Tumor Biol. (2015) 36:6589–6602
DOI 10.1007/s13277-015-3639-0

REVIEW

Current position of TNF-α in melanomagenesis

Iuliana Nenu[1] · **Diana Tudor**[1] · **Adriana Gabriela Filip**[1] · **Ioana Baldea**[1]

Received: 22 March 2015 / Accepted: 3 June 2015 / Published online: 18 August 2015

Abstract Melanoma is one of the most heterogeneous and immunogenic forms of cancer. Both tumor and stroma cells synthesize many cytokines involved in rapid development and metastasis. One of these cytokines from the tumor milieu is tumor necrosis factor-alpha (TNF-α), which seems to have an intricate role in melanomagenesis. Initially, it was found that TNF-α can induce apoptosis of tumor cells through both extrinsic and intrinsic pathways, in contrast with later studies that revealed its protumoral activity. TNF-α is involved in inflammation, inducing the secretion of survival molecules like antiapoptotic proteins, proangiogenetic factors and metastasis markers. Although there are many therapeutic strategies against melanoma, the prognosis of advanced stages remains poor, due to several tumor resistance mechanisms. TNF seems to be a negative prognostic factor in melanoma surgery and correlates with chemotherapy resistance. However, high intratumoral levels of TNF-α might be beneficial for immunotherapy. Researchers may redirect their studies in the future by double activating of the proinflammatory molecule TNF-α and the immune cells in order to obtain an antitumoral response in metastatic melanoma.

Keywords Cutaneous melanoma · Inflammation · Immunotherapy · TNF-α

Iuliana Nenu and Diana Tudor contributed equally to this work.

✉ Adriana Gabriela Filip
gabriela.filip@umfluj.ro; adrianafilip33@yahoo.com

[1] Department of Physiology, University of Medicine and Pharmacy, 1-3 Clinicilor Street, Cluj-Napoca, Romania

Introduction

The association between inflammation and cancer development has been attested for more than 100 years ago, when Virchow observed the presence of the leukocytes in the tumor microenvironment. Lately, it was revealed that skin injury initiates inflammatory remodeling and renewal processes. Persistence of any deregulation of the cytokine/chemokine profile at the inflammation site may be the onset of various pathologies including cancer [1], since disturbance of these paths may lead to oncogenesis. The same pathway is involved in melanomagenesis. Ultraviolet radiation (UVR) from the sun exposure accounts for developing of 65 % of melanomas worldwide [2].

Cutaneous melanoma, first described by the French physician Rene Laennec, is an abnormal proliferation of melanocytes. It is the most harmful type of environmental skin cancer due to its rapid development and metastasis [3]. If diagnosed early, melanoma can be cured by surgical resection with almost 80 % effectiveness. However, once metastases occur, it is largely refractory to existing therapies, including newly developed forms of immunotherapy. Median survival time for patients with stage IV (metastatic) melanoma is approximately 9 months, and 3 years survival rates are less than 15 % [4].

Different molecular pathways are involved in melanomagenesis: (1) B-Raf (BRAF), one of the most intensively studied protein in melanoma biology, is a member of the Raf kinase family of growth signal transduction protein kinases and plays an important role in regulating the mitogen-activated protein kinase (MAPK) signaling pathway. MAPK regulates cell division, differentiation, and proliferation [5]. (2) Other deregulated pathways in melanomagenesis are PI3K-Akt pathway and signal transducer and activator of transcription 3 (STAT3) [6, 7]. (3) Different inflammatory molecules play a critical role in the processes of skin carcinogenesis

such as nuclear factor kappa B (NF-κB), hypoxia inducible factor-1 alpha (HIF-1α), cyclooxygenase-2 (COX-2), interleukin 1 (IL-1), and tumor necrosis factor-alpha (TNF-α) [1].

These molecules are involved in malignant conversion, tumor dissemination/metastasis, and angiogenesis. Understanding the interactions between the inflammatory molecules and the tumor microenvironment may lead to the development of more effective antimelanoma therapies.

A plethora of therapies against metastatic melanoma have emerged, including (1) MAPK pathway inhibitors, (2) anticytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), and (3) programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1) pathway blocking antibodies, with promising perspectives [8, 9]. Different approaches are also studied: (1) immune key players involved in carcinogenesis, (2) antiangiogenic therapies, and (3) combined chemotherapy protocols [10]. Nevertheless, melanoma resistance mechanisms hinder the efficacy of both old and new therapeutic approaches.

A paradigm in the melanoma development has recently emerged, the parallel between pregnancy and cancer [4, 11]. Similarities between the mechanisms involved in the maternal-fetus tolerance and the tumor escape from the host's immune cells have been found. Melanoma cells induce the secretion of sex hormones, corticotropin-releasing hormone (CRH), and other endocrine products that help the tumor evade the immune attack. Starting from this, new research approaches may identify promising targeted therapies against the immune tolerance in melanoma.

Aberrant production of TNF in pregnancy seems to be associated with obstetric complications such as recurrent fetal loss, gestational diabetes mellitus, hypertensive syndromes, and fetal growth restriction [12]. Since evidence supports an unusual resemblance between cancer development and pregnancy, TNF-α may be an important link between these two concepts, as it seems to be involved in both of them. Thus, TNF-α may be in the future one the key of elucidating the mechanisms of pregnancy and cancer, the bridge between life and death.

TNF is a pleiotropic cytokine with abundant roles in the organism. Some of them are synthesized in Table 1. Studies have located TNF-α as a central inflammatory mediator in carcinogenesis. Through binding to specific receptors, TNF-α can induce more than five pathways that end up with inflammation, apoptosis, proliferation, invasion, angiogenesis, metastasis, or morphogenesis. These pathways lead to contradictory effects: antiapoptotic and proapoptotic [24] known to be TNF-α dose-dependent, summarized in Fig. 1. Also, previous studies reported that TNF-α activates apoptosis, proliferation, and morphogenesis at the same time, and only after reaching an equilibrium between these signals, one of these processes will be instigated [25]. Even though it is still hard to estimate where the pathologic activity ends and the beneficial one starts, current studies tried to develop higher efficiency therapies based on the antitumor activity of TNF-α. In addition, TNF orchestrates the pathological response in pregnancy, therefore being a solid bridge between the mechanisms of pregnancy and cancer.

Protumoral mechanisms of TNF-α

TNF-α, also known in literature as cachectin, is a carefully studied cytokine identified for the first time in 1970s by Carwell et al. [26]. Although its name implies cytotoxic activity against cancer cells, abundant data attest TNF-α as a tumor promoter. Indeed, the proinflammatory effect of TNF-α was found to be important for the early stages of tumor promotion [27]. Produced mainly by macrophages and other cells from the tumor milieu, TNF-α participates in the inflammatory processes present in cancer development such as proliferation, enhancing epithelial–mesenchymal transition (EMT), metastasis, and angiogenesis [28]. Besides tumor progression, invasion, and angiogenesis, TNF-α can induce metastasis progression in melanoma cells by activating the NF-κB [29].

The intricate mechanisms of TNF-α are mainly mediated through the receptor TNF-receptor 1 (TNFR1) binding, located on almost all cells, with the exception of erythrocytes. It can be activated by both forms of TNF, soluble and transmembranal. TNF-receptor 2 (TNFR2) plays an accessory role of regulating the actions of TNFR1 [30]. On one hand, TNFR1 can induce cell survival by activating NF-κB and the mitogenic pathway of c-Jun N-terminal kinases (JNK) and, on the other hand, can induce apoptosis by the recruitment and activation of caspases [13]. The second mechanism is discussed in the next part of our review.

The proinflammatory cascade of TNF-α enhances the production of different molecules: growth factors, metalloproteinases, prostaglandins, leucotrienes, and inflammatory cytokines involved in cancer progression. The effects of TNF-α signaling pathway are mainly due to the activation of the protein complex nuclear factor kappa-light-chain-enhancer of activated B cells, also known as NF-κB [31]. NF-κB is responsible for starting the DNA transcription in order to promote the survival of the cells exposed to different harmful agents. NF-κB has been positioned as a crossroad between inflammation and cancer [32]. Thus, TNF-α signaling induces the NF-κB modulated switch from the proapoptotic to the antiapoptotic pathway in melanoma development.

TNF-α binding to its receptor TNFR1 starts the recruitment of two opposite proteins: TNFR-associated death domain-associated protein (TRADD) and tumor receptor-associated factor 2 (TRAF-2). Once activated, TRADD and TRAF-2 mediate both apoptosis and cell survival [33]. TRADD can bind to caspase-8 and initiate cell death, or it can bind to

Table 1 Main tumor and normal cell sources of TNF-α secretion, with consequent effects and relationship with different therapeutic approaches of this inflammatory molecule

Study type	Cells		Secretion trigger	Effects of TNF-α	Therapy effects of TNF-α secretion
	Type	Name			
In vivo [13].	Tumor	Tumor-associated macrophages (TAMs)—melanoma	BRAF inhibitor vemurafenib	Tumor proliferation	Chemoresistance
In vivo [14].	Tumor	B lymphocytes—squamous skin carcinoma, prostate carcinoma	Carcinogen, DMBA/TPA	Enhance protumor immunity, cancer-related chronic inflammation and tumor development in a genetic model of squamous carcinogenesis	Tumor development
In vitro [15].	Tumor	Endothelial cells, stromal cells	Chemotherapeutic agents	Increased production of CXCL1 and CXCL2, leading to myeloid cell recruitment and increased cell survival	Chemoresistance
In vitro [15].	Normal	Human-immortalized fibroblasts (hTERTBJ1 cells)—pancreatic adenocarcinoma	Induced overexpression of TNF-α	Reduced levels of inflammatory cytokines	Improved chemotherapeutic regimen
In vitro [16].	Normal	Murine 3T3-F442A preadipocyte cell line cocultured with human breast cancer cell line ZR 75.1	Tumor cells	Strong suppressor of adipogenesis	Obesity, chronic inflammation, poor prognosis
In vivo [17].	Normal	Cardiomyocytes—mini-pigs	Coronary microembolization	Cardiomyocyte apoptosis	Anti-TNF therapy suppresses cardiomyocyte apoptosis and improves early cardiac function
Clinical study [18].	Normal	Keratinocytes	Minimally painful mechanical and electrical stimuli	Trauma-related pain and hyperalgesia	Inflammation, pain
In vitro [19].	Normal	BV2 microglial cell line	Bisphenol A (BPA, 2,2-bis (4-hydroxyphenyl) propane)	Proinflammatory actions (NF-κB pathway)	Development of neurodegenerative disease
In vitro [20].	Normal	Human adrenocortical cells	Toll-like receptors 2 and 4	Proinflammatory actions (NF-κB pathway)	Sepsis
Clinical study [21].	Normal	Mesangial cells—diabetic nephropathy	Insulin resistance	- Cytotoxic to glomerular, mesangial and epithelial cells - Alteration of glomerular microcirculation - Renal hypertrophy	Antibodies against TNF-α reduced albuminuria and improved histological lesions
In vivo [22].	Normal	Renal parenchymal cells—mice with nephroangiosclerosis	Angiotensin II	Hypertension	Blood pressure elevation
In vitro [23].	Normal	Osteoclasts	Autocrine/paracrine regulation of bone remodeling	Bone resorption	Pathologic bone lesions

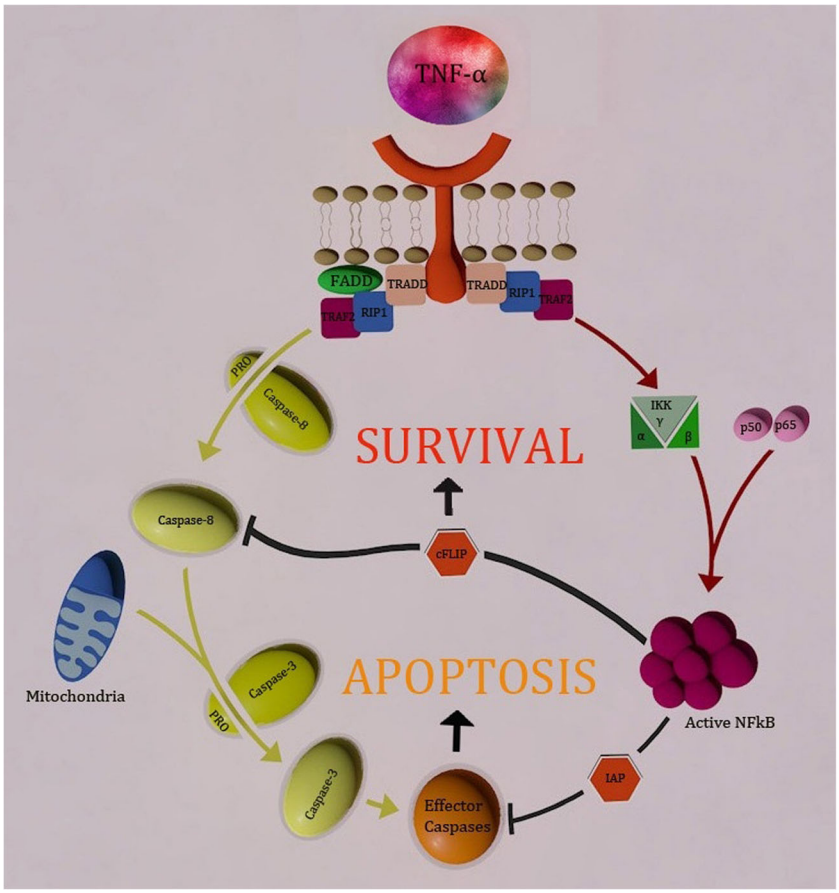

Fig. 1 The vicious circle of TNF-α effects. TNF-α binds to its specific receptor from the melanoma tumoral cell in order to activate its dual effects. TNFR is composed of three major subunits TRADD, RIP1, and TRAF-2. On one hand, this complex can lead to cell death by biding with FADD, forming the complex II of death and activating the caspases. And on the other hand, the TNFR complex leads to the activation of the IKK, with consequent activation of the NF-κB. The result is the upregulation of the antiapoptotic genes, which encodes antiapoptotic proteins such as c-FLIP and IAP that counteract specific caspase activation. In melanomagenesis, it could not be established when the protumor and antitumor mechanisms of TNF-α begin and end, in order to develop a suitable therapeutic action. *TNFR* tumor necrosis factor receptor, *TRADD* TNF receptor 1-associated death domain protein, *RIP1* receptor-interacting protein, *TRAF2* TNFR-associated factor 2, *IAP* inhibitor of apoptosis, *c-FLIP* caspase-8 (FLICE) inhibitory protein, *IKK* inhibitory protein of NF-κB kinase

TRAF-2 eventually leading to the activation of the IKK complex and NF-κB.

The upregulation of TRAF-2 with consequent NF-κB activation leads to the transcription of several antiapoptotic molecules such as the inhibitor of apoptosis (IAP) and caspase-8 (FLICE) the inhibitory protein (c-FLIP) [34, 35]. Antiapoptotic proteins promote cancer cell survival and proliferation. Their inhibition represents a promising target for oncological therapy.

The NF-κB complex consists of five transcription factors: RelA/p65, RelB, c-Rel, p50 (NF-κB1/p105 precursor), and p52 (NF-κB2/p100 precursor) that contain a Rel homology domain (RHD) in the N-terminal region. RHD mediates dimerization and DNA binding [36]. NF-κB is maintained in an inactive form by inhibitory kinases in the cell cytoplasm (IκBα, IκBβ, IκBε) [37]. TNF-α stimulation triggers the secretion of inhibitory protein of NF-κB kinase (IKK), activating the inflammatory molecules of the NF-κB complex. There are two ways of activating the NF-κB: the canonical and noncanonical pathways, detailed in Fig. 2. Elevated levels of nuclear canonical and/or noncanonical forms of NF-κB are present in a variety of cancers [38, 39]. The loss of p53 function in melanoma and other cancers releases IKK, with consequent NF-κB activation. Therefore, the transcriptional induction of IKK might represent a novel pathway through which the loss of p53 function contributes to tumorigenesis, being another possible target [40, 41].

BRAF is a mitogen protein, involved in cell survival. The activating mutation of BRAF is present in more than 50 % of melanomas [42]. The most common mutation is the substitution of glutamic acid with valine in the position 600 (V600E). Activation of oncogenic BRAF leads to signaling via MEK and ERK with consequent excessive cell proliferation and survival [43].

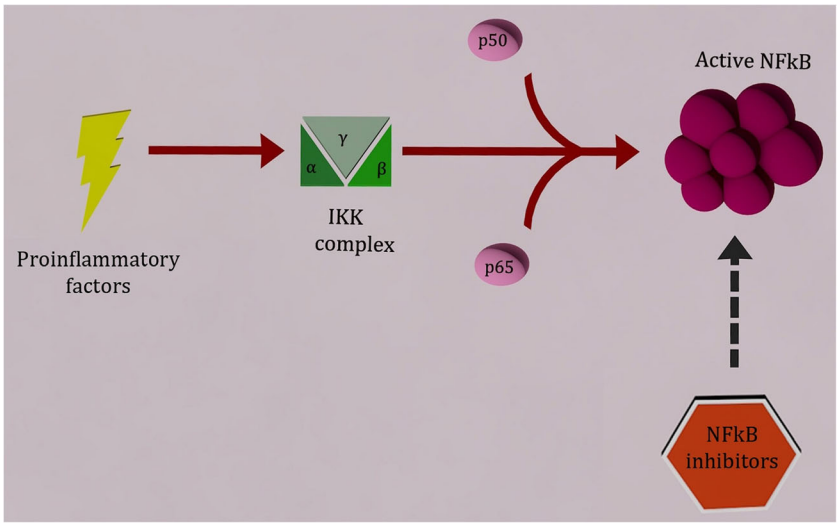

Fig. 2 The NF-κB cascade activation. The IKK kinase complex, made of the three subunits IKKα, IKKβ, and IKKγ (NEMO), is the core of the NF-κB cascade activation. Inflammatory stimuli such as different cytokines, bacterial or viral products determine the phosphorylation of the IκB molecules, the inhibitors of NF-κB, with consequent polyubiquitination and destruction by the proteasome. The free NF-κB form, consisted of its two dimmers p50 and p65, enters the nucleus and activates the process of transcription of a variety of genes participating in the inflammatory processes. *NF-κB* nuclear factor kappa-light-chain-enhancer of activated B cells, *IKK* inhibitory protein of NF-κB kinase, *IκB* inhibitory kinases of NF-κB

BRAF inhibitors were shown to trigger increased TNF secretion from the tumor microenvironment leading to resistance to therapy [44, 45]. In the future, the association of BRAF inhibitors with drugs that target TNF might be mandatory in order to achieve a stable therapy response. Mutant BRAF in human melanoma lines indirectly activates NF-κB by upregulating its activators, inflammatory cytokines like TNF-α and IL-1α/β, as well as different chemokines [46].

Besides cell survival and proliferation enhanced by NF-κB, TNF-α also stimulates the secretion of active matrix metalloproteinase-2 (MMP-2), an enzyme that degrades collagen type IV. As basement membrane components, type IV collagen and laminin are potential substrates for MMP-2, and activation of a type IV collagenase by this cytokine may provide a mechanistic explanation for the role of TNF-α during metastasis and angiogenesis [47]. Another effect mediated by TNFR1 signaling is the activation of the JNK cascade [48]. Once activated, JNK phosphorylates the activator protein 1 (AP-1) transcription factor, leading to transcriptional upregulation of the AP-1-responsive genes, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), MMP-3, and MMP-9, involved in proliferation, differentiation, apoptosis, leading to inflammation, angiogenesis as well as tumor invasion [49].

Signal transducer and activator of transcription (STAT) proteins are important key players in cancer progression [50]. STAT3 is constitutively activated in melanoma and upregulates one of the most proangiogenic factors known as the vascular endothelial growth factor (VEGF). STAT is also responsible for the activation of the matrix metalloproteinases MMP-2 and MMP-9 [51, 52]. Interestingly, increased STAT3 activation can silence Fas activation leading to a TNF-deregulated proapoptotic response [53]. Other studies revealed that STAT3 constantly maintains NF-κB activation in different tumors increasing their development [54].

It seems that many proinflammatory molecules and transcription factors act synergistically in order to induce tumor invasion, progression, and metastasis. TNF-α signaling stands in the core of many of these inflammatory responses. It is difficult to establish the intricate pathways of NF-κB and TNF-α involved in melanoma progression. There are more than 150 genes governing the NF-κB activation, with TNF-α being its main enhancer. In this respect, scientists attest TNF-α with its key player NF-κB as a double-edged sword in melanomagenesis.

Melanogenesis

Besides the mentioned pathways involved in melanomagenesis, scientists have lighted melanin as a major contributor to the aggressiveness of cutaneous melanoma.

Melanogenesis is a complex process in which the melanin pigment is produced by melanocytes. The skin relies on the production of melanin to provide photoprotection and thermoregulation when exposed to solar radiation [55]. UVR-

induced DNA damage in melanocytes and keratinocytes is based on an increased ROS and cytokine formation (TNF-α, IL-1α, IL-6, IL-8, GM-CSF), which in melanomas contributes to therapy resistance [56].

The melanogenic pathway depends on tyrosinase, a glycoprotein enzyme, located in the melanosomal membrane, which converts L-tyrosine into L-dihydroxyphenilalanine (L-DOPA) [57]. L-DOPA is oxidized to dopaquinone, further transformed to leukodopachrome, followed by several oxidation-reduction reactions that end by eumelanin formation [58]. Both L-tyrosine and L-DOPA are key hormone-like regulators that stimulate melanogenesis and also enhance metastatic capability of melanoma cells [59]. Other melanogenesis biomarkers are S100-beta (the melanocyte lineage/differentiation antigen) and melanoma inhibitory activity (MIA), used as indicators of therapy response and prognostic in melanoma [60].

Melanocytes, normal and malignant, are a target for the proopiomelanocortin (POMC)/corticotropin releasing hormone (CRH)-derived neuropeptides: α-melanocyte stimulating hormone (α-MSH), β-endorphin, and adrenocorticotropic hormone (ACTH) and also synthetize these peptides. They act as endocrine, autocrine, and paracrine hormones and bind to the melanocortin 1 receptor (MC1-R), a member of the family of G-protein receptors, present on the melanocyte membrane [3]. Activation of the MC1-R stimulates melanogenesis.

Production of POMC/CRH and expression of the corresponding receptors on the skin cells is upregulated by the proinflamatory interleukin 1 and UVR exposure. This CRH/POMC skin system seems to function similarly to the hypothalamic-pituitary-adrenal axis. Cutaneous POMC peptides neutralize noxious environmental and internal stimuli, enhance pigmentation, stimulate cell proliferation and differentiation, and decrease immune reactions [61–63]. The glucocorticoid hormones, both systemic and secreted by the skin cells, decrease the immune response and attenuate the local CRH/POMC system activity. In melanoma, this factors contribute to immune evasion of the tumor cells [63].

MC1-R genetic polymorphisms are in control of ethnic differences of pigmentation and different responses to UVR exposure, respectively [64]. The variations in human skin inducible pigmentation by UVR are partially due to the existence of the redhead/MC1-R allele. Epidemiologically, there is a strong association between MC1-R loss-of-function allele and the risk for malignant transformation of epidermal melanocytes, suggesting that MC1-R functions as a melanoma susceptibility gene [65].

In human melanoma, increased melanogenesis was correlated with increased levels of tyrosinase and MC1-R [66].

ACTH and α-MSH activate the adenylate-cyclase enzyme, with consequent intracellular cyclic adenosine monophosphate (cAMP) increased levels and the activation of protein kinase A (PKA). The phosphorylation of cAMP response element (CREB) by PKA acts as a transcription factor in genes like microphthalmia-associated transcription factor (MITF) [67]. MITF regulates the melanocyte development, melanosome transport, and the expression of antiapoptotic B-cell lymphoma-2 (BCL-2) protein [4]. Both MITF and BCL-2 are important melanoma biomarkers. Depending on the tumor stage, MITF has been associated with both poor and better prognosis of melanoma, while increased BCL-2 expression was associated with poor prognosis [68].

The melanin pigment acts as a free radical scavenger and chelates chemotherapeutic agents. This leads to a reduced efficacy of radiotherapy, photodynamic therapy (PDT), and chemotherapy in melanoma. Inhibition of melanogenesis [69–71] by blocking tyrosinase or chelating the copper ions (tyrosinase cofactor) sensitized melanoma cells to cyclophosphamide and potentiated immuno-toxic activities of IL-2-activated lymphocytes [3, 12]. Addition of L-tyrosine in the melanoma cells' medium lead to enhanced melanin synthesis and important changes in the cyto-arhitecture, correlated with more aggressive tumors [5]. In a clinical study on cutaneous melanomas, metastases were significantly correlated with shorter disease-free survival and overall survival periods in pigmented compared to amelanotic tumors [72]. Deregulated melanogenesis and pheomelanogenesis were correlated with increased tumor growth [73].

Antitumoral mechanisms of TNF-α

TNF-α is among the most interesting and intense studied cytokines over the past 30 years [74]. This proinflammatory molecule, part of a superfamily composed of 19 ligands and 29 receptors, raised great expectations in developing an antitumor therapy, using its crucial role in apoptosis and necrosis [75]. Preclinical studies (in vitro and in vivo) proved that the benefic potential of TNF-α can be achieved only under controlled situations of acute inflammation.

Apoptosis is a two-step natural process of programmed cell death (PCD) that occurs in multicellular organisms [76, 77]. During the first stage, the nucleus and cytoplasm suffer a condensation, followed by the fragmentation of the cell. In the second stage of apoptosis, apoptotic bodies resulted are shed from epithelial-lined surfaces or phagocytized by other cells [78]. This essential process involved in normal cell turnover can follow an intrinsic or an extrinsic pathway of cell deletion [13, 79].

When initiating the extrinsic pathway of apoptosis for those cells which are no longer required or represent a threat for the organism (tumor cells), activated macrophages, mast cells, and many other cells (fibroblasts, smooth muscle cells, and NK cells) secrete TNF-α. TNF-α binds a TNF-receptor located on the outer side of the membrane. There are two main receptors that can interact with TNF-α: TNFR1 and TNFR2

[80]. The difference between the two receptors is not only in structure (only TNFR1 possesses a death domain) but also in function. However, there is an important cross talk between TNFR1 and TNFR2 in cellular signaling via TRAFs.

In order to initiate apoptosis, the membrane-integrated TNF binds TNFR1, the cytoplasmic death domain (DD) is activated and binds the adaptor proteins called TRADD and Fas associated via DD (FADD). This is only possible with the participation of receptor interacting protein serine-threonine kinase 1 (RIP1), whose DD is able to link the DD-containing death receptors (DRs), like TNFR1, Fas, TNF-related apoptosis-inducing ligand (TRAIL) R1, and TRAIL R2. FADD activates the caspase-8, which leads to the initiation of the apoptosis cascade [81]. Depending on the cell line, TNFR1 involvement is enough for inducing caspase-8 at an adequate level, so that caspase-3 or caspase-7 is activated and the programmed cell death follows its course. If the amount of caspase-8 is insufficient, mitochondrial control intervenes and provides an amplified cell death signal [82].

The intrinsic pathway of apoptosis (mitochondrial apoptosis) is possible with the help of proteins of the BCL-2 family [83]. BCL family of proteins can be divided into proapoptotic (BCL-2-associated X protein (BAX), BCL-2 antagonist/killer (BAK), and BH3-only) and antiapoptotic proteins (BCL-2, BCL-XL, MCL-1, BCL-W, A1) [84]. Common triggers for the mitochondrial apoptosis are represented by radiation, DNA damage, cytokine deprivation, or lack of the survival signal trigger mitochondrial outer membrane permeabilization (MOMP) and release of cytochrome c. This leads to the activation of BAX and BAK proapoptotic proteins and further activation of BH3-only. As a result, antiapoptotic proteins from the BCL family are blocked and mitochondria suffer a permeabilization of the outer membrane with the release of cytochrome c. Cytochrome c binds apoptosis-activated factor 1 (Apaf1) in the cytosol, which determines the blockage of IAPs and caspase-9 activation. Once activated, caspase-9 mediates the activation of caspase-3 and -7; thus, the apoptotic signaling cascade ends. A brief illustration of the mitochondrial pathway is shown in Fig. 3.

Necrosis is a caspase-independent cell death caused by external or internal factors, especially infections, toxins, ischemia, or trauma. Unlike apoptosis, necrosis starts with a depletion of cell energy, which induces metabolic collapse, cell swelling, and irreversible membrane damage with cell rupture [85]. Release of the cellular content, including lysosome enzymes and other harmful chemicals, causes inflammation in necrosis [86]. There are two main cascades leading to necrosis: via DRs and using toll-like receptors (TLRs) [87].

TNF-α is the bridgehead in necrosis, apoptosis, and proliferation, while FADD is the crossroad molecule between apoptosis and necrosis [88]. Following TNF-α binding to TNFR1, TRADD and FADD are recruited. This activates caspase-8-dependent apoptosis. When TRADD binds RIP1 instead of FADD, the necrotic cell death or NF-κB pathway can be activated. Studies showed that necrosis is within RIP1 control [89, 90]. RIP1, among TNFR1 and TRAF2, forms a complex at the membrane level, which activates NF-κB and MAPKs. Activated MAPK affects the mitochondrial integrity and induces necrosis. In the event of caspase inhibition, necrosis is initiated as a backup pathway for the cell death [91]. TLR is another important cascade, in which TLR3 or 4 can also trigger necrosis via RIP1 [92].

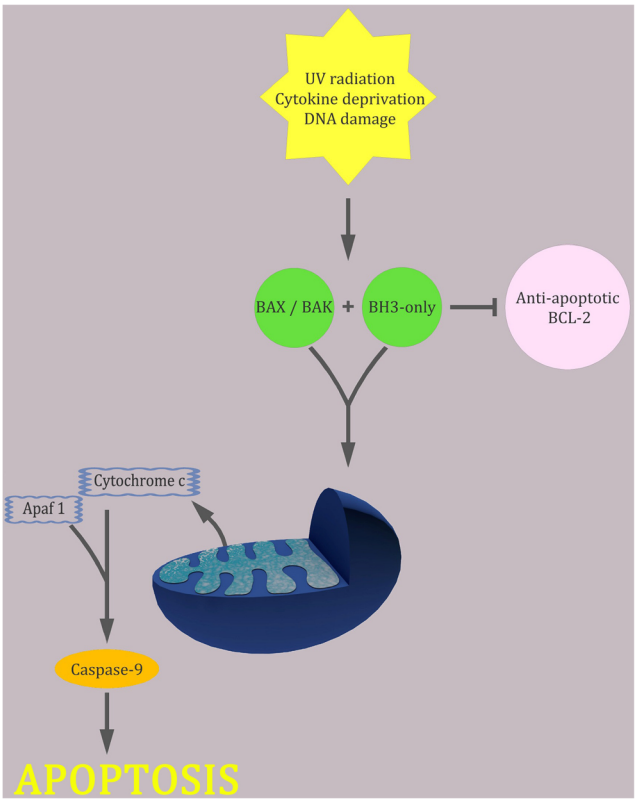

Fig. 3 Mitochondrial apoptosis pathway. UV radiation, DNA damage, and cytokine deprivation lead to the activation of BAX and BAK proapoptotic proteins and further activation of BH3-only. As a result, antiapoptotic proteins from the BCL family are blocked and mitochondrias suffer a permeabilization. Cytochrome c is released from the outer mitochondrial membrane and binds Apaf1 in the cytosol, activates caspase-9, and triggers apoptosis. *BCL family* B-cell lymphoma-2 family, *BAX* BCL-2-associated X protein, *BAK* BCL-2 antagonist/killer, *BH3-only* proapoptotic effector of the BCL family, *Apaf1* apoptosis-activated factor 1

Clinical aspects

Melanoma derives from the pigment cells, melanocytes. These malignant melanocytes are sustained by a vast tumoral stroma consisting of fibroblasts, myofibroblasts, and immune cells such as macrophages, neutrophils, and lymphocytes, responsible for cancer progression.

Prognosis of late-stage melanoma is very poor; therefore, early diagnosis and treatment needs to be taken fast into consideration. Several treatment strategies like surgery, chemotherapy, radiotherapy, and, more recently, immunotherapy can be applied, depending on the clinical stage of melanoma. However, advanced melanoma is capable of developing resistance mechanisms to most of the therapeutic strategies applied, including immunotherapy [4]; thus, the outcome of therapy is largely disappointing. Melanoma resistance mechanisms to therapy involve the development of intratumoral heterogeneity, defined by heterogeneity of melanoma cells and their behavior, the alterations of the microenvironment, and the tumor immune evasion [4].

TNF-α has a complex role in melanomagenesis and is responsible for the tumor response following different oncological therapies. In this review, several antimelanoma therapeutical approaches are taken into consideration in an effort to establish whether TNF-α might have any influence in the treatment outcome. Figure 4 emphasizes the impact of TNF in the most important therapies against melanoma.

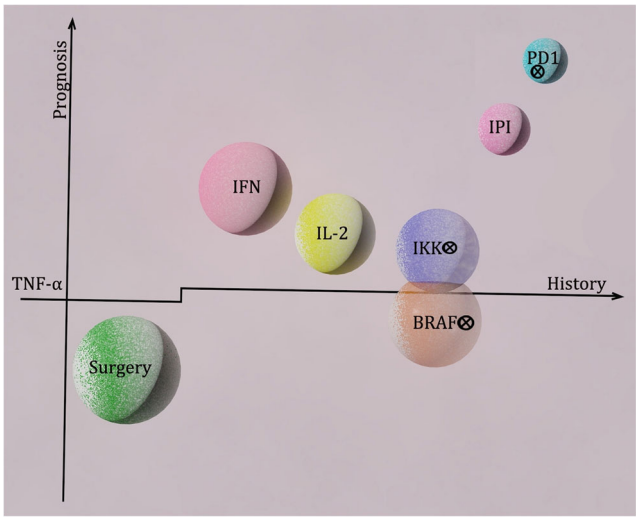

Fig. 4 The implications of TNF-α in antimelanoma therapies. The *horizontal axis* represents the discovery over time of the most important therapies against malignant melanoma. The *vertical axis* highlights the prognosis of patients after various therapies, associated with the presence of TNF-α in the tumor. Thus, it can be seen that TNF may adversely affect surgery, its presence being correlated with a higher recurrence of cancer. It was also demonstrated that BRAF inhibitors cause increased levels of TNF with consequent activation of the proinflammatory and protumoral effects of NF-κB. Thereby, BRAF inhibitors are recommended as combination with other chemotherapeutic drugs that lower the protumoral effects of NF-κB, such as IKK inhibitors. Immunotherapy is a basic weapon against melanoma, since the discovery of interferon to the use of ipilimumab and inhibitors PD-1/PDL-1. One can observe the evolution of these agents, both in time and in terms of improving survival of patients with unresectable melanoma. *IFN* interferon, *IL-2* interleukin-2, *IKK inhibitors* IKK, inhibitory protein of NF-κB kinase inhibitors, *IPI* ipilimumab, *PD-1/PDL-1 inhibitors* programmed cell death protein 1/programmed cell death ligand 1 inhibitors

Surgery and chemotherapy

Surgery represents the golden standard in the melanoma patient management. Depending on the loco-regional invasion, it can usually be applied till stage III [93]. Still, different markers have been assessed to measure the disease recurrence risk. A recent study demonstrated that TNFR2 is the most sensitive marker in relation with the disease progression, being a poor prognostic marker in high-risk surgically resected melanomas [94].

BRAF signaling inhibition induces in melanoma cells a cell cycle arrest with consequent death through apoptosis, thus validating BRAF as an important therapeutic target for stopping the progression of advanced melanoma [95]. In 2011, Chapman and his colleagues conducted a randomized phase III clinical trial, comparing the BRAF inhibitor vemurafenib with dacarbazine in stage IIIC/stage IV unresectable melanoma. Patients with BRAF-positive mutation who received vemurafenib had a relative reduction of 63 % in the risk of death and of 74 % in the risk of tumor progression, which seemed to be remarkable results [96].

In contradiction with the first successful achievements, it was demonstrated that BRAF inhibitors may trigger TNF-α activation of the macrophages from the tumor milieu, further leading to NF-κB proinflammatory actions and eventually treatment failure. NF-κB was the critical transcription factor that blocks apoptosis when BRAF signaling was inhibited [45]. Still, one recent study revealed that IKK inhibitors can inhibit the proinflammatory cytokines from the tumor microenvironment but they cannot be administered alone or in sufficient dosage due to their high toxicity [97]. Thus, it is mandatory to add in future clinical trials drugs that target both the tumor and stromal cells. Other chemotherapeutics that are taken into account in advanced melanoma clinical trials are VEGF inhibitors [98].

Isolated limb perfusion

Isolated limb perfusion (ILP) is one of the loco-regional therapies in melanoma that uses melphalan (used in USA) or melphalan + TNF-α (often used in Europe). The main purpose of ILP is to deliver a high dose of chemotherapy at a high temperature, strictly into the blood stream of the affected limb. Cornett et al. aimed to determine whether TNF-α addition to melphalan in ILP therapy would bring an improvement in the overall response [99]. This prospective trial reported not only the absence of a significant difference between the two treatment strategies but also the increased toxicity because of TNF-α addition. However, the outcome of a 20-year study was that a high-dose of TNF-α is able to prolong local control, without influencing the overall survival [100].

Other studies obtained long-term local control in patients with metastatic melanoma after ILP with TNF-α plus

melphalan [101]. Studies have revealed three important molecules capable of inducing both apoptosis and necrosis (necroptosis), which are TNF-α, TRAIL (or Apo2L), and CD95 ligand [102]. As CD95L and TNF-α induce severe adverse effects after systemic application, TRAIL gave another hope in developing an antitumor therapy. TRAIL is capable of inducing necroptosis in tumor cells, without causing toxicity [103]. γ-irradiation and sodium arsenite treatment increased surface TRAIL-receptor 1 and receptor 2 (DR4/DR5) levels and surface Fas levels, activating apoptosis in melanoma cells via TRAIL [104]. A promising new option for cancer therapy using TRAIL-induced necrosis was suggested by Voigt et al. (2014). The study shows that pronecrotic kinase RIPK-3 is a predictive marker for resistance or susceptibility of tumor cells to TRAIL-induced necrosis, despite TRAIL-receptor [105]. However, many metastatic tumors gained resistance to TRAIL-mediated apoptosis, including late-stage melanoma [106].

Radiotherapy

Radiotherapy in melanoma is associated with poor results. Currently, there is under investigation a radioinducible TNF-α promotor (TNFerade) that can be locally injected in the tumor, thus resembling an antiangiogenic therapy [107]. In the future, it may have promising results in the radiotherapy field, not only in advanced melanoma but also in unresectable esophageal, rectal, and pancreatic cancer. Increased tumor TNF-α was associated with an increase in endothelium apoptosis; therefore, it improved prognosis [88].

Photodynamic therapy

PDT unveils encouraging results on in vitro and in vivo experimental models, as well as few clinical reports, suggesting a possible role in the management of advanced melanoma [108–111]. PDT is an ideal theoretical approach because it induces direct tumor cell photodamage, targets tumor vasculature, and activates the immune response. Due to a limited experience of PDT in melanoma, extensive clinical studies need to be conducted, using selected photosensitizers and standard irradiation protocols and also several combination regimens with chemotherapy or immunotherapy, in order to obtain an established antitumoral effect [107, 112].

Immunotherapy

Still, after decades of chemotherapy, a new era has emerged, the immunotherapy of cancer. Immunotherapy stimulates the patient's immune system to fight harder and smarter against the cancer cells. The latest clinical trials involve anti-CTLA-4 and PD-1/PD-L1 pathway blocking antibodies with very promising future perspectives.

Interferon alpha-2b is the first immunotherapeutic agent, used since 1995. It is able to inhibit DNA/RNA replication and stimulate the fight of the immune system against melanoma cells. High-dose IFNa-2b was introduced as an adjuvant in advanced stages of melanoma (IIB, IIC, and III) or recurrent melanoma. IFN has shown significant progression-free survival (PFS) and overall survival (OS) improvement, especially in patients with lymph node involvement [2].

Serum cytokines were analyzed from melanoma patients treated with IFN and used as biological markers for prognosis. Surprisingly, IFN has induced significant increase in the concentrations of TNF-α, TNF-RII, and multiple other cytokines [113]. These high levels of proinflammatory molecules were correlated with significant enhancements in progression-free survival. PegIFN was approved in 2011 in the treatment of advanced melanoma. PegIFN has a larger molecule which ensures prolonged plasma concentrations, allowing one dose/week, unlike three doses/week of IFN. Studies have shown remarkable improvement in progression-free survival, but no benefit in overall survival for patients treated with PegIFN.

IL-2 was approved as an election treatment for late-stage melanoma in 1998. High doses of IL-2 stimulate T cells, and NK cells attack against malignant cells, inducing cytolysis at the tumor site. Studies on IL-2 biological therapy reported an increase in TNF-α serum levels after IL-2 interruption, in patients that showed clinical response [114]. Even though IL-2 has a higher curative potential than IFN, its administration is limited by the severe acute toxicity (hypotension, acute arrhythmias, hepatic dysfunction, vascular leak syndrome) [115]. Thus, it is mandatory that the patient has a good pulmonary, heart, and kidney status, before starting IL-2 therapy. Moreover, clinical practice showed that patients unresponsive to chemotherapy were unlikely to respond to IL-2. On the other hand, effective therapy methods, like surgery or chemotherapy, were combined with immunotherapy (IFN or IL-2) for a more powerful antitumor effect. Recent studies have proved that cytokines combined with antibodies (F8, L19, FuP) can diminish toxicity and improve the patient survival [116, 117].

Pretto et al. aimed to demonstrate the efficacy of the combined therapy compared to single-agent therapy [118]. The combination therapy consisted of IL-2-based immunocytokines plus a chemotherapeutic drug (dacarbazine or paclitaxel) or IL-2-based immunocytokines plus TNF-based immunotherapy on a murine melanoma (K1735M2). High doses of F8 antibody fused to IL-2 (F8-IL2) focused selectively at the tumor site and were responsible for decreasing the malignant cell proliferation. Dacarbazine or paclitaxel administration increased the effect of F8-IL2, without additional toxicity, but without cure. Furthermore, the combination F8-IL2 + F8-TNF cured completely two out of four mice via necrosis after one intratumoral injection.

Weide et al. analyzed the long-term evolution of 72 melanoma patients with injectable metastases [119]. All patients received high doses of IL-2 intratumoral injections. Thirty patients had recurrence of unresectable distant metastases and required chemotherapy afterwards. Intratumoral IL-2 therapy for stage III melanoma patients with cutaneous metastases (without lymph node involvement) and stage IV melanoma patients with soft tissue metastases (without visceral involvement) showed a great improvement of the survival rate, as well as increased responses to the following chemotherapy.

In the early 2011, FDA approved the use of ipilimumab, a specific inhibitor of CTLA-4 antigen that leads to sustained immune response of the T lymphocytes against melanoma cells. A phase III randomized clinical trial compared ipilimumab with glycoprotein (gp) 100 vaccine [8]. Gp100 was combined with IL-2 in order to obtain such an immune response against cancer [120]. Among 676 patients enrolled in the clinical trial, 403 received ipilimumab associated with gp100, 137 received ipilimumab alone, and the last 136 patients received gp100 alone. It was found that ipilimumab increased the median overall survival with 10.0 months, whether administered alone or in combination.

The PD-1 receptor is a negative regulator of the T cells, silencing their antitumor actions. In 2012, the anti-PD-1 antibody lambrolizumab was tested in phase I trials in patients with advanced melanoma, including those with disease progression while they had been receiving ipilimumab. Following lambrolizumab administration, a high rate of tumor regression was registered. Clinical trials with both ipilimumab and the PD-1 antibody nivolimumab revealed promising results with rapid melanoma regression [121].

Tumor cells use PD-1 and CTLA-4 inhibitory pathways to decrease the immune cell activity [122, 123]. An effective immune antitumor response requires not only the activation of the immune cells but also an increase of their consequent destructive actions.

An in vivo study on mice bearing B16 melanoma revealed that regulatory T infiltrating tumor lymphocytes (Treg) expressed PD-1, CTLA-4, or both markers. Blocking of PD-1 and CTLA-4 can modulate Treg functions, leading to antitumor responses and tumor rejection [124]. In addition, blocking PD-1/PDL-1 bond and CTLA-4 triggers the reactivation of the T lymphocytes with increased secretion of cytokines and inflammatory molecules like IFN, IL-2, and TNF-α with consequent cytotoxic effect. The intratumoral, proinflammatory cytokine TNF-α is essential in order to reorient the lymphocytes against the malignant melanoma cells.

NGR-TNF is a tumor vessel-target peptide conjugate made of TNF-α fused with CNGRCG, a peptide ligand of CD13 expressed by endothelial cells in tumor vessels. Administration of NGR-TNF can alter the endothelial barrier function and facilitate the penetration of the chemotherapeutic drugs in tumors. Based on these findings, a recent pilot phase I trial concerning a combination of TNF peptide conjugate and immunotherapy revealed promising results, with an ex vivo T cell response and long-term overall survival [125].

Conclusion

Although the advances in the skin melanoma therapies have shown great promise, many mutations that induce tumor resistance have also emerged. Thus, the discovery of curative drugs is probably a miles away journey. It could be possible however that the key resides in rediscovering old molecules involved in oncogenesis, particularly melanomagenesis, such as TNF-α and NF-κB and their intricate mechanisms of action, both antiapoptotic and proapoptotic involved in tumor progression. Thus, it is of paramount importance to establish when and where these protumor and antitumor mechanisms begin and end, in order to choose the most suitable therapeutic action.

Acknowledgments The authors are grateful to "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania, for supporting their work through an internal grant program as stated in contract no. 1493/4/28.01.2014. The research was partially funded by POSDRU grant no 159/1.5/S/138776 with title: "Model colaborativ institutional pentru translatarea cercetarii stiintifice biomedicale in practica clinica—TRANSCENT"

Conflicts of interest The authors declare that they do not have any conflict of interest.

References

1. Mantovani A, Garlanda C, Allavena P. Molecular pathways and targets in cancer-related inflammation. Ann Med. 2010;42(3):161–70. doi:10.3109/07853890903405753.
2. Green AC, Williams GM, Logan V, Strutton GM. Reduced melanoma after regular sunscreen use: randomized trial follow-up. J Clin Oncol. 2011;29(3):257–63. doi:10.1200/JCO.2010.28.7078.
3. Whiteman DC, Pavan WJ, Bastian BC. The melanomas: a synthesis of epidemiological, clinical, histopathological, genetic, and biological aspects, supporting distinct subtypes, causal pathways, and cells of origin. Pigment Cell Melanoma Res. 2011;24(5):879–97. doi:10.1111/j.1755-148X.2011.00880.x.
4. Slominski AT, Carlson AJ. Melanoma resistance: a bright future for academicians and a challenge for patient advocates. Mayo Clin Proc. 2014;89(4):429–33. doi:10.1016/j.mayocp.2014.02.009.
5. Wellbrock C, Rana S, Paterson H, Pickersgill H, Brummelkamp T, Marais R. Oncogenic BRAF regulates melanoma proliferation through the lineage specific factor MITF. PLoS One. 2008;16, e2734. doi:10.1371/journal.pone.0002734.
6. Madhunapantula SV, Mosca PJ, Robertson GP. The Akt signaling pathway: an emerging therapeutic target in malignant melanoma. Cancer Biol Ther. 2011;12:1032–49. doi:10.4161/cbt.12.12.18442.
7. Kortylewski M, Jove R, Yu H. Targeting STAT3 affects melanoma on multiple fronts. Cancer Metastasis Rev. 2005;24(2):315–27.

8. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711–23. doi:10.1056/NEJMoa1003466.
9. Keir ME, Liang SC, Guleria I, Latchman YE, Qipo A, Albacker LA, et al. Tissue expression of PD-L1 mediates peripheral T cell tolerance. J Exp Med. 2006;203:883–95.
10. Kottschade LA, Suman VJ, Perez DG, et al. A randomized phase 2 study of temozolomide and Bevacizumab or nab-paclitaxel, carboplatin, and Bevacizumab in patients with unresectable stage IV melanoma: a north central cancer treatment group study, N0775. Cancer. 2013;119(3):586–92.
11. Enninga EAL, Holtan SJ, Creedon DJ, et al. Immunomodulatory effects of sex hormones: requirements for pregnancy and relevance in melanoma. Mayo Clin Proc. 2014;89(4):520–35.
12. Bates MD, Quenby S, Takakuwa K, Johnson PM, Vince GS. Aberrant cytokine production by peripheral blood mononuclear cells in recurrent pregnancy loss? Hum Reprod. 2002;17(9): 2439–44.
13. Van Herreweghe F, Festjens N, Declercq W, Vandenabeele P. Tumor necrosis factor-mediated cell death: to break or to burst, that's the question. Cell Mol Life Sci. 2010;67(10):1567–79. doi: 10.1007/s00018-010-0283-0.
14. Schioppa T, Moore R, Thompson RG, Rosser EC, Kulbe H, et al. B regulatory cells and the tumor-promoting actions of TNF-α during squamous carcinogenesis. Proc Natl Acad Sci U S A. 2011;108(26):10662–7. doi:10.1073/pnas.1100994108.
15. Acharyya S, Oskarsson T, Vanharanta S, Malladi S, Kim J, et al. A CXCL1 paracrine network links cancer chemoresistance and metastasis. Cell. 2012;150(1):165–78. doi:10.1016/j.cell.2012.04.042.
16. Dirat B, Bochet L, Dabek M, Daviaud D, Dauvillier S, et al. Cancer-associated adipocytes exhibit an activated phenotype and contribute to breast cancer invasion. Cancer Res. 2011;71(7): 2455–65. doi:10.1158/0008-5472.CAN-10-3323.
17. Chen ZW, Qian JY, Ma JY, Chang SF, Yun H, Jin H, et al. TNF-α-induced cardiomyocyte apoptosis contributes to cardiac dysfunction after coronary microembolization in mini-pigs. J Cell Mol Med. 2014;18(10):1953–63. doi:10.1111/jcmm.12342.
18. Eberle T, Doganci B, Krämer H, Fechir M, Wagner, et al. Mechanical but not painful electrical stimuli trigger TNF alpha release in human skin. Exp Neurol. 2010;221(1):246–50. doi:10.1016/j.expneurol.2009.11.008.
19. Zhu J, Jiang L, Liu Y, Qian W, Liu J, et al. MAPK and NF-κB pathways are involved in bisphenol a-induced TNF-α and IL-6 production in BV2 microglial cells. Inflammation. 2015;38(2): 637–48. doi:10.1007/s10753-014-9971-5.
20. Kanczkowski W, Zacharowski K, Wirth MP, Ehrhart-Bornstein M, Bornstein SR. Differential expression and action of toll-like receptors in human adrenocortical cells. Mol Cell Endocrinol. 2009;300(1–2):57–65. doi:10.1016/j.mce.2008.10.028.
21. Fernández-Real JM, Vendrell J, García I, Ricart W, Vallès M. Structural damage in diabetic nephropathy is associated with TNF-α system activity. Acta Diabetol. 2012;49(4):301–5. doi: 10.1007/s00592-011-0349-y.
22. Zhang JD, Patel MB, Griffiths R, Dolber PC, Ruiz P, Sparks MA, et al. Type 1 angiotensin receptors on macrophages ameliorate IL-1 receptor-mediated kidney fibrosis. J Clin Invest. 2014;124(5): 2198–203. doi:10.1172/JCI61368.
23. Azuma Y, Kaji K, Katogi R, Takeshita S, Kudo A. Tumor necrosis factor-alpha induces differentiation of and bone resorption by osteoclasts. J Biol Chem. 2000;275(7):4858–64.
24. Gaur U, Aggarwal BB. Regulation of proliferation, survival and apoptosis by members of the TNF superfamily. Biochem Pharmacol. 2003;66(8):1403–8.
25. Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol. 2003;3:745–56.
26. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, et al. An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci U S A. 1975;72(9):3666–70.
27. Mueller MM. Inflammation in epithelial skin tumours: old stories and new ideas. Eur J Cancer. 2006;42:735–44.
28. Ji H, Cao R, Yang Y, Zhang Y, Iwamoto H, et al. TNFR1 mediates TNF-α-induced tumour lymphangiogenesis and metastasis by modulating VEGF-C-VEGFR3 signalling. Nat Commun. 2014;5:4944. doi:10.1038/ncomms5944.
29. Hsu H, Xiong J, Goeddel DV. The TNF receptor 1-associated protein TRADD signals cell death and NF-kappa B activation. Cell. 1995;81(4):495–504.
30. Idriss HT, Naismith JH. TNF alpha and the TNF receptor superfamily: structure-function relationship(s). Microsc Res Tech. 2000;50(3):184–95.
31. Rundhaug JE, Fischer SM. Molecular mechanisms of mouse skin tumor promotion. Cancers (Basel). 2010;2(2):436–82. doi:10.3390/cancers2020436.
32. Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, et al. NF-κB functions as a tumour promoter in inflammation-associated cancer. Nature. 2004;431:4461–6.
33. Hsu H, Shu HB, Pan MG, Goeddel DV. TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. Cell. 1996;84(2):299–308.
34. Zhou J, Yuen NK, Zhan Q, Velazquez EF, Murphy GF, et al. Immunity to the melanoma inhibitor of apoptosis protein (ML-IAP; livin) in patients with malignant melanoma. Cancer Immunol Immunother. 2012;61(5):655–65. doi:10.1007/s00262-011-1124-1.
35. Tian F, Lu JJ, Wang L, Li L, Yang J, et al. Expression of c-FLIP in malignant melanoma, and its relationship with the clinicopathological features of the disease. Clin Exp Dermatol. 2012;37(3): 259–65. doi:10.1111/j.1365-2230.2011.04238.x.
36. Sen R, Baltimore D. Multiple nuclear factors interact with the immunoglobulin enhancer sequences. Cell. 1986;46:705–16.
37. Bonizzi G, Karin M. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. Trends Immunol. 2004;25(6):280–8.
38. Williams JA, Thomas AM, Li G, Kong B, Zhan L, et al. Tissue specific induction of p62/Sqstm1 by farnesoid X receptor. PLoS One. 2012;7(8), e43961. doi:10.1371/journal.pone.0043961.
39. Cao Y, Luo JL, Karin M. IkappaB kinase alpha kinase activity is required for self-renewal of ErbB2/Her2-transformed mammary tumor-initiating cells. Proc Natl Acad Sci U S A. 2007;104(40): 15852–7.
40. Yang J, Pan WH, Clawson GA, Richmond A. Systemic targeting inhibitor of kappaB kinase inhibits melanoma tumor growth. Cancer Res. 2007;67(7):3127–34.
41. Gu L, Zhu N, Findley HW, Woods WG, Zhou M. Identification and characterization of the IKKalpha promoter: positive and negative regulation by ETS-1 and p53, respectively. J Biol Chem. 2004;279:52141–9.
42. Wellbrock C, Hurlstone A. BRAF as therapeutic target in melanoma. Biochem Pharmacol. 2010;80(5):561–7. doi:10.1016/j.bcp.2010.03.019.
43. McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Wong EW, et al. Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochim Biophys Acta. 2007;1773(8):1263–84. doi:10.1016/j.bbamcr.2006.10.001.
44. Lito P, Rosen N, Solit DB. Tumor adaptation and resistance to RAF inhibitors. Nat Med. 2013;19(11):1401–9. doi:10.1038/nm.3392.
45. Gray-Schopfer VC, Karasarides M, Hayward R, Marais R. Tumor necrosis factor-alpha blocks apoptosis in melanoma cells when

BRAF signaling is inhibited. Cancer Res. 2007;67(1):122–9. doi:10.1158/0008-5472.CAN-06-1880.
46. Castelli C, Sensi M, Lupetti R, Mortarini R, Panceri P, et al. Expression of interleukin 1 alpha, interleukin 6, and tumor necrosis factor alpha genes in human melanoma clones is associated with that of mutated N-RAS oncogene. Cancer Res. 1994;54(17):4785–90.
47. Han YP, Tuan TL, Wu H, Hughes M, Garner WL. TNF-α stimulates activation of pro-MMP2 in human skin through NF-κB mediated induction of MT1-MMP. J Cell Sci. 2001;114(Pt 1):131–9.
48. Zhang JY, Adams AE, Ridky TW, Tao S, Khavari PA. Tumor necrosis factor receptor 1/c-Jun-NH2-kinase signaling promotes human neoplasia. Cancer Res. 2007;67(8):3827–34.
49. Coussens LM, Tinkle CL, Hanahan D, Werb Z. MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. Cell. 2000;103(3):481–90.
50. Iliopoulos D, Hirsch HA, Struhl K. An epigenetic switch involving NF-kappaB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. Cell. 2009;139(4):693–706. doi:10.1016/j.cell.2009.10.014.
51. Niu G, Wright KL, Huang M, Song L, Haura E, Turkson J, et al. Constitutive Stat3 activity up-regulates VEGF expression and tumor angiogenesis. Oncogene. 2002;21(13):2000–8.
52. Xie TX, Wei D, Liu M, Gao AC, Ali-Osman F, Sawaya R, et al. Stat3 activation regulates the expression of matrix metalloproteinase-2 and tumor invasion and metastasis. Oncogene. 2004;23(20):3550–60.
53. Alshamsan A, Hamdy S, Haddadi A, Samuel J, El-Kadi AO, Uludağ H, et al. STAT3 knockdown in B16 melanoma by siRNA lipopolyplexes induces bystander immune response in vitro and in vivo. Transl Oncol. 2011;4(3):178–88.
54. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nat Rev Cancer. 2009;9(11):798–809. doi:10.1038/nrc2734.
55. Lin JY, Fisher DE. Melanocyte biology and skin pigmentation. Nature. 2007;445(7130):843–50. doi:10.1038 /nature05660.
56. Liebel F, Kaur S, Ruvolo E, Kollias N, Southall MD. Irradiation of skin with visible light induces reactive oxygen species and matrix-degrading enzymes. J Investig Dermatol. 2012;132(7):1901–7. doi:10.1038/jid.2011.476.
57. Slominski A, Tobin DJ, Shibahara S, Wortsman J. Melanin pigmentation in mammalian skin and its hormonal regulation. Physiol Rev. 2004;84(4):1155–228.
58. Videira IF, Moura DF, Magina S. Mechanisms regulating melanogenesis. An Bras Dermatol. 2013;88(1):76–83.
59. Slominski A, Zmijewski MA, Pawelek J. L-tyrosine and L-dihydroxyphenylalanine as hormone-like regulators of melanocyte functions. Pigment Cell Melanoma Res. 2012;25(1):14–27. doi:10.1111/j.1755-148X.2011.00898.x.
60. Utikal J, Schadendorf D, Ugurel S. Serologic and immunohistochemical prognostic biomarkers of cutaneous malignancies. Arch Dermatol Res. 2007;298(10):469–77. doi:10.1007/s00403-006-0726-5.
61. Slominski A, Wortsman J, Luger T, Paus T, Solomon S. Corticotropin releasing hormone and proopiomelanocortin involvement in the cutaneous response to stress. Physiol Rev. 2000;80:979–1020.
62. Slominski A, Zbytek B, Nikolakis G, Manna PR, Skobowiat C, et al. Steroidogenesis in the skin: implications for local immune functions. J Steroid Biochem Mol Biol. 2013;137:107–23.
63. Slominski AT, Zmijewski MA, Zbytek B, Tobin DJ, Theoharides TC, Rivier J. Key role of CRF in the skin stress response system. Endocr Rev. 2013;34(6):827–84. doi:10.1210/er.2012-1092.
64. Park HY, Kosmadaki M, Yaar M, Gilchrest BA. Cellular mechanisms regulating human melanogenesis. Cell Mol Life Sci. 2009;66(9):1493–506. doi:10.1007/s00018-009-8703-8.
65. Baldea Ioana. Melanocyte pygmentation—friend or foe on the route to melanoma, breakthroughs in melanoma research, Dr Yohei Tanaka (Ed.), ISBN: 978-953-307-291-3, In Tech. 2011. doi:10.5772/20157. Available from: http://www.intechopen.com/books/breakthroughs-in-melanoma-research/melanocyte-pygmentation-friend-or-foe-on-the-route-to-melanoma.
66. Slominski A, Ermak G, Wortsman J. Modification of melanogenesis in cultured human melanoma cells. In Vitro Cell Dev Biol Anim. 1999;35(10):564–5.
67. Schallreuter KU, Kothari S, Chavan B, Spencer JD. Regulation of melanogenesis–controversies and new concepts. Exp Dermatol. 2008;17(5):395–404. doi:10.1111/j.1600-0625.2007.00675.x.
68. Maddodi N, Setaluri V. Prognostic significance of melanoma differentiation and trans-differentiation. Cancers (Basel). 2010;2(2):989–99. doi:10.3390/cancers2020989.
69. Slominski A, Paus R, Mihm MC. Inhibition of melanogenesis as an adjuvant strategy in the treatment of melanotic melanomas: selective review and hypothesis. Anticancer Res. 1998;18(5B):3709–15.
70. Slominski A, Zbytek B, Slominski R. Inhibitors of melanogenesis increase toxicity of cyclophosphamide and lymphocytes against melanoma cells. Int J Cancer. 2009;124(6):1470–7. doi:10.1002/ijc.24005.
71. Wood JM, Jimbow K, Boissy RE, Slominski A, Plonka PM, Slawinski J, et al. What's the use of generating melanin? Exp Dermatol. 1999;8(2):153–64.
72. Brożyna AA, Jóźwicki W, Carlson JA, Slominski AT. Melanogenesis affects overall and disease-free survival in patients with stage III and IV melanoma. Hum Pathol. 2013;44(10):2071–4. doi:10.1016/j.humpath.2013.02.022.
73. Mitra D, Luo X, Morgan A, et al. A UV-independent pathway to melanoma carcinogenesis in the redhair-fairskin background. Nature. 2012;491(7424):449–53. doi:10.1038/nature11624.
74. Aggarwal BB, Gupta SC, Kim JH. Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. Blood. 2012;119(3):651–65. doi:10.1182/blood-2011-04-325225.
75. Roberts NJ, Zhou S, Diaz Jr LA, Holdhoff M. Systemic use of tumor necrosis factor alpha as an anticancer agent. Oncotarget. 2011;2(10):739–51.
76. Yuan J, Horvitz HR. A first insight into the molecular mechanisms of apoptosis. Cell. 2004;116(2 Suppl):S53–6. **1 p following S59**.
77. Degterev A, Yuan J. Expansion and evolution of cell death programmes. Nat Rev Mol Cell Biol. 2008;9(5):378–90. doi:10.1038/nrm2393.
78. Indran IR, Tufo G, Pervaiz S, Brenner C. Recent advances in apoptosis, mitochondria and drug resistance in cancer cells. Biochim Biophys Acta. 2011;1807(6):735–45. doi:10.1016/j.bbabio.2011.03.010.
79. Krammer PH. CD95's deadly mission in the immune system. Nature. 2000;407:789–95.
80. Naudé PJ, den Boer JA, Luiten PG, Eisel UL. Tumor necrosis factor receptor cross-talk. FEBS J. 2011;278(6):888–98. doi:10.1111/j.1742-4658.2011.08017.x.
81. Kurokawa M, Kornbluth S. Caspases and kinases in a death grip. Cell. 2009;138(5):838–54. doi:10.1016/j.cell.2009.08.021.
82. Brenner D, Mak TW. Mitochondrial cell death effectors. Curr Opin Cell Biol. 2009;21(6):871–7. doi:10.1016/j.ceb.2009.09.004.
83. Danial NN, Korsmeyer SJ. Cell death: critical control points. Cell. 2004;116(2):205–19.
84. Pradelli LA, Bénéteau M, Ricci JE. Mitochondrial control of caspase-dependent and -independent cell death. Cell Mol Life Sci. 2010;67(10):1589–97. doi:10.1007/s00018-010-0285-y.

85. Fiers W, Beyaert R, Declercq W, Vandenabeele P. More than one way to die: apoptosis, necrosis and reactive oxygen damage. Oncogene. 1999;18(54):7719–30.
86. Proskuryakov SY, Konoplyannikov AG, Gabai VL. Necrosis: a specific form of programmed cell death? Exp Cell Res. 2003;283(1):1–16.
87. Festjens N, Vanden Berghe T, Vandenabeele P. Necrosis, a well-orchestrated form of cell demise: signalling cascades, important mediators and concomitant immune response. Biochim Biophys Acta. 2006;1757(9–10):1371–87.
88. Vanden Berghe T, van Loo G, Saelens X, Van Gurp M, Brouckaert G, et al. Differential signaling to apoptotic and necrotic cell death by Fas-associated death domain protein FADD. J Biol Chem. 2004;279(9):7925–33.
89. Holler N, Zaru R, Micheau O, Thome M, Attinger A, et al. Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. Nat Immunol. 2000;1(6):489–95.
90. Harper N, Hughes M, MacFarlane M, Cohen GM. Fas-associated death domain protein and caspase-8 are not recruited to the tumor necrosis factor receptor 1 signaling complex during tumor necrosis factor-induced apoptosis. J Biol Chem. 2003;278(28):25534–41.
91. Lin Y, Choksi S, Shen HM, Yang QF, Hur GM, Kim YS, et al. Tumor necrosis factor-induced nonapoptotic cell death requires receptor-interacting protein-mediated cellular reactive oxygen species accumulation. J Biol Chem. 2004;279(11):10822–8.
92. He S, Liang Y, Shao F, Wang X. Toll-like receptors activate programmed necrosis in macrophages through a receptor-interacting kinase-3-mediated pathway. Proc Natl Acad Sci U S A. 2011;108(50):20054–9. doi:10.1073/pnas.1116302108.
93. Balch CM, Soong S, Ross MI, Urist MM, Karakousis CP, et al. Long-term results of a multi-institutional randomized trial comparing prognostic factors and surgical results for intermediate thickness melanomas (1.0 to 4.0 mm). Intergroup melanoma surgical trial. Ann Surg Oncol. 2000;7(2):87–97.
94. Tarhini AA, Lin Y, Yeku O, LaFramboise WA, Ashraf M, et al. A four-marker signature of TNF-RII, TGF-α, TIMP-1 and CRP is prognostic of worse survival in high-risk surgically resected melanoma. J Transl Med. 2014;12:19. doi:10.1186/1479-5876-12-19.
95. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med. 2010;363(9):809–19. doi:10.1056/NEJMoa1002011.
96. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, et al. BRIM-3 study group. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med. 2011;364(26):2507–16. doi:10.1056/NEJMoa1103782.
97. Smith MP, Sanchez-Laorden B, O'Brien K, Brunton H, Ferguson J, et al. The immune microenvironment confers resistance to MAPK pathway inhibitors through macrophage-derived TNFα. Cancer Discov. 2014;4(10):1214–29. doi:10.1158/2159-8290.CD-13-1007.
98. Fruehauf J, Lutzky J, McDermott D, Brown CK, Meric JB, et al. Multicenter, phase II study of axitinib, a selective second-generation inhibitor of vascular endothelial growth factor receptors 1, 2, and 3, in patients with metastatic melanoma. Clin Cancer Res. 2011;17(23):7462–9. doi:10.1158/1078-0432.CCR-11-0534.
99. Cornett WR, McCall LM, Petersen RP, Ross MI, Briele HA, et al. American college of surgeons oncology group trial Z0020. Randomized multicenter trial of hyperthermic isolated limb perfusion with melphalan alone compared with melphalan plus tumor necrosis factor: American college of surgeons oncology group trial Z0020. J Clin Oncol. 2006;24(25):4196–201.
100. Deroose JP, Burger JW, van Geel AN, den Bakker MA, de Jong JS, et al. C. Radiotherapy for soft tissue sarcomas after isolated limb perfusion and surgical resection: essential for local control in all patients? Ann Surg Oncol. 2011;18(2):321–7. doi:10.1245/s10434-010-1400-x.
101. Deroose JP, Grünhagen DJ, van Geel AN, de Wilt JH, Eggermont AM, Verhoef C. Long-term outcome of isolated limb perfusion with tumour necrosis factor-α for patients with melanoma in-transit metastases. Br J Surg. 2011;98(11):1573–80. doi:10.1002/bjs.7621.
102. Jouan-Lanhouet S, Arshad MI, Piquet-Pellorce C, Martin-Chouly C, Le Moigne-Muller G, et al. TRAIL induces necroptosis involving RIPK1/RIPK3-dependent PARP-1 activation. Cell Death Differ. 2012;19(12):2003–14. doi:10.1038/cdd.2012.90. **Epub 2012 Jul 20**.
103. Lemke J, von Karstedt S, Zinngrebe J, Walczak H. Getting TRAIL back on track for cancer therapy. Cell Death Differ. 2014;21(9):1350–64. doi:10.1038/cdd.2014.81.
104. Ivanov VN, Zhou H, Hei TK. Sequential treatment by ionizing radiation and sodium arsenite dramatically accelerates TRAIL-mediated apoptosis of human melanoma cells. Cancer Res. 2007;67(11):5397–407.
105. Voigt S, Philipp S, Davarnia P, Winoto-Morbach S, Röder C, et al. TRAIL-induced programmed necrosis as a novel approach to eliminate tumor cells. BMC Cancer. 2014;14:74. doi:10.1186/1471-2407-14-74.
106. Hersey P, Zhang XD. How melanoma cells evade trail-induced apoptosis. Nat Rev Cancer. 2001;1(2):142–50.
107. Mauceri HJ, Beckett MA, Liang H, Sutton HG, Pitroda S, et al. Translational strategies exploiting TNF-alpha that sensitize tumors to radiation therapy. Cancer Gene Ther. 2009;16(4):373–81. doi:10.1038/cgt.2008.86.
108. Baldea I, Filip AG. Photodynamic therapy in melanoma—an update. J Physiol Pharmacol. 2012;63:109–18.
109. Barge J, Decréau R, Julliard M, Hubaud JC, Sabatier AS, Grob JJ, et al. Killing efficacy of a new silicon phthalocyanine in human melanoma cells treated with photodynamic therapy by early activation of mitochondrion-mediated apoptosis. Exp Dermatol. 2004;13(1):33–44.
110. Davids LM, Kleemann B, Kacerovská D, Pizinger K, Kidson SH. Hypericin phototoxicity induces different modes of cell death in melanoma and human skin cells. J Photochem Photobiol B. 2008;91(2–3):67–76. doi:10.1016/j.jphotobiol.2008.01.011.
111. Calzavara-Pinton PG, Venturini M, Sala R. Photodynamic therapy: update 2006. Part 2: clinical results. J Eur Acad Dermatol Venereol. 2007;21(4):439–51.
112. Nenu I, Popescu T, Aldea MD, Craciun L, Olteanu D, Tatomir C, et al. Metformin associated with photodynamic therapy—a novel oncological direction. J Photochem Photobiol B. 2014;138:80–91. doi:10.1016/j.jphotobiol.2014.04.027.
113. Yurkovetsky ZR, Kirkwood JM, Edington HD, Marrangoni AM, Velikokhatnaya L, et al. Multiplex analysis of serum cytokines in melanoma patients treated with interferon-alpha2b. Clin Cancer Res. 2007;13(8):2422–8.
114. Blay JY, Favrot MC, Negrier S, Combaret V, Chouaib S, Mercatello A, et al. Correlation between clinical response to interleukin 2 therapy and sustained production of tumor necrosis factor. Cancer Res. 1990;50(8):2371–4.
115. Foletto MC, Haas SE. Cutaneous melanoma: new advances in treatment. An Bras Dermatol. 2014;89(2):301–10.
116. Christ O, Seiter S, Matzku S, Burger C, Zöller M. Efficacy of local versus systemic application of antibody-cytokine fusion proteins in tumor therapy. Clin Cancer Res. 2001;7(4):985–98.
117. Schwager K, Hemmerle T, Aebischer D, Neri D. The immunocytokine L19-IL2 eradicates cancer when used in combination with CTLA-4 blockade or with L19-TNF. J

Investig Dermatol. 2013;133(3):751–8. doi:10.1038/jid.2012.376.

118. Pretto F, Elia G, Castioni N, Neri D. Preclinical evaluation of IL2-based immunocytokines supports their use in combination with dacarbazine, paclitaxel and TNF-based immunotherapy. Cancer Immunol Immunother. 2014;63(9):901–10. doi:10.1007/s00262-014-1562-7.
119. Weide B, Eigentler TK, Pflugfelder A, Leiter U, Meier F, Bauer J, et al. Survival after intratumoral interleukin-2 treatment of 72 melanoma patients and response upon the first chemotherapy during follow-up. Cancer Immunol Immunother. 2011;60(4):487–93. doi:10.1007/s00262-010-0957-3.
120. Schwartzentruber DJ, Lawson DH, Richards JM, Conry RM, Miller DM, et al. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. N Engl J Med. 2011;364(22):2119–27. doi:10.1056/NEJMoa1012863.
121. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013;369(2):122–33. doi:10.1056/NEJMoa1302369.
122. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol. 2008;26:677–704. doi:10.1146/annurev.immunol.26.021607.090331.
123. Pentcheva-Hoang T, Egen JG, Wojnoonski K, Allison JP. B7-1 and B7-2 selectively recruit CTLA-4 and CD28 to the immunological synapse. Immunity. 2004;21(3):401–13.
124. Binder DC, Schreiber H. Dual blockade of PD-1 and CTLA-4 combined with tumor vaccine effectively restores T-cell rejection function in tumors—letter. Cancer Res. 2014;74(2):632. doi:10.1158/0008-5472.CAN-13-2216. **discussion 635. Epub 2014 Jan 9**.
125. Parmiani G, Pilla L, Corti A, Doglioni C, Cimminiello C, et al. A pilot phase I study combining peptide-based vaccination and NGR-hTNF vessel targeting therapy in metastatic melanoma. Oncoimmunology. 2014;3(11), e963406.