Abstract. One of the major hallmarks of many cancer cells is dedifferentiated cells (immature cells) with little or no resemblance to normal cells. Besides the poor differentiation, malignant cells also have important features such as aggressiveness and resistance to different therapeutics. Differentiation potentiators hold great promise for cancer treatment. Dimethyl sulfoxide (DMSO) is a well-characterized pharmaceutical solvent. It is used as a component of numerous cancer therapeutic approaches, including cancer treatment and several approved cancer immune therapeutics such as Car-T cell therapy and the FDA-approved drug Mekinist (trametinib DMSO) for melanoma treatment. It is also biologically recognized as a pharmaceutical solvent and cryoprotectant. In the current literature, there are no mentions of DMSO’s possible ability to potentiate therapeutic activity as a component of these cancer treatments. This review aimed to summarize scientific evidence and substantiate the concept that DMSO can contribute positively to the overall efficacy of cancer treatment as an adjuvant that is safe, inexpensive, and an effective differentiation-inducing therapeutic agent.

Differentiation is the cellular developmental process whereby cells change in form and develop specialized functions. The tumor cell differentiation stage is a crucial aspect of defining histopathological malignancies. The differentiation process is typically unidirectional in normal cells; however, cancer cells, like stem cells, have shown that this process can be reversible, be dedifferentiated or re-differentiated. Higher degrees of differentiation pose a better prognosis than a low degree and are strongly associated with tumor behavior, invasiveness, and resistance to cancer therapy (1).

Treating cancers through the induction of cell differentiation has been an attractive and practical approach (2). Reagents, such as all-trans-retinoic acid (ATRA), nerve growth factor (NGF), dimethyl sulfoxide (DMSO), vitamin D3, 12-O-tetradecanoylphorbol 13-acetate (TPA), peroxisome proliferator-activated receptor-gamma (PPAR-γ), hexamethylene-bis-acetamide (HMBA), transforming growth factor-beta (TGF-β), butyric acid, cAMP, and vesnarinone, have been extensively studied for their differentiation-inducing ability on cancer cells in in vitro models and validated in preclinical studies and human trials (3-5). Notably, combining differentiation agents with conventional medicine such as chemotherapy or radiation therapy, can potentiate the treatment effect seen in patients with advanced cancer (6, 7). One example of the most commonly used cellular differentiation treatment for cancer is ATRA, a well-known drug for certain dermatological diseases, and a...
redifferentiation agent for hematological malignancies and thyroid cancer for over 20 years (8, 9). When using retinoic acid in combination with cytotoxic chemotherapies for acute promyelocytic leukemia (APML), the remission rates progressively improve from around 50% to more than 90% in newly diagnosed APML patients (3, 10). Despite the success of ATRA, numerous challenges remain for cancer redifferentiation therapy, particularly in solid tumors. There is a lack of understanding of the biology of the normal differentiation pathway; the mechanisms responsible for the inhibition of differentiation vary among different tumor types and patients. Many potential therapeutic agents have been demonstrated to induce differentiation in experimental models but failed to develop into approved drugs (11).

A potential chemical of interest that fits this bill is Dimethyl sulfoxide (DMSO). As an amphipathic agent, DMSO is widely used as a solvent for water-insoluble molecules, cryopreserving agents, and cell therapies (12). It has been used as a cell differentiation inducer, free radical scavenger, and radioprotectant (13-15). In addition, various pharmaceutical and therapeutic properties of DMSO, such as anti-inflammatory, antiviral, antifungal, antibacterial, local, and systemic analgesia, and membrane penetration enhancement, have been applied in preclinical applications (16, 17). DMSO is used as a drug delivery vehicle for various human and animal conditions, including gastrointestinal diseases, amyloidosis, dermatological disorders, traumatic musculoskeletal disorders, brain edema, rheumatologic diseases, soft tissue injuries of chemotherapeutic drugs, and radiotherapy (18-25). FDA has approved DMSO for treating interstitial cystitis in the United States (26).

Previously, our group reported the excellent safety profile and efficacy of DMSO for palliative care and pain control in advanced cancer patients (27-30). Recently, DMSO has been included in biological cancer treatment and several FDA-approved cancer immune therapeutic modalities such as Car-T cell therapy and melanoma drug Mekinist (trametinib DMSO) (31). However, besides its recognized biological role as a pharmaceutical solvent and cryoprotectant, there was no mention of DMSO’s possible ability to potentiate therapeutic activity as a component of these cancer treatments.

In this current review, we summarize experimental and clinical evidence to support the implementation of DMSO as a safe, effective, and affordable differentiation inducer to potentiate the efficacy of cancer therapeutic modalities.

**DMSO as a differentiation-inducing/signal-transducing agent in experimental cancer studies.** In 1971, a series of experimental studies documented DMSO’s properties of viable differentiation-inducing activities in erythroleukemic cells (32-34). In these studies, DMSO induced differentiation by altering gene expression via regulating DNA and protein interactions through inducing conformational changes. Abnormal cell differentiation, particularly the suppression of terminal cell differentiation, exists in all tumors, especially leukemia. Tumor suppressors are vital in the gateway to terminal cell differentiation. Teimourian et al. studied the differentiation-inducing effects of DMSO and ATRA through the phosphatase and tensin homolog gene (PTEN) (35). The researchers inhibited PTEN tumor suppressor gene expression by siRNA to investigate the effect of potentiating cell survival and inhibiting apoptosis on HL-60 cell differentiation by DMSO and ATRA. The results showed that PTEN siRNA significantly increased HL-60 cell differentiation in the presence of DMSO and ATRA (35). At the same time, the presence of siRNA hampered the accumulation of apoptotic cells during incubation. The study suggested that adding DMSO could increase the efficacy of differentiation therapy through the manipulation of PTEN for acute myelogenous leukemia.

Peripheral blood leukocytes from a patient with APML are predominantly promyelocytes. When DMSO was added in the culture medium, cells were induced to differentiate into mature types of granulocytes, including myelocytes, metamyelocytes, and segmented neutrophils. All 150 clones developed from the HL-60 culture showed similar morphological differentiation with functional maturity, causing leukemic cells to lose their proliferative properties in the presence of DMSO (36). The induction of leukemic cell differentiation into mature cells is a major strategy for treating leukemia. Since differentiated leukemic cells lose their proliferative and tumor-forming abilities, different differentiation inducers have been extensively studied as valuable candidates for leukemia treatment. The study by Hong-Nu et al. (37) showed that the combination of TNF-α with DMSO had a synergic effect on HL-60 cell differentiation by increasing CD11b expression and cell population in the G1 phase through the activation of the ERK pathway. The results of this study also suggest that TNF-α synergistically increases DMSO-induced differentiation of HL-60 cells through the activation of the ERK/MAPK-signaling pathway.

In another study, the same group of researchers demonstrated that DMSO induced up-regulation of the tumor suppressor PTEN by activating NF-kB (38). It is proposed that the degradation of phosphatidylinositol 3,4,5-trisphosphate (PIP3) to phosphatidylinositol 4,5-bisphosphate inhibits the activity of PI3K. PIP3 is an essential regulator of cell growth and survival through Akt, expressed in HL60 cells (39). Cancer survival signals are mediated mainly by PI3K/Akt (40); hence this pathway may contribute to a resistant phenotype. Therefore, an increased expression of the tumor suppressor PTEN could lead to the inhibition of Akt phosphorylation, allowing HL60 cells to differentiate into neutrophil-like cells.

The highly aggressive prostate cancer cells utilize androgen receptor (AR) to signal their growth and metastasis. Despite the treatment advancement achieved through androgen
deprivation therapy, the recurrence of castration-resistant prostate cancer (CRPC) cannot be prevented. DMSO has been shown to suppress AR levels in the CRPC cell lines by decreasing the expression of hetero-nuclear ribonucleoprotein H1 (41). Treatment with low dose DMSO (0.1-1%) did not exhibit any cytotoxicity or changes in cell viability; minimal cytotoxicity was observed when DMSO concentration increased to 2.5% in a 96-h treatment. These clinical doses of DMSO caused a significant (p<0.01) decrease in the migratory ability of CRPC cell lines, suggesting that DMSO may decrease the metastatic ability of CRPC cells (41).

Low-dose DMSO significantly enhanced the antiproliferative effect of interferon-alpha (IFN-α) in several human lung adenocarcinoma cells in vitro and in vivo studies (42). DMSO, along with other anti-cancer drugs, hexamethylene bisacetamide (HMA), doxorubicin, and 5-fluorouracil (5-FU), have been tested on adenocarcinoma cell lines (PC9 & PC14) (43). Different concentrations of DMSO induced morphological changes in the adenocarcinoma cells, with 1% of DMSO causing cells to become cuboidal, polygonal, and adhere closely to each other. Additionally, when tested in conjunction with IFN-α, DMSO increased the sensitivity of the cancer cells compared to other drugs, and also increasing alkaline phosphatase activity (42). Alkaline phosphatase is a marker of type II pneumocyte maturation and differentiation (44), indicating that DMSO could potentiate cancer treatments in lung cancer patients. Another in vitro study compared the differentiation-inducing effect of DMSO and retinoic acid on a polyclonal human ovarian cancer cell line (HOC-7) (45). DMSO caused elevation of membrane-associated staining epidermal growth factor-receptor (EGF-R) and desmplakins I and II (DPI+II) (46). After treatment, evaluation with ELISA and western blotting revealed that both DMSO and retinoic acid caused down-regulation of Myc oncoproteins, with DMSO causing a more significant reduction, leading to a decrease in cell growth. Interestingly, only treatment with DMSO caused increased epithelial cell differentiation.

DMSO and butyrate were studied for their effects on four human intestinal tumor cell lines in vitro (47). The growth of all four of these tumor cell lines was significantly inhibited, and doubling times increased by twofold in the presence of 2 mM butyrate and 2% DMSO. Their lectin-binding properties were evaluated using flow cytometric analysis to assess the effects on modulating cell gene expression. All four cell lines showed an increased lectin binding, indicating a differentiation-inducing effect of butyrate and DMSO on these cell lines (47).

Tsao et al. (48) evaluated three differentiation-modifying agents, sodium butyrate, DMSO, and retinoic acid, on the human rectal adenocarcinoma cell line (HRT-18) on cell growth, morphology, carcinoembryonic antigen content, cell surface membrane-associated enzyme activities, and glycoprotein profiles in vitro. All tested agents caused a marked reversible increase in doubling times, decreased saturation densities, and a markedly reduced colony-forming efficiency. DMSO caused a significant reduction in carcinoembryonic antigen levels and alkaline phosphatase activity, whereas it was shown to increase with butyrate (48).

The most attractive potential application of DMSO in cancer treatment is as an adjuvant in immunotherapy. Jiang et al. (49) proposed a possible implementation of DMSO to induce anti-tumor immunity during chemotherapy. After treating Hepa1-6 cells with 2% DMSO in the culture medium, there was reduced proliferation with no significant apoptosis or decreased viability. After seven days of treatment with DMSO, the proliferation rate of Hepa1-6 cells was restored in a DMSO-free medium. However, their gene expression profile showed an irreversible alteration in more than 1,000 genes, suggesting that treating viable cells with DMSO may alter biological features by inducing anti-tumor immunity in vivo.

In the hepatocellular carcinoma cell line Huh7, regular culture in the absence of DMSO initially formed tightly packed monolayers, but was compromised on day 10 with extensive cell death (50). In the presence of 1% DMSO, the monolayers were composed of mono- and binucleated cells with primary hepatocytes features. The addition of DMSO has been shown to significantly increase the expression of three hepatocellular differentiation markers, including human albumin, A1AT mRNA, and HNF4-α (50). In a subsequent study, Huh7 cells treated with DMSO had increased ability to metabolize drugs, as evidenced by increased levels of various drug-metabolizing enzymes (51). Additionally, DMSO exposure inhibits cell division, arrests the cell cycle

| Table 1. Safety and toxicity of dimethyl sulfoxide (DMSO) usage. |
|---------------------|---------------------|
| **Category**        | **Risks**            |
| Oral delivery       | (LD₅₀ 14,500-28,300 mg/kg, rat) |
| Transdermal delivery| (LD₅₀ 40,000 mg/kg, rat) |
| SC injection        | (100% at <0.5 ml; <15% at 2.0 ml) |
| IV injection        | IV injectables should limit DMSO use to <10% |
| Toxicity            | Well characterized ADME profile |
|                     | Half-life of parent compound and its metabolite of nine hours |
|                     | Essentially non-toxic by all routes of administration |
|                     | Not carcinogenic, mutagenic or a reproductive toxin (CMR) |
|                     | Does not bioaccumulate |
|                     | Primary excretion through the kidney, secondary elimination through the respiratory tract and bile |

SC: Subcutaneous; IV: intravenous; ADME: absorption, distribution, metabolism, and excretion.
DMSO can modulate cell differentiation even in precursor compounds in the cytoplasm (actin) and cytoskeleton (alpha-actin), typical differentiation phenotypes in rhabdomyosarcoma cell lines (53).

The above experimental research indicates a robust differentiation-inducing activity of DMSO in different cancer cell lines, suggesting a possible practical application of this already approved pharmaceutical solvent, cryoprotectant, and drug as adjuvant therapy in conventional cancer treatment.

**DMSO safety profile.** DMSO is mainly used as a cryoprotective agent and a pharmaceutical solvent in many compounds. When used clinically, it has a very good safety profile. A single dose of iodogen dissolved in DMSO up to 30.0 mg/kg, over 3,000 times the dose in potential human applications, appears safe, with a projected LD<sub>50</sub> of 60.0 mg/kg in mice (54). The adverse effect of DMSO that may hamper its application is the garlic or onion-like odor and taste. It can persist in the body for up to 2 days. DMSO by itself is odorless, but the pulmonary excretion as dimethyl sulfide causes such malodor (25, 55). DMSO also releases histamine, leading to flushing and allergic reactions, which can lead to potential adverse human effects at higher concentrations (56). When DMSO was administered intravenously, side effects in patients, such as nausea and vomiting, were sometimes observed. However, these symptoms usually disappeared shortly after infusion and were less frequent at lower dosages (57). The most serious reported adverse effect associated with DMSO injection was intravascular hemolysis when a 40% or higher concentration solution was applied. This pathological hemolysis is caused by increased osmotic pressure on erythrocytes through elevated DMSO concentrations (58). This potential adverse effect can be avoided by infusing DMSO at a concentration of 30% or less (59).

Regarding dermatological side effects, the most reported symptoms related to topical application are rash, dry skin, and contact dermatitis. However, the reactions are usually mild and brief and do not re-appear with conditional treatment (60). When comparing adverse event rates, there was no difference in G0/G1 state, remaining viable in culture without splitting for over 60 days, and increases cell differentiation characterized by an increase in liver-specific genes (51).

Prados *et al.* (52) demonstrated that the addition of 1.25% DMSO can modulate cell differentiation even in rhabdomyosarcomas, poorly differentiated malignant tumors. After culturing these tumor cells with DMSO for 8 h, there was a significantly increased expression of desmin, and after 12 h, there was a significant increase in the expression of precursor compounds in the cytoplasm (actin) and cytoskeleton (alpha-actin), typical differentiation phenotypes in rhabdomyosarcoma cell lines (53).

The above experimental research indicates a robust differentiation-inducing activity of DMSO in different cancer cell lines, suggesting a possible practical application of this already approved pharmaceutical solvent, cryoprotectant, and drug as adjuvant therapy in conventional cancer treatment.
between the trial DMSO drug, placebo, or DMSO vehicle when looking at the skin, gastrointestinal, or cardiac events (61). No cases of lens changes in humans have been reported with prolonged administration of large amounts of DMSO in systemic, topical, or local ophthalmic treatment (62). Therefore, DMSO was proven to have little to no toxicity when used in clinically adequate doses. The pharmacological safety profile of DMSO is listed in Table I (14).

For the past two decades, the registration number of DMSO-based pharmaceutical agents, over-the-counter (OTC) drugs, and medical devices (31) has increased globally. The application of DMSO as an active ingredient or excipient in drug formulations has expanded to topical, oral, and parenteral products (63). DMSO (10%) is commonly used as a cryoprotective agent (CPA), added to the culture media for preserving and storing biological tissues (64). It acts as a penetrating cryopreservation agent to increase the porosity of the cellular membrane, allowing water to flow more freely through the membrane, avoiding ice crystal formation (65). DMSO has remained the gold standard CPA for many different cell types over other agents such as glycerol and polyethylene glycol (66). FDA has approved DMSO as a CPA for sperm, eggs, stem cells, bone marrow cells, and organs for transplant (16). In the concentration ranging from 5% to 17%, DMSO is a critical CPA in many cell therapy products, such as CAR-T, melanoma treatment Mekinist, and stem cell transplantation (31). Table II summarizes the pharmaceutical formulations that incorporate DMSO as an excipient (14).

**Discussion and Perspectives**

DMSO is a very versatile compound that is currently widely used in tissue/organ preservation and as a cryoprotectant for biologic therapy. There is also a huge potential for medical uses of DMSO in cancer management, including penetration-enhancing and solvent excipients in cancer therapeutics, pain control, palliative care, and treatment of tissue injuries due to radiotherapy and chemotherapy. Numerous experimental and clinical data suggest a possible productive role of DMSO as an active drug or adjuvant therapeutic agent to improve the effectiveness of existing cancer therapies and control abnormal cell differentiation, in particular inhibition of end-cell differentiation, in tumors (67).

Based on the available published research data, DMSO might be implemented as an effective, safe, and inexpensive differentiation-inducing therapeutic agent to enhance the overall efficacy of the established conventional and complementary cancer treatments. Besides the documented robust differentiation-inducing activities, DMSO has also demonstrated several anti-cancer properties that could further benefit cancer patients, including suppressing proliferation and inducing apoptosis (68-70).

Although clinical research on DMSO has regained some enthusiasm in the past 20 years, the development of DMSO as an active pharmaceutical drug or adjuvant therapeutic has not been attractive to the pharmaceutical industry, mainly because of its generic status. DMSO will remain as an important component in the most sophisticated modern cell therapeutics in stem cell transplantation and immunotherapy. Currently, DMSO is widely used in various pharmaceutical preparations to enhance the solubility of drugs, leading to the delivery of a higher concentration of medication to the targets (71).

Since DMSO is inexpensive and non-patentable, pharmaceutical companies lack the financial incentive to develop this therapeutic agent in cancer clinical applications. Future non-profit and doctors-driven explorative and translational clinical investigations are needed to prove and promote the practical implementation of DMSO as a possible adjuvant drug, analgesic, and palliative care therapy for cancer patients.

**Conflicts of Interest**

No competing financial interests exist in relation to this study.

**Authors’ Contributions**

All Authors contributed equally to the writing and submission of the manuscript.

**References**


41 Khurana N, Kim H, Khan T, Kahhal S, Bukvic A, Abdel-Mageed AB, Sikka SC and Mondal D: Low dose dimethyl sulfoxide (DMSO) downregulates the expression of androgen receptor (AR) and ar-variant 7 (AR-V7) in castration resistant prostate cancer (CRPC) cells. Cancer Res 82(12_Supplement): 2446-2446, 2022. DOI: 10.1158/1538-7445.AM2022-2446


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