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Is cancer vaccination feasible at older age?

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Abstract

Age-related defects of the immune system are responsible for T cell unresponsiveness to cancer vaccination at older age. Major immune defects at older age are lack of naïve T cells, impaired activation pathways of T cells and antigen-presenting cells (APC), and age-related changes in the tumor microenvironment (TME). This raises the question whether cancer vaccination is feasible at older age. We compared various cancer vaccine studies at young and old age, thereby focusing on the importance of both innate and adaptive immune responses for cancer immunotherapy. These analyses suggest that creating an immune-stimulating environment with help of the innate immune system may improve T cell responses in cancer vaccination at older age.

INTRODUCTION

Cancer is an age-related disease. Since the elderly population is increasing, we can expect an increase in the number of cancer patients and mortality. In contrast to primary tumors which can often be removed by surgery, followed by radiation, chemo- or adjuvant therapy, for metastases there is no cure¹. It has been shown in mice and humans that cancer vaccines expressing tumor-associated antigens (TAA) targets metastases with high specificity²⁻⁴. However, vaccines are less effective at old than at young age⁵⁻⁷. This is caused by major age-related defects in immune responses resulting in short lasting and weak T cell responses to TAA. Ironically, the importance of the age factor in cancer vaccination is totally ignored in human clinical trials. Analysis of various vaccine studies in preclinical cancer models at young and old age, showed that vigorous anti-tumor most innate responses could be obtained by tailoring vaccination to older age, while T cell responses were hardly detectable. Therefore, we questioned whether T cell responses by cancer vaccination could be improved at older age. To answer this question, we reviewed adaptive and innate immune responses in elderly and cancer patients, and compared vaccine studies in preclinical models at young and old age. In this review, we propose new approaches to improve innate and adaptive immune responses against cancer at older age through immunotherapy.

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Immune deficiencies in elderly

Major immune defects at older age are lack of naïve T cells, impaired activation pathways of T cells and antigen-presenting cells (APC), and age-related changes in the tumor microenvironment (TME). While T cell unresponsiveness is the most significant defect in the immune system at older age, also innate immune responses are affected by aging although this seems less abundant than the adaptive immune responses.

Adaptive immune system

Lack of naïve T cells (react for the first time to new antigens) and an increase in the number of memory T cells (react to previously exposed antigens) is one of the most significant changes in the immune system at old compared to young age. It has been suggested that continual activation of the immune system by new antigens during the life span would lead to a depletion of naïve T cells from the thymus, and a clonal expansion of memory T cells⁸. With the involution of the thymus almost complete at the age of 60 years, new naïve T cells at old age are now generated at a much lower frequency than at young age⁹. The host is then dependent on the pool of naïve T cells generated earlier in life. Analogous to the situation in humans, a decrease of naïve T cells and an increase of memory T cells have also been described for aging mice⁹. Other possible causes for diminished T cell responses in aged humans and mice have been described, such as defects in TCR/CD3-mediated phosphorylation events or aberrant regulation of tyrosine kinases associated with the TCR¹⁰ and a decrease in $\alpha\beta$ repertoire of the TCR¹¹. The TCR is expressed by T cells, and is required for recognition of foreign antigens in association with self-major-histocompatibility complex (MHC) molecules, presented by APC to the immune system, and for subsequent activation of T cells. Another molecule important for T cell activation is CD28. CD28 is expressed at the cell membrane of T cells, and is the ligand for co-stimulatory molecule B7, expressed on APC. Clinical studies have documented that high proportions of CD8 T cells that lack CD28 are correlated with reduced antibody response to influenza vaccination¹¹. Also in mice, CD8 T cells lacking CD28 expression have been reported¹². Moreover, it has been shown that CD28-lacking CD8 T cells can suppress antigen-specific cytotoxic T lymphocyte (CTL) responses¹³.

In addition to the problems at the level of T cells, defects in cytokine production have been observed in aged humans. An example is a human vaccine study in which significantly lower interleukin (IL)-2 was produced by T cells of older individuals stimulated with an influenza vaccine *in vitro* compared to those of young individuals⁵. Similarly, significantly lower IFN γ was produced by peripheral blood nuclear cells (PBNC) from elderly individuals immunized with an influenza vaccine compared to young individuals. IL-2 promotes T cell activation and proliferation, as well as release of interferon (IFN) γ by T cells. The lower IL-2 production following *in vitro* stimulation with the influenza vaccine may explain the lower IFN γ production. IFN γ is involved in activation of dendritic cells (DC). These DC are important for CTL priming.

Innate immune system

The innate immune system is affected by aging as well, although this seems less abundant than the effect on the adaptive immune system. This includes natural killer (NK) cells, natural killer T (NKT) cells, $\gamma\delta$ T cells, dendritic cells, macrophages and neutrophils.

NK cell function has been extensively analyzed in relation to aging in human and mice. Although NK cell function and number is decreased at old compared to young mice, such as the production of IFN γ , IL-2 or perforin, in healthy human centenarians NK cell cytotoxicity by activation with IL-12, IFN α , and IFN γ is well preserved, but somewhat decreased in less healthy elderly^{14,15}. In our studies we found that the production IFN γ by NK cells induced by vaccination with an attenuated *Listeria monocytogenes* was almost as good in old as in young mice¹⁶.

NKT cells belong to the innate immune system because of their early response to infection and cancer. Although they have characteristics of T cells, they share some functional and phenotypical characteristics with NK cells¹⁷. They represent a heterogeneous population of CD4+, CD8+ or CD4-CD8- cells, but most characteristic of NKT cells is their invariant TCR V α 14J α 281/V β 8.2, or V β 7, or V β 2 in mice and V α 24J α Q/V β 11 in humans. While the number of NKT cells increases with age, their production of Th1 cytokines decreases with age¹⁸. However, NKT cells bearing TCR $\gamma\delta$ strongly increases with age and their functions are well preserved in very old mice and humans¹⁹. When activated with α galactosylceramide (α GC), NKT cells communicate with NK cells through the production of cytokines²⁰.

DCs are affected by aging as well. They play an important role in T cell activation, but the age-related effects on DC described are variable. It has been reported that blood DC from old individuals can still function as powerful antigen-presenting cells when exposed to purified protein derivate (PPD) of *Mycobacterium tuberculosis* or influenza vaccine²¹, while others have shown that DCs from aged individuals are more mature and have impaired ability to produce IL-12²², or that secretion of tumor necrosis factor (TNF) α and IL-6 significantly increased upon stimulation with lipopolysaccharide (LPS) and ssRNA in DC of aged compared to young individuals²³. Also, others describe that no major effects on the numbers of DC was observed by aging, but their capacity to phagocytose antigens and migration was impaired at older age²⁴.

Macrophages play an important role in the clearance of infections. Crosstalk between innate and adaptive immune responses exists through shared receptors such as toll-like receptors (TLR). It has been reported that Toll-like receptor (TLR) signaling in macrophages is defective at older age^{25,26}. Ageing of mice has been associated with an increase in the number of bone marrow macrophages that have an impaired ability to respond to infections, while cytokines such as circulating IL-6 or IL-10 increases with age upon stimulation with LPS^{27,28}.

Myeloid derived suppressor cells (MDSC) are a heterogeneous population of myeloid progenitor cells, i.e., immature macrophages, granulocytes, and dendritic cells (DC) that are endowed with a robust immunosuppressive activity^{29,30}. They migrate from the bone

marrow in to the blood circulation upon infections or cancer. Evidence exists that the number of MDSC in blood increases with age³¹. Flu is a major problem in the elderly because their immune system is significantly impaired compared to young adults. It has been shown that accumulation of MDSC increases in the lung in response to Influenza A virus infections, in correlation with suppression of CD4 T cell responses, which negatively influences the course of the disease³². The increase in the number of MDSC at older age might contribute to the lower capacity of elderly compared to young adults to clear the infection. We found that MDSC play a central role in cancer immunotherapy and could be used to improve T cell responses by Listeria-based vaccination at young and old age¹⁶. (For more detail see “MDSC under cancer vaccination at older age”)

Immune deficiencies in cancer patients

We expect that these age-related defects in the adaptive and innate immune system also play a role in cancer vaccination, because cancer patients are usually old. In addition to the age related changes, also tumor-induced immune suppression, genetic instability, and expression levels of antigens/receptors involved in T cell stimulation/inhibition contribute to T cell unresponsiveness at all ages.

Adaptive immune system

CTL, recognizing tumor-associated antigens (TAA) in association with major histocompatibility complex (MHC) molecules on the tumor cells through their T cell receptor, and expected to destroy tumor cells when exposed simultaneously to both TAA/self-MHC complexes and co-stimulatory molecules, are often found at the site of the tumor, but have evidently been unable to destroy the tumor cells in cancer patients³³. Multiple possible causes have been described for this unresponsiveness of the CTL in cancer patients (for a review see Gravekamp⁶). This includes decreased expression of MHC, TAA, or co-stimulatory molecules by tumor cells, and immune suppression induced by the primary tumors. In humans and mice, many tumors secrete lymphokines or factors that inhibit vaccine-induced T cell and NK cell responses. Examples are transforming growth factor (TGF) β , IL-6, IL-10, cyclooxygenase-2 (COX-2) and its products prostaglandine E2 (PGE₂), PD1-ligand, or indolamine 2,3-dioxygenase (IDO)³⁴.

Inducible T_{regs} play an important role in suppression of the immune system in cancer patients, through the production of soluble factors such as IL-10 and TGF β or through direct cell-cell contact, resulting in the inhibition of T cell and NK cell responses³⁵⁻⁴⁰. Moreover, evidence exists that the number of T_{regs} increases with age but not their function⁴⁰.

Innate immune system

The innate immune system plays an important role in the TME. For instance myeloid-derived suppressor cells (MDSC), suppress T cell and NK cell responses by the production of IL-6, IL-10, TGF β , reactive oxygen species (ROS), inducible nitric oxide synthase (iNOS) or arginase^{29,30}, or contributes to the expansion of regulatory T cells (T_{regs}) in the tumor TME⁴¹, while tumor associated macrophages (TAM) and M2 macrophages strongly suppress T cell responses through the production of IL-6, IL-10, TGF β in the TME⁴². IL-6 is particularly interesting because IL-6 levels increase with age. IL-6 is a potent regulator of

DC differentiation *in vivo*⁴³ and is able to initiate the expression of STAT3 in dendritic cells (DC). However, high levels of STAT3 can prevent the maturation from DC and subsequent presentation of antigens⁴³, resulting in T cell inhibition. Interestingly, it has been reported that the TME changes with age, i.e. it appeared that the number of MDSCs in the TME increases with age, and that this contributed to the T cell unresponsiveness at older age⁴⁴. So far, little research has been performed on MDSC and T cell unresponsiveness in relation to aging. Our laboratory has analyzed MDSC in young and old mice with metastatic breast cancer and found that the number of MDSC was five times higher in old than in young mice¹⁶. Because of their increased numbers in patients and mice with cancer at older age, and because they play central role in immune suppression in the TME, MDSC are highly attractive for cancer immunotherapy, particularly at old age. We found that MDSC play a central role in *Listeria*-based cancer immunotherapy at young and old age¹⁶ (for more detail see below under cancer vaccination at older age).

NK cell depletion *in vivo* leads to a poor control of tumor growth in various cancer models, indicating the importance of NK cells in anti-tumor responses and tumor surveillance⁴⁵⁻⁴⁹. Evidence exists from mice and humans that NK cells alter with age, but that they still function at older age. However, the effect of aging on NK cells against cancer has been far less extensively studied than T cells. A few reports describe that NK cells of elderly had a lower ability to respond to IL-2, lower spontaneous cytolytic activity towards tumors than young adults¹⁹. However, NK cells can also be used to kill tumor cells through other pathways than perforin-mediated tumor cell destruction. For instance, a clinical trial is ongoing with bortezomib which sensitizes tumor cells for TRAIL- and FasL-mediated destruction by NK cells in cancer patients between 20-70 years (NCT00720785)⁴⁹. We found NK cell responses (producing IFN γ) *in vivo* in old mice with metastatic breast cancer after vaccination with pcDNA3.1-Mage-b⁵⁰. Also NKT cells have anti-tumor activity in mice, including lung and hepatic cancer metastases when activated by α galactosylceramide (α GalCer), by secreting large amounts of IFN γ and IL-4, resulting in activation of other cells of the immune system including NK cells^{51,52}. In a phase I clinical trial with α GalCer in patients with solid tumors, the effect was dependent on the high number of NKT cells present pretreatment⁵³. Since the number of NKT cells increases with age and have anti-tumor reactivity, α GalCer could be a potential candidate to activate NKT cells against cancer at older age.

Cancer vaccination at old age

Cancer is a disease of the elderly. More than 50% of all cancer patients are 65 years or older. The vaccine studies discussed below show that cancer vaccination is less effective at old than at young age, but that tailoring cancer vaccination to older age is feasible. Moreover, innate immune responses may be a potential target for improving immunotherapy against cancer at older age.

The research group of Provinciali reported that immunization with a highly engineered mammary adenocarcinoma TS/A-IL-2, protected both young and old mice from TS/A challenge, which was not possible without IL-2⁵⁴. CD4 and CD8 T cells were present in tumors of young but hardly detectable in tumors of old mice, while macrophages and

neutrophils were detected at both ages. However, protective memory responses that could reject tumor cells upon re-challenge of tumor-free mice was only obtained in young mice. Another study of the group of Provinciali showed that vaccination with pCMV-neuNT against Her2/neu-expressing breast tumor cells (TUBO) completely protected young mice but only 60% of the old mice from TUBO challenge, and correlated with proliferation of spleen cells of young compared to old mice, in vitro upon re-stimulation with the Her/2 neu antigen⁵⁵.

Also the group of Lustgarten found that cancer vaccination was less effective at old than at young age. They showed that young but not old mice developed long-lasting memory responses to a pre-B-cell lymphoma (BM-185). However, inclusion of CD80 to the BM-185 cell line (BM-185-CD80) plus agonist anti-OX40 or anti-4-1BB (receptor for co-stimulation on T cells) mAb induced equally strong long-lasting memory responses at young and old age, suggesting the involvement of T cell responses⁵⁶. Also in another study they found that adding anti-OX40 or anti-4-1BB mAb to a DC vaccine, resulted in vigorous anti-tumor responses in a syngeneic TRAMP-C2 model at young and old age, while without anti-OX40 or anti-4-1BB, protection was significantly better in young than in old mice⁵⁷. Moreover, immunization of young and old mice with DC-TRAMP-C2 vaccine plus anti-OX40 or anti-4-1BB mAb resulted in improved CTL responses to apoptotic TRAMP-C2 cells in vitro upon re-stimulation, compared to the same vaccination without OX40 or anti-4-1BB mAb at old age, but the CTL responses were less vigorous compared to the same immunizations at young age. Grolleau-Julius et al⁵⁸ showed that vaccination with a DC-OVA vaccine derived from young mice was less effective against B16-OVA melanoma tumors in old than in young mice, indicating the altered TME at older age and its effect on vaccination. Also the group of Zhang found that the TME was altered at old compared to young age. They demonstrated that the number of MDSC increased in the tumor environment of old compared to young mice, and that this contributed to the age-related T cell unresponsiveness⁴⁴.

In our laboratory, we developed a DNA vaccine of Mage-b (pcDNA3.1-Mage-b) and tested this vaccine at young and old age in two syngeneic metastatic mouse breast tumor models, 4T07cg and 4T1, both overexpressing Mage-b in metastases and primary tumors⁵⁰. Vaccination of both models with Mage-b was highly effective against metastases and young age but not at old age, and this correlated with strong Mage-b-specific T cell responses in vitro and in vivo at young but not at old age⁵⁰. Interestingly, we found that Mage-b vaccination activated macrophages and NK cells (producing IFN γ) in old mice⁵⁰.

In another more recent vaccine study with Mage-b delivered through a highly attenuated *Listeria monocytogenes* we found a dramatic effect on the metastases in the 4T1 model at young age⁵⁹. This was due to an intimate relationship between *Listeria*, MDSC and cancer. We found that *Listeria* infected MDSC, which in turn selectively migrated to the TME^{16,60} most likely through attractants such IL-6, granulocyte macrophage colony-stimulating factor (GM-CSF), A100⁶¹. Once at the tumor site, *Listeria* is able to spread from MDSC into tumor cells through a cell-to-cell spread mechanism, characteristic for *Listeria*^{16,60}, and then kill the tumor cells through high levels of reactive oxygen species (ROS). Moreover, *Listeria*-infected tumor cells became a highly sensitive target for *Listeria*-activated T cells

and NK cells⁵⁹. We also found that *Listeria* infected MDSC with equal efficacy at young and old age, indicating that MDSC are an attractive target for *Listeria*-based immunotherapy at all ages. Indeed, using a semi-therapeutic immunization protocol (one immunization before and two after tumor development) *Listeria*-based vaccination was equally effective against metastatic breast cancer at young and old age¹⁶. A nearly complete elimination of the metastatic breast cancer was observed at both ages. We found that *Listeria* reduced the number of MDSC in blood and primary tumors, and induced IL-12 production in a sub population of the remaining MDSC at young and old age. This resulted in strong NK and T cell responses to *Listeria* at both ages. We concluded that targeting MDSC, an important arm of the innate immune system, by *Listeria*-based immunotherapy is important for improving T cell responses in young and old mice with metastatic cancer. In other words, the intimate relationship between *Listeria*, MDSC and cancer resulted in improved T cell responses at young and old age. Finally, we developed a clinical more relevant therapeutic immunization protocol. Initially, we applied immunizations with high doses of *Listeria* at one-week time intervals, but little effect was observed against the tumor and metastases, mainly due to immune suppression in the TME. However, when frequent immunizations with doses of *Listeria* were applied every other day, we observed a nearly complete elimination of the metastases¹⁶. Whether this correlated with improved innate and adaptive immune responses is currently under investigation.

With respect to human clinical trials of cancer vaccines or cancer immunotherapies, some effects on tumor progression, generation of T cell responses, or improved overall survival have been observed. For instance, clinical trials with TAA such as MAGE had effect on metastases^{3,4}. Various types of vaccines have been tested such as tumor cell lysates, peptide-based vaccines, vector-based vaccines, as well as RNA-based vaccines or *Listeria*-based vaccines. Most recently, immunotherapies have been developed using antibodies that block the interaction between PD-1 and PD-1 ligand. This interaction strongly inhibits T cell responses. Blocking this interaction resulted in human clinical trials to reduced tumor growth in patients with various cancers. Since immune suppression is one of the major problems in cancer vaccination, anti-PD-1 antibody treatment combined with vaccination has great potential. Another new development is the generation of genetically engineered T cells, i.e. patient's T cells have been uploaded with the TCR (with help of viruses) against TAA such as NY-ESO-1, LAGE-1 or MAGE-A3 or other TAA ex vivo and then re-injected into the patients. The results were variable, i.e. in some patients a dramatic success was observed while in some other patients serious side effects against the heart were observed. However, these clinical trials are still ongoing and their results will be published over the next few years. An overview from published human clinical trials with cancer vaccines or immunotherapies is shown in Table 1. As discussed above, the age factor is totally ignored in human clinical trials with cancer vaccines or immunotherapy. Our results suggest that adapting vaccines to older age may improve the outcome of the treatment.

CONCLUSIONS AND NEW APPROACHES

Activation of the innate immune system should be included as a potential candidate for improving immunotherapy at older age. This is based on the following conclusions. While the effect of cancer vaccination on growth of tumors and metastases could be strongly

improved by tailoring the vaccine to older age, as shown in the preclinical studies analyzed here, in most cases improvement was not the result of T cell activation but rather the result of other immune cells stimulated by the vaccine. Although various functions of NK and NKT cells are decreased at old age, it is far less dramatic than the age-related decline in T cell function, and both cells play an important role in anti-tumor responses. Moreover, we have shown that conversion of MDSC into an immune-stimulating phenotype resulted in improved T cell responses at young and old age. Below, new strategies to improve innate and adaptive immune responses against cancer at older age through immunotherapy are proposed below and summarized in Figure I.

Analysis of the cancer vaccine studies strongly suggests that innate immune responses should be considered as a potential target for improvement of immunotherapy against cancer at older age. For instance, NK cells and TCR $\gamma\delta$ NKT cells could be activated by attenuated *Listeria* and/or α GalCer and exhibit anti-tumor activity. Since the number of TCR $\gamma\delta$ NKT and NK cells also increases with age, *Listeria*-based vaccination in combination with α GalCer could enhance vaccine-induced T cell responses against cancer at older age. Improved innate immune responses may help improving adaptive immune responses.

One of the most important players in immune suppression in the TME is MDSC. Since their number increases with age, MDSC will be an important target for immunotherapy, particularly at older age. Not only *Listeria* has an effect on MDSC, but also CpG ODN, vitA, and several chemotherapeutics may decrease the number or polarize MDSC into an immune-stimulating phenotype⁶². It has been shown that CpG seems especially good at enhancing cellular and humoral immunity and promoting Th1-type responses in old mice⁶³. Immune suppression in the TME is often induced by cytokines and factors produced by MDSC and the tumor cells. For instance, IL-6 is such a cytokine produced by many cancers, MDSC and tumor-associated macrophages (TAM), and strongly suppresses T cell responses in the TME. Moreover, IL-6 recruits MDSC to the TME. We have shown that Curcumin, an Indian spice, improved vaccine efficacy of *Listeria*-Mage-b through reduced IL-6 production and improved T cell responses to Mage-b at young age⁶⁴. Since the IL-6 production and the MDSC population increases in the TME with age, a similar approach might reduce the T cell unresponsiveness in the TME also at old age.

Also other strategies may be used to activate T cell at older age. For instance, naïve T cells could be recruited by IL-7 at old age⁶⁵. However lack of naïve T cells is not the only hurdle to overcome. TAA are weakly immunogenic and T cells need help to become activated against TAA expressed by cancer cells. As shown in the studies discussed here, just adding IL-2 to TS/A tumor cells will improve anti-tumor responses but not memory responses to the tumor at old age. The best results so far has been shown by the group of Lustgarten by activating T cells against cancer through vaccination plus co-stimulation using anti-OX40 or 1-4BB mAb at young and old age. Also, elimination of T_{regs} could improve T cell activation at older age.

Non-immunological approaches might be used to fight metastatic cancer at young and old age. We have shown that an attenuated *Listeria monocytogenes* with help of MDSC can be used to deliver tumor-killing agents such as radioactivity selectively to the metastases and

tumor in a mouse model of pancreatic cancer⁶⁰. This resulted in an almost complete elimination of the pancreatic cancer, without severe side effects. This was possible because low doses of radioactivity were sufficient to kill the tumor cells without harming normal tissues through the selective delivery of radioactivity, by *Listeria* with help of the MDSC, to the TME. Also other non-pathogenic bacteria are currently under investigation for the delivery of tumor-killing agents selectively into tumor cells such as *Lactococcus lactis* and *E.coli*^{66,67}. Our results suggest that such an approach could be effective at young and old age. Moreover, if metastases and tumors are mostly eliminated by such treatment, immune suppression will be at its lowest point and follow up with a targeted immunotherapy will greatly profit from such opportunity.

In conclusion, despite all the obstacles that need to be overcome, cancer vaccination is potentially the most promising and benign approach to reduce morbidity and mortality by cancer at all ages. While cancer vaccination has limited success against late stage tumor development, they can be particularly effective where almost all other therapies struggle, i.e. against metastases and recurrence of cancer. The vaccine studies analyzed here show that improvement of vaccine efficacy at older age is possible, but that in addition to activation of T cells, the innate immune system also should be included as an important target for immunotherapy against cancer at older age. Finally, the results of these studies demonstrate the need of testing and tailoring cancer vaccines to older age in preclinical models before entering the clinic.

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Highlights

#16

MDSC are an important target to improve cancer vaccination at young and old age.

#34

Immune suppression in the tumor microenvironment is a major problem in cancer vaccination

#57

OX-40 improves co-stimulation and T cell activation at young and old age

#62

Various agents that blocks migration of MDSC

#81

Anti-PD1 antibody has great potential for cancer immunotherapy

Figure 1. Improvement of adaptive immune responses with help of innate immune responses through cancer immunotherapy

Various approaches are proposed that may improve innate immune responses and subsequently adaptive immune responses through cancer immunotherapy at older age. This includes activation of innate immune responses by elimination or conversion of myeloid-derived suppressor cells (MDSC) through attenuated *Listeria*, chemotherapeutics or CpG, or activation of co-stimulatory molecules through OX-40 or anti-4-IBB, or activation of natural killer T (NKT) cells and natural killer (NK) cells through α galactosylceramide (α GalCer) or bacterial products. Improved innate immune responses may lead to improved

T cell responses. In addition, T cell responses can be further improved at older age by recruiting naïve T cells through interleukin (IL)-7, or by depleting regulatory T cells (T_{regs}), tumor-associated macrophages (TAM) or M2 macrophages, or by generating memory T cells at young age when plenty naïve T cells are available (and boost memory T cells) and recall these memory T cells at old age, or by reducing immune suppressive cytokine IL-6 by Curcumin. Finally, combining immunotherapy with a non-immunological therapy such as killing tumor cells by delivery of anti-cancer agents through bacterial vectors to the tumor and metastases, or chemotherapy targeted to tumors through paramagnetic nanoparticles may lead to greater effect of the immunotherapy. Once the growth of tumor and metastases are strongly reduced, immune suppression will be at its lowest point, and follow up with a targeted immunotherapy will greatly profit from such opportunity. As we have shown earlier, delivery of foreign antigens into tumor cells and APC through *Listeria* activates T cells also at older age.

Table 1

Cancer immunotherapies in human clinical trials

VACCINE/IMMUNOTHER	PHASE	TUMOR	RESULTS SO FAR	REF
<i>Tumor cells or tumor cell lysates</i>				
OncoVAX	III	Colon	Significant improvement in DFS and OS in stage II	68-70
Renale	III	Renal	Significant improvement in DFS and OS	71,72
GVAX	III	Prostate	Failed to improve OS vs docetaxel	73
GVAX	III	Prostate	Failed. Higher death rate in vacc+doc vs doc alone	73
<i>Peptides</i>				
Provenge	III	Prostate	4.1 mos improvement vs placebo	74,75
Oncophage	III	Melanoma	Prolonged OS in M1a or M1b	76
Gp100:209-217	III	Renal	No difference in DFS and OS	77
Stimuvax	IIb	Lung	Improvement versus BSC in locoregional stage IIIB	78
<i>Vectors</i>				
PSA-TRICOM	II	Prostate	8.5 mos OS improvement vs Placebo	72
PSA-TRICOM	II	Prostate	16.4 mos OS improvement in HPS>18 mos	79
PANVAC-VF	III	Pancreatic	Failed>OS. Pts with life expectancy < 3mos	73
RNA mRNA from pancreatic cell lines	I/II	Prostate	Immunological response	80
<i>Attenuated live bacteria</i> ¹ Listeria-E7	I/II	Cervix (invasive)	Flu-like symptoms; Detection of E7-specific T cell responses	81
<i>Blocking PD-1-PD-1 ligand</i> Anti-PD-1 antibodies	I	Melanoma, NSCLC Prostate, Renal, Colorectal	Tumor reduction in 1 out of 4-5 patients	82

OS=overall survival; PFS= Progression-free survival; HPS=Halabi-predicted survival; DFS=disease-free survival