REVIEW ARTICLE

Therapeutic vaccines based on genetically modified *Salmonella*: a novel strategy in cancer immunotherapy

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KEY WORDS

cancer immunotherapy, *Salmonella typhimurium* VNP20009, vaccine vector

ABSTRACT

In the course of evolution, bacteria from the genus *Salmonella* adapted to survive and multiply in a vertebrate host. Skillful use of bacterial interactions with the host immune system became the basis for the development of modified *Salmonella*-based therapeutic vaccines. Bacterial genome can be modified to reduce toxicity and to develop or enhance therapeutic activity. *Salmonella*-based therapeutic vaccines are an attractive and novel alternative for conventional cancer treatment (chemotherapy, radiotherapy, and passive immunotherapy). Live bacteria have the natural ability to sense external environment and penetrate the target tissue. Appropriate strains of *Salmonella*, infused into experimental animal tumor model, accumulate selectively in solid tumors and inhibit their growth. Moreover, the bacteria can reach tumor areas that are inaccessible for other, passively diffusing therapeutics, e.g., ischemic areas. Thus, bacteria can produce and locally release a natural or recombinant anticancer agent, which enhances their therapeutic effect.

S. typhimurium VNP20009 strain is safe, which has been documented in clinical trials. However, the expected therapeutic benefit has not been observed, presumably due to insufficient tumor colonization by bacteria. To enhance colonization of solid tumors, VNP20009 bacteria have been equipped with the ability to express on the surface an antibody fragment specific for carcinoembryonic antigen present on human tumor cells. Additionally, to potentiate antitumor activity, the genetic material of VNP20009 has been engineered to overproduce an endogenous proapoptotic protein, which targets cancer and immune cells promoting tumor growth.

Rationale behind *Salmonella*-based anticancer therapy

Radio- and chemotherapy of cancer is not fully effective and metastases remain the major cause of death from cancer. Low selectivity for tumor cells markedly reduces the benefit of these therapies. The mechanism of action based on such biological process as cell proliferation cannot provide cytotoxicity limited to tumor tissue, resulting in devastating side effects. Selective cancer immunotherapy should overcome these drawbacks, with the inhibition of metastases being the main target.

The history of tumor immunotherapy began over 130 years ago when physicians started to treat cancer patients with microorganisms. The progress in immunological paradigms on the role of the immune system in cancer progression and development in genetic engineering allowed to rediscover the strategy. Preclinical and clinical studies showed that tumor antigen-targeted therapies alone are not as effective as expected. Limited accessibility of tumor tissue for effector cytotoxic cells, lack of costimulation leading to anergic state and tolerance of T-helper cells, reduced expression of major histocompatibility complex (MHC) molecules on tumor cells and immunosuppresive tumor environment resulted in low effectiveness of immunotherapies based on the stimulation of tumor antigen-specific responses.[1] Numerous strategies have been used to stimulate local immune response and intensify cross-presentation of tumor antigens,

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e.g., cryoablation or administration of in vitro preactivated dendritic cells directly into the tumor.[2,3] As an alternative and supplementation, nonspecific boosting therapies are being developed. An example of such therapy is administration of high doses of cytokines, which, however, causes severe adverse effects.

Bacteria offer unique advantages unavailable to passive therapeutics and superior to other immunotherapeutics. Bacteria are motile, penetrate tumor tissue, and serve as biofactories producing a therapeutic agent. Properly equipped bacteria deliver a local danger signal to the immune system prompting effective antitumor immunity.

Live attenuated *Salmonella typhimurium* (*S. typhimurium*) inhibits tumor growth and promotes survival of tumor-bearing mice. Numerous strains were shown to selectively colonize solid tumors in animal models: the amount of bacteria in tumor exceeds 1000 to 10,000 times the number of bacteria in the spleen and liver (organs naturally occupied by *Salmonella* during systemic infection). As a result, therapeutic action is focused on the tumor and the rest of the body is exposed to moderate side effects. Modern genetic manipulation tools supported by relatively well known genetics and physiology of genus *Salmonella* allow to introduce sophisticated modifications into bacterial phenotype to improve safety and efficacy. The prevailing laboratory strains used for therapeutic vaccine design are derivatives of *S. typhimurium* SL14 028, SL7207, and SL1344 lineages. Their genetic material was modified by in vitro selection and genetic engineering in order to: 1) control pathogenicity and 2) enhance antitumor activity. Proper modifications maximize the benefit from features inherent to pathogen and minimize toxicity.

Ability to accumulate in solid tumors as well as a number of other features make *S. typhimurium* an excellent therapeutic modality. *Salmonella* invades, survives, and multiplies inside the cells of an infected organism. Therefore, it can serve as a vehicle that delivers a therapeutic cargo to cells in the form of coding DNA (cDNA). Coded genes are expressed either by bacterial or eukaryotic translational apparatus in order to yield an active protein product. There is no need to enclose any preformed drug. Instead, the recipe in the form of genetic material is amplified, preferably after bacteria reach the tumor, to release a therapeutic protein locally within the tumor. To date, supplied cDNA have coded tumor-specific or tumor-associated antigen, prodrug converting enzyme, cytokine, apoptosis-inducing factor, antiangiogenic factor, or regulatory RNA (**TABLE 1**).[4-10] Rational design of the expression system enables to produce a favorable form of protein, which is either transported to bacterial cell surface or secreted outside bacteria. The exact form profoundly affects the outcome of a therapeutic intervention. *S. typhimurium* has also been used in gene therapy as a vehicle for the delivery of genetic material, which is then expressed by eukaryotic translational apparatus (**TABLE 1**).

Therapeutic bacterial vaccine vector should not trigger strong antibacterial immune response, so that bacteria are not quickly eliminated from the body after repeated administration. Repeated doses may be necessary to achieve full therapeutic effect and will be effective only in the case of their poor immunogenicity. *Salmonella* fulfills this requirement due to attenuating mutations that minimize systemic inflammatory responses. Moreover, various mutations result in altered tropism toward infected populations of cells and different immunogenicity.[11,12] By introducing different modifications, an adequate balance between attenuation and immunogenicity can be achieved. As for any other vaccination, anticancer therapeutic vaccine should properly polarize the response, in this case – towards cellular immunity. As an intracellular pathogen, *Salmonella* triggers Th1 cellular immune response; however, further modifications leading to increased polarization towards cellular immunity are needed.

Tumor targeting and tumor growth inhibition were studied in murine tumor models after systemic or local *S. typhimurium* administration. Bacteria injected intravenously colonize distant solid tumors and metastases inaccessible by other routes. Alternatively, bacteria are injected into tumor mass or given through oral gavage to treat intestine cancer lesions. Since the latter possibility requires transgenic or orthotopic tumor models, it has been extremely poorly explored. Most of the results come from less demanding transplantable tumor models. The primary objective of both local and systemic administration is to maximize tumor colonization. Local accumulation of bacteria leads to acute inflammation focused at tumor site. Cancer cells are frequently recognized by the immune system as nonself, but the absence of danger signal precludes proper reactivity. The local occurrence of both signals at tumor site is a prerequisite for the induction of effective immunity.

The suspected mode of tumor growth inhibition by *S. typhimurium* is a combination of direct cytotoxicity towards infected tumor cells and anticancer immunity evoked by acute inflammation. In the course of infection, *Salmonella* exports effector proteins which interact with host cells and influence immune response. *Salmonella*-infected macrophages and epithelial cells undergo apoptosis followed by IL-1β release.[13-15] Cytotoxicity towards cancer cells seems to be a minor component of tumor growth control because of attenuated virulence. Apoptosis of infected cancer cells can trigger antitumor immunity through facilitating tumor antigen cross-presentation.[16,17] In this process, antigens originating from proteins produced outside professional antigen presenting cells (APC) are processed and presented on MHC class I molecules to cytotoxic T cells (CTL). The antigenic material is acquired by APC via phagocytosis of apoptotic cells. Cross-presentation results in cross-stimulation

TABLE 1 Efficacy of *Salmonella* in animal tumor models

Salmonella strain	Tumor model	Therapeutic regimen	Outcome
S. typhimurium SL7207 with murine prostate stem cell antigen expressing plasmid[4]	s.c. TRAMPC1 murine prostate carcinoma cells in C57Bl/6 mice	oral gavage of bacteria in 2-week intervals followed by injection of tumor cells	over 50% of mice remained tumor-free
S. typhimurium YS1646 (VNP20009) with plasmid coding shRNA for STAT3 and *S. typhimurium* MVP728 with survivin expression plasmid[5]	s.c. B16F10 melanoma in C57Bl/6 mice	i.v. followed by oral gavage (4 days later)	decreased phospho-STAT3 levels in tumor F4/80+ macrophages, enhanced tumor-specific cytotoxicity, tumor growth inhibition
S. typhimurium SL3261 expressing tumor antigen TRP2 fused to heat shock protein 70[6]	s.c. B16F10 melanoma in C57Bl/6 mice	3 bacterial oral gavages in 10-day intervals followed by B16F10 s.c. injection 7 days after last bacteria dose; therapeutic settings: B16F10 s.c. inoculation followed by 4 oral doses of bacteria in 5-day intervals	tumor rejection in 75% of mice in prophylactic settings; significant suppression of tumor growth after therapeutic administration
S. typhimurium A1-R[7]	XPA-1 human pancreatic cancer cells injected into the spleen of nude mice	3 intrasplenic bacteria injection at day 3, 10, and 17 after cancer cell injection	suppression of tumor growth in the spleen and liver metastases
S. typhimurium SL7207 carrying TRAIL and apoptin genes[8]	human gastric cancer cell line SGC-7901 injected s.c. in Balb/c nude mice	intratumoral injection 7 days after tumor implantation, repeated every 7 days	cancer cell apoptosis in tumor tissue and tumor regression
S. typhimurium VNP20009 expressing TRAIL[9]	attenuated mammary carcinoma cell line 4T1/red s.c. injected at mammary fat pad of Balb/c mice	i.v. injectiat 21 days after tumor implantation	tumor growth retardation and improved survival death risk reduced up to 76%
S. typhimurium VNP20009 producing IL-18[10]	s.c. CT26 colon carcinoma in Balb/c mice	i.v. injectiat 14 days after tumor implantation	enhanced antitumor activity, tumor growth inhibition; leukocyte infiltration into tumor

Abbreviations: IL-18 – interleukin 18, i.v. – intravenous, s.c. – subcutaneous

(cross-priming) or tolerance (cross-tolerance), depending on the presence of costimulatory signal, which is dependent on APC maturity.[18,19] The molecules of bacterial origin (pathogen-associated molecular patterns – PAMP) stimulate APC maturation to immunostimulatory phenotype.[19] Mature APC produce interleukin (IL)-12, tumor necrosis factor α (TNF-α), IL-1β, IL-6, interferon (IFN) α and β, activating T cells and IL-12 is crucial for the polarization of the immune response towards Th1 cellular immunity. Therefore, a danger signal promotes costimulation and effective CTL response. *Salmonella* outer membrane protein A (OmpA) induces the maturation of bone marrow derived dendritic cells.[20] Taken together, the application of therapeutic *Salmonella* strain provides both increased amount of apoptotic cancer cells and immune-stimulating signals.

Cross-presentation of tumor-associated antigen and CTL stimulation were detected after local *Salmonella* administration. Mice were bearing subcutaneous melanoma B16-OVA tumors, expressing a model antigen, i.e., ovalbumin (OVA), and *S. typhimurium* was injected into tumors. Production of anti-inflammatory cytokines (IL-10, IL-13, IL-4 and transforming growth factor β) in tumor-draining lymph nodes decreased to a similar level as in non-tumor-bearing mice; proinflammatory IL-1β and IFN-γ were elevated. Dendritic cells isolated from tumor-draining lymph nodes from mice that received bacteria had a more activated phenotype, cross-presented MHC class I-restricted OVA epitope, and induced lymphocyte proliferation and IFN-γ release. Skin depigmentation indicated breaking tolerance to tumor-associated self antigens (common with B16).[21]

Instead of picking up and processing antigens by themselves, dendritic cells can receive peptide antigens through gap junctions from other cells. Saccheri et al.[22] showed that *Salmonella*-infected melanoma cells restored the ability to form functional gap junctions with DC, resulting in the presentation of transferred antigen.[22]

The mechanism of *S. typhimurium* preferential accumulation in solid tumors Numerous strains of *S. typhimurium* selectively colonize solid subcutaneous tumors and hepatic or pulmonary metastases in mice.[23,24] Defective metabolism, chemotaxis, motility, and local immunosuppression account for preferential accumulation of *Salmonella* in solid tumors. Motile and facultatively anaerobic *Salmonella* penetrate tumor tissue better than passively diffusing therapeutics, also into necrotic tumor areas distant from the vasculature.[24,25] The majority of strains are auxotrophic, i.e., dependent on external sources of essential metabolites (amino acids, aromatic compounds, or purines). For example, chemical mutagenesis, followed by selection for decreased toxicity, increased adherence to cancer cells and enhanced tumor targeting, resulted in *S. typhimurium* A1-R strain, auxotrophic for leucine and arginine.[26] Hence the explanation of tumor targeting refers to tumor tissue as a source of nutrients highly concentrated in tumors. Chemotaxis is essential

TABLE 2 Modified bacteria stains used in anticancer clinical trials (source: http://clinicaltrials.gov)

Official title	Condition	Intervention	Status
A Phase 2, Randomized, Multicenter, Open-Label Study of the Efficacy and Immune Response of the Sequential Adminstration of GVAX Pancreas Vaccine (with Cyclophosphamide) Alone or Followed by CRS-207 in Adults With Metastatic Pancreatic Adenocarcinoma	pancreatic cancer	GVAX pancreas vaccine, CRS-207 (attenuated *Listeria monocytogenes*), cyclophosphamide	recruiting participants
A Randomized, Single Blind, Placebo Controlled Phase 2 Study to Assess the Safety of ADXS11-001 for the Treatment of Cerrvical Intraepithelial Neoplasia Grade 2/3	cervical intraepithelial neoplasia	ADXS11-001 – live attenuated *L. monocytogenes* expressing E7 antigen fused to listeriolysin (Lovaxin C)	recruiting participants
A Phase II Evaluation of ADXS11-001 (NSC 752 718, BB-IND#13,712) in the Treatment of Persistent or Recurrent Squamous or Non-Squamous Cell Carcinoma of the Cervix	cervical cancer	ADXS11-00	recruiting participants
Phase I Safety Study of Costridium Novyi-NT Spores in Patients With Treatment-refractory Solid Tumor Malignancies	solid tumor malignancy	*Clostridium novyi*-NT spores	recruiting participants
A Phase 1 Study of an IL-2 Expressing, Attenuated *Salmonella Typhimurium* in Patients With Unresectable Hepatic Spread From Any Non-Hematologic Primary	cancer of the liver, hepatoma, biliary cancer	Live attenuated *Salmonella typhimurium* × 4550 strain expressing IL-2	recruiting participants
A Phase 1, Open-Label, Dose-Escalation, Multiple Dose Study of the Safety, Tolerability, and Immune Response of CRS-207 in Adult Subjects With Selected Advanced Solid Tumors Who Have Failed or Who Are Not Candidates for Standard Treatment	malignant epithelial mesothelioma, adenocarcinoma of the pancreas, non-small-cell lung carcinoma, adenocarcinoma of the ovaries	CRS-207 – live attenuated *Listeria monocytogenes* expressing human mesothelin	terminated
Phase 1 Dose-Escalation Study of Safety and Tolerability of Intravenous CRS-100 in Adults With Carcinoma and Liver Metastasis	liver neoplasm, carcinoma	CRS-100 – live attenuated *L. monocytogenes*	completed
A phase I Trial of a Live, Genetically Modified *Salmonella Typhimurium* (VNP20 009) for the Treatment of Cancer by Intravenous Administration	cancer, neoplasm, neoplasm metastasis	*Salmonella typhimurium* VNP20009	completed

to initiate accumulation.[25] Chemotactic response is triggered through specific receptor stimulation. *Salmonella* senses serine, aspartate, and ribose/galactose gradient, which guide it to tumors; moreover, inactivation of ribose/galactose receptor leads to the accumulation of bacteria in quiescent tumor areas.[27] These areas are ischemic, starved for nutrients, and therefore have limited responsiveness to chemotherapy. Modifications of bacterial capacity to react towards specific nutrients represent a strategy to direct *Salmonella* to specific quiescent tumor regions.

Modified bacteria in anticancer clinical studies Bacteria of the genus *Salmonella*, *Shigella*, and *Listeria* are potential vehicles for the delivery of therapeutic agents to human cells. Intracellular pathogens not only survive inside the cells but also reside there being capable of protein synthesis. The response against intracellular pathogens is polarized towards cellular response and can foster effective antitumor immunity. The toxicity of bacterial therapy can be controlled through the administration of antibiotics.

Preclinical results from murine tumor models encouraged human trials (TABLE 2), and so far two modified intracellular bacteria have been safely administered to cancer patients: *Listeria monocytogenes* and *S. typhimurium*. *L. monocytogenes* attenuated strain CRS-207 (Cerus Corp., Concord, United States) is intended for the delivery of human tumor-specific antigens to dendritic cells for MHC class I presentation. Bacteria secretes the antigen due to its fusion with a portion of ActA, a *Listeria* protein released inside the infected cells. Mesothelin, expressed on mesotheliomas, pancreatic adenonarcinomas, most of ovarian cancers, and 50% of lung adenocarcinomas elicited mesothelin-specific immunity when delivered by CRS-207,[28] and phase I clinical study is being conducted. Advaxis Inc. (New Brunswick, United States) develops a therapeutic cancer vaccines based on attenuated *L. monocytogenes* strain producing tumor-specific antigens fused to truncated fragment of *Listeria* protein, listeriolysin. Fifteen women with advanced cervical cancer were treated with Lovaxin C, which produces E7 antigen of HPV-16 origin and targets cervical carcinoma. The overall survival of 6 patients was improved and in 4 of 13 evaluated patients a reduction of tumors was observed.

The anaerobic *Clostridium novyi* NT (strain devoid of major systemic toxin gene) spores are predisposed to target hypoxic areas of tumors. The extent of toxicity is correlated with tumor size. Toxicity of this intervention results from spore germination and increases with the progression of hemorrhagic necrosis within the tumor. Spores are extremely sensitive to oxygen and do not germinate in aerobic conditions; therefore, well-oxygenated areas of tumors are not affected directly by the oncolytic bacteria.[29]

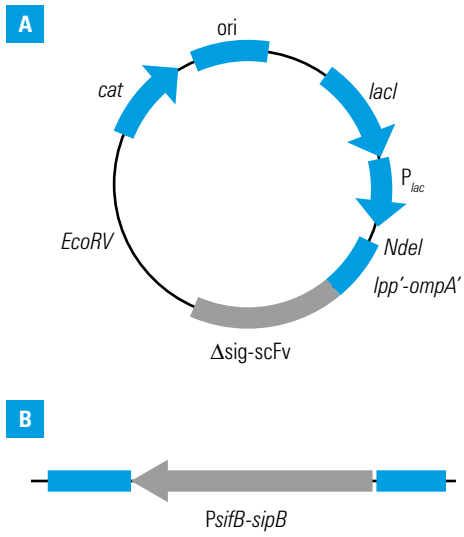

FIGURE 1
Modifications introduced to improve *S. typhimurium* VNP20009 tumor targeting and enhance its antitumor activity[33]
A schematic diagram of pMoPac2 derivative plasmid expressing scFv
B schematic diagram of the expression cassette inserted into VN20009 chromosome
Abbreviations: *cat* – chloramphenicol acetyltransferase, *lacI* – lac repressor, *lpp-ompA* – fusion of major lipoprotein signal sequence fragment and portion of the outer membrane protein A, ompA – outer membrane protein A, ori – origin of replication, P_{lac} – lac promoter, P*sifB* – *sifB* promoter sequence, scFv – single chain antibody, *sipB* – sequence coding proapoptotic protein SipB

S. typhimurium VNP20009 was intended for the targeting of cancer through preferential accumulation in solid tumors, in order to deliver a therapeutic agent. The safety issues were addressed by modifications of the bacteria making it dependent on the external source of metabolites and lowering the ability to induce septic shock. VNP20009 is a genetically modified strain with attenuated virulence and confirmed safety profile for intravenous administration in humans. It has been shown to preferentially colonize solid tumors in mice and dogs.[30,31] In contrast to modified *L. monocytogenes*, VNP20009 load in the liver and spleen after systemic administration in tumor-bearing mice is around thousand times lower than in the solid tumor. The system for surface expression of heterologous proteins in gram-positive *L. monocytogenes* is not available at present, thus facilitation of targeting via tumor antigen binding is not possible.

Two major mutations in VNP20009 determine attenuation of the virulence. Mutation in *purI* gene results in purine deficiency and limited replication rate. The risk of lipopolysaccharide induced septic shock is limited due to partial deletion of *msbB* gene, which decreases the ability of lipopolysaccharide to stimulate TNF-α production. Phase I clinical trial conducted on 24 patients with advanced melanoma was not satisfactory regarding preferential localization of VNP20009 within the tumor lesion, observed in other mammals.[32] Only 3 of 18 tumor biopsies contained viable bacteria and tumor growth regression was not observed. These studies, however, led to the conclusion that intravenous administration of large amounts of VNP20009 is well tolerated, which confirms the fact that VNP20009 is a safe vaccine vector and can be used in humans. A high proportion of bacteria accumulated in the tumor is necessary to boost the efficiency of immunotherapy and to minimize adverse effects.

To improve selective accumulation in solid tumors, we modified VNP20009 to express a single chain antibody fragment (scFv) specific for carcinoembryonic antigen (CEA) present on tumor cells.[33] Tumor-specific antigen is used to concentrate bacteria in the tumor. CEA is expressed on human gastrointestinal tract carcinomas, pancreatic, nonsmall cell lung, and breast cancers. Gastrointestinal cancers account for 25% of neoplastic deaths in the West; in Poland gastrointestinal cancer rates are likely to increase[34] and still represent a clinical challenge. To enhance antitumor activity, an additional copy of *sipB* gene was inserted into VNP20009 chromosome, which codes proapoptotic protein – *Salmonella* invasion protein B (SipB) (**FIGURE 1**). A suggested mode of action of the new therapeutic strain is presented in **FIGURE 2**. The antibody fragment is exposed on the surface of bacteria coupled to outer membrane protein OmpA, which is a member of the PAMP family able to induce dendritic cell maturation and activate natural killer cells through TLR-2 signaling. VNP20009 expressing anti-CEA scFv on the cell surface, administered by oral gavage, accumulated in the upper gastrointestinal tract of CEA transgenic mice and preferentially localized to CEA-expressing transplantable tumors after intravenous injection. Immunization with the modified bacteria led to substantial inhibition of tumor growth and more than doubled the survival time in an MC38CEA tumor transplantation model. Inhibition of tumor growth correlated with mobilization of CD3+ T cells and macrophages at the tumor site.

Future perspectives are to define the effector immune cell populations associated with murine transplantable tumor growth control after administration of modified VNP20009. Toxicology studies of the modified strain on large mammals (pigs) and nonhuman primates are essential before proceeding to clinics. The optimization of scFv expression system will further improve therapeutic effectiveness, safety, and stability of the modification. Due to the modular character of the system, it can be adapted to target other tumors and to deliver various genes or gene products to augment immunity or deliver therapeutic agents to tumors. Our murine tumor model was based on CEA antigen expressed on cancer cells and CEA-specific antibody variable fragment on VNP20009 surface. The genetic modification is possible to equip bacteria with the antibody of other specificity in order to target a solid tumor expressing the relevant antigen.

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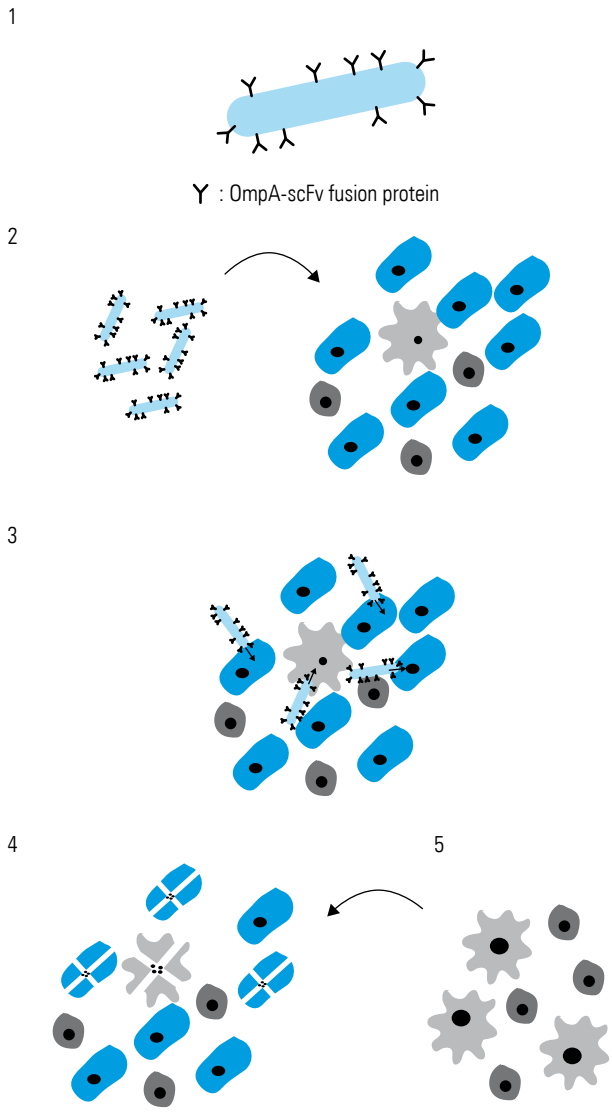

FIGURE 2 *S. typhimurium* VNP20 009 was modified to increase anticancer activity. VNP20 009 with surface expression of antibody fragment (scFv) specific for tumor-related antigen (CEA) (1) and overexpression of proapoptotic protein accumulates in CEA-expressing tumors (2). Bacteria infect cancer cells and tumor-associated macrophages (3), induce apoptosis (4), migration of immune cells (5), and systemic tumor-specific response is triggered.

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Terapeutyczne szczepionki oparte na modyfikowanych genetycznie szczepach bakterii *Salmonella* – nowa strategia w immunoterapii nowotworów

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immunoterapia nowotworów, *Salmonella typhimurium* VNP20009, wektor szczepionkowy

STRESZCZENIE

Bakterie z rodzaju *Salmonella* nabyły w czasie ewolucji umiejętność przetrwania i namnażania się w organizmach kręgowców. Umiejętne wykorzystanie interakcji bakterii z układem immunologicznym gospodarza stało się podstawą rozwoju szczepionek terapeutycznych w oparciu o zmodyfikowane genetycznie szczepy *Salmonella*. Materiał genetyczny tych bakterii może być tak modyfikowany, aby zmniejszyć toksyczność bakterii oraz wzmocnić lub dodać oczekiwane cechy terapeutyczne. Terapeutyczne szczepionki *Salmonella* są atrakcyjną i nowatorską alternatywą dla konwencjonalnych terapii przeciwnowotworowych (chemioterapii, radioterapii i biernej immunoterapii). Żywe bakterie mają naturalną zdolność do chemotaksji i penetrowania docelowej tkanki. Określone szczepy *Salmonella* podane dożylnie do organizmu zwierząt doświadczalnych, u których rozwija się nowotwór, gromadzą się wybiórczo w guzach litych i ograniczają ich wzrost. Co więcej, bakterie *Salmonella* zasiedlają obszary guza niedostępne dla innych, biernie przenoszonych leków, np. obszary niedokrwione. Umożliwia to bakteriom produkowanie i miejscowe uwalnianie naturalnych lub rekombinowanych czynników przeciwnowotworowych, co zwiększa ich efekt terapeutyczny.

S. typhimurium VNP20009 jest szczepem bezpiecznym, co udokumentowano w badaniach klinicznych. Nie zaobserwowano jednak oczekiwanych efektów terapeutycznych, prawdopodobnie ze względu na niewystarczające zasiedlanie guzów nowotworowych przez bakterie. Aby zwiększyć kolonizację guzów litych, bakterie VNP20009 zostały wyposażone w zdolność do powierzchniowej ekspresji fragmentu przeciwciała swoistego dla antygenu CEA (*carcinoembryonic antigen*) obecnego na ludzkich komórkach nowotworowych. Dodatkowo, dla wzmocnienia lokalnej odpowiedzi przeciwnowotworowej, materiał genetyczny VNP20 009 został zmieniony tak, aby umożliwić nadprodukcję endogennego proapoptotycznego białka *Salmonella*, którego celem są komórki nowotworowe i komórki układu immunologicznego wspierające wzrost nowotworu.

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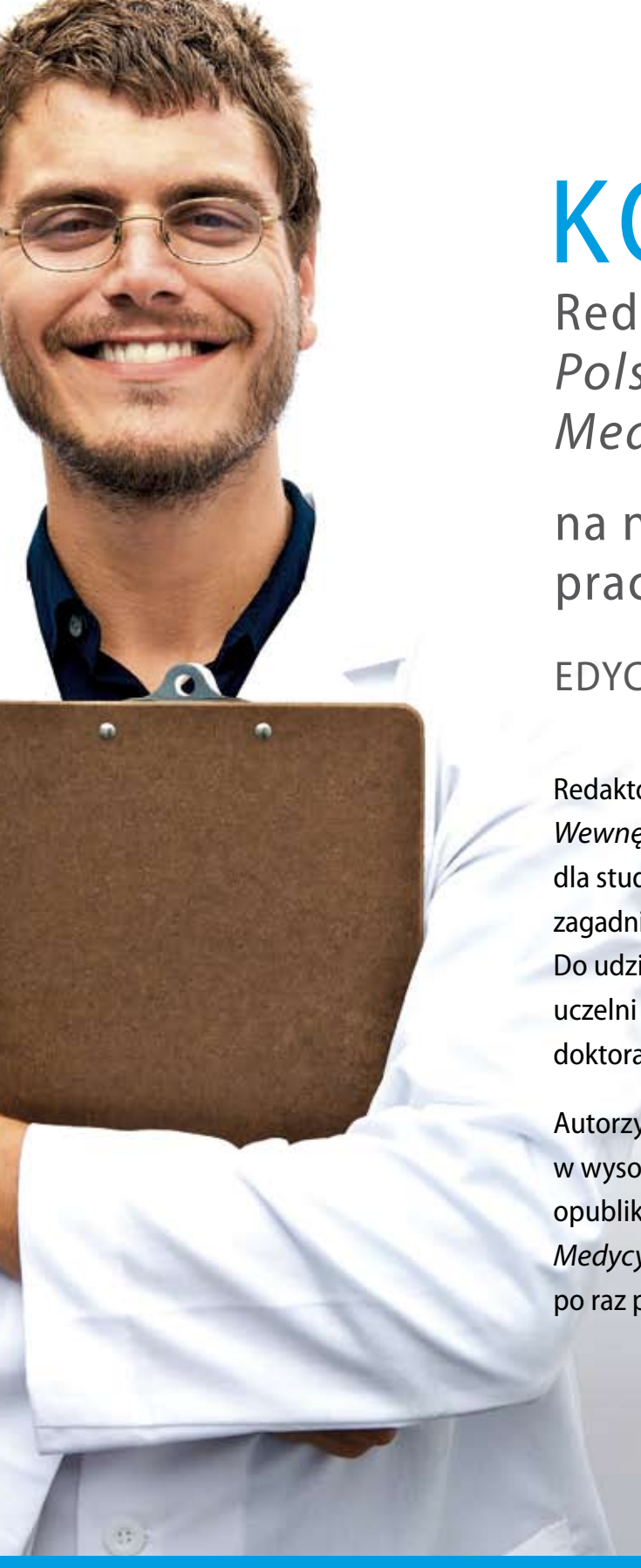

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