Dual Induction of Mitochondrial Ferroptosis and Apoptosis in Acute Myeloid Leukemia

Kazuharu Kamachi[1], Hiroki Akiyama[1,2], Zhao Ran[1], Zhang Liang[1], Saurabh Kumar Gupta[1], Lauren B. Ostermann[1], Michael Andreeff[1], Jo Ishizawa[1]

1. Section of Molecular Hematology and Therapy, Department of Leukemia, University of Texas MD Anderson Cancer Center, Houston, TX
2. Department of Hematology, Institute of Science Tokyo, Tokyo, Japan

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Introduction

-**Apoptosis resistance** is a major driver of relapsed/refractory AML. Targeting non-apoptotic forms of regulated cell death offers the potential to overcome this resistance.

-**Ferroptosis** is characterized by iron-dependent lipid peroxidation (LP), primarily suppressed by **GPX4**. In AML, ferroptosis uniquely relies on mitochondrial LP, termed "**mitochondrial ferroptosis**" (Leukemia 2024[1]).

-Both apoptosis and ferroptosis are regulated by mitochondria in AML cells, however, **the molecular crosstalk between ferroptosis and apoptosis** remains largely unexplored.

Aim

To investigate the anti-AML efficacy and molecular interactions of dual induction of ferroptosis and apoptosis, a process we define as "Ferro-Apoptosis".

GPX4-i + BCL2-i → "Ferro-Apoptosis"

GPX4 inhibition → **Apoptosis ?**

Ferroptosis ? ← **BCL2 inhibition**

Methods

[Reagents]
GPX4 specific inhibitor; ML210 , BCL2 specific inhibitor; Venetoclax (VEN), Lipophilic antioxidants; α-tocoperol (αToc), Ferroptosis specific inhibitor; Ferrostatin-1 (Fer-1), Mitochondrial antioxidants; MitoQ

[Cells]
MOLM13 (VEN-sensitive, VEN-S), OCI-AML3 (VEN-resistant, VEN-R), Mouse Embryonic Fibroblast (MEF); Wild type (WT), BAX/BAK double knock out (DKO), Primary AML patient samples

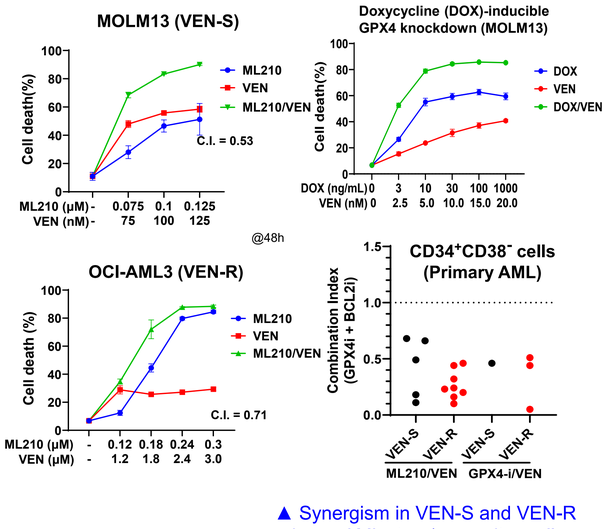
[Flow cytometry]
AnnexinV/DAPI cell death assay, C11 BODIPY581/591 lipid peroxidation assay, Mitochondrial cytochrome c (CytC) detection[2]

[Western blot]
Cleaved-caspase 3 (c-Casp3), β-actin

[Glutathione (GSH) Quantification]
GSSG/GSH Quantification Kit (Dojindo)

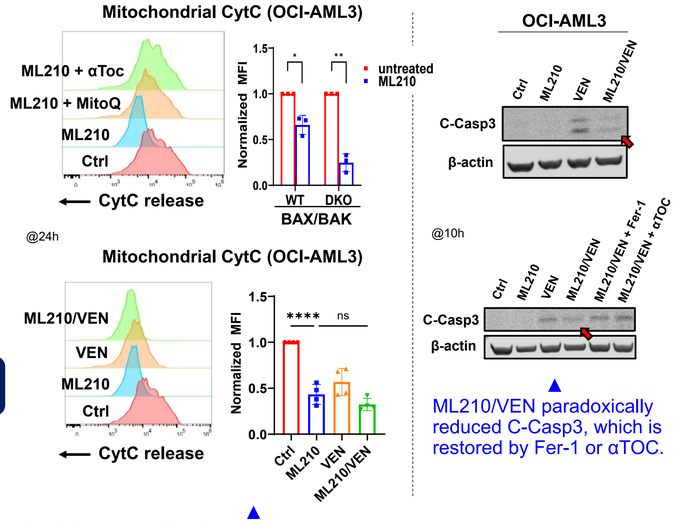
Results

Synergistic Cell Death by "ferro-apoptosis" in AML, regardless of VEN-sensitivity



▲ Synergism in VEN-S and VEN-R primary AML stem/progenitor cells

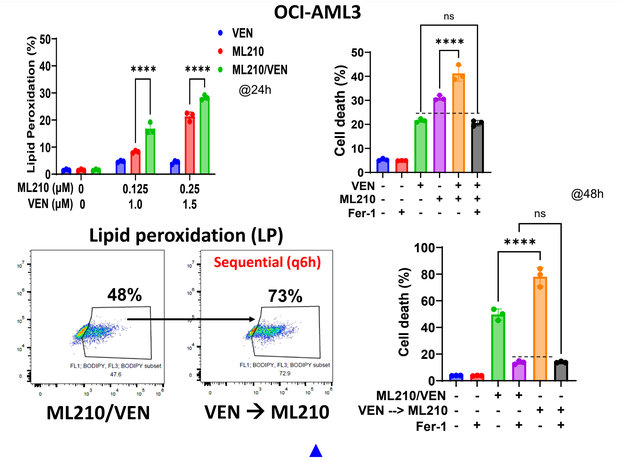
GPX4 Inhibition Suppresses Apoptosis, while Releasing Cytochrome C



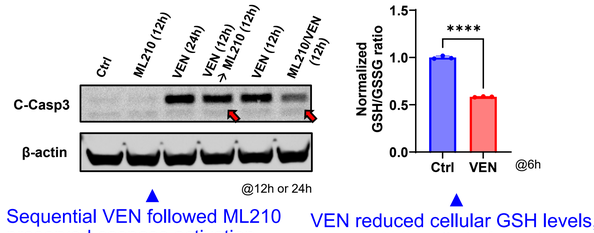
▲ ML210/VEN paradoxically reduced C-Casp3, which is restored by Fer-1 or αTOC.

▲ ML210 induced BAX/BAK-independent CytC release.
ML210/Ven did not further increase CytC release.

BCL2 Inhibition Primes AML to a Pro-ferroptotic State: Sequential Strategy (Apoptosis → Ferroptosis) Works



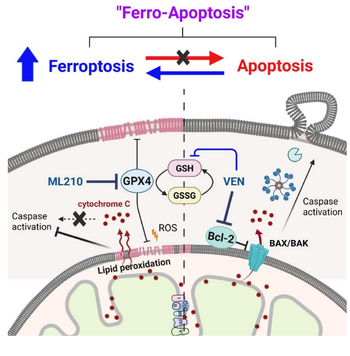
▲ ML210/VEN enhanced LP and cell death; sequential VEN → ML210 further enhanced LP and cell death, which was completely blocked by Fer-1.



▲ Sequential VEN followed ML210 preserved caspase activation.

▲ VEN reduced cellular GSH levels, priming AML cells to ferroptosis.

Summary



Uni-directional Synergy in "Ferro-Apoptosis"

-GPX4 inhibition suppresses apoptosis

-BCL2 inhibition enhances ferroptosis

-Sequential induction of apoptosis followed by ferroptosis maximizes ferro-apoptosis

References

[1] Akiyama H., et al. Leukemia. 2024 Apr;38(4):729-740
[2] Thijssen, R., et al. Blood, 2021;137(20), 2721–2735.

Acknowledgements

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