HHS Public Access

Author manuscript

*Curr Opin Immunol*. Author manuscript; available in PMC 2014 June 11.

Published in final edited form as:

*Curr Opin Immunol*. 2011 August ; 23(4): 555–560. doi:10.1016/j.coi.2011.05.003.

# The Impact of Aging on Cancer Vaccination

**Claudia Gravekamp**

## Abstract

Cancer vaccination is less effective at old than at young age, due to T cell unresponsiveness, caused by various age-related changes of the immune system. This includes lack of naïve T cells, defects in activation pathways of T cells and antigen-presenting cells (APC), and age-related changes in the tumor microenvironment. Although evidence exists that also natural killer (NK) and natural killer T (NKT) cells of the innate immune system change with age, comparison of various studies involving adaptive and innate immune responses in elderly and cancer patients, as well as cancer vaccination at young and old age in this review, indicates that also innate immune responses should be tested as a potential candidate to improve immunotherapy against cancer at older age.

## Introduction

Cancer is a disease of the elderly. With the increase in the elderly population, we can expect an increase in the number of cancer patients and mortality. For most cancers, primary tumors can be removed by surgery, followed by radiation, chemo- or adjuvant therapy. However until today, these therapies are ineffective against metastases [1]. In contrast, cancer vaccination shows great promise against metastases but is hampered by the fact that vaccines are less effective at old than at young age, due to T cell unresponsiveness [2–4]. Analysis of various vaccine studies in preclinical cancer models at young and old age, showed that vigorous anti-tumor responses could be obtained by tailoring vaccination to older age, but in most cases T cell responses were hardly detectable. Therefore, we questioned the feasibility of T cell activation in the tumor microenvironment by vaccination at older age, and whether activation of innate immune responses against cancer could be a more feasible approach since innate immune responses seems less affected by aging than adaptive immune responses. To answer these questions, we reviewed adaptive and innate immune responses in elderly and cancer patients, and comparison of vaccine studies in preclinical models at young and old age. Based on these studies, new approaches to improve adaptive and innate immune responses against cancer through vaccination or immunotherapy, respectively, at older age will be proposed.



**Corresponding address**, Claudia Gravekamp, Albert Einstein College of Medicine, Department of Microbiology and Immunology, 1300 Morris Park Avenue, Forchheimer Bldg, Room 407A, Bronx, NY 10461, claudia.gravekamp@einstein.yu.edu, Phone: 718-430-4048(office)/4067 (lab)/Fax: 718-430-8711.

## Impaired immune responses in elderly

### Adaptive Immune Responses

One of the most important changes in the immune system at older age is the decline in responsiveness of T cells to new antigens. This is mainly caused by strong decrease in the number of naive T cells (capable of reacting to new antigens) and an increase in the number of memory T cells (capable of reacting to previously exposed antigens) at old compared to young age [5,6]. However, also other possible causes for decreased T cell responses in aged humans and mice have been described, such as defects in T cell receptor (TCR)/CD3-mediated phosphorylation events or aberrant regulation of tyrosine kinases associated with the TCR [7], and an age-related decrease in the αβ repertoire of the human TCR [8]. 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. In addition, an age-related decrease in the expression of CD28 on the cell membrane of T cells, which provides a secondary signal for T cell activation when ligated to the B7 molecule on APC has been reported (9–11). Decreased production of interleukin (IL)-2 or interferon (IFN)γ at old compared to young age in individuals vaccinated with influenza virus or in vitro upon stimulation with influenza virus has been shown as well [11, 12].

### Innate Immune Responses

Cumulative evidence indicates that ageing exerts significant effects on all cells of the innate immune system [13]. This includes natural killer (NK) cells, natural killer T (NKT) cells, dendritic cells (DC), macrophages, and neutrophils. NK cells are the most well known cells of the innate immune system. NK cell function has been extensively studied in relation to aging in mice and humans. Although in 25-month-old mice NK cell number and function, such as the production of IFNγ, IL-2 of perforin, is decreased at old compared to 8 week-old mice, it has been reported that in human healthy centenarians NK cytotoxicity by activation with IL-12, IFNα, and IFNγ is well preserved, but somewhat decreased in less healthy elderly [13–15]. In our studies we found that the production of IFNγ by NK cells induced by vaccination with an attenuated *Listeria monocytogenes* was almost as good in old as in young mice (unpublished data).

NKT cells are considered to be a member from the innate immune system because of their early response against infection and perhaps against cancer. They represent a heterogeneous T cell population that shares some functional and phenotypical characteristics with NK cells [16]. It has been reported that the number of NKT cells increases with age [21], while their Th1 cytokines decreases with age [20]. However, liver NKT cells bearing TCRγδ are not only strongly increased in number but also their functions are well preserved in very old mice and humans [17]. It has been reported that NKT cells communicate with NK cells when activated by α-galactosylceramide (GalCer) [19].

DC in blood or Langerhans cells in skin, play a central role in T cell activation, but the results reported so far are variable. For instance, it has been demonstrated 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 vaccine [20,21], while others have shown that DCs from aged individuals are more mature and have impaired ability to produce IL-12 [22], 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 individuals [23]. Aged macrophages and neutrophils have impaired respiratory burst and reactive nitrogen as a result of altered intracellular signaling [24].

## Impaired immune responses in cancer patients

### Adaptive Immune Responses

In cancer patients cytotoxic T lymphocytes (CTL), recognizing tumor-associated antigens (TAA) in association with major histo-compatibility 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 [25]. Multiple possible causes have been described for this unresponsiveness of the CTL in cancer patients [for a review see 4]. 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 ($PGE_2$), PD1-ligand, or indolamine 2,3-dioxygenase (IDO).

Also immune cells in the tumor microenvironment attracted and activated by the primary tumor such as myeloid-derived suppressor cells (MDSC), suppress T cell and NK cell responses by the production of IL-6, IL-10, TGFβ, reactive oxygen species (ROS), inducible nitric oxide synthase (iNOS) or arginase [26•,27•], or contributes to the expansion of regulatory T cells ($T_{regs}$) in the tumor microenvironment [28•], while tumor associated macrophages (TAM) and M2 macrophages strongly suppress T cell responses through the production of IL-6, IL-10, TGFβ in the tumor microenvironment [29]. Interestingly, it has been reported that the tumor microenvironment changes with age, i.e. it appeared that the number of MDSC increases with age, and that this contributed to the T cell unresponsiveness at older age [30]. So far, little research has been performed on MDSC and T cell unresponsiveness in relation to aging. 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 TGFβ or through direct cell-cell contact, resulting in the inhibition of T cell and NK cell responses [31–33]. Moreover, evidence exists that the number of $T_{regs}$ increases with age [34]

### Innate Immune Responses

In vivo depletion of NK cells leads to a poor control of tumor growth in various cancer models, indicating the importance of NK cells in anti-tumor responses and tumor surveillance [35–38]. 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 adults [24]. However, NK cells can also be used to kill tumor cells through other pathways then 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) [39]. We found NK cell responses (producing IFNγ) in vivo in old mice with metastatic breast cancer after vaccination with pcDNA3.1-Mage-b [40•].

Also NKT cells have anti-tumor activity in mice, including lung and hepatic cancer metastases when activated by αGalCer, by secreting large amounts of IFNγ and IL-4, resulting in activation of other cells of the immune system including NK cells [41,42]. In a phase I clinical trial with αGalCer in patients with solid tumors, the effect was dependent on the high number of NKT cells present pretreatment [43]. Since the number of NKT cells increases with age, αGalCer could be a potential candidate to activate NKT cells against cancer at older age.

## Cancer vaccination at older age in preclinical models

More than 50% of all cancer patients are 65 years or older [44]. 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, also innate immune responses may be a potential target for immunotherapy against cancer.

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-2 [45]. 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 antigen [46].

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-OX-40 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 responses [47]. 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 mice [48]. 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 al [49] 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 tumor microenvironment at older age and its effect on vaccination. Also the group of Zhang found that the tumor microenvironment was altered at old compared to young age. They demonstrated that the number of myeloid-derived suppressor cells (MDSC) increased in the tumor environment of old compared to young mice, and that this contributed to the age-related T cell unresponsiveness [48].

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, 4TO7cg and 4T1, both overexpressing Mage-b in metastases and primary tumors [49]. 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 age [40]. Interestingly, we found that Mage-b vaccination activated macrophages and NK cells (producing IFNγ) in old mice [40]. 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 age [50•]. However, we discovered that this was not solely due to Mage-b, but rather to the direct infection and kill of tumor cells by Listeria [50•]. We concluded that this approach could be highly interesting for old age since T cells were not required. Indeed, the Listeria-based vaccine was equally effective against metastatic breast cancer at young and old age, while Mage-b-specific T cells responses were strong at young but almost undetectable at old age (unpublished results). Moreover, also NK cell responses were strongly activated by Listeria at young and old age, and may have contributed to the reduced growth of metastases at both ages as well. Since Listeria-infected tumor cells highly express Listeria proteins, the tumor cells become a highly sensitive target for NK cells and Listeria-specific CTL [50•].

## Conclusions and future prospects

The main conclusion from the studies analyzed here is that also the innate immune system should be considered for testing as a potential candidate for immunotherapy at older age. This is based on the following findings. 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. However, also improvement of T cell activation against cancer through vaccination at older age should be further optimized. Below, new strategies to improve adaptive and innate immune responses against cancer at older age through vaccination or immunotherapy, respectively, are proposed below and summarized in Table I.

As mentioned above, innate immune responses should be considered as a potential target for improvement of immunotherapy against cancer at older age. For instance, NK cells and TCRγδ NKT cells could be activated by attenuated Listeria or αGalCer, since both cell types are present in sufficient numbers at older age, could be activated at older age, and exhibit anti-tumor activity. Not only αGalCer has an effect on MDSC, but also CpG ODN, vitA, and several chemotherapeutica may decrease the number or polarize MDSC into an immune-stimulating phenotype [51•]. It has been shown that CpG seems especially good at enhancing cellular and humoral immunity and promoting Th1-type responses in old mice [52]. Improved innate immune responses may also lead to improved adaptive immune responses. Since the number of MDSC increases with age and contributes to T cell unresponsiveness at older age, and since the number of TCRγδ NKT cells also increases with age, vaccination in combination with αGalCer or CpG, could enhance vaccine-induced T cell responses at older age. Also elimination of immune suppressing TAM and M2 macrophages may lead to improved T cell activation in the tumor microenvironment at young and old age.

T cell could also be activated through other strategies. For instance, the problem of lack of naïve T cells, one of the most important changes at older age, could be avoided by immunizing at young age when sufficient naïve T cells are present, followed by recall at old age to reactivate memory T cells. Such an approach has been successfully used for improving antibody production at older age [53]. Also, naïve T cells could be recruited by IL-7 [54]. 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.

Finally, we have shown that an attenuated *Listeria monocytogenes* can be used to deliver genes directly and selectively in tumor cells in vivo [50•]. Also other nonpathogenic bacteria are currently under investigation for the delivery of genes selectively into tumor cells such as *Lactococcus lactis* and *E.coli* [55•]. Our results suggest that such an approach could be effective at young and old age.

In summary, despite all the obstacles that need to be overcome, vaccination against cancer is potentially the most promising approach. 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 considered as a possible target for immunotherapy against cancer at older age. The advantage of activating adaptive immune responses by vaccination is its prophylactic and therapeutic application, while activating innate immune responses by immunotherapy can only be applied therapeutically. 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.

## References


1. Pardal R, Clarke MF, Morrison SJ. Applying the principles of stem-cell biology to cancer. Nat Rev Cancer. 2003; 3:895–902. [PubMed: 14737120]
2. McElhaney JE, Meneilly GS, Lechelt KE, Bleackley RC. Split-virus influenza vaccines: do they provide adequate immunity in the elderly? Gerontol. 1994; 49:M37–M43.
3. Gravekamp C. The importance of the age factor in cancer vaccination at older age. Cancer Immunol Immunother. 2009; 58:1969–1977. [PubMed: 19259666]
4. Miller RA. The aging immune system: primer and prospectus. Science. 1996; 273:70–74. [PubMed: 8658199]
5. Utsuyama M, Hirokawa K, Kurashima C. Differential age-change in the number of CD4+CD45RA+ and CD4+CD29+ T cell subsets in human peripheral blood. Mech Ageing Dev. 1992; 63:57–68. [PubMed: 1376382]
6. Tamir A, Eisenbraun MD, Garcia GG. Age-dependent alterations in the assembly of signal transduction complexes at the site of T cell/APC interaction. J Immunol. 2000; 165:1243–1251. [PubMed: 10903722]
7. Wack A, Cossarizza A, Heltai S. Age-related modifications of the human alphabeta T cell repertoire due to different clonal expansions in the CD4+ and CD8+ subsets. Int Immunol. 1998; 10:1281–1288. [PubMed: 9786427]
8. Effros RB. Role of T lymphocyte replicative senescence in vaccine efficacy. Vaccine. 2006; 7:599–604. [PubMed: 17014937]
9. Effros RB. Replicative senescence of CD8 T cells: effect on human aging. Exp Ger. 2004; 39:517–524.
10. Filaci G, Fravega M, Negrini S. Nonantigen-specific CD8+ suppressor lymphocytes originate from CD8+CD28- T cells and inhibit both T cell proliferation and CTL function. Hum Immunol. 2004; 65:142–156. [PubMed: 14969769]
11. McElhaney JE, Meneilly GS, Lechelt KE. Split-virus influenza vaccines: do they provide adequate immunity in the elderly? Gerontol. 1994; 49:M37–M43.
12. Quyang Q, Cicek G, Westendorp RGJ. Reduced IFNγ production in elderly people following in vitro stimulation with influenza vaccine and endotoxin. Mech Ageing Dev. 2000; 121:131–137. [PubMed: 11164467]
13. Gomez CR, Nomelli V, Faunce DE, Kovacs EJ. Innate Immunity and Aging. Exp Gerontol. 2008; 43:718–728. [PubMed: 18586079]
14. Ogata K, Yokose N, Tamura H, An E, Nakamura K, Dan K, Nomura T. Natural Killer cells in the late decades of human life. Clin Immunol Immunopath. 1997; 84:269–275.
15. Ravaglia G, Forti P, Maioli F, Bastagli L, Facchini A, Mariani E, Savarino L, Sassi S, Cucinotta D, Lenaz G. Effects of micronutrient status on natural killer immune function in healthy free-living subjects aged >90 y. Am J Clin Nutr. 2000; 71:590–598. [PubMed: 10648276]
16. Emoto M, Kaufmann. Liver NKT cells: an account of heterogeneity. Trends in Immunol. 2003; 24:364–369. [PubMed: 12860526]
17. Mocchegiani E, Malavolta M. NK and NKT cell functions in immunosenescence. Aging Cell. 2004:177–184. [PubMed: 15268751]
18. Plackett TP, Boehmer ED, Faunce DE, Kovacs EJ. Aging and innate immune cells. J Leukoc Biol. 2004; 76:291–199. [PubMed: 15039467]
19. Biron CA, Brossay L. NK cells and NKT cells in innate defense against viral infections. Curr Opin Immunol. 2001; 13:458–464. [PubMed: 11498302]
20. Sauerwein-Teissl M, Schonitzer D, Grubeck-Loebenstin B. Dendritic cell responsiveness to stimulation with influenza vaccine is unimpaired in old age. Exp Ger. 1998; 33:625–631.
21. Sprecher E, Becker Y, Kraal G. Effect of aging on the epidermal dendritic cell population in C57Bl/6J mice. J Invest Dermatol. 1990; 94:247–253. [PubMed: 2299200]

22. Bella D, Bierti L, Presicce P. Peripheral blood dendritic cells and monocytes are differentially regulated in the elderly. Clin Immunol. 2007; 122:220–228. [PubMed: 17101294]

23. Agrawal A, Agrawal S, Cao JN. Altered innate immune functioning of dendritic cells in elderly humans: a role of phosphoinisitide 3-kinasesignaling pathway. J Immunol. 2007; 178:6912–6922. [PubMed: 17513740]

24. Gravekamp C, Bontenbal M, Ronteltap C. *In vitro* and *in vivo* activation of $CD4^+$ lymphocytes by autologous tumor cells. Int J Cancer. 1990; 46:152–154.

25. Gravekamp C. The importance of the age factor in cancer vaccination at older age. Cancer Immunol Immunother. 2009; 58:1969–977. [PubMed: 19259666]

26. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol. 2009; 9:162–174. [PubMed: 19197294] Myeloid-derived suppressor cells (MDSC) strongly suppress T cells and NK cells in the tumor microenvironment, and the number of MDSC in the tumor microenvironment increases with age. Elimination or polarization of MDSC may improve cancer vaccination at young and old age.

27. Ostrand-Rosenberg S, Sinha P. Myeloid-derived suppressor cells: Linking inflammation and Cancer. J. Immuno. 2009; 182:4499–4506. MDSC produce inflammatory lymphokines in the tumor microenvironment that strongly inhibits T cell activation. Elimination of these lymphokines may improve cancer vaccination at young and old age.

28. Dumitriu IE, Dunbar DR, Howie SE, Sethi T, Gregory CD. Human dendritic cells produce TGF-beta 1 under the influence of lung carcinoma cells and prime the differentiation of CD4+CD25+Foxp3+ regulatory T cells. J Immunol. 2009; 182(5):2795–2807. [PubMed: 19234174] $T_{regs}$ strongly inhibit T cells, and their number increases with age. Elimination of $T_{regs}$ may improve cancer vaccination at young and old age.

29. Sica A, Bronte V. Altered macrophage differentiation and immune dysfunction in tumor development. J Clin Invest. 2007; 117:1155–1166. [PubMed: 17476345]

30. Grizzle WE, Xy X, Zhang S, Stockard CR, Liu C, Yu S, Wang J, Mountz JD, Zhang HG. Age-related increase of tumor susceptibility is associated with myeloid-derived suppressor cell mediated suppression of T cell cytotoxicity in recombinant inbred BXD12 mice. Mechanisms of Ageing and Development. 2007; 128:672–680. [PubMed: 18036633]

31. Shimizu J, Yamzaki S, Sakaguchi S. Induction of tumor immunity by removing CD4+CD25+ T cells: a common basis between tumor immunity and autoimmunity. J Immunol. 1999; 163:5211–5218. [PubMed: 10553041]

32. Tanaka H, Tanaka J, Kjaergaard J. Depletion of CD4+CD25+ regulatory T cells augments the generation of specific immune T cells in tumor-draining lymph nodes. J Immunother. 2002; 25:207–217. [PubMed: 12000862]

33. Chen A, Liu S, Park D. Depleting intratumoral CD4+CD25+ regulatory T cells via FasL protein transfer enhances the therapeutic efficacy of adaptive T cell transfer. Cancer Res. 2007; 67:1291–1298. [PubMed: 17283166]

34. Gregg R, Smith CM, Clark FJ, Dunnio D, Khan N, Chakraverty R, Nayak L, Moss PA. The number of human peripheral blood CD4+CD25 high regulatory T cells increases with age. Clin Exp Immunol. 2005; 140:540–546. [PubMed: 15932517]

35. Mocikat R, Braumuller H, Gumy A. Natural killer cells activated by MHC Class Il targets prime dendritic cells to induce protective CD8 T cell responses. Immunity. 2003; 19:561–569. [PubMed: 14563320]

36. Guerra N, Tan YX, Joncker NT, Choy A, Gallardo F, Xiong N, Knoblaugh S, Cado D, Greenberg NR, Raulet DH. NKG2D-defiecient mice are defective in tumor surveillance in models of spontaneous malignancy. Immunity. 2008; 28:571–580. [PubMed: 18394936]

37. Smyth MJ, Crowe NY, Godfrey DA. NK cells and NKT cells collaborate in host protection from methylcholanthrene-induced fibrosarcoma. International Immunology. 2000; 13:459–463. [PubMed: 11282985]

38. Turner JG, Rakhmilevich AL, Burdelya L, Neal Z, Imboden M, Sondel P, Yu H. Anti-CD40 antibody induces antitumor and antimetastatic effects: The role of NK cells. J Immunol. 2001; 166:89–94. [PubMed: 11123280]

39. Hallett WHD, Ames E, Motarjemi M, Barao I, Shankar A, Tamang DL, Sayers TJ, Hudig D, Murphy WJ. Sensitization of tumr clls to NK cell-mdeiated killing by proteosome inhibition. J Immunol. 2008; 180:163–170. [PubMed: 18097016]
40. Castro F, Leal B, Denny A, Bahar R, Lampkin S, Reddick R, Lu S, Gravekamp C. Vaccination with Mage-b DNA induces CD8 T cell responses at young but not at old age in mice with metastatic breast cancer. British Journal of Cancer. 2009; 101:1329–1337. [PubMed: 19826426] CD8 T cells to TAA could not be activated by vaccination at old age and correlated with low vaccine efficacy in preclinical metastatic breast cancer models. However, innate immune responses such as NK cells and macrophages were activated at old age in vivo. These results and other additional reports suggest that innate immune responses could be a potential target for immunotherapy against cancer at older age.
41. Nakui M, Ohta A, Sekimoto M, Sato M, Iwakabe K, Yahata T, Kitamura H, Koda T, Kawano T, Makuuchi H. Potentiation of antitumor effect of NKT cell ligand alpha-galactosylceramide by combination with IL-12 on lung metastasis of malignant melanoma cells. Clin Exp Metastasis. 2000; 18:147–153. [PubMed: 11235990]
42. Nakagawa R, Serizawa I, Motoki K, Sato M, Ueno H, Iijima R, Nakamura H, Shimosaka A, Koezuka Y. Anti-tumor activity of alpha-galactosylceramide, KRN7000, in mice with melanoma B16 hepatic metastases and immunological study of tumor-infiltrating cells. Oncol Res. 2000; 12:51–58. [PubMed: 11132924]
43. Giaccone G, Punt CJ, Ando Y, Ruijter R, Nishi N, Peters M, von Blomberg BM, Scheper RJ, van der Vliet HJ, van den Eertwegh AJ, et al. A phase I study of natural killer T ligand alpha-galactosylceramise (KRN7000) in patients with solid tumors. Clin Cancer Res. 2002; 8:3702–3709. [PubMed: 12473579]
44. Muss HB. Factors used to select adjuvant therapy of breast cancer in the United States: an overview of age, race, and socioeconomic status. J Natl Cancer Inst Monogram. 2001; 30:52–55.
45. Provinciali M, Argentati K, Tibaldi A. Efficacy of cancer gene therapy in aging: adenocarcinoma cells engineered to release IL-2 are rejected but do not induce tumor specific immune memory in old mice. Gene Ther. 2000; 7:624–632. [PubMed: 10819579]
46. Provinciali M, Smorlesi A, Donnini A. Low effectiveness of DNA vaccination against HER2/neu in aging. Vaccine. 2003; 21:843–848. [PubMed: 12547592]
47. Lustgarten J, Dominguez AL, Thomas M. Aged mice develop protective anti-tumor responses with appropriate costimulation. J Immunol. 2004; 173:4510–4515. [PubMed: 15383582]
48. Sharma S, Domiguez AL, Lustgarten J. Aging affect the anti-tumor potential of dendritic cell vaccination, but it can be overcome by costimulation with anti-OX40 or anti-4-1BB. Exp Ger. 2006; 41:78–84.
49. Grolleau-Julius A, Abernathy L, Harning E, Yung RL. Mechanisms of murine dendritic cell antitumor dysfunction in aging. Cancer Immunol Immunother. 2008
50. Kim SH, Castro F, Paterson Y, Gravekamp C. High efficacy of a Listeria-based vaccine against metastatic breast cancer reveals a dual mode of action. Cancer Res. 2009; 69:5860–5866. [PubMed: 19584282] The authors demonstrate that Listeria directly infects and kill tumor cells, and activates NK cells. Based on these results it has been suggested to use Listeria for the selective delivery of any gene into tumor cells, involved in tumor cell destruction. They also demonstrated that tumor cells infected with Listeria highly express Listeria proteins, and change therefore tumor cells into a highly sensitive target for Listeria-specific CTL and NK cells.
51. Lechner MG, Epstein AL. A new mechanism for blocking Myeloidderived suppressor cells by CpG. Clin Cancer Res. 2011; 17:1645–1648. [PubMed: 21288925] MDSC play an important role in T cell unresponsiveness at older age. This article describes various chemotherapeutica as well as CpG caplable of eliminating or blocking the suppressive effect of MDSC in the tumor microenvironment.
52. Maletto B, Ropolo A, Moron V, Pistoresi-Palencia MC. CpG-DNA stimulates cellular and humoral immunity and promotes TH1 differentiation in aged BALB/C mice. J Leukoc Biol. 2002; 72:447–454. [PubMed: 12223511]
53. Stacy S, Infante AJ, Wall K. Recall immune memory: a new tool for generating late onset autoimmune myasthenia gravis. Mech. Ageing Dev. 2003; 124:931–940. [PubMed: 14499498]

54. Tan JT, Dudl E, LeRoy E. IL-7 is critical for homeostatic proliferation and survival of naïve T cells. PNAS. 2001; 98:8732–8737. [PubMed: 11447288]

55. Grillot-Courvalin C, Goussard S, Corvalin P. Bacterial vectors for delivering gene and anticancer therapies. Microbe March. 2011:6. This article describes various vectors that can be used for the delivery of genes into tumor cells. *Lactococcus lactis* and *Escherichia coli* are two potential candidates, in addition to *Listeria monocytogenes*.

**Table I**

Improvement of cancer vaccination or immunotherapy at older age

*Adaptive Immune Responses*

- Generation of memory T cell responses at young age, when sufficient naïve T cells are available, and reactivation of memory T cells at old age
- Recruitment of naïve T cells by IL-7
- Activation of co-stimulatory molecules by OX40 or 1-4BB1
- Activation of Th1 type immune responses by CpG-DNA
- Elimination or polarizing MDSC, TAM or M2 macrophages
- Elimination of $T_{regs}$
- Reduction of lymphokines or factors that inhibits T cell activation

*Innate Immune Responses*

- Activation of NK and NKT cells by bacterial products
- Activation of NKT and NK cells by α-GalCer
- Activation of NK and NKT cells by CpG-DNA
- Sensitizing tumor cells for NK cell-mediated destruction by Bortezomib
- Elimination or polarizing MDSC

*Other Approaches*

- Delivery of genes into tumor cells through bacterial vectors that leads to tumor cell destruction: potential candidates are attenuated *Listeria monocytogenes* or other nonpathogenic bacteria such as *Lactococcus lactis* or *Escherichia coli*.

α-GalCer=α-galactosylceramide, MDSC=myeloid-derived suppressor cells, NK=natural killer, NKT=natural killer T, Th1=T helper 1, TAM=tumor-associated macrophages.