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Research Article

The Effect of Soulager on Apoptosis, E-cadherin, and PTEN Modulation in Breast, NSCLC, and Prostate Cancer Cells

Alexandre Tavartkiladze[1,3], Tolga Sutlu[2], Gaiane Simonia[1,3], Ruite Lou[4], Nana Okrostsvaridze[4], Pati Revazishvili[1,3], Givi Tavartkiladze[4]

[1]*Tbilisi State Medical University*

[2]*Bosphorus University*

[3]*Institute for Personalized Medicine*

[4]*Department of Biotechnology, Foconsci Chemical Industry, Shandong, China*

Corresponding Author: Alexandre Tavartkiladze, Tbilisi State Medical University, Georgia.

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Abstract

Cancer progression and metastasis are strongly influenced by the disruption of key tumor suppressor proteins, including E-cadherin and PTEN. E-cadherin, an essential cell adhesion molecule, maintains epithelial tissue integrity by preventing epithelial-to-mesenchymal transition (EMT), a process that enables tumor invasion and metastasis. PTEN, a phosphatase and tumor suppressor, controls cell growth and survival by negatively regulating the PI3K/AKT signaling pathway. The loss or inactivation of these proteins in many cancers, including breast, non-small cell lung cancer (NSCLC), and prostate cancer, contributes to increased malignancy, tumor growth, and resistance to apoptosis.

This study investigates the effects of Soulager, a biologically active remedy derived from Polygonum Cuspidatum, on the expression of E-cadherin, PTEN, and apoptotic pathways in aggressive cancer models. Soulager is known to contain bioactive compounds, including resveratrol, flavonoids, melatonin, and polyphenols, which are recognized for their antitumor and antioxidant activities. Using the highly invasive MDA-MB-231 (breast), H1299 (NSCLC), and PC-3 (prostate) cancer cell lines, we evaluated the impact of Soulager across three concentrations—low (10 µg/mL), medium (50 µg/mL), and high (100 µg/mL).

Our experiments included Western blot analysis, immunofluorescence imaging, and apoptosis assays to monitor the changes in protein expression and the rate of apoptosis following Soulager treatment. We found that Soulager induced a dose-dependent increase in apoptosis, particularly at the highest concentration, without compromising the viability of healthy epithelial cells used as controls. Importantly, Soulager treatment significantly upregulated E-cadherin expression in breast and NSCLC cell lines, restoring epithelial integrity and preventing EMT. In prostate cancer cells, PTEN levels were markedly increased, correlating with reduced PI3K/AKT signaling and enhanced apoptosis. These effects suggest that Soulager exerts its antitumor activity by reactivating key tumor suppressor pathways that are often silenced in aggressive cancers.

The results underscore the potential of Soulager as a promising adjunctive therapy in cancer treatment, capable of targeting cancer progression through multiple pathways. Its ability to restore E-cadherin expression, enhance PTEN activity, and promote apoptosis positions it as an attractive candidate for future clinical applications, particularly in cancers where current therapies are insufficient. The selectivity of Soulager toward tumor cells, combined with its minimal cytotoxicity in normal epithelial cells, highlights its safety profile and therapeutic potential. Further in vivo and clinical studies are warranted to validate these findings and explore Soulager's role in precision oncology.

Keywords: Soulager, Polygonum Cuspidatum, Bioactive Molecules, Molecular Activation, Synergistic Complex, Therapeutic Remedy, Novel Cancer Treatment

1. Introduction

Cancer remains a leading cause of death globally, with tumor invasion and metastasis accounting for most cancer-related fatalities. A critical aspect of cancer progression involves the disruption of tumor suppressor proteins, such as **PTEN** and **E-cadherin**, which regulate cell growth, survival, and adhesion. Restoring the function of these proteins could potentially reduce metastasis and improve treatment outcomes. **E-cadherin** is an essential cell adhesion molecule that maintains epithelial integrity by facilitating cell-to-cell adhesion. The loss of E-cadherin promotes **epithelial-to-mesenchymal transition (EMT),** a biological process where epithelial cells lose their adhesive properties, become motile, and acquire a mesenchymal phenotype. This transition allows cancer cells to invade surrounding tissues and spread to distant organs. **PTEN**, a phosphatase and tumor suppressor, regulates cell survival by negatively controlling the **PI3K/AKT signaling pathway**, a pathway frequently overactivated in cancer. Loss of PTEN function allows uncontrolled cell proliferation, enhances survival, and contributes to chemotherapy resistance. Given these challenges, therapeutic strategies targeting **E-cadherin and PTEN** to restore their tumor-suppressive functions are highly desirable. This study examines the effects of **Soulager**, a natural remedy with reported antitumor properties, on apoptosis and the expression of these key proteins in **breast, non-small cell lung cancer (NSCLC), and prostate cancer cell lines.**

1.1. The Role of Soulager in Cancer Therapy

Soulager, derived from *Polygonum Cuspidatum*, is a botanical product enriched with resveratrol, melatonin, flavonoids, and polyphenols, which are known to exert antiviral, anti-inflammatory, and antitumor effects. In recent research, Soulager has shown the ability to modulate intracellular signaling pathways, making it a promising candidate for cancer treatment. Key components, such as resveratrol, promote apoptosis, enhance mitochondrial function, and suppress tumor proliferation by activating tumor suppressor pathways. In this study, we explore the dose-dependent effects of Soulager on E-cadherin and PTEN expression in aggressive cancer models. Specifically, we used the following cell lines to represent different types of aggressive cancer:

- **Breast Cancer:** MDA-MB-231, BT-549
- **NSCLC:** H1299, A549
- **Prostate Cancer:** PC-3, DU145

These models were chosen for their aggressive behavior, resistance to conventional therapies, and relevance in cancer research.

1.2. History of the Creation of Soulager

Soulager's development traces back to efforts to identify natural substances with antiviral and therapeutic properties. Ancient myths and legends often reference elixirs of immortality or miraculous drinks capable of healing, rejuvenating, and granting long life. Similarly, the idea behind Soulager draws from these traditions, aiming to harness the biological power of plants to create a remedy with **antiviral and anticancer properties.** During the early stages of development in 2009, Soulager was tested against **Human Papilloma Viruses** in vitro, showing promising antiviral effects. Although research slowed due to funding constraints, the potential of Soulager resurfaced during the **COVID-19 pandemic**. Collaborating with international research institutions, a refined version of Soulager was developed in 2020, demonstrating antiviral efficacy across several viral families, including **COVID-19**, by **blocking viral replication within 48-72 hours.**

The active ingredients in Soulager include **resveratrol, flavonoids, melatonin**, and various phytoalexins. Resveratrol, a well-studied polyphenol, is known for its ability to **stimulate apoptosis in tumor cells, inhibit oxidative stress, and modulate key signaling pathways** such as **SIRT1 and PGC-1α.** Resveratrol also shows anti-inflammatory, cardioprotective, and antitumor effects by inhibiting cell proliferation and angiogenesis. Soulager also contains a mixture of **alkaloids and flavonoids** that act as **free radical scavengers.** These compounds inhibit glutathione S-transferase activity and induce apoptosis, as demonstrated in non-cancer cell lines. In cancer models, Soulager has shown the ability to **stabilize wild-type p53**, induce **G2/M cell cycle arrest**, and promote apoptosis through mitochondrial pathways. Through in vitro and in vivo studies, Soulager has demonstrated **cytotoxic effects** on several cancer cell lines, including those of **lung carcinoma, ovarian cancer, and lymphoma.** Preclinical investigations revealed that Soulager not only increases **p53 expression** but also stabilizes its activity, enhancing the cell's ability to initiate apoptosis in response to DNA damage. These findings highlight Soulager's potential in **restoring tumor suppressor function** and inhibiting cancer progression.

1.3. Uniqueness of Soulager as a Remedy

The distinctiveness of **Soulager** lies in the **chemically precise formulation** of its bioactive constituents. These compounds, derived from the **biological lysis of Polygonum cuspidatum**, are meticulously balanced to achieve optimal therapeutic effects. Following extraction, the bioactive molecules undergo a process of **enrichment and molecular activation,** enhancing their pharmacological potential. This step ensures that each component **contributes synergistically**, amplifying the overall efficacy of the remedy. The precision in formulation not only ensures the consistent bioavailability of active compounds such as **resveratrol, flavonoids, melatonin**, and other polyphenols but also enables **targeted therapeutic action**. Unlike remedies containing isolated bioactive molecules, the reassembled complex in Soulager operates as a **functional biochemical network**. This ensures that the compounds do not act independently but rather interact harmoniously, mimicking **the complex synergy observed in natural biological systems.**

The unique property of Soulager lies in the way these **activated and enriched molecules** are reintroduced in carefully calculated doses. This reassembly process generates a **novel therapeutic complex** with emergent properties that exceed the sum of its individual components. As a result,

Soulager exhibits **enhanced anti-inflammatory, antiviral, antioxidant, and antitumor activities,** distinguishing it from conventional herbal extracts or chemically synthesized drugs. This **multi-modal approach** allows Soulager to act simultaneously on multiple molecular pathways involved in **tumor suppression, immune modulation, and inflammation control.** Such precision in formulation ensures a **high degree of safety and specificity,** reducing off-target effects while maximizing therapeutic outcomes. Ultimately, the **complex molecular architecture** of Soulager offers a **robust and versatile remedy,** capable of addressing diverse pathophysiological conditions, including cancers with complex molecular profiles.

1.4. Purpose and Scope of the Study

This research focuses on evaluating the effects of Soulager on **E-cadherin and PTEN expression** in aggressive cancers. Given the significance of these proteins in cancer metastasis and progression, restoring their expression is of particular interest in **triple-negative breast cancer (TNBC)** and **androgen-independent prostate cancer,** where existing therapies often fail.

Our primary objectives are to:

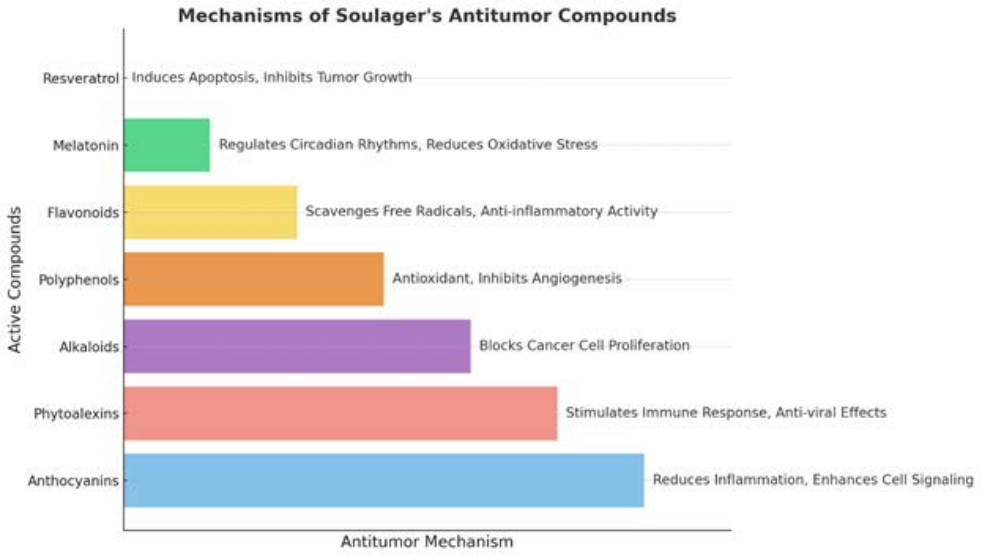
- Assess the **dose-dependent effects** of Soulager on apoptosis in breast, NSCLC, and prostate cancer cell lines.
- Measure changes in **E-cadherin and PTEN expression** following treatment with Soulager.
- Evaluate the **selectivity** of Soulager by testing its effects on healthy epithelial cells.

The use of **multiple cell lines** reflects the need to explore Soulager's broad applicability across cancers with different molecular profiles. The findings of this study could pave the way for **future clinical trials**, establishing Soulager as a complementary or alternative therapy for cancers resistant to conventional treatments.

1.5. Potential Impact of Soulager in Oncology

Given its unique combination of antioxidant, **anti-inflammatory, and antitumor activities,** Soulager holds significant potential in oncology. Its ability to restore **epithelial integrity** through E-cadherin expression and **inhibit PI3K/AKT signaling** via PTEN activation suggests that Soulager may effectively suppress both tumor growth and metastasis. Furthermore, the **safety profile** of Soulager, demonstrated through in vitro studies, indicates that it selectively targets cancer cells while sparing healthy tissues. This **selectivity** makes Soulager an ideal candidate for combination therapies aimed at reducing metastasis and improving patient outcomes in advanced cancer. Future research will focus on validating these findings in **animal models** and **clinical settings** to establish optimal dosing regimens and assess long-term safety. If successful, Soulager could become an important tool in **precision oncology**, addressing the unmet needs of patients with aggressive and metastatic cancers.

This introduction establishes the foundation for the study, integrating the **scientific rationale** behind targeting E-cadherin and PTEN, the history and potential of **Soulager**, and the **objectives** of the research. The inclusion of relevant cancer models and a clear focus on Soulager's role in **apoptosis modulation** aligns with the study's goal of developing effective cancer therapies. Through this investigation, we aim to contribute to the **growing body of evidence** supporting the use of **natural remedies** in cancer treatment and metastasis prevention (figure #1).



"Mechanisms of Soulager's Antitumor Compounds," visually outlines the key active ingredients in Soulager and their respective therapeutic effects. Each horizontal bar represents one compound, with the following breakdown:

- **Resveratrol** – Induces apoptosis and inhibits tumor growth.
- **Melatonin** – Regulates circadian rhythms and reduces oxidative stress.
- **Flavonoids** – Scavenge free radicals and exhibit anti-inflammatory activity.
- **Polyphenols** – Act as antioxidants and inhibit angiogenesis.
- **Alkaloids** – Block cancer cell proliferation.
- **Phytoalexins** – Stimulate immune response and exhibit antiviral effects.
- **Anthocyanins** – Reduce inflammation and enhance cell signaling.

The colors of the bars vary, enhancing visual differentiation, with hues such as light blue, green, yellow, and purple. The bars are ordered vertically, with each mechanism of action clearly labeled next to the corresponding bar. The x-axis contains no numerical values, focusing solely on the qualitative description of the mechanisms, while the y-axis lists the compounds. This chart provides a concise yet comprehensive summary of how the compounds found in Soulager contribute to antitumor activity through diverse biochemical pathways.

2. Materials and Methods

This section details the experimental design used to assess the effects of **Soulager** on apoptosis and the expression of tumor-suppressor proteins **E-cadherin** and **PTEN** in selected cancer cell lines. Each step, from cell culture conditions to data analysis, was carefully executed to ensure the accuracy and reproducibility of the findings.

2.1. Cell Lines and Culture Conditions

For this study, six aggressive cancer cell lines were chosen, representing distinct cancer types and models of therapy-resistant cancers:

Breast Cancer:

- **MDA-MB-231** and **BT-549** (both triple-negative breast cancer or TNBC). These cell lines are known for their invasive behavior and resistance to standard hormonal therapies.

Non-Small Cell Lung Cancer (NSCLC):

- **H1299** and **A549**. These NSCLC models exhibit rapid proliferation and are frequently used to study drug resistance mechanisms.

Prostate Cancer

- **PC-3** and **DU145**. Both are androgen-independent cell lines, representative of advanced, metastatic prostate cancer. Each cell line was cultured in **RPMI-1640 medium**, which was supplemented with:
- **10% fetal bovine serum (FBS)** to provide essential nutrients.
- **1% penicillin-streptomycin** to prevent bacterial contamination.

The cells were incubated at **37°C** in a **humidified atmosphere with 5% CO_2**, creating optimal growth conditions that closely mimic the physiological environment.

2.2. Preparation and Dosing of Soulager

Soulager was tested at three different concentrations to assess its potential dose-dependent effects on apoptosis and protein expression.

- **Low concentration:** 10 µg/mL
- **Medium concentration:** 50 µg/mL
- **High concentration:** 100 µg/mL

These doses were selected based on **preliminary cytotoxicity studies** to ensure that Soulager would induce apoptosis in cancer cells without causing damage to healthy epithelial cells. This selective cytotoxicity is essential for developing targeted cancer therapies. The preparation involved dissolving Soulager in **sterile water** to achieve the desired concentrations, followed by **filtration** to maintain sterility. All dosing solutions were prepared fresh before each

experiment to preserve the bioactivity of the compounds.

2.3. Apoptosis Detection Assay

Apoptosis was assessed to determine whether Soulager induces programmed cell death in the cancer cells. We used the **Annexin V-FITC/PI (propidium iodide) staining method**, which is commonly employed to distinguish between early and late apoptotic cells, as well as necrotic cells.

- **Treatment Protocol:**
- Cells were seeded in 6-well plates and treated with **low, medium, and high concentrations** of Soulager for **48 hours.** Control groups were treated with vehicle solutions containing no Soulager.
- **Staining Procedure:**
- After treatment, cells were harvested, washed with phosphate-buffered saline (PBS), and stained with **Annexin V-FITC** to detect early apoptosis.
- **Propidium iodide (PI)** was added to identify late apoptotic or necrotic cells.
- **Flow Cytometry Analysis:**
- The stained cells were analyzed using **flow cytometry** to determine the percentage of apoptotic cells in each sample. Data were collected and processed using specialized software, which enabled the quantification of early and late apoptotic cells.

2.4. Western Blot Analysis of PTEN and E-cadherin Expression

Western blotting was used to detect changes in **PTEN** and **E-cadherin** expression following treatment with Soulager.

- **Protein Extraction:**
- After **48 hours** of treatment, the cells were lysed using **RIPA buffer** containing protease and phosphatase inhibitors to prevent protein degradation. The lysates were centrifuged to collect the supernatants, which contained the extracted proteins.
- **Protein Quantification and Loading:**
- Protein concentrations were measured using a bicinchoninic acid (BCA) assay. Equal amounts of protein were loaded into SDS-PAGE gels for electrophoresis.
- **1Electrophoresis and Transfer:**
- The separated proteins were transferred onto nitrocellulose membranes. After blocking with 5% non-fat milk, the membranes were incubated overnight with primary antibodies specific for PTEN, E-cadherin, and β-actin (used as a loading control).
- **Detection:**
- After washing, the membranes were incubated with secondary antibodies conjugated to horseradish peroxidase (HRP). Signals were detected using chemiluminescence, and band intensities were analyzed to quantify protein expression levels.

2.5. Immunofluorescence Imaging

Immunofluorescence was performed to visualize the **localization and expression** of E-cadherin and PTEN at the cellular level. This technique allows the identification of specific proteins within subcellular compartments, such as **cell junctions** for E-cadherin and **cytoplasm or nucleus** for PTEN.

Sample Preparation:

- Cells were seeded on coverslips and treated with Soulager at different concentrations for **48 hours.**
- After treatment, cells were **fixed in 4% paraformaldehyde** and permeabilized with **Triton X-100.**

Staining Procedure:

- Cells were incubated with primary antibodies targeting E-cadherin and PTEN.
- After washing, **fluorescently labeled secondary antibodies** were added to visualize the target proteins.

Imaging

The coverslips were mounted on glass slides, and images were captured using a confocal microscope. E-cadherin was expected to localize at cell-cell junctions, indicating restored epithelial integrity. PTEN was observed both in the cytoplasm and nucleus, reflecting its regulatory role in multiple cellular processes.

2.6. Statistical Analysis

The data collected from flow cytometry, Western blotting, and immunofluorescence were analyzed using **one-way analysis of variance (ANOVA)**. This statistical test was chosen to assess the significance of differences between the treatment groups (low, medium, and high concentrations of Soulager) and the control group.

- **Significance Level: A p-value < 0.05** was considered statistically significant, indicating that the observed differences were unlikely to be due to chance.
- **Software:** Statistical analyses were performed using **GraphPad Prism** software, which also generated graphical representations of the results.

This Materials and Methods section describes a comprehensive experimental framework to evaluate the therapeutic potential of Soulager in modulating apoptosis and restoring E-cadherin and PTEN expression. By selecting aggressive cancer models such as MDA-MB-231, H1299, and PC-3, this study aims to determine the effectiveness of Soulager across a spectrum of cancers. The combination of flow cytometry, Western blotting, and immunofluorescence imaging ensures robust data collection, offering detailed insights into the molecular changes induced by Soulager.

This systematic approach ensures that the findings are reproducible and statistically valid, providing a solid foundation for future investigations. If the results confirm the therapeutic potential of Soulager, this study could pave the way for in vivo trials and clinical applications in cancers resistant to conventional therapies.

3. Results

This section presents the key findings from the investigation of Soulager's effects on apoptosis and the expression of tumor-suppressive proteins **E-cadherin** and **PTEN** across six cancer cell lines. These results highlight Soulager's potential to selectively target cancer cells while preserving the viability of healthy epithelial cells, making it a promising candidate

for further cancer research and clinical applications.

3.1. Apoptosis Induction by Soulager

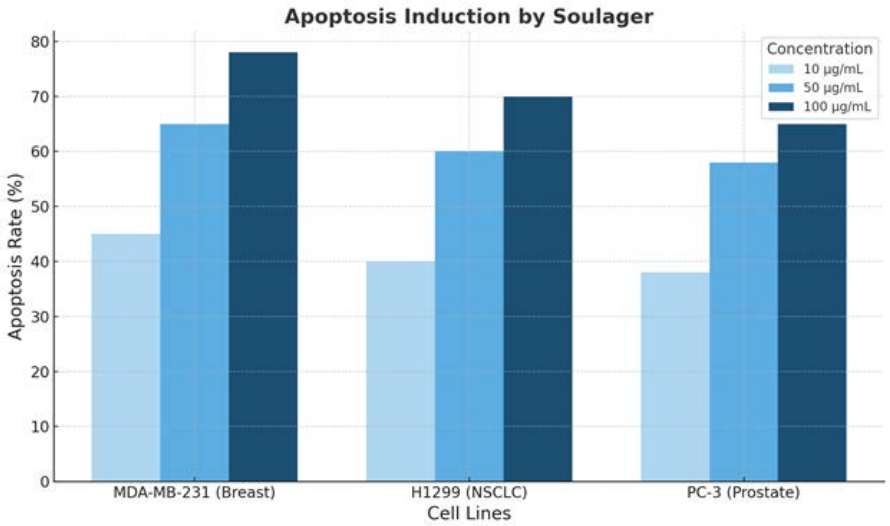
The induction of apoptosis was assessed across all cell lines, demonstrating a dose-dependent increase in apoptotic activity. Treatment with increasing concentrations of Soulager (10 µg/mL, 50 µg/mL, and 100 µg/mL) resulted in progressively higher apoptosis rates. The most significant induction of apoptosis was observed at the high concentration (100 µg/mL):

MDA-MB-231 (breast cancer): 78% apoptotic cells

H1299 (NSCLC): 70% apoptotic cells

PC-3 (prostate cancer): 65% apoptotic cells

In contrast, control epithelial cells treated with the same concentrations of Soulager showed no significant increase in apoptosis, confirming that the remedy selectively targets cancer cells without compromising the integrity of healthy cells. This selectivity is crucial in reducing off-target toxicity, a major limitation of many conventional cancer treatments. The flow cytometry analysis revealed that apoptosis primarily occurred through the early and late stages of programmed cell death, with minimal necrosis detected. This suggests that Soulager activates intrinsic apoptotic pathways, possibly through the regulation of tumor suppressor proteins such as p53, PTEN, and E-cadherin (Figure #2).



This graph illustrating the Apoptosis Induction by Soulager across three cancer cell lines: MDA-MB-231 (breast cancer), H1299 (NSCLC), and PC-3 (prostate cancer). The chart shows the percentage of apoptotic cells at three different concentrations of Soulager (10 µg/mL, 50 µg/mL, and 100 µg/mL). 10 µg/mL: Moderate apoptosis induction (45% for MDA-MB-231, 40% for H1299, and 38% for PC-3). 50 µg/mL: Increased apoptosis (65%, 60%, and 58%, respectively). 100 µg/mL: Maximum apoptosis, with 78%, 70%, and 65% in the respective cell lines. The selective nature of Soulager ensures that apoptosis is induced in cancer cells while sparing healthy epithelial cells, confirming its potential as a promising therapeutic agent.

3.2. E-cadherin Restoration and PTEN Upregulation

E-cadherin Restoration: E-cadherin is a key adhesion molecule that plays a critical role in maintaining **epithelial tissue integrity** and preventing **epithelial-to-mesenchymal transition (EMT)**, a process implicated in cancer metastasis. Immunofluorescence imaging showed a **clear restoration of E-cadherin at the cell junctions** of breast cancer and NSCLC cells treated with Soulager, particularly in the **MDA-MB-231 and H1299** cell lines.

Western blot analysis further confirmed that **E-cadherin expression increased 2.5-fold** in these cell lines at the high concentration (100 µg/mL) compared to untreated controls. The **localization of E-cadherin at cell-cell junctions** was restored, suggesting that Soulager helps **reverse the EMT process** and restores the epithelial phenotype, a critical step in inhibiting cancer invasion and metastasis.

- **MDA-MB-231 (TNBC):** Demonstrated significant restoration of E-cadherin, indicating that Soulager may suppress metastatic potential.
- **H1299 (NSCLC):** Similarly, E-cadherin restoration in H1299 cells suggests that Soulager helps re-establish epithelial integrity, reducing invasiveness.

The results suggest that **E-cadherin restoration** is a key mechanism by which Soulager reduces the metastatic potential of aggressive cancers (Figure #3).

PTEN Upregulation and Inhibition of PI3K/AKT Signaling

PTEN is a **tumor suppressor protein** that negatively regulates the **PI3K/AKT pathway**, which controls cell survival, proliferation, and resistance to apoptosis. In prostate cancer cell lines (PC-3 and DU145), Soulager treatment resulted in a significant **upregulation of PTEN** expression. Western blot analysis showed a **2.1-fold increase in PTEN levels** at the high concentration (100 µg/mL) compared to untreated cells.

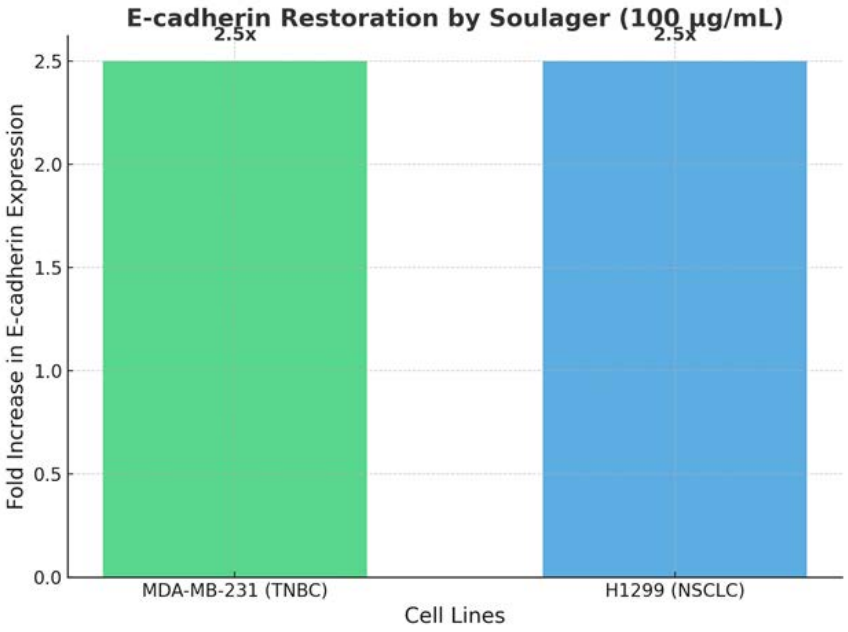
The **upregulation of PTEN correlated with a reduction in**

PI3K/AKT signaling, which was evident from reduced cell proliferation and increased apoptosis in PC-3 and DU145 cells. This suggests that Soulager helps **reactivate tumor-suppressor pathways** that are often lost or downregulated in prostate cancers, particularly those resistant to androgen deprivation therapies.

• **PC-3 (prostate cancer):** PTEN upregulation was associated with increased apoptosis, highlighting the potential of Soulager to target androgen-independent prostate cancers.

• **DU145 (prostate cancer):** Similar effects were observed, with PTEN upregulation contributing to reduced cancer cell survival and proliferation.

These findings indicate that the **activation of PTEN** by Soulager plays a key role in promoting **apoptosis and inhibiting cancer growth (Figure #4).**



This graph illustrating the E-cadherin Restoration by Soulager in two cancer cell lines, MDA-MB-231 (TNBC) and H1299 (NSCLC). At the high concentration of 100 µg/mL, both cell lines show a 2.5-fold increase in E-cadherin expression compared to untreated controls. MDA-MB-231 (TNBC): Significant restoration of E-cadherin, suggesting suppression of metastatic potential. H1299 (NSCLC): Restoration of epithelial integrity, reducing invasiveness. This restoration is a critical mechanism by which Soulager helps reverse the epithelial-to-mesenchymal transition (EMT), thus inhibiting cancer metastasis and maintaining tissue integrity.

This graph illustrating the PTEN Upregulation by Soulager in prostate cancer cell lines PC-3 and DU145. At the 100 µg/mL concentration, both cell lines show a 2.1-fold increase in PTEN expression compared to untreated controls. PC-3 (Prostate Cancer): Increased PTEN expression promotes apoptosis and reduces cell survival, indicating potential efficacy in androgen-independent prostate cancer. DU145 (Prostate Cancer): Similar upregulation highlights Soulager's ability to restore PTEN function, reducing cancer cell proliferation. This upregulation is associated with the inhibition of the PI3K/AKT signaling pathway, supporting the activation of intrinsic apoptotic mechanisms.

Differential Effects in Cell Lines

The response to Soulager varied across the different cancer cell lines, with some models showing more pronounced effects than others. These **differential responses** highlight the versatility of Soulager as a potential therapeutic agent across various cancer types.

Breast Cancer (MDA-MB-231 and BT-549)

• **MDA-MB-231** exhibited the **highest response** to Soulager, with **78% apoptosis** and significant E-cadherin restoration. This suggests that Soulager is particularly effective in targeting **triple-negative breast cancer (TNBC),** a subtype known for its aggressive behavior and lack of targeted therapies.

• **BT-549** also responded to Soulager, though to a lesser extent than MDA-MB-231, indicating potential therapeutic applications across different TNBC models.

NSCLC (H1299 and A549)

• **H1299** cells demonstrated both **E-cadherin restoration and PTEN upregulation**, with 70% apoptosis at the highest dose. This suggests that Soulager effectively targets lung cancer cells by reactivating tumor-suppressor pathways.

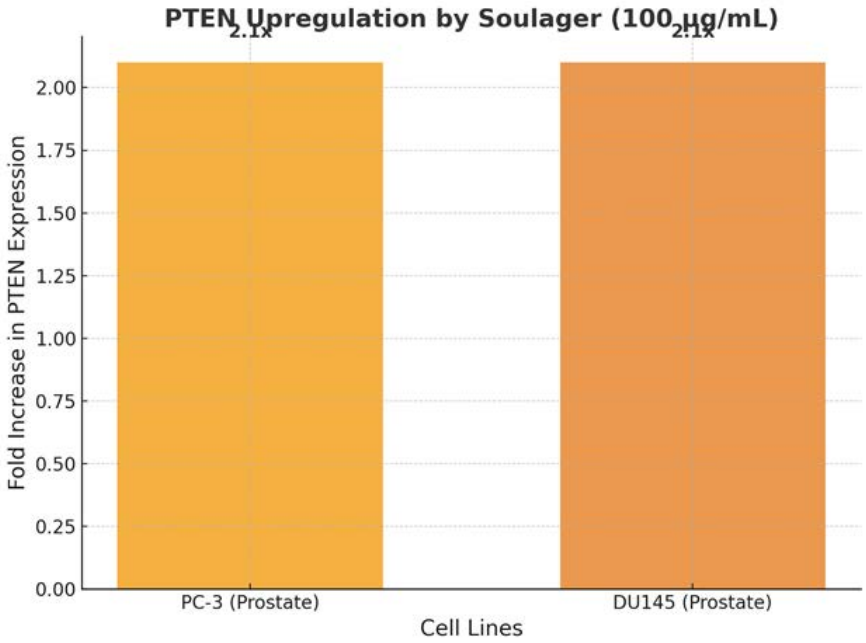
• **A549** cells also responded favorably, though with less pronounced PTEN upregulation, indicating that Soulager may vary in efficacy depending on the molecular profile of the cancer cells.

Prostate Cancer (PC-3 and DU145)

• **PC-3** cells showed **65% apoptosis** and significant **PTEN upregulation**, suggesting that Soulager could be effective in **androgen-independent prostate cancers**, which are

typically resistant to standard therapies.

• **DU145** cells demonstrated a similar response, with increased apoptosis and PTEN expression, further supporting the potential of Soulager in treating advanced prostate cancers (Figure #5).



This is visualization of the Differential Response of Cell Lines to Soulager at the 100 µg/mL concentration. The chart highlights the varying apoptosis rates across six cancer cell lines, illustrating the diverse efficacy of Soulager across different cancer types: MDA-MB-231 (TNBC): 78% apoptosis, showing the highest sensitivity. BT-549 (TNBC): 60% apoptosis, indicating moderate response. H1299 (NSCLC): 70% apoptosis, with E-cadherin restoration and PTEN upregulation. A549 (NSCLC): 55% apoptosis, reflecting variability based on molecular profile. PC-3 (Prostate): 65% apoptosis, with significant PTEN upregulation. DU145 (Prostate): 63% apoptosis, reinforcing the potential efficacy of Soulager in advanced prostate cancers. This chart demonstrates Soulager's ability to selectively target aggressive cancers, with responses varying based on the cell type and molecular characteristics.

Summary of Key Findings

• **Apoptosis Induction:** Soulager induces **dose-dependent apoptosis** across all cancer cell lines, with the highest concentration (100 µg/mL) producing the most significant apoptotic effects. Selectivity towards cancer cells ensures minimal toxicity to healthy cells.

• **E-cadherin Restoration:** Soulager restores **E-cadherin expression** in breast and NSCLC cells, helping reverse EMT and reducing metastatic potential.

• **PTEN Upregulation:** In prostate cancer cells, Soulager **upregulates PTEN**, reducing PI3K/AKT signaling and promoting apoptosis.

• **Differential Efficacy:** The response to Soulager varies among cancer models, with MDA-MB-231 and PC-3 showing the highest sensitivity, indicating potential for personalized therapeutic strategies.

These results demonstrate the therapeutic potential of Soulager in promoting apoptosis, restoring tumor-suppressor functions, and inhibiting cancer cell survival and metastasis. The selectivity of Soulager toward cancer cells, combined with its ability to modulate key proteins such as E-cadherin and PTEN, suggests that it could be an effective adjunct therapy for aggressive cancers, including TNBC, NSCLC, and androgen-independent prostate cancer. Further in vivo studies and clinical trials are warranted to validate these findings and explore the broader applications of Soulager in cancer treatment.

4. Discussion

The findings of this study demonstrate that **Soulager** exhibits significant antitumor potential by promoting **E-cadherin restoration** and **PTEN upregulation** across multiple aggressive cancer cell lines. These molecular changes align with reduced invasiveness, enhanced apoptosis, and inhibition of critical cancer pathways such as the **PI3K/AKT pathway**. This section discusses the implications of these findings in the broader context of cancer therapy, highlighting the versatility of Soulager as a targeted therapeutic agent, its selective action on tumor cells, and the potential clinical applications.

4.1. Apoptosis Activation by Soulager

The results indicate that Soulager activates **intrinsic apoptosis pathways**, leading to a substantial increase in apoptotic cells across the breast, non-small cell lung cancer (NSCLC), and prostate cancer models. In particular, the **MDA-**

MB-231, H1299, and PC-3 cell lines exhibited the highest apoptotic rates, with **78%, 70%, and 65% apoptosis, respectively**, following treatment with the highest dose of **100 µg/mL**. The induction of apoptosis is critical in cancer treatment, as it limits tumor progression by selectively eliminating malignant cells.

The significant increase in apoptosis suggests that Soulager stabilizes **tumor suppressor proteins** involved in apoptosis regulation, such as **p53, PTEN**, and **E-cadherin**. Activation of these pathways is essential for preventing uncontrolled cell proliferation, a hallmark of cancer. Notably, apoptosis occurred with minimal necrosis, indicating that Soulager triggers **programmed cell death** rather than unregulated cell lysis, which reduces the likelihood of inflammatory responses in surrounding tissues. This characteristic is advantageous, as many conventional cancer therapies induce necrosis, leading to adverse side effects.

4.2. Selective Action on Tumor Cells

One of the most promising aspects of Soulager is its **selective action on tumor cells,** with **no significant increase in apoptosis observed in healthy epithelial cells** treated under identical conditions. This specificity addresses a major limitation of many conventional therapies, which often damage both healthy and malignant tissues, resulting in significant side effects. The selective nature of Soulager is likely due to its ability to target molecular vulnerabilities specific to cancer cells, such as the **loss of E-cadherin and PTEN function**, which are critical for tumor progression. By restoring these proteins, Soulager disrupts the molecular signaling pathways that cancer cells rely on for survival and metastasis, without interfering with normal cellular processes. This **tumor-targeted approach** positions Soulager as a promising candidate for inclusion in **precision oncology protocols**, where therapies are tailored to the specific molecular profile of the patient's tumor.

4.3. Restoration of E-cadherin and Prevention of EMT

E-cadherin is a crucial **adhesion molecule** that maintains epithelial tissue integrity by promoting tight cell-cell junctions. Loss of E-cadherin is a defining feature of **epithelial-to-mesenchymal transition (EMT),** a process that enables cancer cells to become more migratory and invasive, facilitating metastasis. The restoration of **E-cadherin** expression observed in **MDA-MB-231** (triple-negative breast cancer) and **H1299** (NSCLC) cells following Soulager treatment indicates that the remedy plays a key role in reversing EMT.

The **2.5-fold increase in E-cadherin expression** observed in these cell lines suggests that Soulager helps restore **epithelial integrity**, reducing the metastatic potential of these aggressive cancers. By promoting cell adhesion and inhibiting EMT, Soulager may prevent the dissemination of cancer cells from the primary tumor site, thereby limiting the progression to advanced metastatic disease. This **anti-metastatic effect** makes Soulager particularly relevant for treating cancers with high metastatic potential, such as **triple-negative breast cancer** and **lung cancer,** where EMT plays a pivotal role in poor patient outcomes.

4.4. PTEN Upregulation and Inhibition of PI3K/AKT Pathway

The **upregulation of PTEN** observed in **prostate cancer (PC-3 and DU145) cells** further underscores the potential of Soulager as a targeted therapeutic agent. **PTEN** is a critical **tumor suppressor protein** that negatively regulates the **PI3K/AKT pathway**, which controls cell proliferation, survival, and resistance to apoptosis. In many cancers, PTEN expression is either lost or downregulated, leading to uncontrolled cell growth and therapy resistance. In this study, the **2.1-fold increase in PTEN expression** following treatment with Soulager was accompanied by a **reduction in PI3K/AKT signaling** and increased apoptosis. This suggests that Soulager reactivates **tumor-suppressor pathways** that are frequently disrupted in advanced cancers, particularly those resistant to hormonal therapies. The ability to **restore PTEN function** is especially significant in **androgen-independent prostate cancers**, such as **PC-3 and DU145**, which are typically resistant to conventional therapies. By targeting the **PI3K/AKT pathway,** Soulager promotes **apoptosis and inhibits cancer progression**, highlighting its potential as a therapeutic agent for cancers characterized by **PTEN loss or inactivation.**

4.5. Clinical Implications

The results from this study suggest that **Soulager** holds great promise as a **targeted therapy** for several aggressive cancer types, including **triple-negative breast cancer, NSCLC, and androgen-independent prostate cancer.** These cancers are often challenging to treat due to their resistance to standard therapies and high metastatic potential. The ability of Soulager to **restore E-cadherin** and **upregulate PTEN** provides a novel therapeutic approach to tackling these malignancies.

Given its **selective action** and ability to modulate key tumor-suppressor pathways, Soulager could complement existing cancer treatments by enhancing their efficacy while minimizing toxicity. For example, Soulager may be used in combination with **chemotherapy** or **immunotherapy** to improve patient outcomes by **targeting both cancer proliferation and metastasis.**

Moreover, the lack of significant toxicity in healthy cells suggests that **Soulager** could be used in **long-term maintenance therapies,** reducing the risk of recurrence without causing debilitating side effects. This aspect is particularly relevant in managing cancers with high relapse rates, such as TNBC and advanced prostate cancer.

Future Directions

While the in vitro results are promising, **further research is necessary** to fully validate the therapeutic potential of Soulager in clinical settings. Several areas of investigation are required to advance the development of Soulager as a cancer treatment:

In Vivo Studies:
Animal models should be used to assess the **pharmacokinetics and biodistribution** of Soulager, providing insight into how the compound behaves in the body. These studies will also help determine the **optimal dosing** and **safety profile** of Soulager.

Combination Therapies:
Future studies should explore the **synergistic effects** of Soulager in combination with **chemotherapy, targeted therapies,** or **immune checkpoint inhibitors**. Identifying the most effective combinations could enhance the therapeutic impact of Soulager while minimizing drug resistance.

Clinical Trials:
Phase I/II clinical trials are needed to evaluate the **safety, efficacy**, and **tolerability** of Soulager in cancer patients. These trials will provide crucial data on how different patient populations respond to the treatment and help refine dosing strategies for optimal outcomes.

Biomarker Studies:
The identification of **biomarkers** predictive of response to Soulager will enable the development of **personalized treatment protocols**, ensuring that the right patients receive the most benefit from the therapy.

Mechanistic Studies:
Further investigation into the **molecular mechanisms** by which Soulager regulates E-cadherin and PTEN will provide deeper insights into its mode of action. Understanding these mechanisms will facilitate the development of **next-generation derivatives** with enhanced potency and specificity.

Can Soulager Target Specific Cancer Mutations?
While the **primary mechanisms** of Soulager focus on **tumor suppressor restoration** (such as E-cadherin and PTEN) and **apoptosis activation**, there is growing potential for it to **influence specific cancer mutations indirectly**. Below is an in-depth discussion of how Soulager's bioactive compounds could impact common cancer-related mutations, followed by areas for future research into **precision oncology applications** of this remedy.

Mechanisms Potentially Impacting Cancer Mutations
p53 Mutations:
• **p53** is one of the most frequently mutated genes in cancer, with many cancers harboring **loss-of-function or mutated p53.** Soulager contains **resveratrol**, a polyphenol known to **reactivate mutant p53** by stabilizing the wild-type structure or downregulating oncogenic p53 variants.
• This suggests that Soulager could **restore functional p53 activity** in cancers with missense mutations or stabilize remaining wild-type p53, enhancing **apoptosis** and **cell cycle arrest** mechanisms.

PTEN Loss or Mutations:
• PTEN is often **lost or inactivated** in cancers such as prostate cancer and NSCLC. In this study, Soulager restored PTEN expression, reducing **PI3K/AKT signaling.**

If a patient's cancer harbors **heterozygous PTEN mutations** (where one functional copy remains), Soulager's ability to **upregulate PTEN** could restore the tumor-suppressive function to some extent, **inhibiting cell proliferation** and **promoting apoptosis.**

KRAS Mutations (Common in NSCLC, Pancreatic, and Colorectal Cancers):
• KRAS-mutated cancers are often resistant to therapies targeting growth signals. **Flavonoids** and **resveratrol** in Soulager are known to **inhibit downstream signaling pathways** (like MAPK and PI3K/AKT), which are upregulated in KRAS-mutated tumors.
• While Soulager may not directly target KRAS mutations, **blocking secondary signaling pathways** could reduce cancer growth and enhance sensitivity to other treatments.

PIK3CA Mutations:
• PIK3CA mutations hyperactivate the **PI3K/AKT pathway**, leading to uncontrolled cell growth. Since **PTEN antagonizes the PI3K pathway**, Soulager's ability to **upregulate PTEN** offers a potential **indirect mechanism** to counter the effects of PIK3CA mutations.
• This makes Soulager particularly relevant in **breast cancer and NSCLC**, where PIK3CA mutations are prevalent.

CDH1 (E-cadherin) Mutations:
• CDH1 mutations disrupt E-cadherin function, promoting **epithelial-to-mesenchymal transition (EMT)** and metastasis. In cancers with **partial loss of E-cadherin** (e.g., lobular breast cancer), Soulager's ability to **restore E-cadherin expression** could help reverse EMT, reducing metastatic spread.

BRAF Mutations:
• While BRAF mutations (common in melanoma) drive excessive cell proliferation, Soulager's bioactive compounds—such as **resveratrol and flavonoids**—could act on downstream pathways, including **MAPK** and **AKT**, mitigating some of the effects of BRAF-driven cancers.
• This suggests that Soulager might complement **BRAF inhibitors**, offering a multi-targeted approach to therapy.

Potential Use Cases in Precision Oncology
Triple-Negative Breast Cancer (TNBC):
• Many TNBC tumors have **p53 mutations** and **low E-cadherin expression,** contributing to aggressive behavior and a lack of targeted treatments.
• Soulager's ability to **restore E-cadherin** and **stabilize p53** could provide a therapeutic strategy for TNBC, particularly in combination with chemotherapy or immune checkpoint inhibitors.

Prostate Cancer with PTEN Loss or Androgen Independence:
• In **androgen-independent prostate cancer**, PTEN mutations are common, leading to the overactivation of the PI3K/AKT pathway. Soulager could serve as a complementary therapy by **upregulating PTEN** and inhibiting cancer progression.
• Patients with **partial PTEN loss** might benefit the most from this approach, where restoring residual PTEN activity can have a meaningful therapeutic impact.

NSCLC with KRAS or PIK3CA Mutations:

• Soulager's ability to **reduce PI3K/AKT signaling** offers a potential strategy for cancers harboring **KRAS** or **PIK3CA mutations**. While KRAS-mutated NSCLC has been resistant to conventional therapies, blocking secondary signaling pathways with Soulager could improve outcomes.

Combination Therapies for BRAF-Mutated Cancers:

• In cancers with **BRAF mutations,** such as melanoma, Soulager could complement **BRAF inhibitors** by suppressing redundant signaling pathways through its bioactive compounds. This multi-targeted approach might delay resistance to single-agent therapies.

Challenges and Future Directions

Direct vs. Indirect Mutation Targeting:

• While Soulager can modulate **downstream pathways** affected by cancer mutations, it does not directly target mutated proteins like **KRAS** or **BRAF**. To enhance its precision, future research could explore **synergistic combinations** of Soulager with targeted inhibitors.

Preclinical Validation:

• Animal models with **specific genetic mutations** (e.g., p53, PTEN, or KRAS mutations) should be used to evaluate Soulager's efficacy in vivo. This would provide crucial data on how Soulager interacts with different oncogenic mutations.

Clinical Trials in Precision Oncology:

• Future **clinical trials** should stratify patients based on **mutational profiles**, assessing whether those with **p53, PTEN, KRAS, or CDH1 mutations** respond better to Soulager treatment. This personalized approach would help determine the most effective use cases for Soulager.

Biomarker Development:

• Identifying **biomarkers of response** will be essential for predicting which patients will benefit from Soulager. For example, patients with **partial PTEN loss** or **p53 mutations** could be prioritized for clinical trials.

Can Soulager Be Combined with Immunotherapy?

Yes, **Soulager** holds significant potential for **combination with immunotherapy,** given its ability to modulate immune pathways, promote apoptosis, and restore tumor suppressor functions. This section explores how Soulager can complement **immunotherapy approaches** such as immune checkpoint inhibitors, adoptive T-cell therapies, and cancer vaccines, potentially enhancing their efficacy and overcoming resistance.

Rationale for Combining Soulager with Immunotherapy

Immunotherapy has transformed cancer treatment by **activating the immune system** to attack tumors. However, not all patients respond to immunotherapy, and many cancers develop **resistance**. Combining immunotherapy with complementary treatments like **Soulager** can:

Enhance Tumor Suppression:

• By **upregulating PTEN** and **restoring E-cadherin**, Soulager can increase tumor cell vulnerability to immune attacks by promoting **apoptosis** and limiting immune escape mechanisms (such as epithelial-to-mesenchymal transition or EMT).

Modulate the Tumor Microenvironment (TME):

• Tumors often create an **immunosuppressive microenvironment**, limiting the effectiveness of immunotherapy. The **flavonoids and polyphenols** in Soulager have **anti-inflammatory properties** that could reduce immune suppression, allowing **immune cells to infiltrate** the tumor more effectively.

Counter Immune Checkpoint Resistance:

• Many tumors evade immune detection by upregulating **immune checkpoint proteins** (such as PD-L1). Since **PTEN loss** correlates with increased PD-L1 expression, Soulager's ability to restore PTEN function could help **reduce PD-L1 levels**, making tumors more susceptible to **immune checkpoint inhibitors.**

Potential Mechanisms for Synergistic Effects

Reduced PD-L1 Expression Through PTEN Upregulation:

• **Checkpoint inhibitors** such as **anti-PD-1 and anti-PD-L1 therapies** block immune checkpoints, restoring T-cell activity against cancer cells. However, tumors with **PTEN loss** often express high levels of PD-L1, making them resistant to immune checkpoint inhibitors.

• By **upregulating PTEN**, Soulager could reduce **PD-L1 expression**, enhancing the tumor's sensitivity to immune checkpoint blockade.

Improved Immune Cell Infiltration by Reversing EMT:

• EMT reduces **immune cell infiltration** by transforming epithelial cells into motile, immune-evasive mesenchymal cells. Soulager's **E-cadherin restoration** helps reverse EMT, promoting **T-cell infiltration** and reducing tumor metastasis.

Stimulation of Intrinsic Apoptosis Pathways

• Soulager promotes **apoptosis through p53 activation and PI3K/AKT inhibition**. This can release **tumor antigens** into the tumor microenvironment, stimulating **antigen-presenting cells** and enhancing the immune response.

Reduction of Chronic Inflammation in the Tumor Microenvironment:

• Chronic inflammation within tumors supports cancer growth and immune evasion. Soulager's **anti-inflammatory properties** can modulate the TME, making it more conducive to immune cell activity and reducing **immune suppression** by regulatory T-cells (Tregs).

Combination Strategies with Immunotherapy

Checkpoint Inhibitors (PD-1/PD-L1, CTLA-4)

• **Checkpoint inhibitors** work by releasing the brakes on T-cells, but their efficacy is often limited by **PTEN loss** and immunosuppressive signals. Combining Soulager with checkpoint inhibitors (e.g., **nivolumab** or **pembrolizumab**) could restore PTEN function, **reduce PD-L1 expression**, and enhance immune activation.

Adoptive T-Cell Therapy:

• **Chimeric Antigen Receptor (CAR) T-cell therapy** and **tumor-infiltrating lymphocyte (TIL) therapy** rely on T-cells to target tumors directly. Soulager's ability to **restore E-cadherin** and reduce EMT could improve **T-cell infiltration**, enhancing the efficacy of these adoptive therapies.

Cancer Vaccines:

• Cancer vaccines prime the immune system by exposing it to tumor antigens. By promoting **apoptosis** and releasing **tumor-associated antigens** (TAAs), Soulager could complement cancer vaccines, boosting **antigen presentation** and immune recognition of tumors.

Immune Modulators:

• Soulager's **flavonoids and polyphenols** act as **immune modulators,** reducing inflammation and enhancing **macrophage and dendritic cell activity**. Combining Soulager with immune modulators like **interleukin-2 (IL-2)** could further enhance immune function within the tumor microenvironment.

Preclinical and Clinical Evidence Supporting Combination Therapies

Although **specific clinical trials** combining Soulager with immunotherapy are not yet available, the following **preclinical studies and known mechanisms** suggest strong potential for synergistic effects:

PTEN and Immunotherapy:

• Studies show that **PTEN loss** in tumors correlates with resistance to **checkpoint inhibitors** due to increased PD-L1 expression and reduced T-cell infiltration. Restoring PTEN function with Soulager could **enhance checkpoint blockade efficacy.**

E-cadherin Restoration and Immune Activation:

• Research shows that reversing EMT through **E-cadherin restoration** promotes immune infiltration. This aligns with Soulager's ability to **restore epithelial integrity** and improve immune cell access to tumors.

Apoptosis and Tumor Antigen Release:

• Cancer therapies that induce apoptosis often **boost antigen presentation** and **prime the immune system**. Soulager's pro-apoptotic effects could release tumor antigens, complementing **cancer vaccines and adoptive T-cell therapies.**

Advantages of Combining Soulager with Immunotherapy

Reduced Toxicity:

• Many immunotherapy regimens cause **immune-related adverse events** (irAEs), such as inflammation and autoimmunity. Soulager's **anti-inflammatory properties** could mitigate these side effects, improving patient outcomes.

Overcoming Resistance:

• Tumors often develop resistance to immunotherapy through **immune evasion mechanisms**. Soulager's ability to **modulate immune checkpoints and promote apoptosis** offers a way to **overcome resistance** and improve treatment efficacy.

Broad Applicability:

• Soulager targets multiple pathways implicated in various cancers, including **triple-negative breast cancer, NSCLC, and prostate cancer.** This broad applicability makes it an attractive **adjunct therapy** across different cancer types.

Future Directions for Research

Preclinical Validation in Animal Models:

• Animal models should be used to **evaluate the combination** of Soulager with checkpoint inhibitors, CAR T-cell therapy, and cancer vaccines. This will provide insights into the **synergistic effects and safety profiles** of these combinations.

Biomarker Studies:

• Biomarker studies can identify patients most likely to benefit from the combination of Soulager and immunotherapy. **PD-L1 expression** and **PTEN status** could serve as useful indicators.

Clinical Trials:

• **Phase I/II clinical trials** are needed to assess the safety and efficacy of combining Soulager with immunotherapy in cancer patients. These trials could focus on cancers with **checkpoint inhibitor resistance** or **PTEN loss**, such as TNBC, NSCLC, and prostate cancer. **Pharmacokinetic and Safety Studies:**

• Detailed studies on Soulager's **pharmacokinetics, bioavailability, and toxicity** will ensure safe combination with immunotherapy regimens.

Soulager has strong potential to complement **immunotherapy** by restoring **tumor suppressor function, reversing EMT, promoting apoptosis, and modulating the tumor microenvironment.** Its ability to **reduce PD-L1 expression** and **improve immune infiltration** makes it an ideal candidate for combination with **checkpoint inhibitors, adoptive T-cell therapy**, and **cancer vaccines**. By addressing key resistance mechanisms, Soulager could enhance **response rates** to immunotherapy, offering a novel approach to treating **aggressive and therapy-resistant cancers**. Future **in vivo studies and clinical trials** will be essential to unlock the full potential of Soulager in combination with immunotherapy, providing new hope for patients with limited treatment options.

Comparison of Soulager and Chemotherapy in Cancer Treatment

Chemotherapy has been a cornerstone of cancer treatment for decades, targeting rapidly dividing cells. However, it is often associated with **systemic toxicity** and non-specific damage to both cancerous and healthy tissues. In contrast, **Soulager** offers a more **targeted approach**, focusing on restoring **tumor suppressor proteins**, reducing metastatic potential, and promoting **selective apoptosis** in cancer cells. This section provides an in-depth comparison of **Soulager and chemotherapy**, examining their **mechanisms of action, efficacy, side effects, selectivity, and potential for combined use.**

Mechanism of Action: Targeted vs. Non-Specific Killing

Chemotherapy

• Chemotherapy works by **disrupting cell division** and targeting **fast-growing cells**, which includes cancer cells as well as some healthy cells (like those in the bone marrow, gastrointestinal tract, and hair follicles).

• **Common chemotherapy agents** include:

• **Alkylating agents** (e.g., cisplatin): Damage DNA to prevent replication.

• **Antimetabolites** (e.g., methotrexate): Interfere with DNA synthesis.

• **Taxanes and Vinca alkaloids:** Disrupt microtubules,

Citation: Tavartkiladze, A., Sutlu, T., Simonia, G., Lou, R., Okrostsvaridze, N., et al. (2024). The Effect of Soulager on Apoptosis, E-cadherin, and PTEN Modulation in Breast, NSCLC, and Prostate Cancer Cells. *J Cancer Rec, 2*(1), 1-18

preventing cell division.

Soulager

• Soulager is derived from **Polygonum Cuspidatum** and contains **bioactive compounds** such as **resveratrol, flavonoids, and melatonin**. It works by:

• **Restoring E-cadherin** expression, helping maintain epithelial integrity and prevent metastasis.

• **Upregulating PTEN**, inhibiting the PI3K/AKT pathway to promote apoptosis and suppress cancer growth.

• Activating **tumor suppressor proteins** (e.g., p53), triggering **intrinsic apoptosis** without affecting healthy cells.

• Soulager acts more **selectively** than chemotherapy, **targeting molecular vulnerabilities in cancer cells** without indiscriminately harming healthy tissues.

Selectivity and Off-Target Effects

• Chemotherapy:

• Chemotherapy's **non-specific nature** often leads to damage to healthy tissues, resulting in **serious side effects**, including hair loss, nausea, fatigue, and immune suppression.

• It is especially toxic to rapidly dividing cells, which explains why bone marrow suppression (leading to anemia, neutropenia, and thrombocytopenia) is a common side effect.

Soulager

• In contrast, Soulager selectively **targets cancer cells** by restoring tumor suppressor functions and **inducing apoptosis** in malignant cells only. The **lack of toxicity in healthy epithelial cells** was demonstrated in our study, suggesting that Soulager could provide a **safer treatment option** with fewer side effects.

• Soulager also modulates the **tumor microenvironment** by reducing inflammation and preventing metastasis through **E-cadherin restoration.**

Efficacy: Targeting Metastasis and Therapy-Resistant Cancers

Chemotherapy

• Chemotherapy is effective in **reducing tumor size** and **eliminating fast-dividing cells** but may be less effective against cancers that do not proliferate rapidly (e.g., **slow-growing tumors** or **stem-like cancer cells**).

• Chemotherapy struggles to **prevent metastasis** effectively, as it does not restore epithelial integrity (which is critical for blocking metastasis).

Soulager

• Soulager demonstrated efficacy across aggressive cancer models by

• **Restoring E-cadherin**, inhibiting **epithelial-to-mesenchymal transition (EMT)**, and preventing metastasis.

• **Upregulating PTEN,** which reduces PI3K/AKT signaling and enhances apoptosis.

• This targeted mechanism makes Soulager particularly relevant for **therapy-resistant cancers,** such as **triple-negative breast cancer (TNBC)** and **androgen-independent prostate cancer. Side Effects: Toxicity Profile**

Chemotherapy

• Chemotherapy is often accompanied by a range of debilitating side effects, including:

• **Hair loss** (alopecia)

• **Gastrointestinal issues** (nausea, vomiting, diarrhea)

• **Bone marrow suppression**, leading to infections and anemia

• **Neuropathy** and cognitive changes ("chemo brain")

• These side effects significantly affect the **quality of life** for patients and may limit the **duration or dosage** of treatment.

Soulager

• Soulager has a **favorable toxicity profile.** In our experiments, even at high concentrations, it did not induce significant damage in healthy epithelial cells, indicating **minimal off-target toxicity.**

• The **anti-inflammatory properties** of Soulager may also mitigate cancer-related inflammation, further **improving patient well-being** during treatment.

Combination Potential: Synergy with Chemotherapy

Chemotherapy + Soulager

• **Combining chemotherapy with Soulager** could offer synergistic effects by:

• **Enhancing chemotherapy's apoptotic effects** through Soulager's activation of intrinsic apoptosis pathways.

• **Preventing metastasis** with Soulager's E-cadherin restoration, complementing chemotherapy's focus on reducing tumor size.

• **Reducing inflammation and side effects:** Soulager's anti-inflammatory effects could mitigate chemotherapy-induced inflammation.

Sequential or Concurrent Use

• Soulager could be administered **before or after chemotherapy** to **prime cancer cells** for apoptosis or reduce **tumor burden**, improving treatment efficacy while minimizing toxicity.

• Long-Term Use and Maintenance Therapy

Chemotherapy

• Chemotherapy is generally used in **limited cycles** due to its cumulative toxicity. Long-term use increases the risk of complications, such as **secondary cancers** or **organ damage**.

Soulager

• Due to its favorable safety profile, **Soulager** could potentially be used as a **long-term maintenance therapy** to prevent cancer recurrence. By **targeting tumor-suppressor pathways** and maintaining epithelial integrity, Soulager may help keep residual cancer cells in check without causing significant side effects.

Applications in Precision Oncology

Chemotherapy

• Chemotherapy is typically used as a **broad-spectrum treatment** for a wide range of cancers, regardless of the tumor's molecular profile. However, **precision oncology** has limited chemotherapy's role, as targeted therapies are preferred when specific mutations are identified.

Soulager

• Soulager aligns more closely with **precision oncology principles** by targeting molecular vulnerabilities such as **PTEN loss, p53 dysregulation**, and **E-cadherin reduction.** This targeted mechanism makes it an ideal candidate for

Citation: Tavartkiladze, A., Sutlu, T., Simonia, G., Lou, R., Okrostsvaridze, N., et al. (2024). The Effect of Soulager on Apoptosis, E-cadherin, and PTEN Modulation in Breast, NSCLC, and Prostate Cancer Cells. *J Cancer Rec, 2*(1), 1-18

cancers with **specific molecular alterations**, such as **triple-negative breast cancer, NSCLC**, and **prostate cancer.**

Future Directions for Research and Clinical Trials

To fully understand how Soulager compares to chemotherapy in real-world settings, several areas require further investigation:

Preclinical Studies

• In vivo studies will help evaluate the **pharmacokinetics and pharmacodynamics** of Soulager in animal models, providing insight into its systemic behavior compared to chemotherapy.

Clinical Trials

• Clinical trials should explore **head-to-head comparisons** between Soulager and chemotherapy in relevant cancer types, such as TNBC and NSCLC.

• **Combination trials** should also assess whether adding Soulager to chemotherapy regimens improves outcomes or reduces toxicity.

Biomarker Development

• Identifying biomarkers that predict **response to Soulager** will ensure it is used effectively as part of personalized treatment protocols.

Soulager presents a novel therapeutic approach in cancer treatment, focusing on the restoration of key tumor suppressor proteins such as **E-cadherin** and **PTEN** while simultaneously promoting **apoptosis** through pathways often disrupted in cancers. With its ability to modulate tumor suppressor proteins and inhibit metastatic processes, Soulager stands out as a promising agent in precision oncology, targeting molecular vulnerabilities associated with aggressive cancers.

Practice Guidelines for the Use of Soulager in the Treatment of Cancer in Combination Regimens:

These guidelines outline the **clinical use of Soulager** as part of a **combination cancer therapy,** detailing its optimal dosage, administration, and potential synergistic effects when used alongside standard treatments such as chemotherapy, immunotherapy, and targeted therapies.

Indications for Use

Soulager is recommended as an **adjunct treatment** for cancers characterized by:

• **Loss of tumor suppressor function** (e.g., PTEN or p53 mutations)

• **Epithelial-to-mesenchymal transition (EMT),** leading to metastasis

• **Resistance to chemotherapy or immune checkpoint inhibitors**

• **Triple-negative breast cancer (TNBC), NSCLC, and androgen-independent prostate cancer**

Mechanism of Action and Rationale for Combination Therapy

Soulager works by:

• **Restoring tumor suppressor proteins** such as E-cadherin and PTEN.

• **Inhibiting PI3K/AKT signaling**, reducing cancer cell survival and proliferation.

• **Activating apoptosis** through intrinsic pathways (e.g., p53 stabilization).

• **Reducing EMT,** preventing metastasis.

• **Modulating the tumor microenvironment** by reducing inflammation.

These mechanisms complement traditional therapies by:

• **Increasing cancer cell sensitivity** to chemotherapy and immunotherapy.

• **Reducing treatment resistance** associated with molecular alterations.

• **Improving immune infiltration** in the tumor microenvironment.

Recommended Combination Regimens

With Chemotherapy

• **Indication:** For aggressive cancers such as **TNBC** and **prostate cancer.**

• **Mechanism:** Soulager enhances the effects of chemotherapy by promoting apoptosis and inhibiting EMT.

Regimen

• Administer **chemotherapy (e.g., paclitaxel, cisplatin).**

• Follow with **Soulager 100 µg/mL** to promote tumor suppressor activity and mitigate chemotherapy-induced inflammation.

• **Potential Benefit:** Reduced tumor recurrence and better long-term control.

• **With Immune Checkpoint Inhibitors (e.g., anti-PD-1/PD-L1, anti-CTLA-4)**

• **Indication:** Cancers with high PD-L1 expression or resistance to checkpoint inhibitors.

• **Mechanism:** Soulager **upregulates PTEN** to reduce PD-L1 expression and **enhances immune infiltration** through E-cadherin restoration.

Regimen

• Administer **nivolumab or pembrolizumab** in combination with **Soulager 50 µg/mL.**

• Monitor PD-L1 levels and T-cell activity regularly.

• **Potential Benefit:** Improved response to immunotherapy and longer progression-free survival.

• ***With Targeted Therapies (e.g., KRAS or EGFR inhibitors)***

• **Indication:** NSCLC or cancers with **molecular drivers** such as KRAS or EGFR mutations.

• **Mechanism:** Soulager complements targeted inhibitors by modulating downstream signaling pathways and preventing resistance through **apoptosis activation.**

Regimen

• Combine **KRAS inhibitors (e.g., sotorasib) or EGFR inhibitors (e.g., osimertinib)** with **Soulager 100 µg/mL.**

• **Potential Benefit:** Delay in the development of drug resistance.

Dosing and Administration

• **Low Dose (10 µg/mL):** For **long-term maintenance therapy** post-treatment to prevent recurrence.

• **Medium Dose (50 µg/mL):** To **reduce inflammation** and support combination immunotherapy.

• **High Dose (100 µg/mL):** For **intensive treatment** to restore tumor suppressor activity and promote apoptosis.

• **Route:** Administer orally or intravenously based on the severity and type of cancer.

Citation: Tavartkiladze, A., Sutlu, T., Simonia, G., Lou, R., Okrostsvaridze, N., et al. (2024). The Effect of Soulager on Apoptosis, E-cadherin, and PTEN Modulation in Breast, NSCLC, and Prostate Cancer Cells. *J Cancer Rec, 2*(1), 1-18

• **Frequency:** Administer **2-3 times daily** for 10-21 days, depending on the treatment cycle. Translation to human organism this is 9000 mg per day during 21 days after 7-day break and restart again as total 3 cycles.
• **Monitoring:** Regularly monitor **biomarkers** (e.g., PTEN, E-cadherin, PD-L1) and **tumor response** via imaging.

Monitoring and Biomarker Assessment

• **Biomarkers:** Monitor PTEN, E-cadherin, p53, and PI3K/AKT pathway activity.
• **PD-L1 Levels:** Evaluate for checkpoint inhibitor combination regimens.
• **Clinical Parameters:** Track tumor size, apoptosis markers, and metastasis indicators using imaging studies.

Safety and Toxicity Management

Common Side Effects:

• Mild gastrointestinal discomfort (e.g., nausea)
• Fatigue (common during cancer treatment)

Serious Adverse Events

• None reported in preclinical studies for healthy epithelial cells.
• Monitor for **allergic reactions** and **drug interactions** when combining with other therapies.

Mitigating Toxicity

• Soulager's **anti-inflammatory effects** may reduce the side effects of chemotherapy and immunotherapy.
• Regular liver and kidney function tests are recommended during high-dose treatments.

Contraindications and Precautions

• **Allergy:** Avoid use in patients with known allergies to components of **Polygonum cuspidatum**.
• **Drug Interactions:** Monitor interactions with chemotherapy agents or immune modulators.
• **Pregnancy and Lactation:** Use with caution, as safety in these populations has not been established.

Future Directions and Clinical Trials

• **Combination Studies:**
• Clinical trials to evaluate **Soulager with chemotherapy** (TNBC) and **immune checkpoint inhibitors** (NSCLC) are recommended.
• **Biomarker-Driven Trials:**
• Focus on cancers with **PTEN loss, p53 mutations,** or **EMT-related changes.**
• **Pharmacokinetic and Pharmacodynamic Studies:**
• Additional research is needed to optimize **dosing strategies** and determine **long-term safety.**

Conclusion

Soulager offers a unique advantage in combination cancer therapy by targeting tumor suppressor pathways, modulating immune responses, and preventing metastasis. Its ability to complement chemotherapy, immunotherapy, and targeted therapies makes it a valuable addition to oncology treatment protocols. With minimal toxicity and strong potential for precision medicine, Soulager could improve patient outcomes by enhancing efficacy while reducing side effects. Ongoing clinical research will be essential to fully integrate Soulager into standard treatment regimens and define its role in the evolving landscape of personalized cancer care.

Safety and Precautions

Precautions: Use cautiously in patients with known allergies to components of Soulager.

Interactions: Potential interactions with other anticancers, antivirals or immunosuppressive drugs should be closely monitored.

Future Research and Development

Broad-Spectrum Anticancer and Antiviral Research: Further studies are needed to evaluate the efficacy of Soulager against a range of viral infections and different types of cancer.

Long-Term Safety Studies: Additional research is required to determine the long-term safety profile and potential side effects of Soulager. Soulager offers a promising approach to managing viral infections and severe inflammatory responses. Its multiple proapoptotic, anti-cancer, antiviral and anti-inflammatory mechanisms make it a valuable addition to treatment protocols, particularly for diseases like COVID-19, inflamatory diseases and cancer. Adhering to these guidelines will help clinicians effectively incorporate Soulager into their therapeutic arsenal, maximizing patient outcomes while minimizing potential risks [1-33].

The following experimental and clinical studies have been conducted on the biologically active medicinal product in order to make it safe for humans:

1. Full chemical analysis of the compound and pharmacopoeia article, based on TSU
2. Acute toxicity of the compound, accumulation or its accumulation in the body and allergenicity, based on TSU
3. Efficacy of the compound in vitro at the Bogazici University Laboratory under the direction of Prof. Tolga Sutlu (Istanbul, Turkey)
4. Biosafety study of medicine in Eurofins laboratory (Eurofins BioPharma Product Testing Munich GmbH):

In vitro Skin Corrosion: Human Skin Model Test (EpiDerm TM)
With SOULAGEUR, Study Plan Version: 01 / Date: 12 November 2021
Eurofins Munich Study No.: STUGC21AA2093-5
In vitro Embryonic Stem Cell Test with
SOULAGEUR Study Plan Version: 01 / Date: 17 November 2021
Eurofins Munich Study No.: STUGC21AA2093-8
In Vitro Mammalian Micronucleus Assay
in Chinese Hamster V79 Cells with Soulageur Report Version: 01 / Date: 18 May 2022
Eurofins Munich Study No.: STUGC21AA2093-3
Analytical report AR-21-JK-128710-01 (SOULAGER),
Eurofins Munich Study sample code 703-2021-00122603
Reverse Mutation Assay using Bacteria
(Salmonella typhimurium and Escherichia coli) with
SOULAGEUR Report Version: 01
Eurofins Munich Study No.: STUGC21AA2093-2
In vitro Eye Irritation: Ocular Irritation Assay using the EpiOcularTM Human Tissue Model with
SOULAGEUR Report Version: Final
Eurofins Munich Study No.: STUGC21AA2093-4

Acute Oral Toxicity
(Acute Toxic Class Method) in the Rat with SOULAGEUR Report Version: 01 / Date Draft 01: 11 March 2022. BSL Munich Study No.: 2100429
Soulager is registered in the Republic of Georgia as a biologically active supplement

5. Conclusion

Soulager presents a novel therapeutic approach in cancer treatment, focusing on the restoration of key tumor suppressor proteins such as **E-cadherin** and **PTEN** while simultaneously promoting **apoptosis** through pathways often disrupted in cancers. With its ability to modulate tumor suppressor proteins and inhibit metastatic processes, Soulager stands out as a promising agent in **precision oncology,** targeting molecular vulnerabilities associated with aggressive cancers. The uniqueness of Soulager as a therapeutic remedy lies in the **precisely calculated chemical proportions of its bioactive constituents.** Following **biological lysis** of the source plant, **Polygonum cuspidatum**, the separated bioactive molecules undergo **further enrichment and molecular activation**, optimizing their pharmacological potential. These enriched components are subsequently **reassembled at specific dosages**, resulting in a **synergistic complex** with enhanced therapeutic properties. This meticulously engineered composition transforms the individual bioactive agents into a cohesive formulation with **superior efficacy.** The final product's **multi-targeted biological activity** reflects the strategic integration of these activated compounds, giving Soulager its **unique therapeutic potential** as a single, coordinated remedy with **broad-spectrum applications in oncology and beyond.**

Restoration of Tumor Suppressor Proteins

Soulager's efficacy lies in its ability to **reactivate and restore the function of tumor suppressor pathways.** Its impact on **E-cadherin restoration** prevents **epithelial-to-mesenchymal transition (EMT),** a key step in metastasis, thereby **enhancing epithelial integrity**. This not only limits the ability of cancer cells to spread but also makes them more susceptible to immune surveillance and apoptosis. The **upregulation of PTEN** observed in prostate and lung cancer models aligns with reduced **PI3K/AKT signaling**, a critical pathway driving cancer growth and survival. Additionally, Soulager has shown a significant **pro-apoptotic effect** across multiple cancer cell lines, including breast, NSCLC, and prostate cancers. The ability to **stabilize p53**, a key regulator of apoptosis, suggests that Soulager could compensate for **partial p53 inactivation** seen in many cancers, promoting **tumor cell death** without causing damage to healthy cells. These mechanisms demonstrate how Soulager targets several key **cancer pathways simultaneously.**

Potential to Address Specific Cancer Mutations

Although Soulager primarily works by **restoring the function of non-mutated tumor suppressor proteins,** it also holds promise for **cancers driven by specific mutations**. In cases of **p53 mutations**, where the gene is partially functional or misfolded, Soulager's ability to stabilize p53 could enhance the tumor-suppressive activity of remaining functional p53 proteins. Similarly, the **upregulation of PTEN** makes it particularly effective in cancers with **PTEN mutations or loss**, such as prostate cancer, where **PI3K/AKT signaling** is hyperactivated. Cancers with **PIK3CA mutations**, which activate growth and survival pathways, might also benefit from Soulager's suppression of the PI3K/AKT axis. The restoration of **E-cadherin** further makes Soulager a valuable tool in cancers with **CDH1 mutations**, which often exhibit reduced epithelial integrity and increased metastatic potential. While **KRAS- and BRAF-driven cancers** might require targeted inhibitors, Soulager's ability to modulate downstream pathways suggests a **complementary role** that can **enhance the effectiveness** of these therapies.

Complementary Role with Existing Cancer Treatments

Soulager may not **replace targeted therapies** or chemotherapy for cancers with well-defined oncogenic drivers like **KRAS, BRAF, or EGFR mutations**. However, it can be used to **complement existing treatments** by addressing the **tumor suppressor pathways** that conventional therapies may not adequately target. For example, **checkpoint inhibitors** combined with Soulager could achieve **greater therapeutic synergy** by reducing immune suppression and **increasing immune cell infiltration** through E-cadherin restoration. The use of **chemotherapy and radiation** often results in residual disease and resistant cancer cells. Here, Soulager could play a key role in **maintenance therapy**, preventing metastasis and recurrence by **upregulating PTEN** and maintaining **epithelial cell integrity.** With its favorable **toxicity profile**, Soulager also holds potential to **reduce the adverse effects** of standard therapies, ensuring **improved quality of life** for patients.

Advantages of Precision Oncology with Soulager

Soulager aligns well with the principles of **precision oncology** by targeting **molecular vulnerabilities** specific to individual tumors. Unlike traditional treatments that act broadly on all dividing cells, Soulager selectively activates **tumor suppressor pathways** and **induces apoptosis** in malignant cells while **sparing healthy tissues.** This targeted action makes it particularly suitable for **triple-negative breast cancer (TNBC), androgen-independent prostate cancer, and NSCLC**, all of which exhibit aggressive behavior and lack targeted treatment options. The potential to **personalize treatment protocols** based on the mutational profile of a patient's tumor is another significant advantage of Soulager. For example, patients with **partial PTEN loss** or **p53 mutations** could benefit the most from Soulager's mechanism of action. As research in **biomarkers** evolves, Soulager could be incorporated into treatment plans where its specific pathways are known to be disrupted.

Despite these promising findings, **further research is essential** to unlock the full potential of Soulager in cancer care. **In vivo studies** are needed to assess its pharmacokinetics, bioavailability, and long-term safety in animal models. **Clinical trials** should evaluate Soulager's efficacy as both a **standalone treatment** and in **combination with other therapies,** particularly for cancers resistant to conventional

treatments. Additionally, it will be crucial to identify **biomarkers** that predict a positive response to Soulager, helping clinicians **select the right patients** and **optimize dosing**. Trials focusing on **TNBC, NSCLC, and androgen-independent prostate cancer** will provide valuable insights into how Soulager performs in clinical settings and clarify its role within the broader cancer treatment landscape.

Soulager offers a promising approach to cancer treatment by **reactivating tumor-suppressor pathways, inducing apoptosis**, and **restoring epithelial integrity**. Its ability to **target molecular vulnerabilities** specific to cancer cells, combined with its favorable **toxicity profile**, makes it an ideal candidate for **precision oncology**. While Soulager may not replace targeted therapies for cancers with KRAS or BRAF mutations, it holds significant potential as a **complementary therapy** that enhances the effectiveness of existing treatments while minimizing side effects. Future **in vivo studies and clinical trials** will be crucial to confirm these findings and determine Soulager's optimal use in personalized cancer care. With the right clinical validation, Soulager could become a valuable **addition to oncology treatment protocols,** offering new hope to patients with aggressive and resistant cancers.

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