Combination Therapies Targeting Apoptosis Pathways in Paediatric AML

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INTRODUCTION

- It has been highlighted recently the need for age-specific therapies for AML patients, with paediatric AML having a different mutational landscape compared with adult AML[1,2].
- Using the **TARGET AML** transcriptomic data, the group identified gene expression anomalies in the molecular function cell death and survival pathways particularly in AML patients who subsequently relapsed.

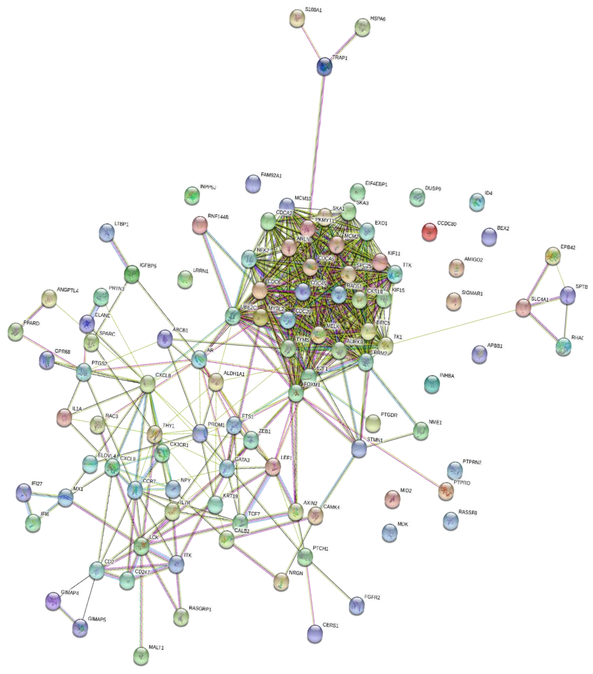


Figure 1: Pathways identified as deregulated using Ingenuity Pathway Analysis in AML patients..

The interactions of the genes provide evidence that the apoptotic pathways are deregulated

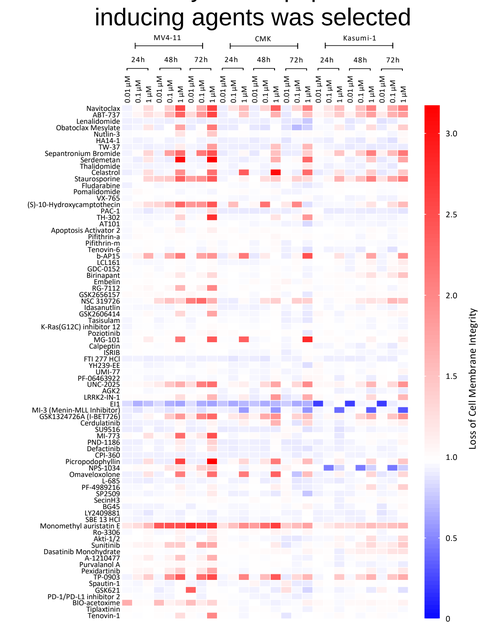
AIMS

Using drug-repurposing strategy, we aim to identify novel combination therapies with the promise of providing alternative more effective and less toxic induction therapy options.

METHODS AND MATERIALS

Single agent Screen

Library of 83 apoptotic-inducing agents was selected



0.1 μM as the optimum dose to be taken forward for the combination screen.

Combination Screen

Using all-pairs testing algorithm, 83 library compounds were tested by using ten compounds per well (over 160 wells).

Well-155

Each of the ten compounds in had minimal or no effect as single agents on the viability of either cell line, however, a combination of two or more of the compounds resulted in a substantial increase in cell death.

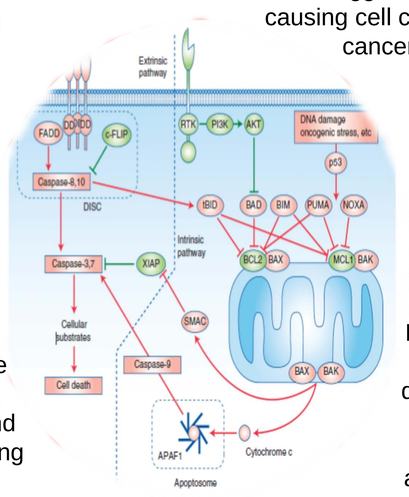
The 10 compounds were de-convoluted to identify a possible synergistic pair/triple combinations.

Mechanism of 10 Apoptotic Drugs

Akti-1/2 AKT inhibitor

LY294002 PI3K inhibitor

Nutlin-3 and MI-773
Restore p53 function by binding to MDM2, to block its interaction with p53



Purvalanol A
Is a specific CDK inhibitor which triggers apoptosis by causing cell cycle arrest in cancer cells

Poziotinib and SecinH3
Target EGFR

ABT-737
BCL-2 (BH3) inhibitor, disrupts BCL-2/BAX and induces apoptosis via the intrinsic apoptotic pathway

SU9516
Induces mitochondrial injury and cytochrome c release, and apoptosis in association with pronounced down-regulation of the antiapoptotic protein Mcl-1

RESULTS

Single agent Screen

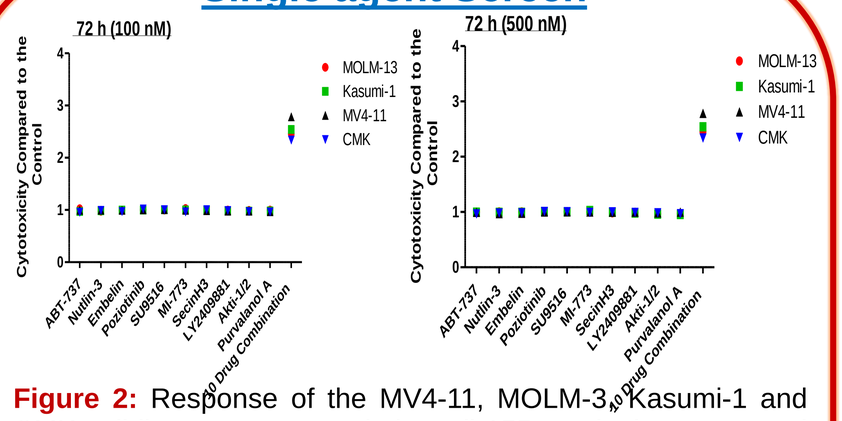


Figure 2: Response of the MV4-11, MOLM-3, Kasumi-1 and CMK cell lines to compounds in well-155 as single agents and in the 10-drug combination.

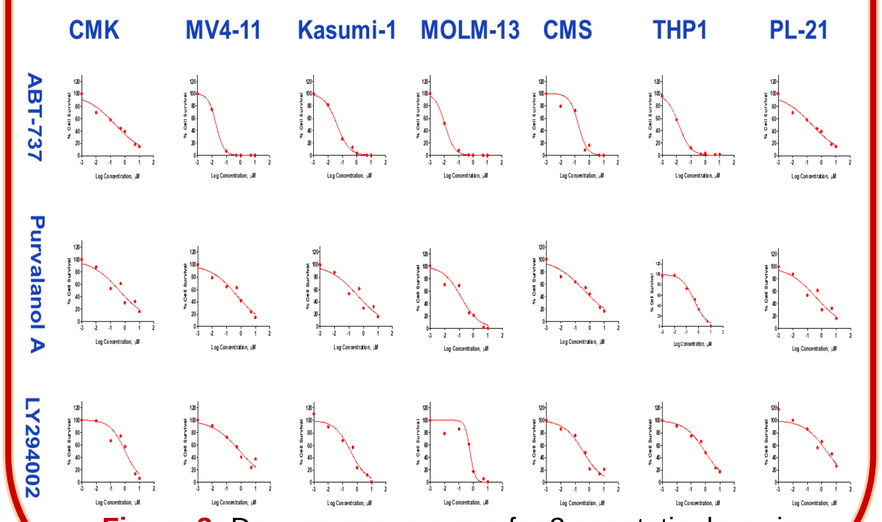


Figure 3: Dose response curve for 3 apoptotic drugs in 7 paediatric cell lines in order to determine IC20 values

Double agent Screen

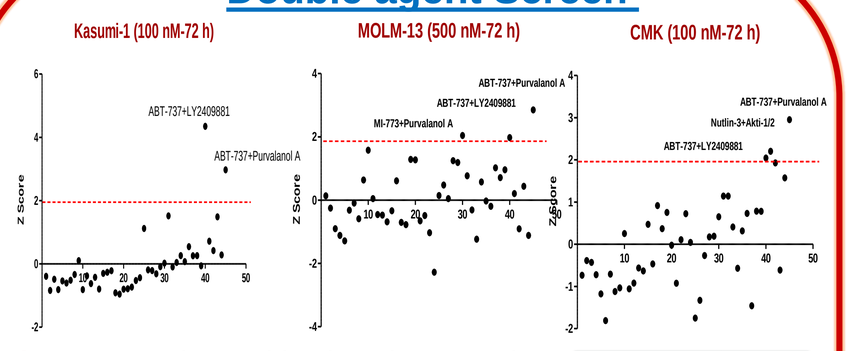


Figure 4: Pair combination therapy of 10 apoptotic drugs in Kasumi-1, MOLM-13, and CMK cells after 72 h. A well was considered 'successful' if each drug individually demonstrated a Z-Score <2, while the combination exhibited a Z-Score >2.

Double Combinations	Combination Index
ABT-737+Purvalanol	0.0643
ABT-737+LY2409881	0.1973
ABT-737+Poziotinib	0.6421

Triple agent Screen

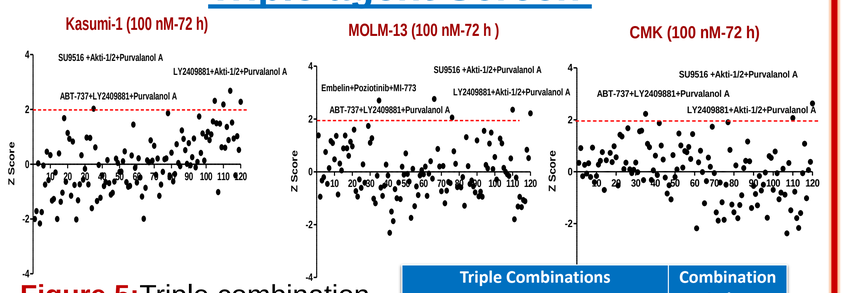


Figure 5: Triple combination therapy of 10 apoptotic drugs in CMK Kasumi-1, and MOLM-13 cells after 72 h.
A well was considered 'successful' if each drug individually demonstrated a Z-Score <2, while the combination exhibited a Z-Score >2.

Triple Combinations	Combination Index
ABT-737+LY2409881+Purvalanol A	0.1547
SU9516 +Akti-1/2+Purvalanol A	0.1585
LY2409881+Akti-1/2+Purvalanol A	0.2632

CONCLUSIONS

- The screen identified two possible 'novel' drug pairing, with BCL2 inhibitor ABT-737, combined with either a CDK inhibitor Purvalanol A, or AKT/ PI3K inhibitor LY294002. **(ABT-737+ Purvalanol A) (ABT-737+ LY294002).**
- Three possible triple combinations were identified **(LY2409881+Akti-1/2+Purvalanol A, SU9516+Akti-1/2+Purvalanol A, and ABT-737+LY2409881+Purvalanol A)**, which will be taken forward for examining their efficacy at varying concentrations and dosing schedules, across multiple paediatric AML cell lines for optimisation of maximum synergy.
- Our combinations showed interesting therapeutic potential, which to our knowledge has not been reported by other groups investigating these drug pairings. We believe that our combination screening approach has potential for future use with a larger cohort of drugs including FDA approved compounds and patient material.

REFERENCES

1.Bolouri H, Farrar JE, Triche T Jr, *et al*. The molecular landscape of pediatric acute myeloid leukemia reveals recurrent structural alterations and age-specific mutational interactions. Nature Medicine, 2018.
2.Young CS, Clarke KM, *et al*. Decitabine-Vorinostat combination treatment in acute myeloid leukemia activates pathways with potential for novel triple therapy. Oncotarget, 2017.

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