Identification of an effective ribonucleoside analogue and immunomodulatory imide drug (IMiD) combination for Multiple Myeloma

Weijie Wang[1,2], Lissa Hooi[1,2], Widanalage Sanjay Prasad De Mel[3], Wee Joo Chng[1,3], Steven T. Rosen[4], Dean Ho[5,6], Edward Kai-Hua Chow[1,2,5]

[1.] Cancer Science Institute of Singapore; [2.] Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore; [3.] Department of Haematology-Oncology, Nation University Cancer Institute, Singapore, National University Health System, Singapore; [4.] City of Hope, USA; [5.] Department of Bioengineering, National University of Singapore; [6.] The N.1 Institute for Health, National University of Singapore

Overview

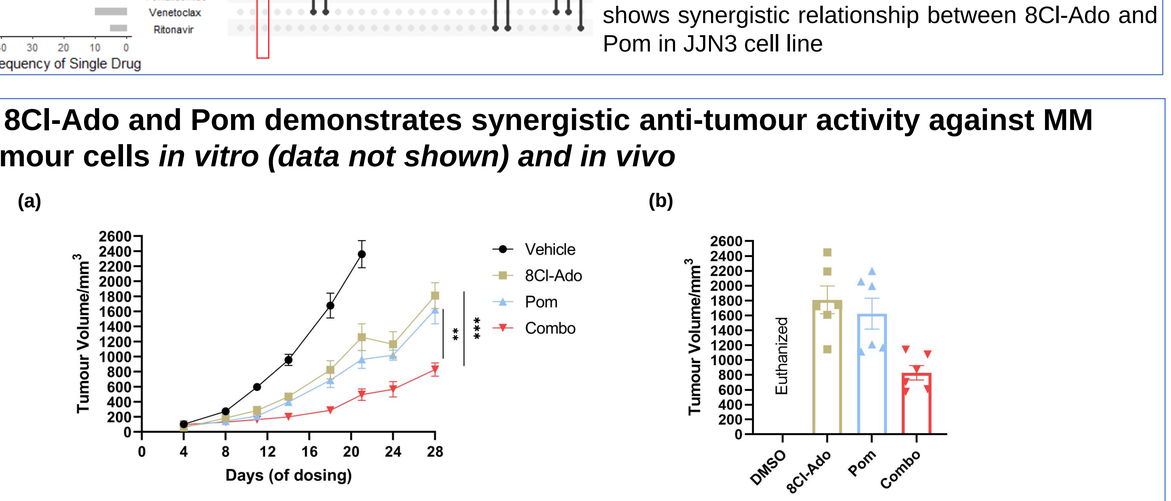
- We applied quadratic phenotypic optimisation platform (QPOP), a phenotypic-analytical hybrid multidrug interrogation platform developed by our lab to identify 8-Chloroadenosine and Pomalidomide as an effective novel drug combination against Multiple myeloma.
- The synergy of this combination has been validated *in vitro and in vivo.*
- This combination works potentially through:
- Downregulation of c-Myc and its downstream targets
- Alteration of the metabolic profiles, particularly glutamine metabolism
- Stimulation of release of reactive oxygen species (ROS) and induction of apoptosis
- This study also reinforces the idea of targeting MM through inhibition of glutamine uptake, which has been implicated in several published literature

Introduction

Multiple myeloma (MM) is a cancer disease characterised by malignant plasma cells, a type of white blood cells secreting immunoglobulins. It accounts for 10% of all haematological malignancies, ranked the second most common blood cancers only to Non-Hodgkin's Lymphoma worldwide.[1]

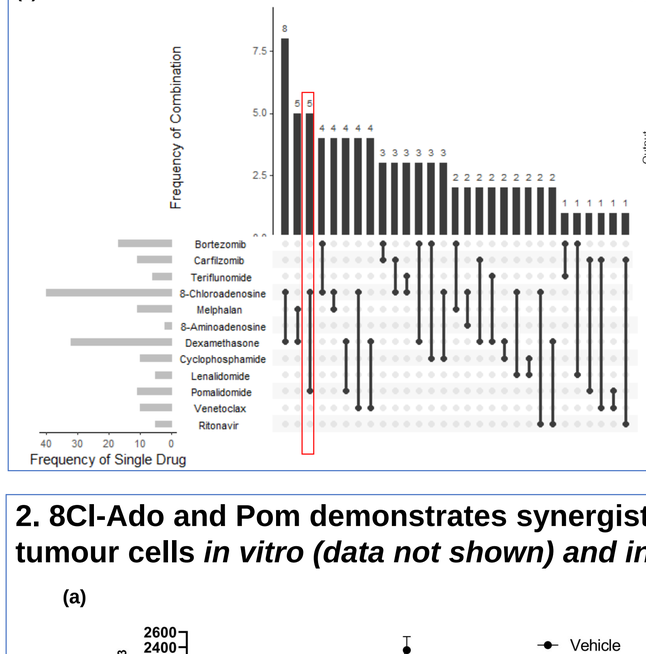
Despite the advance in treatment strategies, MM is still an incurable disease, with no established curative treatments available. One main issue associated with this is the development of resistance to the current therapeutic approach. An effective strategy for overcoming drug resistance is to use combination therapy. Rapid advance in existing classes of treatments as well as the emergence of novel therapeutic approach has provided a large number of potential drug candidates to be used as combinatory therapies in second-line treatments.[2] Hence, out study aims to **(1)** apply a small dataset rational design approach (Quadratic Phenotypic Optimization Platform, QPOP)[3,4] to identify effective drug-combinations in MM, **(2)** validate their efficacy *in vitro* and *in vivo*, and to **(3)** interrogate the molecular mechanisms underlying the selected drug combination in MM.

Methods



Results

1. QPOP identifies 8-Chloroadenosine (8Cl-Ado) and Pomalidomide (Pom) as a top-ranking drug combination against MM cells *in vitro*



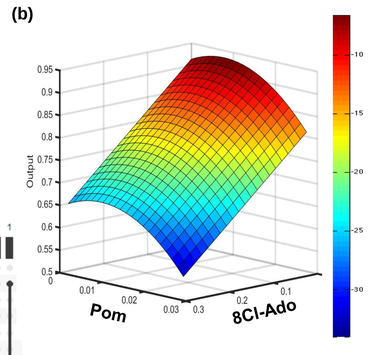


Figure 1. (a) Frequency of the drugs and combinations appearing in the top 20 ranked two-drug combinations across 4 MM cell lines. 8Cl-Ado+Pom ranks the 2nd among all combinations in the table.

(b) QPOP-derived parabolic response surface map shows synergistic relationship between 8Cl-Ado and Pom in JJN3 cell line

2. 8Cl-Ado and Pom demonstrates synergistic anti-tumour activity against MM tumour cells *in vitro (data not shown) and in vivo*

Figure 2. (a) Tumour volumes and **(b)** end volume of Vehicle, 8Cl-Ado, Pom and Combo in H929 xenograft NSG mice (n=6); vehicle control was euthanized on Day 21 and all other groups were euthanized on Day 28. ***p<0.01, ***p<0.001*

3. Combination treatment of 8Cl-Ado and Pom leads to increased apoptosis

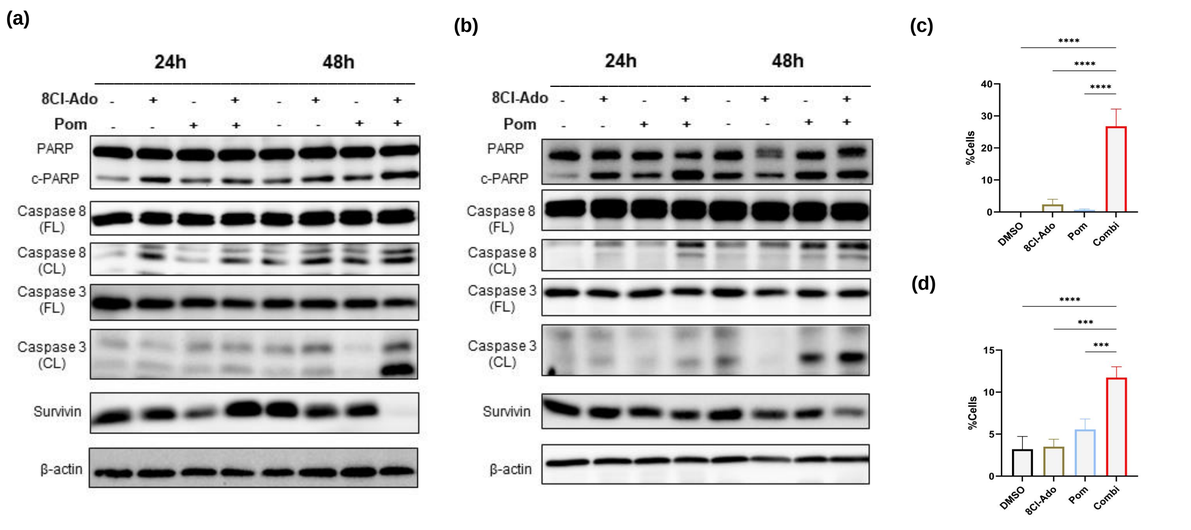


Figure 3. Immunoblots of **(a)** H929 and **(b)** JJN3 show increased activation of apoptotic markers and downregulation of the anti-apoptotic protein survivin. Increased proportion of apoptotic cells in **(c)** H929 and **(d)** JJN3 is detected by Annexin V/PI staining at 72h. **p<0.05, **p<0.01, ***p<0.001, ****p<0.0001*

Results (continued)

4. Combination treatment of 8Cl-Ado and Pom exerts their anti-myeloma effects through c-Myc downregulation

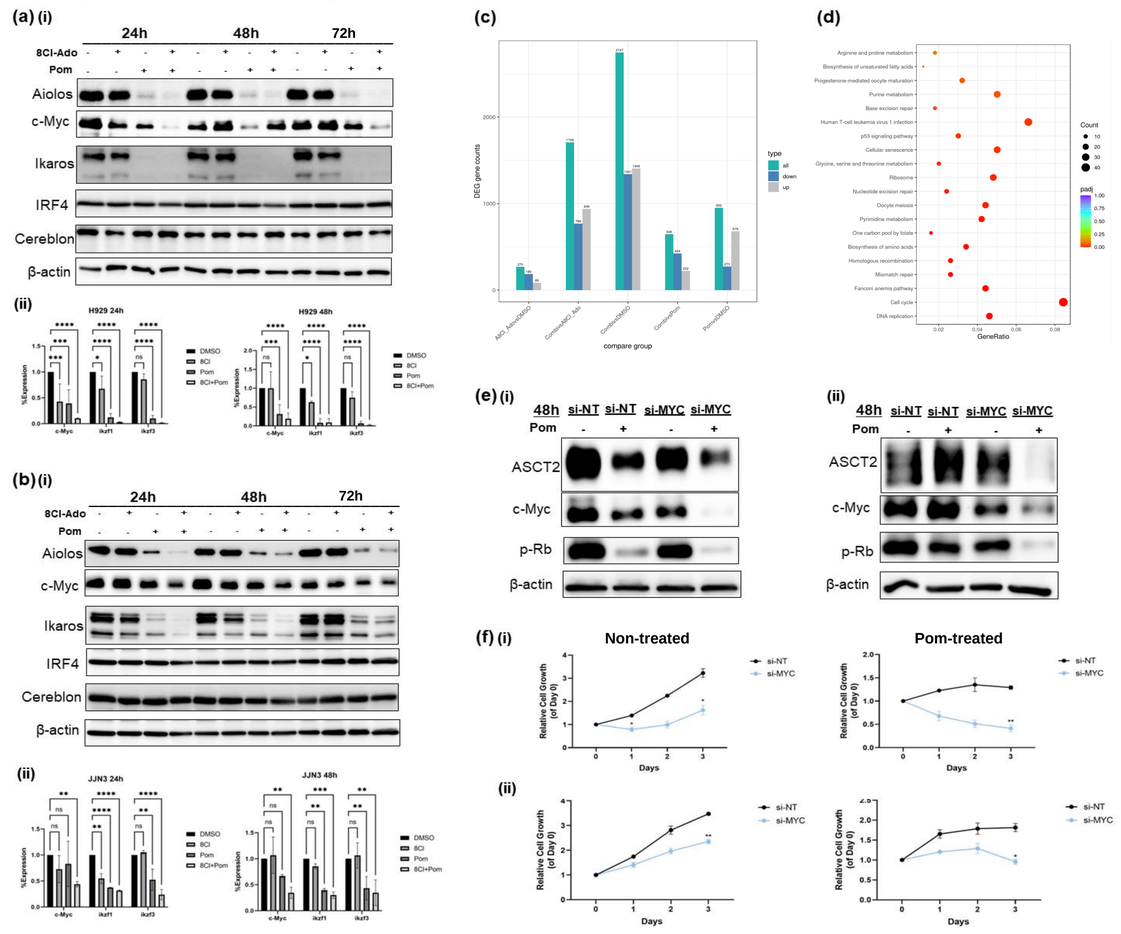


Figure 4. Immunoblots of **(a)(i)** H929 and **(b)(i)** JJN3 and densitometric quantification of immunoblots (**(ii) and (iv)**) confirm the downregulation of the CRBN-IKZF signalling pathways, leading to significant downregulation c-Myc. **(c)** Combination treatment induces greater transcriptional change in terms of differentially-expressed genes. **(d)** KEGG enrichment analysis reveals the combination treatment induces transcriptional alterations in a c-Myc-regulated pathways. **(e)** Transient silencing of c-Myc in **(i)** H929 and **(ii)** JJN3 enhances the effect of Pom, mimicking the synergistic effects of 8Cl-Ado+Pom. **(e)** Transient silencing of c-Myc suppresses proliferation of **(i)** H929 (non-treated and Pom-treated) **(ii)** JJN3 (non-treated and Pom-treated) . **p<0.05, **p<0.01, ***p<0.001, ****p<0.0001*

5. Combination treatment of 8Cl-Ado and Pom alters the metabolic profiles of MM cells

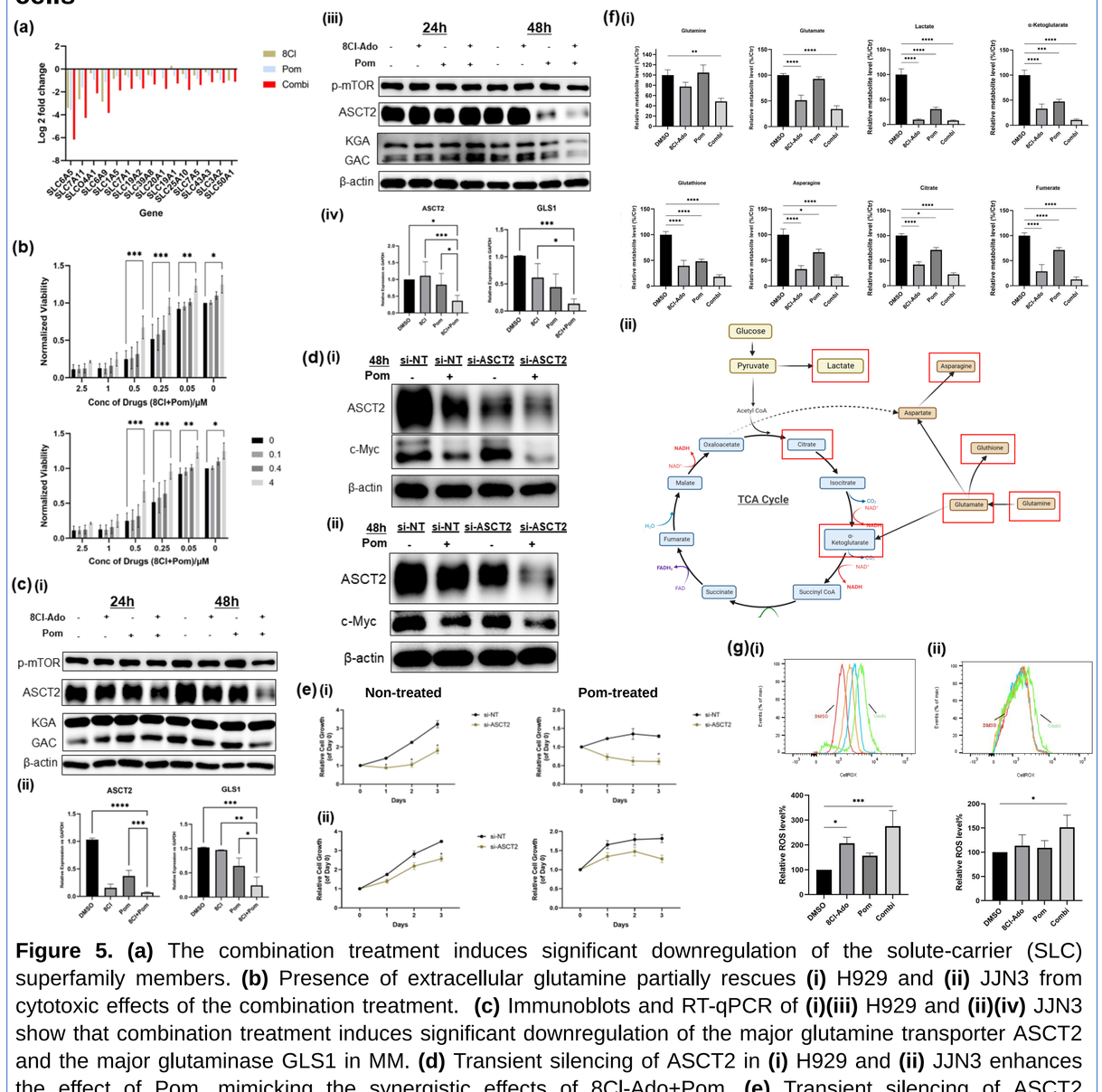


Figure 5. (a) The combination treatment induces significant downregulation of the solute-carrier (SLC) superfamily members. **(b)** Presence of extracellular glutamine partially rescues **(i)** H929 and **(ii)** JJN3 from cytotoxic effects of the combination treatment. **(c)** Immunoblots and RT-qPCR of **(i)(iii)** H929 and **(ii)(iv)** JJN3 show that combination treatment induces significant downregulation of the major glutamine transporter ASCT2 and the major glutaminase GLS1 in MM. **(d)** Transient silencing of ASCT2 in **(i)** H929 and **(ii)** JJN3 enhances the effect of Pom, mimicking the synergistic effects of 8Cl-Ado+Pom. **(e)** Transient silencing of ASCT2 suppresses proliferation of **(i)** H929, and Pom-treated H929, **(ii)** JJN3 and Pom-treated JJN3. **(f)(i)** LC-MS analysis reveals that the combination treatment reduces the cellular metabolic activities significantly in H929. **(ii)** Diagrammatic illustration of metabolites altered by the combination treatment in H929. **(g)**Combination treatment increases intracellular reactive oxygen species (ROS) level in (**i**) H929 and **(ii)** JJN3, which could be the concomitant result of downregulation of the antioxidant glutathione. **p<0.05, **p<0.01, ***p<0.001, ****p<0.0001*

Conclusions

- QPOP analysis identifies that 8Cl-Ado and Pom work synergistically against MM *in vitro* and *in vivo*
- This novel combination induces apoptosis and suppresses proliferation via c-Myc downregulation, leading to subsequent downregulation of pathways regulated by this transcription factor
- Combination treatment alters the metabolic profiles in MM cells, particularly suppressing glutamine uptake and metabolism. As ASCT2 and GLS1 are transcriptionally regulated by c-Myc, these alterations are potentially the concomitant results of upstream c-Myc degradation
- Future work : Comparison of expression of selective markers (e.g. Ki67, c-Myc, cleaved caspases, ASCT2) *in vivo*; Incorporation of more MM patient samples to identify specific biomarkers for this combination; Construction of Splice-switch oligonucleotide (SSO) to test *in vitro* and *in vivo*

References

1.Ludwig, Heinz et al. *The oncologist* vol. 25,9 (2020): e1406-e1413.
2.Bayat Mokhtari, Reza et al. *Oncotarget* vol. 8,23 (2017): 38022-38043.
3. Rashid, Masturah Bte Mohd Abdul et al *Science translational medicine* vol. 10,453 (2018): eaan0941.
4. Goh, Jasmine et al. *Science translational medicine* vol. 14,667 (2022): eabn7824.