A Review of Novel Methods of the Treatment of Cancer by Bacteria

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Authors’ contributions

This work was produced in collaboration with all authors. Author ZK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MH and SK have edited the text. All authors have read and approved the final version.

ABSTRACT

Nowadays, the attention of nanoparticle-bearing bacteria as drug carrier is increasing with decreasing side effects in the treatment of cancer. Nanoparticles have better release and selectivity with less toxicity. Due to the cost-effectiveness of using bacteria and the conditions for the growth of drug delivery with nanoscale particles that is less in size and less toxic, it has been the goal of drug delivery. Targeted drug delivery with nano carriers is possible to control the location, timing, and speed of drug release. Anaerobic gram-negative bacteria such as Salmonella and E. coli and also beneficial intestinal bacteria, including bacterial Bifidium have been used in the treatment of cancer that are in clinical trials of laboratory studies. In this review article, we are trying to draw up new related patterns that provide effective ways for research and future achievements by reviewing novel bacteria-targeted drug delivery methods.

Keywords: Cancer cell; targeted drug delivery; nanoparticles; carrier; probiotic; drug release.

1. INTRODUCTION

Cancer is a group of diseases that are characterized by the loss of control of the growth, division, and invades other tissues [1]. Surgery, chemotherapy, radiation therapy, immunotherapy, and gene therapy, which is the same as bone marrow transplantation. All these therapies have side effects on other healthy tissues of the body. Also may be Side effects of the drugs can include nausea, hair loss, and fatigue [2]. Although primary tumors can be treated by surgery or chemical treatments, the cataracts that reach the stage of metastasis are resistant
to treatment. One strategy to overcome the problem is the use of nanotechnology which can help to development of medicine delivery systems [3]. In addition, these methods have no adequate specificity on cancerous cells and in addition they have adverse effects on normal cells. According to the new knowledge on the molecular pathogenesis of cancer, other therapeutic strategies such as nanobodies, monoclonal antibodies [4]. Bacterial cancer therapy is a concept more than 100 years old, it is still in early development. While the use of many passive therapeutics is hindered by the complexity of tumor biology, bacteria offer unique features that can overcome these limitations, spore and anaerobic bacteria are use in cancer therapeutic methods [5].

Medicinal drug-borne bacteria Early diagnosis and recovery during cancer, helpful help. Attention is given to making new cancer drugs that can make cellsCancer targets and prevent their growth and reduce the side effects of medications [6]. Microbial metabolism, motility and sensitivity immunogenicity, and preferential accumulation within the anoxic tumour environment [7]. Advances in biotechnology and molecular techniques have made it easier than ever to engineer bacteria as both therapeutic agents themselves and as therapeutic vectors. lead to site-specific treatment, highly focused on the tumor and safe to other tissues [8]. This article aims to present a current applications and novel progresses of using apoptin as tumor-specific killer agent and will provide an insight into the possible mechanism of action. Finally we will discuss a new direction for the therapeutic uses of viruses and viral proteins for the treatment of different kinds of cancers.

2. STRATEGY IN CANCER BY BACTERIAL THERAPIES

The American physician William Coley injected live bacteria into cancer tumours. Coley Vaccine (Mixed Bacterial Vaccine), He developed a vaccine in the late 1800s with the help of two killed bacterial species streptococcus pyogenes and Serratia used to treat patients who were suffering from sarcomas, carcinomas, lymphomas and related diseases [9,10]. Natural anticancer drug by bacteria Bacterial products like endotoxins (Lipopolysaccharides) have to some extent already been tested for cancer treatment [11]. Anaerobic bacteria are demonstratively well-suited as natural anticancer agents due to their intrinsic cytotoxicity, motility, tumour specificity and capacity for therapeutic gene expression [12].

3. ENVIRONMENTAL SENSING

Quorum sensing (QS) describes bacterial cell-cell communication where gene expression responds to changes in cell density, monitored through autoinducers such as acyl homoserine lactones (AHLs), The LuxI/LuxR regulatory system is one of the most commonly described [13]. A similar system was also constructed in S. typhimurium strain A1, using QS to amplify the activation of arabinose-mediated production of a fluorescent reporter [14].

4. TREATMENT: IMMUNOTHERAPY

Recently, a number of anaerobic bacterial species Salmonella and Clostridia have emerged as agents with high antitumour potential. Nonpathogenic (bifidobacteria, lactobacilli pathogenic, clostridiaand, Escherichia), there by promoting tumour lysis or the stimulation of immune responses against a tumour. Clostridium novyi demonstrated significant anti-tumor effects [15]. Induces enhanced immune response to cancer tissue. Active component – complex reflects the immune response to the pathogen multitude of cytokine casudiverse cellular & humoral immune responses [16].

Immunotherapy involves the administration of helpful bacteria through a catheter into the to trigger the immune system to attack the cancer cells. Bacillus Calmette-Guerin (BCG) is a type of bacteria used in this therapy. New treatments are being investigated for bladder cancer. Targeted therapies are directed at limiting growth of cancer cells [17].

5. ANTIBODY-DRUG CONJUGATES (ADC)

Development of monoclonal antibodies targeting various antigens highly expressed on malignant cells is a novel approach. ADC represents a broad class of effective biopharmaceutical agents designed for targeted therapy including cancer. Antibodies may target malignant cells that overexpress a specific antigen. Currently, a majority of antibody drug conjugates are in different stages of clinical trials and some biologics are still in early stages of development. Robertson et al. studied the application of a recombinant human interleukin 18 (rHL-18), a cytokine with antitumor activity conjugated to Rituximab [18].
In another study, DM1, a cytotoxic agent that binds and destabilizes microtubule activity was studied by Lopus et al. The researchers established the application of antibody-DM1 conjugates in cancer therapy. Such antibody-drug conjugates were formulated as Trastuzumab emtansine (T-DM1) consisting of monoclonal antibody trastuzumab (Herceptin) conjugated to DM1 [19,20].

Trastuzumab emtansine has been recently approved by FDA for breast cancer treatment. T-DM1 targets epidermal growth factor receptor 2 (HER2) overexpressed in aggressive breast cancer. Trastuzumab emtansine destroys cancer cells due to interaction of microtubules and DM1 [21].

6. TREATMENT: PROBIOTICS

There are few studies showing benefit of probiotics; however, there are theoretical reasons why altering the bacteria in the intestine might modulate symptoms. Probiotics are generally safe so there is little harm in trying them. A Japanese study that analyzed the micro-genome using terminal restriction fragment length polymorphism and next-generation sequencing analyses noted that several bacterial genera (Actinomyces, Atopobium, Fusobacterium, and Haemophilus) as well as several individual species (including Bacteroides fragilis and Streptococcus gordonii) were significantly associated with patients with known colorectal carcinoma but not in control patients without the disease [22].

Treating the cells with combined Lactobacillus plantarum and 5-FU led to caspase-induced apoptosis and demonstrated further potential anticancer effects by inactivating the cells’ Wnt/β-catenin signaling pathway [23].

In addition, combinations of Bifidobacterium infantis with Lactobacillus acidophilus were also proven to be effective in reducing the incidence of necrotizing enterocolitis (NEC) and NEC-associated mortality in critically ill neonates [24].

7. ACTIVE DELIVERY

Bacteriacean can be exploited as delivery agents for anticancer drugs, and as vectors for gene therapy. Spores of anaerobic bacteria can be used for the aforementioned strategies because only spores that reach an oxygen starved area of a tumour will germinate, multiply and become active [25].

Here are lots of applications of minicells reported as delivery for therapeutics, nucleic acids, and other bioactive compounds to target cells. MacDiarmid introduced minicells generated by Shigella flexneri, Pseudomonas aeruginosa, Salmonella typhimurium, Escherichia coli, and Listeria monocytogenes with diameter about 400 nm [26]. In addition, Lactobacillus rhamnosus and Lactobacillus acidophilus could generate minicells with nanosize as well [27,28].

Vogel, et al. monoclonal antibodies have the ability to bind to specific tumor antigens. For example, the trastuzumab antibodies have been used in the treatment of breast cancer and it attaches to the Her2 receptor and exhibits high expression (about 27-37%) in breast cancer patients [29].

Bacterial spores have also been exploited as delivery agents for anticancer agents, cytotoxic peptides, therapeutic proteins, and as vectors for gene therapy. A summary of relevant clinical trials using bacteria is shown in Table 1.

8. NANOPARTICLE PLATFORMS FOR ANTICANCER DRUG DELIVERY

Nanoparticle drug delivery platforms have been in center of focus of researchers. Many solid tumors such as breast, lung, prostate, and colon cancers have unique structural features including the hyper permeable vasculature and impaired lymphatic drainage, hence, tumor tissues are quite permeable to macromolecules and nanocarriers [34,35]. The use of metal nanoparticles, such as gold and silver, is used to target the drug. Conjugation of the carrier with targeting compounds (targeting ligands), such as antibodies, can be achieved [36].

The use of nanoparticles as carriers increases penetration, decreases the power of liberation, reduces the toxicity and reduces the side effects of drugs on healthy people [37].

Nanoparticulate systems for drug delivery can be produced by two different methods. The first method of nanosuspension production involves the breaking down of bigger particles to nanosize using highpressure homogenization of drug suspensions in the presence of surfactants [38]. The second method involves crystallization that build the nanoparticles up from the supersaturated solution state, or solid nanoparticles [39].
9. LIPOSOMES

The use of lipid nano-carriers, including liposomes, has been considered due to low toxicity and increased drug delivery. Direct introduction of therapeutic DNA into target cells. Creation of an artificial lipid sphere with an aqueous core liposome [43]. Gupta et al. Evaluated the efficacy of *Escherichia coli* liposome for the transfer of cancer cells molecules as drug carriers [44].

Krishnan et al. Exitated liposomes from *Archei bacteria, Methanobrevibacter smithii* Strong adjuvants on CD8 stimulate and respond. They showed that they can be used to make an anticancer vaccine [45].

Drug-loaded microbubbles are attractive and ultrasound-responsive drug carriers may be very beneficial for drug targeting to intravascular targets [46].

Targeted and ultrasound-triggered liposomes co-modified with single stranded DNA aptamers can target platelet-derived growth factor receptors (PDGFRs) expressed in breast cancer cells. Poly(NIPMAM-co-NIPAM) as the thermos sensitive polymer can sensitize these liposomes at high temperature [47].

10. BACTERIA AS VECTORS FOR GENE THERAPY OF CANCER

Various bacterial species including *Salmonella spp.*, *L. monocytogenes* and *E. coli* have been examined as bactofection vectors. Bactofection of mammalian cells applies to both active invasion of non-phagocytic mammalian cells (e.g., tumor cells), and also ‘passive’ uptake by phagocytic immune cells [48].

*Clostridia* are Grampositive, spore-forming, obligate anaerobes and many strains are pathogenic. It has previously been established for many species of Clostridia that when injected in spore form, they will only germinate once in an appropriate anaerobic environment in more recent years, tumor-specific, including *Bifidobacterium, Salmonella, Escherichia coli, Vibrio cholerae* and *Listeria monocytogenes* [49].

Lechardeur et al. Connected to the surface of the cells expressing the diphereria poison, it enters the HB-EGF2-mediated endothelium-induced endothelium and undergoes several catalytic changes after translation, and ultimately inhibits protein synthesis, causes cell lysis and induces apoptosis [50].

Another sample of Clostridium perfringens endotoxin, this toxin has been investigated ongastric, chest and clone cancers. However, the efficacy of treatment for a long time and lack of toxicity in the living creature is further investigated [51]. The biological nature of bacterial vectors means that many of the
inherently beneficial traits of viral vectors are retained. Bacterial presence in excised patient tumors was first reported in the 1950s while the correlation between bacterial infection and tumor regression dates back to the nineteenth century when regression of certain tumors were noted in patients with streptococcal and clostridial infections[52].

Nowadays, many of these therapies are being used in clinical settings, including the checkpoint inhibitors monoclonal antibodies anti cytotoxic T-Lymphocyte associated protein 4 (CTL-4) and programmed death protein 1 (PD1). They have been shown to increase survival in patients with metastatic melanoma [53]. But their mechanism of action decreases immunotolerance with systemic administration. The latter may cause autoimmune adverse effects, limiting its use only for specific patients [54]. In the last few decades, experimental studies and clinical trials have been aimed to assess bacteria therapeutic functions [55,56]. Bacteria selective replication within the tumor microenvironment gives them antitumor effect and minimizes systemic adverse effects. On the other hand, expression of multiple ligands, immunostimulants, cytokines and tumor antigens can be achieved through gene manipulation to increase the therapeutic effect against specific tumors [57].

11. NATURAL ANTICANCER DRUG BY BACTRIA

In 1941, one of the first candidates for antineoplastic antibiotics, actinomycin, was isolated from the culture of Streptomyces antibioticus; however, due to its lack of specificity and violent toxic effects, its use in humans had many limitations. Later, it was possible to isolate actinomycin C from cultures of Streptomyces chrysomallus, followed by actinomycin D with more significant activities in neoplasms although its use would continue to be limited due to the severe toxicity [58]. Among these classes, the most relevant are anthracyclines, which were isolated from Streptomyces peucetius in the early 1960s and referred to as doxorubicin and daunorubicin. Only two more anthracyclines reached clinical approval; idarubicin and valrubicin [59].

12. NATURAL ANTICANCER DRUG BY STREPTOMYCN

Mithramycin (MTM), a natural product of soil bacteria from the Streptomyces genus, displays potent anticancer activity but has been limited clinically by severe side effects and toxicities. Engineering of the MTM biosynthetic pathway has produced the 3-side-chainmodified analogs MTM SK (SK) and MTM SDK (SDK), which have exhibited increased anticancer activity and improved therapeutic index [60].

13. NATURAL ANTICANCER DRUG BY Serratia marcescens

Prodigiosin pigmentation was first detected in 1902 in Serratia marcescens, and its chemical structure was identified by Reapoport and Holden [61]. Prodigiosin (C_{20}H_{25}N_{3}O) is one of the components of the family of statin drugs that has antibacterial, antifungal, antimalarial, antibiotic, immunosuppressive, and anti-cancer effects in addition to lowering blood cholesterol [62].

Natural pigments are synthesized by living organisms, such as plants, animals, fungi and bacteria [63] [Table 2].

<table>
<thead>
<tr>
<th>Strains</th>
<th>Anti-tumor drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast</td>
<td>Beta-glucan</td>
</tr>
<tr>
<td>Lechevalieria</td>
<td>Becatecarin</td>
</tr>
<tr>
<td>Micromonospora sp.</td>
<td>Eco-4601</td>
</tr>
<tr>
<td>Salinispora tropica</td>
<td>NPI-0052</td>
</tr>
<tr>
<td>Chromobacterium violaceum</td>
<td>Romidepsin</td>
</tr>
<tr>
<td>Taxus cuspidata</td>
<td>Taxol</td>
</tr>
<tr>
<td>Streptomyces alanosinus</td>
<td>Spicamycine</td>
</tr>
<tr>
<td>Streptomyces peucitius</td>
<td>Amrubicin hydrochloride</td>
</tr>
<tr>
<td>Streptomyces sp.MDG-04-17-069</td>
<td>Tartrolon D</td>
</tr>
<tr>
<td>Streptomyces chartreusis</td>
<td>Elsamirucini</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>Prodigiosin</td>
</tr>
</tbody>
</table>

However, microorganisms versatility remains a feature that may show encouraging results in the future [Table 3] with significant improvements in cancer diagnosis and treatment.

14. IMMUNOSTIMULATION IN TUMOR MICROENVIRONMENT

There is no bacterium capable of completely inhibiting tumor growth just through colonization.[65] However, it represents an important prospect for cancer treatment as an immunostimulator or as a vector for therapeutic components that can be released inside a tumor [66,67] Table 4.
### Table 3. Current clinical trials to evaluate bacteria use in cancer treatment

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Indication</th>
<th>Clinical phase</th>
<th>NCT identification</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium novyi-NT</em></td>
<td>Patients with malignant solid tumors refractory to treatment</td>
<td>Phase I</td>
<td>NCT01924689</td>
</tr>
<tr>
<td><em>Lm-LLO-E7</em></td>
<td>Patients with grade 2 cervical intraepithelial neoplasia</td>
<td>Phase II</td>
<td>NCT01116245</td>
</tr>
<tr>
<td></td>
<td>Patients with non-small cell lung carcinoma, HPV positive</td>
<td>Phase II</td>
<td>NCT02531854</td>
</tr>
<tr>
<td></td>
<td>Patients with anorectal carcinoma</td>
<td>Phase II</td>
<td>NCT02399813</td>
</tr>
<tr>
<td></td>
<td>Patients with HPV positive oropharyngeal cancer</td>
<td>Phase II</td>
<td>NCT02002182</td>
</tr>
<tr>
<td></td>
<td>Patients with high risk of locally advanced cervical cancer</td>
<td>Phase III</td>
<td>NCT02853604</td>
</tr>
<tr>
<td><em>CRS-207</em></td>
<td>Adults with previously treated pancreatic adenocarcinoma</td>
<td>Phase II</td>
<td>NCT02004262</td>
</tr>
<tr>
<td><em>ADU-623</em></td>
<td>Patients with malignant pleurethelial melanoma</td>
<td>Phase I</td>
<td>NCT01675765</td>
</tr>
<tr>
<td><em>ADXS31-142</em></td>
<td>Patients with ovarian or peritoneal cancer</td>
<td>Phase I/II</td>
<td>NCT02575807</td>
</tr>
</tbody>
</table>

**CRS**: human papilloma virus

### Table 4. Pre-clinical studies for evaluation of molecular antitumor effects made by genetic engineering bacteria [64]

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Molecule</th>
<th>Most relevant results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VNP20009</td>
<td>CCL21</td>
<td>Increased intratumoral production INFg, CXCL9 and CXCL1</td>
<td>Loeffler et al.[68]</td>
</tr>
<tr>
<td>VNP20009</td>
<td>LIGHT (TNFSF14)</td>
<td>Prominent reduction in tumor growth was observed. Evidenced with an inflammatory infiltrate (B lymphocytes, CD4+, CD8+ in models treated with this bacterium</td>
<td>Loeffler et al.[69]</td>
</tr>
<tr>
<td>VNP20009</td>
<td>IL-18</td>
<td>Inhibition of tumor growth was observed. Evidenced with a leukocytic infiltrate (especially NK cells) and increased secretion of INF-g, TNF-a, IL-1b and GM-CSF</td>
<td>Loeffler et al.[70]</td>
</tr>
<tr>
<td>VNP20009</td>
<td>FASL</td>
<td>Significant reduction of tumor size was observed in primary tumors and lung metastases, increasing neutrophil recruitment</td>
<td>Loeffler et al.[71]</td>
</tr>
<tr>
<td>VNP20009</td>
<td>TRAIL</td>
<td>TRAIL expression increased tumor cells apoptosis dependent on caspase 3 and 8</td>
<td>Ganai et al.[72]</td>
</tr>
<tr>
<td><em>S. choleraesuis</em></td>
<td>Endostatine</td>
<td>Inhibition of tumor growth was observed in 40%-70%. Evidenced with a decrease in intratumoral microvasculature, VEGF expression and increase in T CD8+ lymphocyte recruitment</td>
<td>Lee et al.[73]</td>
</tr>
<tr>
<td><strong>S. choleraesuis</strong></td>
<td>Thrombospondin</td>
<td>Selective colonization was observed in a 1000:1 to 10000:1 ratio with respect to liver and spleen. Evidenced with inhibition of tumor growth and increase in survival by angiogenic effects</td>
<td>Lee et al.[74]</td>
</tr>
<tr>
<td><strong>Nula phoP/phoQ LH430</strong></td>
<td>RNAi-STAT3</td>
<td>RNAI inhibited significantly tumor growth, the number of metastatic lesions decreased, increased survival rate in animal models</td>
<td>Zhang et al.[75]</td>
</tr>
<tr>
<td><strong>S. typhi Ty21</strong></td>
<td>VEGFR-2</td>
<td>Vaccination for this molecule showed inhibition of tumor growth, decreased metastasis growth and prevented new spontaneous metastasis, increasing survival rate in models</td>
<td>Niethammer et al.[76]</td>
</tr>
<tr>
<td><strong>aroA SL7207</strong></td>
<td>PSA-CtxB*</td>
<td>This vaccine administration conjugated with Salmonella showed protective effects by reducing tumor size in 8-14 days since its inoculation. This mechanism depends on T CD8+ lymphocyte activity and a prototype of the E. coli Hemolysin secretion system</td>
<td>Fensterle et al.[77]</td>
</tr>
</tbody>
</table>

**Clostridium**

| **C. beijerinckii** | NR | Nitroreductase activity increased *in vitro* antitumor activity of CB in 1954, by a factor of 22 | Lemmon et al.[78] |
| **C. beijerinckii** | Citosine deaminase | Tumor cells sensitivity to 5-fluorocytosine increased by 500 times | Fox et al.[79] |
| **C. sporogenes** | IL-12 | Increased selective secretion of INF-γ with effects on tumor growth, without signs of toxicity | Zhang et al.[75] |
| **C. novyi-NT** | AC anti-HIFa | A heterologous gene transfer was satisfactory in this bacterium. Showing increased antibody secretion (with adhesion capacity and specificity) | Groot et al.[80] |

**Listeria monocytogenes**

| **Lm-LLO-E7** | HPV16-E7* | This therapy induced regression in 75% of tumors expressing E7 antigen. This response depends on TCD4+ and TCD8+ lymphocytes and INFγ secretion | Gunn et al.[81] |
| **ADXS31–164** | HER-2/neu (Human)* | An increase in TCD8/Tregs ratio was observed with this therapy. It also prevented more breast tumor formation and delayed more metastasis growth than other vaccines based on this bacterium | Shahabi et al.[82] |
| **LM-LLO-Mage-b/2nd** | MAGE-b* | The most effective vaccine for breast tumors, decreasing number of metastasis by 96%, correlating to a strong CD8+ lymphocytic response in spleen after restimulation with antigen use | Kim et al.[83] |
| **Lm-LLO-HMW-MAA-C** | HMW-MAA | This therapy immunization prevented tumor growth not only in models that expressed the antigen, but in melanoma, renal carcinoma and breast carcinoma. TCD4+ and TCD8+ lymphocytes were needed to achieve this | Maciag et al.[84] |
15. OTHER BACTERIA UNDER STUDY

Research for bacteria use in cancer treatment is not limited to the cited genres. Lactococcus lactis NK34, generally used as a probiotic, showed significant antitumor activity against lung, colorectal, gastric and breast cancers on in vitro models [85]. These effects appear to be mediated by an increase in tumor expression of p21 and p53 leading to apoptosis [86,87]. Intratumor Streptococcus pyogenes was employed in pancreatic cancer models and complete tumor regression was observed and associated to cytokine release and immune cell infiltration [88]. Recently, Bacillus subtilis and Bifidobacterium infantis are being included in preclinical studies to find more evidence supporting bacteria as lifesaving prospects [89,90].

16. CONCLUSION

The use of a variety of bacteria for treating cancer based on basic knowledge of cancer in Phase 3 clinical trials of cancer patients. Due to the inability of conventional treatments in advanced tumor stages, resistance to treatment and the lack of specificity of these treatments, hope with the advancement of studies in this field, a new dimension to cancer treatment is to be created. This feature can also allow bacteria to cross physiological barriers and accumulate in cellular regions that are either distant and inaccessible for passive therapeutics or quiescent and unresponsive to chemotherapy. For example, motile strains of Salmonella were shown to effectively penetrate tumor tissue in vitro. The main advantage of treating bacteria is its selective colonization in tumor tissue, which reduces its toxicity. This is a direct Oncolytic effect on proliferation and immunostimulation in cancerous tissues. Despite the lack of significant effects in the initial models and multiple side effects, these obstacles have been overcome. Antitumor effects and their long half-lives are critical variables in future research protocols and clinical trials. However, the adaptability of microorganisms remains a feature that may show some encouraging results in the future.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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84.


85.


86.


87.