Cancer cell line profiling of an indirect Myc modulator

Mayank NK Choudhary[1], Francisco Caballero[2], Angela N. Koehler[2]

[1]Department of Biotechnology, Indian Institute of Technology Madras, India

[2]Chemical Biology Program, Broad Institute of MIT & Harvard, Cambridge, MA

INTRODUCTION

The Myc transcription factor is a master regulator, modulating up to a third of the transcriptome. One of the most common oncogenic events in human malignancies, is c-Myc dysregulation. The oncoprotein is aberrantly active in a wide varieties of human cancers and is also a driver of the malignant phenotype. c-Myc dysregulation, which may occur through several mechanisms, may result in uncontrolled cell proliferation, alterations in the apoptotic pathway, genomic instability, escape from immune surveillance, growth factor independence, and immortalization. These different cellular fates are determined, in part, by c-Myc protein expression levels, which are regulated through several transcriptional and post-transcriptional pathways.

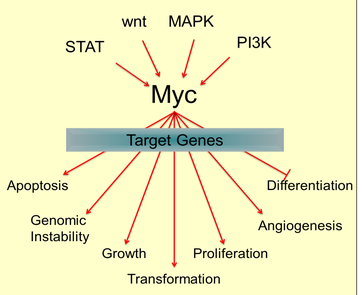


Fig 1: The very many functions of Myc

Cancer cell lines were also profiled to assess their viability against BRD4132. The goal of this study is to help us gain insights on the effects that certain genomic aberrations impart on human cancers, thereby making them sensitive to certain molecules. We specifically study a Human Burkitt's Lymphoma cell line, Namalwa, wherein enhancers that normally drive constitutive expression of immunoglobin genes now lead to overexpression of *MYC* proto-oncogene, in lymphoma cells.

Myc besides being a formidable therapeutic target, also has an essential function in normal physiology, thus creating the need for strategies specifically targeting cancer milieu. Inactivation of c-Myc driven transcription in tumour cells, using a small molecule may result in an effective approach to developing candidates for cancer therapeutics and clarifying the potential of Myc as a therapeutic target.

FOREGROUND & AIMS

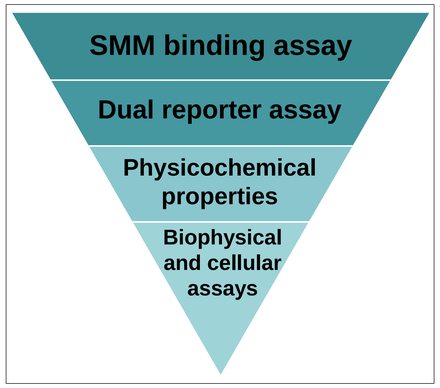


Fig 2: **The road to the selection of BRD4132,** from a collection of 46,176 compounds.

The focus of this research was centered around the effects of BRD4132, and circles around three main objectives:

1. To ascertain the mode of action of BRD4132.
2. To gain insights into the impact of BRD4132 on cell viability across a diverse set of cell lines and analyze the most sensitive and most resistant cell lines.
3. To study the effect of BRD4132 on the cell viability of Namalwa wherein Myc is known to be overexpressed, always.

METHODS AND RESULTS

I. Myc Dual Reporter Assay

Myc dual reporter assay is used to monitor the activity of Myc-regulated signal transduction pathways.

The Myc-responsive construct encodes a firefly luciferase gene under the control of a minimal CMV promoter and tandem repeats of the E-box sequence.

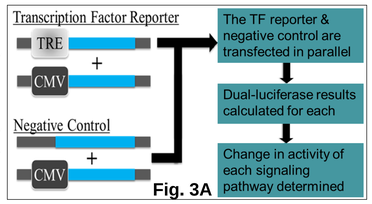


Fig. 3A

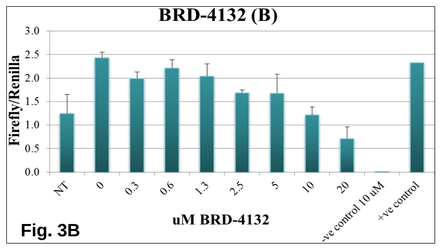


Fig. 3B

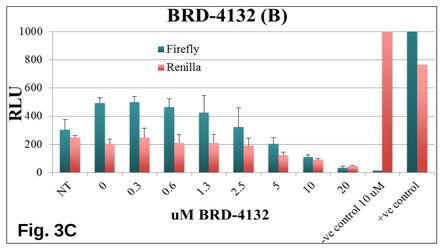


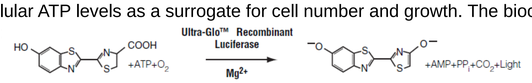
Fig. 3C

Fig 3: The Myc Dual Reporter Assay, works as shown in **Fig.3A** The gradual lowering of signal (**Fig. 3B, Fig. 3C**), demonstrates inhibition of Myc-activated transcription by BRD4132.

II. Cell Viability Profiling of 789 Cell Lines over 25 Lineages

Cells seeded at 500 cells/well → Incubated overnight → Compound treated in 16-pt doses → Cell viability assessed after 3 day treatment

Cell Titer-Glo measures cellular ATP levels as a surrogate for cell number and growth. The biochemical reaction that facilitates this assay is:



The cell viability data is averaged from a single run per cell line across duplicate assay plates.

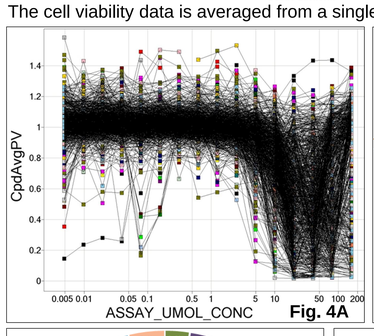


Fig. 4A

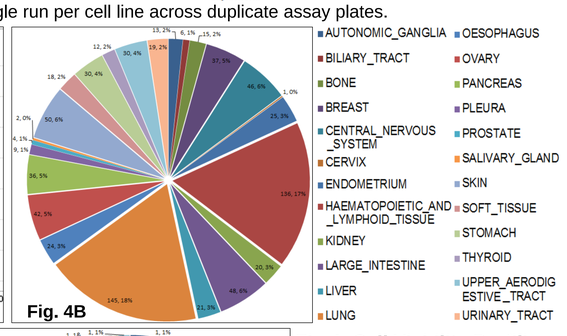


Fig. 4B

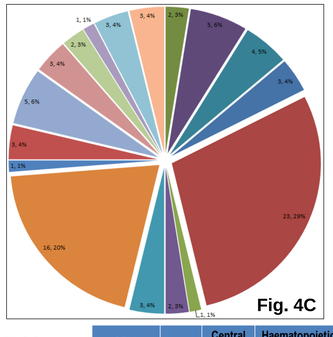


Fig. 4C

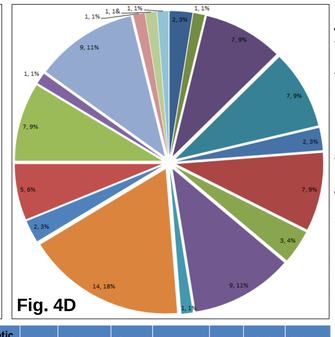


Fig. 4D

Fig 4: Cell Viability Profiling The dose-response curve of the 789 cell lines across 25 diverse cell lineages (**Fig. 4A**) at 16-pt dose concentrations, the over-all distribution (**Fig. 4B**), and the 80 most sensitive (**Fig. 4C**) and 80 most resistant (**Fig. 4D**) cell lines to the compound, BRD4132.

Table 1

Relative Increase

Relative Decrease

CELL LINEAGE	Breast	Central Nervous System	Haematopoietic and Lymphoid Tissue	Liver	Large Intestine	Oesophagus	Pancreas	Skin	Soft Tissue	Urinary Tract
TOTAL 789	4.69	5.83	17.24	2.66	6.08	3.04	4.56	6.34	2.28	2.41
TOP 80	6.25	5	28.75	3.75	2.5	1.25	0	6.25	3.75	3.75
BOTTOM 80	8.75	8.75	8.75	1.25	11.25	2.5	8.75	11.25	1.25	0

Table 1: An analysis of the distribution of cell lineages with respect to their sensitivity to BRD4132

III. Dose and time-dependent cell viability assay of a Human Burkitt's Lymphoma cell line, Namalwa

Data is averaged from a single run across triplicate assay plates.

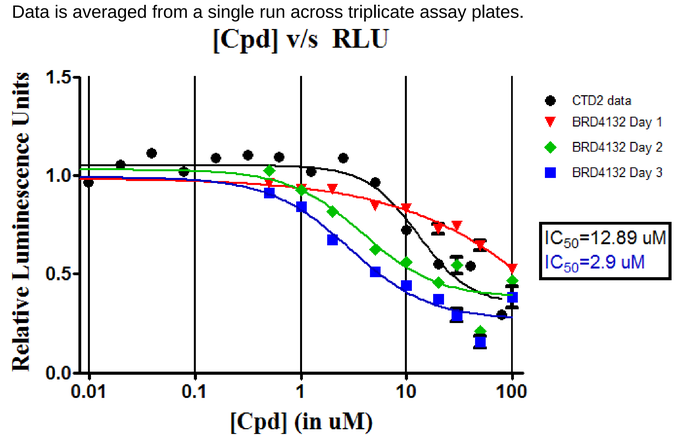


Fig. 5: Cell viability assay of Namalwa cells, in 8-pt doses varied over 24, 48 & 72hrs of compound treatment demonstrates significant growth inhibition.

CONCLUSION

1. BRD4132 indirectly modulates Myc by inhibiting Myc-mediated transcription as shown by the dual reporter assay.
2. Profiling studies establish most sensitive and resistant cancer cell lineages to BRD4132.
3. Partial inhibition of lymphoma cell growth observed in Namalwa.
4. Namalwa found to be more sensitive than originally established by the CTD[2] data, with an AUC and IC_{50} lower than earlier reported.

FUTURE WORK

1. Myc protein and transcript levels, at different dose/time points to be assayed to gain insights into the mechanism of action.
2. Post-translational modifications status at different dose/time points to be assayed to characterize Myc-GSG2 interaction, after a kinase profiling suggests a novel mechanism, involving GSG2.
3. Cell viability in the most sensitive and resistant cell line across different time points (24hrs, 48hrs, 72hrs) to be performed.

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