In Vitro Studies of the Aurora-Kinase Inhibitor MLN8237 in Prostate Cancer Cell Lines

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Abstract

After first-line hormone deprivation treatments, a majority of malignant prostate cancers reemerge with aggressive, castrate-resistant properties. Of these cases, around 20% exhibit little to no androgen receptor signaling and are phenotypically similar to small-cell prostate carcinomas. Small-cell variants have been found to express unusually high levels of Aurora-kinase A, a protein involved in mitotic regulation. In this study, we hypothesized that MLN8237, an Aurora-A inhibitor previously found to be cytotoxic against multiple myeloma cell lines\(^3\), would produce similar effects when tested in vitro on DU145, LNCaP, and PC3 prostate cancer cell lines. After a number of preliminary measures, we ultimately treated the three lines at dose concentrations ranging from 0.1 to 10µM and analyzed the inhibitor’s effects on cell proliferation by WST absorbance assay. Although cell proliferation was not reduced by >50% in any of these instances, the potential efficacy of MLN8237, in higher dosages or as demonstrated in the future by its effect on xenograft models of small-cell prostate carcinomas, remains to be fully uncovered.

Introduction

A pervasive group of diseases with nearly 200,000 diagnoses and 30,000 resulting deaths annually, cancers of the prostate are generally under the regulatory influence of androgen receptors (ARs). The attachment of testosterone and its more potent counterpart, dihydrotestosterone, induces the AR to bind to the androgen response components of various genes implicated in prostate cell proliferation and survival. Accordingly, the first line of therapy utilized in all prostate cancers is androgen deprivation, commonly through surgical or chemical castration\(^1\) Though such therapies are effective in reducing tumor size, in a majority of cases, malignant cells adapt and develop castrate-resistant phenotypes. Around 80% of these aggressive variants still exhibit AR signaling. The remaining minority, however, are similar to small-cell carcinomas, which are characterized by little to no PSA expression, frequent bone metastases, and poor prognosis. In addition, they are commonly AR-negative and thus do not respond to hormone therapies. The development of a novel therapeutic approach for small-cell prostate carcinomas and
their phenotypically similar counterparts has thus become a matter of great clinical
importance.

Notably, Aurora kinase A, one of a family of serine-theonine kinases involved
in the regulation of mitosis, is expressed in unusually high levels in models of the
aggressive, small-cell variant of the disease (Aparicio, 2010). Encoded on
chromosome 20q 13.2, Aurora-A has regulatory functions within the centrosome
and in spindle formation throughout the mitotic process. In malignant cells, Aurora-
A is present in a pervasive and dysfunctional manner, leading ultimately to
chromosomal instability. The inhibition of Aurora-A has been found to interfere
with chromosomal alignment and lead ultimately to polyploidy and cell death²,
preventing a novel therapeutic concept that has only begun to be fully explored.
MLN8237, the first orally available, Aurora-A specific inhibitor, was found by
Görgün et al. to both inhibit the proliferation of and induce apoptosis in multiple
myeloma cells³. In this study, we hypothesize that MLN8237 will similarly inhibit
the proliferation of small-cell type prostate cancer cells and thus attempt to extend
the emerging therapeutic potential of the drug by seeking effective dosages of the
inhibitor in vitro against the proliferation of DU145, LNCaP, and PC3 cell lines, with
plans to further test an effective dosage on xenograft models that better represent
the small-cell variant of the disease.

Methods

Cell Culture

Three human prostate cancer cell lines were obtained from the American
Type Culture Collection (ATCC). DU145 (HTB-81) are non-hormone sensitive cells
derived from a brain metastasis of prostate carcinoma. LNCaP (CRL-1740) cells
were obtained from a lymph-node metastasis of prostate carcinoma and are
responsive to 5-alpha-dihydrotestosterone⁴. PC3 (CRL-1435) are epithelial cells
derived from a bone metastasis of grade IV prostate adenocarcinoma. The cell lines
were maintained in an RPMI medium supplemented with 10% fetal bovine serum
and 1% Pen-Strep antibiotic and incubated in an Incu-Safe copper alloy stainless
incubator (SRS) at 37°C, 5% CO2.

WST Assay

At the outset, we sought to determine the optimal number of cells to plate to
achieve an initial logarithmic growth phase for inhibitor performance by WST
absorbance assay, a reliable and relatively expeditious method of determining the
level of mitotic activity within a culture. Three 96 well plates, one per cell line, were
seeded with cell counts of 1,000, 5,000, 10,000, 20,000, 30,000, and 40,000 and
allowed to incubate for 72 hours. For the assay, 10 uL of WST-1 proliferation
reagent (Roche, 05 015944 001) per well were added to each plate. WST-1 contains
tetrazolium salts, which are cleaved to formazan by enzymes prevalent within
actively dividing cells. The amount of formazan dye formed can be read as
absorbance of a particular wavelength, which directly correlates to the number of mitotically active cells present. Proliferation was assessed with a Perkin-Elmer Envision plate reader at 450 nm wavelength after 0.5, 1, 2, and 4 hours of incubation with the reagent.

As an additional condition for inhibitor performance, the WST assay for optimal incubation time for the plated cells (for logarithmic phase growth) before drug treatment was later performed. 10,000 cells per well were again seeded in each well of three 96 well plates, one per cell line. Cell proliferation readings were taken over a four-day period at 24, 48, 72, and 96 hours after plating, each consisting of four separate, 10uL reagent per well WST-1 incubations (0.5 h, 1h, 2h and 4h) assessed per day.

For the final cell proliferation assay, viability of the cells was assessed 48 hours after the initial treatment using the cell proliferation reagent WST-1 and a Perkin-Elmer Envision plate reader. 10uL of the reagent were added to each well of the six plates, which were then allowed to incubate for 2 hours before absorption data was collected with the Envision reader.

MLN8237-Preparation and Treatment of Cells

The aurora-kinase inhibitor MLN8237 was obtained in powdered form from Selleck Chemicals (1028486-01-2) and reconstituted in dimethyl sulfoxide; the drug was stored at -20°C until use. Three stock concentrations of MLN8237 (0.01 mM, 0.1mM, 10mM) and a DMSO control solution (0.01 mM) were prepared (DMSO is used as a vehicle for drug absorption).

Cells seeded at 5,000 or 10,000/well were allowed to incubate 24 or 48 hours before treatment with MLN8237 at 0.0001uM, 0.001uM, 0.01uM, 0.1uM, 0.5uM, 1uM, 4uM and 10uM concentrations, as reported to be effective in prior literature. Additional wells for media and DMSO controls were seeded in the same fashion. Cells were treated for 24 or 48 hours (media +/- MLN8237 changed daily) and their proliferation measured using WST assay as described above.

Results

1. Optimization of Assay: Initial cell concentration, incubation time and WST incubation time

PC-3, LNCaP and DU145 cells were seeded in 96 well plates at 1,000, 5,000, 10,000, 20,000, 30,000, and 40,000 cells per well and incubated for 72 hours. At 72 hours, WST reagent was added and incubated for 1, 2 or 4 hours (Figure 1.). Each data point represents the average of results obtained for 3 wells.

In the determination of the initial number of cells to seed, relatively similar proliferation curves were observed as expected. As the measured absorbance data
implies, we selected 10,000 cells per well, where the cells, in general, achieve logarithmic growth, as a uniform count to be used in the MLN8237 treatment.

**Figure 1:** Optimization Assay: Determining the initial number of cells to seed

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2. **MLN8237 treatment dose response curves**

PC3, LNCaP, and DU145 cells were seeded in 96 well plates at 10,000 cells per well. MLN8237 was added at 0.0001, 0.001, 0.01, 0.1, 0.5 and 4uM at 24 or 48 hours from seeding. WST reagent was added at 72 hours and incubated for 0.5, 1, 2 and 4 hours prior to the absorbance measurement. Results are shown in Figure 2.

The initial drug treatment attempt, without other preliminary measures, yielded little to no change in cell proliferation. Because little difference in dose response was observed between the four WST incubation times among all cell lines, only the 2 hour readings are shown:

**Figure 2:**

![Dose Response-24h Treatment](image.png)

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As indicated by the dosage response curves, the measured absorbance data (indicated by percentiles of the absorbance value of the control), did not suggest a significant change in cell proliferation (>50% change) in any instance.

We, in addition, determined that treatments would be performed at 48 hours and 72 hours after seeding, with a single absorbance reading taken after two hours of WST incubation (Fig. 3):

**Figure 3:** Optimization of WST incubation time
Despite preliminary measures intended to create the best possible imitation of the proliferative conditions of aggressive prostate cancer cells within the human body, as well as promising results with MLN8237 in vitro in previous studies of other cancers\textsuperscript{3}, our results did not indicate a change in cell proliferation at or above 50% in any instance (Fig. 4):

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**Figure 4:** Absorbance values after principal MLN8237 treatment

**DU145- 5,000 Cells/well**

![Graph showing absorbance values for DU145-5,000 Cells/well](image)

**DU145- 10,000 Cells/well**

![Graph showing absorbance values for DU145-10,000 Cells/well](image)

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Discussion

Our findings fundamentally present two possibilities. On one hand, the compound obtained may be inactive. To test this possibility we will measure changes in phosphor-AURKA by western blot in treated and untreated cells. If we see no change, we will test the compound in cell lines previously reported to be sensitive to the drug (e.g. U226).

It is possible that the prostate cancer cell lines are not dependent on AURKA for survival and proliferation, though DU145, LNCaP, and PC3 are not representative
of the phenotype that we are interested in. We thus intend to test the drug in short-term small cell prostate carcinoma xenograft cultures. MLN8237 may be inherently ineffective against Aurora kinase-A in the tested cell lines due to the cells' ability to resist or hinder the inhibition of the phosphorylation of Aurora-A. In another light, it may prove beneficial to simply test higher dosages.

In consideration of the previous successes of MLN8237, as well as the moderate degree of activity it displayed in our study and its ease of administration, the therapeutic potential of the inhibitor remains promising and relevant in cancers of the prostate and beyond.

References