Spy1 Levels Predict Sensitivity of Refractory Multiple Myeloma to Therapy

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Spy1 Levels Predict Sensitivity of Relapsed Multiple Myeloma to Therapy

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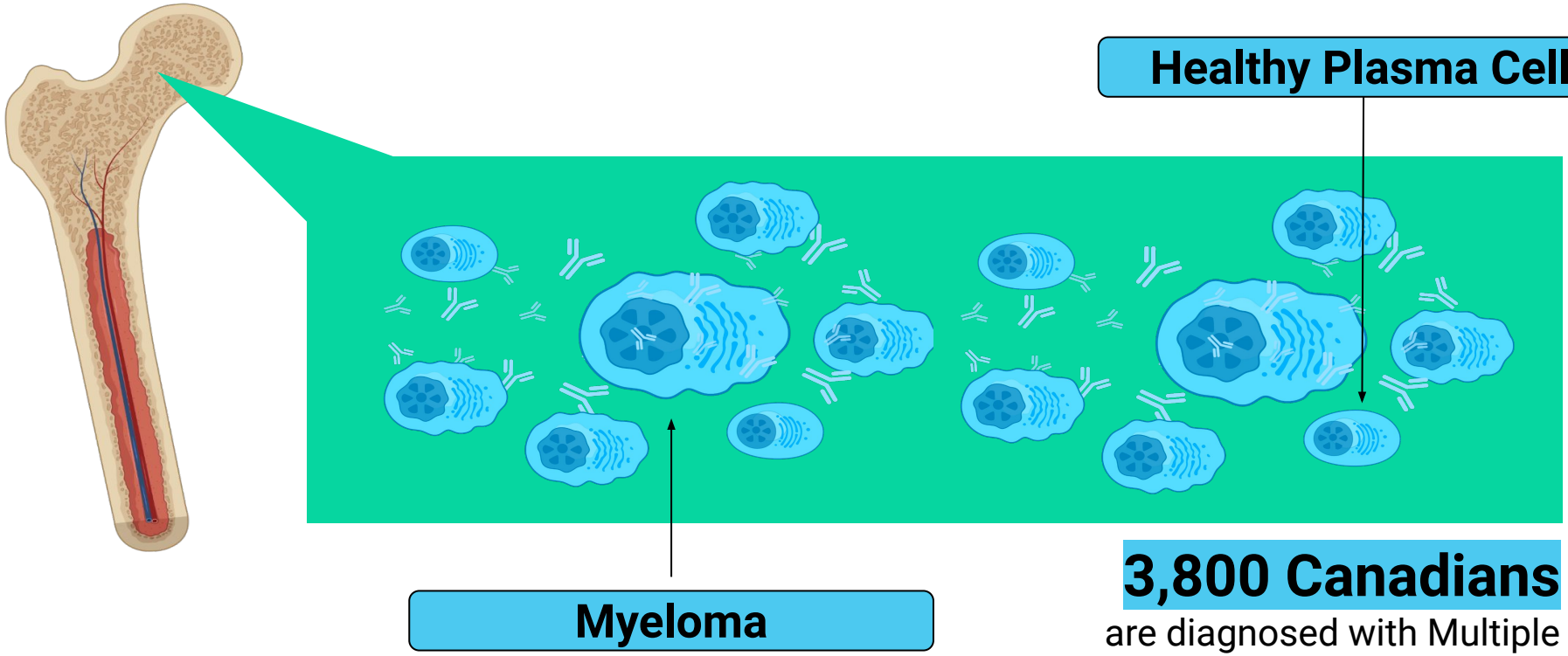
University of Windsor

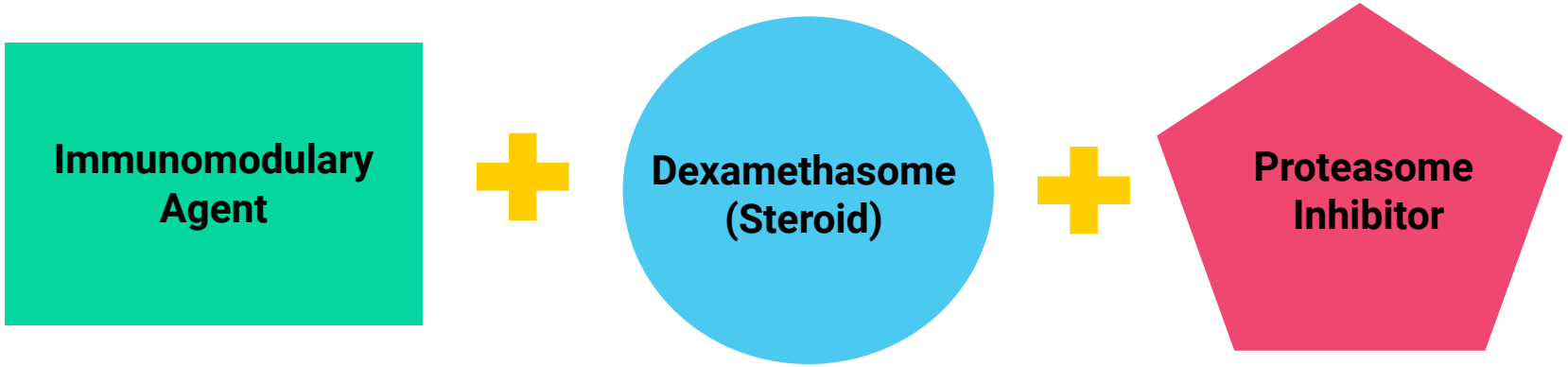
What is Multiple Myeloma (MM)?



3,800 Canadians
are diagnosed with Multiple Myeloma every year.[1]

1. CCS (2022). https://cancer.ca/en/cancer-information/cancer-types/multiple-myeloma/statistics

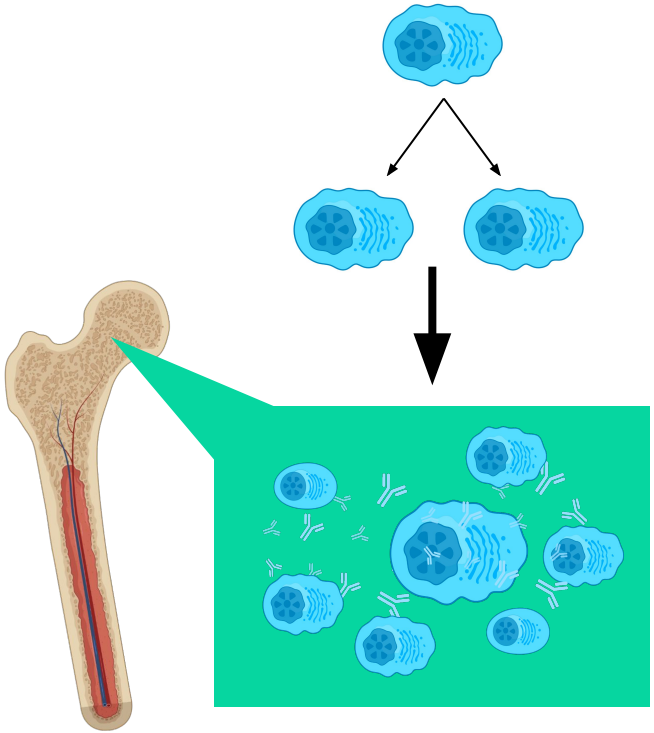
Current Treatment of MM

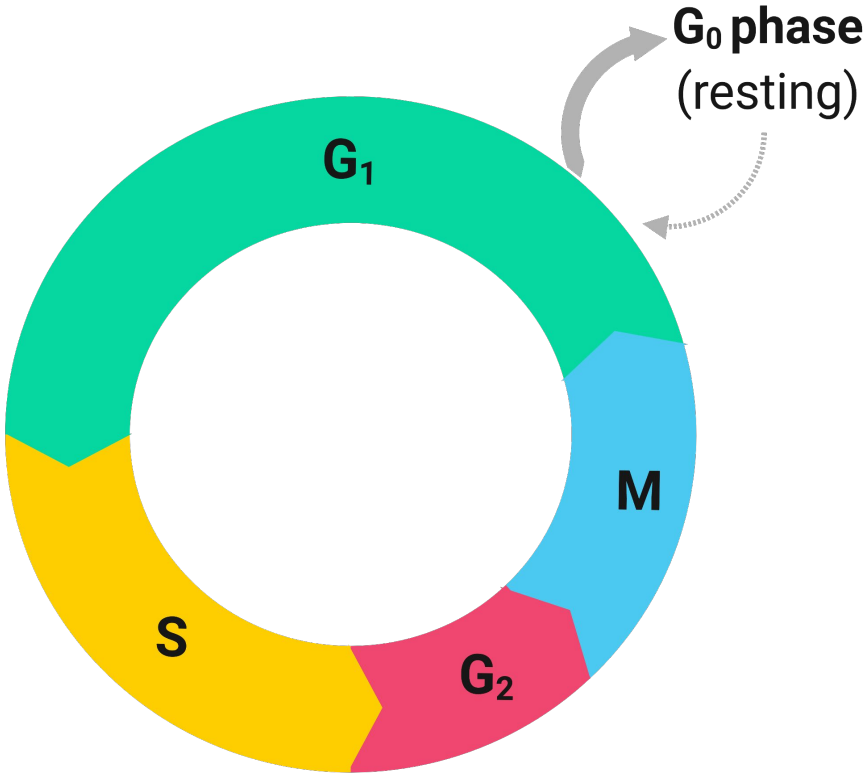


- **Most MM patients will eventually relapse** with a more aggressive and untreatable form of the disease.
- In Canada, the **5-year net survival** for patients newly diagnosed and treated with the current standard of care is **44%**.[1]

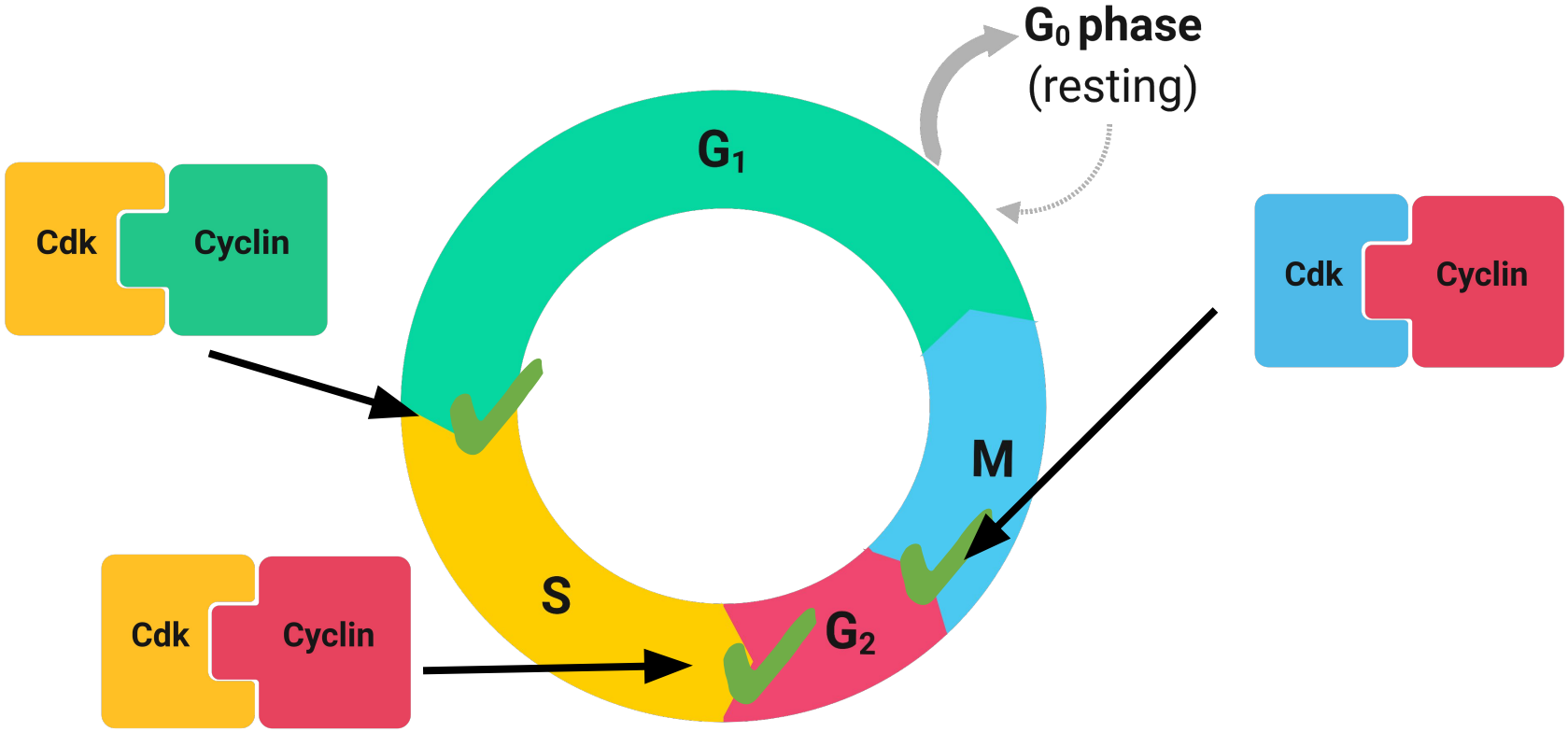
1. CCS (2022). https://cancer.ca/en/cancer-information/cancer-types/multiple-myeloma/statistics

The Role of Cell Cycle in the Progression of MM





The Role of Cell Cycle in the Progression of MM

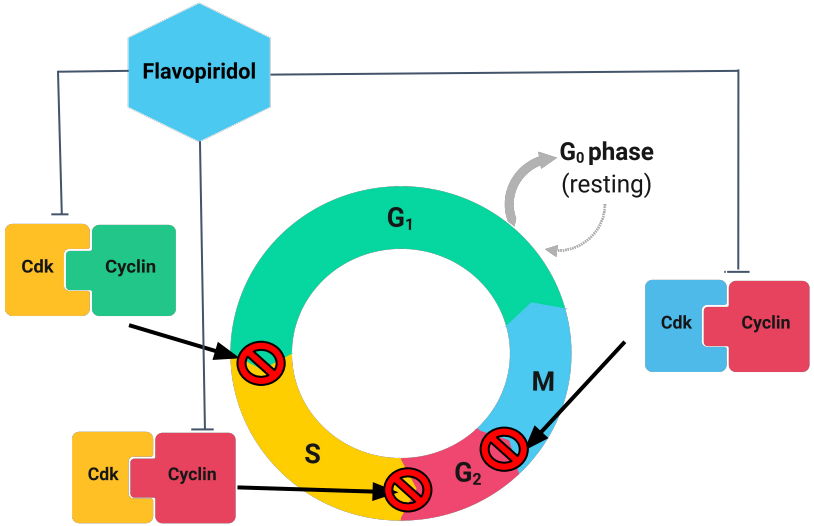


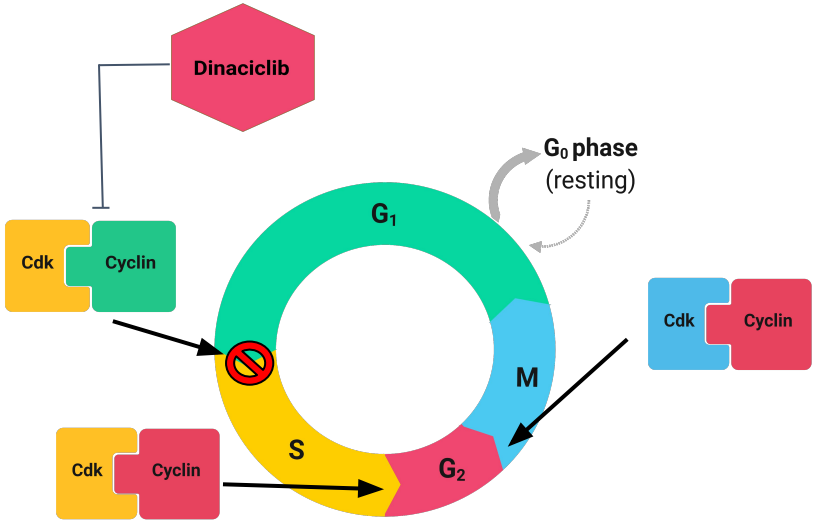
Cdk Inhibitors & Cell Cycle Progression

Pan-Cdk inhibitors, such as Flavopiridol & Purvalanol A, inhibit <u>all</u> Cdks.

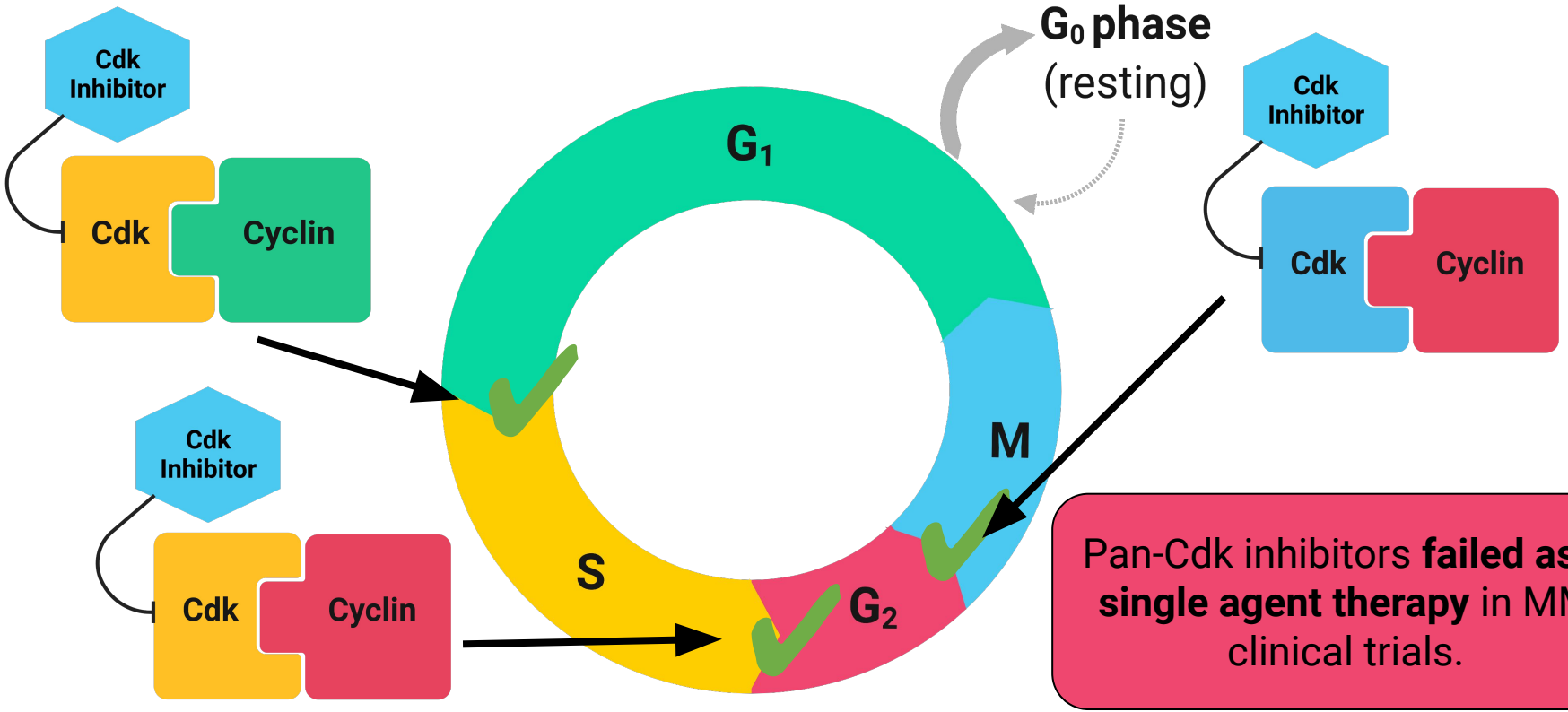
NEW

Specific Cdk inhibitors, such as dinaciclib, inhibit <u>select</u> Cdks.



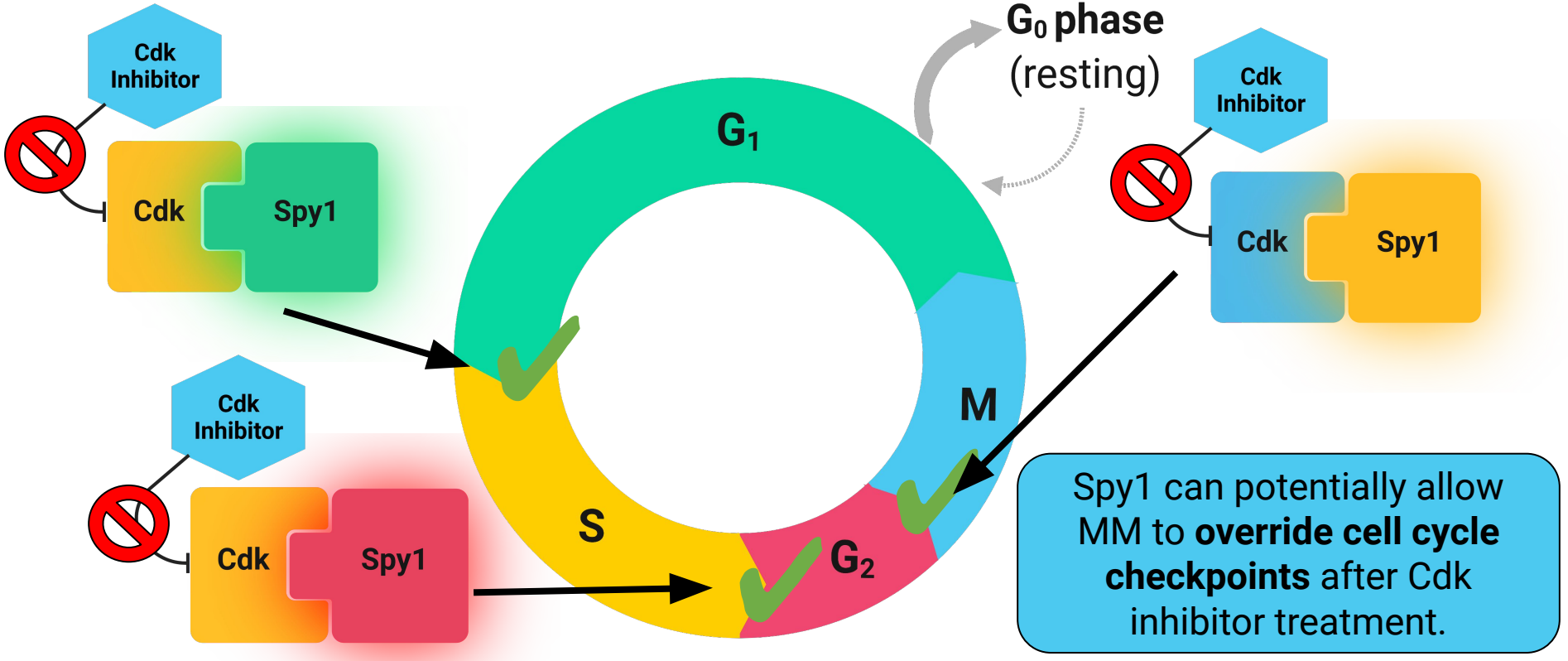


Can Cdk Inhibitors Improve Therapy Response in MM?



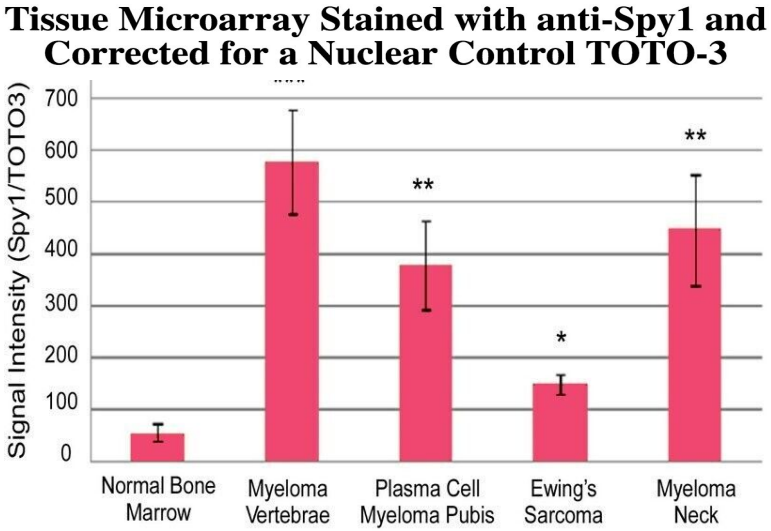
Pan-Cdk inhibitors **failed as a single agent therapy** in MM clinical trials.

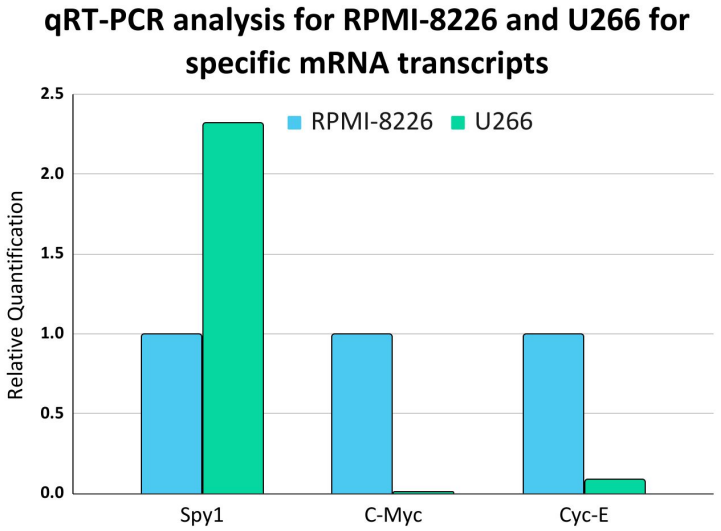
The Cyclin-Like Protein Spy1 & Multiple Myeloma



Spy1 can potentially allow MM to **override cell cycle checkpoints** after Cdk inhibitor treatment.

Spy1 is highly expressed in MM tissue samples and human cell lines.





MM Patient Sample Collection Flowchart

MM PATIENT
- CONSENT
- BONE MARROW BIOPSY

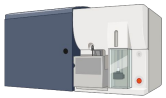
DR. WOLDIE NOTIFIES THE LAB

PREPARE FOR SAMPLE PICKUP (WHO, WHERE, WHEN)

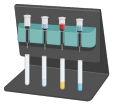
SAMPLE PROCESSED AND FROZEN

CD138+/CD38+ CELLS ARE SORTED OUT

FACS

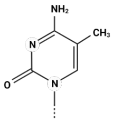


MACS



FUNCTIONAL ASSAYS

EPIGENETICS



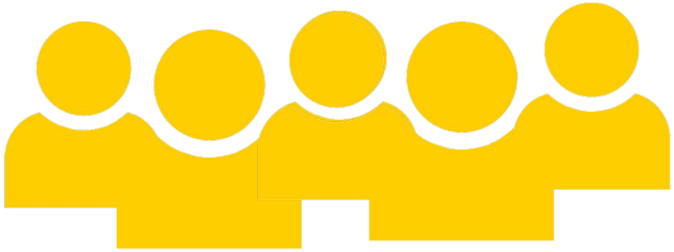
ZEBRAFISH XENOGRAFT

RNA SEQUENCING

MM Clinical Data

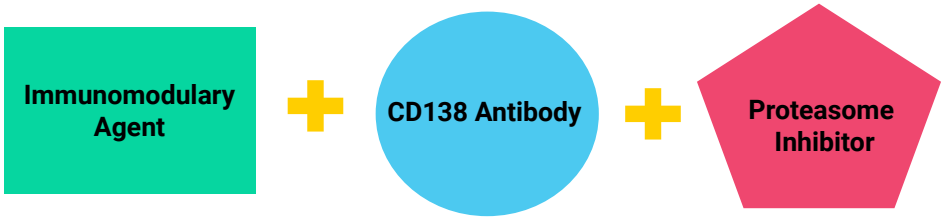
PATIENT DEMOGRAPHIC

- 5 Patients
- M:F 1.5
- Median Age: 59 (50-84)
- 3 Relapsed MM



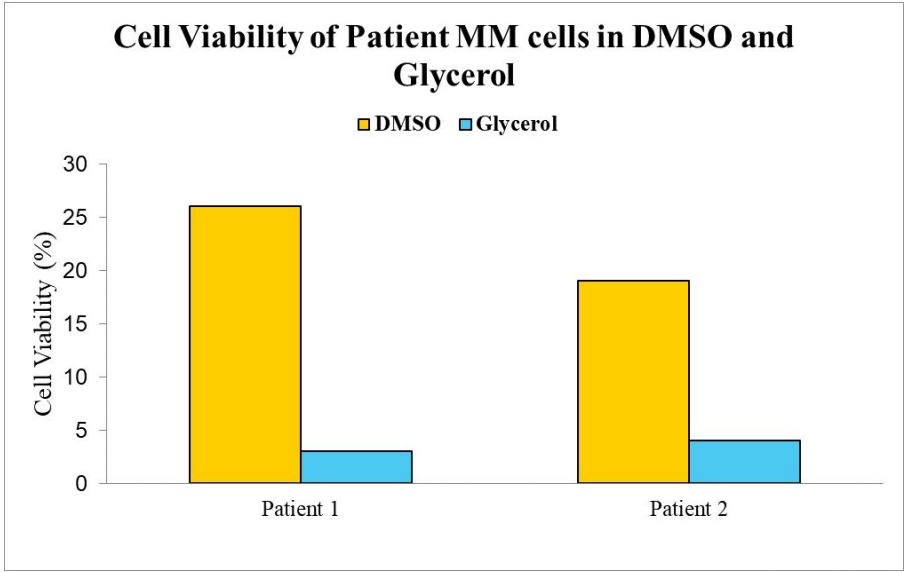
TREATMENT

- All had proteasome inhibitor
- 2 had proteasome inhibitor + immunomodulator
- 2 had CD138 Monoclonal Antibody



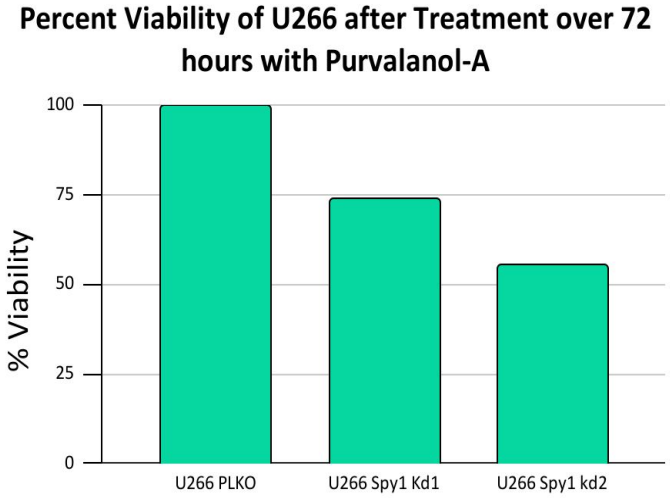
Comparing cell viability of patient samples stored using different cryopreservation protocols.

Higher cell viability for patient samples stored in DMSO compared to Glycerol.



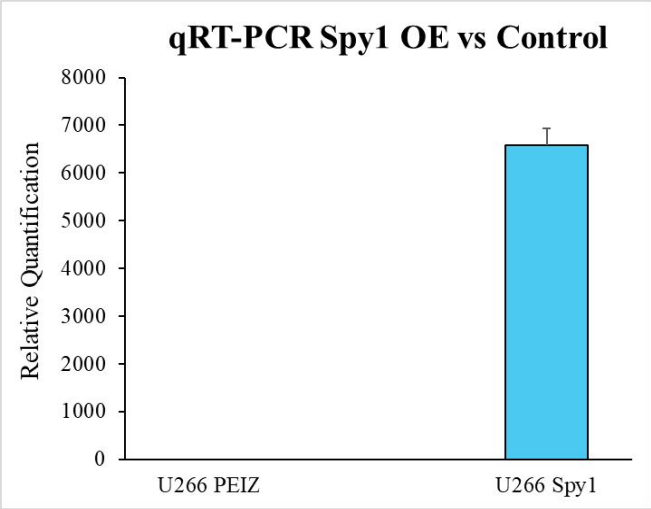
Next Steps: EDTA vs No EDTA

Knockdown of Spy1 in a human MM cell line shows an increased sensitivity to Pan-Cdk inhibitor treatment.

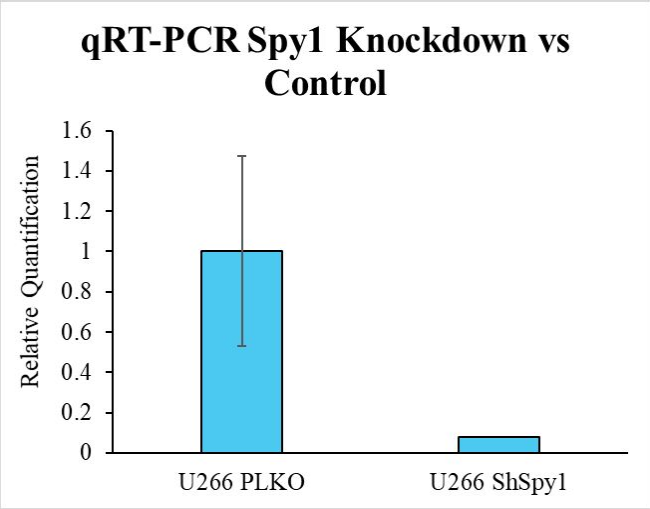


U266 cells are B Lymphocytes derived from the peripheral blood of a human MM patient.

qRT-PCR analysis of Spy1 gene expression after lentiviral infection.

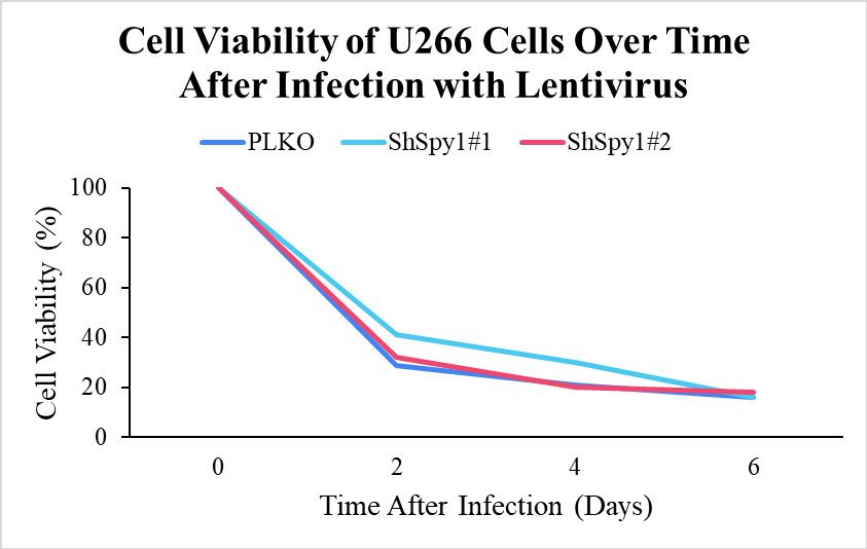


Successful Spy1 overexpression

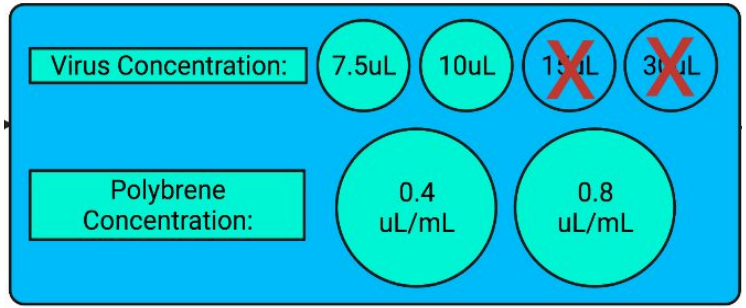


Successful Spy1 knockdown

Cell viability over time of U266 cells after infection with lentivirus.



Low cell viability after lentiviral infection



Optimization of infections using different concentrations of virus and polybrene

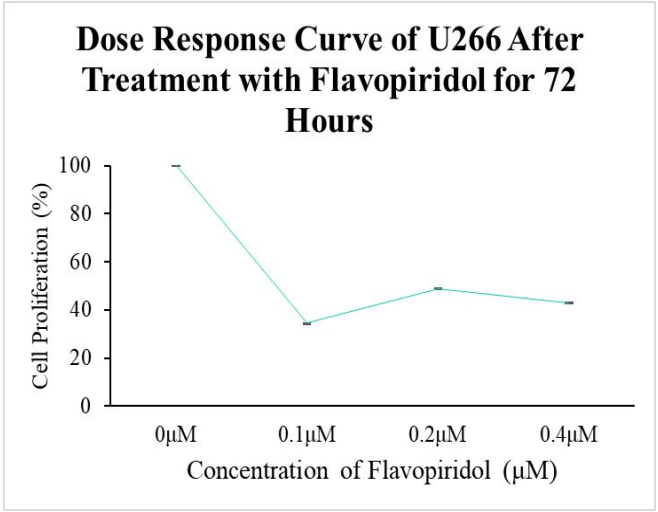
Determination of IC50 values for Flavopiridol and Dinaciclib in U266 cells.

Alamar Blue

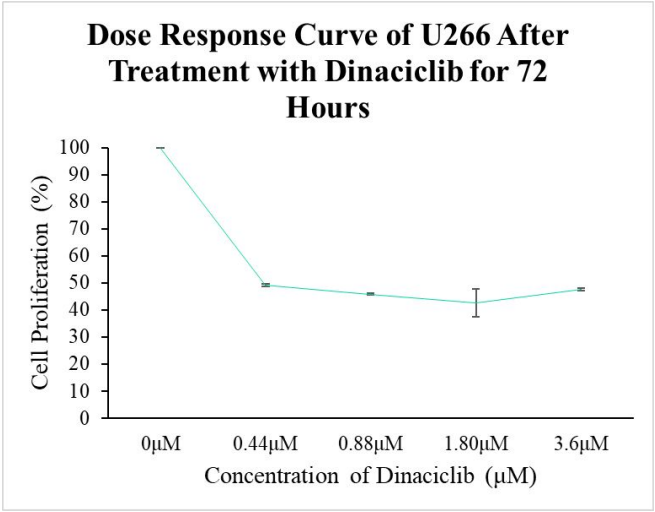
CDK Inhibitor treatment (0hrs, 24hrs, 48hrs)

Add Alamar Blue (72 hrs)

Read Absorbance at 570/585 nm

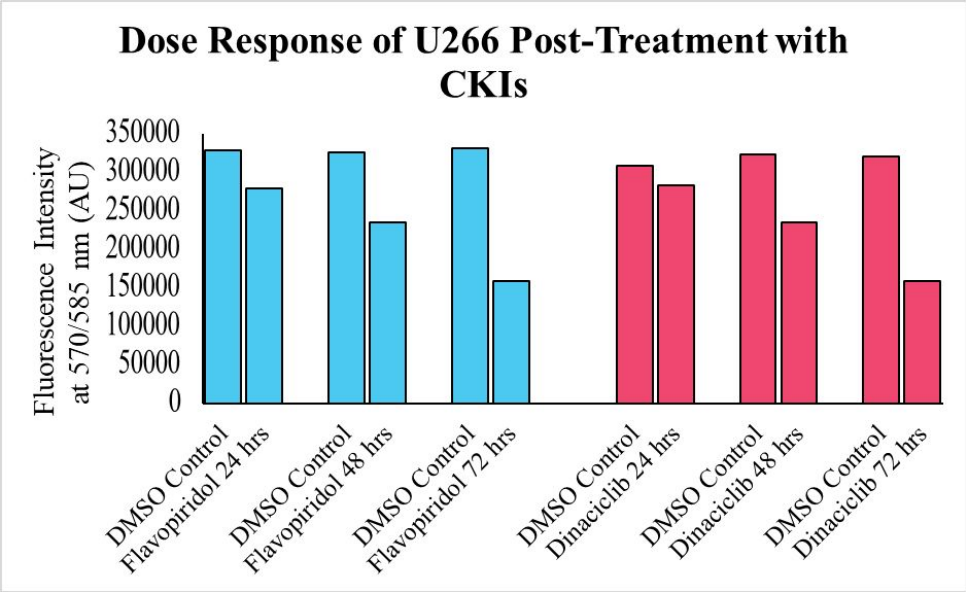


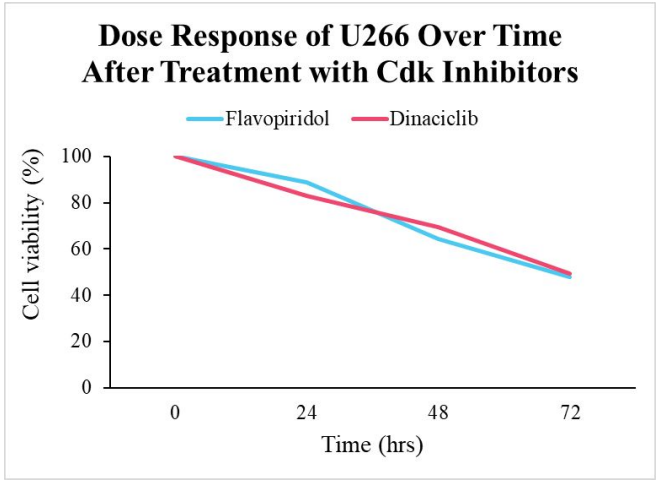
IC50: 0.2μM



IC50: 0.44μM

Proliferation time course of U266 cells after treatment with Flavopiridol vs Dinaciclib.

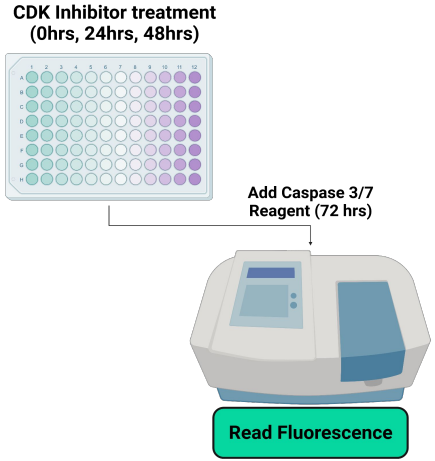


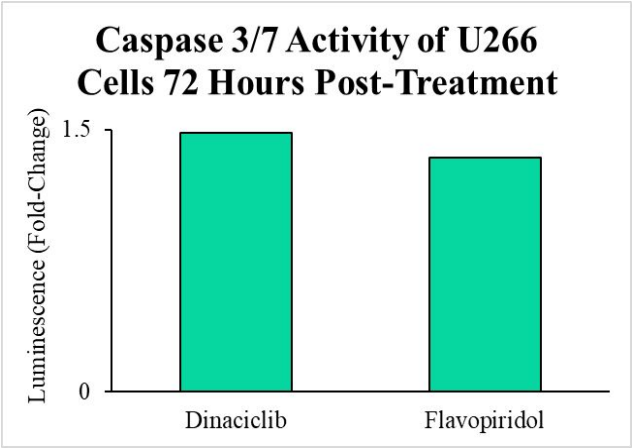


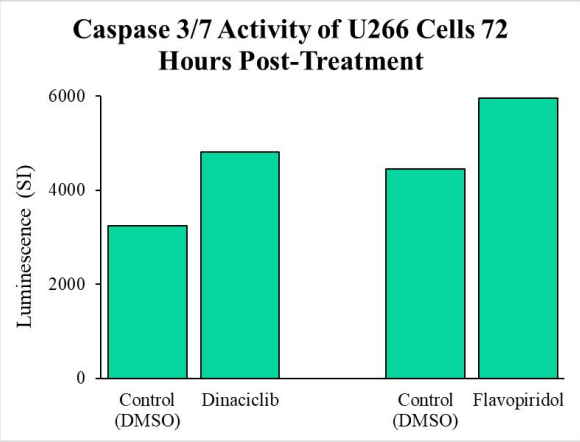
No significant difference in pan vs specific in U266 cell proliferation.

Cell death in U266 cells treated with Flavopiridol vs Dinaciclib.

Caspase 3/7 Assay







No significant difference in cell death in U266 cells treated with Flavopiridol vs Dinaciclib.

Work in Progress

Spy1-bound Cdks may be a potent therapeutic direction for MM.

More research needs to be conducted to characterize the response of MM to pan vs specific Cdk inhibitors.

Optimization of the storage and processing of MM patient samples will be beneficial for future research.

Acknowledgements

Special thanks to Dr. Porter, Dr. Woldie, Jillian Brown, Dr. Lubanska, Dr. Hudson, Dr. Fifield, Dr. FIdalgo, and to my fellow Porter lab rats!