# Genome wide RNAi screening to uncover sensitivities to GSK-3 inhibition

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## Abstract

Glycogen Synthase Kinase-3, or GSK-3 is a protein crucial for numerous cellular processes, including apoptosis and cellular signaling. Moreover, GSK-3 plays an important role in tumorigenesis, thereby emphasizing the need for further understanding of GSK-3, GSK-3 inhibition and the effects of GSK-3 inhibition on cellular progression. As 35% of the human kinome interact with GSK-3, inhibition of GSK-3 is thought to be detrimental to cells, as GSK-3 inhibition causes an inability for various cellular processes, which can be lethal. Therefore, sensitivities to GSK-3 inhibition were determined through the use of a genome-wide RNAi screen, which coupled the knockdown or upregulation of various genes with GSK-3 inhibition. A kinome screen will be performed with 467 genes as a precursor to the genome wide screen, with 1500 cells/well as the optimal cell density and 0.15 ul as the optimal lipid concentration. The most efficacious and potent drug as determined using an $IC_{50}$ was NCGC00261619-01 and will be used in the kinome screen. If the kinome screen is robust, a genome wide screen will follow. Determining the effects of gene knockdown on GSK-3 inhibition through the use of advancements in the understanding of the human genome allows for the development of drugs that will be able to antagonize GSK-3 inhibition, which can play a large role in tumorigenesis, and therefore can lead to the development of novel treatments in the clinical setting.

## Introduction

Glycogen Synthase Kinase-3, or commonly known as GSK-3, is an enzyme with a plethora of associations with numerous biological processes, including apoptosis, cell signaling, and gene transcription, among others. Specifically, GSK-3 has been shown to play an immense role in tumorigenesis but can also be involved negative control of tumor growth.

GSK-3 is expressed in all human tissues, and normally functions through the phosphorylation of serine-threonine residues on substrates. Due to both the abundance of GSK-3 and its function as the keystone of a diverse set of cellular processes, determining the sensitivities associated with the inhibition of this kinase will enable both the salutary and adverse effects of GSK-3 overexpression and lack of GSK-3 to be uncovered. GSK-3 is estimated to interact with 35% of the human kinome, therefore the effect of GSK-3 inhibition could be have a large magnitude, due to the many other kinases GSK-3 interacts with may also be affected (Thorne et al. 2015).

In addition, RNAi screening, which has exponentially grown over the past 10 years, can assist in the development of genome-wide screens. A genome wide RNAi screen allows for the analysis of the inhibition or knockdown of multiple genes, and the effects of gene knockdown (Sharma and Rao, 2009). A genome wide screen of GSK-3 beta inhibition would determine whether various gene knockdowns coupled with GSK-3 beta inhibition using small molecules have a synergistic or antagonist effect on cell viability. As a whole, the analysis of the effects of GSK-3 inhibition genome-wide can be facilitated by a high-throughput RNAi screen, which would use developments in the understanding of the human genome to provide insight into GSK-3 inhibition for future developments in the clinical aspects of cancer treatment.

## Methods

**Tissue Culture:** Human High Grade Serous Ovarian cancer cells were cultured in a 225 cm2 flask (Corning, Corning, New York, USA) with media containing McCoy's 5A (ATCC, Manassas, Virginia, USA), and 10% FBS (Sigma-Aldrich, St. Louis, Missouri, USA). The cells were incubated at 37˚C and 5% CO2.

**Cell Density:** Cells were placed into a white, opaque bottom 384-well plate (Corning, Corning, New York, USA), and grown in 40 microliters of media. 16 columns were used, with every two columns containing a certain cell count. The cell counts used were 500, 750, 1000, 1500, 2000, 2500, 3000, and 4000 cells/well. After microscopic analysis, the most optimal cell count for lipid optimization was determined to be 1500 cells/well.

**Lipid Optimization**: 20 columns totaling 320 wells were used of a white, opaque bottom 384-well plate, with each column designated a Lipofectamine RNAiMax concentration (Invitrogen, Carlsbad, California, USA). Columns 1-2 and 18-20 were designated to only have cells, and the remaining columns were split into 5 sections of three columns, with the 5 sections being 0.02 ul, 0.05 ul, 0.1 ul, 0.15 ul, and 0.2 ul. Each column within a section was given one of three siRNA complexes to be transfected, the negative control, PLK1 inhibitor, and "killer". 1500 cells were then added to each well as 1500 cells/well was the determined cell count from the cell density optimization procedures. The cells were then incubated and afterwards, CellTiter-Glo (Promega, Madison, Wisconsin, USA) was used to indicate cell viability and the most optimal lipid concentration.

## GSK-3 inhibitors

| Sample ID | Sample Name | SMILES |
|---|---|---|
| NCGC00025233-04 | SB-415286 | C=1C=CC(=C(C1)C2=C(C(=O)NC2=O)NC=3C=CC(=C(C3)Cl)O)[N+](=O)[O-] |
| NCGC00033879-01 | Bikinin | OC(=O)CCC1=O)NC1=CC=C(Br)C=N1 |
| NCGC00250379-10 | CHIR99021 | CC1=C[N+](C(=N1)C2=C(N=C(NCCNC3=CC=C(C=N3)C#N)N=C2)C4=CC=C(C)l)C=C4Cl |
| NCGC00261619-01 | SB 216763 | CN1C=C(C2=CC=CC=C12)C3=C(C(=O)N(C3=O)C4=C(Cl)C=C(C=C1)C=C4 |
| NCGC00345024-01 | GSK3 inhibitor | O=C/1N(Cl\C=C)C(=O)C(=C1C2=CN=C3C=CC=C[N]23)/C4=C[N]5CCN(CC6=C5C4=CC=C6)C(=O)N7CCOCC7 |
| NCGC00346638-04 | TWS 119 | NC1=CC(=CC=C1)C2=CC3=C([NH]2)N=CN=C3OC4=CC=CC(=C4)O |

**Figure 1. GSK-3 inhibitors tested**

## Dose Responses in SKOV-3

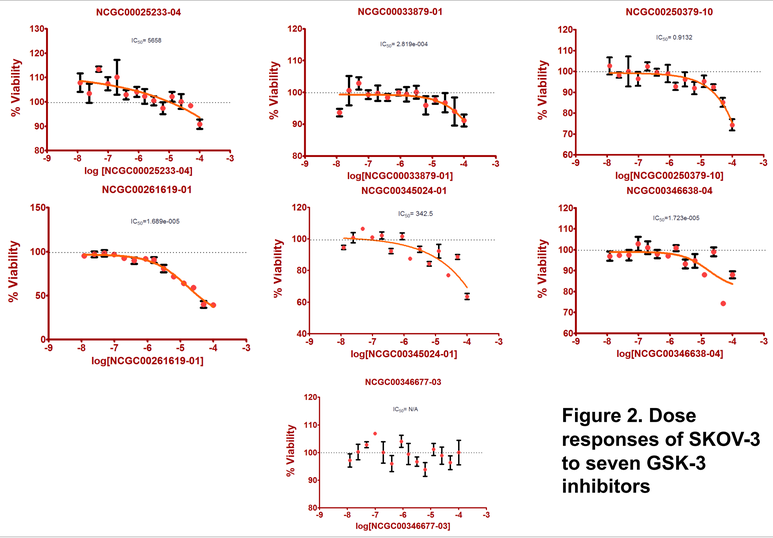
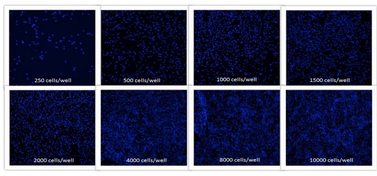


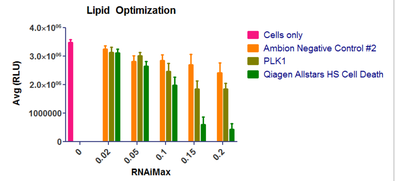

**Figure 2. Dose responses of SKOV-3 to seven GSK-3 inhibitors**

## Cell Number and Lipid Optimization

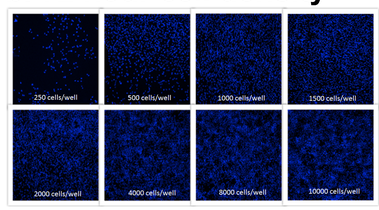
### a. 48 hour cell density




### c. Lipid Optimization




### b. 72 hour cell density




### d. Nuclei/CTG count

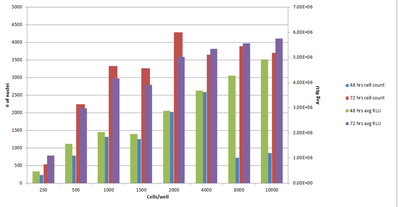



**Figure 3. Cell Number and Lipid Optimization Results**

## Results and Conclusion

- The cell density and lipid concentration were optimized for SKOV-3 cells for the primary genome wide screen on GSK-3 sensitivities in SKOV3 cells.
- A genome-wide screen can only be performed if the kinome screen is robust, as a high reproducibility of the screen on the 467 genes in the human kinome will justify a genome wide screen to uncover sensitivities to GSK-3 inhibition

## Pilot Screens

**Kinome Screen**

- 467 genes in the human kinome with 3 siRNAs against each gene
- Each kinome will be run with and without the GSK-3β inhibitor
- Hits will be chosen depending on whether they are more than two standard deviations from the median, using negative normalized data that is normalized to the median of the negative control

**Genome-wide screen**

- **Genome wide screens will be initiated after robustness and reproducibility has been determined by replicate kinome screens**
- Around 20000 genes will be used
- Off-target effects will be determined by the Haystack analysis to ensure the effect of the siRNA KD is against its intended target

## Optimization Results

- The most optimal cell density as determined through imaging and subsequent analysis was 1500 cells/well
- The most optimal lipid concentration, using the 1500 cells/well concentration was 0.15 μl, as it caused the most cell death with PLK1 when compared to the cell death caused by Qiagen Allstars HS Cell Death and Ambion Negative Control #2
- NCGC00261619-01 was chosen as the drug for the genome wide screen due to the potent yet efficacious nature of the drug when compared to the other drug candidates through an $IC_{50}$