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Combination of physical activity, nutrition, or other metabolic factors and vaccine response

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1. ABSTRACT

A number of lifestyle factors that reduce cancer risk in the primary prevention setting may be potential new targets for use in combination with cancer vaccines. This review discusses the modulation of energy balance (physical activity, calorie restriction, and obesity prevention), and the supplementation with natural and synthetic analogs of vitamins A and E, as potential interventions for use in combination with cancer vaccines. Additionally, the pharmacologic manipulation of nutrient metabolism in the tumor microenvironment (e.g., arachidonic acid, arginine, tryptophan, and glucose metabolism) is discussed. This review includes a brief overview of the role of each agent in primary cancer prevention; outlines the effects of these agents on immune function, specifically adaptive and/or anti-tumor immune mechanisms, when known; and discusses the potential use of these interventions in combination with therapeutic cancer vaccines. Modulation of energy balance through exercise and strategies targeting nutrient metabolism in the tumor microenvironment represent the most promising interventions to partner with therapeutic cancer vaccines. Additionally, the use of vitamin E succinate and the retinoid X receptor-directed rexinoids in combination with cancer vaccines offer promise. In summary, a number of energy balance- and nutrition-related interventions are viable candidates for further study in combination with cancer vaccines.

2. INTRODUCTION

Following completion of primary therapy, a limited number of interventions have demonstrated success in reducing the risk of recurrence of the primary tumor, preventing a secondary malignancy and increasing survival (1). Therapies targeting the immune system offer promise in controlling micro-metastases and increasing survival (2-5), but are likely to yield greater success if used in combination with other strategies that may either slow tumor growth or increase the efficacy of therapeutic cancer vaccines. New targets for use in combination with cancer vaccines need to be identified and explored.

A number of lifestyle factors that reduce cancer risk in the primary prevention setting may be promising candidates such as the modulation of energy balance, as well as supplementation with vitamins and minerals. Changes in energy balance (i.e. physical activity, calorie restriction, and obesity prevention) alter cancer risk. Physical activity significantly reduces the risk of several types of cancer. The data on this are most consistent for colon and breast cancer in humans (6) and animals (7;8). Calorie restriction also consistently and significantly reduces the risk of tumor formation in a variety of animal models (8;9). At the other end of the energy balance spectrum, obesity increases cancer risk and mortality in humans (10;11) and in animal models (8;12-17). With respect to vitamins and minerals, the vitamins A, B_6, B_{12}, C, D, E, folate, selenium, and zinc have been shown to reduce the risk of cancer in humans (recently reviewed in (18-22)).

The biological mechanisms underlying the cancer preventive effects of both changes in energy balance and of specific nutrients differ based on the intervention or agent, and are reviewed in detail elsewhere (23-29). A variety of biological mechanisms have been proposed to explain the relationship between changes in energy balance and cancer prevention including: 1) alterations in growth factors; 2) reductions in reproductive and metabolic hormones; 3) enhanced antioxidant defense mechanisms; and 4) enhanced immune function. The chemopreventive activity of many nutrients is achieved through the inhibition of proliferation, promotion of differentiation, and/or induction of apoptosis of tumor cells, as well as increased antioxidant, anti-inflammatory, and immunomodulatory activities. An understanding of the immunoregulatory capabilities of these lifestyle interventions is particularly relevant if these are to be used in combination with cancer vaccines and will be explored in this review.

The target population to receive therapeutic cancer vaccines is likely an older cohort since 60% of all newly diagnosed malignant tumors and 70% of all cancer deaths occur in persons 65 years and older (30). It is well documented that components of both the innate and adaptive immune system decline with advancing age (31-33) and that the T cell population is most affected by the aging process (32). Immunosenescence has been hypothesized to contribute to a greater risk of tumor formation and infection with advancing age, and may contribute to a less effective response to vaccination. In particular, the age-associated decline in T cell function has been associated with thymic involution and atrophy, as well as acquired defects in the bone marrow stroma, hematopoietic stem cell populations, and peripheral lymphoid tissues (34). Therefore, the energy balance- and nutrition-related intervention strategies proposed need to be effective within the context of an aging immune system in order to benefit the patient population most likely to receive cancer vaccines.

The scope of this review was narrowed by only including lifestyle interventions that have shown efficacy in primary prevention coupled with those that regulate immune function. Using these two criteria, several traditional cancer prevention interventions emerge as promising strategies for use in combination with various cancer vaccine platforms. In this review, changes in energy balance are discussed in combination with therapeutic vaccines. The use of systemic supplementation with natural and synthetic analogs of vitamins A and E are also reviewed. Additionally, the pharmacologic manipulation of nutrient metabolism in the tumor microenvironment (e.g., arachidonic acid, arginine, tryptophan, and glucose metabolism), all of which have been shown to contribute to immunosuppression in cancer, are discussed. This review includes a brief overview of the role of each agent in primary cancer prevention; outlines the effects of these agents on immune function, in particular adaptive and/ or anti-tumor immune mechanisms, when known; and discusses the potential use of these interventions in combination with therapeutic cancer vaccines (Tables 1-3). There is a paucity of work in the nexus between nutrition, energy balance, and cancer vaccines. Therefore, the goal of this review is to discuss the rationale for using these prevention strategies in combination with cancer vaccines and to outline key questions that need to be addressed to incorporate these interventions with cancer vaccines in the future.

3. CHANGES IN ENERGY BALANCE

3.1. Physical activity

3.1.1. Role of physical activity in cancer prevention

An *International Agency for Research on Cancer (IARC) Working Group on the Evaluation of Weight Control and Physical Activity* concluded that consistent epidemiologic evidence exists demonstrating that physical activity reduces the risk of some forms of cancer. The evidence is conclusive for a protective effect of physical activity on colon and postmenopausal breast cancer risk, and is mounting for a protective effect of physical activity on endometrial, ovarian, and prostate cancers (35). Some of the reported benefit of physical activity on cancer risk reduction is independent of weight loss. These data suggest that the impact of physical activity on cancer risk reduction may not be solely due to the prevention of obesity (another potential modifiable risk factor that may negatively impact response to vaccine discussed in a later section), but rather due to a cancer preventive effect specifically of exercise.

A variety of animal models have been used to explore the effects of exercise on carcinogenesis including

Table 1. Summary of the effects of changes[1] in energy balance and the impact of nutrients on anti-tumor activity, immune function and use in combination with vaccines in preclinical studies.

	Anti-Tumor Activity			Immune Function						Use with Cancer Vaccine	
				NK Cell Function		T Cell Function					
	Prevention	Therapy/ Survival	Ref.[2]	Cytotoxicity	Ref.[2]	Proliferation	Cytokine Production	Cytotoxicity	Ref.[2]		Ref.[2]
Changes in Energy Balance											
Physical Activity	+ +	+	7;35-38; 41-46; 48-58	+ +	68-70; 72; 354	+/-	+	ND	72;76-78; 81-83;85; 90;94;95; 102;104; 355	ND	
Calorie Restriction[3]	+ +	+/-	8;9; 115-125	(-)	139; 140	+/-	+/-	ND	82; 130-138; 168;356	ND	
Obesity	(-)	(-)	8;12-14; 16;17; 357;358	(-)	168-170	(-)	+/-	ND	169;174; 175;359	ND	
Impact of Nutrients											
Retinoids and Carotenoids											
Retinoid Analogs[4]	+ +	+	223;224; 233-236; 268	+	257; 259; 265	+	+	+	251-254; 257-259	+	266; 268
Synthetic Rexinoids[5]	+ +	+ +	242-248; 250;270	ND		ND	ND	ND		ND	
Tocopherols and Tocotrienols											
Alpha-Tocopherol	ND	ND		ND		+	+	ND	290-292	ND	
Vitamin E Succinate (VES)	+ +	+ +	274-281; 293	ND		ND	ND	ND		+ +	294-296

[1] Changes in activities outlined in this table are designated with the following symbols: (-) = negative effect, +/- = inconsistent results, + = mild/moderate stimulatory effect, + + = strong stimulatory effect, ND = No data available, [2] Ref. = References, [3] Only studies utilizing adult onset calorie restriction are reviewed in this table, [4] Retinoid Analogs (all-trans-, 9-cis-, and 13-cis-retinoic acid), [5] Synthetic Rexinoids (Bexarotene (LGD1069) and LG100268).

Table 2. Summary of the effects of changes[1] in energy balance and the impact of nutrients on anti-tumor activity, immune function and use in combination with vaccines in human studies

	Anti-Tumor Activity			Immune Function						Use with Cancer Vaccine	
				NK Cell Function		T Cell Function					
	Prevention	Therapy/ Survival	Ref.[2]	Cytotoxicity	Ref.[2]	Proliferation	Cytokine Production	Cytotoxicity	Ref.[2]		Ref.[2]
Changes in Energy Balance											
Physical Activity	+ +	+	35; 105-107	+ +	59-66; 73;74	+/-	+/-	ND	59;64;75;79; 80;82;84; 86-89;91-93;100;101	ND	
Calorie Restriction[2]	ND	ND		(-)	177-179	+/-	ND	ND	173;176	ND	
Obesity	(-)	(-)	10;11;35; 144-147; 180-193; 195-222	+/-	165-167	(-)	+/-	ND	149-158; 166;171-173	ND	
Impact of Nutrients											
Retinoids and Carotenoids											
Retinoid Analogs[3]	+ +	+	223; 237-240	ND		+	ND	ND	267	ND	
Synthetic Rexinoids[4]	ND	+	249; 260-263	ND		ND	ND	ND		ND	
Tocopherols and Tocotrienols											
Alpha-Tocopherol	+/-	ND	231; 271-273	+	288	+	+	+	282;283; 287;288	ND	
Vitamin E Succinate (VES)	ND	ND		ND		ND	ND	ND		ND	

[1] Changes in activities outlined in this table are designated with the following symbols: (-) = negative effect, +/- = inconsistent results, + = mild/moderate stimulatory effect, + + = strong stimulatory effect, ND = No data available, [2] Ref. = References, [3] Only studies utilizing adult onset calorie restriction are reviewed in this table, [4] Retinoid Analogs (all-trans-, 9-cis-, and 13-cis-retinoic acid), [5] Synthetic Rexinoids (Bexarotene (LGD1069) and LG100268).

chemically-induced, transplantable, and spontaneous tumor models (7). In these studies, the effect of exercise on intestinal tumor incidence and multiplicity have been the best characterized, with a protective effect of exercise observed in most reports. Early studies showed a significant reduction in the incidence of carcinogen-induced tumors in exercising animals (36-39). Several subsequent studies have examined polyp development following exercise training in the APC^{Min} mouse, a model in which an APC tumor suppressor gene mutation results in multiple intestinal polyps (40). Although some variability has been reported due to the type of exercise and gender of the animals studied, collectively these studies show a decrease in polyp number in exercising male animals (41-44). A protective effect of exercise on mammary tumor incidence, multiplicity, growth rate and/or survival has also been reported (45-52). In additional to colon and breast cancer models, exercise has been shown to be effective in reducing tumor incidence or burden in carcinogen-induced pancreatic (53;54) and liver (55-57) neoplasias. One study has shown that exercise delays tumor growth and enhanced regression of an allogeneic tumor (a murine T cell

Table 3. Summary of the immunosuppressive effects of metabolic factors in the tumor microenvironment and the use small molecule inhibitors in combination with cancer vaccines

Metabolic Factor	Key Pathway(s)	Cellular Distribution	Metabolic Changes	Mechanisms of Immune Suppression	Inhibitor	Use with Cancer Vaccine	References
Arachidonic Acid	COX-2	Tumor TAMs	↑ PGE_2 ↑ Angiogenesis	↓ B and T cell proliferation ↓ NK cell cytoxicity ↑ Tregs ↓ DC function ↓ TH1 and ↑ TH2 cytokines	Celecoxib	Yes	297;300;301;304;308
Arginine	ARG1 iNOS ODC	Tumor TAMs MDSCs	↓ Arginine ↑ Polyamines	↓ TCR CD3 zeta chain expression ↓ IL-2R signaling ↓ IL-2 ↑ Effector T cell apoptosis	NCX-4016	Yes	297;313;316;319;321;322
Tryptophan	IDO	Tumor TAMs MDSCs DCs	↓ Tryptophan ↑ Kynurenines	↓ TCR CD3 zeta chain expression ↑ Tregs ↑ TGF-beta ↑ IL-10	1-MT	Yes	297;325;333;334
Glucose	Glut-1 MCT-4 HIF-1-alpha	Tumor	↓ Glucose ↓ pH ↑ Hypoxia ↑ Angiogenesis	↓ Effector T cell function	ND[1]	ND[1]	337;342;348;350

[1] ND = No data available.

lymphoma cell line) (58). Thus, exercise has been shown to be effective in reducing the number and size of tumors in a number of models.

Numerous mechanisms have been proposed to explain the relationship between exercise and cancer prevention (29), including an exercise-induced stimulation of anti-tumor immunity. Little work has been done to examine those components of the immune system that are most likely mediating anti-tumor immunity or the effects of exercise on antigen-specific immune responses. However, the work in this area is promising and suggests that an exercise-induced enhancement of immune function may be one of the mechanisms underlying the protective effect of exercise on tumor formation.

3.1.2. Modulation of immune function by physical activity

The current theory to explain the relationship between exercise and immune function is the Inverted J Hypothesis (59). This hypothesis proposes that regular, moderate exercise enhances immune function and in turn, reduces the susceptibility to cancer. In contrast, sedentary behavior, at one end of the curve, and overtraining, at the opposite end of the curve, both lead to suppressed immunity and elevated risk of tumor development. In terms of anti-tumor immunity, NK cell function has been studied in response to exercise to a greater extent than either $CD4^+$ and/or $CD8^+$ T cell function. Little work has been done examining the effect of exercise on antigen-specific T cell functions such as cytokine production, proliferation, and/or cytotoxicity.

Overall, moderate exercise enhances NK cell activity, although there are some inconsistencies in the literature. Several cross-sectional studies have demonstrated higher NK cell function in trained athletes as compared to sedentary individuals (60;61). Longitudinal studies have shown that aerobic training over a period of several months in untrained individuals enhances NK cell activity in humans (59;62-66) and experimental animals (67-72). The beneficial effect of long-term aerobic training on NK cell function (four to seven months) has also been observed in postmenopausal breast cancer survivors (73;74), suggesting that exercise may benefit those patients who may be immunosuppressed following adjuvant therapy. However, another study in breast cancer survivors reported that eight weeks of aerobic training had no effect on NK cell function (61). The lack of a statistically significant effect of exercise on NK cell cytotoxicity in the latter study may be due to a relatively short training period. Recent studies suggest that the duration of the training period influences the effect of exercise on splenic NK cell cytotoxicity in mice. A signficant beneficial effect of running was observed only after 11 weeks of voluntary running (not at six or eight weeks) with the greatest increase in NK cell cytotoxicity observed following 15 weeks of training (72).

The effect of regular moderate exercise on mitogen-induced T cell proliferation has been examined in many studies which have yielded inconsistent results. Some studies have reported an increase (64;75-82), a decrease (83;84), or no effect (59;64;85-93) of regular, moderate exercise on T cell proliferative responses to mitogens in both humans and in experimental animals. Part of the heterogeneity of proliferative responses reported in the literature may be due to variability in the timing of lymphocyte collection with respect to the last exercise bout; to varying intensity and duration of exercise employed in the different studies; and to the origin of lymphoid tissue. With respect to the latter, it has been shown that lymphocytes isolated from different lymphoid tissues are differentially impacted by exercise training. For example, eight weeks of training has been shown to enhance the concanavalin A (Con A)-induced proliferation of T cells collected from the peripheral blood but not the spleen of hamsters (85). Additionally, exercise in rats resulted in an increase in Con A-induced lymphocyte proliferation in the mesenteric lymph nodes, but not the spleen (81). These results are consistent with recent findings in which T cell proliferative responses following six weeks of voluntary exercise differed by tissue type, with exercise significantly enhancing Con A-induced lymphocyte proliferation in

intestinal lymphocytes but not splenocytes (82). These data suggest that voluntary exercise enhances T cell proliferation in some but not all lymphoid organs. This is an important distinction since many clinical studies only collect peripheral blood from patients. The assessment of immune status based on the response of one population of lymphoid cells (e.g., PBMC) following an intervention such as exercise may not be representative of the effect on all lymphoid cells and should be taken into consideration.

In tumor-bearing animals, splenic Con A-induced lymphocyte proliferation was increased following exercise training in two studies (94;95). In the first study, increased lymphocyte proliferation was correlated with reduced tumor growth and increased survival (94). However in the second report, final tumor volumes did not differ between the exercising and control animals despite an increase in T cell proliferative responses in the exercise group (95). A study in early-stage breast cancer patients three to six months following chemotherapy found that women who participated in a six-month exercise program showed a greater percentage of $CD4^+CD69^+$ cells and a greater level of mitogen-induced proliferation at the end of the intervention (96). The results from these limited studies suggest that exercise may enhance T cell proliferation under conditions where immunosuppressive factors, i.e. the presence of a tumor or following chemotherapy, are exerting an inhibitory effect on the immune system.

There have been considerably fewer studies examining the effect of exercise on antigen-specific T cell function. Most of these have explored the interaction between exercise, aging and T cell function; however, none to date have examined the effect of exercise on the generation of tumor-specific T cells. One line of evidence to suggest that regular, moderate exercise can stimulate antigen-specific immunity is the observation that the incidence and duration of upper-respiratory tract infections is significantly lower in postmenopausal women who are moderately active as compared to sedentary controls (63;64;97). Although no adaptive immune endpoints were measured in any of these studies, adequate cellular immune responses play a critical role in the clearance of viral infections of the respiratory tract (98;99). Two other studies that have examined the effect of exercise on antigen-specific T cell responses have reported a stimulatory effect. Physically fit older men (age 60-79) had significantly greater delayed-type hypersensitivity (DTH) reaction to keyhole limpet hemocyanin (KLH) and higher anti-KLH antibody titers than sedentary controls (100). Older men and women (age 65 or older) who were active or moderately active prior to influenza immunization had greater *in vitro* antigen-specific T cell proliferation and antibody titers than sedentary subjects (101). Furthermore, several studies in animals have demonstrated a beneficial effect of exercise on T cell function in aged mice. Kohut and colleagues demonstrated that eight weeks of exercise prior to herpes simplex virus-1 (HSV-1) infection enhanced *in vitro* HSV-1 specific cytokine production (IL-2 and IFN-gamma) in older (16-18 months) but not younger mice (2-4 months) (102;103).

Recent studies conducted to assess the effect of exercise on antigen-specific immunity in young, non-tumor bearing animals have demonstrated that eight weeks of voluntary running prior to vaccination with either a protein or viral based vaccine enhances antigen-specific immune responses. Specifically, antigen-specific proliferation of $CD4^+$ T cells collected from the spleens and inguinal lymph nodes of animals vaccinated subcutaneously with a protein-based vaccine (ovalbumin plus lymphotactin) was significantly higher in exercising animals (104) Additionally, eight weeks of training prior to vaccination enhanced antigen-specific splenic $CD4^+$ T cell proliferation following vaccination with a pox virus based vaccine (recombinant vaccinia/fowlpox NP34 plus recombinant fowlpox GMCSF) (72). In subsequent studies, the minimum length of training time needed to enhance antigen-specific immune responses in C57BL/6 mice was shown to be eight weeks. Importantly, initiating exercise concurrently with the administration of the primary vaccination did not yield significant increases in $CD4^+$ T cell proliferation (72). These data suggest that a training period of eight weeks prior to the primary vaccination is required to achieve the stimulatory effect of exercise on adaptive immune function and that exercise can be used effectively in combination with vaccination.

3.1.3. Physical activity in combination with cancer vaccine

No studies to date have combined moderate physical activity with the administration of a therapeutic cancer vaccine. However, this combinatorial approach seems promising for several reasons. Moderate exercise alone following cancer treatment has been shown to decrease recurrence and increase survival in cancer patients. Compelling findings from the Nurses Health Study, one of the largest prospective investigations examining chronic disease risk factors in women, demonstrated that women who exercised for the equivalent of walking 3-5 hours per week at an average pace had a 50% reduction in breast cancer mortality risk (105). Importantly, women who exercised for the equivalent of walking 1-3 hours per week had a 20% reduction in breast cancer mortality risk, suggesting that modest increases in physical activity can have a profound impact on clinical outcomes. A second report examining physical activity and colorectal cancer outcomes from the Nurses Health Study found similar results. Female nonmetastatic colorectal cancer patients who exercised for the equivalent of walking six or more hours per week at an average pace had approximately a 50% reduction in both colorectal cancer-specific and overall mortality (106). A third study of patients enrolled in an adjuvant chemotherapy trial for stage III colon cancer who exercised for the equivalent of walking six or more hours per week at an average pace had a 47% improvement in disease free survival compared to sedentary patients (107). In addition to the robust effect of exercise on clinical outcomes, exercise interventions in women with breast cancer have been shown to be safe, have had high compliance levels and result in improved fitness and quality of life (108;109). These data suggest that combining an exercise intervention with other therapeutic strategies, such as cancer vaccine treatment, may be relatively easy to implement and confer significant benefit to the patient independent of any enhancement of vaccine efficacy.

Numerous cancer vaccine platforms have already been shown to stimulate tumor-antigen specific immune responses (110-113) and increase disease free survival (4;114) in cancer patients over 65 years old. Moderate exercise has also been shown to enhance the antigen-specific immune responses in aged humans and animal models. Furthermore, moderate exercise can be used effectively in combination with a variety of vaccination protocols to enhance antigen-specific T cell responses in preclinical models. Combined, these data suggest that moderate physical activity is a likely candidate to partner with therapeutic cancer vaccine treatment to enhance the vaccine efficacy. Studies are currently underway to examine this combination in preclinical animal models.

3.2. Calorie restriction

3.2.1. Role of calorie restriction in cancer prevention

The best studied alteration of energy balance in experimental tumor models is on the energy intake side of the equation, specifically involving obesity prevention through calorie restriction (CR). Studies in rodents have most often intiated CR early in life (at the time of weaning) and maintained the CR for the life of the animal. This long term CR inhibits the incidence and growth rate of a variety of spontaneous neoplasias in experimental cancer model systems, including tumors arising in several genetically altered mouse models (e.g., p53-deficient mice, APC^{min} mice and *Wnt-1* transgenic mice); as well as carcinogen and radiation induced cancer (recently reviewed in (8;9)). Additionally, several studies have shown that CR initiated in adult animals in mid to late life also reduces the incidence (115-118) and/or delays the onset (118;119) of spontaneous tumors. CR has also been shown to increase survival in most tumor models (120-125). Thus, the inhibitory action of CR on carcinogenesis is effective in several species for a variety of tumor types and importantly, when intiated in either early and late life.

3.2.2. Modulation of immune function by calorie restriction

Long term CR throughout the life course prevents a variety of age-associated decrements in immune function (117;126-129), and is an intriguing model to explore the effects of aging on the immune system. However, two aspects of long term CR make it difficult to translate into a viable intervention for use in humans: 1) the early age of onset of CR in animal models and 2) the duration of this exposure over the life course. CR started in older animals and/or shorter duration of CR (weeks to months) are both more appropriate model systems to investigate the effects of CR on immune function, particularly if CR is being considered as a potential intervention for use in combination with therapeutic cancer vaccines. Much less is known about the effects of CR initiated in mid-life on immune function. Adult onset CR initiated at 12 months of age (117;130) and at 17 months of age (131) and lasting for up to eight months increased splenic mitogen-induced proliferation and increased the percentage of splenic T cells in mice (116). Additionally, 27 month old mice restricted at 12 months of age had significantly higher allogeneic T cell responses compared to their *ad libitum* fed, age-matched controls (116;130). Although a limited number of studies have explored the immune sequelae of CR started in mid life, the results to date suggest that a beneficial effect on immune function can be achieved when CR is initiated in older animals.

In contrast, studies exploring the effects of shorter term CR (up to six months) on immune function in animals of varying ages have reported inhibitory effects. A dose dependent inhibition of antigen-specific T cell proliferation following eight weeks of either mild (20%) or severe (50%) CR was observed in normal C57BL6 mice (132). This CR-induced inhibition of T cell proliferation was due to both a deficit in the antigen presenting capabilities of macrophages and proliferative capacity of T cells. In one study, moderate CR significantly reduced mitogen-induced and allogeneic T cell proliferation during the first month of restriction, but after six months on the diet the proliferative responses of CR and *ad libitum* animals were no longer different (131), suggesting that the time of exposure to CR may influence immune responsiveness. Additionally, in several rodent autoimmunity models, short-term CR in young animals decreases antigen-specific proliferation of T cells, as well as decreases IFN-gamma, IL-12 and autoantibody production (133;134). Futhermore, 40% CR beginning at six weeks of age and lasting for several months, dampens autoreactive lymphocyte proliferation and cytokine production (IL-2 and IFN-gamma) by both $CD4^+$ and $CD8^+$ lymphocytes in the autoimmune prone $(NZBxNZW)_{F1}$ (B/W) murine model of systemic lupus erythematosis (135-137). Using autoimmune prone (B/W) mice another group has shown decreased mRNA expression of IL-6 and TNF-alpha, and increased expression of TGF-beta in splenocytes harvested from CR animals (138). Finally, four to eight weeks of moderate (30%) to severe (50%) CR significantly lowered splenic NK cell cytotoxicity (139;140). These data suggest that short-term CR in non-aged animals may suppress a number of effector cell functions. Futhermore, these results suggest that a longer duration of exposure to CR (greater than six months) may be necessary to achieve the stimulatory effects on immune function. Additional studies are needed to further characterize the effects of adult onset CR on antigen-specific T cell functions and NK cell cytotoxicity to determine what factors (e.g., age of the animal at the onset of CR, duration of CR exposure, severity of CR, etc...) influence immune responses.

3.2.3. Calorie restriction in combination with cancer vaccine

No studies to date have implemented CR prior to the administration of a therapeutic cancer vaccine. In our view, CR may be an effective strategy to reduce weight in overweight and obese individuals (discussed in the next section). However, additional studies are needed to futher examine the effects of short term CR on immune function in middle-aged normal and overweight animals before any recommendations regarding its use in combination with therapeutic vaccines can be made.

3.3. Obesity: prevention and reversal

3.3.1. Links between obesity and cancer

Obesity continues to be one of the leading health issues facing the nation. The prevalence of obesity has risen significantly over the past several decades and there is no indication that this trend is declining (141;142). In 2004, 66.3% of adults were overweight (body mass index or BMI = 25.0-29.9 kg/m^2), 32.2% were obese (BMI > 30.0 kg/m^2), and 4.8% were extremely obese (BMI > 40 kg/m^2) (142). This trend in the prevalence of obesity is related to an increased incidence of a myriad of chronic diseases, including cancer (143). A body of convincing epidemiologic evidence has accumulated suggesting that overweight or obesity increases risk of several types of cancer including colon, breast (in postmenopausal women), endometrial, ovarian, kidney, esophageal, pancreatic and gallbladder (35;144-147). Furthermore, data from a large cohort study of more than 900,000 adults in the U.S. has demonstrated that in both men and women, increased BMI is associated with increased mortality from tumors of the esophagus, colon, liver, gallbladder, pancreas, and kidney, as well as death due to non-Hodgkin's lymphoma and multiple myeloma (11). In this same cohort, increased BMI is associated with increased mortality from cancers of the stomach and prostate in men; and mortality from cancers of the breast, uterus, cervix, and ovary in women (11). In addition to providing recommendations on physical activity, the *IARC Working Group on the Evaluation of Weight Control and Physical Activity* concluded that excess body weight and physical inactivity account for approximately a quarter to one third of cancers of the colon, breast, endometrium, kidney and esophagus (35). Thus, excess adiposity and physical inactivity appear to be the most important avoidable causes of these cancers.

3.3.2. Obesity and immune function

Although obesity is believed to adversely impact immunity, the effect of overweight and obesity on the function of specific immune cell types has not been well studied. However, obesity has been consistently associated with a state of low grade, chronic inflammation (148;149). Cross-sectional analyses show elevated levels of C-reactive protein (CRP) (150;151), tumor necrosis factor alpha (TNF-alpha) (152-154), and IL-6 (155) in the serum of overweight and obese individuals. A variety of pro-inflammatory markers are produced in adipose tissue and increase with increasing adiposity, including: TNF-alpha, IL-6, monocyte chemotatic protein-1 (MCP-1), inducible nitric oxide synthase (iNOS), TGF-beta-1, and plasminogen activator inhibitor type 1 (PAI-1) (149). Macrophages are the main source of pro-inflammatory cytokines in the adipose tissue of obese subjects (156-158) and the number of adipose tissue macrophages increases with increasing body fat (156;157). Increased expression of inflammation-specific genes by adipose tissue macrophages has been shown in obese mice preceding the development of insulin resistance, a well-documented consequence of obesity (155). Both TNF-alpha and IL-6 block insulin action by triggering key steps in the insulin signaling pathway (155;159;160). In the study by Xu and colleagues, obese mice treated with rosiglitazone (an insulin-sensitizing drug) had a decreased expression of inflammation-specific genes in adipose tissue macrophages (157), suggesting an additional feedback loop between insulin signaling and inflammatory cytokines. Currently it is uncertain if the chronic elevation of circulating pro-inflammatory cytokines observed in obese subjects cause and/or contribute to any of the obesity-induced changes in innate and adaptive immune function (discussed below) or if the elevation of inflammatory cytokines and alterations in immunity are both downstream consequences of obesity.

In addition to the elevation of inflammatory markers in obesity, several reports have documented an obesity-induced impairment in innate immunity. Wound healing is significantly delayed and the reported incidence of wound complications is significantly greater in obese patients compared with normal-weight patients (161-163). Additionally, the incidence of nosocomial infections is higher in overweight and obese patients compared with normal-weight patients (161) and obese burn patients have an increased risk of infection and bacteremia during recovery (164). Taken together, these data suggest that several components of innate immunity are adversely impacted by obesity and the surgical resection of a primary tumor may be more complicated in obese patients.

The effect of obesity on NK cell function has been examined in several studies, but the results are inconsistent. Two cross-sectional studies have reported no statistically significant difference in NK cell cytotoxicity between obese subjects and lean controls (165;166). In another report, the influence of obesity on NK cell function differed by age. Obesity had no effect on NK cell cytotoxicity in younger subjects, but obese subjects over 60 years old had lower NK cell function than the lean age-matched controls (167). In animal studies, diet-induced obese animals have lower NK cell function than lean control animals (168;169). Moreover, in a metastasis model, the number of experimental lung colonies was significantly higher in obese mice compared to lean controls (170). Depletion of NK cells with anti-asialo-GM1 antibody led to increased metastases in both control and obese mice, but eliminated the obesity-induced difference in tumor metastasis (170). These results demonstrate that the obesity-induced increase in metastasis in this model is mainly due to impaired NK cell function in obese mice. These data suggest that obesity adversely impacts NK cell function in mice. However, additional studies are needed to further characterize the effects of overweight and obesity on NK cell function in both humans and animal models.

Little work has been done to date to identify the effect of obesity on adaptive immune function. Phenotypic studies have shown increased numbers of $CD19^+$ and $CD3^+$ cells among obese individuals. The

increase in $CD3^+$ T cells is due to a selective increase in $CD4^+$ T cells (166;171). There are conflicting results on the effects of obesity on the $CD8^+$ T cell population. One report showed no effect of obesity on the $CD8^+$ T cell population (84) while another showed a decreased frequency of $CD8^+$ T cells (171). Despite increases in the number of $CD4^+$ T cells, their functional capacity is impaired by obesity. Reduced mitogen-induced T cell proliferation has been observed in obese subjects (166;172;173) and in experimental animals (174;175). Additionally, obese children and adolescents have impaired DTH responses compared to normal weight controls (172). Finally, a recent study has demonstrated that obesity impairs antigen-specific $CD4^+$ T cell proliferation and cytokine production following vaccination with a pox virus based vaccine (169). Taken together, these preliminary results suggest that T cell function is impaired by obesity. Therefore, the generation of adequate immunological response to common vaccinations (e.g., flu, tetanus, etc…), and potentially, therapeutic cancer vaccines may be significantly impaired by obesity.

If obesity impairs innate and adaptive immune function, several critical questions need to be addressed. First, can weight loss reverse the adverse effects of obesity on the immune system? Second, does the method of weight loss, i.e. CR (diet), exercise or a combination of CR and exercise, differentially alter the immunological response to weight loss? A few studies have examined immunological endpoints following weight loss in obese subjects using either CR, exercise or a combination of CR and exercise and have reported inconsistent results. Obese subjects maintained on a very low calorie diet for 12 weeks lost a significant amount of body weight and had increased mitogen-induced T cell proliferation after 12 weeks on the diet (173). Similarly, overweight rats that were calorie restricted had a significantly higher number of splenic $CD4^+$ T cells and enhanced mitogen-induced proliferation compared to overweight *ad libitum*-fed rats (168). However, in another study, obese women completing a 26-week weight loss program involving severe CR followed by a period of incremental refeeding had a significant loss in body weight and percent body fat, but also had a significant decline in DTH responsiveness (176). Importantly, DTH responses remained suppressed for eight months after the end of the diet in those subjects who regained less than 40% of their original weight (176). Additionally, obese women who completed a 12-week weight loss program using a low calorie diet (50% reduction in calories) had a 30-35% reduction in the number of circulating NK cells that persisted following 35 days of normal food intake (177). Finally, one report showed that repeated cycles of weight loss were associated with lower NK cell function in postmenopausal, obese women (178).

In contrast, studies that have incorporated exercise into the weight loss program have reported better immune responses than CR alone. For example, in one report obese women were randomized to one of four groups: control, exercise (walking 45 minutes, 5 days/wk); CR (1200-1300 kcal/day) or a combined CR and exercise group. Women in the CR and CR plus exercise groups lost weight and lowered their percent body fat, however, only the obese exercisers had significantly lower upper respiratory tract infections (84). In another study, obese women were randomized into CR (925 kcal/day) or CR plus exercise (925 kcal/day plus 20 minutes aerobic activity 3 days/wk) groups for eight weeks. Women in the CR only group had significantly lower NK cell function following the diet, but the CR plus exercise group had no decrements in NK cell function (179). In animal studies, exercise but not weight loss enhanced NK cell cytotoxicity and Con A-induced proliferation of splenic lymphocytes in obese Zucker rats (174). These limited results suggest that the incorporation of physical activity into a weight loss regimen may enhance a broader repertoire of immunological responses than dieting alone.

3.3.3. Weight loss strategies in combination with cancer vaccine

No studies to date have implemented weight loss strategies (either via CR, increased physical activity or a combination of both) prior to the administration of a therapeutic cancer vaccine. However, reducing body weight and/or percent body fat may provide a better host environment to generate antigen-specific vaccine responses since obesity had been linked to adverse clinical outcomes, as well as potentially impaired immune function.

In numerous studies, being overweight at the time of diagnosis has been identified as a predictor of adverse clinical outcomes in women with breast cancer (10). To date, 39 studies have examined the relationship between obesity and risk of recurrence or survival in breast cancer patients. A significant association between obesity and recurrence or survival for breast cancer was observed in 31 of these studies (180-185); and the negative effects of obesity on recurrence and survival were observed in both pre and postmenopausal women (185-187). Weight gain after diagnosis is also related to poorer survival in breast cancer patients (183;188;189). Weight gain after diagnosis is commonly reported in breast cancer patients, particularly those receiving adjuvant chemotherapy (190-193). Furthermore, several studies have demonstrated that a return to pre-diagnosis body weight seldom occurs in breast cancer patients (188;194), suggesting that the adverse effects of obesity, at least the risk associated with post-diagnosis weight gain, impact the vast majority of breast cancer patients.

Obesity is also predictive of other less favorable breast cancer clinical outcomes and related co-morbidities. Obesity is associated with more advance stage of disease at diagnosis (195-198), greater lymph node involvement (199-201), and greater risk of contralateral breast cancer in pre (202) and postmenopausal (202-204) women. Obese women also have greater wound complications (e.g., slower healing

and greater infections) and lymphedema following breast cancer surgery (205;206).

Although less well studied, similar patterns are emerging between obesity and clinical outcomes related to prostate and colorectal cancer. A number of large cohort studies have demonstrated a clear association between obesity and prostate cancer mortality (11;207-209). In addition, several of these studies have also demonstrated that obesity is strongly correlated with more aggressive disease (207-210). Obese prostate cancer patients have higher grade and/or stage tumors and higher rates of positive surgical margins at the time of radical prostatectomy (210-214), and are at greater risk for biochemical failure following surgery (210;214). Two recent studies have demonstrated that obese men are at an increased risk of developing metastases (215;216). Finally, several recent studies have shown a positive association between obesity at the time of diagnosis and prostate cancer recurrence (210;217-219).

Less data is available regarding the impact of obesity on clinical outcomes related to colorectal cancer. In a randomized adjuvant chemotherapy trial for colorectal cancer, obese women had 34% greater risk of overall mortality and 24% increased risk of recurrence than normal weight women (220). In a second trial investigating clinical outcomes in subjects from the National Surgical Adjuvant Breast and Bowel Project, obesity at the time of diagnosis was associated with increased risk of colon cancer recurrence and death (221). Lastly, in an Australian cohort, incremental increases in percent body fat were positively associated with colorectal-specific mortality (222). Together these data suggest that obesity is associated with adverse clinical outcomes following diagnosis of breast, prostate and colon cancer. Reduction in body weight either through moderate calorie restriction and/or physical activity following diagnosis may reduce the risk of these cancer-related events. However, no studies to date have addressed this issue.

The adverse effects of obesity on recurrence, survival, and other clinical outcomes, and the potential for obesity to impair immunological response to vaccine provide compelling rationale for reducing obesity in cancer patients in general, but also specifically prior to receiving a therapeutic vaccine. However, it may not be feasible to recommend weight loss in overweight and obese patients until after the completion of adjuvant therapy given the potential for adverse side effects associated with treatment (e.g., nausea, fatigue, etc…). Furthermore, the few studies that have explored the effect of weight loss on immune endpoints in obese subjects suggest that weight loss achieved via dieting versus dieting in combination with exercise may have different consequences on immune function, with exercise providing greater benefit. Given the number of potential cancer patients who are likely to be overweight or obese at the time of diagnosis or become obese following treatment, future studies are warranted to first, determine the mechanisms by which obesity impairs immune function; and second, determine if these immunological impairments can be reversed though weight loss or other pharmacological interventions that can be used prior to or in combination with vaccine to enhance vaccine efficacy.

4. Impact of Nutrients

4.1. Retinoids and carotenoids

4.1.1. Role of retinoids and carotenoids in cancer prevention

Vitamin A and its natural analogues, all-trans, 9-cis, and 13-cis-retinoic acids, have been shown to promote the differentiation of normal and neoplastic cells, *in vitro* and *in vivo* (223;224). The carotenoids are fat-soluble, antioxidant compounds (xanthophylls, carotenes, and lycopene) that are found in green and yellow leafy vegetables and serve as precursors to vitamin A and its derivatives. Epidemiological studies indicate that diets deficient in the retinoids and carotenoids or individuals with low serum retinol levels are associated with increased relative risk of cancer (225-227). However, large, randomized studies of dietary supplementation with beta-carotene reported significant increases in lung cancer incidence and mortality in heavy smokers and asbestos workers (228-231). These data led the Institute of Medicine (IOM) to conclude that beta-carotene supplementation is not advisable for the general population, but may be appropriate in vitamin A deficient populations (232).

In preclinical studies, a liposomal formulation of all-trans retinoic acid (ATRA) was shown to induce sustained remission of acute promyelocytic leukemia in immunocompetent but not immunodeficient mice, suggesting an immunological mechanism of action (233). The use of 9-cis-retinoic acid (9-cis-RA) has been shown to inhibit the growth of ER^+ (234;235) and ER^- (236) mammary tumors in the rat. Clinical trials of 13-cis-retinoic acid (13-cis-RA, Isotretinoin, Accutane, Hoffman-La Roche Inc.) show regression of oral leukoplakia (237) and significant reductions in the incidence of secondary squamous cell carcinomas of the head and neck (238;239). A topical formulation of 9-cis-RA (Alitretinoin gel 0.1%, Panretin, Ligand Pharmaceuticals) is FDA approved for the treatment of AIDS-related Kaposis's sarcoma (KS) where it is thought to inhibit the proliferation and neoangiogenesis of KS lesions (240).

To avoid the toxicities associated with the retinoids (241), synthetic analogues that preferentially bind to the retinoid X receptor (RXR) termed "rexinoids", LGD1069 (Bexarotene, Targretin, Ligand Pharmaceuticals) and LG100268, were developed. These synthetic analogues show preventive and therapeutic efficacy against animal models of ER^+ (242-245) and ER^- (244;246-248) mammary carcinogenesis. In a phase II clinical trial of metastatic breast cancer patients, Bexarotene (Targretin, LGD1069) was shown to benefit 20% of the patients without significant

toxicity (249). Even greater promise may lie in the new rexinoid, LG100268, which has been shown to synergize with the selective estrogen receptor modulators, Arzoxifene and Acolbifene (244;248), and may be more potent and less toxic than LGD1069 due to its greater specificity of RXR binding (250).

4.1.2. Modulation of immune function by retinoids and carotenoids

There is support in the literature for a diverse immunomodulatory role of the retinoids. Preclinical studies suggest that ATRA therapy promotes Th2-mediated immune responses (251-253). Vertuani and colleagues (254) demonstrated that the retinoids, ATRA and 9-cis-RA, stabilized the class I major histocompatibility complex (MHC) and enhanced both MHC class I restricted peptide-specific lysis by $CD8^+$ cytotoxic T lymphocytes (CTLs) and NK cell cytotoxicity of neuroblastoma cells. Treatment with ATRA works additively with IFN-gamma (255) and high dose gamma irradiation (256) to upregulate human leukocyte antigen (HLA) class I and intercellular adhesion molecule 1 (ICAM-1) on human cervical carcinoma cell lines. Against a panel of human uveal melanoma cells, ATRA treatment was shown to induce G1 arrest, apoptosis, and sensitize cells to MHC class I restricted CTL killing and NK mediated lysis (257). Furthermore, the *in vitro* proliferation of T cells in response to anti-CD3/anti-CD28 stimulation is significantly improved with ATRA (258). Following chemotherapy in advanced ovarian cancer patients, the combination of low-dose IL-2 with 13-cis-RA was shown to reduce circulating vascular endothelial growth factor (VEGF) levels, increase lymphocyte and NK cell numbers, and significantly improve both progression-free and overall survival (259).

Bexarotene (Targretin) is FDA approved for the treatment of early (260) and advanced (261) cutaneous T cell lymphoma (CTCL). Both Bexarotene (Targretin) and Alitretinoin (Panretin) enhance the sensitivity of T- and B-leukemia cells to denileukin diftitox (ONTAK) due to upregulation of the α/p55/CD25 and β/p75/CD122 chains of the high-affinity IL-2 receptor (262). The combination of Bexarotene (Targretin) and ONTAK (Panretin) is currently being evaluated in clinical trials of patients with relapsed or refractory CTCL (263).

4.1.3. Use of retinoids and carotenoids in combination with cancer vaccine

Several lines of evidence suggest that the retinoids may be effective adjuncts to antigen-specific immunotherapy. The expansion of $Gr\text{-}1^+/CD11b^+$ myeloid-derived suppressor cells (MDSCs) in tumor-bearing hosts are reported to be a major mechanism of tumor-associated immunosuppression (264). ATRA therapy has been shown to improve antigen-specific T cell responses through reductions in the $Gr\text{-}1^+/CD11b^+$ MDSC population in both mice (265;266) and humans (267). In an adoptive transfer model, ATRA therapy reduced the proportions of $Gr\text{-}1^+/CD11b^+$ MDSCs with concomitant increases in the proportions of $CD11c^+/I\text{-}Ab^+$ dendritic cells (DCs), $F4/80^+$ macrophages, and $Gr\text{-}1^+/CD11b^-$ granulocytes (266). Moreover, the loss of $Gr\text{-}1^+/CD11b^+$ MDSCs was associated with improvements in $CD4^+$- and $CD8^+$-mediated immune responses. ATRA therapy was shown to significantly enhance the antitumor efficacy of two vaccine strategies: 1) a peptide-based vaccine against C3 fibrosarcomas, and 2) a DC-based vaccine against MethA sarcomas (266).

In myeloid leukemia, reciprocal DNA translocations result in the formation of unique tumor-associated antigens such as the promyelocytic leukemia-retinoic acid receptor-α (PML-RARA) fusion protein which represents over 95% of the fusion proteins found in acute promyelocytic leukemia patients (268). Padua and colleagues (268) developed a DNA-based vaccine that linked the PML-RARA oncogene with fragment c of the tetanus toxin (PML-RARA-FrC). The DNA vaccine successfully targeted the oncoprotein and combination therapy with ATRA was shown to improve survival, induce antibody production against RARA, and increase serum IFN-gamma levels *in vivo* (268).

These studies provide mechanistic insights and demonstrate the potential of combination immunotherapy with retinoids such as ATRA; however, significant toxicities associated with the use of the retinoids (269) have led to the development of the synthetic rexinoids with fewer RAR-mediated side effects. The rexinoids show tremendous potential for chemoprevention, but have not been tested in combination with cancer vaccines (personal communication with Dr. Michael Sporn). Interestingly, the rexinoid, Bexarotene (Targretin, LGD1069) has been reported to inhibit cyclooxygenase-2 (COX-2) expression through transrepression of the AP-1 transcription factor in both normal and malignant mammary tissue (270). As pharmacologic inhibition of COX-2 expression has been shown to synergize with a variety of anti-tumor vaccine strategies (see Arachidonic Acid Metabolism), the potential for combination immunotherapy with the rexinoids is intriguing.

4.2. Tocopherols and tocotrienols

4.2.1. Role of tocopherols and tocotrienols in cancer prevention

Vitamin E, a fat-soluble antioxidant vitamin found in plant oils, grains, and seeds, is comprised of a family of four tocopherols and four tocotrienols. The tocopherols have been shown to scavenge free radicals, prevent lipid peroxidation in cell membranes and low-density lipoprotein cholesterol, and inhibit production of the nitrosamines, carcinogens derived from dietary nitrites. Epidemiological studies offer mixed results with some trials suggesting that alpha-tocopherol supplementation may protect against cancers of the prostate (231) and upper gastrointestinal tract (271) in high-risk populations. The Heart Outcomes Prevention Evaluation (HOPE) trial observed a protective effect of vitamin E supplementation (400 IU/day of RRR-alpha-

tocopheryl acetate) on incident cases of lung cancer; however, vitamin E supplementation was not shown to be protective against prostate or colorectal cancers (272). In a phase II clinical trial of the Community Clinical Oncology Program (CCOP), alpha-tocopherol supplementation (800 IU/day for 24 weeks) resulted in clinical or histological responses in 61% of patients with premalignant oral leukoplakia (273).

Vitamin E succinate (VES) is a redox-inactive ester analog of vitamin E and a potent chemopreventive agent in many types of cancer with remarkable specificity for malignant tissues (274;275). In prostate cancer cells, VES induces apoptosis by suppressing NF-kB activity, inhibiting the interaction between Bcl-XL and Bcl-2 proteins, and sensitizing androgen-dependent prostate cancer cells to androgen deprivation *in vitro* (276;277). Malafa and colleagues (278) demonstrated that VES induced caspase 4-dependent apoptosis and inhibited lung metastases in human prostate tumors growing in SCID mice. In malignant mesothelioma cells, VES activates the expression of death receptors 4 and 5 (DR4 and DR5); thereby, rendering malignant mesothelioma cells sensitive to TNF-related apoptosis-inducing ligand (TRAIL) mediated apoptosis in a p53-dependent manner (279). The antitumor activity of VES has also been associated inhibition of angiogenesis through the suppression of VEGF expression in B16F10 melanoma (280) and MDA-MB-231 breast cancer cells (281).

4.2.2. Modulation of immune function by tocopherols and tocotrienols

There is evidence from clinical trials that vitamin E supplementation may boost immune function, particularly in high-risk elderly populations. Meydani and colleagues (282) evaluated the effects of vitamin E supplementation on indices of cell-mediated immunity in a randomized, double-blinded, placebo-controlled trial of healthy older subjects. Supplementation with 30 days of vitamin E (dl-alpha-tocopheryl acetate) at 800 IU/day was shown to increase DTH responses, Con A-stimulated T cell proliferation, and IL-2 release (282). In a subsequent randomized, double-blinded, placebo-controlled trial of elderly subjects, vitamin E supplementation for 4 months at 200 mg/day was shown to enhance DTH responses and antibody responses to hepatitis B and tetanus toxoid vaccination (283). In this study, subjects in the highest tertile of serum alpha-tocopherol concentration (> 2.08 mg/dL) following supplementation had the highest antibody titers to hepatitis B and DTH responses (283). These findings are consistent with a study in a Japanese cohort of nursing home residents in which pre-vaccination vitamin E status was positively associated with response to the influenza vaccine (284). In yet a third supplementation study by Meydani's group, nursing home residents receiving one year of vitamin E supplementation (200 IU/day) were significantly protected from upper respiratory tract infections such as the common cold (285). However, this effect was not observed in another randomized trial of non-institutionalized elderly individuals receiving vitamin E supplementation (200 mg/day) for up to 15 months (286).

In advanced colorectal cancer patients, short-term, high-dose vitamin E supplementation (750 mg/day for 2 weeks) increased $CD4^+/CD8^+$ T cell ratios, increased T cell production of IL-2 and IFN-gamma, and significantly improved NK function through induction of NKG2D expression (287;288). The beneficial effects of vitamin E supplementation on cellular and humoral immunity appear to be dose-dependent as De Waart and colleagues (289) reported that 3 months of supplementation with 100 mg/day of dl-alpha-tocopheryl acetate did not significantly alter mitogen-activated lymphoproliferative responses or antigen-specific IgG or IgA antibody titers.

Several hypotheses have been forwarded to explain the effects of vitamin E supplementation on age-associated decrements in cell-mediated immunity. In a recent study, *in vivo* vitamin E supplementation of aged mice improved the development of an effective immune synapse between naive $CD4^+$ T cells and APCs resulting in improved T cell signaling and activation (290). Han and colleagues (291) reported that vitamin E supplementation in aged mice resulted in up-regulation of IL-2 and down-regulation of IL-4 expression following anti-CD3/anti-CD28 stimulation suggesting a shift in Th1/Th2 balance in the T cells of older mice receiving vitamin E *in vivo*. Another hypothesis is based on the observation that macrophages from aging animals produce more prostaglandin E_2 (PGE_2) which has been shown to inhibit the proliferative capacity and IL-2 secretion of T cells, *in vitro*. In co-culture experiments, vitamin E supplementation and the cyclooxygenase inhibitor, indomethacin, were shown to improve T cell responsiveness in old mice primarily by reducing macrophage-derived PGE_2 production (292). Similarly, VES is hypothesized to inhibit the initiation and progression of lung cancer through its ability to suppress COX-2 activity and PGE_2 production in phorbol ester-stimulated human lung epithelial cell lines (293). Thus, the synthetic rexinoid, Bexarotene, and the esterified vitamin E analog, VES, both appear to mediate at least some of their chemopreventive and immunomodulatory effects through the inhibition of COX-2 activity and PGE_2 production in target tissues.

4.2.3. Use of tocopherols and tocotrienols in combination with cancer vaccine

To date, three preclinical studies have evaluated VES in combination with DC-based cancer vaccines. Ramanathapuram and colleagues (294) demonstrated that DCs pulsed with tumor cell lysate and injected either intratumorally or subcutaneously in combination with locally or systemically administered VES resulted in significant growth inhibition of established Lewis lung carcinomas. In this study, the adjuvant effect of VES was shown to outperform that of cyclophosphamide. One of the impediments to the clinical applicability of VES is its requirement for ethanol, DMSO, or sesame oil for solubilization. A

more soluble formulation of vitamin E succinate, vesiculated alpha-tocopherol succinate (V-alpha-TOS), was shown to be soluble in aqueous solutions and enhance the anti-tumor efficacy of DC-based vaccines against 4T1 breast (295) and Lewis lung (296) carcinomas. In both studies, non-matured DCs incubated with the lysates of V-alpha-TOS-treated tumor cells were shown to mature as evidenced by the upregulation of costimulatory molecules (CD40, CD80, and CD86) and the secretion of IL-12p70 (295;296). Two mechanisms were proposed to explain how V-alpha-TOS improves DC-based immunotherapy: 1) V-alpha-TOS directly lyses tumor cells thereby cross-priming DCs through antigen cascade, and 2) V-alpha-TOS induces the expression of heat shock proteins 60, 70, and 90 in tumor cells which present a "danger" signal to antigen presenting cells (APCs) leading to their activation (295;296). The improved solubility and demonstrated adjuvant characteristics of V-alpha-TOS make this compound a promising candidate for additional research and clinical development.

5. OTHER METABOLIC FACTORS

5.1. Introduction to other metabolic factors

Thus far, this review has focused on systemic modulation of energy balance and nutritional status to optimize innate and adaptive immune function and responses to antigen-directed cancer immunotherapy (Tables 1 and 2). However, there is a growing body of evidence demonstrating that nutrient metabolism at the level of the tumor microenvironment (tumor cells, stroma, and tumor-draining lymph nodes) is employed by the growing tumor to induce peripheral immune tolerance and tumor escape (reviewed in (297)). Below, we have reviewed complex immunosuppressive networks in which arachidonic acid metabolism, the catabolism of the amino acids arginine and tryptophan, and the depletion of glucose from the tumor microenvironment contribute individually, and in combination to inactivate effector T cell function and promote a tolerogenic state (Figure 1). Where available, we have provided examples of small molecule inhibitors that target these pathways and their utility in combination with cancer vaccines (Table 3).

5.2. Arachidonic acid metabolism

Arachidonic acid is a 20-carbon polyunsaturated fatty acid hydrolyzed from membrane phospholipids by the enzyme phospholipase A_2 (298). The cyclooxygenase-2 enzyme, COX-2, is the rate-limiting step in the metabolism of arachidonic acid to the prostaglandins (PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2) and the thromboxanes (TXA_2) (298). Overexpression of COX-2 activity has been reported in preneoplastic lesions as well as gastric cancers and carcinomas of the lung, colon, and breast (299). The synthesis of PGE_2 contributes to tumor promotion and progression through its effects on apoptosis, inflammation, angiogenesis, tumor invasiveness, and immunosuppression (298). Tumor or macrophage-derived PGE_2 production induces immunosuppression by impairing B and T cell proliferation, inhibiting NK cell cytotoxicity, and inducing FoxP3 expression and regulatory T cell (T_{reg}) function in naïve $CD4^+CD25^-$ cells (298;300;301). Additionally, PGE_2 inhibits the production of TNF-alpha while promoting the expression of the immunosuppressive cytokine, IL-10 (302). Clinically, COX-2 and PGE_2 expression were correlated with reduced Th_1 and increased Th_2 serum cytokine levels and impaired DC and T cell function in breast cancer patients (303). Thus, selective COX-2 inhibition is reported to reverse tumor immunosuppression by reducing intratumoral $CD4^+CD25^+FoxP3^+$ T_{reg} cells, increasing the production of IFN-gamma by tumor infiltrating lymphocytes (TILs), and normalizing the balance between Th_1 (IL-12) and Th_2 (IL-10) cytokines (297;298;301;304).

Stolina and colleagues (304) were the first to report that COX-2 inhibition could promote anti-tumor immunity. In this study of Lewis lung carcinoma, the selective COX-2 inhibitor, SC-58236, led to a reduction in IL-10, an increase in IL-12 production by APCs, significant tumor growth inhibition, and prolonged survival (304). Subsequent animal studies demonstrated that selective COX-2 inhibition significantly improves the efficacy of anti-tumor vaccines to inhibit tumor growth and promote survival in murine models of mesothelioma (305), breast (306;307), lung (301;306), and familial adenomatous polyposis (FAP) (308). Due to the safety concerns regarding the selective COX-2 inhibitors (309-311), efforts are now being made to develop small molecule inhibitors that target the prostaglandin receptors (EP1-4) through which PGE_2 is known to induce cell signaling such as the induction of FoxP3 expression in T_{reg} cells (297). Thus, the available evidence provides ample rationale for combining strategies for targeted inhibition of arachidonic acid metabolism such as COX-2 inhibition or PGE_2 receptor antagonism with antigen-directed cancer immunotherapy.

5.3. Arginine metabolism

L-arginine is a conditionally essential amino acid and its local catabolism by tumor cells, tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs) is reported to modulate tumorigenesis and immune function (297;312;313). The catabolism of arginine is catalyzed by two classes of enzymes: 1) arginase (ARG) encoded by the ARG1 and ARG2 genes, and 2) nitric-oxide synthase (NOS) encoded by the NOS1 (neuronal or nNOS), NOS2 (inducible or iNOS), and NOS3 (endothelial or eNOS) (297;313). As a component of the urea cycle, the ARG1 isoform is highly expressed in the liver where it catalyzes the conversion of L-arginine to L-ornithine and urea (297). The enzyme ornithine decarboxylase (ODC) acts on L-ornithine to initiate the first step in the biosynthesis of the polyamines (putrescine, spermidine, and spermine), a class of organic by-products of L-arginine catabolism known to act as endogenous tumor promoters (314). The intracellular accumulation of polyamines have been reported to be a consequence of

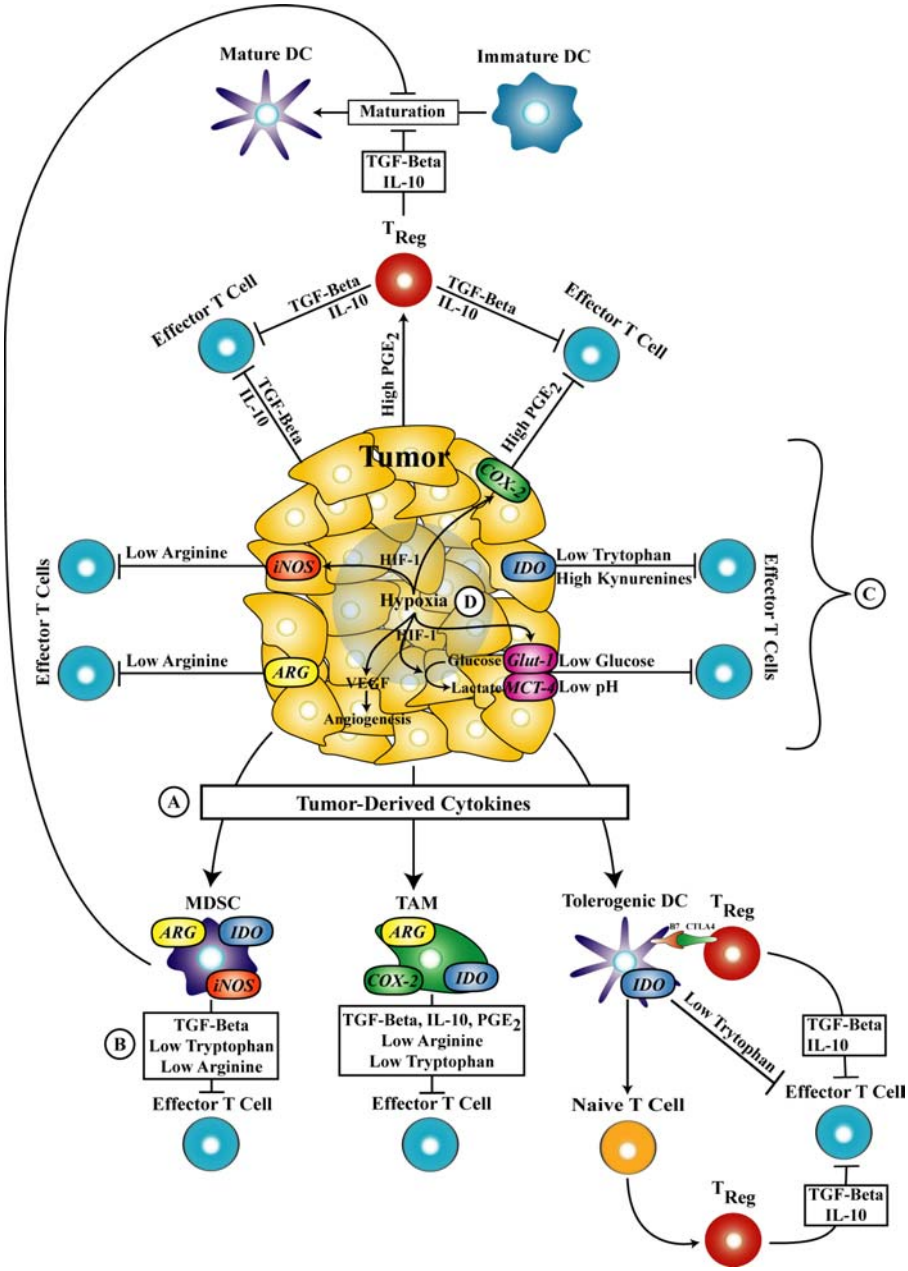

Figure 1. Nutrient metabolism in the tumor microenvironment contributes to immunosuppression and tumor escape from immune surveillance. A) Tumor-derived cytokines and others soluble factors such as interleukin-10 (IL-10), transforming growth factor-beta (TGF-beta), vascular endothelial growth factor (VEGF), prostaglandin E_2 (PGE_2), and colony stimulating factor (CSF) promote the expansion of immunosuppressive cell populations such as myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), and tolerogenic dendritic cells (DCs). B) Both tumor cells and immunosuppressive cell types can inhibit $CD4^+$ and $CD8^+$ effector T cells through the: 1) release of TGF-beta and IL-10, and 2) activation of $CD4^+/CD25^+/FoxP3^+$ regulatory T (T_{REG}) cells. C) The enzymatic activity of indoleamine 2,3-dioxygenase (IDO), inducible nitric oxide synthase (iNOS), arginase I (ARG), and cyclooxygenase-2 (COX-2) found in tumor cells and tolerogenic cells contributes to effector T cell inactivation by depleting local concentrations of nutrients such as tryptophan, arginine, and producing the immunosuppressive factor, PGE_2. D) Hypoxia generated in rapidly growing tumors further induces a tolerogenic state in the tumor microenvironment in a HIF-1-dependent manner by depleting glucose concentrations through increased uptake and metabolism, lowering the pH through lactate production, depleting arginine concentrations through upregulation of iNOS, and increasing the production of PGE_2 through COX-2 induction, and increased angiogenesis through transcriptional activation of VEGF expression.

elevated ARG and ODC-mediated arginine catabolism in tumor cells (312;315). Consistent with these findings, elevated ARG activity has been reported in patients with non-small cell lung cancer (NSCLC), skin, colorectal, breast, and prostate cancer (313). However, other studies suggest that MDSCs and TAMs are the more significant source of tumor-associated arginine catabolism (316-319).

Myeloid-derived suppressor cells, expressing ARG and/or NOS activity, induce arginine catabolism in the tumor microenvironment and contribute to tumorigenesis and immunosuppression by several mechanisms (Figure 1). First, MDSC- or TAM-derived ARG1 activity has been shown to inhibit the function of adjacent effector T cells by down-regulating the expression of the T cell receptor (TCR) CD3 zeta chain (313;318;320). Second, Chang and colleagues (316) demonstrated that murine macrophages overexpressing ARG1 promote the proliferation of ZR-75-1 breast cancer cells through the conversion of L-arginine to L-ornithine and the subsequent activation of polyamine biosynthesis. This proliferative effect was inhibited by the arginase inhibitor, L-norvaline (316). These observations gain support from findings in patients with metastatic renal cell carcinoma (RCC) in which the arginase activity of peripheral blood mononuclear cells (PBMCs) was significantly higher while TCR CD3 zeta chain expression, IL-2, and IFN-gamma production were significantly lower in RCC patients compared to age- and gender-matched healthy controls (321). Depletion of the $CD11b^{+}/CD14^{-}$ subpopulation from RCC samples restored T cell proliferative capacity, IL-2 and IFN-gamma production, and TCR CD3 zeta chain expression suggesting a role for MDSC-derived ARG1 activity in local immunosuppression (321). Third, the catabolism of L-arginine via the iNOS pathway results in the production of nitric oxide (NO) and L-citrulline. The NO produced by MDSCs abrogates the effector function of adjacent T cells by inhibiting IL-2 receptor signaling and IL-2 production (reviewed by (313)). Finally, activation of both the ARG1 and iNOS enzymes synergize to induce effector T cell apoptosis through the previously described mechanisms and the production of cytotoxic peroxynitrites (313;317;319).

De Santo and colleagues (322) demonstrated that T cell proliferation and cytotoxicity were inhibited by $CD11b^{+}$ MDSCs expressing both ARG and NOS. In this study, an inhibitor of ARG and NOS, nitroaspirin (NCX-4016), was shown to reverse the effects of MDSC-mediated immunosuppression. In tumor-bearing mice, the combination of NCX-4016 and DNA-based vaccination increased the number of tumor-specific CTLs and significantly extended survival (322). These findings suggest a role for combining pharmacological inhibitors of L-arginine catabolism with cancer immunotherapy. As with celebrex, NCX-4016 targets multiple immunosuppressive pathways by inhibiting COX-1, COX-2, ARG1, and iNOS activities in the absence of the gastrointestinal toxicities observed with traditional aspirin.

5.4. Tryptophan metabolism

Local concentrations of the essential amino acid tryptophan can be depleted through the activity of the cytosolic enzyme indoleamine 2,3-dioxygenase (IDO) which catalyzes the conversion of tryptophan to its major metabolites, known collectively as the kynurenines (297). Munn and colleagues were the first to show that IDO-mediated typtophan catabolism induced peripheral immune tolerance in pregnant mice carrying allogeneic concepti (323). A specific, pharmacologic inhibitor of IDO, 1-methyl-tryptophan (1-MT), was shown to reverse maternal tolerance in this model (323). As illustrated in Figure 1, localized immunosuppression leading to tumorigenesis and immune escape is hypothesized to be the result of cumulative tryptophan catabolism by IDO-positive cell types in the tumor microenvironment including tumor cells, stroma, tolerogenic DCs, MDSCs, and TAMs (297).

Mechanistic studies suggest that IDO-mediated tryptophan catabolism regulates adaptive T cell immunity (324). Fallarino and colleagues (325) demonstrated that the absence of tryptophan and the presence of the immunotoxic kynurenines can inhibit the cytotoxic effector function of murine $CD8^{+}$ T cells through down-regulation of the TCR CD3 zeta chain. Prolonged tryptophan deprivation and exposure to kynurenine metabolites, or co-incubation with IDO-expressing DCs induces a TGF-beta dependent regulatory phenotype in naïve $CD4^{+}CD25^{-}$ T cells (326). Antigen-specific anergy is thought to be mediated by IDO-expressing tolerogenic DCs which have been found to accumulate in the tumor draining lymph nodes of both mice (327) and humans (297). The cytotoxic T lymphocyte antigen-4 (CTLA-4) on T_{regs} engages B7 receptor molecules on tolerogenic DCs to promote the release of IFN-gamma which acts in an autocrine feedback loop to activate IDO expression in tolerizing DCs (297). Immunosuppression in the tumor microenvironment is maintained when IDO-positive DCs promote T_{reg} expansion, differentiation, and local production of the immunosuppressive cytokines, IL-10 and TGF-beta (297).

A large number of human tumors are reported to constitutively express IDO including but not limited to prostatic, colorectal, pancreatic, cervical, and ovarian carcinomas (327) and the level of IDO expression is negatively correlated with survival in colorectal (328) and ovarian (329) cancer patients. The serum ratio of kynurenine to tryptophan, an indirect marker of IDO activity, has been shown to increase with age (330) and predict all-cause mortality in nonagenarians (331). Murine MC57G fibrosarcoma cells transfected with IDO inhibit $CD8^{+}$ T cell function *in vitro* (332) and IDO-transfection of P815 mastocytoma cells prevents the rejection of P815 tumors in mice vaccinated against the P1A tumor associated antigen (327). The IDO inhibitor, 1-MT, inhibits the growth of Lewis lung carcinomas (LLC) by antagonizing the immunosuppressive effects of IDO-positive mononuclear cells in LLC tumors and

the tumor-draining lymph nodes (333). Recently, tumor-induced COX-2 activity was shown to positively regulate IDO expression in a murine model of mammary carcinogenesis, PyV MT mice (334). The combination of celecoxib plus a DC-based vaccine improved IFN-gamma production and cytotoxicity of $CD8^+$ T cells from the tumor-draining lymph nodes, slowed the growth of mammary tumors, prevented lung metastases, and improved survival (334). These data suggest a role for the pharmacologic management of immunosuppression with selective inhibitors of inflammation (e.g., COX-2 with celecoxib) and/or tryptophan metabolism (e.g., IDO with 1-MT) in combination cancer immunotherapy.

5.5. Glucose metabolism

In an experiment comparing the gene expression profiles of naïve and primed $CD8^+$ effector CTLs, the effector state was associated with a significant increase in the expression of genes encoding glycolytic enzymes such as α-enolase, pyruvate kinase, triosephosphate isomerase, hexokinase II, and 6-phosphofructokinase type C (335). Consistent with these findings, effector $CD8^+$ T cells display greater glucose uptake, a higher glycolytic rate, and increased lactate production than naïve cells (336). In this study, glucose deprivation was shown to inhibit IFN-gamma gene expression suggesting that $CD8^+$ effector T cell function is uniquely sensitive to glucose availability (337). Despite the greater glucose uptake and utilization by effector versus naïve T cells, tumor cells are reported to take up and metabolize 10 to 100 fold more glucose per unit of time (336).

Enhanced glucose metabolism and angiogenesis are hallmarks of the cellular response to hypoxia in cancer. The central regulator of this response is the transcription factor hypoxia inducible factor 1 (HIF-1) which functions as a heterodimer between HIF-1-alpha and HIF-1-beta (338). Under normoxic conditions, intracellular HIF-1-alpha concentrations are regulated through interaction with the von Hippel-Lindau (VHL) tumor suppressor protein which induces ubiquitination and proteosomal degradation of HIF-1-alpha (339). Under hypoxic conditions (Figure 1), HIF-1-alpha is stabilized, heterodimerizes with HIF-1-beta, and binds to hypoxia response elements in the promoter regions or enhancer elements of genes involved in glucose metabolism (glucose transporters and glycolytic enzymes), angiogenesis (VEGF), inflammation (COX-2), and arginine metabolism (iNOS) (340-345). Similarly, under normoxic conditions in VHL-deficient renal cell carcinomas, HIF-1-alpha is stabilized (339) and glucose uptake is increased (346). Thus, tumors adapt to hypoxia by shunting ATP production away from the tricarboxylic acid (TCA) cycle and oxidative phosphorylation in favor of glycolysis (342). The microenvironments of many solid tumors are reported to be acidic (347) and the greater reliance on glycolysis contributes to this acidification by increasing the export of lactic acid into the tumor microenvironment (348). An acidic microenvironment may also contribute to metastasis by increasing the expression and activity of extracellular matrix-degrading proteases such as the collagenases (349).

Collectively, these findings suggest that hypoxia in the developing tumor acts as a master regulator of glucose metabolism, inflammation, angiogenesis, and metastasis. As such, HIF-1-alpha-directed immunotherapy offers the specificity of targeting hypoxic or VHL-deficient tissues and the opportunity to modulate glucose uptake and metabolism, neovascularization of the tumor, and the immunosuppressive effects of PGE_2 production and arginine depletion (Figure 1). This strategy is plausible given that HIF-1-alpha antisense gene transfer was shown to significantly enhance B7-1-mediated immunotherapy of EL-4 tumors in preclinical studies (350). Alternative immunotherapeutic strategies have targeted HIF-1-alpha-regulated gene products such as carbonic anhydrase IX, involved in pH control (351). Another strategy may lie in the use of NSAIDs such as ibuprofen which have been shown to upregulate the VHL protein leading to down-regulation of HIF-1-alpha, the Glut-1 transporter, VEGF, and the VEGF receptor, Flt-1 (352;353).

6. FUTURE RESEARCH DIRECTIONS

Reduction of obesity is an important health issue; however, additional preclinical and clinical work is needed to determine the effects of weight loss either through CR or exercise on immune function before any recommendations can be made about initiating weight loss prior to receiving a cancer vaccine. Modulation of energy balance through exercise is the most promising energy balance related intervention to partner with therapeutic cancer vaccines. Exercise interventions are safe, have high compliance levels and have significant positive effects on clinical outcomes in cancer patients. Exercise training has also been shown to have stimulatory effects on NK cell function, and antigen-specific T cell proliferation and cytokine production. These data suggest that combining exercise interventions with therapeutic cancer vaccine strategies may enhance vaccine efficacy and improve clinical outcomes in cancer patients. Future studies should focus on exploring the effects of combining physical activity with known cancer vaccine platforms to determine the dose, duration and frequency of exercise needed to achieve the maximum stimulatory effects of exercise on anti-tumor immune function.

Synthetic analogs of vitamins A and E are shown to have significant chemopreventive and immunomodulatory activity. Preliminary data suggests that vesiculated vitamin E succinate may synergize with cancer immunotherapy while the RXR-directed rexinoids offer intriguing potential in this regard. A robust and growing body of literature suggests that the pharmacologic manipulation of nutrient metabolism in the tumor microenvironment may offset

immunosuppressive mechanisms activated by the growing tumor and tolerogenic cell populations induced by tumor-derived cytokines. Several promising intervention strategies for translational development in combination cancer immunotherapy include COX-2 inhibition with celebrex or PGE_2 receptor antagonism, the use of NO-aspirin (NCX-4016), and the IDO inhibitor, 1-MT. Finally, the diverse repertoire of metabolic, immunosuppressive, and angiogenic mechanisms induced by tumor hypoxia make HIF-1α-directed immunotherapy a potential target for future vaccine development.

In summary, changes in energy balance via exercise, supplementation with specific nutrients (e.g., synthetic analogs of vitamins A and E) and modulation of nutrient metabolism in the tumor microenvironment all are viable candidates for further study in combination with cancer vaccines.

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