Epithelial ovarian carcinoma is the most lethal gynecological cancer due to its silent onset and recurrence with resistance to chemotherapy. Overexpression of oncogene c-Myc is one of the most frequently encountered events present in ovarian carcinoma. Disrupting the function of c-Myc and its downstream targets is a promising strategy for cancer therapy. In this work, we aimed to evaluate the potential effects of small molecule c-Myc inhibitor, 10058-F4, on ovarian cancer cells and the underlying mechanisms by which 10058-F4 exerts its actions. Using Cellometer image cytometry for cell cycle and Annexin V apoptosis assays, flow cytometry, MTT assay, and colony formation, we found that 10058-F4 significantly inhibited cell proliferation of both SKOV3 and Hey ovarian cancer cells in a dose dependent manner through induction of apoptosis and cell cycle G1 arrest. Treatment with 10058-F4 reduced cellular ATP production and ROS levels in SKOV3 and Hey cells. Consistently, primary cultures of ovarian cancer treated with 10058-F4 showed induction of caspase-3 activity and inhibition of cell proliferation in 15 of 18 cases. These novel findings suggest that targeting c-Myc-Max heterodimerization could be a potential therapeutic strategy for ovarian cancer.

**1. ABSTRACT**

**2. CELLOMETER IMAGE CYTOMETRY METHOD**

- Pipette 20 µL of sample into disposable counting chamber
- Insert chamber in Cellometer

**3. IMAGE CYTOMETRIC ANALYSIS OF CELL CYCLE**

- Bright-field image
- Propidium iodide fluorescence image

**Cell Cycle Analysis**

- The Hey and SKOV3 cells are treated with different dosages of 10058-F4 for 24 hours
- The cells are collected and washed, and then resuspended in Annexin V binding buffer
- The supernatant is replaced with Annexin V-FITC and PI from the Nexcelom Apoptosis kit
- The cells are allowed to incubate for 15 min in the dark before Cellometer Vision image cytometric analysis
- The results are exported to FCS Express for cell population analysis

**4. IMAGE CYTOMETRIC ANALYSIS OF APOPTOSIS**

**Increasing Dosage**

**Apoptosis Analysis**

- The Hey and SKOV3 cells are treated with different dosages of 10058-F4 for 24 hours
- The cells are collected and washed, and then resuspended in Annexin V binding buffer
- The supernatant is replaced with Annexin V-FITC and PI from the Nexcelom Apoptosis kit
- The cells are allowed to incubate for 15 min in the dark before Cellometer Vision image cytometric analysis
- The results are exported to FCS Express for cell population analysis

**5. EFFECT OF 10058-F4 ON HEY AND SKOV3 CELL CYCLE USING CELLOMETER VISION**

**Hey**

- The results showed that 10058-F4 induced cell cycle arrest in G0/G1 phase for both Hey and SKOV3 cells
- The Hey cell cycle G0/G1 phase increased from 45.36% to 56.78%, and the SKOV3 G0/G1 cell population increased from 46.87% to 61.80%, where the 50 µM showed the highest arrest
- The results indicated that 10058-F4 has the ability to arrest the growth of Hey and SKOV3 cells by inhibiting c-Myc, which inhibited the proliferation of the cancer cells

**SKOV3**

- The results showed that 10058-F4 induced cell cycle arrest in G0/G1 phase for both Hey and SKOV3 cells
- The Hey cell cycle G0/G1 phase increased from 45.36% to 56.78%, and the SKOV3 G0/G1 cell population increased from 46.87% to 61.80%, where the 50 µM showed the highest arrest
- The results indicated that 10058-F4 has the ability to arrest the growth of Hey and SKOV3 cells by inhibiting c-Myc, which inhibited the proliferation of the cancer cells

**6. EFFECT OF 10058-F4 ON HEY AND SKOV3 APOPTOSIS USING CELLOMETER VISION**

**Hey**

- The results showed that 10058-F4 induced apoptosis in both Hey and SKOV3 cells
- The Hey apoptotic cell population (UR) increased from 5.16% to 12.11%, while the SKOV3 apoptotic cell population increased from 5.51% to 18.03%
- The results indicated that 10058-F4 inhibition of c-Myc induced increase in early apoptotic cells that are Annexin V positive only
- In addition, the late apoptotic cells (UR) increased from 3.83% to 13.50% for Hey and 6.08% to 14.53% for SKOV3

**7. EARLY AND LATE APOPTOTIC CELL POPULATION COMPARISON**

**8. SUMMARY AND CONCLUSION**

- By plotting the early and late apoptotic cell population percentages for Hey and SKOV3, a clear dose response trend can be observed
- Both early (Annexin V) and late (Annexin V + PI) population showed increase at similar rate for the increasing dosage of 10058-F4
- By utilizing Cellometer Vision CBA, each cycle and apoptosis analysis can be rapidly performed on Hey and SKOV3 cancer cell lines
- The effect from the c-Myc inhibitor 10058-F4 was clearly observed from the G0/G1 cell cycle arrest, as well as the increase in early and late apoptotic cells
- The Cellometer Vision CBA Image Cytometry method can be easily utilized to measure fluorescent cell-based assays such as cell cycle, apoptosis, fluorescent protein expression, autophagy, and cell viability
- The ability to export the data to FCS Express can facilitate simple data analysis and reporting to efficiently generate results for publications