Coley's immunotherapy revived: Innate immunity as a link in priming cancer cells for an attack by adaptive immunity

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There is no doubt that immunotherapy lies in the spotlight of current cancer research and clinical trials. However, there are still limitations in the treatment response in certain types of tumors largely due to the presence of the complex network of immunomodulatory and immunosuppressive pathways. These limitations are not likely to be overcome by current immunotherapeutic options, which often target isolated steps in immune pathways preferentially involved in adaptive immunity. Recently, we have developed an innovative anti-cancer immunotherapeutic strategy that initially elicits a strong innate immune response with subsequent activation of adaptive immunity in mouse models. Robust primary innate immune response against tumor cells is induced by toll-like receptor ligands and anti-CD40 agonistic antibodies combined with the phagocytosis-stimulating ligand mannan, anchored to a tumor cell membrane by biocompatible anchor for membrane. This immunotherapeutic approach results in a dramatic therapeutic response in large established murine subcutaneous tumors including melanoma, sarcoma, pancreatic adenocarcinoma, and pheochromocytoma. Additionally, eradication of metastases and/or long-lasting resistance to subsequent re-challenge with tumor cells was also accomplished. Current and future advantages of this immunotherapeutic approach and its possible combinations with other available therapies are discussed in this review.

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Introduction

The history of cancer immunotherapy spans more than 100 years from the earliest forms using bacteria for immune system activation to current, highly sophisticated immune checkpoint inhibitors or monoclonal antibody therapy [1,2]. Despite the potential of modern medicine and science, cancer treatment remains challenging, and no universal approach and solution exists. The first immunotherapy was pioneered by William Coley, who stimulated the immune system by using intratumoral injections of the inactivated gram-positive bacteria Streptococcus pyogenes and gram-negative bacteria Serratia marcescens – so called 'Coley’s toxins' [3]. Although the underlying mechanism was not understood at that time, Coley achieved an immune response against sarcomas, resulting in a strong tumor burden reduction and even tumor elimination in some patients. Later on, a more clear understanding of this treatment response came through the discovery of pathogen-associated molecular patterns (PAMPs) and their receptors (pattern recognition receptors, RRs) [4].

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Subsequently, the discovery of various new groups of tumor antigens and their matching receptors on immune cells opened another door to cancer immunotherapy and represented a promising avenue for cancer elimination and recurrence prevention [5]. However, some discouraging results from vaccine-based clinical trial studies [6,7] led to the discovery of new immunosuppressive mechanisms by which tumor cells provide unique and strong protection against immune attack [8,9]. Thus, current cancer immunotherapies are heavily focused on the elimination of immunosuppressive mechanisms, specifically on the use of checkpoint inhibitors and activators [10,11]. However, their systemic application is associated with lower efficacy (only about 20% of patients overall respond to these drugs in some cancers [12]) and a simultaneous risk of autoimmunity [13]. In contrast to some current immunotherapy trends heavily oriented towards adaptive immunity, here we present a new immunotherapeutic approach tested in several different types of mouse tumor models, which affects the immune system on several levels with initial activation of innate immune followed by activation of adaptive immunity. This immunotherapy is predicted to be highly effective in a broad range of tumors, including immunologically “cold” tumors which are challenging for most current immunotherapies.

The immune system in cancer pathogenesis

The immune system is the body’s defense system protecting against infection and diseases. It can be categorized into 2 cooperative arms: (1) nonspecific, innate immunity and (2) specific, adaptive immunity [14].

Innate immunity represents the first line of host defense and provides nonspecific protection through several mechanisms, including physical barriers, such as epithelial cells with close cell-cell contact, as in the gastrointestinal tract, respiratory tract and genitourinary tract. Innate immunity also involves proteins and bioactive small molecules either permanently present in biological fluids, like complement proteins and defensins, or secreted upon activation, like cytokines, chemokines, lipids, and enzymes. Complement proteins represent a far-reaching and potent mechanism of innate immune defense. Complement is a hierarchical system of more than 30 plasmatic and surface proteins involved in initial pathogen identification, which results in the release of proinflammatory mediators, pathogen opsonization, and targeted lysis of the pathogen surface [14].

The last and very crucial component of the innate immunity defense involves receptors or cytoplasmic proteins binding specific molecular patterns (ligands) expressed by invading microbes. Recognition of these patterns (PAMPs), by innate immune cell receptors (PRRs), results in the release of inflammatory mediators and subsequent elimination of pathogens by attracted effector immune cells [4].

Adaptive immunity, the second arm of the immune system, is specific for its targeted antigens. The adaptive response is mainly based on the activation of T and B cells. The effector portion of adaptive immunity can be divided into 2 main parts, humoral immunity involving the production of antibodies by B cells and cell-mediated immunity involving the action of T cells. Moreover, adaptive immunity provides immunologic memory, which enables rapid and often long-lasting responses in the case of re-exposure to the same pathogen [14].

The role of the immune system in cancer pathogenesis has been intensively studied for many decades and is usually referred to as cancer immunosurveillance [15]. The relationship between tumors and immunity is based on a delicate balance that controls whether a tumor will progress or will get eliminated. This unique balance is known as immunoeediting [15,16]. All immune cells or immune mechanisms interact with a tumor in specific ways, and their role is mostly ambivalent regarding tumor progression or elimination [17].

Innate immunity can affect tumors on several levels. Various studies have demonstrated that tumor cells are able to activate complement, which can lead to their elimination and to enhancement of T and B cell activation [18]. However, complement can also demonstrate a pro-tumor effect when its activation increases vascular permeability, histamine release, and the generation of radical oxygen species (ROS), thereby promoting a microenvironment favorable for tumor progression [19,20]. Natural killer (NK) cells, another type of innate immune cells, are able to recognize tumors based on their aberrant or reduced major histocompatibility complex class I (MHC I) [21]. However, even here, the potential role of NK cells in tumor progression is questionable, since NK cells can be a source of IFN-gamma and promote resistance to adaptive immunity [22]. Neutrophils, the most abundant innate immunity granulocytes, are known to promote cancer progression through granular proteases supporting tumor growth, invasion, and spread of metastases [23]. Nevertheless, based on studies with neutrophils isolated from human donors, they also possess some unique mechanisms to recognize and eliminate tumor cells [24]. Dendritic cells (DCs) represent an important and unique communication bridge between innate and adaptive immunity. DCs might also be polarized by the tumor microenvironment into cells with pro-tumorigenic and anti-tumorigenic functions. The most obvious anti-tumorigenic function is their ability to present tumor antigens to T cells and so initiate their activation, however, their polarization into an immunosuppressive subset of regulatory DCs may have a significant effect on tumor growth and progression [25]. Typical tumor polarization can also be observed in macrophages. Macrophages infiltrating tumors, so-called tumor-associated macrophages (TAM), can be classified based on their function as M1 and M2 macrophages. M1 macrophages exert an anti-tumor function, whereas M2 macrophages are associated with pro-tumor features [26].

Like innate immunity, adaptive immunity also affects tumor pathogenesis, mainly via tumor neoantigens presented by antigen presenting cells (APCs) to T and B cells. This can result in T cell-mediated lysis of cancer cells or in the formation of tumor-specific antibodies. However, here the tumor microenvironment can also cause polarization of adaptive immune cells. This can lead to the accumulation of suppressive regulatory T and B cells (Tregs, Tbregs) in a tumor, where their presence correlates with tumor progression [27,28].

From the examples discussed above, it is clear that the immune system has an important function in tumor pathogenesis. Both arms have an unquestionable ability to recognize and eliminate tumor cells. However, immune cells are constantly challenged by multiple tumor immune escape mechanisms. These mechanisms can establish an immunosuppressive environment in tumors by promoting the production of cytokines such as interleukin 10 (IL-10), transforming growth factor beta (TGF-beta), vascular endothelial growth factor (VEGF), or immunoregulatory molecules such as programmed cell death 1 (PD-1), programmed cell death 1-ligand (PD-L1), and indoleamine-2,3-dioxygenase (IDO) [29]. The effect of an immunosuppressive environment on immune cells with their subsequent polarization towards a pro-tumorigenic function was already discussed previously (M2 macrophages, regulatory DCs, Tregs, and Tbregs). Simultaneously, tumor cells can also alter their antigenicity (disable recognition by APCs) [29], release tumor-derived exosomes (leading to downregulation of T cell and NK cell-mediated antitumor immunity) [30], or use specialize tumor stroma to pose an additional immune challenge [31].

Despite all these tumor immunosuppressive mechanisms, there is a strong potential in using the immune system as a tool for cancer treatment. However, we have to fully understand and be aware of this fragile relationship between tumors and the
immune system in order to develop an effective therapeutic strategy.

Activation of innate immunity as an initial step of successful immunotherapy

Innate immunity represents an old and conserved evolutionary mechanism of body protection. Since the tumor environment and tumor cells themselves are very unstable with constant changes in antigenicity, the nonspecific and rapid recognition that innate immunity uniquely offers represents a major advantage compared to the specific and delayed adaptive immune response. Therefore, activation of innate immunity could be the optimal initial therapeutic option for a broad range of tumors.

Our confidence that innate immunity may be a potent tool for initial cancer treatment arises from a study where Cui and his colleagues described a mouse model with a unique mutation, SR/CR, where innate immune cells showed a remarkable ability to recognize and eliminate a broad range of tumors [32]. This brought us to the idea that effective recognition of tumor cells by innate immunity could result in a strong antitumor effect and lead to the elimination of the tumor. Since innate immune cells can demonstrate difficulty in tumor cell recognition, we decided to support this recognition by artificially anchoring PAMPs to the tumor cell surface. Such tumor cell opsonization leads to their effective recognition and elimination by phagocytic cells [33]. After a series of optimization experiments, we determined the critical importance of combining two groups of PAMPs, anchored ligands stimulating phagocytosis and soluble toll-like receptor (TLR) ligands. This combination results in strong tumor infiltration by inflammatory cells [33] with subsequent extensive primary tumor burden reduction and, in most of the experimental animals, even a complete eradication of tumors [33-36].

This therapy is directly targeted via intratumoral application, which significantly decreases any side effects and results in the concentration of administered substances needed for effective activation of immunity. This proposed immunotherapy corresponds well with a new paradigm of intratumoral immunization [37-39]. Detailed steps of our immunotherapy paradigm as well as results related to the treatment of various tumors using this immunotherapy approach are summarized in subsequent sections.

Artificial opsonization of tumor cells by ligands stimulating phagocytosis

The concept of tumor cell artificial opsonization by phagocytosis-stimulating ligands originated from the essential nature of various ligands in the process of pathogen recognition by the innate immune system [40]. Thus, several individual ligands stimulating phagocytosis were tested in an effort to induce a strong anti-cancer innate immune response, namely laminarin, mannan, formyl-methionyl-leucyl phenylalanine (f-MLF), and zymosan A. Simultaneously, heat killed bacteria (Mycobacterium tuberculosis and Stenotrophomonas maltophilia), representing a complex group of ligands stimulating phagocytosis, were tested as well [34]. Even though whole bacteria present a complex combination of PAMPs (including multiple phagocytosis-stimulating ligands) and should logically result in strong immune system stimulation, the use of individual ligands stimulating phagocytosis was found to yield a more potent immune anti-tumor response. Specifically, with mannan, we achieved the best therapeutic effects.

The identification of the most potent ligand stimulating phagocytosis was just the first step in the successful artificial opsonization of tumors by PAMPs. The mechanism of anchoring phagocytosis-stimulating ligands to tumor cells also played a key role. We tested various methods of anchoring phagocytosis-stimulating-ligands, namely charge interaction, use of the hydrophobic anchors BAMDope (biocompatible anchor for membrane/dioleylphosphatidylethanolamine), and covalent binding based on the heterobifunctional crosslinker SMCC (succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate) [33-36]. The best results were achieved with a BAM anchor containing one fatty acid aliphatic chain.

The anti-cancer effect is dependent on the recognition of mannan anchored by BAM (mannan-BAM) (Fig. 1A) via mannan binding lectin (MBL) (Fig. 1B). This recognition process is complement dependent (does not work in heat-inactivated serum); however, it is not the final membrane attack complex that kills the tumor cells. Mannan-BAM triggers lectin complement pathway activation and results in iC3b opsonization of tumor cells. This tumor cell opsonization attracts the attention of phagocytic cells, mainly neutrophils, and those eliminate tumor cells by a process called frustated phagocytosis. During frustrated phagocytosis, neutrophils, in an attempt to ingest objects that are too big to be internalized, form clefts or pockets filled with enzymes and products of oxidative burst. These clefts and pockets, located in the space between the neutrophil and the target, lead to the destruction of the target [34,36]. Even though neutrophils were identified as key cells in this therapy, macrophages and NK cells are also able to recognize iC3b opsonized tumor cells (Fig. 1C) and participate in their elimination [33-35].

TLR ligands in the therapeutic mixture: The right combination and the right timing

Ligands stimulating phagocytosis (mannan) are not the only important component of presented immunotherapy. To stimulate massive tumor inflammatory infiltration, mannan must be combined with other PAMPs/TLR ligands. After extensive studies, we demonstrated that it was essential to use mannan with a mixture of several TLR ligands to achieve the best tumor infiltrating results, specifically a combination of resiquimod (R-848) (TLR7/8 ligand), polyinosinic:polycytidylic acid (poly(I:C)) (TLR3 ligand) and lipoteichoic acid (LTA) (TLR2 ligand) [35].

Moreover, TLR ligands require a specific application schedule to prevent the development of TLR resistance. Accordingly, we focused on the optimization of a precise delivery scheme, which resulted in a consensus of 5-day-long gaps between 3-day application pulses. Our findings regarding the application complexity of TLR ligands and the risk of resistance are consistent with Trinchieri and Sher's previous reports [41] as well as with the findings of Bourquin et al. [42]. We are confident that the correct timing of TLR ligands is necessary not only to ensure the innate immunity-based attack but also to initiate involvement of adaptive immunity. The application scheme mimics a vaccination scheme and can provide the required time and conditions for tumor antigen presentation and transition of the signal to adaptive immunity.

Adaptive immunity involvement as a second step of effective immunotherapy

Although this immunotherapy approach is based on the initial activation of innate immunity, there is undeniable evidence of adaptive immunity involvement. This can be explained by the interaction of multiple factors, which create ideal conditions for innate and adaptive immune cell communication. Phagocytic cells, involved in the initial stage of the therapy, present antigens to T cells in draining lymph nodes. Moreover, TLR ligands, besides their positive effect on tumor leukocyte infiltration, also promote maturation of DCs. DCs are known for their key role in antigen presentation and adaptive immunity activation.
Furthermore, we also observed strong IFN-gamma production and establishment of the type 1 T helper cells (Th1) immune response [34,36].

The involvement of adaptive immunity was verified both in vitro and in vivo. In vitro quantification assessed the response of CD4+ and CD8+ cells to antigenic stimulation [35]. In vivo verification focused on resistance to tumor cell re-challenging experiments [34,35] and measurement of anti-metastatic effects in mouse models [35,36].

**Adaptive immune activation is dependent on the tumor environment and can be increased by modification of the therapeutic mixture**

Our immunotherapy approach revealed excellent therapeutic results in murine melanoma B16-F10 and murine sarcoma S-180 models [33-35]. Subsequently, this approach was tested in a murine pancreatic adenocarcinoma model (Panc02) and pheochromocytoma model. In these models, we also observed a strong initial reduction of tumor growth; however, there was a decreased effect on survival when compared to previously tested murine models [35,36]. This result in the pancreatic adenocarcinoma model was expected, since this tumor is considered to be highly resistant against any kind of treatment [43]. Also, pheochromocytomas, specifically metastatic pheochromocytomas, are resistant against conventional treatments [44]. Moreover, there is very limited knowledge about the immunology of pheochromocytomas, with isolated reports describing a low neoepitope burden and low mutation burden, which may suggest a low immunogenicity of these tumors [45,46].

It can be concluded that the initial phase of the innate immune attack is not dependent on the tumor type; however, the subsequent activation of adaptive immunity and its effect on survival is dependent on the tumor environment, which is specific for each tumor type.

To address the effect of the tumor environment on adaptive immunity activation in pancreatic adenocarcinoma and subsequent partial resistance against tested immunotherapy, we decided to modulate this tumor environment by combining immunotherapy with immune checkpoint inhibitors or immune activators. Since the pancreatic adenocarcinoma model is known to have high levels of infiltrating Tregs [47], we used anti-CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) for their depletion to see if this led to a significant difference in response to the tested immunotherapy. Although anti-CTLA-4 added to the therapeutic mixture improved the initial reduction of tumor growth, the survival of the tested mice was not affected.

In the second step, anti-PD-1 antibody was tested. Anti-PD-1 is a checkpoint inhibitor positively affecting T cell activation and function [48]. However, anti-PD-1 antibody did not have any effect on tumor growth or survival of the animals. This failure can be explained by a low expression of PD-L1 in pancreatic adenocarcinoma cells [49].

Subsequently, anti-CD40 (immunostimulant) was tested in pancreatic adenocarcinoma and pheochromocytoma murine models to improve the activation of the adaptive immunity. Anti-CD40 is an agonistic antibody that mimics CD40 ligands (CD40L, CD154) expressed on CD4+ cells. Anti-CD40 binds to CD40 receptor which are present on DCs and other myeloid cells [50]. Specifically, the ligation of CD40 with CD40 receptor on DCs leads to the upregulation of MHC molecules, CD80/CD86 costimulatory molecules, and the production of IL-12. This DC activation enhances antigen presentation and induces an effective T cell based anti-tumor response [51,52].
The enhancement of the therapeutic mixture with anti-CD40 resulted in 80% complete elimination of pancreatic adenocarcinoma tumors with resistance against tumor re-challenging after 120 days [35]. In the pheochromocytoma mouse model, anti-CD40 enhancement resulted in 62.5% complete elimination of subcutaneous tumors with complete resistance against tumor re-challenging. Additionally, the enhanced effect of mannan-BAM + TLR ligands + anti-CD40 therapy on activation of the specific part of immunity was also verified in metastatic pheochromocytoma. In metastatic pheochromocytoma, significant growth reduction and even complete elimination of distant liver lesions was observed and was dependent on the presence of CD8+ T cells [36]. The main mechanisms of the enhanced therapeutic mixture are shown in Fig. 2.

Overall, the combination of mannan-BAM + TLR ligands with checkpoint inhibitors (anti-CTLA-4, anti-PD-1) did not show improvement in the therapeutic outcome compared to anti-CD40. This can be explained by the fact that checkpoint inhibitors mainly target isolated steps in the adaptive immune response cascade with lack of innate immune system stimulation. On one hand, the therapeutic response to checkpoint inhibitors is dependent on previously activated innate immunity and on the presence of tumor immunosuppressive mechanisms. On the other hand, anti-CD40 acts simultaneously on the level of the innate and adaptive immunity, which supports the immune response in a more complex matter. Moreover, the synergy of anti-CD40 with R-848 and poly(I:C) that results in extensive expansion of APCs and CD8+ memory cells is another example of the advantage of anti-CD40 compared to checkpoint inhibitors [53].

Application of CD40 antibodies and FDA-approved TLR ligands in cancer therapy

Interestingly, several innate immunity-based immunotherapies including above mentioned TLR ligands and anti-CD40 antibodies are currently being tested in clinical trials or used in clinical practice.

Six anti-CD40 antibodies have been investigated in clinical trials: ADC-1013 (Janssen/Alligator), APX005M (Apexigen), CDX-1140 (Celldex), Chilob7/4 (University of Southampton), SEA-CD40 (Seattle Genetics), and Selsecrelumab (Roche) [54]. However, most of anti-CD40 antibodies alone have shown minimal rates of objective tumor response in patients. On the other hand, the combination of anti-CD40 with either chemotherapy (carboplatin, paclitaxel, gemcitabine) or immunotherapy (CTLA-4, PD-1, PD-L1 antibodies) has shown more promising results compared to anti-CD40 antibodies or chemotherapy alone [54].

In the case of TLR ligands, 3 types are already used in cancer therapy. Imiquimod, a TLR7 agonist, has been approved by Food and Drug Administration (FDA) as a therapeutic agent for basal cell carcinoma [55]. Bacillus Calmette-Guérin (BCG) has been shown to potently activate TLR2 and TLR4. This attenuated strain of Mycobacterium bovis is used as an immunotherapeutic agent against bladder carcinoma [56,57].

Monophosphoryl lipid A, a modified derivate of Salmonella minnesota endotoxin and a TLR4 agonist, has also been approved by FDA as an adjuvant for use in vaccines against human papillomavirus type 16 and 18 (Cervarix®) [58,59]. Moreover, many TLR ligands have been investigated in clinical trials and have been reviewed elsewhere [60-63].

Potential combination with other therapeutic approaches

Combination therapy is becoming the hallmark of successful cancer treatment. The complexity of this disease requires complex treatment solutions, especially in the case of enlarged inoperable tumors or multiple organ lesions (metastases). The described immunotherapy based on PAMPs-related artificial opsonization of tumor cells could potentially be combined with stereotactic radiotherapy, radiofrequency ablation, cryoablation, and/or chemotherapy with significant beneficial results.

The most important factor that has to be considered before the application of combination therapy is the right timing and order of the individual therapies. Even though, there are no conclusive data in this research area, it is clear that the mechanisms of action of each individual chemotherapeutic agents and immunotherapeutic modulators has to be considered prior their combination to ensure the ideal timing. Since chemotherapy and ra-
diotherapy may have significant negative impacts on the viability of immune cells [64,65]. We envisage that the application of the described immunotherapy prior to chemotherapy or radiotherapy will achieve the best therapeutic results. Prior application of immunotherapy based on artificial opsonization of tumor cells by PAMPS will vaccinate the organism against tumor antigens and support the development of memory cells specific to these antigens. Memory cells are known for their increased resistance against toxicity caused by radiotherapy [66] or chemotherapy [67,68] since they are in a quiescent G0 state hidden in the body [69]. There are several studies conducted in humans or animal models which can possibly support this view. Based on the results from the clinical studies, patients initially treated with immunotherapy (antigen pulsed DCs) followed by chemotherapy had better clinical outcomes than patients who received chemotherapy alone [70,71]. In animal models, chemotherapy (gemcitabine + cisplatin) given after immunotherapy (adenoviral vector expressing IFN-a) had also better antitumor effect than either chemotherapy or immunotherapy alone [72].

On the other hand, when immunotherapy introduced by our group will be used in the opposite sequence (immunotherapy that comes after chemotherapy and radiotherapy), the immune system may be more or less impaired by physical or chemical attack, which prevents its effective activation and subsequent tumor cell elimination. Even though the white blood cell count can be normalized after chemotherapy or radiotherapy, the function of the immune system can be seriously affected in the long term. Moreover, most of the chemical or physical interventions can lead to a simultaneous massive release of tumor antigens, which can result in the establishment of high zone tolerance [73,74]. Furthermore, released adenosine and TGF-beta contribute to the suppressive effect on the immune system [75]. Under these circumstances, the chance of establishing a strong immune response may be very limited.

Even though the above-mentioned studies suggest the prior application of immunotherapy to be more beneficial, the timing of individual therapies still remains inconclusive and will need to be deeply investigated prior further clinical applications.

**Perspective**

Although the described immunotherapy can eliminate metastases and prolongate the survival of experimental animals, the size of the metastases is still a significant limitation for the therapeutic response. While small metastases can be completely eradicated with this therapy, advanced metastases are only slowed in growth. Therefore, future research has to be focused on the application of this immunotherapy in advanced metastatic disease and investigation of involved immune mechanisms in order to improve the therapeutic effect. Below we describe several approaches that can be studied for future improvement of this immunotherapy.

**Intra-metastatic injection of the therapeutic mixture**

In cases of metastatic disease presenting with a limited number of lesions, the individual lesions can be located and targeted with a dose of immunotherapy applied directly into the lesions involving interventional radiology techniques.

**Adaptive transfer of innate immune cells into the metastases**

Adaptive transfer of activated innate immune cells into the organ lesions can support the therapeutic response of uncontrolled metastases. This approach can represent a better option than the intra-metastatic injections of the therapeutic mixture mentioned above. With multiple injections of TLR ligands, the risk of side effects, such as fever or septic shock [76], can be increased. Since the main purpose of TLR ligands in this therapeutic mixture is to attract innate immune cells into the tumor and metastases, the direct adoptive transfer of innate immune cells into the metastases combined with mannann-BAM can overcome the challenges involved with multiple injections of TLR ligands.

**Development of combination therapy with chemotherapy and/or radiotherapy involvement**

Combination therapy is another possibility for improving the therapeutic response of advanced metastases. Specifically, immunotherapy applied in primary tumors with subsequent application of systemic chemotherapy or radiotherapy affecting distant tumors (metastases) may result in a synergistic effect and lead to complete elimination of advanced metastases.

**Study of involved mechanisms**

Acquiring a deeper understanding of the immune mechanisms involved in the eradication of tumors is one of the most important steps towards therapeutic improvement. Therefore, future studies should also focus on the investigation of innate and adaptive immune cell involvement and activation during immunotherapy application.

**Conclusions**

The immune system represents the most complex and effective tool which our bodies possess to fight cancer. Current cancer immunotherapies are mainly focused on activation of adaptive immunity, and the complex activation initiated by innate immunity is missing. Here, we demonstrate that effective cancer immunotherapy requires phased activation of both immune parts, innate immunity and adaptive immunity, to achieve the complex immune response against tumors. Our proposed immunotherapy involves an initial attack of innate immunity with subsequent involvement of adaptive immunity, which was demonstrated in tumor mouse models. In the future, this unique concept of complex immune system activation can result in effective treatments for patients with inoperable tumors or can be used to shrink tumors prior to surgery. More importantly, this therapy can vaccinate organisms and result in elimination not only of primary tumors but also metastases, with future protection against recurrent disease. The proposed immunotherapy can be also potentially combined with other therapies such as chemotherapy or radiotherapy to yield an increased therapeutic effect. These qualities make this immunotherapeutic approach an outstanding candidate for the treatment of a broad range of tumors.

However, the main upcoming challenge will be to prove this therapeutic effect in clinical translation.

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**Declaration of Competing Interest**

The authors have nothing to disclose.

**References**


