Targeting STAT3 and P300 in Treatment Resistant Multiple Myeloma to Inhibit MYC Expression and Decrease Cellular Viability

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Abstract

Multiple myeloma arises due to genetic and epigenetic alterations in cellular pathways that converge on ectopic MYC expression. The aberrant activation of the oncogenic transcription factor STAT3 has been of interest, given microenvironment IL6 secretion is known to activate STAT3 and confers therapeutic resistance. Additionally, MYC translocations in multiple myeloma led to an increased proximity of the MYC gene to super-enhancer regions, where histone acetyltransferase P300 is preferentially localized. ChIP-seq data for the B lymphoblastoid line GM12878 showed 66.2% (16,062 / 24,257) of STAT3-bound sites also had P300 binding (ENCODE). This study aims to test the hypothesis that the combination of the P300 inhibitor GNE781 with the STAT3 inhibitor atovaquone or pyrimethamine, would synergize to inhibit MYC expression and decrease viability of multiple myeloma cells. We utilized JJN3 myeloma cell lines, in which the endogenous MYC loci had a modified green fluorescent protein (GFP) with a two-hour half-life knocked-in using CRISPR/Cas9 (Steinberger et al, Cell Chemical Biology, 2019). Cells were treated with various dosages of EP300 and STAT3 inhibitors and were monitored daily over a 72-hour time course for GFP expression and relative viable cell number. Combining EP300 inhibitor at 25nM GNE781 and STAT inhibitor at 20uM atovaquone/5uM pyrimethamine results in decreased cell viability and GFP expression after 72 hours. This time frame is consistent with the transcriptional inhibitor's time requirement to inhibit transcription and for its target mRNA to decay. Atovaquone and pyrimethamine demonstrated viability and GFP downregulation at their lowest therapeutic doses, indicating a potential synergistic effect with the GNE781. To explore this further, additional combinations of both drugs should be tested, including higher doses of atovaquone and pyrimethamine. Additionally, it underscores the potential value of studying the effects of different dosage combinations of these drugs on multiple myeloma cell lines.

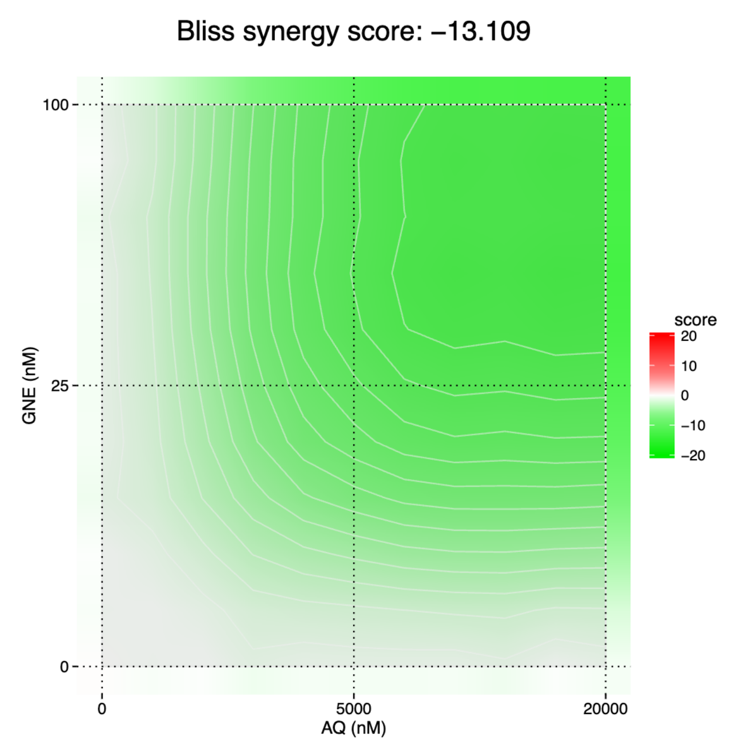
Introduction

- Multiple myeloma is an incurable plasma cell malignancy, which occurs due to dysregulation of various signaling pathways such as the NF-κB and JAK/STAT pathways (Nelson et.al, 2011).
- In multiple myeloma, complex rearrangement results in an increase in the proximity of the MYC protein to super-enhancers, thus resulting in an elevation in MYC expression.
- Pharmacologically targeting super-enhancers is now being considered as a potential therapy for MM patients.
- EP300 is preferentially localized to myeloma super-enhancer sites and aberrant activation of STATs has been implicated in promoting tumor growth, survival, and immune invasion, therefore EP300 (GNE-781) and STAT inhibitors (Atovaquone and Pyrmethamine) are the specific agents that were examined in this study.

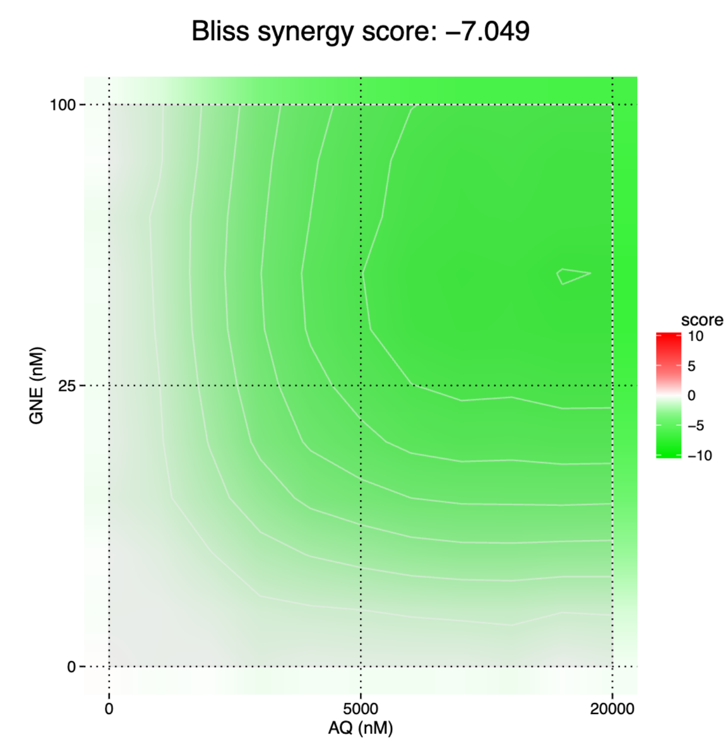
Objective

The primary objective of this study is to explore the synergistic effects of the combination of EP300 and STAT3 inhibitors on MYC Expression and cellular viability.

Cell Viability Results

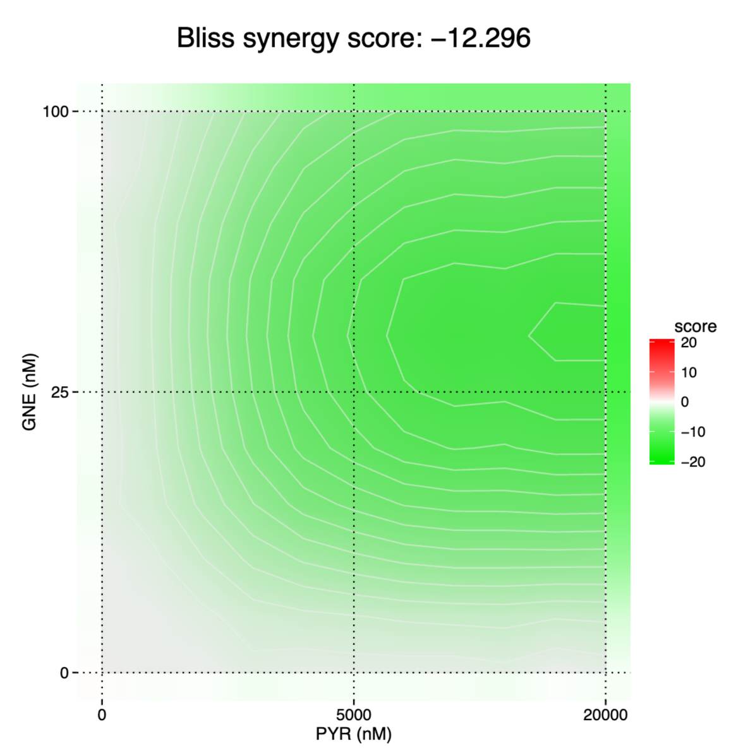


Cell Viability Synergy Score of GNE781-Atovaquone on Day 1

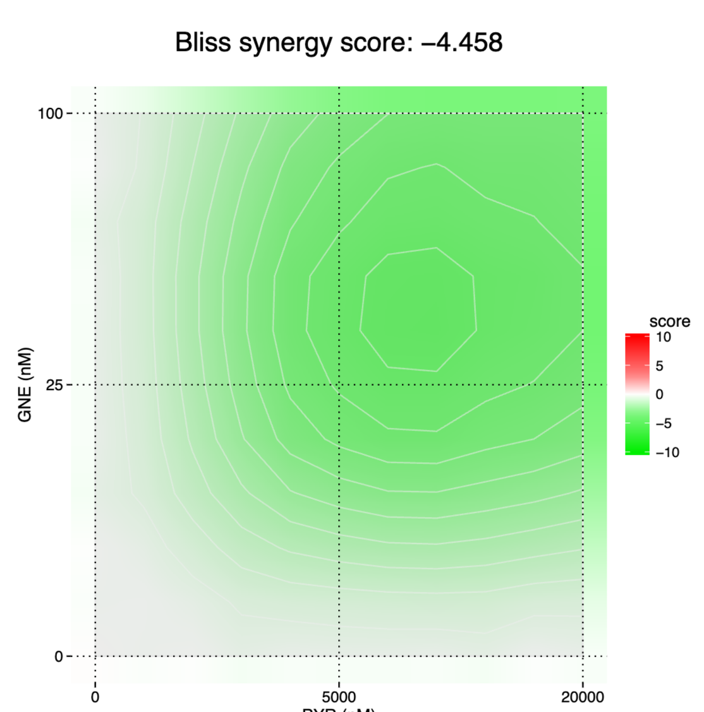


Cell Viability Synergy Score of GNE781-Atovaquone on Day 2

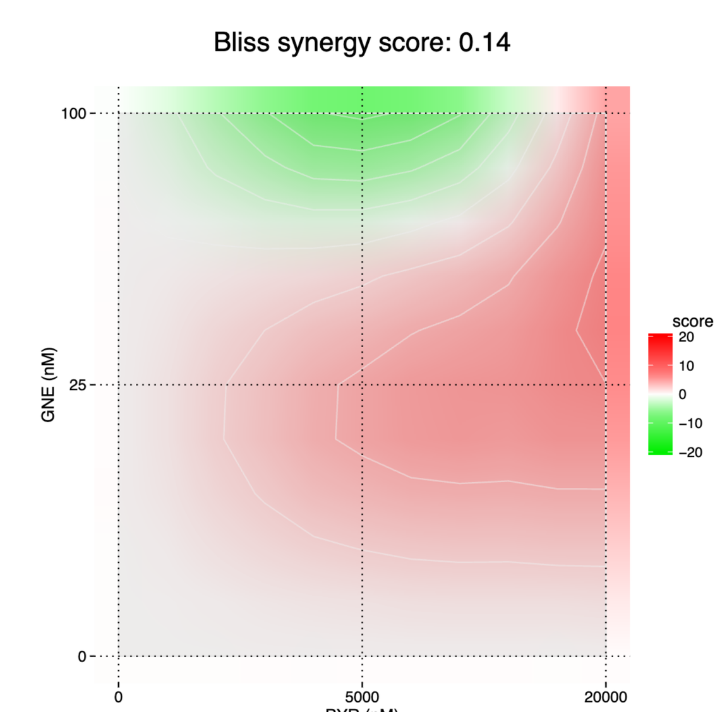
Cell Viability Synergy Score of GNE781-Atovaquone on Day 3



Cell Viability Synergy Score GNE781-Pyrmethamine of on Day 1

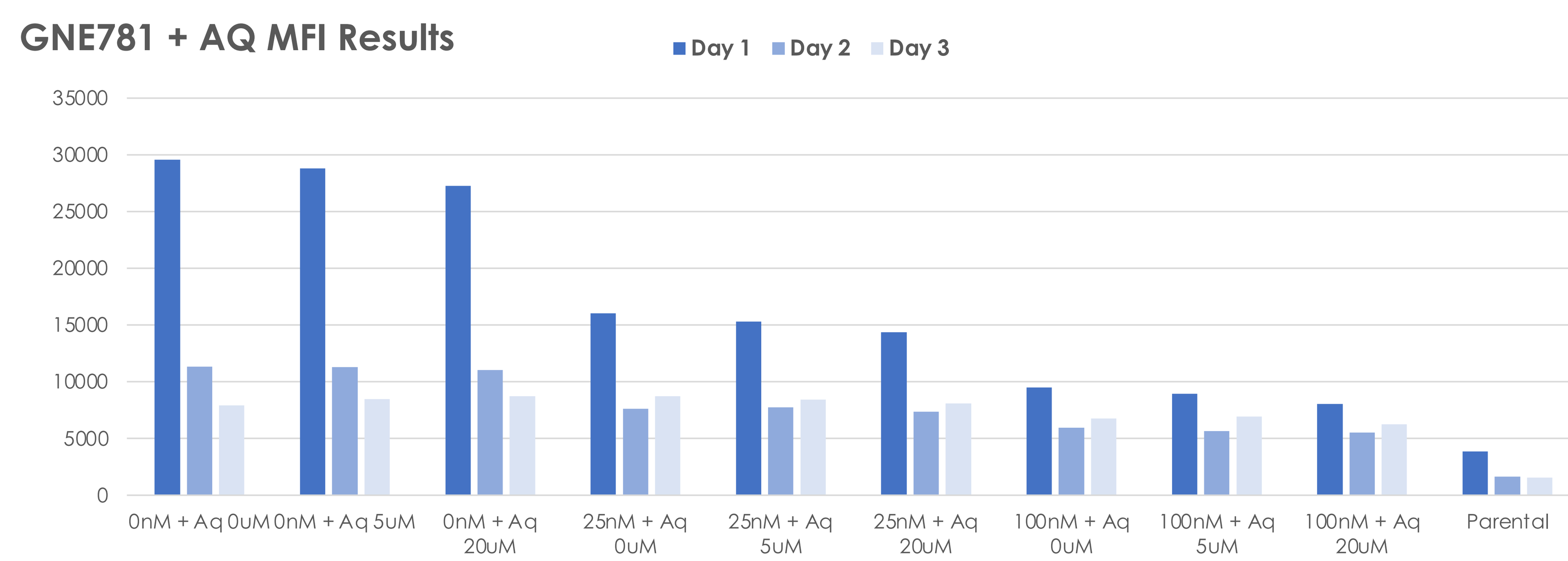


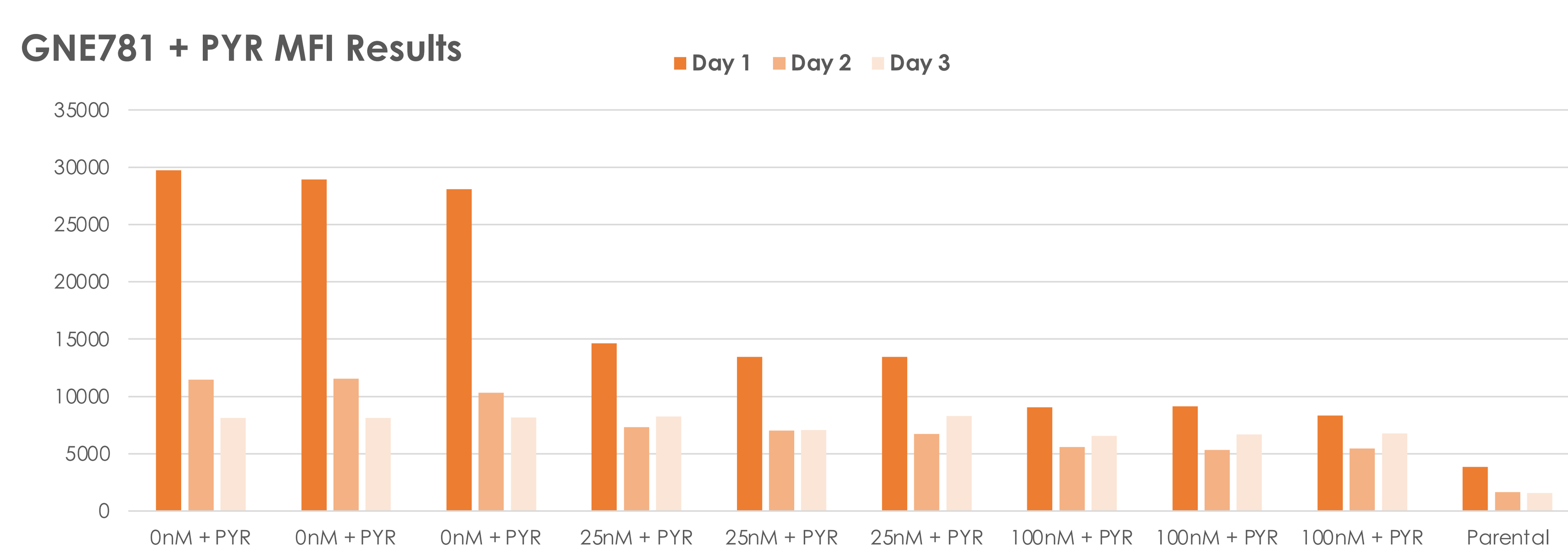
Cell Viability Synergy Score of GNE781-Pyrmethamine on Day 2



Cell Viability Synergy Score of GNE781-Pyrmethamine on Day 3

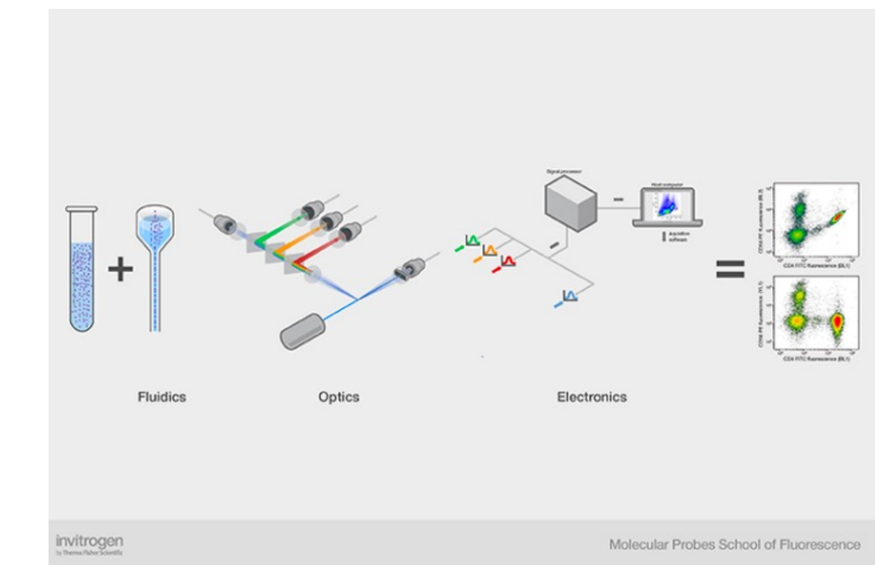
Mean Fluorescent Intensity (MFI) Expression Results

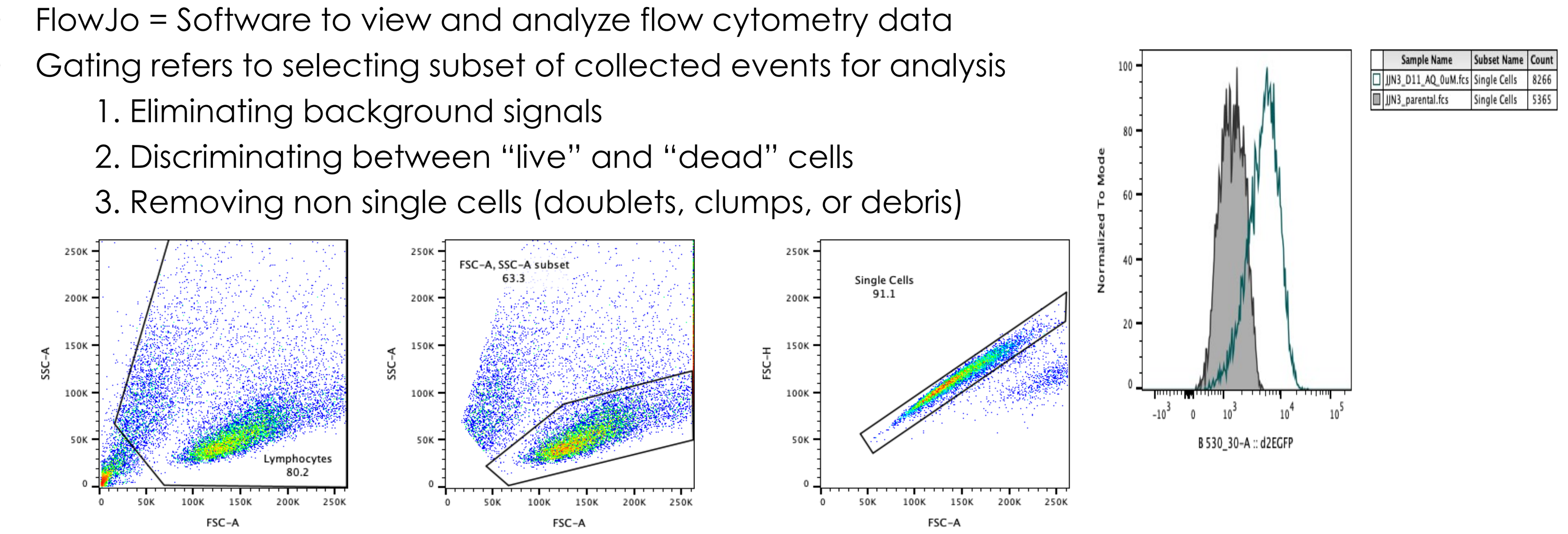




Materials and Methods

- Technique used to analyze and quantify characteristics of individual cells or particles in fluid sample
- Measurement of fluorescence and light scattering properties of cells, utilizing a combination of lasers, optics and detectors to analyze and sort cells
- CRISPR/Cas9 somatic cell engineering techniques were used to develop JJN3 cell line, where MYC expression can be measured with a surrogate reporter, Green Fluorescent Protein (GFP) (Steinberger et.al, 2019).
- FlowJo = Software to view and analyze flow cytometry data
- Gating refers to selecting subset of collected events for analysis
    1. Eliminating background signals
    2. Discriminating between "live" and "dead" cells
    3. Removing non single cells (doublets, clumps, or debris)





Discussion/Conclusion

The findings of this study reveal a notable decrease in MYC expression and cell viability when various doses of the EP300 inhibitor are combined with STAT inhibitors. Specifically, our repeated measurements consistently showed a significant reduction in cytotoxicity and MYC expression at dosages of 25nM GNE781, in conjunction with 5uM of both Atovaquone and Pyrimethamine, following a 72-hour incubation period.

Moreover, the analysis of our data using the SynergyFinder software unveiled intriguing results. The combination of GNE781 with Atovaquone yielded a Bliss score of 0.70, while its combination with Pyrimethamine resulted in a higher Bliss score of 1.36, again, at the 72-hour time point. These outcomes suggest a time-dependent enhancement in the synergy of cell viability. The significance of the 72-hour mark lies in its alignment with the typical duration required for transcriptional inhibitors to suppress transcription and for the associated target mRNA to degrade.

Of particular interest, Atovaquone and Pyrimethamine exhibited reductions in viability and GFP expression at their lowest therapeutic doses, hinting at a potential synergistic interaction with GNE781. Future research endeavors could delve deeper into exploring additional dosages that might further potentiate synergy in terms of MFI expression and cytotoxicity. Additionally, it would be valuable to investigate the effects of these drug combinations on other types of cell lines, broadening our understanding of their potential applications.

References

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