Investigating the potential of EO3001 as a therapeutic agent for clear cell ovarian cancers harboring ARID1A mutations

The Pulmonary Metastasis Assay (PuMA): A Tool to Study Novel Therapies

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INTRODUCTION

Clear cell ovarian cancer (CCOC) is a chemoresistant subtype of ovarian cancer, accounting for approximately 10% of all epithelial ovarian carcinoma (EOC) cases in North America and up to 30% of EOC cases in Japan. Recent research underscores the significance of hematogenous spread in ovarian cancer metastasis, a factor previously neglected due to the absence of suitable models for vascular ovarian cancer metastasis. **ARID1A** inactivation has been identified as a key oncogenic event (65%) in CCOC, playing a pivotal role in CCOC development. Recent studies showed that ARID1A-mutated cancer cells heavily rely on oxidative phosphorylation (OXPHOS) for energy.[1]

EO3001, a synthetic drug studied in various cancers, selectively transports Cu(II) to mitochondria, where it accumulates and induces mitochondrial ROS, which triggers cuproptosis. Recent studies indicate heightened sensitivity of ARID1A-deficient cells to EO3001. ***(Figure 1)***

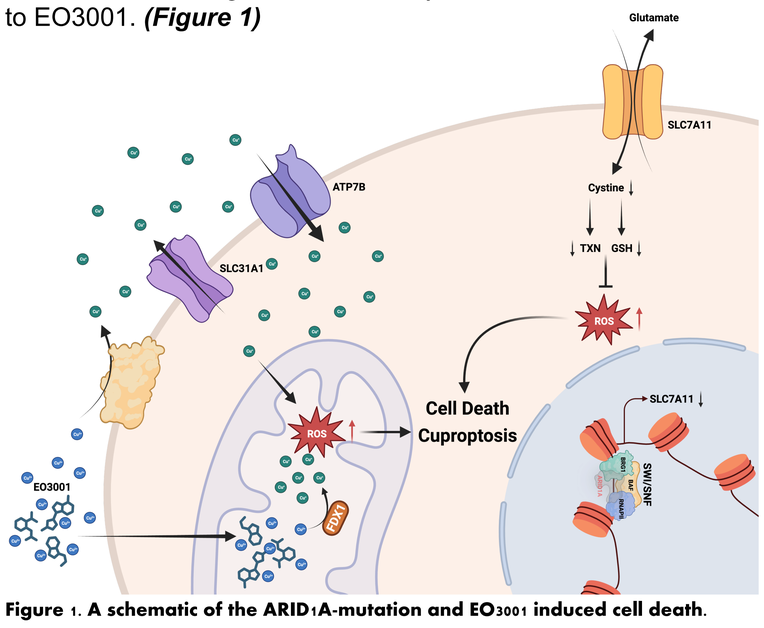

Figure 1. A schematic of the ARID1A-mutation and EO3001 induced cell death.

Nowadays, a lot of *in vitro* and *in vivo models* have been developed for cancer researches. In-vitro studies, which use cells derived from humans or animals in 2D and 3D cultures, are relatively cheap and easy to access but fail to capture the complexity of organ systems and the appropriate microenvironment, making them less translatable to humans. In contrast, in-vivo studies with animal models address these shortcomings by providing a more complex biological environment, but they are more time and resource-intensive and raise significant ethical concerns.

The Pulmonary Metastasis Assay (PuMA), originally designed for sarcomas, offers a more biologically relevant environment than traditional 2D and 3D culture models for studying the underlying biology and therapy response of CCOC, and addressing the gaps left by earlier models.[2] ***(Figure 2)***

Method	2D Culture	3D Culture (Organoid)	Animal	Pulmonary Metastasis Assay (PuMA)
Strength	Low Cost Easy to Access High throughput	Cell Heterogeneity Cell communication Microenvironment	Real physiology Cell Heterogeneity Metastasis Complex ECM	Medium Cost Easy to Access Metastasis Cell Heterogeneity
Weakness	Defined ECM Microenvironment Metastasis	Medium-High Cost Microenvironment Low throughput Metastasis	Very High Cost Extra Training Ethical Problems Extra Care Low throughput	Ethical Problems

Figure 2. A Comparison of Models Currently Used in Cancer Research.

REFERENCE

1. Cochrane, D. R. et al. Clear cell and endometrioid carcinomas: are their differences attributable to distinct cells of origin? J Pathol 243, 26-36, doi:10.1002/path.4934 (2017).
2. Au - Lizardo, M. M. & Au - Sorensen, P. H. Practical Considerations in Studying Metastatic Lung Colonization in Osteosarcoma Using the Pulmonary Metastasis Assay. JoVE, e56332, doi: 10.3791/56332 (2018).

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MATERIALS AND METHODS

Cell Line

1. Generation of fluorescent tagged RMG1 and JHOC5 cell lines -/+ mutant ARID1A using CRISPR/Cas9.
2. *In vitro* Assays to study the EO3001 drug toxicity on different cell lines with +/- ARID1A.

The Pulmonary Metastasis Assay (PuMA) Early-Harvesting vs. Late-Harvesting

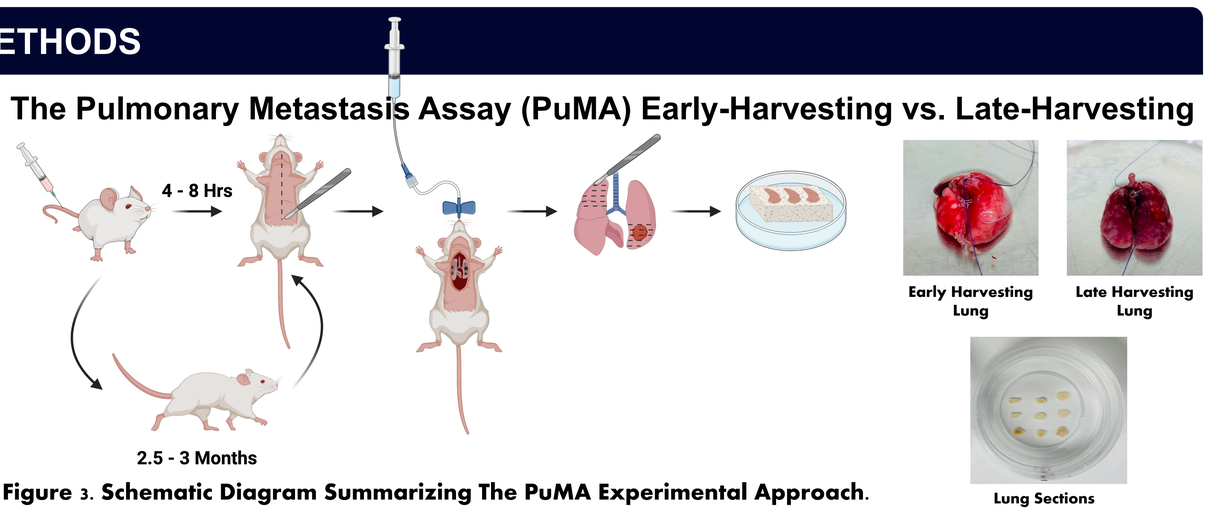

Figure 3. Schematic Diagram Summarizing The PuMA Experimental Approach.

CCOC cells with fluorescent tags were injected into mice through tail vein injection and allowed to settle for hours to days.
The lung was then inflated with special agarose gel dissolved in media, solidified, and sectioned into 3 * 4 mm pieces.
The lung sections were grown on sponge soaked with PneumaCult™ media and treated with either vehicle or EO3001 for up to 21 days, fluorescent metastatic cells were able to interact with this framework and subsequently develop into metastatic colonies, and the growth of cancer cells was visualized using epifluorescence or confocal microscopy. ***(Figure 3)***

RESULTS AND DISCUSSION

1.1 PuMA: a promising model developed and optimized for studying CCOC cell lines ex-vivo

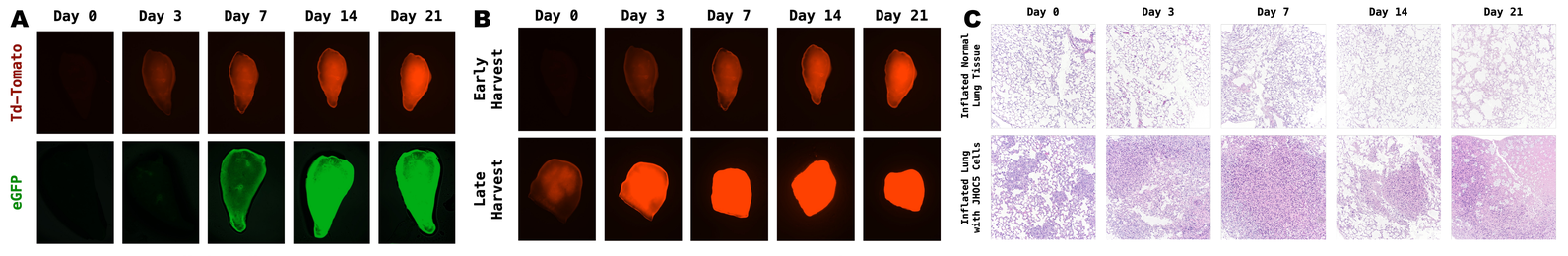

Figure 4.1. RMG1 cells labelled with Td-Tomato and eGFP in PuMA (A); A Comparison of Early-Harvesting and Late-Harvesting PuMA in RMG1 and JHOC5 (B); H&E Staining of Normal Inflated Lung Tissue and Late-Harvesting PuMA with JHOC5-Td-Tomato (C & D).

1.2 ARID1A-mutant CCOC cell lines are selectively, and significantly, more sensitive to EO3001 *in vitro*, under ambient conditions, with a lesser effects observed under hypoxia.

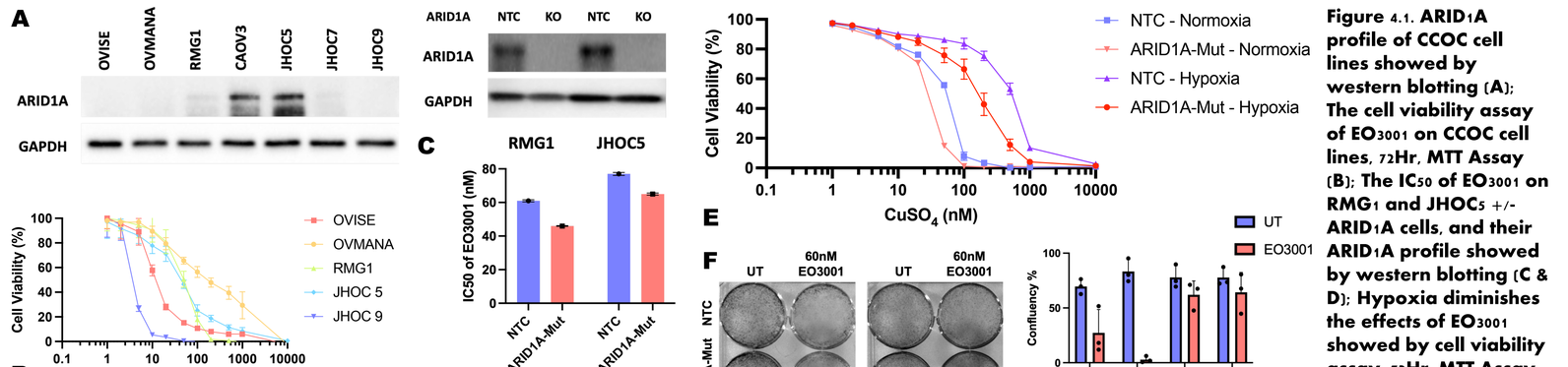

Figure 4.1. ARID1A profile of CCOC cell lines showed by western blotting (A); The cell viability assay of EO3001 on CCOC cell lines, 72Hr, MTT Assay (B); The IC50 of EO3001 on RMG1 and JHOC5 +/- ARID1A cells, and their ARID1A profile showed by western blotting (C & D); Hypoxia diminishes the effects of EO3001 showed by cell viability assay, 72Hr, MTT Assay (E), Crystal Violet Assay and its normalization (F & G).

1.3 ARID1A-Mut CCOC cell lines grown in PuMA show higher sensitivity to EO3001.

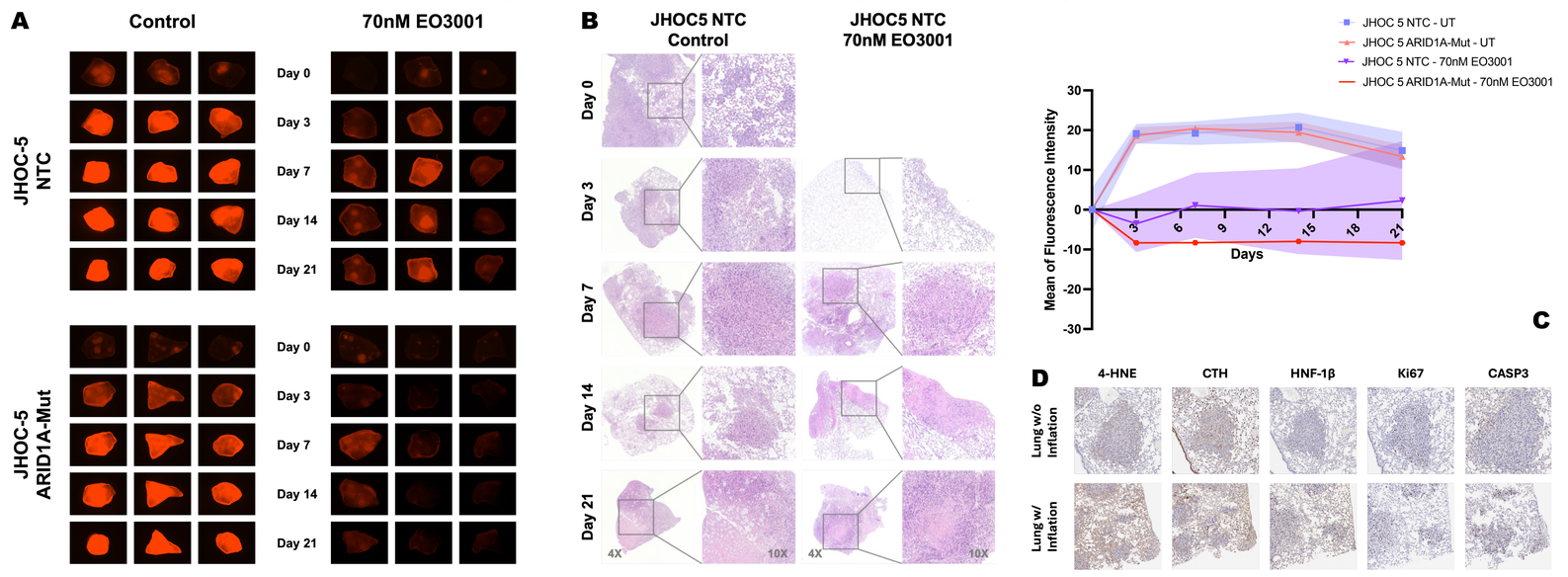

Figure 4.2. The growth of JHOC5 +/- ARID1A cells in the PuMA model over time showed by serial images, 3 months post injection, NRG mice (A) and the quantification by the mean of the fluorescence intensity (C); H&E staining of NTC Un-Treated and EO3001 Treated Lung Sections in serial images (B); 4-HNE, CTH, HNF1β, Ki-67, Cleaved Caspase 3 - IHC stained sections from the PuMA assay (D).

CONCLUSION

EO3001 has demonstrated promising therapeutic potential in ARID1A-deficient cells, both in vitro and ex vivo. However, hypoxia significantly diminishes the efficacy of EO3001, potentially due to alterations in glycolysis and oxidative phosphorylation (OXPHOS). By targeting the dependence of ARID1A-deficient clear cell ovarian carcinoma (CCOC) on OXPHOS, EO3001 could offer a viable therapeutic approach.
In addition to its therapeutic potential, EO3001's effects have been studied using PuMA, a robust ex vivo model. Early-harvesting PuMA provides comprehensive insights, but late-harvesting PuMA stands out due to its enhanced visualizability, ease of quantification, minimal optimization requirements, and broader applicability. This versatility and efficiency make late-harvesting PuMA an excellent choice for diverse research domains.

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