

Coley's Lessons Remembered: Augmenting Mistletoe Therapy

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Abstract

The following four observations point in the same direction, namely that there is an unleveraged potential for stimulating the innate immune system against cancer: (1) experimental treatments with bacterial extracts more than 100 years ago by Coley and contemporaries, (2) a positive correlation between spontaneous regressions and febrile infection, (3) epidemiological data suggesting an inverse correlation between a history of infection and the likelihood of developing cancer, and (4) our recent finding that a cocktail of pattern recognition receptor ligands (PRRLs) can eradicate solid tumors in cancer mice if applied metronomically. Because the main immunostimulating component of mistletoe extract (ME), mistletoe lectin, has been shown to be a PRRL as well, we suggest to apply ME in combination with additional PRRLs. Additional PRRLs can be found in approved drugs already on the market. Therefore, augmentation of ME might be feasible, with the aim of reattaining the old successes using approved drugs rather than bacterial extracts.

Keywords

Coley's toxin, spontaneous regression, mistletoe lectin, pattern recognition receptor ligand, cancer immunotherapy, mistletoe therapy

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Introduction

Coley's Fever Therapy Using Bacterial Extracts

More than 100 years ago, William B. Coley and others applied fever-inducing bacterial extracts to cancer patients, with sometimes astounding success. His revolutionary attempts to treat cancer were based on older publications from Busch¹ and from Fehleisen² in Germany, who had observed that sometimes a feverish erysipelas infection can lead to softening, shrinkage and even clearance of solid tumors in cancer patients. Busch, in 1867, infected his first cancer patient deliberately by direct contact with an erysipelas patient, and Fehleisen, a couple of years later, applied living bacteria from bacterial cultures to patients, both with substantial risk from the resulting high-grade infection. Coley, starting in the 1890s, took a step forward and reduced danger by using heat-killed *Streptococcus pyogenes* and *Serratia marcescens*.

Coley felt that successful treatment correlated with the height of fever. His daughter, Helen Coley-Nauts, reviewed his patient records and determined that successful treatment correlated with four additional parameters: extract preparation procedure, length and frequency of application, and application close to the tumor.^{3,4}

Coley adjusted dosage on a per patient basis. He started with a low dose of bacterial extract and titrated upward to achieve a body temperature of 39°C or more. As the fever readiness varied, final dosages could be very different for different patients. If during repeated applications the resulting fever decreased, he increased dosage, and vice versa. After morning injection, fever generally peaked two to three hours later and gradually fell over the day, leaving patients afebrile in the evening, such that the patient could recover overnight. The best results were achieved by inducing fever 2 to 3 times per week, over months, starting peritumoral, intravenous (i.v.), or intramuscular injections and possibly continuing with regular intratumoral (i.t.) application. Coley cautioned that the first application should never be i.t. because the resulting reactions could be severe, including tumor lysis syndrome and circulatory collapse.^{3,4}

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Contemporary physicians put Coley's method to the test. Nicholas Senn of Rush Medical College in Chicago reported overall failure of the method in 1895. Caulkins of Watertown, New York, reported a large number of successful outcomes. Matagne in Belgium, who used fresh extracts rather than preparations from other labs, claimed he had treatment successes similar to those reported by Coley; he published his observations in low-impact French and Belgian journals of limited distribution.⁵ Christian and Palmer reported a spectacular cure in 1928.⁶ At a symposium on Ewing's sarcoma in 1934, a form of cancer at that time most often lethal because of metastatic spread at diagnosis and before surgery, Coley presented 44 cases, of which 12 had been treated by other physicians with radiation or surgery or both, but not fever therapy, and 32 patients treated by Coley. In the first group, no patient reached 5 years of survival, whereas in Coley's group, 12 were alive after 5 years (0% vs 38%).

In an extensive review of the Coley story, Starnes collected 170 cancer patient records (121 sarcomas, 43 carcinomas and myelomas, and six melanomas) of late-stage, inoperable tumors, which were treated using Coley's extract but not by radiation, and calculated a remission rate of 64% and 5-year survival of higher than 44% (some patients were lost on follow-up).⁷ This is a remarkable achievement in cases of advanced disease.

Clearly, Coley could not induce remissions in all patients. His approach was critically patient oriented and required continuously adjusting the protocol in response to the febrile response of the individual patient. This proved labor intensive and difficult to standardize. Also, over time, he continued to develop and alter his regimen, both in its route of administration as well as the composition and manufacturing process of the bacterial extracts. In contrast, radiation offered a tissue-oriented protocol, valid for every case, with predictable, immediately verifiable effects and did not require laborious treatment over months. Even though at the time of Coley's death in 1936 it was clear that radiation was not the final answer to treating cancer, it remained attractive and Coley's method fell out of favor, with one exception.

Klyuyeva and coworkers in Russia, in the 1940s, treated cancer patients using not bacterial but *Trypanosoma* extracts, in a manner similar to Coley. Their work was stimulated by Coley's publications, but also by the observation that cancer patients in Brazil almost never had a positive Machado reaction, a blood test indicating a past or ongoing *Trypanosoma* infection (Chaga's disease), an otherwise endemic infection, indicating some protection from cancer by the infection. From a collection of case studies, it appears that this group also achieved interesting positive results⁸ (the book can be downloaded from <http://www.fevertherapy.eu>).

In the 1960s and 1970s, when it became apparent that chemotherapy, like radiotherapy before, can be a two-edged

sword in cancer therapy and Helen Coley-Nauts had published extensive reviews about her father's work, interest in Coley's method experienced a modest revival. Some remissions could be achieved, but overall, Coley's remarkable success rates could not be repeated. In retrospect, several most likely determining aspects of Coley's method were not considered: for instance, length and frequency of treatment as well as height of fever.⁹

To this day, there is no widely accepted molecular explanation for the remarkable regressions Coley without doubt had achieved.

Spontaneous Regressions

More than 1000 case studies on spontaneous regression and remission from cancer can be found in the literature.⁹ This, certainly, is only the tip of the iceberg, for several possible reasons. In some cases, the physicians responsible might not see a regressed patient again; in other cases, they might not rule out effective adjuvant treatment of some kind, or may not be literate enough or may not have taken the effort to write a report. If one reads publications on spontaneous regressions carefully, in a large number of cases an antecedent feverish infection is reported. However, in most cases, this correlation is reported only cursorily, without highlighting possible causality between infection and regression. Reviews on spontaneous regression reported this correlation for between 28%¹⁰ and 80%¹¹ of cases, again without elaborating on the correlation. These are most likely low estimates because many original authors might not have reported an antecedent infection.

Protective Effects of Acute Infections With Respect to Cancer

It is well known that about 15% to 20% of all cancers result from chronic viral infections.¹² However, chronic and acute infections are in this context entirely different entities: whereas chronic infections downregulate the immune system, acute infections upregulate it. The observed inverse correlation between feverish infection and spontaneous regression is related to acute, usually febrile and fully resolved, infections.⁹

If this time correlation is causal, it could be protective. Indeed, more than 30 studies confirm that a personal history of feverish infections provides some protection from developing cancer.¹³ We have no explanation for this inverse correlation other than that a feverish infection results in some pattern recognition receptor ligand (PRRL)-induced clearance from precancerous or transformed cells.

We hypothesize that these three observations—Coley's treatments, correlation between feverish infection and spontaneous regression, and inverse correlation between infection and cancer risk—share an immunological mechanism.

Discussion

An Immunological Interpretation

We proposed in 2008 that the molecular trigger responsible for spontaneous remissions, epidemiological protection, and Coley's results are pathogenic PRRs, in particular pathogen-associated molecular pattern, toll-like receptor (PAMP, TLR ligands).¹⁴ According to this hypothesis, Coley's toxin is a PAMP mix.

Pattern recognition receptors (PRRs), including transmembrane PRRs such as TLRs (TLR-1 to TLR-12) and C-type-lectin receptors (Dectin-1, Dectin-2) as well as intracellular PRRs (NOD1, NOD2, NALP3, ISD, RIG-1, MDA5) are preferentially found on and within professional antigen-presenting cells such as dendritic cells (DCs) and macrophages. These immune cells act at the interface of the innate and the adaptive immune system. On encounter with antigen and PAMP at the same time, DCs are activated (licensed), migrate to regional draining lymph nodes, where they in turn activate antigen-specific T- and B-cells. PAMP-activated DC also can mount NK (natural killer) cell helper responses in a dose-dependent manner.¹⁵

It is important to note that neither antigen alone nor PAMP alone is sufficient for DC activation. On the contrary, antigen presenting DC unengaged by PAMP or other PRRs are inducers of T-cell tolerance against the respective antigens. In contrast, T-cells activated properly through costimulatory DC signals such as CD80, CD86, ICOSL, CD137, and OX40 in combination with DC-generated cytokines undergo clonal expansion. They either differentiate into CD8+ cytotoxic T-cells (CTLs) able to kill infected (eg, virus) or cancer cells or differentiate into type 1 or type 2 T-helper cells (TH1 or TH2), depending on the pathogen. TH1 cells can induce B-cells to produce IgG antibodies; TH2 cells can induce B-cells to produce IgM and subsequently IgA, IgE, and IgG antibodies (humoral response).

Some T-cell types, when activated, can express TLR, thus becoming capable of interpreting PAMP on their own. This TLR expression, however, is transient and downregulated after a few days, unless they receive continuous PAMP stimulation.¹⁶ In mice, B-cells producing IgM, IgG1, and IgG2c can express TLR, and responses are largely TLR dependent, whereas IgE producing B-cells are TLR independent. In humans, analogous results have been found.¹⁷ It has been speculated whether TLR expression on cellular components of the adaptive immune system can provide additional checkpoints to monitor ongoing infection.¹⁸ These results suggest that to resemble a proliferative infection, continuous provision of PAMP is required.

In many cancer patients, tumor-specific T-cells can be found, indicating that neoplastic cells are, in principle, not invisible to the immune system. However, those T-cells usually are not fully activated but either enter a state of exhaustion, characterized by the expression of inhibitory

receptors such as PD-1 and cell cycle arrest because of persistent exposure to antigen as in the case of chronic disease,¹⁹ or tumor-specific T-cells enter a state of anergy, characterized by decreased expression of interleukin (IL)-2, interferon (IFN)- γ , and tumor necrosis factor (TNF)- α . Anergy can be caused by lack of correct costimulation through antigen-presenting cells (tumors do not produce PAMP) or by inhibitory stimulation from regulatory T-cells (Treg) or myeloid-derived suppressor cells (MDSCs) engaged by the tumor environment (tumor escape). Both anergy and exhaustion can be reversed *in vivo*.^{20,21} Naïve T-cell susceptibility to suppression by Tregs is controlled by PAMP.²² Infection in a cancer patient presumably can revive anergic or exhausted T-cells by PAMP-activated DCs, with different T-cell clones targeting pathogen and neoplastic cells. Repeated exposure to a collection of PAMPs can decrease MDSC numbers in cancer mice,¹³ potentially suppressing tumor escape.

The advantage of a PAMP fever therapy over other tumor-antigen-based experimental therapies is that particular tumor antigens neither have to be extracted nor enriched and not even characterized. PAMP-activated DCs are, as far as we know, not antigen-selective and collect all antigens indiscriminately, including tumor antigens. Therefore, antigenic drift, a problem for other tumor-antigen therapies, should not occur with augmented mistletoe therapy (AMT).

Recently, the manipulation of immune checkpoints such as CTLA-4 and PD-1 by antibodies has led to the expectation of an imminent revolution in clinical oncology.²³ Both CTLA-4 and PD-1 are expressed on Tregs; many tumors recruit large numbers of Tregs both inside and within the micro-environment. Blockade of CTLA-4 with the antibody drug ipilimumab improved survival in patients with advanced melanoma.²⁴ Blocking interaction between PD-1 and its natural ligand PD-L1 by antibodies such as nivolumab and pembrolizumab has demonstrated durable remissions in several forms of cancer and has been approved for the treatment of melanoma and non-small-cell lung cancer. However, these immunotherapies can lead to severe and even fatal toxicities, which require immunosuppression.²⁵ Similar adverse events have not been observed under mistletoe therapy (MT) or AMT so far.

PAMPs have widely been tested in clinical trials against cancer, without much success,²⁶ but only as single substances, for short periods, and mostly in patients with compromised immune systems. Arguably, these regimens were not inspired by any lesson learned from Coley's and Klyuyeva's seminal work. Rather, they follow the medicinal-chemical paradigm, which expects rapid visible benefit. In contrast, and in line with Coley's work, we proposed that PAMP should be applied in combination and over a number of weeks,¹⁴ preferably in patients with uncompromised immune function. In cancer-bearing mice, we found preliminary evidence supporting this hypothesis: tumor

development was slowed with single PAMP substances and cured with a PAMP combination.¹³ PAMPs were applied ten times over three weeks after tumor outgrowth, the equivalent of a month in humans, as was common practice in Coley's days. Combining PRRLs is also suggested by PRR studies: NOD1 stimulation can potentiate TLR responses upon *Helicobacter pylori* infection.¹⁸

Mistletoe and Bacterial Extracts in Present Day Practice

MT is used as adjuvant or palliative treatment for thousands of cancer patients each year in Europe. According to Schwabe Arzneiverordnungsreport (Springer, Heidelberg) ME has been applied about 10 000 times per day in Germany alone in 2015. Several reviews consistently report improved quality of life, improved tolerance of conventional cancer treatments, and limited survival advantage.^{27,28} Disease responses—that is, stable disease and remissions—are exceptions and presumably associated with higher than usual doses. Although early reports suggested a dosage optimum of mistletoe lectin (ML), with higher doses leading to immune suppression in mice,²⁹ this could not be confirmed in humans during two decades of clinical ME application. Therefore, the note on this alleged adverse effect was removed from the German MT-Leitlinien (guidelines).

Generally ME appears too weak an immune stimulator compared with bacterial extracts when applied as approved by subcutaneous (s.c.) injections. Nevertheless, ME seems to have some unleveraged potential when used multimodally (both s.c. as well as i.v. and i.t.), with high dosage, and for a prolonged period in immune-competent patients.^{30,31} In some German private clinics, the application of bacterial extracts has been retained to this day. Treatments have hardly been investigated systematically (see Jacobson et al³² for an exception), so average outcomes are unknown. Again, treatments are short compared with Coley's concept, and patients usually are immunocompromised by prior treatment. Regressions, let alone cures, seem to be rare, though not impossible.³³ Bacterial extracts used in these clinics are usually manufactured according to the German principle of "Therapiefreiheit" (§13.2 AMG), under which physicians, on a patient-by-patient basis, are allowed to manufacture therapeutic products in appropriate laboratories, and applied according to "Heilversuch" (individual compassionate use, §80 AMG). These extracts have recently come under increasing scrutiny by the authorities. Bacterial extracts in general face major approval obstacles. For instance, even for established fermentation in medicinal facilities, it is not easy to maintain constant batch-to-batch quality and composition. Thus, alternatives are required.

Mistletoe Extract Immunogenicity

ME has been shown to be remarkably immunogenic both in vitro³⁴ and in vivo.³⁵ Its strong immunogenicity has been a conundrum, but recent studies offer an explanation. The main immune stimulating component of ME is ML, a protein dimer with 254 (chain-A) and 264 amino acids (chain B). The 3D structure of mistletoe lectin chain-A is identical to shiga-toxin chain-A produced by *Shigella dysenteriae*,¹³ an often lethal bacterial toxin. Both amino acid sequences, however, have almost no homology. Mistletoe most likely in the far past has captured a bacterial toxin by horizontal gene transfer. Over time, the amino acid sequences of both proteins have evolutionarily diverged. This is an exceptional case of structural conservation over hundreds of millions of years. The adamant structural conservation of ML chain-A over eons indicates that this protein serves some important purpose for the plant, which is as yet unknown. ML has been shown to be a TLR ligand as well,³⁶ another indication for its pathogenic origin.

The manufacturer's instructions for all ME preparations recommend low-dose s.c. application to avoid significant reactions at the injection site (a limited local inflammation is tolerated) and avoid systemic reactions. Fever induction is only achieved with upfront s.c. application of higher than commonly used ME doses, in mistletoe-naïve patients, and can be typically repeated for two to three applications. During this initial time, ML induces production of neutralizing antibodies,³⁷ which might explain the decreasing fever-inducing capability. Fever kinetics differ between ME and bacterial extracts. Whereas fever induced by bacterial extracts or lipopolysaccharide (LPS) peaks two to three hours after injection and decreases over another six to 10 hours (endotoxin fever), fever induced by ME peaks after about 12 to 16 hours and falls back to a normal circadian pattern after about three days (mistletoe fever). This is true for commercial brands such as Abnobaviscum,³⁸ Helixor, and Iscador with s.c. application. From the perspectives of fever surveillance and patients' metabolic burden, shorter fever is preferable. It should be mentioned that Iscador applied i.v. off-label induces a LPS-like fever with endotoxin fever kinetics and does so over many applications without developing fever resistance. Iscador has a particularly high content of endotoxins because its manufacturing process involves fermentation.³⁹ As a corollary, we believe that ME, though clearly not potent enough to produce disease responses on its own and in low doses, might nevertheless be valuable as a basis to revive Coley's concept and success.

During a normal infection and in applying bacterial extracts, most likely several PAMPs play their role in concert in activating DCs. Our mouse experiments have shown that a mix of three PAMPs could eradicate established tumors, when applied repeatedly, whereas a single PAMP

Table 1. Selection of PRRL Substances and Manufacturers.

PRRL (Receptor)	Manufacturer	GMP/GLP
CRX-527, CRX-675 (TLR-4)	Corixa/GSK	GMP
CpG ODN (TLR-9)	Invivogen	GLP
dSLIM (TLR-9)	Mologen	GMP
E5564 (TLR-4)	Eisai	GMP
Imiquimod (TLR-7/8)	Invivogen	GLP
Loxoribine (TLR-7)	Invivogen	GLP
LPS (TLR-4)	Sigma/Medgamal	GLP/GMP
LTA <i>Staphylococcus aureus</i> (TLR-2/6)	Sigma	GLP
MALP-2 (TLR-1/2)	Enzo	GMP
MPL (TLR-4)	Corixa/GSK	GMP
PAM2CSK4 (TLR-2/6)	Invivogen	GLP
poly-AU (TLR-3)	Ipsen-Beaufour	GMP
poly-ICLC (TLR-3)	Hiltonol/Oncovir	GMP
poly-U _s 21+DOTAP (TLR-7)	Innate Pharma	GMP
Resiquimod (TLR-7/8)	Enzo	GMP
Vaximmune (TLR-9)	Coley-Pharma/ Aventis	GMP
Zyosan (Dectin-1, TLR-2)	Invivogen	GLP
Stimuvax (contains MPL, TLR-4)	Biomira/Merck	GMP

Abbreviations: PRRL, pattern recognition receptor ligands; GMP, good manufacturing practice; GLP, good laboratory practice.

could only slow tumor growth.¹³ Thus, we believe that ME could be augmented by other PAMPs and should be applied in a metronomic (high-frequency application) setting.

Several PRRLs can be purchased from high-quality manufacturers (good laboratory practice registered) for use in preclinical experiments; however, only a few PRRLs have good manufacturing practice quality registration. These are patented PRRLs, usually available only for clinical trials initiated by the manufacturers and, therefore, not available in larger quantities for the general public (see Table 1). In addition, use of PRRLs in humans must be preceded by extensive preclinical tests in laboratory animals, requiring years of preclinical work.

To bypass these obstacles, we suggest that MT should be augmented with approved drugs (augmented mistletoe therapy, AMT). These drugs should, according to their directions of use leaflets, contain PAMP and induce fever as an usually unwanted, but in this context “on target,” side effect (see Table 2 for a selection of drugs). We suggest that there should be adherence to a regimen that respects the main lessons we can learn from Coley’s and Klyuyeva’s experiments—namely to apply AMT frequently over longer periods, for example, two to three times per week over several weeks. In principle, this approach could be considered in a primary treatment setting (ie, neoadjuvant) rather than as adjuvant or palliative therapy alone. Although this might appear to be a far-fetched goal, we believe that it should not

be impossible to reclaim Coley’s successes, now with a much better immunological understanding.

From individual cases, we have preliminary indications that bacterial extracts can partially be replaced by products listed in Table 2, both with respect to fever induction in humans (see Table 3) and disease responses. In two cases, AMT (using Freund’s adjuvant) was given intratumorally, with convincing disease responses. In a third case, an elderly gentleman with a large axillary metastasis of melanoma was treated with multimodal MT (both i.v. and i.t.), during which the tumor continued to increase; when MT was combined with Colibiogen for i.t. injection over several weeks, the tumor reduced steadily (submitted for publication).

Response Criteria

After treatment with bacterial extracts, in vascular, ulcerating, or fungating tumors, rapid degradation can occur, often with sloughs. In less-vascular tumors, changes observed are softening and reduction in size. Following i.t. injection, a transient increase in size, with skin becoming red and tense, may be observed. Transient tumor size increase is a common observation in immunotherapy and led to revised RECIST (response evaluation criteria in solid tumors) response criteria called immune-related response criteria (irRC).^{40,41} An activated immune reaction can lead to a massive influx of immune cells into the tumor (tumor-infiltrating lymphocytes), comprising up to 40% of tumor volume. More tumor-infiltrating lymphocytes correspond with better prognosis.^{42,43} This influx can lead to a temporary enlargement, which, without biopsy, can hardly be distinguished from malignant growth. It has been observed that final tumor responses after immunotherapy can happen after a time lag of up to 12 weeks, so one of the main differences between RECIST and irRC is to wait up to 12 weeks before a decision on treatment continuation or discontinuation is taken. Physicians experienced in bacterial fever therapy gauge positive response by general patient condition, for example, pain decrease, improvement of blood markers, and improved energy and/or mobility and appetite. In cases of palpable tumors softening is a sign of response. We would expect similar observations during AMT treatment.

Fever will be accompanied by a marked leukocytosis and other markers of acute phase response. Increased cytokine markers of the innate immune system include TNF- α , IL-1, IL-1 β , IL-6, IL-12, and IFN- γ . Inflammatory markers and markers of immunosuppression such as IL-10 and TGF (transforming growth factor)- β should decline over time. The so-called neutrophil-lymphocyte ratio should fall below 4.⁴⁴ These laboratory markers for innate stimulation can be monitored over the first weeks and might indicate beneficial treatment response; however, this has to be investigated further.

Table 2. Approved Drugs Most Likely Containing PRRLs, Judged by Content and Side Reactions Described in the Respective Instruction Leaflets.

Brand	Manufacturer	Ingredients	Main Indication	Fever Reported as Adverse Event	Approved for Cancer Therapy
BCG vaccine	CC-Pharma Medac	Attenuated live <i>Mycobacterium bovis</i>	Vaccine	Yes	Yes
Broncho-Vaxom	Eurim Pharm	Lyophilized bacterial extract from <i>Haemophilus influenzae</i> , <i>Diplococcus pneumoniae</i> , <i>Klebsiella pneumoniae</i> and <i>K ozeanae</i> , <i>Staphylococcus aureus</i> , <i>Streptococcus pyogenes</i> and <i>viridans</i> , <i>Neisseria catarrhalis</i>	Respiratory infections	No	No
CADI05	Immuvac	Autoclaved <i>Mycobacterium indicus pranii</i>	Leprosy	Yes	Yes
Cholera vaccine	Wyeth	Inactivated cholera bacteriae	Vaccine	Yes	No
Colibiogen inject	Laves	Metabolic products from <i>Escherichia coli laves</i>	Colon infections	No	Yes
Detox	Biomira Inc	MPL (monophosphoryl-lipid-A) and cell wall extracts from <i>Mycobacterium phlei</i>	Adjuvant	Yes	No
Flulaval	GSK	Inactivated influenza virus	Vaccine	Yes	No
Iscador	Weleda	Mistletoe extract	Immune stimulant	Yes	Yes
Ixiaro	Novartis-Behring	Inactivated Japanese encephalitis virus	Vaccine	Yes	No
JE-VAX	Sanofi-Pasteur	Inactivated Japanese encephalitis virus	Vaccine	Yes	No
Lektinol	Rottapharm	Mistletoe lectin	Cancer	Yes	Yes
MPL	Corixa	MPL from <i>Salmonella minnesota</i>	Adjuvant	No	No
Picibanil	Chugai	Lyophilized <i>Streptococcus pyogenes</i>	Cancer	Yes	Yes
Pollinex	Bencard	Pollen allergens and MPL	Allergies	No	No
Polyvaccinum forte	IBSS biomed (Poland)	Inactivated extract from <i>Staphylococcus aureus</i> , <i>Staphylococcus epidermidis</i> , <i>Streptococcus salivarius</i> , <i>Streptococcus pneumoniae</i> , <i>Streptococcus pyogenes</i> , <i>Escherichia coli</i> , <i>K pneumoniae</i> , <i>H influenzae</i> , <i>Corynebacterium pseudodiphtheriticum</i> , <i>Moraxella catarrhalis</i>	Chronic and recidivistic inflammatory process of the respiratory tract, bladder and endometrium	Yes, up to 8 hours	No
Pyrogenalum	Medgamal (Russia)	LPS from <i>Salmonella typhi</i>	Nerve trauma, prostatitis, urethritis, uveitis, latent TBC	Yes, up to 8 hours	No
StroVac	Strathmann	Inactivated <i>Escherichia coli</i> , <i>Morganella morganii</i> , <i>Proteus mirabilis</i> , <i>K pneumoniae</i> , <i>Enterococcus faecalis</i>	Recidivistic bladder inflammation	Yes, up to 40°C	No
Typhoral	Novartis-Behring	<i>Salmonella typhi</i> ; apathogenic live germs	Vaccine	Yes	No
YF-VAX	Sanofi-Pasteur	Yellow fever virus	Vaccine	Yes	No
Zylexis	Pfizer	Inactivated <i>Parapoxvirus ovis</i>	Veterinary drug, immune stimulant	No	No

Abbreviations: PRRL, pattern recognition receptor ligands; LPS, lipopolysaccharide.

Table 3. Partial Replacement of Bacterial Extracts by Approved Drugs in About 80 Patients: Fever Induction.

	Fever Induction
Bacterial extract	+++
Strovac	–
Strovac + reduced amount of bacterial extract	++
Strovac + Colibiogen + reduced amount of bacterial extract	+++
Mistletoe extract	+
Mistletoe extract + Colibiogen	+

Risk of Severe Adverse Reactions

Life-threatening pathogenic infections are usually accompanied by high fever, whereas successful containment and removal of pathogens by the immune system aligns with fever decline; so fever is often felt to be “guilty by association.” Cancer treatments using cytokines such as TNF, ILs, and IFNs were sometimes associated both with fever and severe adverse reactions.⁴⁵⁻⁴⁷ In these experiments, cytokines, which physiologically are excreted locally in very small amounts, were applied systemically in large nonphysiological amounts—a situation distinct from the application of PRRLs, upon which the immune system can regulate cytokine production and distribution itself. Hence, unease with inducing fever may be understandable. Yet fever induced by PRRL-containing vaccines does not persist in the same manner as fever caused by a progressive infection. The deeply rooted suspicion of fever in the minds of many physicians is not justified. Treatment-related elevation of body temperature will come down in a predictable fashion and dose dependently. Dosage will be decided on a per-patient basis, starting with very low dosage. The final therapeutic dosage can be determined in such a way as to shape rise and decline within eight to 12 hours. Physicians with long experience in the application of bacterial extracts report that the first few fever inductions can be burdensome, yet alleviated by aids such as hot-water bottles, whereas later inductions are more reconcilable.

Over 30 years and over several thousands of applications, Coley reported six treatment-related fatalities in his own department and three more from colleagues. He assumed that these nine cases were “probably or possibly” caused by the treatment with bacterial extracts; all patients had inoperable late-stage tumors. Two i.v.-injected patients died from embolism; three patients got a too high initial dosage, one of the three directly into the tumor; three patients died from kidney failure, most likely caused by tumor lysis syndrome; in one case, a second injection was given during high fever. Hence, six out of the nine fatal results could have been avoided. The three cases of assumed tumor lysis syndrome likely would have been treatable in

modern clinical settings. German physicians, who applied fever therapy in hundreds of cancer patients using Vaccineurin off-label in the 1970s and 1980s, and at that time approved drug containing bacterial extracts, did not report a single fatal event. In one case, an anaphylactic reaction was observed after initial i.v. application of a bacterial extract at high dosage (personal communication to UH), whereas Coley recommended starting with s.c. injections and low i.v. doses. Again, the natural course of a pathogenic infection should be borne in mind, where systemic pathogenic load does not increase in an instant, but gradually.

If all relevant aspects are considered, although fever therapy is a considerable treatment burden and needs careful medical monitoring by experienced physicians, it is unlikely to do harm. Fever is an evolutionarily conserved mechanism, in the service of maintaining species survival over million years. Yet any therapy involving fever, including AMT, will be no walk in the park. It requires a motivated patient and a dedicated physician.

Implementing Augmented Mistletoe Treatment

Health insurances in Germany and Switzerland either do not cover inpatient treatment with the explicit aim of mistletoe treatment induction or cover only for a limited time period of one to three weeks, depending on health insurance. During this time frame, starting with a very low dose, the goal would be to find the dose appropriate for each patient. Outpatient treatment is feasible, provided appropriate medical backup to monitor treatment reactions is available. Ideally, treatment should start before surgery, when tumor antigen levels can be expected to be high. Postsurgery treatment should range from a minimum of two weeks to an optimum of three to eight weeks and even more for large inoperable tumors. Regular treatment boosts of one to two weeks’ duration each might be advisable. Whereas dosages for ME alone or for drugs listed in Table 2 are well established, combining these drugs may require some adjustment.

Patients who are unable or unwilling to accept the treatment burden of two to three high fever applications per week might still benefit from one fever-inducing application per week and one or two subfebrile low-dosage applications to keep the innate immune system stimulated. Although Coley emphasized induction of high fever as a prerequisite for successful therapy, it may turn out that with optimized AMT, an intermittent subfebrile PAMP application may be beneficial as well.

Conclusions

The lessons that can be learned from experimental fever-inducing vaccinations against cancer done about 100 years ago (Coley) are very clear, yet have not been developed into

present therapies. It is now clear that the innate immune system must be harnessed robustly to fully activate tumor-specific T-cells by PAMP-alerted DC. We should stimulate frequently over longer periods because the innate immune system has no memory and requires to be kept alert by metronomic stimulation. This approach contrasts with mainstream treatments that aim for rapid disease response (“hit fast and hard”), for example, using antibiotics against bacterial pathogens or chemotherapy against cancer. The conventional paradigm and a widespread aversion against fever are in our eyes the main reasons why the Busch-Coley treatment never has received the scrutiny it deserves.

It is not yet established whether high fever is a critical requirement for successful AMT because some remissions induced by ME did not involve prolonged fever periods^{30,31}; yet fever helps to increase the level of tumor antigens^{48,49} (the same might be true for hyperthermia⁵⁰⁻⁵³) and activates DCs.⁵⁴

The present mistletoe treatment regimen is located between the two extreme paradigms: the pharmacological paradigm on one hand and Coley’s very long immune stimulations on the other. Traditional ME is usually given at low doses (avoiding febrile reactions) in a low-frequency setting over long periods of time (years) and assumes a cumulative benefit in immune competence. Coley’s results indicate that a high-frequency setting under appreciation of fever over weeks is preferable. With higher initial ME doses, fever reactions can be elicited over the first few treatments, and experienced physicians in some clinical centers apply this, often in a multimodal setting (eg, s.c., and i.v. and i.t.). Maintaining fever reactions beyond this time window of fever readiness is possible if ML is combined with PAMPs. ME alone contains at least one PAMP in substantial amounts—namely, mistletoe lectin; common brands in addition contain endotoxins and viscotoxins, likely contributing to the overall immune stimulation ME provides. The application of several PAMPs at once, rather than a single PAMP, has been shown to induce synergistic effects,⁵⁵⁻⁶⁰ leading to complete remissions in cancer-bearing mice¹³; therefore, augmentation of mistletoe lectin seems obvious. Several approved PAMP-containing drugs are available, where fever is reported as a common side reaction in the respective instruction leaflets (see Table 2); in this context, this side reaction has to be interpreted as welcome rather than adverse. These drugs could be tested for ME augmentation, and they are very low cost compared with other cancer treatments. We suggest using MT as a basis for applying a PAMP cocktail because MT is one of the few cancer therapies where a PAMP substance (ML) is causally involved and because there is a rich body of experience with ME in central Europe; thus, respective physicians know how to handle fever inductions running over long time periods.

To the best of our knowledge, there is no principal obstacle to repeat Coley’s successful treatments, now

with a much better understanding on the molecular level and improved technologies for therapy surveillance and progress monitoring. Whereas MT is usually applied in an adjuvant or palliative setting, AMT might even be applicable as neoadjuvant treatment in some settings and possibly primary cancer therapy in amenable forms of cancer.

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