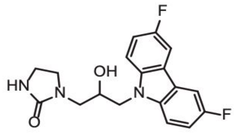
We use two drugs to target the circadian rhythm network on a breast cancer cell line: MDA-MB-231. The goal of this experiment is to determine at what concentration of drugs would inhibit the growth of 50% of cells.



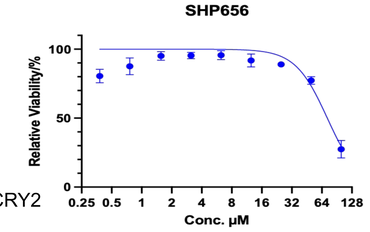
MG132: inhibitor of the 20S-proteasome



IC50: 357.6 nM



SHP656: stabilizer of the circadian gene CRY2



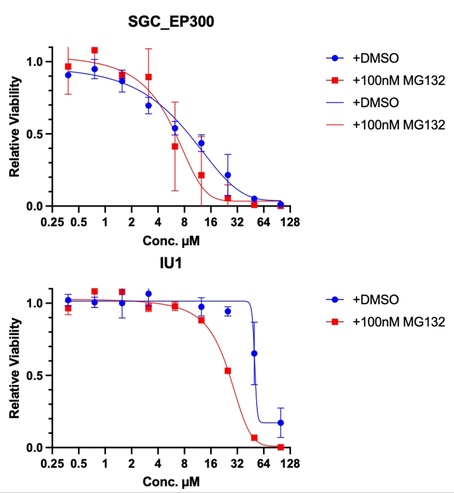
IC50: 72.52 μM

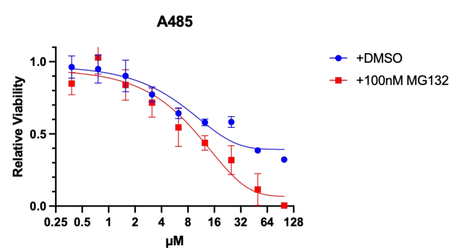
Healthy Growing Cells

Dead Cells upon drug treatment

Using Drugs as Tools for Biological Discovery

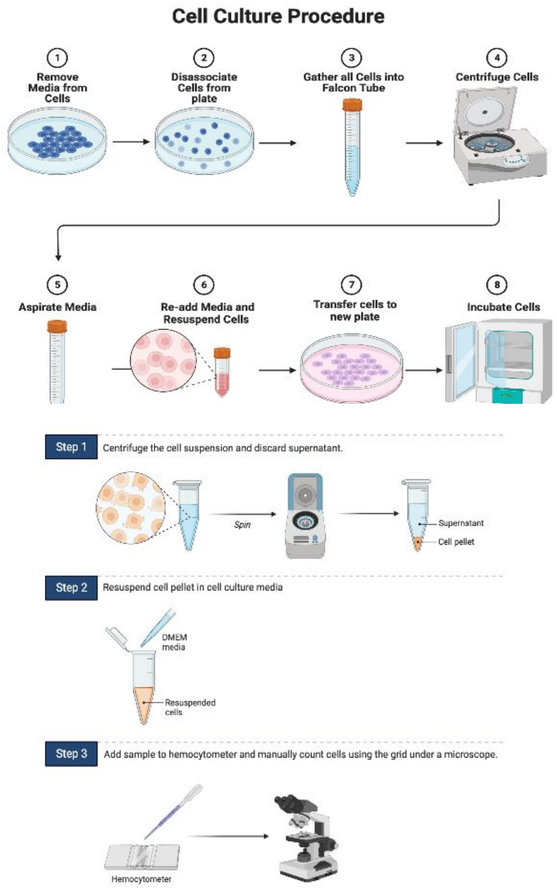
- By using drugs of known targets, we can determine which regulatory pathways work towards the same physiological processes.
- We use three drugs to test which pathways the 20S-proteasome is collaborating with.





- **SGC-CBP300**: inhibitor of the bromodomain of CREB-binding protein
- **A485**: inhibitor of the histone acetyltransferase subunit of EP300
- **IU1**: inhibitor of the ubiquitin-specific protease 14 (USP14)

Important Techniques



Summary

- MG132 can inhibit breast cancer cell growth by 50% at 357.6 nM, which is within clinical-relevant range.
- CRY2 inhibitor SHP656 as a single agent can only inhibit breast cancer cell growth at high concentration (>50 μM).
- By testing synergistic effect of drugs of known targets, we found that USP14 and EP300 might work in the same pathway in regulating protein homeostasis in cancer cells.

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