

Introduction

Methylation-induced silencing of gene promoters is a key mechanism in numerous cancer types, and the anti-cancer activity of agents that modulate DNA methylation has been evaluated in preclinical and clinical settings. In this study, we investigated the effects of a combination regimen of 5-fluorodeoxycytidine (FdC) and Tetrahydrouridine (THU) on tumor cell growth by inhibiting DNA methyltransferase 1 (DNMT1) and decreasing LINE1 promoter methylation.

Materials and Methods

**Patients and Sample Collection**

- All enrolled patients and healthy donors gave informed consent for study participation and were enrolled using institutional review board-approved protocols.
- Patients received treatment in clinical trials at the U.S. Army Institute of Infectious Diseases (USAMRIID), Bethesda, MD; Stanford University Hospital, Stanford, CA; and the University of California, San Diego, CA.
- Cytokine gene promoter methylation was analyzed in patient samples collected from the clinical trials.

**Cell Lines**

- Bladder cancer cell line EJ6 and breast cancer cell line MDA-MB-231 were grown in DMEM or RPMI 1640 supplemented with 10% FCS at 37°C.
- Epstein-Barr virus-transformed B cells (negative control) were obtained from the American Type Culture Collection (ATCC). Jurkat cells (positive control) were obtained from the ATCC.

**Sample Preparation**

- Cells were collected and isolated from paracentesis specimens from patients enrolled on a Phase 1/2 clinical trial.
- DNA was isolated from the samples using the EZ DNA Methylation Kit (Zymo Research) and bisulfite converted using the EZ DNA Methylation Kit (Zymo Research).
- Real-time PCR was performed to analyze LINE1 methylation.

**Immunohistochemistry**

- Paraffin-embedded tumor tissues were stained with anti-p16 antibody and analyzed using the CellTracks® Analyzer II system.
- Images were captured and automatically presented in gallery format.

**CTC Enumeration and Identification of p16-positive CTCs in Patients’ Blood**

- Blood samples were collected into CellSave® tubes (Veridex) and processed within 96 hr.
- CTCs were enumerated and identified using the CellSearch System.

**Discussion**

- The combination of FdC and THU demonstrated cytotoxicity and allowed sufficient time for demethylation to occur.
- Decitabine was used as the positive control.

**Conclusion**

- The combination of FdC and THU was effective in inhibiting tumor cell growth and decreasing LINE1 promoter methylation.
- Further studies are needed to evaluate the efficacy and safety of this combination regimen in clinical settings.