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Pathogen-Associated Molecular Pattern in Cancer Immunotherapy

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ABSTRACT: Observations from different research frontiers—epidemiological data, case studies on spontaneous regressions from cancer, clinical studies, tumor immunology—indicate that exposure by vaccination or infection to pathogen-associated molecular patterns (PAMP) can have beneficial effects on neoplastic diseases, both prophylactically and therapeutically. These effects have not yet been harnessed to their full extent for the prophylaxis and therapy of cancer. Here, we summarize clinical, epidemiological, and experimental data and discuss the role of PAMP in cancer therapy.

KEY WORDS: PAMP, cancer, DC maturation, fever, neoplasm, regression, tumor, oncology, Toll ligand

I. INTRODUCTION

Over time, cells within a growing tumor acquire hundreds or thousands of mutations.[1] Although some mutations shape the known cancer phenotype characterized by uncontrolled proliferation, induction of angiogenesis, diminished control by local tissue factors, and the eventual appearance of metastasis, others might not serve any purpose and just represent nonlethal damage. All mutations lead to the expression of potentially antigenic gene products. Since the first tumor antigen was identified, in 1991,[2] many more tumor antigens have been detected, isolated, and, in some cases, cloned. They can be classified into five principal categories:

1. Differentiation antigens that are normally expressed only in the early stages of development (e.g., Melan-A/MART-1).
2. Mutational antigens expressed as a result of mutational damage accumulated in cancer cells.
3. Overexpressed proteins again resulting from mutational damage and altered transcriptional control (e.g., HER-2/neu).
4. Testis antigens (e.g., NY-ESO-1).
5. Viral antigens in the case of virally induced cancers.

To date, no single tumor antigen characterizing all tumors has been identified, but some antigens are characteristic of particular forms of cancer, for example, HER-2 for about 30% of breast cancers. Yet, T-cell recognition of tumors can be based on a panel of antigens consisting both of known and unknown antigens, the latter probably arising by chance from somatic mutations.[3] The relative contribution of distinct T-cell specificities to overall tumor recognition can vary over time.[3] This suggests a profound individuality in tumor antigens between patients and may be one explanation why no vaccination strategy, so far, has been very promising.[4] In this review, we discuss whether and how pathogen-associated molecular patterns

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(PAMP) might be utilized to improve cancer vaccination outcomes.

II. TUMORS ARE OFTEN RECOGNIZED BY THE IMMUNE SYSTEM

Long-ignored observations that the presence of high numbers of tumor-infiltrating lymphocytes (TILs) around and within tumor tissue correlates with a high survival rate[5] have been confirmed, almost half a century later, in more than 3400 patients with cancer of the breast, bladder, colon, prostate, ovary, rectum, and brain.[6] A particularly striking difference was observed in a study of ovarian cancer in which the overall 5-year survival rates of patients with TILs and no TILs were 38% and 4.5%, respectively, and the comparable survival rates in those treated by debulking and platinum-based chemotherapy were 73.9% and 11.9%.[7] Indeed, evidence that the presence, location, and density of T cells within colorectal tumors is a better predictor of survival of patients than tumor staging by size and spread challenges the prevailing clinical paradigm.[8] Together, these observations indicate that tumors, in many cases, are antigenic and recognized by tumor-specific T cells.

III. REQUIREMENT FOR INFLAMMATORY ENVIRONMENT IN CANCER TREATMENT

A large portion of TIL consists of tumor-specific T cells, but these T cells must be activated for successful immune attack. Unfortunately, such activation is usually absent in cancer. According to the "danger" concept proposed by Matzinger, tumor rejection is driven by the amount of danger signals that tumor tissue provide, which usually is low. Danger signals, according to this hypothesis, can be products of cell injury or necrotic cell death, as well as signals that are generated by concurrent bacterial infection and inflammation.[9]

Most, if not all, T-cell responses *in vivo* are initiated by mature dendritic cells (DC). DC maturation in the lymph nodes, after antigen capturing, depends on inflammatory signals such as tumor necrosis factor (TNF)-α, interleukin-1 and -6 (IL-1, IL-6), and transforming growth factor (TGF)-β in the microenvironment, and, as a major prerequisite, various molecules unique to pathogens termed pathogen-specific molecular patterns (PAMP). Components of PAMP include lipopolysaccarides (LPS), characteristic of bacterial cell walls; unmethylated CpG-islands, characteristic of bacterial DNA; bacterial heat shock proteins (HSP); and double-stranded RNA, as found in some viruses. Table 1 lists some known PAMP and the respective human Toll-like receptors (TLR) that they bind to. TLRs are type I membrane glycoproteins on the surfaces of DC (TLR1, -2, -4, -5, -6, -10) or in intracellular compartments, such as the endosomes and endoplasmatic reticulum of most cell types (TLR3, -7, -8, -9). There are at least 11 different TLRs in humans, and alone or preferably together with inflammatory cytokines they effectively induce DC maturation. Besides PAMP, DC maturation can be induced by signaling from NK cells, from T-helper cells via CD40L, by Prostaglandin E2, and by antigen–antibody complexes.[10] In contrast to non-PAMP signals, however, PAMP act as canonical danger signals and are probably the most effective inducers of DC maturation. Cancer cells, by nature, do not provide PAMP, and tumor antigen alone, without an associated danger signal, normally keeps DC immature and in a tolerizing state.

A literature survey from two areas of research, which on first impression appear unrelated—namely, spontaneous tumor regression and cancer epidemiology—supports the danger model and provides evidence that PAMP might indeed play an important role in tumor rejection. In the following, we argue that bacterial infections, in particular those inducing fever, establish an "inflammatory environment" required for a fully effective immune attack.

IV. THE RELATION OF SPONTANEOUS REGRESSIONS OF CANCER TO INFECTION

The literature on spontaneous regression and remission of cancer[11–15] provides no consistent explanation for the cause and initiating events of this puzzling phenomenon. In a recent retrospective analysis, it was shown that a large proportion

TABLE 1
Examples of Characterized PAMP[16,17]

PAMP	Source	Binding to human Toll-receptor
Triacyl lipopeptides	Bacteria, mycobacteria	1, 2
Pam3Cys	Synthetic	1, 2
Diacyl lipopeptides	*Mycoplasma*	2, 6
Lipoteichoic acids	Group B *Streptococcus, Listeria*	2, 6
Zymosan	*S. cerevisiae*	2, 6
LPS, LPS analogs	Gram-negative	4
Mannan	*C. albicans*	4
MPL	Synthetic	4
Taxol	*Taxus brevifolia*, synthetic	4
Flagellin	Flagellated bacteria	5
Profilin	*Toxoplasma gondii*	11
Uropathogenic bacteria	Not determined	11
dsRNA, Poly(I:C)	Viruses	3
ssRNA	RNA viruses	7, 8
Imidazoquinolines (imiquimod, resiquimod)	Synthetic	7, 8
Unmethylated CpG	Bacteria, mycobacteria	9
Synthetic oligos	Synthetic	9
Polyglucan	Gram-positive	2
Porins	*Neisseria*	2
Lipoarabinomannan	Mycobacteria	2
Phospholipomannan	*C. albicans*	2
Glucuronoxylomannan	*Cryptococcus neoformans*	2, 4
tGPI-mutin	*Trypanosoma*	2
Glycophosphoinositollipids	*Trypanosoma*	2
Hemozoin	*Plasmodium*	9
Hemagglutinin	Measles virus	2
HSP60, HPS70	Bacterial, host	4
Fibrinogen	Host	4
Envelope proteins	RSV, MMTV	4

of regressions—from 25% to 80%—was preceded by a febrile infection.[18] It may reasonably be assumed that the actual proportion is even higher because it is highly likely that in many published original case studies and reviews, infections were under-reported owing to a lack of awareness of any association. The concept that bacterial infections and associated fever could modify the progress of a tumor has not yet been considered in the design of immunological cancer therapy because the evidence from spontaneous regression case studies is anecdotal and has never been rigorously tested in a modern clinical study. Additional evidence, however, can be gained from epidemiological studies.

V. PROTECTION FROM CANCER THROUGH BACTERIAL INFECTION

Epidemiological data, summarized in Table 2, indicate that certain infections and vaccinations may prevent the development of cancer. Data for 6 of the 20 studies listed in Table 2 included age-matched controls[19–24] and can be considered statistically robust; these are the more recent studies in this table. Six of the studies used control groups that were not age matched,[25–30] 5 were anecdotal observations[31] or date back to pre-war times[32–35] and may or may not be biased, and 3 were experiencing spontaneous regressions[11,14,36] and are thus casual observations. Taken together, in our opinion, these

TABLE 2
Epidemiological and Clinical Data Supporting the Hypothesis of Beneficial Effects of Infections on Prognosis

Observation	Effect—prophylactic/therapeutic	Year	Pathogen	Refs.
Lower risk of cancer in syphilitic prostitutes	Prophylactic	1725	*Treponema pallidum*	35
Collection of 302 cases of spontaneous regression (44 complete remissions); 27/302 cases accompanied by infection (9%), 69 cases where "incomplete operation [was] often accompanied by post-operative fever" **(28%)**	Therapeutic	1918	Diverse	11
Low risk of cancer in tuberculosis patients	Prophylactic	1929	*Mycobacterium tuberculosis*	32
Lower risk of cancer in malaria patients	Prophylactic	1929	*Plasmodium falc., malariae, vivax*	33, 34
In a cohort of 300 cases of childhood leukemia, 26 spontaneous remissions were observed. 21/26 **(80%)** were accompanied by infection	Therapeutic	1951	Diverse	36
In 62/224 cases of spontaneous regression **(28%)** either an infection or a persistent temperature elevation was observed prior to regression	Therapeutic	1971	Diverse	14
Occasional remissions in Hodgkin's lymphoma after measles attack	Therapeutic	1971	*Morbillivirus*	31
Patients developing empyema after lung cancer surgery have improved 5-year survival (50%, n = 18 vs 22%, n = 411)	Therapeutic	1972	Diverse	25
Lower cancer incidence after Herpes infections	Prophylactic	1987	Herpes simplex	26
Post-transfusional hepatitis in patients with acute myelogeneous leukemia doubles survival rate	Therapeutic	1982, 1992	Hepatitis viruses	27, 28
A total of 255 carcinoma cases were interviewed using a standard questionnaire. Population and hospital controls were matched to the cases for age, sex, and region of residence at the time of the interview. A history of common colds or gastroenteric influenza prior to the interview was found to be associated with a decreased cancer risk	Prophylactic	1991	Common cold viruses	30

Italy in the period 1890–1960: each 2% reduction in number of infectious diseases was followed by a 2% increase in in tumors about 10 years later	Prophylactic	1998	Diverse	29
68 well-documented cases of spontaneous regression from melanoma, preceded in 21 (31%) cases by a febrile infection	Therapeutic	1998	*Streptococcus pyogenes* (9/21 cases)	19
Statistically significant inverse association between a reported history of infections and glioma/ meningioma (RR = 0.72, age and gender-matched population control of 1509 cases)	Prophylactic	1999	Diverse	20
Inverse correlation between melanoma risk and number of recorded infections on one hand and between melanoma risk and fever height on the other hand, leading to a combined reduction of melanoma risk of about 40% for people with a history of three or more infections with high fever above 38.5°C (age- and gender-matched population control)	Prophylactic	1999	Diverse	21
More than a twofold higher incidence of cancer in Europe, GUS/Russia, and the United States compared to Africa and Asia of 381 vs 156 (10 most prominent cancer forms, age-standardized rate per 100,000 population; in Africa and Asia a significantly higher rate of infections is assumed to occur)	Prophylactic	2003	Diverse	22
Prior immunization of melanoma patients with vaccinia or BCG is associated with better survival (age-matched controls)	Prophylactic	2005	Vaccinia, BCG vaccine	23
The 10-year survival for patients with osteosarcoma with infection within one year after surgery (n = 41) was 84.5% compared to 62.3% in the noninfected group (n = 371)	Therapeutic	2007	Diverse	24

Note: RR = relative risks.

studies present enough evidence to justify further evaluation of the hypothesis that these effects are principally mediated by PAMP, as DC-activating and fever-inducing effects of PAMP are well established. Furthermore, the wide diversity of pathogens involved, including bacteria, viruses, trypanosoma, and plasmodia, strongly suggests that some common denominator factors, such as Toll-receptor ligands, are involved.

These immune effects may affect tumors both prophylactically and therapeutically (Table 2). In the latter case, the effect can be observed as spontaneous tumor regression or remission or as improved survival rate after infection. Effects can be very powerful and lead, in some fortunate patients, to complete erradication of established tumors. Beneficial effects of infections on cancer have, overall, long been ignored, with the exception of a few trials of treatments using bacterial extracts that started at the beginning of the 20th century and continued sporadically until the early 1990s.[18] TLR and TLR ligands were unknown at that time, but in the last few years, PAMP have been investigated in modern clinical trials. Both the early and recent clinical experiments are summarized below.

VI. COLEY'S TOXIN

In the first part of the 20th century, thousands of cancer patients were treated by injection of a bacterial extract. This treatment, invented by Busch[37] in 1868 and later exploited by Coley[38,39] was based on the chance observation that cancer patients with a feverish infection sometimes underwent regressions. The predominant infectious condition associated with regression was erysipelas, caused by *Streptococcus pyogenes*. In contrast to most other infectious pathogens, *S. pyogenes* delivers superantigens, which are able to expand a large fraction of T-cell clones nonspecifically and are thus able to mediate an exceptionally vigorous immune attack. Coley and co-workers used heat-sterilized extracts of *S. pyogenes* and *Serratia marcescens*, which were injected once or twice a week over several weeks. Coley mainly treated sarcoma patients, since this was the predominant form of cancer referred to his department, but other cancers were treated as well. The dosage of the bacterial preparation was increased for each patient until a marked temperature elevation was achieved, possibly reaching or exceeding 39°C.

A retrospective analysis[40] showed that Coley achieved a 5-year survival rate above 44%, a striking result considering that these were mostly patients with late stage cancers who had failed to respond to previous treatments. If those patients in whom the treatment was the longest are considered, with injections given over 6 months or more, an astonishing 80% of those with inoperable sarcoma of soft tissues survived more than 5 years.[39]

In the 1960s and 1970s, commercial preparations of Coley's extract appeared on the market (MBV, Vaccineurin) and were tested on small patient groups but without the success previously reported by Coley (reviewed in Ref. 41). It should be noted, however, that in contrast to the strategy adopted by Coley, treatment with these agents was of relatively short duration, and no apparent attention was given to achieving a particular body temperature. In addition, most of the patients had previously received chemotherapy with its obvious immunocompromising effects. Coley's results remain unexplained and unexploited until today.

VII. OTHER INFECTIOUS AGENTS USED TO TREAT CANCER

An unexpectedly low risk of cancer in tuberculosis patients was demonstrated in an extensive review in 1929[32] and led to the therapeutic use of Bacille Calmette-Guerin (BCG), a vaccine derived from pathogenic *Mycobacterium bovis*. BCG was applied with minor success in colon cancer patients[42] and with very variable but generally limited success in melanoma patients.[43] On the other hand, instillation of BCG into the bladder is currently the treatment of choice for superficial bladder carcinoma. Ten years after treatment, the rates of recurrence were 94% for surgery alone compared to 50% for surgery plus BCG.[44] The precise mode of action of intravesical BCG is unclear, but local inflammation with the generation of various cytokines certainly occurs.

Salmonella abortus equi endotoxin was administered intravenously in patients with colorectal and non–small cell lung cancer. In the subgroup with colorectal cancer (27 patients), one complete

and two partial remissions were observed, but fever was regarded as an unwanted side effect and treated with ibuprofen.[45]

A heat-killed preparation of *Mycobacterium vaccae*, a nonpathogenic mycobacterium isolated from soil, has been used with some success in the treatment of melanoma,[46] prostate cancer,[47] and lung cancer.[48] The latter study showed no effect of immunotherapy on squamous carcinoma of the lung but a significant prolongation of life (a mean of 135 days) in patients with inoperable adenocarcinoma. The benefit difference between the two tumor types strongly indicates that the effect of immunotherapy was not due to confounding factors.

VIII. THE ROLE OF ELEVATED BODY TEMPERATURE

Not all of the above-mentioned forms of immunotherapy induce significant fever. For example, *Mycobacterium vaccae* (SRL172) rarely causes any rise in body temperature, suggesting that bacterial preparations can have a therapeutic effect without the need to induce fever. On the other hand, many infections reported to have a beneficial effect on cancer do lead to elevated body temperature, and it is therefore possible that such beneficial effects of certain bacterial preparations might be accentuated by the induction of fever. Several mechanisms, some inducing fever and others not, may possibly act together in a synergistic manner.

Cancer cells are often more vulnerable to heat than normal cells and die from necrosis to a larger extent.[49] Necrotic cancer cell debris can supply tumor antigens. External antigens are usually major histocompatibility (MHC) class II restricted, but DC have a specialized capability to internalize antigen–HSP complexes and present respective antigens in an MHC I context[50] (cross-presentation). This mechanism might fortify immunity to pathogens that avoid "professional" antigen-presenting cells (APC), but the same mechanism could potentially counterbalance escape by MHC I down-regulation on the surface of cancer cells. Exposure of DC to stressed cells[51] or necrotic tumor cells[52] alone can induce DC maturation.

Some of the effects generated by endogenous fever can be provided by externally applied heat, usually called hyperthermia. DC treated with fever-like heat (41°C, 6 hours) were significantly more effective compared to non–heat-treated DC in stimulating T cells both in the presence and absence of antigens.[53] Hyperthermia has been shown to increase the number of TILs.[54]

Taken together, these findings suggest that fever is capable of liberating more antigens, in both quantity and diversity, from tumors, thereby generating pleiotropic immune-stimulating effects.

IX. THE ROLE OF HEAT-SHOCK PROTEINS

Heat-shock proteins are among the most ubiquitious proteins in cells, providing about 5% of the entire cellular protein. For a long time, their sole proven function was to chaperone protein folding and to determine the shape of folded proteins, but since HSP genes lie within the MHC gene complex, other functions appeared possible. In this context, it has been demonstrated that HSP, which are normally situated in the cytoplasm or located in organelles such as the nucleus or mitochondria, can appear on the outer side of membranes of stressed cells, including cancer cells, and, by means of protein-binding domains similar to those of MHC I and MHC II molecules, can display host-cell peptides but of different length than those presented by MHC molecules. HSP72–peptide complexes, but not empty HSP72 molecules, on the surface of cancer cells can serve as antigens, which can bind to DC through Toll-like receptors[55] and directly activate tumor-specific T cells[56] and natural killer (NK) cells.[57] HSP–peptide complexes are probably the main active cross-priming molecules.[58] Interestingly, although non-lethal heat shock enhances synthesis of HSPs within both cancer cells and normal cells, they are only expressed on the surfaces of the latter.[59,60] Thus, higher temperatures could very well enhance the effects mediated by HSP.

HSP can increase the "danger appearance" of tumors[61] since HSP70 is a natural ligand of mammalian Toll-like receptor 4 (TLR4). At least some HSPs can bind to certain PAMP, for example, LPS,[62] and enhance PAMP signaling in APC,[63] perhaps by lowering the threshold of PAMP detection.[64] HSPs are abundant proteins in the cell and are the major protein species

released into the extracellular compartment when cells die by necrosis (but not apoptosis), leading to the maturation of DC.[65] Extracellular HSP can induce macrophages to produce proinflammatory cytokines (IL-1β, TNF-α, IL-12, GM-CSF)[65] and C-C-chemokines (MCP-1, MIP-1, RANTES).[66] Thus fever both stresses and damages tumor cells, giving rise to the display of immunogenic HSP–peptide complexes and increased host danger signals, respectively.

Immunotherapeutic agents based on HSPs have been used in clinical trials on cancer patients. This approach has been pioneered by Srivastava[4] and requires surgical removal of tumor tissue, subsequent extraction of antigen-bearing HSPs, and vaccine preparation. In contrast to other vaccination strategies using specific, known tumor antigens, this approach has the advantage that antigens do not have to be characterized, that it is individualized rather than generic, and that a multiplicity of antigens is used rather than one or just a few. The putative beneficial effect of elevated body temperature has not, however, been considered so far in this vaccination strategy.

X. PAMP IN CLINICAL TRIALS

In recent years, PAMP have been investigated in clinical trials (Table 3). The focus, until today, has been on mononucleotide- and oligonucleotide-derived agents, with the exception of MPL, a lipid-A derivative, which is used to vaccinate against human papilloma virus infection and which might indirectly protect against cervical cancer. Most of these agents target TLR9 and are in Phase I and Phase II stages. Early results show some improvement over standard therapy, with final results yet to be published. In one of the largest trials so far, including 112 melanoma patients treated with CPG-7909 (Coley Pharmaceutical, Canada) in combination with chemotherapy, the 1-year survival rate was 50% vs. 36% for chemotherapy alone.[67] A Phase III trial with CpG-7909 used as monotherapy for patients with unresectable or metastatic melanoma is ongoing. Patients recruited into these studies had usually received prior treatment, including cytotoxic chemotherapy and had advanced disease—factors likely to reduce the efficacy of immunotherapy.

XI. CAN WE DO BETTER?

In our opinion, the full potential of PAMP has, to date, not been harnessed. First, in contrast to the experiments done by Coley, who used a complex bacterial extract, modern clinical trials are usually based on single agents. There are good reasons for this, imposed by regulative authorities who demand clear structure–function relationships, as well imposed by financial arguments, since each substance to be combined in a drug requires expensive preparation based on good manufacturing practice (GMP) criteria. However, a combination of PAMP might be more powerful, since we know from vaccine preparations that the substitution of live attenuated or dead vaccines by more defined antigens is often accompanied by a substantial loss in immunogenicity. A study in nonhuman primates showed that ligands targeting both TLR7 and TLR8 and thus activating both myeloid and plasmacytoid DC, induce stronger T-cell responses than CpG-DNA targeting TLR9 alone.[68] Yellow fever vaccine, one of the most effective vaccines available, which is able to induce immunological memory detectable 35 years after a single vaccination, activates DC by signaling through multiple TLR (TLR2, -7, -8, -9).[69] Combinations of TLR ligands have been found to exert synergistic DC-inducing effects *in vitro*.[70] Also, recent studies usually limit the number of treatments from the beginning, whereas Coley treated his patients with bi-weekly injections, often over several months, without a prior fixed limit on the number of injections. The latter practice is in more accord with the danger model, which suggests to repeat boosting, since local APCs lack memory and therefore need to be alerted repeatedly.[71] Both danger signals and antigens are needed to maintain a vigorous immune response, whereas prolonged stimulation with antigens alone can impair T-cell differentiation.[72]

Second, the occurrence of fever is, in general, regarded as a side effect or adverse effect[73,74] and treated by fever-lowering drugs.[45] TLR ligands often induce fever, and limiting the dose when fever occurs likely leads to suboptimal treatment. Instead, since fever has pleiotropic immune-stimulating and antigen-providing effects, the opposite should be aimed at, namely, to maintain or even augment fever, even if this requires enhanced

clinical care, including monitoring of the circulation during periods of high fever. Experience with Vaccineurin, a fever-inducing *Streptococcus pyogenes* extract used over several decades in some private clinics in Germany show that these fever periods are usually short (about 6–10 hours) and safe when induced in the absence of proliferative infection (Wöppel, Hufeland Klinik, personal communication). The limited success of tumor-vaccination strategies in general[4] might have been improved if elevated body temperature had been considered.

Third, PAMP are probably more effective when injected proximal to the tumor site rather than systemically, since adjuvant properties of CpG DNA were markedly improved when applied in close proximity to antigen.[76,77] The same reasoning suggests that it might be better to administer PAMP both before and after surgery rather than just after surgery, in order to maximize the immune response when antigen load is high and thereby enhance the effectiveness of subsequent residual therapy.

Fourth, antagonistic effects of prior or concurrent chemotherapy with PAMP therapy should be considered. Since the immune-compromising effects of chemo- or radiotherapy might interfere with optimal immunotherapy, it might be advisable to consider PAMP therapy as monotherapy or together only with other immunity-enhancing measures, such as cytokine or antibody therapy. Also, it might be beneficial to commence treatment early in the course of the disease, when tumor load is small, rather than in advanced disease. For ethical reasons, appropriate therapeutic windows will need to be defined. One example of such a window might be a slowly progressive disease such as prostate cancer in which bacterial extracts and vaccination strategies could be applied for some months before more drastic measures are considered. Another therapeutic window might be cancers with a very bad prognosis, such as those of the liver, pancreas, or irresectable and metastatic melanoma, where mainstream medicine has very little to offer.

Fifth, evaluation criteria for cancer immunotherapy might need reconsideration. The usual primary objective is to determine tumor response by RECIST (Response Evaluation Criteria in Solid Tumors). However, classical midpoint evaluations based on assessing regressions and remissions might not be appropriate in cancer vaccination trials, since some vaccinated patients without any classical response do show long-term stabilization of disease.[78] It might be better to consider so-called secondary objectives, such as toxicity, time to progression, survival, and effect on quality of life, with particular emphasis on survival and quality of life.

Clinical trials involving PAMP commenced only a few years ago, but considering these agents merely as adjuvants may result in the underestimation of their full potential, which is still to be uncovered.

XII. SEARCH STRATEGY AND SELECTION CRITERIA

Data for this review were identified by searches of MEDLINE, PubMed, and references from relevant articles.

REFERENCES

1. Loeb LA, Loeb KR, Anderson JP. Multiple mutations and cancer. Proc Natl Acad Sci U S A. 2003;100: 776–81.
2. VanderBruggen P, Traversari C, Chomez P, Lurquin C, DePlaen E, VandenEynde B, Knuth A, Boon T. A gene encoding an antigen recognized by cytolytic T-lymphocytes on a human melanoma. Science. 1991; 254:1643–7.
3. Lennerz V, Fatho M, Gentilini C, Frye RA, Lifke A, Ferel D, Wolfel C, Huber C, Wolfel T. The response of autologous T cells to a human melanoma is dominated by mutated neoantigens. Proc Natl Acad Sci U S A. 2005;102:16013–8.
4. Srivastava PK. Therapeutic cancer vaccines. Curr Opin Immunol. 2006;18:201–5.
5. Black MS, Opler S, Speer S. Structural representations of tumor-host relationships in gastric carcinoma. Surg Gynec Obstet. 1956;102:599–603.
6. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3:991–8.
7. Zhang L, Garcia JRC, Katsaros D, Gimotty PA, Massobrio M, Regnani G, Makrigiannakis A, Gray H, Schlienger K, Liebman MN, Rubin SC, Coukos G. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med. 2003;348: 203–13.
8. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoue F, Bruneval P,

Cugnenc PH, Trajanoski Z, Fridman WH, Pages F. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science. 2006;313:1960–4.
9. Matzinger P. An innate sense of danger. Semin Immunol. 1998;10:399–415.
10. Schuurhuis DH, Fu N, Ossendorp F, Melief CJM. Ins and outs of dendritic cells. Int Arch Allergy Immunol. 2006;140:53–72.
11. Rohdenburg G. Fluctuations in the growth energy of malignant tumors in man, with especial reference to spontaneous recession. J Canc Res. 1918;3:193–225.
12. Boyd W. The spontaneous regression of cancer. Springfield (IL): Charles C. Thomas; 1966.
13. Everson TC, Cole WH. Spontaneous regression of cancer. Philadelphia: J. B. Saunders & Co; 1968.
14. Stephenson HE, Delmez JA, Renden DI, Kimpton RS, Todd PC, Charron TL, Lindberg DA. Host immunity and spontaneous regression of cancer evaluated by computerized data reduction study. Surg Gynecol Obstet. 1971;133:649–55.
15. Challis GB, Stam HJ. The spontaneous regression of cancer. A review of cases from 1900 to 1987. Acta Oncol. 1990;29:545–50.
16. Guy B. The perfect mix: recent progress in adjuvant research. Nat Rev Microbiol. 2007;5:505–17.
17. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006;124:783–801.
18. Hobohm U. Fever therapy revisited. Br J Cancer. 2005;92:421–5.
19. Maurer S, Koelmel KF. Spontaneous regression of advanced malignant melanoma. Onkologie. 1998;21: 14–8.
20. Schlehofer B, Blettner M, Preston-Martin S, Niehoff D, Wahrendorf J, Arslan A, Ahlbom A, Choi WN, Giles GG, Howe GR, Little J, Menegoz F, Ryan P. Role of medical history in brain tumour development. Results from the international adult brain tumour study. Int J Cancer. 1999;82:155–60.
21. Koelmel KF, Pfahlberg A, Mastrangelo G, Niin M, Botev I, Seebacher C, Schneider D, Lambert D, Shafir R, Kokoschka E, Kleeberg U, Henz B, Gefeller O. Infections and melanoma risk: results of a multicenter EORTC case study. Melanoma Res. 1999;9:511–9.
22. Stewart BW, Kleihues P. World Cancer report: World Health Organization. Lyon (France): IARC Press; 2003.
23. Koelmel KF, Grange JM, Krone B, Mastrangelo G, Rossi CR, Henz BM, Seebacher C, Botev IN, Niin M, Lambert D, Shafir R, Kokoschka EM, Kleeberg UR, Gefeller O, Pfahlberg A. Prior immunisation of patients with malignant melanoma with vaccinia or BCG is associated with better survival. An European Organization for Research and Treatment of Cancer cohort study on 542 patients. Eur J Cancer. 2005;41:118–25.
24. Jeys LM, Grimer RJ, Carter SR, Tillman RM, Abudu A. Post operative infection and increased survival in osteosarcoma patients: are they associated? Ann Surg Oncol. 2007;14:2887–95.
25. Ruckdeschel JC, Codish SD, Stranahan A, McKneally MF. Postoperative empyema improves survival in lung cancer. Documentation and analysis of a natural experiment. N Engl J Med. 1972;287:1013–7.
26. Grossarth-Maticek R, Frentzel-Beyme R, Kanazir D, Jankovic M, Vetter H. Reported herpes-virus-infection, fever and cancer incidence in a prospective study. J Chronic Dis. 1987;40:967–76.
27. Rotoli B, Formisano S, Martinelli V, Nigro M. Long-term survival in acute myelogenous leukemia complicated by chronic active hepatitis. N Engl J Med. 1982; 307:1712–3.
28. Treon SP, Broitman SA. Beneficial effects of post-transfusional hepatitis in acute myelogenous leukemia may be mediated by lipopolysaccharides, tumor necrosis factor alpha and interferon gamma. Leukemia. 1992;6:1036–42.
29. Mastrangelo G, Fadda E, Milan G. Cancer increased after a reduction of infections in the first half of this century in Italy: etiologic and preventive implications. Eur J Epidemiol. 1998;14:749–54.
30. Abel U, Becker N, Angerer R, Frentzel-Beyme R, Kaufmann M, Schlag P, Wysocki S, Wahrendorf J, Schulz G. Common infections in the history of cancer patients and controls. J Cancer Res Clin Oncol. 1991; 117:339–44.
31. Zygiert Z. Hodgkin's disease: remissions after measles. Lancet. 1971;1:593.
32. Pearl R. Cancer and tuberculosis. Am J Hyg. 1929; 9:97–162.
33. Braunstein A. Krebs und Malaria. Zeitschrift fuer Krebsforschung. 1929;29:330–3.
34. Braunstein A. Experimentelle und klinische Grundlagen fuer Malariabehandlung des Krebses. Zeitschrift fuer Krebsforschung. 1929;29:468–90.
35. Deidier. Dissertation Medecinal et Chirurgical sur les Tumeurs. Paris; 1725.
36. Diamond LK, Luhby LA. Pattern of 'spontaneous' remissions in leukemia of the childhood, observed in 26 of 300 cases. Am J Med. 1951;10:238ff.
37. Busch W. Aus der Sitzung der medicinischen Section vom 13. November 1867. Berliner Klinische Wochenschrift. 1868;5:137.
38. Coley WB. The treatment of malignant tumors by repeated inoculations. Am J Med Sci. 1893;105: 487–511.
39. Nauts HC, McLaren JR. Coley toxins—the first century. Advances in experimental medicine and biology. 1990;267:483–500.
40. Wiemann B, Starnes CO. Coley's toxins, tumor necrosis factor and cancer research: a historical perspective. Pharmacol Ther. 1994;64:529–64.
41. Hobohm U. Fever and cancer in perspective. Cancer Immunol Immunother. 2001;50:391–6.
42. Vermorken JB, Claessen AM, van Tinteren H, Gall HE, Ezinga R, Meijer S, Scheper RJ, Meijer CJ, Bloemena E, Ransom JH, Hanna MG, Pinedo HM. Active specific immunotherapy for stage II and stage III human colon cancer: a randomised trial. Lancet2. 1999;353:345–50.

43. Tan JK, Ho VC. Pooled analysis of the efficacy of bacille Calmette-Guerin (BCG) immunotherapy in malignant melanoma. J Dermatol Surg Oncol. 1993; 19:985–90.
44. Patard J, Moudouni S, Saint F, Leclercq NR, Manunta A, Guy L, Ballanger P, Lanson Y, Hajri M, Irani J, Guillé F, Beurton D, Lobel B, Lobel B; The members of the group Necker. Tumor progression and survival in patients with T1G3 bladder tumors: multicentric retrospective study comparing 94 patients treated during 17 years. Urology. 2001;58:551–6.
45. Otto F, Schmid P, Mackensen A, Wehr U, Seiz A, Braun M, Galanos C, Mertelsmann R, Engelhardt R. Phase II trial of intravenous endotoxin in patients with colorectal and non-small cell lung cancer. Eur J Cancer. 1996;32A:1712–8.
46. Maraveyas A, Baban B, Kennard D, Rook GA, Westby M, Grange JM, Lydyard P, Stanford JL, Jones M, Selby P, Dalgleish AG. Possible improved survival of patients with stage IV AJCC melanoma receiving SRL 172 immunotherapy: correlation with induction of increased levels of intracellular interleukin-2 in peripheral blood lymphocytes. Ann Oncol. 1999;10:817–24.
47. Hrouda D, Baban B, Dunsmuir WD, Kirby RS, Dalgleish AG. Immunotherapy of advanced prostate cancer: a phase I/II trial using Mycobacterium vaccae (SRL172). Br J Urol. 1998;82:568–73.
48. Stanford JL, Stanford CA, O'Brien ME, Grange JM. Successful immunotherapy with Mycobacterium vaccae in the treatment of adenocarcinoma of the lung. Eur J Cancer. 2008;44:224–7.
49. Trieb K, Sztankay A, Amberger A, Lechner H, Grubeck-Loebenstein B. Hyperthermia inhibits proliferation and stimulates the expression of differentiation markers in cultured thyroid carcinoma cells. Cancer Lett. 1994;87:65–71.
50. Srivastava P. Roles of heat-shock proteins in innate and adaptive immunity. Nat Rev. 2002;2:185–94.
51. Gallucci S, Lolkema M, Matzinger P. Natural adjuvants: endogenous activators of dendritic cells. Nat Med. 1999;5:1249–55.
52. Sauter B, Albert ML, Francisco L, Larsson M, Somersan S, Bhardwaj N. Consequences of cell death: exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. J Exp Med. 2000;191:423–34.
53. Basu S, Srivastava PK. Fever-like temperature induces maturation of dendritic cells through induction of hsp90. Int Immunol. 2003;15:1053–61.
54. Burd R, Dziedzic TS, Xu Y, Caligiuri MA, Subjeck JR, Repasky EA. Tumor cell apoptosis, lymphocyte recruitment and tumor vascular changes are induced by low temperature, long duration (fever-like) whole body hyperthermia. J Cell Physiol. 1998;177:137–47.
55. Asea A, Rehli M, Kabingu E, Boch JA, Bare O, Auron PE, Stevenson MA, Calderwood SK. Novel signal transduction pathway utilized by extracellular HSP70: role of toll-like receptor (TLR) 2 and TLR4. J Biol Chem. 2002;277:15028–34.
56. Rivoltini L, Castelli C, Carrabba M, Mazzaferro V, Pilla L, Huber V, Coppa J, Gallino G, Scheibenbogen C, Squarcina P, Cova A, Camerini R, Lewis JJ, Srivastava PK, Parmiani G. Human tumor-derived heat shock protein 96 mediates in vitro activation and in vivo expansion of melanoma- and colon carcinoma-specific T cells. J Immunol. 2003;171:3467–74.
57. Botzler C, Issels R, Multhoff G. Heat-shock protein 72 cell-surface expression on human lung carcinoma cells is associated with an increased sensitivity to lysis mediated by adherent natural killer cells. Cancer Immunol Immunother. 1996;43:226–30.
58. Binder RJ, Srivastava PK. Peptides chaperoned by heat-shock proteins are a necessary and sufficient source of antigen in the cross-priming of CD8+ T cells. Nat Immunol. 2005;6:593–9.
59. Udono H, Srivastava PK. Heat shock protein 70-associated peptides elicit specific cancer immunity. J Exp Med. 1993;178:1391–6.
60. Multhoff G, Botzler C, Wiesnet M, Müller E, Meier T, Wilmanns W, Issels RD. A stress-inducible 72-kDa heat-shock protein (HSP72) is expressed on the surface of human tumor cells, but not on normal cells. Int J Cancer. 1995;61:272–9.
61. Fuchs EJ, Matzinger P. Is cancer dangerous to the immune system? Semin Immunol. 1996;8:271–80.
62. Byrd CA, Bornmann W, Erdjument-Bromage H, Tempst P, Pavletich N, Rosen N, Nathan CF, Ding A. Heat shock protein 90 mediates macrophage activation by Taxol and bacterial lipopolysaccharide. Proc Natl Acad Sci U S A. 1999;96:5645–50.
63. Osterloh A, Kalinke U, Weiss S, Fleischer B, Breloer M. Synergistic and differential modulation of immune responses by Hsp60 and lipopolysaccharide. J Biol Chem. 2007;282:4669–80.
64. Osterloh A, Breloer M. Heat shock proteins: linking danger and pathogen recognition. Med Microbiol Immunol. 2008;197:1–8.
65. Basu S, Binder RJ, Suto R, Anderson KM, Srivastava PK. Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa-B pathway. Int Immunol. 2000;12:1539–46.
66. Wang Y, Kelly CG, Singh M, McGowan EG, Carrara AS, Bergmeier LA, Lehner T. Stimulation of Th1-polarizing cytokines, C-C chemokines, maturation of dendritic cells, and adjuvant function by the peptide binding fragment of heat shock protein 70. J Immunol. 2002;169:2422–9.
67. Romagne F. Current and future drugs targeting one class of innate immunity receptors: the Toll-like receptors. Drug Discov Today. 2007;12:80–7.
68. Wille-Reece U, Flynn BJ, Lore K, Koup RA, Kedl RM, Mattapallil JJ, Weiss WR, Roederer M, Seder RA. HIV Gag protein conjugated to a Toll-like receptor 7/8 agonist improves the magnitude and

quality of Th1 and CD8+ T cell responses in non-human primates. Proc Natl Acad Sci U S A. 2005; 102:15190–4.
69. Querec T, Bennouna S, Alkan S, Laouar Y, Gorden K, Flavell R, Akira S, Ahmed R, Pulendran B. Yellow fever vaccine YF-17D activates multiple dendritic cell subsets via TLR2, 7, 8, and 9 to stimulÜate polyvalent immunity. J Exp Med. 2006;203:413–24.
70. Napolitani G, Rinaldi A, Bertoni F, Sallusto F, Lanzavecchia A. Selected Toll-like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells. Nat Immunol. 2005;6:769–76.
71. Matzinger P. The danger model: a renewed sense of self. Science. 2002;296:301–5.
72. Zajac AJ, Blattman JN, Murali-Krishna K, Sourdive DJ, Suresh M, Altman JD, Ahmed R. Viral immune evasion due to persistence of activated T cells without effector function. J Exp Med. 1998;188:2205–13.
73. Parmiani G, Testori A, Maio M, Castelli C, Rivoltini L, Pilla L, Belli F, Mazzaferro V, Coppa J, Patuzzo R, Sertoli MR, Hoos A, Srivastava PK, Santinami M. Heat shock proteins and their use as anticancer vaccines. Clin Cancer Res. 2004;10:8142–6.
74. Choudhury A, Mosolits S, Kokhaei P, Hansson L, Palma M, Mellstedt H. Clinical results of vaccine therapy for cancer: learning from history for improving the future. Adv Cancer Res. 2006;95:147–202.
75. Hoffman ES, Smith RE, Renaud RC Jr. From the analyst's couch: TLR-targeted therapeutics. Nat Rev Drug Discov. 2005;4:879–80.
76. Klinman DM, Barnhart KM, Conover J. CpG motifs as immune adjuvants. Vaccine. 1999;17:19–25.
77. Davis HL, Weeratna R, Waldschmidt TJ, Tygrett L, Schorr J, Krieg AM. CpG DNA is a potent enhancer of specific immunity in mice immunized with recombinant hepatitis B surface antigen. J Immunol. 1998;160:870–6.
78. Pardoll DM. Therapeutic vaccination for cancer. Clin Immunol. 2000;95:S44–62.