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Review Article

Application of Salinomycin in Treatment of Different Types of Cancer

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ABSTRACT

Cancer occurs when the delicate balance between proliferation of cell and regulation is disturbed. Genes that inhibit tumors stop working when proto-oncogenes turn into oncogenes, which results to uncontrolled cell growth. KRAS, MYC, and HER2 are oncogenes. Cancer cells control potassium K^+ levels in order to fight against treatments. This equilibrium is upset by salinomycin, it brings K^+ ions and forces them out of the cell. Particularly in cancer stem cells (CSCs), this effects survival signals, manufacturing of proteins, and mitochondrial function, result in apoptosis. CSCs depend on ABC transporters for resistance to drugs, which salinomycin inhibits, rendering them susceptible to treatment. It blocks Wnt/β-catenin and Hedgehog signaling pathways, preventing cancer cell proliferation. Salinomycin also causes ER stress and cell death by upsetting Ca^{2+} homeostasis. The SlnR pathway, which governs antibiotic production in *Streptomyces albus*, regulates its biosynthesis. Salinomycin's ability to inhibit multiple cancer mechanisms renders it an appealing anticancer agent. While challenges in manufacture, purification, and toxicity must be addressed, the present data shows the possibility of salinomycin as a beneficial supplement to cancer therapy. Despite exhibiting positive effects in severe cancer cases, its use in medicine is still restricted due to concerns about possible damage to healthy cells. The goal of study is to implement salinomycin in clinical practice.

Keywords: *Streptomyces albus,* salinomycin, cancer stem cells (CSCs), antibiotic, antimicrobial peptide, cancer treatment.

INTRODUCTION

Streptomyces genus generates antimicrobial peptides (Hu and Ochi, 2001). The whole genome sequence of *S. albus* J1074 was obtained with a length of 6,841,649 base pairs and 5,832 genes encoded, its genome is the shortest of all the genus *Streptomycetes*. The entire transcriptomes of *S. albus* J1074 were sequenced at different times to determine when the living thing experienced an initial metabolic shift from the exponential to the stationary phase (Zaburannyi *et al*., 2014). *Streptomyces lividans* and *Streptomyces coelicolor A3* can produce secondary metabolites as a result of a particular str mutation that confers resistance to streptomycin (Hesketh and Ochi, 1997). Antibiotics often come from *actinomycetes*, especially *Streptomyces*, which emphasizes the enormous potential of compounds generated from bacteria (Schallmey *et al*., 2004). Numerous enzymes and antibacterial substances are made by *Bacillus* species, including *Bacillus amyloliquefaciens* and *Bacillus subtilis*, which opens up a possible treatment option for infections in people and animals (Magashi *et al*., 2019). Creation of antimicrobial peptides like the recently identified bacteriocins as alternatives to antibiotics (Seal

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et al., 2018). Salinomycin biosynthesis gene cluster, important enzymes, and regulatory factors of polyketone skeleton chain synthesis, sought to offer a few approaches to enhance salinomycin manufacture through secondary metabolism regulation technology and production process of salinomycin in *Streptomyces albus* (Guo Songsong *et al*., 2024). Salinomycin is a new polyether antibiotic that is extracted by solvent extraction and silica or alumina gel chromatography from *Streptomyces albus* (ATCC 21838). Acidic chemical (molecular formula C42H70O11) is effective against mycobacteria, gram-positive bacteria, and some filamentous fungi, which makes it a good treatment for cancer (Miyazaki *et al*., 1974).

Salinomycin targets cancer stem cells to solve the problem of resistance to drugs in cancer treatment. It originates from *Streptomyces albus* microbe by tank fermentation process. It functions via limiting processes that cause tumor formation, such as the production of the adenosine triphosphate–binding cassette transporter and interfering with other signals like Akt, Wnt/β-catenin, Hedgehog, and Notch (Dewangan *et al*., 2017). Salinomycin is a monocarboxylic polyether antibiotic that was derived from *Streptomyces albus*. It works by inducing necrosis, autophagy, and apoptosis in a bid to exclusively destroy cancer stem cells. Salinomycin can reverse the immune inhibitory stop cell division, attack, and migration in different cancer, hence limiting tumor growth and metastasis. Salinomycin is therefore a possible remedy for cancer (Wang et al., 2021). The function of slnR in S. albus salinomycin synthesis using overexpression, complementation, and gene deletion Salinomycin production basically stops when the S. albus chromosome's slnR gene is replaced however, salinomycin production returns when slnR is complemented, revealing that slnR is a beneficial regulator of salinomycin production (Zhu et al., 2017). Cancer is one of the top cause’s deaths worldwide, demanding a continuous search for new and efficient medicines. Salinomycin, polyether ionophore antibiotic produced by Streptomyces albus, is now recognized as a viable candidate due to its influential anticancer activities, especially against cancer stem cells (CSCs). The aim of study to investigate salinomycin's potential as a therapeutic agent for a range of cancers, with a focus on its mechanism of action, efficiency and clinical applications, to understand the processes by which salinomycin exhibits its anticancer properties. To examine potential medicinal benefits of salinomycin in conjunction with developed cancer therapies.

STREPTOMYCES ALBUS: BIOLOGY AND GENETICS

Gram-positive *Streptomyces albus* bacteria are mainly isolated from soil, which is a dynamic medium with little food (Wang *et al*., 2021). This organism has ability to generate and release antibiotics. One of the antibiotics made from *S. albus*, salinomycin, has been used extensively in agriculture to treat cancer because of its capacity to impede the growth of a variety of Gram-positive bacteria (Gumila *et al*., 1997). SlnR has been shown to be an advantageous pathway-specific regulator for salinomycin biosynthesis and could be able to affect the gene expression of genes in the *S. albus* salinomycin biosynthesis cluster (Zhu *et al*., 2017). Within *Streptomyces*, the two component system (TCS) is the most prevalent pleiotropic regulator a considerable portion of TCS controls both morphological differentiation and antibiotic synthesis. For instance, TCS was reported to adversely regulate ACT synthesis in *S. coelicolor* A3, but the system's activator signal was concealed (Chang *et al*., 1996). Linear + linear homologous recombination-mediated recombination (LLHR) provides gene clusters that facilitate the biosynthesis of natural products from predigested bacterial genomic DNA. The salinomycin gene clusters (salO-orf18) from *Streptomyces albus* (*S. albus*) DSM41398 was isolated into three distinct pieces using LLHR. Red/ET was subsequently utilized to put the fragments together into an entire gene cluster (106 kb) which was subsequently expressed in the recombinant host *Streptomyces coelicolor (S. coelicolor*) A3. First to report that LLHR was used to clone a sizable genomic area from a Gram-positive strain (Yin *et al*., 2015).

SALINOMYCIN: STRUCTURE, MECHANISM AND ANTIMICROBIAL ACTIVITY

The monocarboxylic polyether ionophore-containing antibiotic 751-Da has an average acidity. Salinomycin is a type of membrane ionophore that exhibits a preference for potassium ions over alkali ions (Fuchs *et al*., 2009). SAL has strong anticancer effects that inhibit the growth of a variety of tumor cells, such as those that are multidrug resistant (MDR), and it has the ability to solely destroy cancer stem cells (CSCs) (Jędrzejczyk *et al*., 2022). Salinomycin structural derivatives improved selectivity and efficacy against cancer stem cells, and for their ability to probe the molecular mechanism underlying the observed phenotypic effects. Salinomycin's accessible functional groups can be adapted synthetically. A library of analogues was created, and its cytotoxicity and phenotypic the precision in breast cancer cells were used to determine structure activity relationships. Enhanced selectivity and activity distinguish 20-O-Acylated derivatives from other. Mechanistically, modifications that directly interfere with either of salinomycin's two

main ion coordinating motifs the C11 ketones and the C1 carboxylate significantly lower activity, illustrating the significance of the drug's ionophore capabilities (Borgström *et al*., 2017).

Salinomycin Structure

Carboxylic acid, hydroxyl groups, ketones, tetrahydropyran and tetrahydrofuran rings, and other common physicochemical components are required for these compounds' abilities. Sal is a tiny molecule (751 Da) that contains a carboxylic acid that has an affinity for divalent (Fe^{2+}, Ca^{2+}, Mg^{2+}) and monovalent (K^+, Na^+, Cs^+) cations (Versini *et al*., 2018). The sodium salt complex structure of salinomycin has polar inner core which is made up of atoms of oxygen and one carboxylic group, along with its lipophilic surface make it a good option for moving monovalent cations across lipid cytoplasm and mitochondrial membranes, particularly H^+, Na^+, and K^+. Because of the creation of bonds of hydrogen among the group of carboxylic acids on the opposite side of the molecule and the two hydroxyl groups that are on the other, salinomycin and its salts have a pseudo cyclic structure. The polyether skeleton of the pseudo-cyclic structure, like other polyether ionophores like monensin, lasalocid acid, and narasin, may develop complexes with metal cations. Metal complexes might be transferred through biological cell membranes because to the salinomycin molecule's lipophilic exterior (Huczynski, 2012). Salinomycin in a stable pseudo-cyclic shape. Non-covalent "head-to-tail" hydrogen bonds connect the hydroxyl group on one side of the molecule and the carboxyl group on the other to form salinomycin. The hydroxy groups at locations C9 and C28 are vital because they establish a network of hydrogen bonds with the carboxylate moiety, this keeps the metal cation enclosed in the head-to-tail conformation during membrane crossing. Of special significance is the hydrogen link that links the carboxylic and the C28 hydroxy group, because it must be broken in order for metal ions to be caught and released. Apart from the carboxylate and cyclic ethers, the C11 ketones is the only functional group that is actively involved in the coordination of metal ions. Well-defined alterations to the C-ring conformation through modification of the C-ring olefin, or through the re-hybridization of the C20 carbon when combined with changes to the C20 carbon's oxidation state (Borgström *et al*., 2017).

Salinomycin as Anticancer Agent

When applied to the uveal melanoma cell lines and a xenograft mouse model, salinomycin significantly reduced both growth and survival. In addition, in a mouse model of uveal melanoma liver metastasis, salinomycin effectively inhibited hepatic metastasis and substantially reduced CSCs (cancer stem cells). Twist1 was essential for the removal of CSCs and the inhibition of migration and invasion in uveal melanomas when salinomycin was used (Zhou *et al*., 2019). Using the HCC cell lines Huh7, LM3, and SMMC-7721 as well as a subcutaneous tumor model in mice, the potential of salinomycin (Sal) to enhance the susceptibility of hepatoma cells to conventional chemotherapeutic agents like 5-FU. Both in vitro and in vivo, the combination of Sal with 5-FU had a synergistic antitumor impact on liver cancers. The 5-FU-induced rise in CD133+ and EPCAM+ cells, the pathway of Wnt/-catenin activation, and the epithelial–mesenchyme transition were all reversed by sal. Sal and 5-FU together could offer us a fresh way to treat HCC patients by overcoming their drug resistance (Wang *et al*., 2014).

Salinomycin with Combination Therapies

Since tumors are composed of up of several cell populations that have distinct mutations and behaviors, single-agent therapies are inadequate for curing cancer. Combination therapies, that employ different drugs have synergistic mechanisms to boost anti-tumor activity while reducing drug resistance, are critical to attaining effective eradication of cancer. The study of different drug combinations has been rendered easier by recent developments in sustainable Nano-carrier delivery methods, which have also brought light to the effectiveness of salinomycin if combined with other medicines. When salinomycin and dasatinib pair up them can increase DNA damage while regulating levels of anti-apoptotic proteins, leading to cell cycle arrest and improves sensitivity to conventional chemotherapy like doxorubicin and etoposide. Novel delivery methods, such PLGA/TPGS nanoparticles, have shown better therapeutic results if used in combination therapies that target cancer stem cells (CSCs) (Al Faraj *et al*., 2016).

SALINOMYCIN TOXICITY

Salinomycin's EC50 values vary based on the type of cancer cell, and different populations with high levels of cancer stem cells (CSCs) should be eliminated at different concentrations. Salinomycin can be neurotoxic to neural cells when used at high concentrations, although it is not harmful to human mesenchymal stem cells from the bone marrow (hBMSC) if used at quantities higher than 30 μM. In addition, it has been shown that salinomycin inhibits adipocyte development at low doses while exhibiting inflammatory effects on lower concentrations. Taken together, these results highlight the need for cautious dosage and close observation of salinomycin's effects on several human cell types in order to lessen any possible safety concerns related to its therapeutic use (Szkudlarek-Mikho *et al*., 2012).

MECHANISMS OF ACTION

Salinomycin has molecular actions in anti-cancer activity. Some of the reported mechanisms of action include altering signaling pathways starting autophagy, lowering ATP levels and raising ROS production, iron sequestration, DNA damage, inducing ER stress, and, most recently, suppressing CSC marker expression through interaction with nucleolin (Xipell *et al*., 2016).

Effects on Mitochondria and its Role as a Polyether Ionophore

Early in the 1970s, salinomycin whose molecular formula is C42H70O11 was discovered to be a new polyether ionophore antibiotic (Kinashi *et al*., 1973). This substance transports both monovalent and divalent cations, with the former preferring K^+ in organic phases and having antibacterial properties against a range of microbes. Salinomycin's function in ion transport across lipid bilayers as well as its possible modes of action against cancer cells and parasites (Mitani *et al*., 1975). Salinomycin also prevents K^+ uptake and inhibits respiration depending on conditions. It blocks K^+ retention in mitochondria more effectively than Na^+ (Mitani *et al*., 1976). Salinomycin's early effect as a K^+ ionophore on mitochondrial function was studied in human cells. It decreased respiration without inducing ROS and showed similarity to nigericin. Salinomycin had cytotoxic and apoptotic effects on leukemia cells. Apoptosis was induced in mouse cells in a dose-dependent manner (Hofer and Kunemund, 1984). Salinomycin's influence on mitochondria and cancer cells was examined. Salinomycin caused p53 translocation and necrosis in glioma cells (Qin *et al*., 2015).

DNA damage, ROS, and Autophagy Induction

In cancer cells, autophagy performs a dual purpose to avoid and promoting cell death. The application of salinomycin therapy increased the level of production of microtubule-associated protein 1A/1B-light chain 3 (LC3), confirming autophagy being activated, however it may avoid ROS formation and stop cells from going through apoptosis (Li *et al*., 2013). Salinomycin-induced cell death likely partially inhibited by knocking down autophagy protein 7 (ATG7), indicating that autophagy related apoptosis was established (Verdoodt *et al*., 2012). In CSCs, autophagy is crucial. Therefore, the triggering of autophagy related apoptosis may lead to the eradication of CSCs through salinomycin. Various organizations on the complex control of autophagy in malignant cells by salinomycin yet, intracellular oxygen species generation, LC3/autophagy induction, and resultant cell death following single-drug therapy in vitro is absent (Jiang *et al*., 2018). It is generally accepted that this phosphorylation has links to the reaction to DNA damage and chromatin relaxation (Czerwińska *et al*., 2017). Side effect of salinomycin therapy could be DNA damage. More recently, the Law group used RNA sequencing to prove that the combination of salinomycin and dasatinib, a Src kinase inhibitor, shown synergism by straight reducing several pathways in breast cancer cell lines. These pathways included the DNA damage response pathway, the BRCA1 (breast cancer gene 1), and the estrogen-mediated S-phase entry pathway (G1/S arrest) (Bellat *et al*., 2020).

Stress Induction in the Endoplasmic Reticulum (ER)

A luminous salinomycin analogue conjugate, shows salinomycin's anti-CSC action. Salinomycin also causes ER Ca^{2+} release and triggers stress in the ER in breast cancer cells (Huang *et al*., 2018). Dispersion of a fluorescent salinomycin conjugate (SAL-NBD) in the cells during treatment, particularly within the ER and lipids droplet (LD). An order of distribution in cells was also noted, with salinomycin analogues clustering in lysosomes (Huang *et al*., 2018). As cellular Ca^{2+} homeostasis is highly maintained by controlling free as well as bound Ca^{2+} levels in all parts of a cell, disruption of Ca^{2+} signaling sets off ER stress-management reactions, like the response of unfolded proteins (UPR), and the activation of pathways to restore ER equilibrium (Krebs *et al*., 2015). Following therapy with the fluorescent compound of salinomycin, C/EBP homologue protein (CHOP), G protein-coupled receptor 78 (GPR78), and activation transcription factor 6 (ATF6) were observed. Of a breast cancer cell line, CHOP knockdown impaired the conjugate's capacity to inhibit β-catenin, a crucial effector of Wnt signaling (Huang *et al*., 2018).

Wnt Signaling Pathway Suppression

Salinomycin's anticancer activity that affects the Wnt signaling pathway in both patient samples and in vitro reporter systems. They observed that in vitro, salinomycin use had little impact on Fizzled class receptor 5 (Fzd5) but a greatly suppressive effect on Wnt1 and its downstream β-catenin. So salinomycin therapy decreases the phosphorylation process of lipoprotein receptor-related protein-6 (LRP6), which is essential for Wnt signaling. They discovered that primary chronic lymphocytic leukemia (CLL) cells were 100 times prone to undergo cell apoptosis and was susceptible to salinomycin as contrasted with cells from peripheral blood mononuclear cells (PBMC) from healthy donors. This is due CLL cells convey Wnt proteins and have constitutive Wnt activation (Lu *et al*., 2011). Although salinomycin was a cation ionophore and may affect cellular Na^+, K^+, and Ca^{2+} exchange, they added the K^+ ionophore nigericin plus the Ca^{2+} ionophore. Salinomycin and nigericin, 2-Fzd5-activated reporters, were significantly reduced by calcium

ionophore. The known effect of salinomycin is the suppression of the Wnt pathway (Lu and Li, 2014). In NB cells, salinomycin reduced the β-catenin that was phosphorylated by protein kinase A (PKA). B-catenin phosphorylation at Serine 675 by PKA is not related to β-catenin disintegration, rather activates subsequent TCF/lymphoid enhancer factor transcription (Taurin *et al*., 2006).

Lysosomal Iron Sequestration

Ironomycin known as AM5, is a fluorescence amine derivative of salinomycin. The balance of iron was impacted by salinomycin and AM5 in CSC-high cancer cell lines. Salinomycin (0.5 μM) suppresses the translation of the iron transporter ferroportin, treatment resulted in an increase in iron buildup in the lysosome, a decrease in the levels of iron keeper ferritin, and an increase in the level of iron regulatory protein 2 (IRP2). Unusual mechanism for salinomycin's inhibition of breast CSCs, which involves iron sequestration in lysosomes (Hamaï *et al*., 2017). ROS are generated when iron accumulates in the lysosome, and these can cause cell death. Additionally, AM5 exhibited an almost tenfold increase in effectiveness towards HMLER CD24 low cells in contrast to salinomycin, yet retained the CSC-high cell population's selectivity compared to the CSC-low cell populace. the aldehyde dehydrogenase (ALDH) subgroup of breast cancer cells a cell subsection identified for possessing CSC characteristics was significantly more vulnerable to AM5's cytotoxicity (Ginestier *et al*., 2007).

Targets for Intracellular Binding

Numerous cancer forms, such as breast cancer, neuroblastoma (NB), medulloblastoma, pancreatic cancer, colon cancer, prostate cancer, melanoma, lung cancer, are inhibited by salinomycin. Small-molecule inhibitor creation, typical medications developed from natural molecules that found, like salinomycin and thalidomide, were generally aware of their binding targets. By exploiting ferrite-glycidyl methacrylate beads in affinity purification, binding target of thalidomide (Ito *et al*., 2010). Coupled the DARTS method with cross-validation using co-immune-precipitation (co-IP), showed that NCL is most likely a viable intracellular binding site for salinomycin (Wang *et al*., 2019). Salinomycin significantly reduces NB development with an IC50 much fewer then that observed with all presently utilized chemotherapy drugs such NB, for instance, carboplatin (Wickström *et al*., 2007). The multifunction protein NCL is needed for cell division and growth. NCL binds to several proteins, RNA, and DNA. Upon iron chelator therapy, NCL regulates the translation of Matrix Metallopeptidase 9 (MMP9) mRNA (Fähling *et al*., 2005). Target cells of salinomycin could help in its ultimate translation into medical care by potentially categorizing patients by NCL expression in those who react and are resistant to salinomycin therapy. Ion channels in CD133+ and CD133- NB cells are distinct from each other. Greater BKCa, Cav1.3, and Nav1.7 protein expressions in CD133+ enriched NB-CSCs the addition of ionomycin, a Ca^{2+} ionophore, enhanced an ongoing Ca^{2+} influx (Park *et al*., 2010).

MEDICAL APPLICATIONS OF SALINOMYCIN

Reduces the Ovarian Cancer Cells' Stemness Characteristics

With the greatest death rate among gynecological cancers, ovarian cancer is the third most prevalent kind and offers a serious risk to women's health due to its late-stage recognition. The 5-year survival rate for patients is only 25% to 30%. Sal's solubility has been increased when the 1-COOH site is substituted with a photosensitive linker. By reducing intracellular GSH focus, peptide-drug conjugates (PDCs) like Sal-A6 targeting CD44+ have shown improved activity, the ability to dissolve, and target specificity. They have also overcome drug resistance and showed anticancer effects in vivo (Hao *et al*., 2024). Human ovarian epithelial cancer cell line A2780 exhibits apoptosis induction by inhibiting the resistance to multiple drugs protein gp170. OVCAR-8 cell line cell death mediated by caspase in vitro. In the OVCAR-8 cancer of the ovary cell line and it’s resistant to multiple drugs daughter cells, Stat3 inactivates and down regulates Skp-2 (Koo *et al*., 2013).

Influence on Stem Cells' Biological Activity in Liver Cancer

Using phototherapy and chemotherapy is a promising way to fight drug resistance and boost the efficacy of cancer treatment. Powerful cytotoxic drug salinomycin (SAL) attacks cancer cells that are resistant to treatment. For photo-thermal therapy, IR780 iodide is an innovative photosensitive chemical-based with exceptional near-infrared (NIR) light absorption. Successfully enhance drugs target and absorption in liver cancer stem cells (LCSCs) by encasing SAL and IR780 with DSPE-PEG on UCNPs. In the presence of near-infrared light, UISP considerably boosted the MAPK pathway in LCSCs, restricted LCSC migration, and further lowered HCCLM3 viability. These results demonstrate how UISP could enhance treatment outcomes for liver cancer by stimulating the MAPK pathway gradually if exposed to near-infrared radiation (Zhang *et al*., 2024).

Prostate Cancer

Salinomycin (Sal) has been demonstrated to be a promising chemotherapeutic agent for prostate cancer a Nano-carrier (Sal-NPs) was created to extend its systemic circulation and improve its anti-cancer potential. Sal-NPs induced apoptosis in DU145 and PC3 cells via mechanisms like the creation of reactive oxygen species, loss of mineral protein (MMP), and cell cycle arrest. Furthermore, Sal-NPs successfully blocked the migration of cells and prostate adenocarcinoma induction in male rats, showing superiority over salinomycin alone (Kanchan et al., 2024). Prostate Cancer cells LNCaP, DU-145 and PC-3 exhibit growth inhibition. Apoptosis induced by ROS and PARP breakage in PC-3 cells. In PC-3 cells, autophagy and mitophagy responses result in cell death (Jangamreddy *et al*., 2013).

Blood Cancer

A type of blood cancer that originates in the bone marrow, are chemotherapy and bone marrow transplantation. In different kinds of cancer through cell cycle apprehend and apoptosis induction and insulin's role in modulating the metabolism of cancer cells and proliferation varies depending on the type of cancer. These factors may have an impact on the results of leukemia treatment (Alhajamee *et al*., 2024). When vincristine is added to Jurkat cells, salinomycin increases apoptosis (Liu *et al*., 2015). Suppresses the synthesis of ABC transporters in KG-1a cells that resemble human leukemia stem cells, including P-gp, BCRP, and MRP8 (Fuchs *et al*., 2010). Focuses on Wnt signaling in leukemia patients' isolated cells that have chronic lymphocytic leukemia (Lu *et al*., 2011).

Breast Cancer

Cancer stem cells (CSCs), a type of cells found within a primary tumor have the capacity to self-regenerate and specialize into other kinds of cells, causing a mixed tumor. It has been shown that CSCs play a role in every phase of the development of cancer, including the initial development, growth, and metastatic activity of tumors. They also contribute to the recurrence of some cancers and the emergence of drug resistance to chemotherapy (Soni *et al*., 2023). Reduces the percentage of metastases in mice. Kills CSCs. Promotes autophagic response. Damages cellular DNA by increasing the levels of DNA damage protein pH2AX and p53BP1. Blocks the growth of tumors in MCF-7 mammosphere cancer xenograft in nude mice. Targets CSCs via STAT3 in triple-negative breast cancer (Kim *et al*., 2011).

Lung Cancer

In LNM35 and A549 carcinoma cells from humans, caspase-mediated death of cells and prevention of proliferation and invasion. In A549 cells, autophagic response is mediated by ER stress. Via a caspase 3/7-connected cell death mechanism, salinomycin reduced the viability of LNM35 and A549 cells in a concentration and time-dependent manner. Similarly, the development of LNM35 and A549 colony in soft agar was significantly inhibited by salinomycin (2.5–5 μM) for 7 days. The primary cause of lung cancer-related fatalities is metastasis. In this instance, cell invasion and migration were impeded by salinomycin in both time and concentration-dependent ways. In addition, we revealed for the initial time that salinomycin significantly up regulated the levels of the pro-apoptotic protein NAG-1, which prevented lung cancer cells from invading but did not extend their survival (Arafat *et al*., 2013).

Osteosarcoma

Salinomycin prevented osteosarcoma by focusing on its stem cells in vitro and in vivo. It is possible that the Wnt/β-catenin signaling system leads to this exclusion of salinomycin. Salinomycin is an effective blocker of osteosarcoma stem cells, consequently recommending its application in the treatment of osteosarcoma stem cell growth and suggesting the necessity for more clinical testing. Inhibits the tumor remission of human osteosarcoma cells ZOS in mice as well as the osteosarcoma stem cells of U2OS, MG63, and SAOS2 in vitro (Tang *et al*., 2011).

Gastric Cancer

The anticancer drug salinomycin substantially reduced the in vivo tumor volume formed by Wnt1-overexpressing AGS cells. Reduces angiogenesis in vitro by disrupting VEGFR2 signaling in HUVEC cells. Inhibits the neovascularization of mice Matrigel plug test in vivo. Inhibits STAT3 signaling and triggers apoptosis in vitro in human stomach cancer SGC-7901 cells. In vivo proliferation of SGC-7901 tumor xenografts pin BALB/cA mice is inhibited by salinomycin via reduction of CD-31-positive endothelial cells and VEGF, p-VEGFR2, and p-STAT3 production. Suppresses the number of CD44+, Oct4+ gastric CSCs in Wnt1-transfected AGS cancer cells in naked mice (Mao *et al*., 2014).

Uterine Sarcoma

Salinomycin may trigger cancer cells that show resistance to anticancer drugs and apoptosis to undergo apoptosis by overexpressing Bcl-2, P-glycoprotein, or 26S proteasomes that have higher proteolysis activity. Salinomycin triggers a unique apoptotic route that is not dependent on the proteasome, the CD95/CD95L system, the tumor suppression

protein p53, or cascade activation. It also does not result in cell cycle arrest. Reduce the quantity of P-gp expressed in vitro by MDR human uterine sarcoma cells MES-SA/Dx5 (Fuchs *et al*., 2009).

Larynx Cancer

Cancer stem cells, or CSCs, are cancer-initiating cells that are resistant to conventional treatments. Salinomycin targets these types of cells. As an adjunct to regular treatments, salinomycin not just reduces tumor volume but also tumor recurrence. In this work stress responses in primary normal cells and cancer cells, which enhanced salinomycin's ability to selectively target cancer cells. According to our in vitro research, 2-Fluoro 2-deoxy D-glucose or 2-deoxy D-glucose when combined with Salinomycin is fatal to cancer cells, however Oxamate does not enhance Salinomycin's ability to induce cell death (Jangamreddy *et al*., 2015).

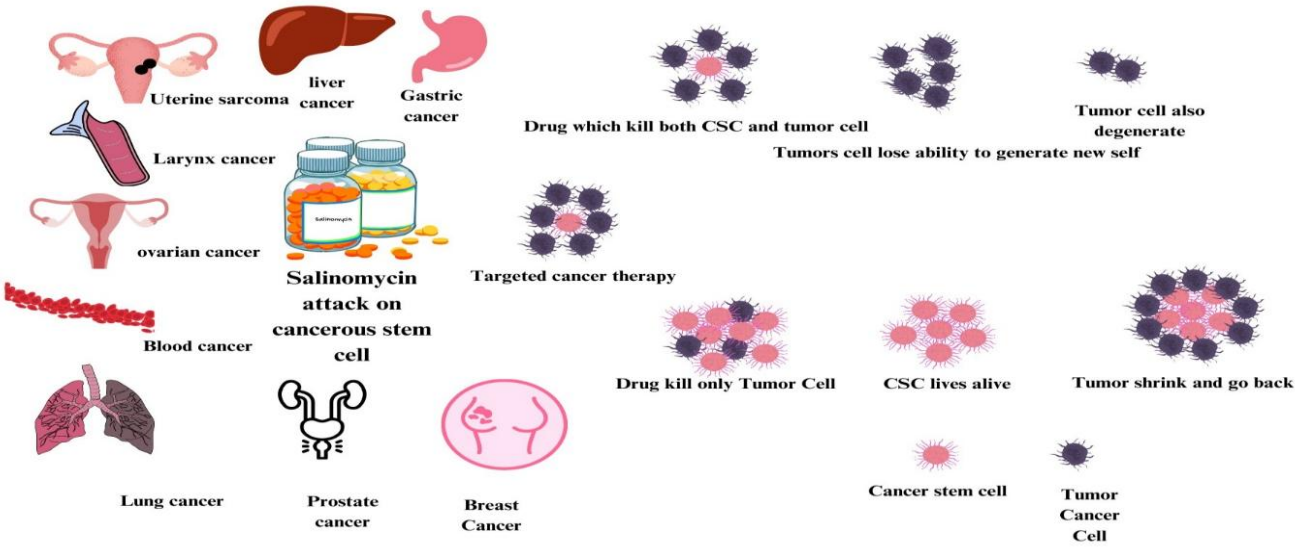

Figure 1. Numerous cancer forms, such as breast cancer, larynx cancer, uterine sarcoma, Gastric cancer, pancreatic cancer, colon cancer, prostate cancer, melanoma, lung cancer, Breast Cancer, Blood cancer, ovarian cancer are inhibited by salinomycin. Figure illustrating how to target CSCs with chemotherapy medications as a potential cancer treatment.

Table 1. Comparative Analysis of Salinomycin's efficacy across Different Cancer Types

Cancer Type	Effects of Salinomycin	Challenges	Strategies to Overcome Challenges	Reference
Breast Cancer	Inhibits HER2-positive & TNBC cells, induces autophagy, DNA damage, kills CSCs	Toxic to healthy cells, poor solubility and bioavailability	Develop targeted drug delivery systems (e.g., nanoparticles), structural modifications for better selectivity	(Malekmarzban, 2023)
Glioblastoma	Enhances chemo/radiotherapy, induces apoptosis, limits migration & invasion	Limited by blood-brain barrier (BBB) penetration, requires more efficient drug delivery methods	Use BBB-penetrating nanoparticles or conjugates; localized delivery approaches	(Norouzi *et al*., 2021).
Ovarian Cancer	Prevents chemoresistance with cisplatin, targets CSCs	Tumor heterogeneity, hard to target peritoneal metastases	Use peritoneal-specific delivery systems; develop subtype-specific formulations	(Zou *et al*., 2022)
Pancreatic Cancer	Reduces tumor size, inhibits metastasis	Dense stroma, poor vascularization, evolving chemoresistance	Use stroma-penetrating drug carriers; combination with stroma-disrupting agents	(Revstedt, 2021)

Colorectal Cancer	Enhances effect of 5-FU, induces apoptosis	Drug delivery optimization and systemic toxicity reduction are essential for clinical development	Combine with targeted chemotherapy; encapsulate in biocompatible carriers	(Zamani *et al*., 2023)
Liver Cancer	Enhances drug uptake in liver CSCs, activates MAPK pathway	Poor delivery to CSCs, drug resistance	Use UCNPs with photothermal/chemo combo; optimize NIR-responsive nanocarriers	(Liu *et al*., 2021)
Uterine Sarcoma	Induces apoptosis in MDR cells via non-canonical pathways	Overexpression of Bcl-2, P-gp, 26S proteasome	Use inhibitors of resistance proteins or combine with apoptosis-inducing agents	(Jiang *et al*., 2024)
Larynx Cancer	Reduces tumor recurrence, targets CSCs	Selective toxicity, needs combination with glycolysis inhibitors	Combine with 2-DG or similar agents for metabolic stress; enhance CSC targeting	(Lin *et al*., 2024)
Gastric Cancer	Inhibits STAT3, VEGFR2, angiogenesis, suppresses gastric CSCs	Requires improved VEGFR2 targeting; tumor heterogeneity	Use VEGFR2-targeting formulations; develop combination therapies to address heterogeneity	(Mao *et al*., 2014)
Osteosarcoma	Blocks stem cell proliferation, inhibits tumor growth	Limited targeting of osteosarcoma stem cells	Focus on Wnt/β-catenin pathway inhibitors; develop osteosarcoma-specific nanocarriers	(Jian *et al*., 2014)
Lung Cancer	Induces apoptosis, inhibits proliferation and EMT, targets lung CSCs	High metastasis rate, multidrug resistance, off-target toxicity	Develop inhalable nanoformulations, co-delivery with resistance inhibitors, enhance tumor specificity	(Malekmarzban, 2023)

Table 2. Medical Applications of salinomycin.

Goal category	**Target demonstrate**	**References**
Breast cancer cells	HMLER cells have human mammalian epithelial cells that have been immortalized & transformed by the expression of the SV40 big T oncogene, hTERT, and H-rasV12. Short hairpin interference with RNA is then used to knock down E-cadherin, causing HMLER-shEcad cells	(Gupta *et al*., 2009)
Lung CSCs	Human lung cancer A549 cells that express Oct-4, Sox2, Nanog, and ALDH1	(Wang, 2011)
Gastric CSCs	Human gastrointestinal cancer cells NCI-N87 and SNU-1 exhibit resistance to 5-fluorouracil and cisplatin and express high amounts of ALDH1, Sox2, Nanog, and Nestin	(Zhi *et al*., 2011)
Osteosarcoma CSCs	Tumorsphere selection, chemotherapeutic selection, Oct-4 and Sox2 expression, and other factors were used to select osteosarcoma CSC from various human osteosarcoma cell lines	(Tang *et al*., 2011)
Colorectal CSCs	CSCs expressing CD133 in human colorectal HT29 and SW480 cells	(Dong *et al*., 2011)
Pancreatic CSCs	Using flow cytometry, pancreatic CSCs were isolated from various human pancreatic carcinoma cell lines based on the expression of CD133	(Zhang *et al*., 2011)

TARGETED DRUG DELIVERY SYSTEMS

Due to its poor water solubility of salinomycin liposomes, micelles, polymeric nanoparticles protect salinomycin by encapsulation (Nanoparticle-based drug carriers) to improve its bioavailability and circulation time. Consequently, there is a need for enhanced targeting to reduce the impact on healthy tissues. In Ligand-mediated targeting specific receptors overexpressed on cancer cells. Target drug having molecules like antibodies that linked to drug guide it to attach to the receptor of CSC protect it during circulation. In response to specific triggers like pH changes, enzymes, or temperature differences found in tumor environments stimuli-responsive systems release salinomycin. Targeted delivery aims that drug reaches its CSC site of action therefore minimizing damage to healthy cells and lowering systemic toxicity and enhance drug accumulation in tumor tissues. Combination therapy with paclitaxel or doxorubicin target cancer stem cells and overcoming drug resistance. Salinomycin-based therapy may be most advantageous for patients with aggressive or drug-resistant malignancies, people with an elevated level of CSC (e.g., breast, pancreatic, and glioblastoma) (Li *et al*., 2025).

Challenges in achieving targeted delivery to tumor sites

Drug penetration is hindered by the biological barriers that tumors create. These consist of the abnormal tumor vasculature, thick blood vessels, and extracellular matrix. Systems for delivering salinomycin must be made to successfully cross these hurdles. Drug delivery system stability and efficacy may be impacted by an acidic or pH-altered tumor microenvironment. Drug localization at the location of the tumor may be enhanced by focusing on these settings. Cancer cells frequently alter their surface markers to become resistant to medications like salinomycin through mechanisms of drug resistance (Tefas *et al*., 2021). The creation of multifunctional carriers that not just transmit salinomycin but also bypass resistance pathways, in this way combination therapies overcome this resistance. Salinomycin does not specifically target cancer cells. It may cause damage in non-cancerous tissues (including the kidneys, liver, or heart) by affecting healthy cells. Developing targeted delivery methods that can administer salinomycin precisely to the cancerous site while ideally avoiding needless exposure of healthy cells. The intricate tumor microenvironment in which cells reside make precise targeting challenging. To deliver salinomycin to cancer stem cells selectively, it is necessary to identify the surface markers that appear on these cells. To find the best targeted ligands, this needs thorough molecular profiling of cancers (Taghipour *et al*., 2023).

Pharmacokinetics and bioavailability of salinomycin

Salinomycin is taken orally have poor water solubility inefficient in absorption from the gastrointestinal tract a small fraction of the administered dose reaches the bloodstream. Liposomes or nanoparticles improve its bioavailability and ensure that drug reaches its target. Salinomycin is distributed to various tissues in the body there is uneven distribution to brain and liver, (fat-solubility) of salinomycin help it to penetrate through cell membrane reaches tumor cells and other tissues. Various enzymes in the liver may break it down into active metabolites, which further enhance its therapeutic benefits. With just a little amount excreted in the urine, salinomycin is mainly removed from the body through the bile (feces). Salinomycin's bioavailability may be increased by chemically altering it to make it more soluble in water. Intravenous Administration ensure a higher concentration reaches the target tissues (Soni, 2023).

Pharmacokinetic and toxicity profiling of salinomycin in preclinical models

To determine the therapeutic window and harmful dose of salinomycin, perform dose-escalation experiments in animal models (such as mice or rats). Using preclinical models, examine salinomycin's absorption, distribution, metabolism, and elimination (ADME). Both toxicity and efficacy are affected by metabolite identification and toxicity profiling (Sitia *et al*., 2022). To evaluate the safety and therapeutic potential of salinomycin, pharmacokinetic and toxicity profiling in preclinical models is essential (Schmohl and Vallera, 2016). Its delayed clearance and mainly fecal excretion should be noted in pharmacokinetic studies that assess its absorption, bioavailability, tissue distribution (particularly tumor targeting), absorption through cytochrome P450 enzymes, and excretion pathways (Azizi *et al*., 2020). Acute toxicity (LD50) chronic and organ-specific toxicity (particularly nephrotoxicity, hepatotoxicity, and cardiotoxicity), genotoxicity, reproductive toxicity, and for a long time carcinogenicity evaluations are all essential components of toxicity profiling (Antoszczak, 2019). To find early indications of toxicity, histopathology examinations and key safety biomarkers are crucial (Januszyk *et al*., 2021). Finding NOAEL and LOAEL values ensures a secure therapeutic window for upcoming clinical applications, and PK/PD modeling can assist in defining ideal dosage schedules. To determine the molecular targets and the main signaling pathways impacted by the medication, RNA sequencing or gene expression microarrays are performed on tumor cells treated with salinomycin (Jangamreddy *et al*., 2015). This will confirm whether salinomycin selectively inhibits CSCs' capacity to form tumors. Better dose plans, enhanced targeting, biomarker

discovery, and eventually the safe and efficient introduction of salinomycin in clinical settings will all be made possible by these research (Dewangan *et al*., 2017).

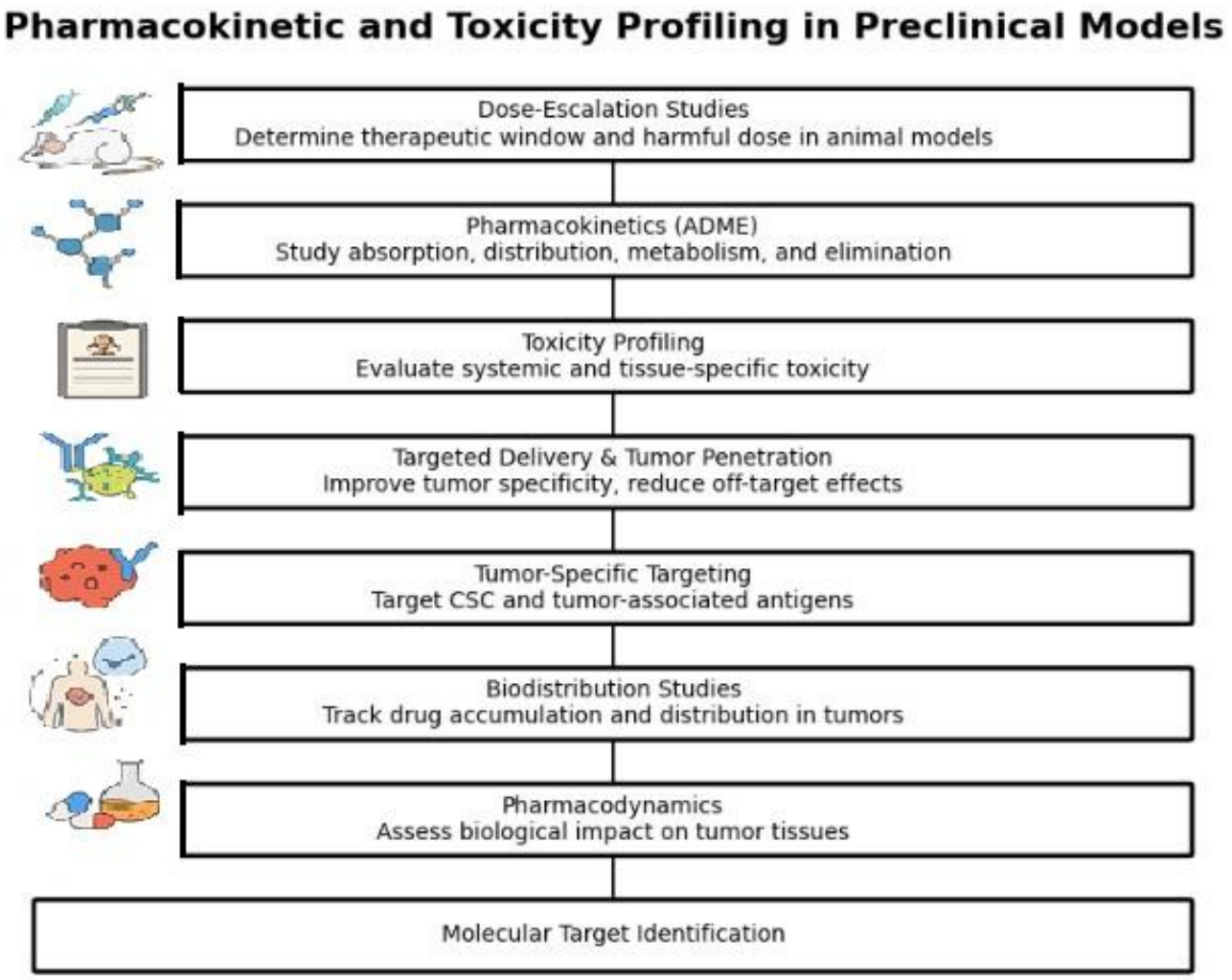

Figure 2. Figure illustrating pharmacokinetic and Toxicity Profiling of salinomycin in Preclinical Models

Perspectives

Since 2009, salinomycin traditionally employed as a treatment of cancer. Its anti-cancer stem cell (CSC) action on different cancer types has been thoroughly studied and confirmed to occur both in vivo and in vitro. The development of innovative drug delivery techniques and salinomycin derivatives as well as conjugates holds potential for raising salinomycin's therapeutic value and creating anticancer drugs with uses in practice (Jiang *et al*., 2018). In order to figure out whether salinomycin is effective towards tumors in humans, more research using orthotopic, likable patient-derived, as well as ex-vivo models is required. Thus, improving research on the processes governing CSCs is essential for improving CSC-targeted therapies and allowing clinical use of salinomycin, as either a stand-alone treatment or in a combination therapy. Furthermore, inadequate screening for CSC status may substantially contribute to failure of therapy and recurrence.

CONCLUSIONS

Salinomycin, produced from *Streptomyces albus*, as a new anticancer drug. This work might help to design more effective cancer medicines by identifying the mechanisms of action. Salinomycin's antimicrobial and anticancer characteristics need more study to fully realize its therapeutic potential. The pharmacokinetics and toxicity of salinomycin, in particular it’s neurotoxic and organ-specific effects, should be determined by future studies. Its association with CSC pathways such as Wnt/β-catenin and STAT3 need more investigation. To improve efficacy, tests should be conducted on delivery methods based on nanoparticles and synergistic medication combinations. Finding predictive biomarkers such as nucleolin or CD133 will help with patient stratification. Additionally, immune-competent

models should be used to evaluate salinomycin's immune-modulatory potential. Additionally, concentrate on improving salinomycin's efficacy, tolerability, and delivery through the use of combination therapies, targeted nanocarriers, and biomarker-guided approaches. Preclinical models such as PDX, organoids, and ADME/toxicity assessments will be safer and more effective in clinical trial designs.

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AUTHOR CONTRIBUTIONS

All the authors contributed equally.

COMPETING OF INTEREST

No conflicts of interest have been disclosed by the authors.

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