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REVIEW ARTICLE

Current advances in microbial-based cancer therapies

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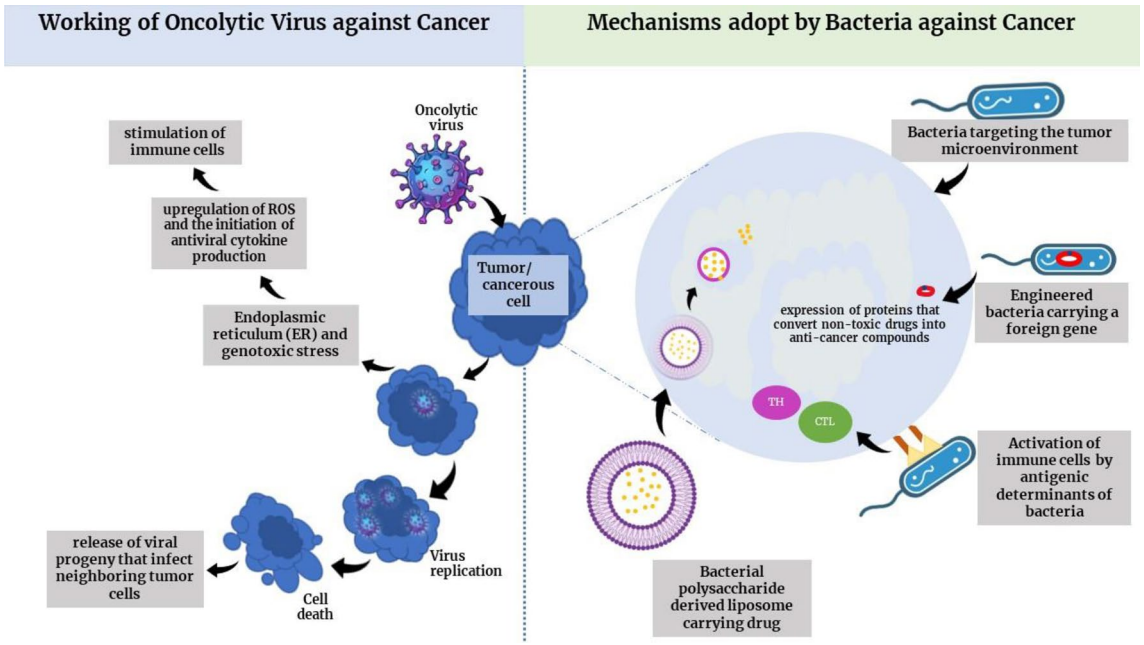
Abstract

Microbes have an immense metabolic capability and can adapt to a wide variety of environments; as a result, they share complicated relationships with cancer. The goal of microbial-based cancer therapy is to treat patients with cancers that are not easily treatable, by using tumor-specific infectious microorganisms. Nevertheless, a number of difficulties have been encountered as a result of the harmful effects of chemotherapy, radiotherapy, and alternative cancer therapies, such as the toxicity to non-cancerous cells, the inability of medicines to penetrate deep tumor tissue, and the ongoing problem of rising drug resistance in tumor cells. Due to these difficulties, there is now a larger need for designing alternative strategies that are more effective and selective when targeting tumor cells. The fight against cancer has advanced significantly owing to cancer immunotherapy. The researchers have greatly benefited from their understanding of tumor-invading immune cells as well as the immune responses that are specifically targeted against cancer. Application of bacterial and viral cancer therapeutics offers promising potential to be employed as cancer treatments among immunotherapies. As a novel therapeutic strategy, microbial targeting of tumors has been created to address the persisting hurdles of cancer treatment. This review outlines the mechanisms by which both bacteria and viruses target and inhibit the proliferation of tumor cells. Their ongoing clinical trials and possible modifications that can be made in the future have also been addressed in the following sections. These microbial-based cancer medicines have the ability to suppress cancer that builds up and multiplies in the tumor microenvironment and triggers antitumor immune responses, in contrast to other cancer medications.

Graphical abstract



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Keywords Microbiome · Cancer therapy · Bacteria-based therapy · Virus-based therapy · Cancer

Introduction

Cancer is publically a serious health problem and is the second major reason for all the mortalities and morbidities around the globe. Based on data gathered by the National Center for Health Statistics, it is estimated that in 2023, there will be 1,958,310 new cases of cancer and 609,820 cancer-related deaths in the United States. Over the period of 2014–2019, the incidence of prostate cancer saw an annual increase of 3%, resulting in an extra 99,000 new cases. In general, though, the incidence trends were more favorable for men compared to women [1]. Apart from the lifestyle choices, genetic factors and environmental reasons, other primary factors behind immensely increasing number of cancer patients are the quick rise of population and to some extent, the lack of research in this regard [2]. The growth and progression of the tumor is usually reliant on several closely related aspects such as the meantime for the cell to mitotically divide, a fraction of the growth, and the entire burden of cancer. The diverse variability of these aspects is the reason behind the different growth rates of different tumors [3, 4]. Cancer treatments that are conventionally employed such as radiotherapy, chemotherapy, and surgery are not completely successful in eliminating the cancer and moreover, are reported to show different side effects. A number of researchers are thus triggered to find other strategies to treat cancer [5]. There is a growing evidence emerging that microbes can be manipulated and can be proved as the best choice for improving cancer treatment [6].

The goal of microbial-based cancer therapy is to treat patients with difficult-to-treat cancers by using infectious microorganisms that are particular to their tumors [7]. Microbial therapies are usually originated from microorganisms that occur in nature and are genetically altered to show a reduction in pathogenicity and an enhancement in anti-cancer efficiency. Microbial therapies can focus on nearly all types of cancer such as solid tumors in the reproductive and digestive system, cancers of bone and blood, sarcoma, and melanoma. By employing several mechanisms, malignant tissues can be eliminated by the use of microbial therapies. Such mechanisms may include the in situ generation of immunostimulatory substances as well as cytolytic compounds in the vicinity of tumor beds [8].

Microbial therapies have the ability to re-sensitize the tumors to the immune system. The tumors are needed to undergo re-sensitization because, within the environment of cancer, the tumors often show the suppression of immune response at their late stage, and lead to the immense proliferation of cancer cells. This problem can be reversed by the employment of microbial therapies as anti-cancer mechanisms are amplified and consequently lead to the clearance of tumor tissue as well as the reduction in rejuvenation of the cancer cells. Moreover, microbial therapies have the ability to cure a number of cancers and make some cancers frequently treatable (Fig. 1). As conventional therapies are unable to meet some of the clinical needs, the microbial therapies can address all the problems associated with the additional treatments. Such problems

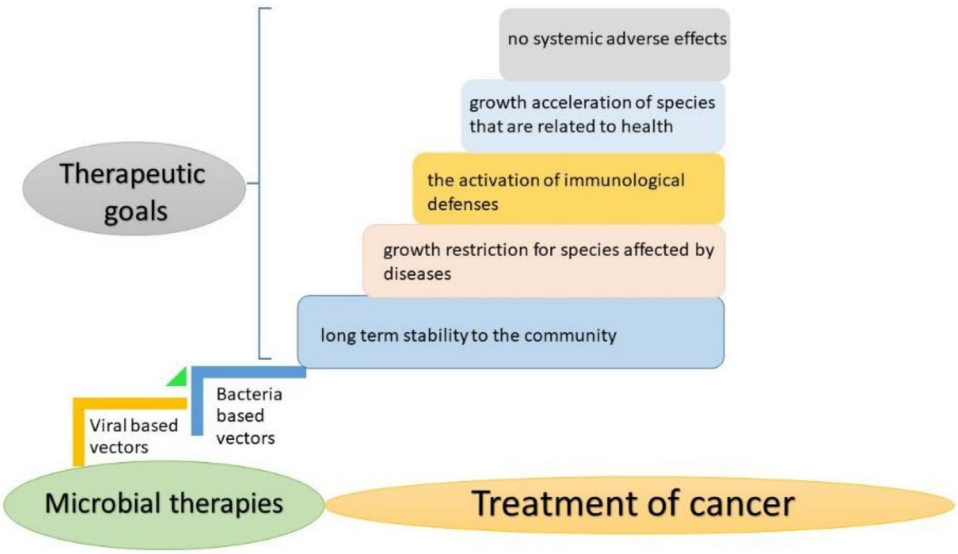

Fig. 1 Objectives of microbial therapies to treat Cancer

may include the ability of some cancers to eliminate the immune response, cancers that can resist a number of medications as well as cancers that show metastasis [9].

The metastasis of the tumors is one of the leading causes of death by cancer and is mostly targeted by these microbial therapies. The cancer phenotypes that show resistance to the anti-cancer drugs and limit the efficiency of small molecules-based treatments can now be treated efficiently by microbial therapies. Viruses that are loaded with carrier-cell or bacteria which are in their motile stage have been designed to pass through the tumorous sites that are otherwise not accessible to the standard small molecules-based drugs [10, 11]. Moreover, microorganisms are engineered in such a way that their cancer-treating abilities are significantly enhanced. For instance, microbial genes can be expressed in a varying manner and this difference is created by the aspects of the environment such as the absence of oxygen or the cancerous properties of cells. A very large dose–response index may be made possible by recombinant microbiota that incorporates genetic characteristics that are turned on or off in the context of cancer-specific alterations, boosting safety while strengthening the anticancer benefits [12]. These factors lead to the potential use of microbial therapies for generating tumoricidal effects that mimic the immunotherapeutic responses and can show a reduction in the damage and toxicity to the tissues.

During recent years, the usage of microbes as a cancer therapy has shown a high success rate owing to the plasticity as well as diversity of prospective microbes. The time it takes to develop a hypothesis and do preclinical proof-of-concept trials before moving on to clinical testing has been significantly shortened because of ongoing advancements in genetic modification, and this trend is anticipated to continue [13]. However, a number of problems have been reported regarding microbial therapeutics, but addressing such concerns will lead to the advancement and unification of the viruses and bacteria concerning research societies. Thus, this paper aims at specifically outlining the main obstacles that microbial therapies still face and what has to be done for the encouragement of better translation into clinical use, particularly when combined with other cutting-edge approaches like cancer immunotherapy.

Microbial therapies against cancer are considered as the older ones, benefits and drawbacks of this field are not well-investigated, because the use of medical tools for research is limited. Recent advancements in the subjects of oncology including microbial pathogenesis, the study of tumors, immunity against cancer and advances strategies employed in molecular biology has been reviewed in this article that will allow to retreat the former concept with advanced perspectives.

Impact of microbes on cancer therapy

The exploration of microbes as potential anti-cancer tools has garnered substantial interest. Once the mucosal barriers are breached, microbes interact with the elements of the immune system of host and lead to a pro-inflammatory effect or the suppression of immune response. The induction of tumorous growth as well as the regulation of various receptors, which are engaged in the activation of NF-κB, is reliant on a number of different inflammatory factors [14]. Within the environment of tumor, the interaction of microbes can lead to either the induction [15, 16] or the elimination of carcinogenesis. A variety of cancers are also described by the presence of specific microbiomes. Various microbiomes that show enhanced activities related to a specific cancer type can also be employed for the early diagnosis of particular carcinoma by acting as a biomarker [17, 18]. The role played by prebiotics and probiotics in the elimination of different types of cancer has been revealed by the recent advancements. Likewise a weakened microbiome holds a high potential in cancer treatment by the regulation of numerous immunomodulatory responses [18]. Different metabolites are secreted by numerous microbes that can cause toxicity to the cancer cells because of the apoptotic and anti-inflammatory properties they exhibit; this feature is adaptable in few microbes for contending counter to other organisms within an environment [19].

There are several cases reported in which the efficiency of the anti-cancer therapies are enhanced by the action of microbes [19, 20]. The activity of IFNγ is enhanced by the employment of *Salmonella* at the site of carcinoma. This augmented action of IFNγ is associated with the mediation of CD8+ and CD4+ cells production. *Salmonella*-based vaccines have also been reported to show anti-tumor properties in mice models [21]. In contrast to the tumorous ones, the healthy tissues of breast are reported to have high number of *Streptococcus* and *Lactobacillus* as these bacterial species are observed to act as the inducer of tumor suppression and also as a natural killer.

Moreover, reduction in the damage to DNA is reported by the *Streptococcus* species [22]. The potential of prebiotics (PEB) and probiotics (POB) to work against colorectal cancer can be manifested by various mechanisms. For instance, the employment of *Lactobacillus rhamnosus* to eliminate the carcinogens can work either by biotransformation of cancer-causing substances or by binding with carcinogens. Butyrate like acidic substances are produced in the gut by PEB and can lead to the regulation of apoptotic cell death, modulation of cells growth and the transformation of ROS. Once the competence of the antibodies that control tumorous growth and the functionality of

NF-κB is increased, it leads to the immunomodulatory effects. These augmentations are reported to be caused mainly by the *L. rhamnosus*, *B. longum*, *B. breve*, and *L. casei*.

The signaling pathways of carcinomas are altered by the action of POBs and can ultimately lead to the apoptotic cell death [18]. Mice models with colorectal cancer are reported to be treated efficiently with the employment of genetically altered strains of *L. monocytogenes*. Such strains are engineered to yield high number of CD8+ cells because of the antigen expression that is related to the suppression of tumors. The memory of T-cells is also improved leading to the reversal of tumor recurrence by employing the species mentioned above. The inhibition of ovarian cancer is also reported by the use of *T. gondii* that has the ability to induce high levels of C8+ and CD4+ T cells. In mice with ovarian cancer, *Listeria monocytogenes* strain boosted macrophage entrance at tumor sites and improved macrophage anticancer activity. In mice having cervical cancer, *L. monocytogenes* produced a comparatively less potent antitumor response because its tumor-suppressive response was dependent on the activity of αβ-T cells [21].

Microbes and their mechanisms for microbial-based cancer therapy

The medical application of microbiotas primarily includes viruses and bacteria [23]. It has been discovered by William Coley's that bacterial extracts can be employed for treating the metastatic cancers, specifically those which are at the last stage. Bacterial species that have more potential for this purpose are *Serratia marcescens* and *Streptococcus pyogenes*. This discovery has led to the emergence of microbial therapeutics against cancer [24]. Till date, a great number of researches have been documented to show the correlation among the microbial species and the regression of tumors [14, 25].

Researchers have contributed significantly in the field of microbial therapeutics against cancer and now advancements are being made in the field of synthetic biology to design the microbes in such a way to show anti-tumor potential [26]. Microbe-based therapies can employ different mechanism to eliminate the tumorous tissues. Such mechanisms may include the ability of re-sensitizing the tumors, augmentation of the anti-tumor effects for stimulating the immune system and the ability to clear the focus of metastatic tumors. Moreover, such therapies may involve the generation of immunostimulatory substances [13].

During the past few years, the research against cancer is also giving attention to the early stage diagnosis of cancer as well as designing new strategies to combat this disease by developing the personalized medicines that target the tumors at the molecular level. All the aforementioned mechanisms are exceptional and hard to attain with other medications and the synthetic drugs. Thus, it has become possible by using these mechanisms, that advanced and centrally important therapeutics can be made against cancer, particularly in order to treat the cancers that show obstinate malignancies [13, 27, 28]. Microbes that are extensively investigated against different cancer types are listed in Table 1 and the mechanisms adopted by viral- and bacterial-based therapies are discussed in the following section.

Bacteria-based cancer therapy

It is essential to use a variety of strategies that particularly target cancer cells by focusing on certain components of the bacteria to progress the development of BBCT (Bacterial-Based Cancer Therapy). Targeting the microenvironment of tumor, secreting cytotoxic chemicals, modifying bacterial virulence agents, and using modified bacterial vectors for the development and discharge of tumoricidal proteins are some of the strategies responsible for anti-cancer action (Fig. 2) [69].

Bacterial targeting of the tumor microenvironment

Bacterial species that are able to survive without oxygen can also target the hypoxic cores of the tumor and thus drives the main attention toward the use of bacterial-based therapy against cancer [29]. The partial pressure of oxygen in the tumor environment is usually below 10 mmHg [70]. In the absence or the limited supply of oxygen, bacterial metabolism results in the production of lactic acid as a by-product. Due to this, acidity is enhanced in the tumor microenvironment [71].

Additionally, the tumor microenvironment has enhanced tissue necrosis, which is the death of tumor cells as a result of nutrition deprivation and unchecked growth [72]. Rapidly growing solid tumors are characterized by hypoxia, which is thought to be caused by the tumor's' growth outside of the range of the available blood supply [73]. Tumors have functionally aberrant blood artery structure, which causes erratic blood flow throughout the tissue and consequently results in oxygen deprivation [74]. Tumors are forced by the hypoxic environment to modify their genetic makeup so that they can withstand the cell death and tissue necrosis that hypoxia causes [75].

MDR1 (a multidrug-resistant gene) and P-glycoprotein genes, which are in charge of the expansion of multidrug resistance to several anticancer medicines, are recognized to be related with greater expression in the hypoxic tumor region [76]. However, the hypoxia brought on by these damaged blood arteries provides an exclusive environment

Table 1 Examples of some of the extensively studied microbes against cancer

Microbe (bacteria/virus)	Strain used	Cancer type	Mechanism observed	References
Salmonella typhimurium	YB1; ST8	Breast cancer, colon cancer	Defects in the synthesis of diaminopimelic acid (DAP)	[29]
	A1-R	Prostate cancer	Auxotrophic strain that is unable to synthesize leucine and arginine	[30]
	VNP20009	Metastatic melanoma, glioblastoma, pancreatic cancer, colon cancer, breast cancer	Production of adenine occurs insufficiently	[31]
	MvP728	Colon carcinoma, DBT glioblastoma, Melanoma	Production of heat shock protein response against tumors	[32]
	VNP20009	Benign tumors caused by neurofibromatosis type 2 (NF2)	Decreased angiogenesis, apid stimulation of Th1 responses, and immunological memory response	[33]
Salmonella typhimurium	X4550	Osteosarcoma	Production of cyclic adenosine monophosphate occurs in a disabled manner	[34]
	SB824	Melanoma	Defective in pathogenicity island	[35]
	MPO378	Breast cancer cell line	insufficient biosynthesis of purine bases	[36]
	FlaB	Colon cancer	Engineered FlaB from *Vibrio vulnificus*-secreting bacteria	[37]
	DP-L4029	Colon cancer, lung cancer	Defects are produced in the surface bound polypeptide	[38]
	(*Luc-S.T*$_{\Delta ppGpp}$)	Diverse cancers	Inhibit tumor metastasis	[39]
Listeria monocytogenes	DP-L4017	Lung cancer	Production of lymphocytes that are specific to the tumor-associated antigens	[40]
	CS-L0001	Colon tumor lung metastases	Cell to cell spreading of tumor is inhibited	[41]
Clostridium novyi	NT	Glioblastomas neurosphere, Colon cancer	Production of toxins that work against cancer	[42]
Streptococcus pyogenes	OK-432	Lymphangioma intraoral ranula	Increase in the count of white blood cells	[43, 44]
Mycobacterium bovis	BCG Pasteur	Bladder cancer	Triggering of immune system, cancerous cells phagocytosis	[45, 46]
Bifidobacterium breve	UCC2003	Head and neck tumor	Active against tumors, cause apoptotic death of cancerous cells	[47]
Lactobacillus rhamnosus	SHA111; SHA112;SHA113	Colorectal cancer, Cervical adenocarcinoma, Breast cancer	Death of cancerous cells due to apoptosis, downregulation of genes that are involved in breast cancer	[48]
Bifidobacterium longum	*B. longum*-C-CPE-PE23	Breast cancer	Suppression of tumor growth without any side effect	[49]
Alphavirus	M1 Strain	Various cancers	Anti-proliferative actions	[50]
Adenovirus	Ad3, Ad35, Ad37, and Ad52	Breast cancer	Coxsackie adenovirus receptor-independent function	[51]
	Ad serotype 3 (Ad3)	Renal cell carcinoma	Expression of decorin is induced in the extracellular matrix assembly	[52]
	Ad type 5	Lung cancer stem cell	Increase in the cytotoxicity due to tumor necrosis factor	[53]

Table 1 (continued)

Microbe (bacteria/virus)	Strain used	Cancer type	Mechanism observed	References
Herpesvirus	HSV-1716	Malignant glioma	necrosis within tumor	[54]
	G207	Anaplastic astrocytoma, glioblastoma, and gliosarcoma	Selective viral replication in dividing (tumor) cells	[55]
	NV1020	Colorectal, gastric, and hepatic cancer	Anti-tumor efficacy causing tumor reduction	[56]
Zika virus	Immune-sensitized ZIKV strain	Glioblastoma multiforme	Tumor clearance	[57]
Measles virus	MV-CEA	Ovarian cancer	Tumor regressions occurred during natural measles infections, immunogenic cell death	[58]
	MV-NIS	Ovarian cancer, multiple myeloma	Infected mesenchymal stem cells directly carried tumor-killing compounds to the ovary	[59]
Newcastle disease virus (NDV)	NDV AF2240	Breast cancer	Cytokine-related apoptosis	[60]
	NDV/FMW	Lung cancer	Cancer cell death due to autophagy or apoptosis	[61]
	NDV/HK84 strain	Hepatocellular carcinoma	Suppression of in vitro migration and invasion of hepatocellular carcinoma cells, reduction of tumor size	[62]
	rNDV-mOX40L	CT26 cell lines	Anti-tumor immunity by stimulating tumor-specific T cells	[63]
	NDV Iraqi strain	Mammalian adenocarcinoma AN3	Reduction in the volume of solid tumor	[64]
	Oncolytic NDV attenuated AMHA1 strain	AMJ13 and MCF7 breast cancer cell lines	Decrease in the glycolysis activity of the NDV infected tumor cells	[65]
Reovirus	Reovirus type 3 Dearing strain (ReoT3D)	Lung cancer	Cytotoxic activity observed in lung cancer cells	[66]
Avian reovirus (ARV)	S1133 strain	Cancer cell lines (A549, B16-F10, and HeLa)	σA structural protein regulates metabolic pathway that can stimulate host cells to produce more energy for virus replication and compete with cancer cells thereby achieving an anti-cancer purpose	[67]
Vaccinia virus	MVA	Melanoma, colon cancer	Adaptive antitumor responses, cytokines response	[68]
	WR	Lung cancer	Apoptosis and necrosis	[68]

for anaerobic bacteria to thrive [77]. As a result, by using microbes as medication and gene delivery vehicles, it is now possible to specifically target the parts of cancers that were previously the most resistant to treatment [78]. It has been demonstrated that the processes for motility and survival of bacteria, as well as how dependent they are on oxygen, are both necessary for their growth and survival in tumors [27]. *Salmonella sp*. and *Clostridium sp.* have been shown to target and multiply preferentially in the core anaerobic region of tumors [79]. So, the problem of specificity in medication and gene delivery for cancer therapy may be resolved by microorganisms.

Bacteriobots

Bacteriobots refer to the devices that are engineered by employing bacteria to be used as a micro-sensor or micro actuators in order to transport different kinds of chemotherapeutics and other curative substances to the inner environment of tumor site [80, 81]. The speed of chemotaxis and their migration to the site of cancer is regulated by designing appropriate bacteriobots. Bacteriobots attack the tumors at targeted site where they get attached to the cancerous cells as shown in Fig. 3. Moreover, they are designed for secreting the anti-tumor substances leading to the death of cancer cells

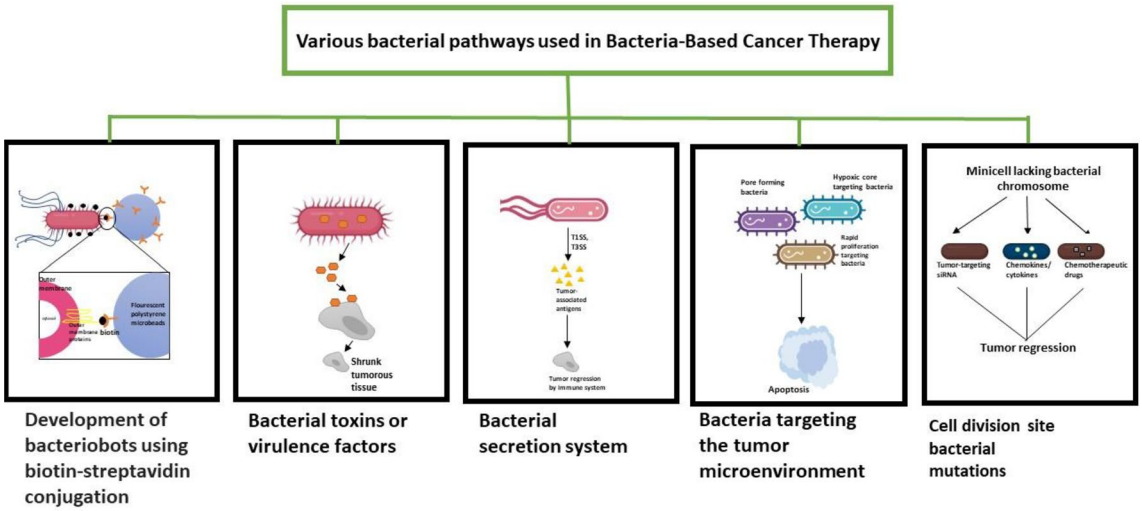

Fig. 2 Pathways used in bacterial-based cancer therapy

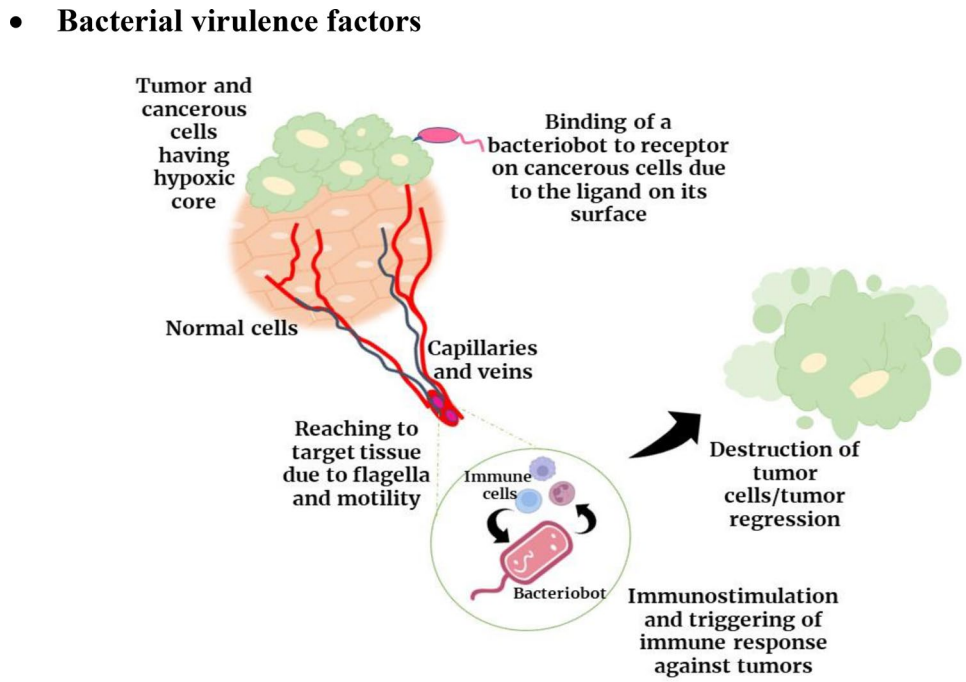

Fig. 3 Representation of bacteriobot employed for bacteriotherapy

[82]. By gauging the binding affinity of streptavidin which is present on the outer surface of liposome which is loaded with drug and biotin which is presented on the proteins' outer surface, the motility of bioengineered bacteriobots has been demonstrated [83]. A number of different bacterial species can be used for designing bacteriobots such as *E. coli*, *S. Typhimurium*, *S. marcescens, and* magnetotactic bacteria. Nevertheless, because of augmented pathogenicity and developed resistance, the applications of bacteriobots are somehow limited. Moreover, particular nutritional needs and problematic expansion can further complicate the applicability. Though, it has been anticipated that by engineering the bacteriobots with those bacteria that can target the tumors, advances in the cancer diagnosis and treatment can be made.

Bacterial virulence factors

Virulence factors refer to the cellular bodies, molecules, and the controlling systems that allow the microbes-derived pathogens to accomplish the growth and colonization in the host as well as immunosuppression. Moreover, the withdrawal of nutrients as well as the entrance and departure from the cells can also be achieved [84, 85]. Therefore, it is necessary to standardize the bacterial virulence in contradiction

of immune structure of host. Nevertheless, a few of the virulence factors might be accountable for the anti-cancerous affect. Therefore, by manipulation or elimination of such factors can decrease the anti-cancerous responses of bacterial species. It is essential to weaken a bacterial strain deprived of changing the anti-tumor response.

The strain VNP20009 of bacteria *Salmonella typhimurium*, which has been extensively investigated because of its anti-cancer affectivity is changed by removing the main genes of virulence. These virulence genes are usually *purI* and *msbB* [86]. A deletion mutation the gene named *msbB* results in the lipid myristoylation. The risk of sepsis can be reduced by a constituent of LPS which can lead to the induction of TNF production. Mutational changes in some genes such as *rfaD* and *rfaG* can lead to the generation of truncated LPS within host, as a result of which toxicity is reduced and anti-cancerous response is generated [87].

Deletion of genes *SpoT* and *relA*- from the species of *Salmonella* results in the production of mutants that are not capable of synthesizing a signaling peptide, ppGpp, which is considered to play a role in expression of genes in bacteria. However, the resulting mutants show reduced toxicity and exhibits anti-cancerous activities. Moreover, they can lead to the activation of IPAF and NLRP3 inflammasomes and various pro-inflammatory cytokines can also get expressed [88].

The deletion of genes that play role in invading cells can lead to the reduced cytotoxic effect of *Listeria monocytogenes*. The secretion of phagolysosomes can also get defected by the deletion of *Hyl* gene [46, 89]. The diffusion across the cells can gets abrogated by the mutation in *ActA and actA genes* [90, 91], and altered variant of *inlA* and *inlb* exhibit the loss of invasion-related characteristics [41, 92]. As depicted in Fig. 4, infection with *Clostridium* and *Cornyebacterium spp*. results in the secretion of several toxins that disrupt intracellular processes, including hemolysins, phospholipases, actin-specific ADP-ribosyltransferase, and others [93, 94].

The bacterial secretion system

Bacteria can utilize their secretion processes for the transportation of virulence factors that can undergo exploitation and manipulation with the aim of being employed in novel anti-cancer therapies. Necessarily, this includes the signaling compounds that are essential for delivery and then their fusion with the therapeutic agents in order to attain an effective delivery of the drugs to their target [95].

Type III secretion system which is referred to as T3SS is most widely applied secretion system in the field of anti-cancer therapeutics. This secretion system works by the injection pf bacteria-derived peptides directly into the cytoplasm [96]. A number of investigations pay attention to the efficiency of T3SS and involve the genetic fusion of T3SS with the antigen, Survivin, that is associated with a tumor, leading to the regression of tumor [97]. Several studies have also reported that TAA/TSA can gets expressed and released via type 1 (T1SS) secretion system of a bacterial specie known as *Salmonella typhimurium* [98]. It has been demonstrated that the development of tumor can be inhibited by the activation of immune response that is mediated by CD8+ T lymphocytes. This activation is usually caused by the releasing of prostate-specific antigen (PSA) from *S. typhimurium* via HlyA (T1SS) system [98].

In an experimental mouse model of fibrosarcoma, the secretion of peptides from the *Listeria monocytogenes* p60 protein imitate the tumor antigen via T3SS of *S.*

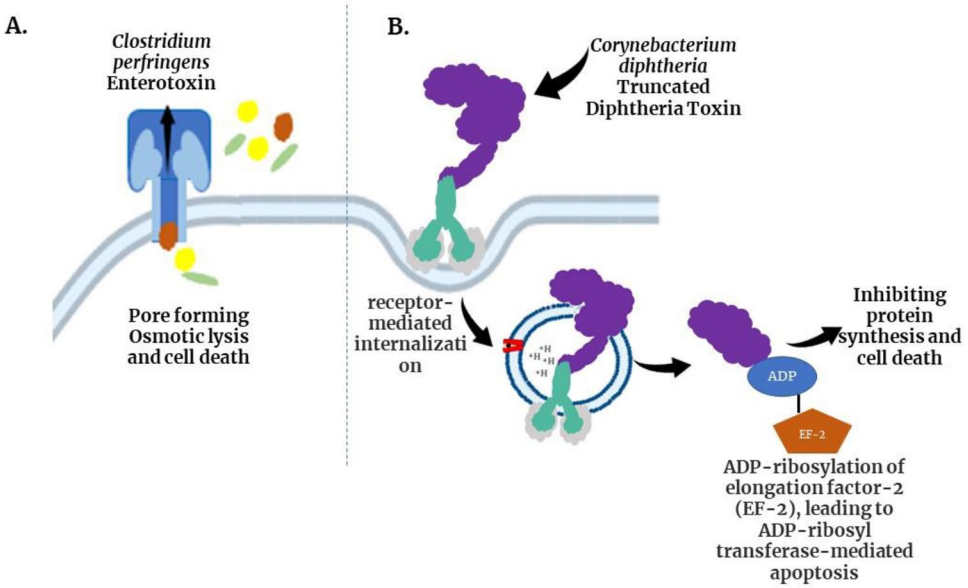

Fig. 4 The impacts of bacterial toxins on cancerous cells. **A** High osmotic pressure, brought by pore-forming toxins such *Clostridium* perfringens enterotoxin, causes apoptosis (CPE). **B** ADP-ribosylation of elongation factor-2 (EF-2) and subsequent apoptosis are caused by the receptor-mediated administration of diphtheria toxin (DT)-based immunotoxin, which inhibits protein synthesis

typhimurium, indicating that 80% of animals inoculated with p60 peptide were immune after being challenged with fibrosarcoma tumor cells [99]. A living strain of *Pseudomonas aeruginosa* has been genetically altered to enter mammalian cells via the T3SS carrying the *Yersinia* (T3SS) YopE and YopH proteins. This strain induces CTL responses in vivo against encroaching malignancies [100].

Bacterial mutations

There exists a number of rod-shaped bacterial species that constitute both Gram-negative and Gram-positive species. All these bacterial groups have been reported to be capable of producing minicells because the cells divide abnormally. Minicells are achromosomal cells that range in size from 100 to 400 nm and are typically created by aberrant cell division in the mother cell poles of rod-shaped bacteria [101]. The minicells produced in turn mimic the usual cell membrane in terms of characteristics. Moreover, they contain the same type of RNA, proteins, and ribosomes but bacterial chromosome is not present in them [97]. By developing the mutational changes in the machinery involved in cell divisions, rod-shaped bacteria like *Salmonella enterica* or *Escherichia coli* can be modified on the genetic level in such a way that the minicells created by them have the chemotherapeutic drugs loaded onto them [102].

Minicells continue to be an effective approach for the delivery of drugs and are considered to be a vital advancement in the field of anti-cancerous research. Minicells still keep all the virulence characteristics that are required for targeting the tumors. Additionally, the gene transfer characteristics of the bacteria are also involved in the targeted delivery of drugs. It has been demonstrated by several in vitro and in vivo studies that the bacteria that reside inside the cells are thought to transfer the genes inside the cells of mammals. *Salmonella*, invasive *E. coli*, *Shigella*, *Listeria*, *Pseudomonas*, and other bacteria have all been investigated and experimented with for their potential as vectors to deliver genes. In order for the transfected genes to be expressed at the cellular level, attenuated bacteria must first release plasmid DNA into the host cells's cytoplasm [103].

Using RNA interference, this can be further tailored to silence genes that encourage tumor growth. Small hairpin RNAs (shRNAs) expressed on a plasmid must be transferred in order to become small interfering RNAs (siRNAs), which subsequently work to encourage the destruction of target mRNA in malignancies. *Listeria monocytogenes* and *S. enterica* ssp. Typhimurium-exhibiting targets, such as CTNNB1, Stat3, or Bcl2, all of which are connected to tumor persistence, have been the subject of some research into this mechanism. Testing has been done on a mutant strain of Salmonella typhimurium VNP20009 that has changed motility as well as other traits including adhesion and invasion of mammalian cell cultures. When compared to controls, the mutant VNP20009 reduced tumor development in schwannoma models and brought about cytokine and immune effector cell profile alterations that were consistent with enhancing innate and adaptive host immune responses. Additionally, the mutant strain caused death in tumor cells, a decline in tumor angiogenesis, and a slowing of the development of the injected schwannoma tumors [102].

Another study by Felgnet et al. [104] showed that the deletion of aroA dramatically increased the virulence of attenuated Salmonella in mouse models. Mutant bacteria lacking aroA elicited increased levels of the proinflammatory cytokine tumor necrosis factor alpha (TNF-α) after systemic application in cancer cells. Thus, introducing the mutations in bacterial strains can possibly offers a promising pathway for treating cancer.

Genetically modified bacteria in cancer therapy

Gene therapy is considered to be a substitutive strategy to cancer therapy and is more focused on the advancements. Gene therapy holds promising benefits such as the specific targeting and the killing of cancerous cells [27, 105]. Bacteria that are genetically altered can also become capable of lowering the pathogenicity as well as increasing the efficiency of anti-tumor response [106].

Currently, several studies have reported the development of novel strategy against cancer by employing the genetically modified bacteria with the aim of expressing cytotoxic agent, anticancer proteins as well as the expression of reporter genes and antigens that are specific to several tumors [107]. It has been reported that within the cancerous tissues, genetically engineered bacterial species show more substantial multiplication as compared to the tissues which are normal [108]. Bacterial species that have the ability to specifically colonize cancerous tissues can be used as a vector for delivering the therapeutic genes. Such bacterial species include strain VNP20009 of *Salmonella typhimurium* serovar and *Clostridium butyricum* M55. Such bacterial species are also free from any sort of complicated immune response or the side effects that can lead to toxicity. But the results obtained from researchers, are however, less favorable than anticipated [27, 109]. Some strains of *Clostridia* such as *C. beijerinckii* and *C acetobutylicum* can be designed successfully for the expressions of genes that code for the particular enzymes of bacteria such as nitroreductase and cytosine deaminase.

Expression of murine tumor necrosis factor alpha (m-TNFα) can also be achieved thus leading to more efficient effects against tumors [110]. It has been investigated by a number of researchers that the factors that can induce

hypoxia can bind to the antibodies that are produced by certain bacteria. An example of such factor is 1α which is involved in the transcription as well as the development of tumor [111]. Clinical trials have shown that the engineered *S. typhimurium* and *Clostridium novyi* NT expressing HlyE or Stx2 (an acidic pH responsive promoter) or recA (a 38 kDa protein absolutely vital for the maintenance and repair of DNA) stimulated the immune system of host for expressing cytokines like interleukin 2 (IL2), IL4, IL18, and CC chemokine 21, and as a result caused the tumors to regress [112]. These investigations revealed that a novel and effective strategy for treating cancer would involve combining bacteriotherapy with radiation, immunotherapy, or chemotherapy.

Clinical trials for bacteria-based therapy

The intricacy of pathways that favors the growth as well as those that control the signaling molecules or neuronal schemes that contribute to the tumor development, can make the cancer a more complicated disease. Therefore, there is a dire need to facilitate the discovery of a drug that can multitarget the tumor sites with reduced side effects. The advancements must be made so that only the cancerous tissues are made target instead of targeting the normal ones [113].

Azurin is considered to be one of the medicines that show no toxicity and also exhibits the ability of targeting multiple sites. In addition to p28, a diverse number of domains are present in Azurin. The drug is highly potent against cancer because of the least development of resistance, no production of toxic side effects and the efficiency that can be demonstrated by clinical trials. In the first phase of clinical trials, one patient of sarcoma and 7 patients of melanoma were investigated along with 1 patient suffering from prostate cancer, 1 patient of pancreatic cancer as 4 colon cancer patients. All the patients were administered with 5 levels of dosage. It was reported that there were no signs of toxicity and the rate at which patients were surviving was increased [114].

A group of researchers conducted a second human study in which 15 patients received brief infusions of p28 three times per week for a total of four weeks. As no patient showed signs of drug toxicity and no immunological reaction was triggered in response to the treatment, it was shown that p28 from azurin blocked p53 ubiquitination [115]. These studies showed that azurin only affects malignant cells and not healthy ones, which may account for the absence of any adverse effects after delivery. Azurin's treatment via twice-weekly injection in healthy individuals with a family history of breast cancer and BRCA1 and BRCA2 polymorphism may be linked to another significant function in the reduction or prevention of cancer initiation [116]. In a clinical trial with 18 pediatric patients who had central nervous system tumors, researchers administered intravenous p28 injections for four weeks on a schedule of three times per week. Children tolerated the phase II indicated dose quite well, and the data showed no toxicity [117]. Every drug's oral administration is essential to its effectiveness, and this process is now ongoing alongside clinical studies.

Virus-based cancer therapy

Cancerous cells can get infected by viruses more significantly in contrast to the normal tissues. As a result of infection, antigens that are associated with tumors get presented leading to the activation of "danger signals" which produce a microenvironment around tumor that shows least immunity. Viruses can also be employed as a transduction vehicle so that immunomodulatory or inflammatory cytokines can get expressed [118]. Recently, with the aim of overcoming these problems, the advancements in medical genetics look forward for increasing the significance and efficiency of a few viruses so that abnormal cells can get infected by different processes like gene deletion or by merging the immune checkpoint inhibitors (ICIs) with the employment of viruses [119].

Oncolytic viruses (OVs) refer to the organisms that are capable of infecting, identifying, and lysing various cells present at the tumor site with the aim of stabilizing and decreasing the progression of tumors. They can exhibit an inherent affinity for cancer cells or can be genetically positioned to recognize particular targets [120]. In clinical studies, a wide range of OVs are under investigation as probable cancer treatments [11]. Additionally, the OVs have the capacity to contribute to the immune system's stimulation against the tumor cells, affecting the emergence of an antitumor response [121].

There is a diverse range of mechanisms by which tumor microenvironment gets invaded. The invasion of the site can trigger the immune system's downregulation and shows a positive effect over the disease's progression [122]. The action of T-cells can be discouraged by preventing the antigen-presenting cells (APCs) from the presentation of tumor-associated antigens [123]. The cytotoxic T lymphocyte-associated antigen 4 and apoptotic cell death protein 1/programmed death ligand 1 (PD-L1), both of which are connected to the downregulation of the inflammatory reaction and immune system homeostasis making a contribution to cell death and suppression of T cell proliferation, can be stimulated abnormally by certain types of tumors [124].

Additionally, an important method of immune evasion is the overproduction of tumor-associated macrophages, which are the primary lymphocytes in the inflammatory response against the tumor and are similar in function and appearance to type M2 macrophages that are in charge of repairing

tissues and controlling immune response. Consequently, the downregulation of inflammation and the aberrant acceleration of tumor growth rates have been linked [125]. In order to change the tumor microenvironment from an immunological desert brought on by evasion processes to an inflammatory condition where the immune cells are capable to eradicate abnormal cells, the clinical usage of OVs becomes increasingly important [126]. Furthermore, the viruses have several mechanisms that could cause infected cells to undergo cell lysis, which would cause the tumor cells to die and boost the effectiveness of the immunotherapy [119].

General mechanism of oncolytic virus in cancer therapy

Oncolytic viruses have the ability of infecting the cancerous cells via targeting specific sites. Such targeted sites are usually the compounds that are secreted by the cancerous cells and include, for instance, prostate-specific antigen, osteocalcin, CD20, folate receptor, human telomerase reverse transcriptase, surface markers as prostate-specific membrane antigen, Her2/neu, and cyclooxygenase-2 [120]. Moreover, it is also possible that the selective targeting of cancerous cells as well as reduction in oncolytic viruses' aggression toward the normal cells can be achieved in a laboratory by deleting the genes from the pathogenic viruses [127]. The route by which OVs are administered are naturally interrelated to the kind of tumor that needs to be treated. It is because that the pathways that are taken by the viruses have an effect over the success rate of the therapy depending upon either there is an access to the virus on-site or not. Moreover, it is also effected by the presence of any naturally occurring blockade toward antigen in host.

The virus can spread by intratumoral, intrathecal, intraperitoneal, or subcutaneous route that offers the regulation of viral amount at tumor site and can result in least toxic side effects. Intravenous route can also be established that is linked with treating distantly metastatic cancers [128]. Depending upon the mechanism that is selected by tumors for invading the immune system, cancerous cells can alter the way in which certain mechanisms are activated, for instance, the alteration of interferon 1 signaling pathway and, protein kinase R in retaliation to the programmed cell death, inflammatory cells' maturation and the infections by virus (Figs. 5, 6). OVs can live longer in cancer cells due to changes in the antiviral response, viral factors that can block apoptotic pathways, completion of their developmental stages, and maturation to the lytic phase [129].

DNA, viral proteins, viral capsid, RNA, and pathogen-associated molecular patterns (PAMPs) are some of the viral structure-associated immune signs, the recognition of which is stimulated by several viruses present in humans [130]. Once the PAMPs are recognized by toll-like receptors (TLRs), dendritic cells can lead to the stimulation of inflammatory agents such as tumor necrosis factor alpha (TNF-alpha) and type 1 interferons. The production of cytokines like interleukin 2 (IL-2) can also get stimulated. Moreover, the inflammatory microenvironment is maintained and the immune cells are recruited [131]. The expression of class 1 major histocompatibility complex in cell membranes, as well as the caspase enzyme's activity and cell apoptosis in various malignancies, are all positively regulated by TNF-alpha in response to viral infection [132]. Through processes that promote necrosis and apoptosis, this interferon can encourage the death of cancer cells. Its antiangiogenic activities can also cause thrombotic events, which may result in the elimination of several vascular channels necessary for the tumor's blood supply [133]. TNF-alpha is also linked to the maturation of antigen-presenting cells, an increase in natural killer cell cytotoxicity, and the activation of T helper cell type 1 (Th1) reactivity [132].

It has been reported by several studies that the activation of T cells' action as well as the cytotoxic lymphocytes' response is related to IL-2, playing a role in natural killer cells' expansion and maturation. CD4+ T cells (TCD4) are also positively regulated. IL-2 also has the ability to regulate the homeostasis and T regulatory cell act, leading to

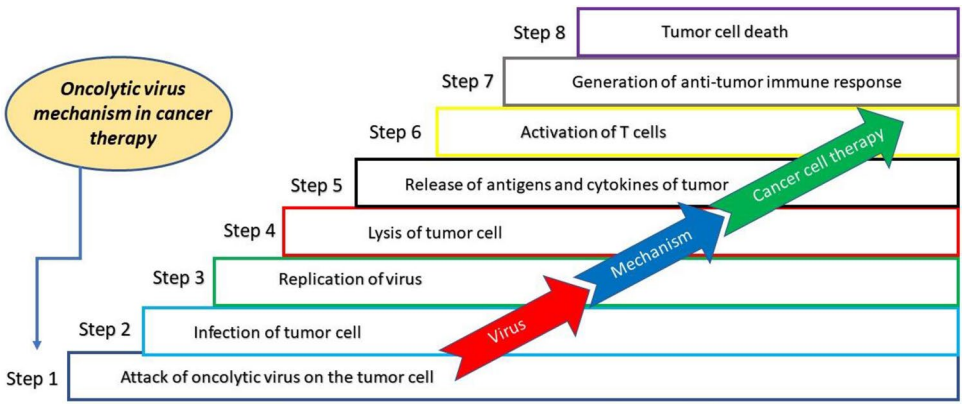

Fig. 5 Mechanism of oncolytic virus in cancer therapy

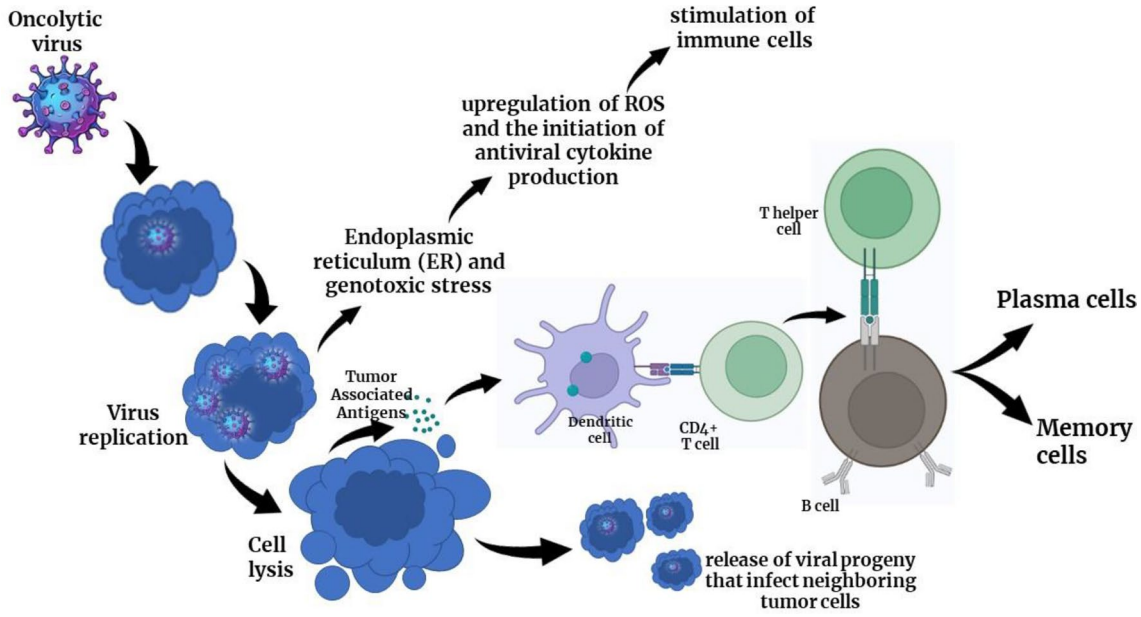

Fig. 6 Attack of oncolytic virus on tumor cell

the production of an inflammatory microenvironment that favors the fight against cancer [134]. The Th1 inflammatory profile was also linked to a decline in T regulatory cells, an increase in TCD4 and TCD8 effector cell counts, stimulation and differentiation of T lymphocytes, and maturation of dendritic cells, all of which help reverse the immunosuppressive condition of a tumor and encourage an inflammatory reaction [134].

Further adding to the destruction brought by the inflammatory reactions, the lytic cell death of the abnormal cells is also affected by another factor that is the action of virus within the cells. A few of the organelles including lysosome, endoplasmic reticulum, or mitochondria lost their functions due to the presence of OVs. Additionally, the presence of viruses can promote the production of reactive nitrogen species leading to the stimulation of oxidative stress. Endoplasmic reticulum stress is also created and these aforementioned stress conditions are linked to an enhancement in the levels of intracellular calcium [131], thus causing the reduction in tumor. It aids in the development of an inflammatory response against the tumor. The use of OVs and cell checkpoint blockers in combination is a crucial strategy to boost the rates of viral persistence in the human body.

The tumor can evade the maturation of T cells and the immune system by negatively regulating PD-L1. In this manner, TCD8 cells against the tumors appeared as a result of PD-L1 suppression, promoting natural killer cell activity, and generating a reaction with a Th1 profile [135]. Additionally, research has shown that administering OVs and monospecific antibodies that block the activity of cytotoxic T lymphocyte-associated antigen 4 increased the efficacy of immunomodulatory treatment [136]. As a result, immune markers for cell damage called damage-associated molecular patterns (DAMPs), such as highly mobile group box 1 protein and Adenosine triphosphate, are produced. The cross-presentation of the DAMPs and the cancer antigens, which results in the continuation of the inflammatory reaction phase, contributes to the antigen presentation and to the promotion of dendritic cell maturation [137]. Therefore, cellular breakdown enables virus liberation in the extracellular space and subsequent septicity of more tumor cells, resulting in a cascade of antitumor effects [130]. In addition, cell death causes the discharge of tumor-associated antigens that can be recognized by immune cells in an inflammatory milieu, causing the OVs to respond by attacking tumor cells even those that are not infected [129].

Oncolytic viruses commonly employed in cancer therapy

The role of various oncolytic viruses (Fig. 7) are as follows.

Adenovirus

When recognized by the immune system, the proteins of adenovirus help the formation of an antiviral response [138]. These viruses have a strong affinity for a variety of organ tissues, including the ophthalmic, respiratory, intestinal, renal, and lymphoid ones, and they can enter host cells using a number of receptors, including the human coxsackie-adenovirus receptor, CD86, CD46, and CD80 [139]. Adenoviruses

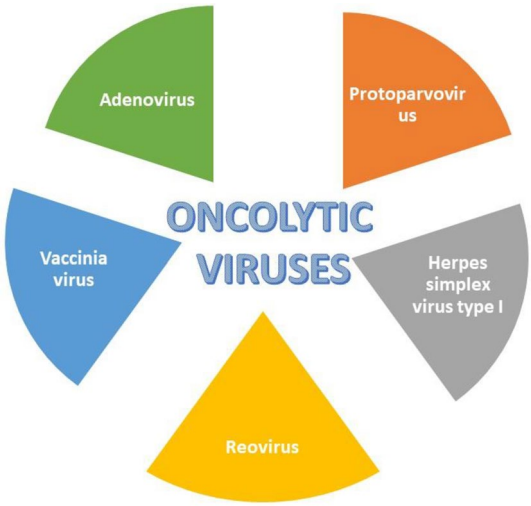

Fig. 7 The various oncolytic viruses that are used in cancer therapy

have also been employed for the creation of numerous immunological therapies [140] because of their propensity to act as viral vectors [138], a variety of routes for cellular entry, resistance toward the chemical or thermal degradation outside the cell, and extensive knowledge of their biology. Beginning inside the cell nucleus, the viral replication process causes the expression and release of certain proteins in the cytoplasm, including E1a and E1b, which are connected to the activation of the pathways of autophagy. Organelles or even the entire cell might die as a result of this mechanism, which causes the creation of some autophagosomes that can subsequently fuse with lysosomes [141]. Additionally, studies have indicated that the expression of E1a in tumor cells may be linked to the induction of autophagic complex synthesis, and E1b may assist the amplification of operations of these complexes, both of which help to stabilize and shrink the tumor [142].

Protoparvovirus

The Parvoviridae family can infect mammalian cells, including human cells, by utilizing fixation factors such as the transferrin receptor or glycosidic compounds like *N*-acetylneuraminic acid. These factors are expressed on the cellular membrane and create a favorable environment for viral attachment within the cell [143]. The primary capsid protein VP1 is a protein that controls the endocytosis process used by protoparvoviruses to enter their host cells. This process also allows for the dissolution of the endocytic vesicle within the cell and additional viral protein delivery in the cytoplasm. Additionally, VP1 possesses nuclear localization cues that help the viral protein to reach the nucleus of cell. From this point on, the virus can survive by remaining dormant till the start of the cellular division when the cellular genome stops replicating, through the action of the protein NS1, and the incorporation of viral material with the hereditary information of the host cell is permitted to ensure viral survival [144].

Through the activity of the NS1 protein inside the cell, H-1PV can cause a condition characterized by elevated oxidative stress by increasing the amounts of nitrogen as well as reactive oxygen species. The control of RNA virus replication, which results in the loss of hereditary material and the stimulation of apoptotic pathways with subsequent cell death, is similarly related to NS1. Additionally, the virus can promote the transport of proteases into the cytoplasm from the lysosome, which results in the necrosis of tumor cell membranes [131].

Vaccinia virus

After smallpox was eradicated in 1796, the scientific application of poxviridae is now focused on developing vaccines and treatments for other pathologies [145]. Pexa-Vec (pexastimogene devacirepvec, JX-594) is a member of this family that has been genetically altered to contain the GM-CSF and the TK gene deletion in order to boost its affinity for tumor cells and restrict its replication to cells that display abnormal levels of TK [146]. The activation and production of GM-CSF and IL-24, two molecules that collectively may help to stabilize and give tumor cell death, were associated to the delivery of VACVs in the tumor environment. Dendritic cells and neutrophils, immune system cells, contribute to the generation of an inflammatory environment that makes it possible to fight the tumor, are associated with the maturation and differentiation of GM-CSF, and IL-24 inhibits tumor angiogenesis, which has a positive impact on the pathways that lead to programmed cell death, the development of an antitumor reaction, and the inhibition of tumor metastases [147].

Reovirus

The Reoviridae family of viruses, which includes the respiratory enteric orphan virus (Reovirus), infects a variety of hosts, including plants, mammals, fungi, and fish [148, 149]. This name was given to the virus as a result of its isolation in the digestive and respiratory tracts and its incapability to set the basis for any recognized human diseases [150, 151]. It's interesting to note that this final quality is also highly associated with the effectiveness of reoviruses in oncolytic therapy. When a study revealed that reoviruses had a tropism for "transformed cells" and that normal cells are resistant to the virus, the first link between reoviruses and an oncolytic function was made in 1977 [152]. Further research was conducted to assess the potential of reoviruses as a cancer treatment substitute as a result of this information.

Herpes simplex virus type I

The alpha-herpesviruses subfamily includes the herpes simplex virus-1 (HSV-1) [153, 154]. Given that it may be easily changed to enhance its oncolytic characteristics and patient safety, its big genome is crucial [153]. HSV-1, unlike reoviruses, is harmful to humans and may infect the skin, mucosa, and central nervous system. This indicates that more transgenes must be deleted and inserted in order to create an effective oncolytic virus therapy [155].

Clinical trials for virus-based therapy

Clinically speaking, the use of viruses to treat cancer is still in its infancy in the modern period. The introduction of wild type viruses at first raised the possibility of unfavorable outcomes brought on by viral replication in healthy tissues. However, the first oncolytic virotherapy product for cancer was approved in 2004 in Latvia and a few other nations. It was called Rigvir (an ECHO-7 virus) [156], an oncolytic picornavirus with some inherent tumor selectivity. The second oncolytic virus was purposefully created to target certain tumor types. This adenovirus, known as H101 (Oncorine), has been utilized in China since 2005 to treat solid tumors.

Notably, neither of these viruses has an arming mechanism. Scientists have been able to harness the immunological properties of oncolytic viruses as a result of the recognition that repurposing the immune system to perform antitumor functions may give a promising method to treating cancer [157]. For instance, adding immunological transgenes like the GMCSF has been a well-liked strategy. One of the earliest oncolytic viruses created to increase immunity was Talimogene Laherparepvec, a herpes simplex-1 virus that codes for GMCSF and is also known as T-vec or Imlygic®. A randomized phase III clinical investigation resulted from its clinical use (OPTiM). Patients with unresectable stage IIIB/C and IV metastatic melanoma who received intratumoral T-vec in this trial showed a 19.3% durable response rate, more than 80% of which were full responses [158]. The Food and Drug Injection (FDA) and European Medicines Agency approved GM-CSF subcutaneous administration in 2015 despite its lower efficacy (1.4% durable response rate and 0.7% complete response) (EMA) [158].

The medical world is optimistic about continuing to discover and enhance oncolytic viruses for cancer therapy, particularly adenoviruses, as a result of this historic approval in Western nations. Later, when the virus was coupled with pembrolizumab in a similar patient population, 62% of the patients' experienced responses, of which 33% were complete. As anticipated, after treatment, a significant amount of cytotoxic T cell infiltration was found in the tumors [159]. T-vec enhanced the response rate of ipilimumab as compared to ipilimumab alone, according to a recent phase II clinical trial in metastatic melanoma patients (38% vs 18%, respectively) [160]. Importantly, adverse events were not compounded, in contrast to combinations of checkpoint inhibitors. This shows that checkpoint inhibition and oncolytic viruses can be used together without posing a risk to patient safety [161].

Future prospects

Apart from the abundant benefits provided by the microbial-based cancer therapy, some of the mechanisms offer several limitations. In order to overcome such hurdles, the next critical step to incorporating microbes into cancer therapy on a regular basis may very well be the careful manipulation of microbes. Moreover, extracellular matrix modulators and capsid modifications must be used to avoid the spread of tumors and the passive targeting. With advances in genomics and molecular biology, it is becoming increasingly possible to tailor microbial-based therapies to individual patients. By analyzing the genetic makeup of a patient's tumor, scientists can design viruses or bacteria that are optimized to target specific molecular features of the cancer cells. This new level of control may be applied to each patient's specific tumor type, thus it suggests enormous applications as a tailored therapy.

Hypoxia is another challenge that is encountered while designing a cancer treatment for a specific type of tumor. The optimum microbial therapy, according to theory, combines a non-pathogenic but effective species with several strains chosen for their particular targets before being paired with other efficient standard therapies for the highest level of efficacy. To target the remaining tumor areas with higher oxygen content, various treatment approaches can be paired with microorganisms. Additionally, Hypoxia-response element containing promoter can be employed to avoid this situation. Microorganisms' genetic adaptability might actually be their greatest asset, enabling fine-tuning of tailored therapy for optimum cytotoxic effects.

There is still a long way to go before the concept of treating cancer using microorganisms as delivery systems reaches the level of acceptance that current standard treatments have. Before microorganisms may be trusted in the field of cancer therapy, toxicity concerns and cultural stigmas must be overcome. More rigorous scientific research must be done in order to eliminate the persistent drawbacks and side effects of bacteriotherapy and virotherapy, which are still considered to be rather innovative fields of medicine. Due to the abundance of favorable means that can be used to target tumors and enhance treatment consequences, the potential of both therapies, however, cannot

be ignored. Few investigations have resulted in clinical trials, despite good in vitro and in vivo results of cancer immunotherapies. The scientific and medical sectors must thus start planning more clinical studies to look into and maximize the effectiveness of these microbial-based cancer medicines. Although microbial-based cancer therapy shows promise, it is still a developing field, and further research is needed to fully understand its potential and optimize its application.

Conclusion

The term "cancer," which refers to any one of a vast range of diseases characterized by the formation of aberrant cells that divide uncontrollably and have the capacity to invade and destroy normal body tissue, is considered as the second-leading cause of mortality in the world. The goal of microbial-based cancer therapy is to treat patients with difficult-to-treat cancers by using tumor-specific infectious microorganisms. Although clinical and preclinical data have demonstrated excellent efficacy for microbe-based cancer therapy, more effective treatment approaches are required to establish long-term tumor control in humans. Thus, different strategies are being used to increase the effectiveness of bacteria and viruses. It is anticipated the decades of work by the scientific and medical community will soon result in better treatment alternatives. We will probably get a better grasp of the unique patient-tumor immune state, which will improve our ability to provide medical care for individuals with advanced stage illness. Probably, the future multimodality approaches will still include bacteria and viruses in order to efficiently achieve the elimination of different types of cancers. Conclusively, microbial-based therapies have the potential to revolutionize cancer treatment in the coming years.

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References

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin. 2023;73(1):17–48.
2. Bernardes N, Seruca R, Chakrabarty AM, Fialho AM. Microbial-based therapy of cancer: current progress and future prospects. Bioeng Bugs. 2010;1(3):178–90. https://doi.org/10.4161/bbug.1.3.10903.
3. McCance KL, Huether SE. Pathophysiology-e-book: the biologic basis for disease in adults and children. Amsterdam: Elsevier Health Sciences; 2018.
4. White SC, Pharoah MJ. Oral radiology-e-book: principles and interpretation. Amsterdam: Elsevier Health Sciences; 2014.
5. Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, Jemal A. Cancer treatment and survivorship statistics, 2016. CA Cancer J Clin. 2016;66(4):271–89.
6. Bhatt AP, Redinbo MR, Bultman SJ. The role of the microbiome in cancer development and therapy. CA Cancer J Clin. 2017;67(4):326–44. https://doi.org/10.3322/caac.21398.
7. Ji H, Yang X. Microbial-based cancer therapy—bugs as drugs: history & the essential role of medical imaging. Cancer Treat Res Commun. 2021;28:100436.
8. Ganai S, et al. In tumors *Salmonella* migrate away from vasculature toward the transition zone and induce apoptosis. Cancer Gene Ther. 2011;18(7):457–66. https://doi.org/10.1038/cgt.2011.10.
9. Brown JM, Wilson WR. Exploiting tumour hypoxia in cancer treatment. Nat Rev Cancer. 2004;4(6):437–47.
10. Bazett M, et al. Harnessing innate lung anti-cancer effector functions with a novel bacterial-derived immunotherapy. Oncoimmunology. 2018;7(3):e1398875. https://doi.org/10.1080/2162402X.2017.1398875.
11. Fu L-Q, et al. Recent advances in oncolytic virus-based cancer therapy. Virus Res. 2019;270:197675. https://doi.org/10.1016/j.virusres.2019.197675.
12. Leber MF, et al. Engineering and combining oncolytic measles virus for cancer therapy. Cytokine Growth Factor Rev. 2020;56:39–48. https://doi.org/10.1016/j.cytogfr.2020.07.005.
13. Forbes NS, et al. White paper on microbial anti-cancer therapy and prevention. J Immunother Cancer. 2018;6(1):1–24. https://doi.org/10.1186/s40425-018-0381-3.
14. Garrett WS. Cancer and the microbiota. Science. 2015;348(6230):80–6. https://doi.org/10.1126/science.aaa4972.
15. Schwabe RF, Jobin C. The microbiome and cancer. Nat Rev Cancer. 2013;13(11):800–12. https://doi.org/10.1038/nrc3610.
16. Vogtmann E, Goedert JJ. Epidemiologic studies of the human microbiome and cancer. Br J Cancer. 2016;114(3):237–42. https://doi.org/10.1038/bjc.2015.465.
17. Bultman SJ. The microbiome and its potential as a cancer preventive intervention. Semin Oncol. 2016. https://doi.org/10.1053/j.seminoncol.2015.09.001.
18. Ambalam P, et al. Probiotics, prebiotics and colorectal cancer prevention. Best Prac Res Clin Gastroenterol. 2016;30(1):119–31. https://doi.org/10.1016/j.bpg.2016.02.009.
19. Zitvogel L, et al. Anticancer effects of the microbiome and its products. Nat Rev Microbiol. 2017;15(8):465–78. https://doi.org/10.1038/nrmicro.2017.44.
20. Routy B, et al. Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. Science. 2018;359(6371):91–7. https://doi.org/10.1126/science.aan3706.
21. Kaimala S, et al. Attenuated bacteria as immunotherapeutic tools for cancer treatment. Front Oncol. 2018;8:136. https://doi.org/10.3389/fonc.2018.00136.

22. Malik SS, et al. Anticarcinogenecity of microbiota and probiotics in breast cancer. Int J Food Prop. 2018;21(1):655–66. https://doi.org/10.1080/10942912.2018.1448994.
23. Elinav E, et al. The cancer microbiome. Nat Rev Cancer. 2019;19(7):371–6. https://doi.org/10.1038/s41568-019-0155-3.
24. Cann SH, Van Netten J, Van Netten C. Dr William Coley and tumour regression: a place in history or in the future. Postgrad Med J. 2003;79(938):672–80.
25. Luginbuehl V, et al. Intracellular drug delivery: potential usefulness of engineered Shiga toxin subunit B for targeted cancer therapy. Biotechnol Adv. 2018;36(3):613–23. https://doi.org/10.1016/j.biotechadv.2018.02.005.
26. Hosseinidoust Z, et al. Bioengineered and biohybrid bacteria-based systems for drug delivery. Adv Drug Deliv Rev. 2016;106:27–44. https://doi.org/10.1016/j.addr.2016.09.007.
27. Nallar SC, Xu D-Q, Kalvakolanu DV. Bacteria and genetically modified bacteria as cancer therapeutics: current advances and challenges. Cytokine. 2017;89:160–72. https://doi.org/10.1016/j.cyto.2016.01.002.
28. Kensler TW, et al. Transforming cancer prevention through precision medicine and immune-oncology. Cancer Prev Res. 2016;9(1):2–10. https://doi.org/10.1158/1940-6207.CAPR-15-0406.
29. Yu B, et al. Explicit hypoxia targeting with tumor suppression by creating an "obligate" anaerobic *Salmonella typhimurium* strain. Sci Rep. 2012;2(1):1–10. https://doi.org/10.1038/srep00436.
30. Zhao M, et al. Tumor-targeting bacterial therapy with amino acid auxotrophs of GFP-expressing *Salmonella typhimurium*. Proc Nat Acad Sci. 2005;102(3):755–60. https://doi.org/10.1073/pnas.0408422102.
31. Zheng JH, Min J-J. Targeted cancer therapy using engineered *Salmonella typhimurium*. Chonnam Med J. 2016;52(3):173–84. https://doi.org/10.4068/cmj.2016.52.3.173.
32. Broadway KM, Scharf BE. *Salmonella typhimurium* as an anticancer therapy: recent advances and perspectives. Curr Clin Microbiol Rep. 2019;6:225–39. https://doi.org/10.1007/s40588-019-00132-5.
33. Ahmed SG, et al. Intratumoral injection of schwannoma with attenuated *Salmonella typhimurium* induces antitumor immunity and controls tumor growth. Proc Nat Acad Sci. 2022;119(24):e2202719119. https://doi.org/10.1073/pnas.2202719119.
34. Sorenson BS, et al. Attenuated *Salmonella typhimurium* with IL-2 gene reduces pulmonary metastases in murine osteosarcoma. Clin Orthop Relat Res. 2008;466:1285–91. https://doi.org/10.1007/s11999-008-0243-2.
35. Jellbauer S, et al. CD8 T-cell induction against vascular endothelial growth factor receptor 2 by *Salmonella* for vaccination purposes against a murine melanoma. PLoS ONE. 2012;7(4):e34214. https://doi.org/10.1371/journal.pone.0034214.
36. Mesa-Pereira B, et al. Improved cytotoxic effects of *Salmonella*-producing cytosine deaminase in tumour cells. Microb Biotechnol. 2015;8(1):169–76. https://doi.org/10.1111/1751-7915.12153.
37. Zheng JH, et al. Two-step enhanced cancer immunotherapy with engineered *Salmonella typhimurium* secreting heterologous flagellin. Sci Transl Med. 2017;9(376):aak9537. https://doi.org/10.1126/scitranslmed.aak9537.
38. Carvalho F, Sousa S, Cabanes D. How *Listeria monocytogenes* organizes its surface for virulence. Front Cell Infect Microbiol. 2014;4:48. https://doi.org/10.3389/fcimb.2014.00048.
39. Yang Z, et al. Engineering bioluminescent bacteria to boost photodynamic therapy and systemic anti-tumor immunity for synergistic cancer treatment. Biomaterials. 2022;281:121332. https://doi.org/10.1016/j.biomaterials.2021.121332.
40. Sewell DA, et al. Recombinant *listeria* vaccines containing PEST sequences are potent immune adjuvants for the tumor-associated antigen human papillomavirus-16 E7. Cancer Res. 2004;64(24):8821–5. https://doi.org/10.1158/0008-5472.CAN-04-1958.
41. Brockstedt DG, et al. Listeria-based cancer vaccines that segregate immunogenicity from toxicity. Proc Nat Aca Sci. 2004;101(38):13832–7. https://doi.org/10.1073/pnas.0406035101.
42. Roberts NJ, et al. Intratumoral injection of *Clostridium novyi*-NT spores induces antitumor responses. Sci Transl Med. 2014;6(249):249ra111. https://doi.org/10.1126/scitranslmed.3008982.
43. Ohta N, et al. Treatments of various otolaryngological cystic diseases by OK-432 1: its indications and limitations. Laryngoscope. 2010;120(11):2193–6. https://doi.org/10.1002/lary.21141.
44. Ohta N, et al. Effects and mechanism of OK-432 therapy in various neck cystic lesions. Acta Oto-laryngol. 2010;130(11):1287–92. https://doi.org/10.3109/00016489.2010.483480.
45. Karabay O, et al. Investigation of the frequency of COVID-19 in patients treated with intravesical BCG. Rev Assoc Med Bras. 2020;66:91–5. https://doi.org/10.1590/1806-9282.66.S2.91.
46. Glomski IJ, Decatur AL, Portnoy DA. *Listeria monocytogenes* mutants that fail to compartmentalize listerolysin O activity are cytotoxic, avirulent, and unable to evade host extracellular defenses. Infect Immun. 2003;71(12):6754–65. https://doi.org/10.1128/IAI.71.12.6754-6765.2003.
47. Wang L, et al. *Bifidobacterium breve* as a delivery vector of IL-24 gene therapy for head and neck squamous cell carcinoma in vivo. Gene Ther. 2017;24(11):699–705. https://doi.org/10.1038/gt.2017.74.
48. Asoudeh-Fard A, et al. *Lactobacillus plantarum* induces apoptosis in oral cancer KB cells through upregulation of PTEN and downregulation of MAPK signalling pathways. Bioimpacts. 2017;7(3):193. https://doi.org/10.15171/bi.2017.22.
49. Shimizu Y, et al. Anti-tumor effect of a recombinant *Bifidobacterium* strain secreting a claudin-targeting molecule in a mouse breast cancer model. Eur J Pharmacol. 2020;887:173596. https://doi.org/10.1016/j.ejphar.2020.173596.
50. Cai J, Yan G. The identification and development of a novel oncolytic virus: alphavirus M1. Hum Gene Ther. 2021;32(3–4):138–49. https://doi.org/10.1089/hum.2020.271.
51. Sato-Dahlman M, LaRocca CJ, Yanagiba C, Yamamoto M. Adenovirus and immunotherapy: advancing cancer treatment by combination. Cancers (Basel). 2020;12(5):1295. https://doi.org/10.3390/cancers12051295.
52. Haviv YS, et al. Adenoviral gene therapy for renal cancer requires retargeting to alternative cellular receptors. Cancer Res. 2002;62(15):4273–81.
53. Davison E, et al. Adenovirus type 5 uptake by lung adenocarcinoma cells in culture correlates with Ad5 fibre binding is mediated by αvβ1 integrin and can be modulated by changes in β1 integrin function. J Gene Med. 2001;3(6):550–9. https://doi.org/10.1002/jgm.223.
54. MacKie RM, Stewart B, Brown SM. Intralesional injection of herpes simplex virus 1716 in metastatic melanoma. Lancet. 2001;357(9255):525–6. https://doi.org/10.1016/S0140-6736(00)04048-4.
55. Mineta T, et al. Attenuated multi-mutated herpes simplex virus-1 for the treatment of malignant gliomas. Nat Med. 1995;1(9):938–43. https://doi.org/10.1038/nm0995-938.
56. Fong Y, et al. A herpes oncolytic virus can be delivered via the vasculature to produce biologic changes in human colorectal

cancer. Mol Ther. 2009;17(2):389–94. https://doi.org/10.1038/mt.2008.240.
57. Nair S, et al. Zika virus oncolytic activity requires CD8+ T cells and is boosted by immune checkpoint blockade. JCI Insight. 2021. https://doi.org/10.1172/jci.insight.144619.
58. Engeland CE, Ungerechts G. Measles virus as an oncolytic immunotherapy. Cancers. 2021;13(3):544. https://doi.org/10.3390/cancers13030544.
59. Mader EK, et al. Mesenchymal stem cell carriers protect oncolytic measles viruses from antibody neutralization in an orthotopic ovarian cancer therapy modelvirotherapy in immune mice using MSC cell carriers. Clin Cancer Res. 2009;15(23):7246–55. https://doi.org/10.1158/1078-0432.CCR-09-1292.
60. Raihan J, et al. Regression of solid breast tumours in mice by Newcastle disease virus is associated with production of apoptosis related-cytokines. BMC Cancer. 2019;19(1):1–13. https://doi.org/10.1186/s12885-019-5516-5.
61. Ye T, et al. Oncolytic Newcastle disease virus induces autophagy-dependent immunogenic cell death in lung cancer cells. Am J Cancer Res. 2018;8(8):1514.
62. Chen L, et al. Oncolytic activity of wild-type Newcastle disease virus HK84 against hepatocellular carcinoma associated with activation of type I interferon signaling. J Clin Transl Hepatol. 2022;10(2):284. https://doi.org/10.14218/JCTH.2021.00284.
63. Tian L, et al. Oncolytic Newcastle disease virus expressing the co-stimulator OX40L as immunopotentiator for colorectal cancer therapy. Gene Ther. 2023;30(1–2):64–74. https://doi.org/10.1038/s41434-021-00256-8.
64. Ali SM, et al. In vivo oncolytic activity of non-virulent newcastle disease virus Iraqi strain against mouse mammary adenocarcinoma. In AIP Conference proceedings. 2021. https://doi.org/10.1063/5.0067194
65. Al-Ziaydi AG, et al. Newcastle disease virus suppress glycolysis pathway and induce breast cancer cells death. Virusdisease. 2020;31:341–8. https://doi.org/10.1007/s13337-020-00612-z.
66. Sei S, et al. Synergistic antitumor activity of oncolytic reovirus and chemotherapeutic agents in non-small cell lung cancer cells. Mol Cancer. 2009;8:1–15. https://doi.org/10.1186/1476-4598-8-47.
67. Hsu C-Y, et al. Oncolytic avian reovirus σA-modulated upregulation of the HIF-1α/C-myc/glut1 pathway to produce more energy in different cancer cell lines benefiting virus replication. Viruses. 2023;15(2):523. https://doi.org/10.3390/v15020523.
68. Guo ZS, et al. The enhanced tumor selectivity of an oncolytic vaccinia lacking the host range and antiapoptosis genes SPI-1 and SPI-2. Cancer Res. 2005;65(21):9991–8. https://doi.org/10.1158/0008-5472.CAN-05-1630.
69. Gupta KH, Nowicki C, Giurini EF, Marzo AL, Zloza A. Bacterial-based cancer therapy (BBCT): recent advances, current challenges, and future prospects for cancer immunotherapy. Vaccines 2021;9(12):1497
70. Wei MQ, et al. Facultative or obligate anaerobic bacteria have the potential for multimodality therapy of solid tumours. Eur J cancer. 2007;43(3):490–6. https://doi.org/10.1016/j.ejca.2006.10.005.
71. Martinez-Outschoorn UE, et al. Cancer metabolism: a therapeutic perspective. Nat Rev Clin Oncol. 2017;14(1):11–31. https://doi.org/10.1038/nrclinonc.2016.60.
72. Forbes NS. Engineering the perfect (bacterial) cancer therapy. Nat Rev Cancer. 2010;10(11):785–94. https://doi.org/10.1038/nrc2934.
73. Sedighi M, et al. Therapeutic bacteria to combat cancer; current advances, challenges, and opportunities. Cancer Med. 2019;8(6):3167–81. https://doi.org/10.1002/cam4.2148.
74. Jain RK, Forbes NS. Can engineered bacteria help control cancer? Proc Nat Acad Sci. 2001;98(26):14748–50. https://doi.org/10.1073/pnas.261606598.
75. Carlisle R, Coussios C-C. Mechanical approaches to oncological drug delivery. Ther Deliv. 2013;4(10):1213–5. https://doi.org/10.4155/tde.13.94.
76. Brown JM, Giaccia AJ. The unique physiology of solid tumors: opportunities (and problems) for cancer therapy. Cancer Res. 1998;58(7):1408–16.
77. Ryan R, et al. Bacterial delivery of a novel cytolysin to hypoxic areas of solid tumors. Gene Ther. 2009;16(3):329–39. https://doi.org/10.1038/gt.2008.188.
78. Cheong I, Zhou S. Tumor-specific liposomal drug release mediated by liposomase. Meth Enzymol. 2009;465:251–65. https://doi.org/10.1016/S0076-6879(09)65013-8.
79. Chakrabarty A. Microorganisms and cancer: quest for a therapy. J Bacteriol. 2003;185(9):2683–6. https://doi.org/10.1128/JB.185.9.2683-2686.2003.
80. Song S, Vuai MS, Zhong M. The role of bacteria in cancer therapy–enemies in the past, but allies at present. Infect Agents Cancer. 2018;13(1):1–7. https://doi.org/10.1186/s13027-018-0180-y.
81. Park SJ, et al. New paradigm for tumor theranostic methodology using bacteria-based microrobot. Sci Rep. 2013;3(1):1–8. https://doi.org/10.1038/srep03394.
82. Han J-W, et al. Active tumor-therapeutic liposomal bacteriobot combining a drug (paclitaxel)-encapsulated liposome with targeting bacteria (*Salmonella typhimurium*). Sens Actuators B Chem. 2016;224:217–24. https://doi.org/10.1016/j.snb.2015.09.034.
83. Park D, et al. Motility analysis of bacteria-based microrobot (bacteriobot) using chemical gradient microchamber. Biotechnol Bioeng. 2014;111(1):134–43. https://doi.org/10.1002/bit.25007.
84. Cross AS. What is a virulence factor? J Crit Care. 2008;12(6):1–2. https://doi.org/10.1186/cc7127.
85. Casadevall A, Pirofski L-A. Virulence factors and their mechanisms of action: the view from a damage–response framework. J Water Health. 2009;7(S1):S2–18. https://doi.org/10.2166/wh.2009.036.
86. Lee C, et al. *Salmonella* induce autophagy in melanoma by the downregulation of AKT/mTOR pathway. Gene Ther. 2014;21(3):309–16. https://doi.org/10.1038/gt.2013.86.
87. Frahm M, et al. Efficiency of conditionally attenuated *Salmonella enterica serovar typhimurium* in bacterium-mediated tumor therapy. MBio. 2015;6(2):e00254-e315. https://doi.org/10.1128/mbio.00254-15.
88. Na HS, et al. Immune response induced by *Salmonella typhimurium* defective in ppGpp synthesis. Vaccine. 2006;24(12):2027–34. https://doi.org/10.1016/j.vaccine.2005.11.031. (**Epub 2005 Dec 1**).
89. Glomski IJ, et al. The Listeria monocytogenes hemolysin has an acidic pH optimum to compartmentalize activity and prevent damage to infected host cells. J Cell Biol. 2002;156(6):1029–38. https://doi.org/10.1083/jcb.200201081.
90. Decatur AL, Portnoy DA. A PEST-like sequence in listeriolysin O essential for *Listeria monocytogenes* pathogenicity. Science. 2000;290(5493):992–5. https://doi.org/10.1126/science.290.5493.992.
91. Camilli A, Tilney LG, Portnoy DA. Dual roles of plcA in *Listeria monocytogenes* pathogenesis. Mol Microbiol. 1993;8(1):143–57. https://doi.org/10.1111/j.1365-2958.1993.tb01211.x.
92. Bakardjiev AI, et al. Listeriosis in the pregnant guinea pig: a model of vertical transmission. Infect Immun. 2004;72(1):489–97. https://doi.org/10.1128/IAI.72.1.489-497.2004.
93. Cheong I, et al. A bacterial protein enhances the release and efficacy of liposomal cancer drugs. Science. 2006;314(5803):1308–11. https://doi.org/10.1126/science.1130651.

94. Chagnon A, et al. Cytotoxicity and reduction of animal cell growth by clostridium M-55 spores and their extracts. Cancer. 1972;29(2):431–4. https://doi.org/10.1002/1097-0142(197202)29:2%3C431::AID-CNCR2820290226%3E3.0.CO;2-Z.
95. Felgner S, et al. Bacteria in cancer therapy: renaissance of an old concept. Int J microbiol. 2016. https://doi.org/10.1155/2016/8451728.
96. Fronzes R, Christie PJ, Waksman G. The structural biology of type IV secretion systems. Nat Rev Microbiol. 2009;7(10):703–14. https://doi.org/10.1038/nrmicro2218.
97. Farley MM, et al. Minicells, back in fashion. J Bacteriol. 2016;198(8):1186–95. https://doi.org/10.1128/JB.00901-15.
98. Fensterle J, Bergmann B, Yone C, et al. Cancer immunotherapy based on recombinant *Salmonella enterica serovar typhimurium* aroA strains secreting prostate-specific antigen and cholera toxin subunit B. Cancer Gene Ther. 2008;15:85–93. https://doi.org/10.1038/sj.cgt.7701109.
99. Roider E, Jellbauer S, Köhn B, et al. Invasion and destruction of a murine fibrosarcoma by Salmonella-induced effector CD8 T cells as a therapeutic intervention against cancer. Cancer Immunol Immunother. 2011;60:371–80. https://doi.org/10.1007/s00262-010-0950-x.
100. Epaulard O, et al. Anti-tumor immunotherapy via antigen delivery from a live attenuated genetically engineered *Pseudomonas aeruginosa* type III secretion system-based vector. Mol Ther. 2006;14(5):656–61. https://doi.org/10.1016/j.ymthe.2006.06.011.
101. Kim S-J, Chang W, Oh M-K. *Escherichia coli* minicells with targeted enzymes as bioreactors for producing toxic compounds. Metab Eng. 2022;73:214–24. https://doi.org/10.1016/j.ymben.2022.08.006.
102. Paton AW, Morona R, Paton JC. Bioengineered microbes in disease therapy. Trends Mol Med. 2012;18(7):417–25. https://doi.org/10.1016/j.molmed.2012.05.006.
103. Grillot-Courvalin C, Goussard S, Courvalin P. Wild-type intracellular bacteria deliver DNA into mammalian cells. Cell Microbiol. 2002;4(3):177–86. https://doi.org/10.1046/j.1462-5822.2002.00184.x.
104. Felgner S, et al. aroA-deficient *Salmonella enterica serovar typhimurium* is more than a metabolically attenuated mutant. MBio. 2016;7(5):e01220-e1316. https://doi.org/10.1128/mbio.01220-16.
105. Ginn SL, et al. Gene therapy clinical trials worldwide to 2017: An update. J Gene Med. 2018;20(5):e3015. https://doi.org/10.1002/jgm.3015.
106. Zhang S, et al. Role of nontoxigenic *Clostridium novyi* in solid tumor therapy. Rev Med Microbiol. 2014;25(3):71–6. https://doi.org/10.1097/MRM.0000000000000005.
107. Zhou S, et al. Tumour-targeting bacteria engineered to fight cancer. Nat Rev Cancer. 2018;18(12):727–43. https://doi.org/10.1038/s41568-018-0070-z.
108. Liu X, et al. Radiotherapy combined with an engineered of *Salmonella typhimurium* inhibits tumor growth in a mouse model of colon cancer. Exp Anim. 2016;65:413–8. https://doi.org/10.1538/expanim.16-0033.
109. Felgner S, et al. Tumour-targeting bacteria-based cancer therapies for increased specificity and improved outcome. Microb Biotechnol. 2017;10(5):1074–8. https://doi.org/10.1111/1751-7915.12787.
110. Theys J, et al. Specific targeting of cytosine deaminase to solid tumors by engineered *Clostridium acetobutylicum*. Cancer Gene Ther. 2001;8(4):294–7. https://doi.org/10.1038/sj.cgt.7700303.
111. Jahanban-Esfahlan R, et al. Modulating tumor hypoxia by nanomedicine for effective cancer therapy. J Cell Physiol. 2018;233(3):2019–31. https://doi.org/10.1002/jcp.25859.
112. Flentie K, et al. A bioluminescent transposon reporter-trap identifies tumor-specific microenvironment-induced promoters in salmonella for conditional bacterial-based tumor therapycancer cell-induced transcriptional response of *Salmonella*. Cancer Discov. 2012;2(7):624–37. https://doi.org/10.1158/2159-8290.CD-11-0201.
113. Avner BS, Fialho AM, Chakrabarty AM. Overcoming drug resistance in multi-drug resistant cancers and microorganisms: a conceptual framework. Bioengineered. 2012;3(5):262–70. https://doi.org/10.4161/bioe.21130.
114. Naffouje S, Goto M, Ryoo I, Green A, Das Gupta TK, Yamada T. A Method of tumor in vivo imaging with a new peptide-based fluorescent probe. Methods Mol Biol. 2022;2394:857–65. https://doi.org/10.1007/978-1-0716-1811-0_45.
115. Warso M, et al. A first-in-class, first-in-human, phase I trial of p28, a non-HDM2-mediated peptide inhibitor of p53 ubiquitination in patients with advanced solid tumours. Br J Cancer. 2013;108(5):1061–70. https://doi.org/10.1038/bjc.2013.74.
116. Fialho AM, Chakrabarty AM. Patent controversies and court cases: cancer diagnosis, therapy and prevention. Cancer Biol Ther. 2012;13(13):1229–34. https://doi.org/10.4161/cbt.21958.
117. Lulla RR, et al. Phase I trial of p28 (NSC745104), a non-HDM2-mediated peptide inhibitor of p53 ubiquitination in pediatric patients with recurrent or progressive central nervous system tumors: a pediatric brain tumor consortium study. Neuro Oncol. 2016;18(9):1319–25. https://doi.org/10.1093/neuonc/now047.
118. de Gruijl TD, Janssen AB, van Beusechem VW. Arming oncolytic viruses to leverage antitumor immunity. Expert Opin Biol Ther. 2015;15(7):959–71. https://doi.org/10.1517/14712598.2015.1044433.
119. Lawler SE, Speranza MC, Cho CF, Chiocca EA. Oncolytic viruses in cancer treatment: a review. JAMA Oncol. 2017;3(6):841–9. https://doi.org/10.1001/jamaoncol.2016.2064.
120. Russell SJ, Peng K-W, Bell JC. Oncolytic virotherapy. Nat Biotechnol. 2012;30(7):658–70. https://doi.org/10.1038/nbt.2287.
121. Desjardins A, Vlahovic G, Friedman HS. Vaccine therapy, oncolytic viruses, and gliomas. Oncology (Williston Park). 2016;30(3):211–8.
122. Vinay DS, et al. Immune evasion in cancer: mechanistic basis and therapeutic strategies. Semin Cancer Biol. 2015. https://doi.org/10.1016/j.semcancer.2015.03.004.
123. Munn DH, Bronte V. Immune suppressive mechanisms in the tumor microenvironment. Current Opin Immunol. 2016;39:1–6. https://doi.org/10.1016/j.coi.2015.10.009.
124. Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. J Clin Oncol. 2015;33(17):1974. https://doi.org/10.1200/JCO.2014.59.4358.
125. Ugel S, et al. Tumor-induced myeloid deviation: when myeloid-derived suppressor cells meet tumor-associated macrophages. J Clin investig. 2015;125(9):3365–76.
126. Rosewell Shaw A, Suzuki M. Oncolytic viruses partner with T-cell therapy for solid tumor treatment. Front Immunol. 2018;9:2103. https://doi.org/10.3389/fimmu.2018.02103.
127. Thorne SH, et al. Rational strain selection and engineering creates a broad-spectrum, systemically effective oncolytic poxvirus, JX-963. J Clin Investig. 2007;117(11):3350–8.
128. Li L, et al. Delivery and biosafety of oncolytic virotherapy. Front Oncol. 2020;10:475. https://doi.org/10.3389/fonc.2020.00475.
129. Kaufman HL, Kohlhapp FJ, Zloza A. Oncolytic viruses: a new class of immunotherapy drugs. Nat Rev Drug Discov. 2015;14(9):642–62. https://doi.org/10.1038/nrd4663.
130. Chiocca EA, Rabkin SD. Oncolytic viruses and their application to cancer immunotherapy. Cancer Immunol Res. 2014;2(4):295–300. https://doi.org/10.1158/2326-6066.CIR-14-0015.
131. Marchini A, et al. Immune conversion of tumor microenvironment by oncolytic viruses: the protoparvovirus H-1PV case

study. Front Immunol. 2019;10:1848. https://doi.org/10.3389/fimmu.2019.01848.
132. Gatti-Mays ME, et al. If we build it they will come: targeting the immune response to breast cancer. NPJ Breast Cancer. 2019;5(1):1–13. https://doi.org/10.1038/s41523-019-0133-7.
133. Shen J, et al. Anti-cancer therapy with TNF α and IFN γ: a comprehensive review. Cell Prolif. 2018;51(4):e12441. https://doi.org/10.1111/cpr.12441.
134. Kim J-H, Lee K-J, Lee S-W. Cancer immunotherapy with T-cell targeting cytokines: IL-2 and IL-7. BMB Rep. 2021;54(1):21. https://doi.org/10.5483/BMBRep.2021.54.1.257.
135. Rajani K, et al. Combination therapy with reovirus and anti-PD-1 blockade controls tumor growth through innate and adaptive immune responses. Mol Ther. 2016;24(1):166–74. https://doi.org/10.1038/mt.2015.156.
136. Zamarin D, et al. Localized oncolytic virotherapy overcomes systemic tumor resistance to immune checkpoint blockade immunotherapy. Sci Transl Med. 2014;6(226):226ra32. https://doi.org/10.1126/scitranslmed.3008095.
137. Khare R, et al. Generation of a Kupffer cell-evading adenovirus for systemic and liver-directed gene transfer. Mol Ther. 2011;19(7):1254–62. https://doi.org/10.1038/mt.2011.71.
138. Lasswitz L, et al. Glycomics and proteomics approaches to investigate early adenovirus–host cell interactions. J Mol Biol. 2018;430(13):1863–82. https://doi.org/10.1016/j.jmb.2018.04.039.
139. Uusi-Kerttula H, et al. Oncolytic adenovirus: strategies and insights for vector design and immuno-oncolytic applications. Viruses. 2015;7(11):6009–42. https://doi.org/10.3390/v7112923.
140. Hendrickx R, et al. Innate immunity to adenovirus. Human Gene Ther. 2014;25(4):265–84. https://doi.org/10.1089/hum.2014.001.
141. Tazawa H, et al. Impact of autophagy in oncolytic adenoviral therapy for cancer. Int J Mol Sci. 2017;18(7):1479. https://doi.org/10.3390/ijms18071479.
142. Rodriguez-Rocha H, et al. Adenoviruses induce autophagy to promote virus replication and oncolysis. Virology. 2011;416(1–2):9–15. https://doi.org/10.1016/j.virol.2011.04.017.
143. Ros C, et al. Protoparvovirus cell entry. Viruses. 2017;9(11):313. https://doi.org/10.3390/v9110313.
144. Marchini A, et al. Oncolytic parvoviruses: from basic virology to clinical applications. Virol J. 2015;12(1):1–16. https://doi.org/10.1186/s12985-014-0223-y.
145. Smith GL, et al. Vaccinia virus immune evasion: mechanisms, virulence and immunogenicity. J Gen Virol. 2013;94(11):2367–92. https://doi.org/10.1099/vir.0.055921-0.
146. Chon HJ, et al. Tumor microenvironment remodeling by intratumoral oncolytic vaccinia virus enhances the efficacy of immune-checkpoint blockade potentiation of immunotherapy by oncolytic vaccinia virus. Clin Cancer Res. 2019;25(5):1612–23. https://doi.org/10.1158/1078-0432.CCR-18-1932.
147. Deng L, et al. An oncolytic vaccinia virus armed with GM-CSF and IL-24 double genes for cancer targeted therapy. OncoTargets Ther. 2020;13:3535. https://doi.org/10.2147/OTT.S249816.
148. Steyer A, et al. High similarity of novel orthoreovirus detected in a child hospitalized with acute gastroenteritis to mammalian orthoreoviruses found in bats in Europe. J clin Microbiol. 2013;51(11):3818–25. https://doi.org/10.1128/JCM.01531-13.
149. Day JM. The diversity of the orthoreoviruses: molecular taxonomy and phylogentic divides. Infect Genet and Evol. 2009;9(4):390–400. https://doi.org/10.1016/j.meegid.2009.01.011.
150. Rosen L, Hovis JF, Mastrota FM, Bell JA, Huebner R. Observations on a newly recognized virus (Abney) of the reovirus family. Am J Epidemiol. 1960;71:258–65. https://doi.org/10.1093/oxfordjournals.aje.a120109.
151. Sabin AB. Reoviruses. A new group of respiratory and enteric viruses formerly classified as ECHO type 10 is described. Science. 1959;130(3386):1387–9. https://doi.org/10.1126/science.130.3386.1387.
152. Hashiro G, Loh PC, Yau JT. The preferential cytotoxicity of reovirus for certain transformed cell lines. Arch Virol. 1977;54(4):307–15. https://doi.org/10.1007/BF01314776.
153. Ma W, He H, Wang H. Oncolytic herpes simplex virus and immunotherapy. BMC immunol. 2018;19(1):1–11. https://doi.org/10.1186/s12865-018-0281-9.
154. Watson G, et al. Sequence and comparative analysis of the genome of HSV-1 strain McKrae. Virology. 2012;433(2):528–37. https://doi.org/10.1016/j.virol.2012.08.043.
155. Watanabe D, Goshima F. Oncolytic virotherapy by HSV. Adv Exp Med Biol. 2018;1045:63–84. https://doi.org/10.1007/978-981-10-7230-7_4.
156. Tanaka R, et al. The efficacy of combination therapy with oncolytic herpes simplex virus HF10 and dacarbazine in a mouse melanoma model. Am J Cancer Res. 2017;7(8):1693.
157. Shafren DR, Au GG, Nguyen T, Newcombe NG, Haley ES, Beagley L, Johansson ES, Hersey P, Barry RD. Systemic therapy of malignant human melanoma tumors by a common cold-producing enterovirus, coxsackievirus a21. Clin Cancer Res. 2004;10(1 Pt 1):53–60. https://doi.org/10.1158/1078-0432.ccr-0690-3.
158. Andtbacka RHI, et al. CALM study: a phase II study of intratumoral coxsackievirus A21 in patients with stage IIIc and stage IV malignant melanoma. Am Soc Clin Oncol. 2013. https://doi.org/10.1200/jco.2013.31.15_suppl.tps3128.
159. De Cicco P, Catani MV, Gasperi V, Sibilano M, Quaglietta M, Savini I. Nutrition and breast cancer: a literature review on prevention, treatment and recurrence. Nutrients. 2019;11(7):1514. https://doi.org/10.3390/nu11071514.
160. Armstrong N, et al. A systematic review of the international prevalence of BRCA mutation in breast cancer. J Clin Epidemiol. 2019;11:543. https://doi.org/10.2147/CLEP.S206949.
161. Yedjou CG, Sims JN, Miele L, Noubissi F, Lowe L, Fonseca DD, et al. Health and racial disparity in breast cancer. In: Breast cancer metastasis and drug resistance: challenges and progress, 2019, pp. 31–49.

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