

Microbes in Oncology: Controllable Strategies for Bacteria Therapy

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Abstract

Bacterial therapy is an emerging method of tumor treatment. By utilizing wild-type bacteria or engineered bacteria to treat solid tumors, bacterial therapy has recently attracted attention due to its high therapeutic specificity. Although many bacterial strains have been tested in animal models or have even advanced to clinical trials, the efficacy of bacterial therapy remains undesirable. The lack of efficient control methods could cause side effects as well as insufficient therapeutic efficiency, both of which are urgent problems for bacterial therapy. Therefore, some studies have constructed bacteria with inducible plasmid or adsorption with responsive nanoparticles, which improved controllability and specificity during bacterial therapy. Herein, we introduce the unique advantages of bacteria in cancer treatment and highlight the issues associated with the application of bacterial therapy, focusing on the incorporation of various methodologies in the advancement of some controllable strategies in bacterial therapy.

Keywords

Bacteria, controllable approaches, inducible promoter, tumor targeting.

Introduction

Although substantial research has been undertaken to develop new therapies against tumors, the ever-increasing number of cancer cases demonstrates an urgent need for novel therapeutic options. The most common modes for the treatment of cancer include surgery, radiotherapy, and chemotherapy. Although the therapeutic efficiency of surgery is obvious, surgery is invasive and is associated with post-operative complications. Although radiotherapy is suitable for the treatment of solid tumors, it is prone to causing treatment tolerance and surrounding tissue damage. Chemotherapy is widely used in tumor treatment, but the off-target effect of chemotherapy drugs can cause serious toxic side effects to normal tissue. In recent years, biological therapy (i.e., gene therapy, immunotherapy, bacterial therapy, etc.) has attracted widespread attention due to its advantage of low toxicity and lasting curative effects (Table 1) [1].

The poorly vascularized microenvironments and high interstitial fluid pressures of solid tumors impede the penetration of traditional therapeutic agents into the centers of the cancerous tissue,

dramatically reducing their anti-tumor effects [2]. However, these same characteristics of tumor microenvironments provide appropriate conditions for some bacteria to colonize, proliferate, or even develop anti-tumor abilities [3]. In this regard, some bacterial strains have been shown to be advantageous as ideal tumor-targeted delivery systems to transport therapeutic payloads into tumors [4].

Therefore, an apt exploitation of bacteria may provide a valuable solution for overcoming the limitations of other conventional therapies. The past decade has seen the rapid development of bacteria in many cancer treatments [5]. Some engineered bacterial strains have been tested in various preclinical setups and clinical trials. Other reviews have already reported the current stage of bacterial therapy [6, 7]. However, certain urgent issues still need to be considered during clinical translation, especially safety concerns and therapeutic efficiency. To avoid injury to normal tissue caused by constant cytotoxin release of engineered bacteria, some novel strategies such as engineered bacteria with inducible plasmids or responsive nanoparticles have been presented, which could be summarized as controllable bacterial therapy. In this mini-review, the history

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Table 1 Comparison of Characteristics of Different Therapeutic Modes

| Therapeutic Modality | Types of Tumors | Advantages | Disadvantages |
|----------------------|---------------------|-----------------------------|--|
| Surgery | Solid tumor | High therapeutic efficiency | Invasive operation, Postoperative complications |
| Radiotherapy | Solid tumor | Local therapy | Therapeutic tolerance, surrounding tissue damage |
| Chemotherapy | All types of tumors | Wide indications | Off-target effect, systemic toxic side effects |
| Biological Therapy | All types of tumors | Curative effect lasting | High cost, dubious curative effect |

of bacterial therapy and the challenges faced will be introduced. Furthermore, the research progress of controllable strategies in bacterial therapy will be reviewed.

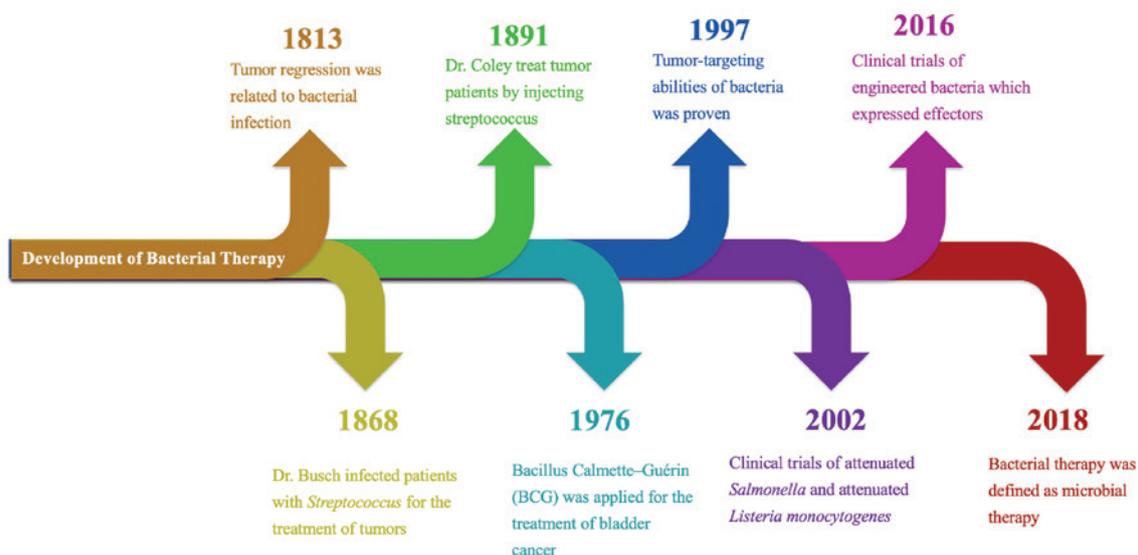
Mechanism and advantages of bacterial therapy

Bacteria have shown great potential in anti-tumor activities since the first documented utilization of therapeutic bacteria to attack cancerous tissue in 1813 (Figure 1). In this case, tumor regression was observed in tumor patients suffering from gas gangrene, which was caused by *Clostridia* sp. [8]. Later, more clinical reports showed that patients with tumors were cured after the administration of live bacteria. In 1868, Wilhelm Busch infected a patient with malignant sarcoma with *Streptococcus pyogenes*, and discovered a significant reduction in the volume of tumor [9]. Subsequently, some scholars reported similar cases. William Coley made significant contributions by applying attenuated bacteria for tumor treatment. He was the first to treat malignant sarcoma by injecting *Streptococcus* into the tumor [10]. At that time, the immune response and high fever caused by bacterial infection were considered to be the main mechanisms for bacterial treatment to achieve anti-tumor effects. With the development of synthetic biology and microbiology, more and more engineered bacterial strains have been developed to improve both the safety and therapeutic efficiency of

bacterial therapy. These bacterial strains have also shown great potential in clinical trials. Fortunately, the mechanism of bacterial therapy has been gradually clarified.

One of the advantages of bacterial therapy is the good specificity due to the tumor targeting of bacteria. Some bacterial strains such as obligate anaerobes have moderately high tumor specificity. Such bacterial strains tend to accumulate and localize in the tumor region which presents a preferable growth environment (low pH, immune-privileged site) for their proliferation [11]. Tumor-targeting bacteria are therefore ideal vehicles to shuttle therapeutic payloads to tumor tissues [12]. Beyond applying their tumor specificity, certain chemical explorations have also been made to conjugate drug-loaded nanomaterials to tumor-targeting bacteria [13].

Another advantage of bacterial therapy is the ability of immunomodulatory activities caused by bacteria [14]. During bacterial therapy, tumor regression occurs in response to multiple therapeutic effects, including adjuvant effects of tumor immune surveillance and direct cytotoxicity of engineered bacteria. As exogenous species, bacteria can intrinsically cause innate immune responses [15]. Inspired by the immunomodulatory activities, some bacterial strains have been engineered to express specific tumor antigens on their surfaces and applied as “cancer vaccines,” activating tumor-specific immune responses [16]. For instance, the CRS-207 vaccine, which was engineered to express mesothelin by deleting the genes of *actA* and *inlB*, demonstrated the capacity to activate the immune response of NK cells and T cells to kill cancer cells [17].

**Figure 1** Timeline of the development of bacterial therapy.

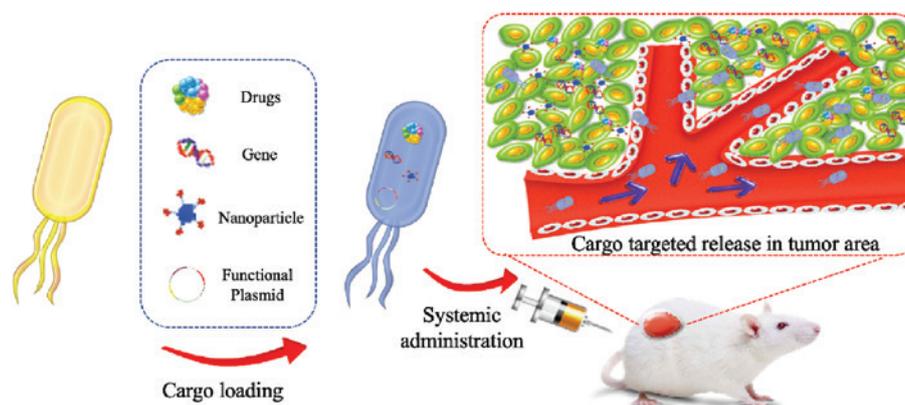


Figure 2 Bacteria as a vector for the delivery of a therapeutic substance. With the characteristics of tumor targeting, bacteria could be applied as a vector for the delivery of drugs, functional nanoparticles, and therapeutics. Also, bacteria which were transformed with functional plasmids carrying gene expression cassettes could express therapeutic proteins.

Since bacteria have the capabilities of tumor targeting and immune activation, the treatment mode of bacterial therapy is diverse. According to existing research, bacteria can be used as special biological carriers to deliver drugs [18], genes [19], and nanoparticles [20, 21] to tumor regions, enhancing the anti-tumor efficiency (Figure 2). Furthermore, some genetically engineered bacteria can even express and release therapeutic molecules in tumor microenvironments to achieve ideal anti-tumor effects [22]. For instance, Schlechte et al. developed engineered *Clostridium* with recombinant plasmid encoding colicin E3, and proved that the recombinant bacteria could express E3 to inhibit protein synthesis of tumor cells [23]. Additionally, Thamm et al. reported that the genetically modified bacteria of *Salmonella typhimurium* VNP20009, which were attenuated by chromosomal deletion of the *purI* and *msbB* genes, resulted in tumor regression in cancerous dog models after weekly or biweekly intravenous administration [24].

Safety concern of bacterial therapy

With the development of biomedical research as well as bacterial behavior and genetic engineering, some attenuated bacteria have been widely applied in clinical applications, such as Bacillus–Calmette–Guérin (BCG). BCG is a classical bacterial vaccine, which is composed of attenuated bovine tuberculosis bacillus [25]. Due to its immunomodulatory capabilities, BCG has been applied in the treatment of bladder cancer. However, it was reported that BCG could cause hematuria and fever during treatment [26].

Safety is a major limitation of bacterial therapy. Further clinical application of utilizing bacteria in cancer treatment has mainly been impeded due to safety concerns. A discontinued phase I trial in 2016 using ADXS11-001 (Axalimogene filolisbac) in human papillomavirus (HPV)-positive oropharyngeal cancer (OPC) once again heightened the importance of safety concerns and received considerable critical attention [27]. The side effects of bacterial therapy

are mainly caused by protein toxins and the payload released by bacteria. To reduce the toxicity of bacteria, some attenuated strains, such as VNP20009 [28], CRS-207 [29], and so on, were developed. To avoid the toxicity of the payload released by engineered bacteria, the process of bacterial therapy, especially the gene expression in engineered bacteria, should be carefully managed.

Controllable bacterial therapy

The most straightforward method to increase anti-tumor activity would be to engineer bacteria-expressing cytotoxic agents [30, 31]. However, a major problem for the application of cytotoxin-expressing bacteria in cancer treatment is their intrinsic toxicity in non-tumoral reticuloendothelial organs, mainly the liver and spleen, due to their initial localization in these organs after systematic administration [32]. The constitutive payload expression would thus inevitably result in hepatic or splenic injury, reducing the therapeutic efficiency of the engineered bacteria. In addition, unlike nanoparticles or other common vectors for drug delivery, bacteria, as live vectors, need to retain their activity and ability to express payloads for the desirable delivery of therapeutic payloads. Some scholars pointed out that constitutive payload expression could cause metabolic burdens, thus decreasing the fitness and therapeutic efficiency of engineered bacteria [33]. With the development of bacterial genetics, the strategy of engineering bacteria with inducible expression system was proposed and it provided a novel idea for controllable bacterial therapy (Table 2).

Chemical controllable strategy

Some efforts have been made to solve the problems by using inducible promoter systems in genetic engineering for better control of bacterial gene expression to decrease toxicity to normal tissues (Figure 2). Chemical-inducible promoter systems are most often used in protein engineering research.

Table 2 Representative Modes of Controllable Bacterial Therapy

| Type | Effector System | Inducible Factor | Characteristics | Refs |
|-----------------------------|---------------------------------------|-------------------|------------------|-------------|
| Chemical controllable | pBAD plasmid | L-arabinose | High efficiency | [31, 35–38] |
| | pET28a plasmid | IPTG | High efficiency | [20] |
| Environment controllable | STM1781 promoter | pH | Safety | [40] |
| | PpepT promoter | Hypoxia | Safety | [43] |
| Physical controllable | recA promoter | Radiation | High specificity | [44] |
| | pBV220 plasmid and gold nanoparticles | Light irradiation | High specificity | [46] |
| Quorum-sensing controllable | Quorum-sensing gene circuit | AHL | Internal trigger | [47–49] |

The expression of the proteins of interest can be controlled by the administration of a chemical compound such as isopropyl β -D-thiogalactoside (IPTG), arabinose, and so on [34]. Based on this principle, some bacterial strains were engineered with a chemical-inducible promoter system to enhance the controllability of the expression of therapeutic molecules in bacterial therapy (Figure 3).

P_{BAD} is a classic inducible plasmid that activates the expression of target genes in response to L-arabinose. In 2007, Stritzker et al. inserted P_{BAD} plasmids encoding luciferase into *Escherichia coli* strain Nissle 1917. The results demonstrated that induced-light emission from the bacterial luciferase within tumor-bearing mice could only be detected after arabinose injection, which provided the fundamental basis for chemical controllable strategy for bacterial therapy [35]. Hereafter, Jung-Joon Min and colleagues performed other research involving the application of P_{BAD} engineered bacteria in tumor treatment [36]. By utilizing the induction system of the L-arabinose-dependent promoter P_{BAD} for gene

activation, the engineered bacteria could express and release therapeutic molecules in the tumor area under the control of arabinose administration and achieve ideal therapeutic efficiency [31, 36–38].

Tumor microenvironment-triggered bacterial therapy

There are significant differences between the tumor microenvironment and the internal environment of the human body in terms of physical and chemical properties, such as hypoxia and low pH [39]. Acidic pH in a tumor microenvironment plays an important role in tumor progression via immune suppression and drug resistance. In another way, it could also be applied as a trigger for controllable bacterial therapy. After screening the *S. typhimurium* library, Fliente et al. found that the STM1787 promoter was sensitive to the acidic microenvironment of tumors. Furthermore, they constructed

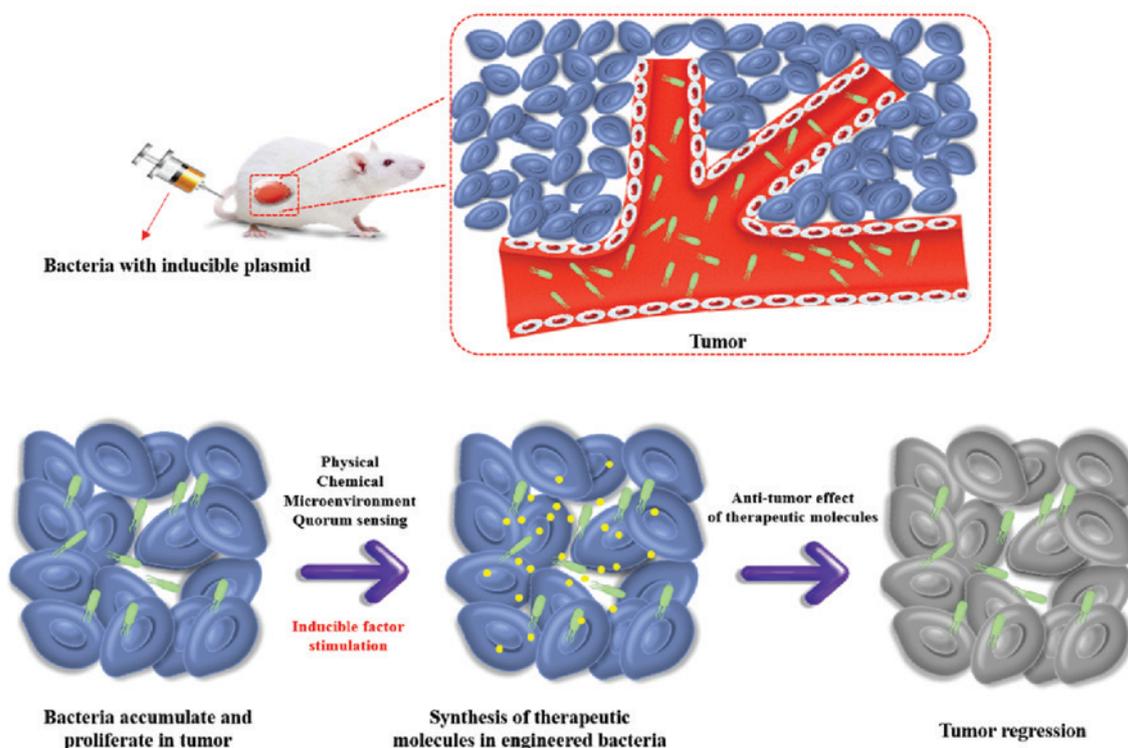


Figure 3 Schematic of controllable bacterial therapy. After the bacteria with the inducible plasmid is administrated systemically, bacteria could accumulate and proliferate in the tumor due to its tumor targeting ability. Then with the stimulation of a physical trigger, a chemical trigger, the conditions of the tumor microenvironment and the quorum sensing, engineered bacteria could synthesis and release the therapeutic molecules *in situ* to realize tumor targeting treatment.

Salmonella which expressed Shiga toxin under the control of the STM1787 promoter. The recombined strains showed dramatic anti-tumor effects *in vitro* and *in vivo*, due to the characteristics of acidic feature in tumor microenvironment [40].

Hypoxia is an acknowledged feature of solid tumors. In this regard, bacteria engineered with a hypoxia-inducible system also has great potential in the treatment of tumors [41, 42]. In one case, by placing a critical gene under a hypoxia-conditioned promoter, *S. typhimurium* strain SL7207 was engineered to survive only in anaerobic conditions (strain YB1) without otherwise affecting its functions. The results revealed that YB1 served as a safe bacterial vector for anti-tumor therapies without compromising other functions or tumor fitness of the bacteria as attenuation methods normally do [43].

Physical controllable strategy

In addition to the strategy mentioned, the physical controllable strategy also shows great potential in controllable bacterial therapy due to its characteristics of good specificity. With the stimulation of physical triggers, anti-tumor agents could be synthesized in tumor areas, which could reduce the toxicity in normal tissues. Therefore, some studies utilized physical stimulation to achieve spatiotemporal control of the gene expression of bacteria for targeted tumor therapy. Nuyts et al. used radiation-induced *recA* promoter to control the expression of *lacZ* (a therapeutic gene). As a result, under the control of *recA* promoter, β -galactosidase activity was significantly increased to improve therapeutic effect after 2 Gy of irradiation [44].

In addition to the method of genetic modification, decoration of responsive nanoparticles also provided a new physical-controllable strategy for bacterial therapy [45]. Fan et al. constructed a thermally biotic/abiotic hybrid system, which was composed of gold nanoparticles and engineered *E. coli* carrying thermally sensitive plasmid pBV220 expressing tumor necrosis factor- α (TNF- α). Based on the tumor targeting of *E. coli*, gold nanoparticles-loaded bacteria could accumulate in tumor tissue after administration orally. Under light irradiation, the photothermal effect produced by gold nanoparticles could activate the expression of TNF- α from engineered *E. coli*, which resulted in ideal local anti-tumor effects. The research implied that the integration of nanomedicine and synthetic biology provide a novel strategy for controllable bacterial therapy [46].

Quorum sensing regulating strategy

Some bacterial strains such as *Salmonella* were proven to express proteins only in tightly packed colonies within tumors, showing a density-dependent characteristic [47]. In 2016, Tal Danino et al. proposed an interesting idea of using the proliferation characteristics of bacteria itself as a trigger for payload release. Exploiting the natural propensity for certain bacteria to colonize tumor sites, they designed a quorum-sensing gene circuit for a transcriptional

program enabling effective bacterial population control and drug release in repeated cycles. When the concentration of acyl-homoserine lactone (AHL) released by bacteria reached a certain threshold, the bacteria would split and promote drug release. After lysis, a few remaining bacteria reproduced and repeated the cycle(s). The results revealed that the circuit-engineered bacteria had notable anti-tumor activity and caused prolonged survival of tumor-bearing mice [48]. Based on the advantages of this system, in 2019, this group engineered *E. coli* to specifically lyse in the tumor area and release the encoded nanobody antagonist of CD47. Compared with conventional systemic treatments, the recombinant strain improved the targeted therapeutic effect of the CD47-antibody, induced tumor regression and systemic anti-tumor immunity. The biological control switch strategy to synchronously lyse at a threshold population density may provide a promising solution to precisely control drug delivery or other therapeutic effects [49].

Prospects of bacterial therapy

With the rapid advancement of cancer research since the 19th century, bacterial therapy has progressed to clinical trials. In 2002, a clinical trial involving bacterial therapy was raised by the National Cancer Institute in USA. In this trial, 24 patients received administration of VNP20009, which is a strain of attenuated *Salmonella*. It was proven that VNP20009 was safe and it could colonize in tumor areas at a high dose of administration [50]. Later, some strains of bacteria engineered with effectors were also applied in clinical trials [51, 52]. Overall, the results of these clinical trials revealed that engineered bacteria were proven to be safely administrated [53, 54]. However, according to these clinical trials, the effects of bacterial therapy alone was not promising.

The pathogenesis of tumors is complex. Monotherapy often fails to achieve the desired therapeutic effect. Therefore, the combination of multiple therapeutic modes is widely applied in clinical practice. Combination therapy can overcome the shortcomings of monotherapy, reduce the therapeutic dose, and improve the sensitivity of tumor treatment. It has been proven that the combination of engineered bacteria and chemotherapy as well as radiotherapy could significantly inhibit the growth of tumors, which is mainly due to the immunity response caused by engineered bacteria in tumor areas, enhancing the sensitivity of traditional cancer treatment and reducing the risk of tumor recurrence [55, 56]. In 2019, a new clinical trial report also confirmed this conclusion. In this study, 35 patients with advanced peritoneal mesothelioma received standard chemotherapy and CRS-207, an attenuated *Listeria monocytogenes* strain expressing mesothelin. As a result, the rate of disease control and therapeutic reactivity reached 89% and 54%, respectively [57]. However, the strategy of bacteria-based combination treatment still requires further optimization in several aspects, including the ratio of engineered bacteria to drugs, the timing of bacterial administration, and so on.

Due to safety concerns, there are still some challenges for bacterial therapy. Since live bacteria could have both therapeutic and toxic effects, it is necessary to maintain the balance of tumor therapeutic outcomes with the risk of possible serious infection caused by bacteria [7]. It may be feasible to monitor the process of bacterial therapy with reporter gene imaging technique. For this purpose, some researchers modified bacteria with various reporter genes, which were monitored by different imaging techniques including optical imaging [58], positron emission tomography [59], and magnetic resonance imaging [60]. In this regard, the imaging signals of bacteria could provide information about their distribution and proliferation, which provides a non-invasive monitoring method for bacterial therapy.

Conclusion

Bacterial therapy shows great potential for application in tumor treatment. However, the relatively low therapeutic efficiency and safety risks halts its further clinical application. One potential solution is to develop controllable strategies for bacterial therapy through engineered bacteria with

inducible plasmid and responsive nanoparticles. Controllable approaches in bacterial therapy present a necessity for achieving better therapeutic outcomes. The approaches mentioned exemplify a methodology for applying synthetic biology to exploit the inherent features of certain bacteria to target, selectively colonize, and eventually exert anti-tumor effects. It is believed that with the continuous development of synthetic biology and molecular cloning technology, more new strains of high efficiency and safety will be developed and applied to clinical practice, which will provide new paths to improve the efficiency of tumor treatment.

Acknowledgments

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