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Liposome-Tagged Salmonella Vnp20009 for Hydroxy Tumor: A Review

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Abstract

CANCER remains an important global health challenge, with traditional treatments such as chemotherapy, radiation, and surgery often limited by their specificity and lack of systemic poisoning. Recent progress in bacterial-mediated cancer therapy, especially using *Salmonella Typhimurium* strain VNP20009, presents a promising option for select targets and their unique ability to eradicate the tumor, sparing healthy tissue. VNP20009 has been genetically amended to enhance its oncolytic properties and immunogenicity, allowing it to preferably replicate in hypoxic tumor microenvironments. Literature was searched across databases (PubMed, ScienceDirect, Google Scholar, ResearchGate) and Google using keywords with Boolean operators. From 2,825 initial records, 60 articles were screened after duplicate removal. Of these, 40 full texts were assessed, and 27 studies (22 human, 5 animal) met the inclusion criteria for the final review. This review examines the medical capacity of liposome-tag VNP20009 in the treatment of hydroxy tumors, which integrates the drug delivery benefits of liposomes with genetically modified bacterial tumor-targeting capabilities. By encapsulating VNP20009 in biocompatible liposomes, we aim to improve the stability, localization, and release of the therapeutic payload within tumor sites, potentially adapting to the treatment efficacy. We analyze preclinical clinical studies that portray the protection, immunogenicity, and effectiveness of this innovative approach, which describe intensive implications to increase therapeutic results in patients with hydroxy tumors. In addition, our reviews highlight the emerging strategies, such as individual medical and combination treatment, which take advantage of current progress in genomics and immunology with bacterial therapy. We conclude by discussing future research directions that can facilitate the translation of this promising medical strategy from experimental structure to clinical applications, which eventually aims to remove the boundaries of traditional cancer remedies and address the existing health disparities in oncology.

Keywords: Bacterial-mediated therapy, *Salmonella typhimurium*, VNP20009, hydroxy tumors, clinical applications, health disparities.

Introduction

Cancer remains an important global health crisis, in which traditional therapeutic modalities such as chemotherapy, radiation, and surgery often exhibit limitations in specificity and systemic toxicity, obstructing their ability to completely eradicate tumors [1]. Recently, novel approaches such as bacterial-mediated cancer therapy have gained

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attention due to their ability to selectively target and eliminate tumor cells while sparing healthy tissues [2]. One such promising candidate is *Salmonella Typhimurium* strain VNP20009, which can naturally target hypoxic tumor microenvironments [3,4]. This strain can also be genetically modified to enhance its therapeutic efficacy.

Cancer remains one of the leading global health challenges, and despite significant advances in conventional therapies such as chemotherapy, radiotherapy, and immunotherapy, limitations including drug resistance, systemic toxicity, and poor tumor-specific delivery persist [5].. To overcome these barriers, researchers have explored innovative strategies that exploit the natural tumor-targeting properties of bacteria, particularly attenuated strains such as *Salmonella typhimurium* VNP20009. This strain demonstrates preferential accumulation within hypoxic tumor microenvironments, making it a promising vector for targeted therapy. However, the efficacy of VNP20009 alone has been limited in clinical applications due to rapid clearance, systemic toxicity, and insufficient therapeutic payload delivery [6].. To address these challenges, liposomal encapsulation has been proposed as a synergistic approach, enhancing bacterial stability, prolonging circulation time, and enabling controlled release of therapeutic agents. Thus, liposome-tagged VNP20009 represents a novel biotherapeutic platform that integrates bacterial tumor-targeting with nanotechnology, potentially improving both safety and efficacy in hydroxy tumor treatment [6].

Cancer treatment has a long history, dating back thousands of years, with ancient civilizations attributing the disease to supernatural causes. Surgical intervention for tumor removal was recorded in ancient Egypt, and Greek physician Hippocrates described cancer as a systemic ailment affecting the whole body [5]. The 20th century marked a turning point with the advent of X-ray techniques and the development of radiation therapy, which became a standard treatment for many cancers. Chemotherapy emerged when certain chemicals were found to inhibit cancer cell growth, leading to the use of alkylating agents and plant-derived compounds like vincristine [6].

Surgical interventions are particularly effective for localized tumors, aiming for complete removal with clear margins. However, not all tumors are amenable to surgery due to factors such as size, anatomical location, or metastasis [7]. Chemotherapy, while effective in some cases, often induces adverse effects due to its impact on healthy cells, including alopecia, nausea, and immunosuppression [8]. Radiation therapy, which damages cancer cell DNA via ionizing radiation, is effective but may cause secondary malignancies and harm adjacent tissues [9]. Despite improvements in survival, many patients suffer from reduced quality of life due to side effects of conventional treatments [10].

Furthermore, significant disparities exist in access to optimal cancer treatment, driven by geographic, socioeconomic, and racial inequalities [11]. The growing cancer burden, especially among aging populations, underscores the urgent need for more effective and accessible therapies. Advances in cancer biology, genomics, and immunology have paved the way for targeted therapies, which inhibit specific molecular pathways implicated in cancer. Examples include HER2-targeting agents for breast cancer and imatinib for chronic myeloid leukemia [12]. However, not all tumors possess actionable mutations, and resistance remains a challenge.

Immunotherapy has transformed cancer treatment by leveraging the immune system. Immune checkpoint inhibitors such as pembrolizumab and nivolumab have shown efficacy in various cancers. CAR T-cell therapy has revolutionized treatment of hematologic malignancies, although universal application and biomarker identification remain ongoing challenges [13]. The integration of genomics and proteomics into oncology allows for personalized treatment plans based on individual tumor profiles, enhancing therapeutic precision and minimizing toxicity [14]. Moreover, combining multiple therapies can help address tumor heterogeneity and resistance. Studies have shown that combining immunotherapy with targeted therapies enhances treatment efficacy in some cancers [15]. Given the high costs and long timelines of new drug development, drug repurposing offers a cost-effective alternative for clinical application [16].

Nanoparticles improve drug solubility, and absorption, and enable site-specific delivery to tumor tissues [17]. Among nanoparticle platforms, liposomes hold significant promise by reducing systemic toxicity and enhancing targeted delivery. This review focuses on liposome-tagged VNP20009 and its application in treating hypoxic tumors, discussing how liposomal encapsulation enhances VNP20009's stability and delivery within the tumor microenvironment. Additionally, the review evaluates preclinical and clinical data to assess immunogenicity and therapeutic efficacy. The objective of this review is to highlight the potential of liposome-tagged VNP20009 to improve treatment outcomes and outline future research directions for clinical translation.

This review also highlights liposome-tagged *Salmonella* VNP20009 as an innovative strategy for targeted hydroxy tumor therapy, offering insights into its mechanisms, therapeutic benefits, and future applications in cancer biotherapy. This review is novel in integrating the emerging concept of liposome-tagging with *Salmonella* VNP20009, highlighting how this dual strategy may overcome

traditional challenges of bacterial therapy such as low efficacy, systemic toxicity, and rapid clearance in hydroxy tumor treatment

Material and Methods

A comprehensive review was conducted to identify, screen, and evaluate relevant literature on liposome-tagged Salmonella VNP20009 for hydroxy tumor therapy. Searches were performed across multiple electronic databases and academic platforms, including Google Scholar, PubMed, ScienceDirect, ResearchGate, and the Google search engine, using a combination of relevant keywords and Boolean operators. An initial total of 2,825 records was retrieved. After removing duplicates, 60 articles were screened based on titles and abstracts, of which 20 were excluded due to irrelevance or insufficient data. The remaining 40 full-text articles were assessed for eligibility according to predefined inclusion and exclusion criteria. Following this assessment, 13 articles were excluded, leaving 27 studies that met the criteria and were included in the final review, comprising 22 human studies and 5 animal studies (Fig. 1) .

Criteria for Inclusion

The inclusion criteria were included which checks the use of liposome-tagged vNP20009 in the treatment of hydroxy tumors or related cancer types. This includes colleague-reviewed articles, clinical Testing, and pre-pregnancy studies that expand the functioning, efficacy and mechanisms associated with this treatment method. Additionally, case studies that provide insight into patient reactions for medical or variation in tumor biology in response to treatment can also be included. Studies discussing the properties of liposomes, engineered *Salmonella* stress, and their co-intent on drug delivery and increased tumor targeting are also necessary. Reviews, meta-analysis, and systematic reviews that address the broader reference to using bacterial therapy in oncology, especially associated with hydroxy tumors, were considered.

Criteria for Exclusion

Conversely, the exclusion criteria focused on studies that do not directly belong to liposome-tagged *Salmonella* VNP20009 or fail to address hydroxy tumors, especially hydroxy tumors. The articles that are not colleague-reviewed are not excluded, such as conference abstracts empirical evidence. In addition, studies that focus on unrelated bacterial therapy were Disregarded, various liposomal formulations, or non-qualified applications. Research that does not provide adequate functioning details or lacks comprehensive evaluation of results related to the medical efficacy of VNP20009 in hydroxy tumors was abandoned. Finally, people who did not meet moral standards in any articles or research practices presenting conflicting data without adequate discussion were not included in this review.

VNP20009: A Genetically Modified Strain of Salmonella typhimurium as a Bacterial Therapeutic Candidate

The discovery of innovative approaches in cancer therapy has led to significant advancements in the application of genetically modified organisms (GMOs) for medical treatment. Among these advances, **VNP20009,** a genetically modified strain of *Salmonella typhimurium*, has emerged as a promising bacterial therapeutic candidate. This engineered strain offers a unique tumor-targeting mechanism by preferentially replicating in hypoxic (low oxygen) tumor environments, thereby reducing damage to normal tissues [18]. The following paragraphs, supported by contemporary research and clinical studies, explore the characteristics, mechanisms, and therapeutic capacities of VNP20009.

Characteristics of VNP20009

VNP20009 is derived from *Salmonella typhimurium*, a Gram-negative bacterium known for its pathogenicity in mammals. Unlike its wild-type counterpart, VNP20009 is attenuated, meaning it is genetically modified to reduce its virulence while retaining potent oncolytic (cancer-killing) properties [19]. This attenuation includes the deletion of multiple virulence genes such as msbB, which encodes enzymes essential for lipid A biosynthesis—a key component of the bacterial outer membrane [20]. These modifications enhance the strain's immunogenicity and reduce toxicity.

Research indicates that VNP20009 stimulates apoptosis (programmed cell death) in tumor cells while simultaneously triggering the host's immune system to target both the bacteria and the tumor [21]. The strain is also engineered for tumor-specific targeting, improving its tumor-homing capacity and reducing systemic toxicity, making it an ideal candidate for further clinical exploration [22, 23].

Mechanism of Action of VNP20009

The therapeutic efficacy of VNP20009 stems from its unique mechanism of action. One of its most significant attributes is its preferential replication within hypoxic zones of solid tumors. These regions develop due to rapid tumor proliferation and compromised vasculature [24]. VNP20009 exploits this microenvironment, selectively colonizing tumor tissues while sparing normal, oxygen-rich organs.

In vivo studies have demonstrated that VNP20009 effectively suppresses tumor progression in various cancer models, including melanoma, breast cancer**,** and colon cancer [25, 26]. The bacterium infiltrates the tumor via leaky vasculature and accumulates in the tumor core, where it

replicates and lyses tumor cells. This lysis releases tumor-associated antigens**,** which further activate the host's immune system [27]. Specifically, VNP20009 induces the recruitment and activation of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells**,** enhancing systemic anti-tumor immunity [28]. This dual-action mechanism comprising direct cytotoxicity and immune activation renders VNP20009 a promising agent for both local tumor control and systemic immunotherapy [29].

Synergistic Effects of Liposome Tagging and Salmonella Therapy

In recent years, the discovery of novel medical strategies aimed at cancer treatment and therapeutic efficacy distribution has attracted significant attention in oncology and immunology. Among these strategies, liposome tagging and *Salmonella*-based therapy have emerged as promising dual approaches with synergistic potential for cancer treatment. This discussion focuses on the integration of liposome technology with *Salmonella*-based therapeutics, examining their combined mechanisms, medical implications, and impact on cancer treatment strategies.

Liposomes are nanoscale vesicles composed of phospholipid bilayers that can encapsulate and deliver various therapeutic agents, including small molecules, proteins, and nucleic acids. Their unique Properties, such as biocompatibility, biodegradability, and surfacemodifiability for targeted delivery make them ideal candidates for enhancing the pharmacokinetics and pharmacodynamics of chemotherapeutic agents [30].

Liposomes can be engineered with tumor-specific surface ligands, facilitating targeted drug delivery to cancer cells while minimizing systemic toxicity. Furthermore, they improve drug solubility and stability, allowing for higher local concentrations of therapeutic agents at the tumor site [31].

Parallel to this, *Salmonella* therapy using attenuated strains of *Salmonella enterica* as oncolytic agents—has gained traction due to its tumor-targeting properties and ability to proliferate within the hypoxic and necrotic regions of tumors [32]. *Salmonella* exhibits strong tumor colonization potential, migration toward nutrient-deficient microenvironments, and the capacity to stimulate robust innate and adaptive immune responses [33].

The combination of liposome tagging and *Salmonella* therapy represents an innovative strategy that leverages the strengths of both modalities. The core concept involves enhancing specificity and efficacy in targeting malignant tissues. By coating or tagging *Salmonella* with liposomes, researchers can manipulate the bacterial surface to improve tumor homing, immune evasion, and controlled pharmacokinetics [34]. Liposomes can also be engineered to carry tumor-specific ligands or monoclonal antibodies, enhancing the selective binding of *Salmonella* to tumor cells and increasing localized bacterial accumulation [35].

This targeted delivery reduces the off-target effects traditionally associated with *Salmonella* therapy, improving safety profiles. In addition, liposomes offer a versatile platform for co-delivering chemotherapeutic agents or immunomodulators alongside bacterial vectors. For example, cytotoxic drugs or cytokine genes encapsulated within liposomes can be co-administered with *Salmonella*, providing a multi-modal anti-cancer strategy [36].

Sequential or concurrent delivery of liposomal payloads with bacterial therapy has demonstrated synergistic effects in preclinical models. Studies indicate that this approach amplifies anti-tumor responses by combining direct oncolysis with enhanced immune-mediated tumor destruction [37]. Liposomal carriers ensure precise delivery of adjunct agents to the tumor, potentially increasing overall therapeutic efficacy (Figure 3) [38].

One of the major benefits of using liposome tagging in *Salmonella* therapy is the opportunity to circumvent immune system clearance. *Salmonella* is rapidly eliminated from circulation by the host immune system, particularly via phagocytosis by macrophages, despite its inherent immunogenicity [39]. Encapsulation within liposomes allows *Salmonella* to evade immune surveillance, leading to prolonged systemic circulation and enhanced bioavailability [40]. Furthermore, liposomes can be engineered to modulate immune recognition, thereby enhancing the therapeutic impact of *Salmonella* [41]. This immune-modulatory strategy not only improves biodistribution but also preserves bacterial viability, enabling sustained oncolytic activity within the tumor microenvironment [42].

Liposome encapsulation enhances the therapeutic profile of *Salmonella* VNP20009 by enabling controlled release of bioactive components at the tumor site. The liposomes provide a protective barrier, prolonging bacterial viability and allowing gradual release in response to the tumor microenvironment's acidic pH and enzymatic activity. Pharmacokinetically, liposome tagging improves systemic stability, reduces premature clearance by the reticuloendothelial system, and enhances circulation time. In terms of biodistribution, liposome-tagged VNP20009 preferentially accumulates in tumor tissues due to the enhanced permeability and retention (EPR) effect and the intrinsic tumor-targeting ability of attenuated ***Salmonella***, thereby minimizing off-target colonization and maximizing therapeutic efficacy**.**

Preclinical models have demonstrated that liposome-tagged *Salmonella* effectively targets tumors and produces improved therapeutic responses

with minimal systemic toxicity [43]. These findings suggest that combining this strategy with additional agents could transform current cancer therapy paradigms. For instance, co-administration with immune checkpoint inhibitors or chemotherapeutic agents can enhance anti-tumor responses while reducing side effects associated with conventional therapies such as chemotherapy and radiation [44].

Several parameters play a crucial role in determining the yield and therapeutic effectiveness of liposome-tagged *Salmonella* VNP20009. The composition and surface charge of liposomes are fundamental, as they enhance stability, protect bacterial vectors, and improve drug delivery by regulating immune evasion and membrane fusion. Similarly, encapsulation efficiency and loading capacity directly influence the concentration of therapeutic payload delivered to the tumor site, with higher encapsulation ensuring a greater therapeutic load reaches its target. The tumor-targeting ability of VNP20009 itself is equally important, as its motility and preference for hypoxic environments enable selective colonization of tumor tissues, thereby enhancing targeting specificity. Moreover, particle size and zeta potential significantly affect biodistribution, circulation time, and tumor penetration, since these physicochemical features determine stability, uptake, and the enhanced permeability and retention (EPR) effect. External delivery factors such as dosage and route of administration also dictate therapeutic efficacy, with optimized regimens minimizing clearance while maximizing tumor uptake. Finally, the timing of delivery is critical, as synchronizing bacterial colonization with controlled drug release ensures peak therapeutic activity and improved treatment outcomes. Together, these parameters collectively enhance the efficiency and yield of therapeutic reactions, establishing liposome-tagged *Salmonella* VNP20009 as a promising platform for targeted tumor therapy.

Preclinical and Clinical Studies

Since its development, *VNP20009*, a genetically attenuated strain of *Salmonella typhimurium*, has undergone extensive preclinical and clinical evaluation for cancer therapy. Early studies in murine models confirmed its safety, revealing minimal systemic toxicity [45]. Subsequent investigations showed that *VNP20009* preferentially colonizes tumors and exerts cytotoxic effects without harming adjacent normal tissues [46]. A Phase I clinical trial in patients with advanced melanoma demonstrated that *VNP20009* could be safely administered intravenously, with some patients showing partial tumor regression [47]. Following this, Phase II trials explored its combination with chemotherapy, reporting improved tumor regression without increased toxicity [48].

Beyond direct tumoricidal action, *VNP20009* significantly alters the tumor immune microenvironment. It promotes dendritic cell maturation and enhances the presentation of tumor antigens to T cells, thereby boosting adaptive immune responses [49]. This immunostimulatory potential is especially promising in the context of modern immunotherapy. Emerging studies suggest synergistic effects when *VNP20009* is combined with immune checkpoint inhibitors such as anti-PD-1 antibodies [50].

Challenges and Future Directions

Despite promising preclinical and early clinical data, several challenges remain. Tumor immune suppression can impair the efficacy of oncolytic bacteria, and a better understanding of the immunological context is needed to optimize therapeutic windows [51]. Additionally, immune tolerance to *VNP20009* over time could reduce its long-term effectiveness, highlighting the need for combination therapies and optimized dosing regimens [52].

Hydroxy tumors, often referred to in the literature as neuroendocrine tumors (NETs) producing hydroxyphenyl compounds, present a complex clinical challenge due to their heterogeneity and variable biological behavior. These tumors arise from neuroendocrine cells located predominantly in the gastrointestinal tract, pancreas, and lungs [53]. NETs can be classified by their functional status (hormone-producing vs. non-functional), histological grade, and metastatic potential [54]. Functional NETs often produce serotonin, catecholamines, and other amines, leading to clinical syndromes like flushing, diarrhea, and hypoglycemia [55]. Non-functional tumors may remain asymptomatic and are often incidentally discovered during imaging for unrelated conditions.

Epidemiological data suggest that while NETs are relatively rare, their incidence is rising due to improved diagnostic modalities and heightened awareness [56]. Women appear to have a slightly higher incidence in some studies. Tumors are graded by mitotic count and necrosis; well-differentiated low-grade tumors have a better prognosis, while poorly differentiated high-grade NETs tend to be aggressive and metastasize early [57].

Diagnostic approaches include imaging techniques such as CT, MRI, and PET scans using radiotracers like 68Ga-DOTATATE, which target somatostatin receptors expressed by NETs [58]. Biochemical markers, particularly chromogranin A (CgA)**,** are widely used due to their sensitivity, though false positives can occur in renal failure or other malignancies [59].

Historically, the treatment of neuroendocrine (hydroxy) tumors involves a multidisciplinary approach, including surgical resection, systemic

therapy, and supportive care. Surgical excision remains the primary and most effective option for localized tumors; however, complete resection is often not feasible, especially in cases of metastatic disease. In such scenarios, medical management becomes essential. Somatostatin analogs, such as octreotide and lanreotide, have been used to control symptoms related to hormone hypersecretion and may exhibit antiproliferative effects [60].

Additionally, targeted therapies including everolimus and sunitinib have demonstrated efficacy in treating advanced neuroendocrine tumors (NETs), particularly in patients resistant to conventional treatments [61]. The management of these tumors also necessitates an understanding of the molecular pathways underlying tumorigenesis. Genetic mutations in genes such as MEN1, TP53, and DAXX/ATRX have been implicated in NET pathogenesis. The menin protein, encoded by MEN1, is involved in regulating gene transcription and cellular proliferation [62]. Mutations in chromatin remodelers like DAXX and ATRX are associated with alternative lengthening of telomeres (ALT), a mechanism that confers survival advantages to tumor cells [63]. These genetic alterations highlight the biological complexity of NETs and represent potential targets for novel therapeutic strategies.

Furthermore, tumor progression and metastasis are heavily influenced by the tumor microenvironment (TME). NETs can alter interactions between tumor cells and the surrounding stroma, affecting processes such as angiogenesis and immune evasion [64]. This highlights the need to explore the TME as a therapeutic target. Immune checkpoint inhibitors (ICIs) are an emerging strategy that harnesses the immune system to combat NETs and have shown promise in early studies [65].

A novel area of interest is the integration of liposome technology with bacterial therapy, which represents a promising direction in biomedical research. Traditional bacterial therapies utilize attenuated or genetically modified bacteria to treat cancer and infections but face challenges such as poor specificity, immune clearance, and systemic toxicity [66]. Liposomes spherical vesicles composed of lipid bilayers offer a versatile drug delivery platform that can improve the therapeutic index of bacterial therapies by reducing associated risks [67].

One of the main advantages of liposomes is their ability to encapsulate both hydrophilic and hydrophobic agents, enabling co-delivery of drugs and genetic material [68]. In the context of bacterial therapy, liposomes can be engineered to transport bacteria within their aqueous core, allowing targeted delivery to tumors or infection sites while minimizing systemic distribution [69]. This targeted approach enhances bacterial accumulation at the desired site and reduces off-target effects, a common limitation in conventional therapies. Moreover, liposomal encapsulation can shield bacteria from the host immune response, prolonging their circulation time in vivo and increasing therapeutic efficacy [70].

Liposomes can also be used to alter bacterial surface properties, aiding in immune evasion and facilitating tissue penetration, including biofilms or chronic lesions that are typically difficult to treat [71]. Furthermore, liposomal delivery systems improve the stability of orally administered bacteria, protecting them from the harsh gastrointestinal environment and enhancing their bioavailability and therapeutic outcomes [72].

In addition, when coupled with specific ligands targeting receptors on epithelial cells, liposome-encapsulated bacteria can achieve better cell uptake, increasing the efficacy of mucosal administration routes [73]. The use of liposomes in combination with bacterial therapy also allows for the inclusion of additional functionalities, such as imaging agents or medical enzymes. This multimodal approach enables bacterial localization and real-time tracking of activity within the body using imaging techniques such as magnetic resonance imaging (MRI) or fluorescence imaging [74]. In the case of cancer therapy, this facilitates monitoring of treatment response and enables real-time adjustment based on observed therapeutic efficacy.

Liposomes are additionally tunable in terms of size, charge, and lipid composition, which allows for adaptation to meet specific requirements of various medical applications [75]. This level of fine-tuning enables the development of liposomes that can increase bacterial adherence to target tissues or modulate the release of encapsulated materials over time. Such controlled release mechanisms are critical in bacterial therapy, ensuring that therapeutic bacteria are delivered when and where they are most needed enhancing the probability of successful treatment outcomes [76].

Another compelling argument for combining liposome technology with bacterial therapy lies in its potential integration with advanced genetic engineering techniques. Rapid progress in synthetic biology enables the design of bacteria with specific therapeutic functions, such as the production of anticancer agents or immune modulators. Using liposomes as delivery vectors, these genetically engineered bacteria can be efficiently directed to pathological sites, playing a dual role in delivering both the therapeutic payload produced by the bacteria and the bacteria themselves [77, 78].

Despite the promising characteristics of this strategy, several challenges must be addressed to fully harness the potential of combining liposome technologies with bacterial treatments. Key issues include stability and encapsulation efficiency, optimizing interactions between liposomes and

bacteria, and evaluating potential immune responses against both components [79]. Additionally, extensive research on the pharmacokinetics and pharmacodynamics of these joint systems is required to tailor dosage and administration strategies to ensure maximum therapeutic efficacy with minimal side effects [80].

The combination of liposome-based techniques with bacterial therapy represents a versatile and powerful approach that enhances delivery, efficacy, and safety. Liposomes can improve therapeutic outcomes in diverse applications, from chronic infections to cancer therapy, by enabling targeted delivery, controlled release**,** and modulated immune responses**.** Continued exploration and development of this integrated platform may revolutionize treatment strategies for a wide range of diseases [81–83].

Limitation

Despite its promise as a tumor-targeting bacterial vector, *Salmonella* VNP20009 presents several limitations that must be critically addressed. First, low clinical efficacy has been reported in early-phase human trials, where tumor colonization and regression were limited compared to encouraging preclinical outcomes. Second, systemic toxicity remains a concern, as bacterial administration can trigger strong immune responses, septic-like symptoms, and off-target inflammation. Third, rapid clearance by the host immune system and the reticuloendothelial system restricts bacterial persistence, reducing the therapeutic window. Furthermore, variability in tumor microenvironment conditions may influence colonization efficiency, leading to inconsistent results across different tumor types. Finally, biosafety concerns and regulatory challenges continue to hinder clinical translation, emphasizing the need for improved genetic engineering, combination strategies (e.g., liposome encapsulation), and more rigorous human trials to optimize safety and efficacy.

Conclusion and Recommendations

The findings of the current review demonstrate that the integration of liposome technology with bacterial therapy, especially genetically modified stress, represents an innovative and promising approach to address the limits of traditional cancer remedies, especially in relation to the laziness of the hydroxy tumor. Liposome encapsulation increases the stability and targeted distribution of VNP20009, adapting its medical effects and reducing systemic toxicity. The unique immunogenic properties of VNP20009, especially its ability to spread in hypoxic tumor microenvironments, complement the advantages provided by liposomes; both direct tumoricidal functions and strong anti-tumors facilitate recruitment of immune responses. Despite the exciting capacity of therapy, this combination includes several challenges, which include customizing frozen parameters, ensuring frequent therapeutic efficacy, and understanding immune interactions. Future research should focus on refining the physical chemical properties of liposomes to enhance the stability and bioavailability of bacteria, as well as other novels that examine the collective effects of VNP20009 in combination with medical terms, such as immunity checkpoint and target treatment. Clinical trials should prioritize the evaluation of safety, immunogenicity, and overall efficacy in various patient populations and tumor types to validate the medical utility of liposome-tag VNP20009. In the light of the data presented, recommendations include promoting cooperation between multi-kissing teams to avail nanotechnology, synthetic biology, and immunotherapy to develop more effective and sewn remedies for patients with hydroxy tumors. In addition, there is an important requirement for the installation of infrastructure that supports the translation of promising pregnant findings in clinical practice, eventually to improve the results of the patient and contribute to the quality of life for those affected by cancer.

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Author's contributions

All authors participated in data analysis, manuscript drafting, and revisions, and they

Consented to assume responsibility for all aspects of this study.

Conflict of interest

All authors declare that they have no conflicts of interest.

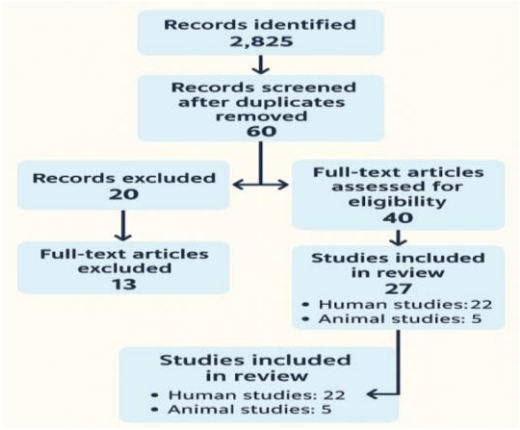

Fig. 1. Methodology of the review article

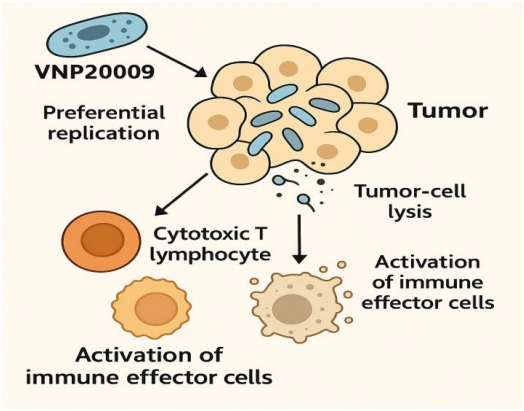

Fig. 2. Mechanism of Action VNP20009 for Hydroxy

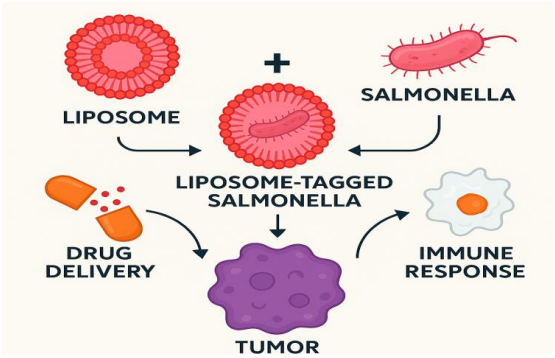

Fig. 3. Synergistic Effects of Liposome Tagging and Salmonella Therapy

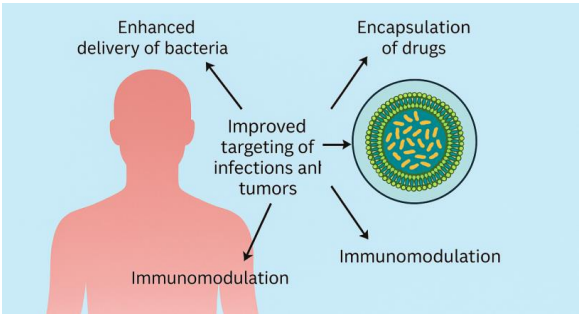

Fig. 4. potential therapeutics application for tumor

References

1. Baskar, R., Lee, K.A., Yeo, R. and Yeoh, K.W. Cancer and radiation therapy: Current advances and future directions. *International Journal of Medical Sciences*, **9**(3), 193–199 (2012). https://doi.org/10.7150/ijms.3635
2. Zhou, S., Gravekamp, C., Bermudes, D. and Liu, K., Tumour-targeting bacteria engineered to fight cancer. *Natural Review Cancer*, **18**(12), 727–743 (2018). https://doi.org/10.1038/s41568-018-0070-z
3. Low, K.B., Ittensohn, M., Le, T., Platt, J., Sodi, S., Amoss, M., Ash, O., Carmichael, E., Chakraborty, A., Fischer, J., Lin, S.L., Luo, X., Miller, S.I., Zheng, L., King, I., Pawelek, J.M. and Bermudes, D., Lipid A mutant Salmonella with suppressed virulence and TNFα induction retain tumor-targeting in vivo. *Natural Biotechnology*, **17**(1), 37–41 (1999). https://doi.org/10.1038/5209
4. Toso, J.F., Gill, V.J., Hwu, P., Marincola, F.M., Restifo, N.P., Schwartzentruber, D.J., Sherry, R.M., Topalian, S.L., Yang, J.C., Stock, F., Freezer, L.J., Morton, K.E., Seipp, C., Haworth, L., Mavroukakis, S., White, D., MacDonald, S., Mao, J., Sznol, M. and Rosenberg, S.A., Phase I study of the intravenous administration of attenuated *Salmonella typhimurium* to patients with metastatic melanoma. *Journal of Clinical Oncology*, **20**(1), 142–152 (2002). https://doi.org/10.1200/JCO.20.1.142
5. Pioner, J.M., Bertorello, R. and Rizzuti, L., Cancer in ancient times: Historical overview. *Journal of Histology Medicine and Allied Sciences*, **73**(2), 123–132 (2018). https://doi.org/10.1093/jhmas/jrx058
6. Bileer, S.A., The evolution of chemotherapy: From nitrogen mustards to targeted therapy. *Journal of Oncology and Pharmacy Practice*, **8**(4), 145–152 (2002). https://doi.org/10.1191/1078155202jp104oa
7. Fitzmaurice, C., Allen, C., Barber, R.M., Barregard, L., Bhutta, Z.A., Brenner, H., Dicker, D.J., Chimed-Orchir, O., Dandona, R., Dandona, L. and Fleming, T., Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life years for 29 cancer groups, 1990–2017. *Lancet Oncology*, **21**(4), 484–503 (2020). https://doi.org/10.1016/S1470-2045(19)30752-2
8. Clamper, J., Li, M. and Sharma, R., Chemotherapy-induced side effects: Mechanisms and clinical management. *Cancer Treatment Review*, **74**, 1–9 (2019). https://doi.org/10.1016/j.ctrv.2019.01.005
9. Liu, J., Bi, K., Yang, R., Li, H., Nikitaki, Z. and Chang, L., Role of DNA damage and repair in radiation cancer therapy: a current update and a look to the future. *International Journal of Radiation Biology*, **96**(11), 1329–1338 (2020). https://doi.org/10.1080/09553002.2020.1818865
10. Chagani, P., Parpio, Y., Gul, R. and Jabbar, A.A., Quality of life and its determinants in adult cancer patients undergoing chemotherapy treatment in Pakistan. *Asia-Pacific Journal of Oncology*, **4**(2), 140–146 (2017). https://doi.org/10.4103/2347-5625.204499
11. Siegel, R.L., Miller, K.D. and Jemal, A., Cancer statistics, 2019. *cancer journal for clinicians*, **69**(1), 7–34 (2019). https://doi.org/10.3322/caac.21551
12. Sharma, P. and Allison, J.P., The future of immune checkpoint therapy. *Science*, **348**(6230), 56–61 (2020). https://doi.org/10.1126/science.aaa8172
13. Fairo, R.M., Lin, Y. and Barrett, D.M., CAR T-cell therapies: Past, present, and future. *Hematology/ Oncology Clinics of North America*, **29**(2), 227–239 (2015).
14. Ginsburg, G.S. and Willard, H.F., Genomic and personalized medicine: Foutionsnda and applications. *Clinical & Translational Research*, **154**(6), 277–287 (2017).
15. Hyman, D.M., Puzanov, I. and Subbiah, V., Molecularly targeted therapies: A framework for combinatorial approaches. *Nat. Rev. Drug Discov.*, **16**(11), 726–741 (2017).
16. Freedman, J.E. and Loscalzo, J., Reengineering the drug development pipeline. *Science Translational Medicine*, **8**(335), 335ps10 (2016).https://doi.org/10.1126/scitranslmed.aad9632
17. Sahni, A., Doggui, S., Ali, J. and Baboota, S., Nanotechnology in cancer therapy: Challenges and future perspectives. *Current Drug Delivery*, **15**(4), 471–486 (2018).
18. Forbes, N.S., Engineering the perfect (bacterial) cancer therapy. *Natural Review of Cancer*, **10**(11), 785–794 (2010). https://doi.org/10.1038/nrc2934
19. Liu, B., Huang, J., Xiao, J., Xu, W., Zhang, H., Yuan, Y., Yin, Y. and Zhang, X., The Streptococcus virulence protein PepO triggers anti-tumor immune responses by reprograming tumor-associated macrophages in a mouse triple negative breast cancer model. *Cell Biosci*ence, **13**(1), 198 (2023). https://doi.org/10.1186/s13578-023-01106-2
20. Suardana, I.W., Wihadmadyatami, H. and Widiasih, DA,. Anticancer activity of the 28.4 kDa protein from *Pediococcus pentosaceus* SR6 in MCF-7 breast cancer cell line. *International Journal of Veterinary Science*, **13**(6), 742-748 (2024). https://doi.org/10.47278/journal.ijvs/2024.158
21. Drake, D., Schreiber, R.D. and Krug, L.M., Bacterial targeting of tumors and activation of innate immunity lead to tumor regression. *Cancer Immunology Research*, **9**(3), 195–203 (2011). https://doi.org/10.1158/2326-6066.CIR-11-054
22. Toso, J.F., Gill, V.J., Hwu, P., Marincola, F.M., Restifo, N.P., Schwartzentruber, D.J., Sherry, R.M., Topalian, S.L., Yang, J.C., Stock, F. and Freezer, L.J., Phase I study of the intravenous administration of attenuated *Salmonella typhimurium* to patients with metastatic melanoma. *Journal of Clinical Oncology*, **20**(1), 142–152 (2002). https://doi.org/10.1200/JCO.20.1.142
23. Sznol, M., Lin, S.L., Bermudes, D., Zheng, L.M. and King, I., Use of preferentially replicating bacteria for the treatment of cancer. *Journal of Clinical Investigation*, **105**(7), 1027–1030 (2000). https://doi.org/10.1172/JCI8679

24. Liu, F., Zhang, L., Hoffman, R.M. and Zhao, M., Vessel destruction by tumor-targeting *Salmonella typhimurium* A1-R is enhanced by high tumor vascularity. *Cell Cycle*, **9**(22), 4518–4524 (2010). https://doi.org/10.4161/cc.9.22.13744

25. Zhao, M., Yang, M., Li, X.M., Jiang, P., Baranov, E., Li, S., Xu, M., Penman, S. and Hoffman, R.M., Monitoring of infection and gene expression with real-time reporting of *Salmonella typhimurium* in living animals. *Natural Medicine*, **11**(8), 899–904 (2005). https://doi.org/10.1038/nm1277

26. Jhao, J.Y., Su, C.L., Liao, L.W., Hsu, H.S., Lin, Y.W. and Chang, W.W., Engineered *Salmonella typhimurium* VNP20009 suppresses metastasis in a mouse model of breast cancer. *Frontier in Microbiology*, **9,** 1680 (2018). https://doi.org/10.3389/fmicb.2018.01680

27. Zhang, Y., Cao, W., Toneri, M., Zhang, N., Kiyuna, T., Murakami, T., Nelson, S.D., Dry, S.M., Li, Y., Li, S., Wang, X., Ma, H., Singh, A.S., Zhang, L., Suetsugu, A., Momiyama, M., Hoffman, R.M. and Zhao, M., Toxicology and efficacy of tumor-targeting *Salmonella typhimurium* A1-R compared to VNP20009 in a syngeneic mouse tumor model in immunocompetent mice. *Oncology & Cancer Research Journal*, **8**(56), 54616–54626 (2017). https://doi.org/10.18632/oncotarget.27558

28. Zheng, J.H., Nguyen, V.H., Jiang, S.N., Park, S.H., Tan, W., Hong, S.H., Shin, M.G., Chung, I.J., Hong, Y., Bom, H.S. and Choy, H.E., Two-step enhanced cancer immunotherapy with engineered *Salmonella typhimurium* secreting heterologous flagellin. *Science Translational Medicine*, **9**(376), eaak9537 (2017). https://doi.org/10.1126/scitranslmed.aak9537

29. Zhou, M., Tang, Y., Xu, W., Hao, X., Li, Y., Huang, S., Xiang, D. and Wu, J., Bacteria-based immunotherapy for cancer: a systematic review of preclinical studies. *Frontier in Immunoogy.*, **14**, 1140463 (2023). https://doi.org/10.3389/fimmu.2023.1140463

30. Allen, T.M. and Cullis, P.R., Liposomal drug delivery systems: From concept to clinical applications. *Advanced Drug Delivery Reviews*, **65** (1), 36–48 (2013). https://doi.org/10.1016/j.addr.2012.09.037

31. Swantara, M.D., Rita, W.S., Dira, M.A. and Agustina, K.K. Effect of the methanol extract of annona squamosa linn leaf on cervical cancer. *International Journal of Veterinary Science* **12(**3), 295-301(2023). https://doi.org/10.47278/journal.ijvs/2022.187

32. Santos, J.C. and Enninga, J., At the crossroads of host and pathogen: the role of *Salmonella* effector proteins in cancer. *rends in Cell Biology*, **30**(8), 635–648 (2020). https://doi.org/10.1016/j.tcb.2020.05.001

33. Moshaikova, O., Wang, H., Bernard, J. and Li, Y., Tumor-targeting bacteria: Genetically modified *Salmonella* for cancer therapy. *International journal of Molecular Science*, **22**(23), 12671 (2021). https://doi.org/10.3390/ijms222312671

34. Anees, M., Ashraf, M.U., Malik, A. and Saleem, M., Liposome-mediated enhancement of bacterial cancer therapy: Synergism between nanotechnology and oncolytic microbes. *Cancer Nanotechnology*, **7,** 3 (2016). https://doi.org/10.1186/s12951-016-0217-z

35. Diah, F.V., Arozal, W., Noviana, D., Andrijono, Winarto, H., Wuyung, P.E., Juniantito, V., Putri, R.T. and Ro, C.B., In Vivo modelling of metastatic ovarian cancer in Wistar rats induced by a carcinogen 7,1 dimethylbenz[a]anthracene. *International Journal of Veterinary Science* **14**(1), 39-47(2025). https://doi.org/10.47278/journal.ijvs/2024.201

36. Qin, S., He, G. and Yang, J., Nanomaterial combined engineered bacteria for intelligent tumor immunotherapy. *Journal of Materials Chemistry B***, 12** (39), 9795–9820 (2024). https://doi.org/10.1039/D4TB01426E

37. Nguyen, V.H., Kim, H.S., Ha, J.M., Hong, Y., Choy, H.E. and Min, J.J., Genetically engineered *Salmonella typhimurium* as an imageable therapeutic probe for cancer. *Cancer Research*, **70**(1), 18–23 (2010). https://doi.org/10.1158/0008-5472.CAN-09-2247

38. Park, S.H., Zheng, J.H., Nguyen, V.H., Jiang, S.N., Kim, D.Y., Szardenings, M., Min, J.H., Hong, Y., Choy, H.E. and Min, J.J., RGD peptide cell-surface display enhances the targeting and therapeutic efficacy of attenuated *Salmonella*-mediated cancer therapy. *International Journal of Biological Sciences*, **6**(11), 1672–1682 (2016). https://doi.org/10.7150/thno.16484

39. Zheng, J.H. and Min, J.J., Targeted cancer therapy using engineered *Salmonella typhimurium*. *Chonnam Medical Journal*, **52**(3), 173–184 (2016). https://doi.org/10.4068/cmj.2016.52.3.17

40. Phuket, N.R.N., Buahom, J., Senaphan, K., Sukon, P., Sringam, S., Angkititrakul, S. and Sringam, P., Serovars and antimicrobial resistance of *Salmonella* isolated from free-living turtles in the turtle village, Northeastern Thailand: One Health Perspective. *International Journal of Veterinary Science*, **14**(1), 212-218(2025). https://doi.org/10.47278/journal.ijvs/2024.229

41. Min, Y., Caster, J.M., Eblan, M.J. and Wang, A.Z., Clinical translation of nanomedicine. *Chemical Reviews*, **115**(19), 11147–11190 (2015). https://doi.org/10.1021/acs.chemrev.5b00116

42. Zhou, S., Gravekamp, C., Bermudes, D. and Liu, K., Tumour-targeting bacteria engineered to fight cancer *Nature Reviews Cancer*, **18**(12), 727–743 (2018). https://doi.org/10.1038/s41568-018-0070-z

43. Kasinskas, R.W. and Forbes, N.S., *Salmonella typhimurium* lacking ppGpp have delayed tumor colonization. *Journal of Bacteriology*, **188**(7), 2725–2732 (2006). https://doi.org/10.1128/JB.188.7.2725-2732.2006

44. Al-Saafeen, B.H., Fernandez-Cabezudo, M.J. and Al-Ramadi, B.K., Integration of *Salmonella* into combination cancer therapy. *Cancers*, **13**(13), 3228 (2021). https://doi.org/10.3390/cancers13133228

45. Selim, L., Ataku, K. and Tharwat, M. Effect of addition of fermented green juice, bacterial inoculant, enzyme or effective microorganisms on fermentation quality of timothy silage. *International Journal of Agriculture and Biosciences*, **13**(2), 216-221(2024). https://doi.org/10.47278/journal.ijab/2024.105

46. Jia, L.J., Xu, H.M., Ma, D.Y., Hu, Q.G., Huang, X.F., Jiang, W.H., Li, S.F., Jia, K.Z., Huang, Q.L. and Hua, Z.C. Enhanced therapeutic effect by combination of tumor-targeting Salmonella and endostatin in murine melanoma model. *Cancer Biology & Therapy*, **4**(8), 840–845 (2005).

47. Liu, L., Li, Q., Chen, C., Xin, W., Han, C. and Hua, Z. Oncolytic bacteria VNP20009 expressing IFNβ inhibits melanoma progression by remodeling the tumor microenvironment. *Science*, **27**(4), 107053 (2024). https://doi.org/10.1016/j.isci.2024.107053

48. Drake, I., Hindy, G., Almgren, P., Engström, G., Nilsson, J., Melander, O. and Orho-Melander, M. Methodological considerations for identifying multiple plasma proteins associated with all-cause mortality in a population-based prospective cohort. *Scientific Reports*, **11**, 6734 (2021). https://doi.org/10.1038/s41598-021-86189-8

49. Lin, Z., Meng, F., Ma, Y., Zhang, C., Zhang, Z., Yang, Z., Li, Y., Hou, L., Xu, Y., Liang, X. and Zhang, X., In situ immunomodulation of tumors with biosynthetic bacteria promote anti-tumor immunity. *Bioactive Materials*, **32**, 12-27 (2023). https://doi.org/10.1016/j.bioactmat.2023.09.007

50. Zhao, M., Liu, C., Liu, Z., Zuo, Y., Chen, C., Shi, S., Shi, X., Xie, Y., Yang, H. and Chen, Y. Myocardium-targeted liposomal delivery of the antioxidant peptide 8P against doxorubicin-induced myocardial injury. *International Journal of Pharmaceutics*, **663,** 124569 (2024). https://doi.org/10.1016/j.ijpharm.2024.124569

51. Corthay, A. How do regulatory T cells work? *Scand. Journal of Immunology*, **70**(4), 326–336 (2009). https://doi.org/10.1111/j.1365-3083.2009.02308.x

52. Leschner, S., Westphal, K., Dietrich, N., Viegas, N., Jablonska, J., Lyszkiewicz, M., Lienenklaus, S., Falk, W., Gekara, N., Loessner, H. and Weiss, S. Tumor invasion of Salmonella is accompanied by strong immune responses. *Cancer Research*, **69**(11), 4827–4832 (2009). https://doi.org/10.1158/0008-5472.CAN-08-3571

53. Modlin, I.M., Oberg, K., Chung, D.C., Jensen, R.T., De Herder, W.W., Thakker, R.V., Caplin, M., Delle Fave, G., Kaltsas, G.A., Krenning, E.P. and Moss, S.F. Gastroenteropancreatic neuroendocrine tumours. *Lancet Oncology*, **9(**1), 61–72 (2008).

54. Yao, J.C., Hassan, M., Phan, A., Dagohoy, C., Leary, C., Mares, J.E., Abdalla, E.K., Fleming, J.B., Vauthey, J.N., Rashid, A. and Evans, D.B. One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors. *Journal of Clinical Oncology*, **26**(18), 3063–3072 (2008).

55. Laban SE, Arafa AA, Ibrahim ES, Eman FE and Khalefa HS, Dry biofilm formation, mono- and dual-attachment, on plastic and galvanized surfaces by *Salmonella typhimurium* and *Staphylococcus aureus* isolated from poultry house. *International Journal of Veterinary Science* **14**(1), 25-31(2025). https://doi.org/10.47278/journal.ijvs/2024.197

56. Chan, D.T., Luk, A.O., So, W.Y., Kong, A.P., Chow, F.C., Ma, R.C. and Lo, A.W. Natural history and outcome in Chinese patients with gastroenteropancreatic neuroendocrine tumours: a 17-year retrospective analysis. *BMC Endocrine Disorders*, **16**, 12 (2016). https://doi.org/10.1186/s12902-016-0091-y

57. Klimstra, D.S., Modlin, I.R., Coppola, D., Lloyd, R.V. and Suster, S. Pathology reporting of NETs. *Cancer*, **116**(13), 2627–2640 (2010). https://doi.org/10.1002/cncr.25020

58. Rufini, V., Calcagni, M.L. and Baum, R.P. Imaging of neuroendocrine tumors. *Seminars in Nuclear Medicine*, **36**(3), 228–247 (2006). https://doi.org/10.1053/j.semnuclmed.2006.04.002

59. Davis, R.A., Patterson, C.J. and Lieu, E. Chromogranin A as a NET biomarker. *Biomarkers in Medicine*, **16**(3), 221–230 (2022).

60. Kvols, L.K., Moertel, C.G., O'Connell, M.J., Schutt, A.J., Rubin, J. and Hahn, R.G. Treatment of the malignant carcinoid syndrome. *The New England Journal of Medicine*, **315**(11), 663–666 (1986).

61. Wells, S.A. and Nevins, J.R. Evolving strategies for targeted cancer therapy—past, present, and future. *The Journal of the National Cancer Institute*, **96**(13), 980–981 (2004).

62. Gourni, E., Waser, B. and Reubi, J.C. The role of MEN1 mutations in endocrine tumor development and progression. *Endocrine Connections*, **10**(4), R47–R59 (2021).

63. Heaphy, C.M., De Wilde, R.F., Jiao, Y., Klein, A.P., Edil, B.H., Shi, C., Bettegowda, C., Rodriguez, F.J., Eberhart, C.G., Hebbar, S. and Offerhaus, G.J. Altered telomeres in tumors with ATRX and DAXX mutations. *Science*, **333**(6041), 425–425 (2011).

64. Cives, M., Pelle, E., Quaresmini, D., Rizzo, F.M., Tucci, M. and Silvestris, F. The tumor microenvironment in neuroendocrine tumors: biology and therapeutic implications. *Neuroendocrinology*, **109** (2), 83–99 (2019)

65. Kalita, R.,, Pegu A. and Baruah, C. Prospects of probiotics and fish growth promoting bacteria in aquaculture: a review. *International Journal of Agriculture and Biosciences*, **12**(4),234-244. (2023). https://doi.org/10.47278/journal.ijab/2023.070

66. Prados-Rosales, R., Baena, A., Martinez, L.R., Luque-Garcia, J., Kalscheuer, R., Veeraraghavan, U., Camara, C., Nosanchuk, J.D., Besra, G.S., Chen, B. and Jimenez, J. Mycobacteria release active membrane vesicles that modulate immune responses in a TLR2-dependent manner in mice. *The Journal of Clinical Investigation*, **121**(4), 1471–1483 (2015).

67. TabibzadehTehrani, P., Nazari, M., Rastgoo, P., Bolouri, N.S., HeydariKarsaf, R., Hadiani, A. and Mohsenipour, Z. Bacterial-based drug delivery systems: A new way to combat infectious disease. *Medicine in Drug Discovery*, 100205 (2025).

68. Allen, T.M. and Cullis, P.R. Liposomal drug delivery systems: From concept to clinical applications. *Advanced Drug Delivery Reviews*, **65** (1), 36–48 (2013).

69. Kumar, R., Singh, A. and Sehrawat, S. Bacterial encapsulation in liposomes for cancer therapy: An evolving frontier. *Expert Opinion on Drug Delivery*, **12**(3), 377–389 (2015).

70. Künkele, K.P., Wesch, D., Oberg, H.H., Aichinger, M. and Supper, V. Vγ9Vδ2 T cells: can we re-purpose a potent anti-infection mechanism for cancer therapy? Cells, **9**(4), 829 (2020).

71. Han, J.W., Choi, Y.J., Cho, S., Zheng, S., Ko, S.Y., Park, J.O. and Park, S. Active tumor-therapeutic liposomal bacteriobot combining a drug (paclitaxel)-encapsulated liposome with targeting bacteria (Salmonella Typhimurium). Sens. *Sensors and Actuators B: Chemical*, **224**, 217–224 (2016).

72. Solfaine, R., Purnama, M.T.E., Maslamama, S.T., Fikri, F. and Hamid, I.S,. *Bdellovibrio bacteriovorus*: a boost for hematological and gut health in *Salmonella enteritidis*-infected mice. *International Journal of Veterinary Science*, **13**(6), 776-781. (2024). https://doi.org/10.47278/journal.ijvs/2024.165

73. Bai, Y., Xu, Y., Liu, X., Liu, Y., Han, X. and Li, Y. Targeted delivery of bacteria: An emerging strategy for cancer therapy. *Journal of Hematology & Oncology*, **13**, 132 (2020). https://doi.org/10.1186/s13045-020-00918-w

74. Chen, C.T., Chen, C.P., Yang, J.C. and Tsai, T. Liposome-encapsulated photosensitizers against bacteria. Recent Pat. *Anti-Infect. Drug Discovery*, **8**(2), 100–107 (2013).

75. Torchilin, V.P. Recent advances with liposomes as pharmaceutical carriers. *Nature Reviews Drug Discovery*, **4**(2), 145–160 (2005).

76. de la Fuente-Núñez, C., Reffuveille, F., Fernández, L. and Hancock, R.E. Bacterial biofilm development as a multicellular adaptation: Antibiotic resistance and new therapeutic strategies. *Current Opinion in Microbiology*, **16**(5), 580–589 (2013).

77. Yang, H., Jiang, F., Ji, X., Wang, L., Wang, Y., Zhang, L., Tang, Y., Wang, D., Luo, Y., Li, N. and Wang, Q. Genetically engineered bacterial protein nanoparticles for targeted cancer therapy. *International Journal of Nanomedicine*, **16**, 105–117 (2021).

78. Chowdhury, S., Castro, S., Coker, C., Hinchliffe, T.E., Arpaia, N. and Danino, T. Programmable bacteria induce durable tumor regression and systemic antitumor immunity. *Nat. Med.*, **25**(7), 1057–1063 (2019).

79. Carpenter, A.W. and Payne, C.K. Imaging and analysis of bacterial interactions with liposomal drug delivery systems. *ACS Infectious Diseases*, **7**(4), 749–758 (2021).

80. Leventhal, D.S., Sokolovska, A., Li, N., Plescia, C., Kolodziej, S.A., Gallant, C.W., Christmas, R., Gao, J.R., James, M.J., Abin-Fuentes, A. and Momin, M. Immunotherapy with engineered bacteria by targeting the STING pathway for anti-tumor immunity. *Nature Communications*, **11**, 2739 (2020).

81. Tang, L., Zheng, Y., Melo, M.B., Mabardi, L., Castaño, A.P., Xie, Y.Q., Li, N., Kudchodkar, S.B., Wong, H.C., Jeng, E.K. and Maus, M.V. Enhancing cancer immunotherapy with nanomedicine. *Nature Reviews Materials*, **7**(3), 189–213 (2022).

82. Barbé, S., Van Mellaert, L. and Anné, J. Liposome-mediated targeting of bacteria to tumors. *Journal of Controlled Release*, **284**, 103–119 (2018).

83. Kim, O.Y., Park, H.T., Dinh, N.T.H., Choi, S.J., Lee, J. and Kim, J.H. Bacterial therapy and immunotherapy: Synergistic approaches for cancer treatment. *Cancers* (Basel), **13**(21), 5472 (2021).