# Targeting beta-adrenergic receptor pathways in melanoma: how stress modulates oncogenic immunity

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**The intricate pathways of the sympathetic nervous system hold an inherently protective role in the setting of acute stress. This is achieved through dynamic immunomodulatory and neurobiological networks. However, excessive and chronic exposure to these stress-induced stimuli appears to cause physiologic dysfunction through several mechanisms that may impair psychosocial, neurologic, and immunologic health. Numerous preclinical observations have identified the beta-2 adrenergic receptor (β2-AR) subtype to possess the strongest impact on immune dysfunction in the setting of chronic stressful stimuli. This prolonged expression of β2-ARs appears to suppress immune surveillance and promote tumorigenesis within multiple cancer types. This occurs through several pathways, including (1) decreasing the frequency and function of CD8 + T-cells infiltrating the tumor microenvironment (TME) via inhibition of metabolic reprogramming during T cell activation, and (2) establishing an immunosuppressive profile within the TME including promotion of an exhausted T cell phenotype while simultaneously enhancing local and paracrine metastatic potential. The use of nonselective β-AR antagonists appears to reverse many chronic stress-induced tumorigenic pathways and may also provide an additive therapeutic benefit for various immune checkpoint modulating agents including commonly utilized immune checkpoint inhibitors. Here we review the translational and clinical observations highlighting the foundational hypotheses that chronic stress-induced β-AR signaling promotes a pro-tumoral immunophenotype and that blockade of these pathways may augment the therapeutic response of immune checkpoint inhibition within the scope of melanoma. *Melanoma Res* 34: 89–95**


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## Introduction

Stress-related stimuli are well recognized to trigger crucial neuroimmunomodulatory networks within the sympathetic autonomic nervous system. These pathways orchestrate a dynamic physiologic response via direct and indirect cross-talk between a host's hypothalamic-pituitary-adrenal (HPA) axis of the central nervous system (CNS) and (1) systemic sympathetic nervous fibers, (2) vascular endothelium, (3) muscle, (4) viscera and (5) primary and secondary lymphoid tissues (bone marrow, thymus, spleen, and lymphatics) [1]. Exposure to stressful stimuli triggers neurohormonal secretion of neurotransmitter catecholamines (acetylcholine, glutamine, epinephrine and norepinephrine) from (1) chromaffin cells in the adrenal medulla, (2) the amygdala of the CNS, and (3) sympathetic nerve endings. These catecholamines then systemically bind to a variety of adrenergic receptors within a myriad of tissue types resulting in physiologic reactions associated with arousal, attention, and the well-known 'fight or flight' response. Despite sharing similar primary neurotransmitter stimuli, an assortment of α and ß adrenergic receptors (more specifically subdivided into α1A, α1B, α1D, α2A, α2B, α2C, β1, β2, and β3) are uniquely distributed across a variety of tissue types, each possessing physiologic implications across all organ systems [2]. These mechanisms provide helpful and even life-saving responses in the acute setting. However, chronic exposure to stress-induced stimuli, triggered mostly through beta-2 adrenergic receptor (ß2-AR) signaling, effectively derails immunologic and neurobiochemical homeostasis resulting in significant detriments on one's psychosocial and immuno-physiologic health [3,4]. Chronic stress-related stimuli are now well established to promote an immune-suppressed environment that results in suboptimal (1) wound healing [5], (2) elimination of infections [6,7], (3) preservation of cardiovascular and metabolic health [8], and (4) ability to clear developing cancer cells [9–11]. The latter observation warrants heightened clinical attention, given the disproportionately elevated psychologic, physiologic, and financial stresses that are commonly faced by individuals living with a cancer diagnosis [12].

From an oncological vantagepoint, the dynamic neurobiochemical pathways associated with chronic stress via increased autonomic nerve conduction in β2-AR and

β3-AR signaling appear to directly and indirectly contribute to the hallmarks of cancer [9–11]. These signals compromise antitumoral immunity and the effectiveness of immune-modulating therapies by promoting tumor growth and metastasis while cultivating an exhausted immunophenotype within the tumor microenvironment (TME) [11,13–19]. Interestingly, blockade of β2-AR pathways has been observed to enhance the anti-tumoral immune response and improve overall survival in murine models as well as patients with melanoma, breast, and prostate cancer [20–25]. Given that patients with melanoma exhibit high levels of chronic stress and their tumors express high levels of β2-AR [26–30], a prospective randomized clinical trial observing the effectiveness of adding a non-selective beta-blocker (propranolol) to a standard-of-care immune checkpoint inhibitor (ICI), pembrolizumab, in patients with treatment-naïve unresectable or metastatic melanoma is ongoing (NCT03384836) [31]. Here we will provide an in-depth review of the underlying immunologic and physiologic impacts of chronic stress signaling pathways in melanoma from a translational and clinical vantagepoint.

## Immunologic implications of ß-adrenergic signaling pathways

The impacts of stressful stimuli on the immune system have clearly demonstrated regulatory mechanisms involving both the innate and adaptive response [1]. Although classically observed within airway smooth muscle tissues, the β2-AR subtype, a G-protein coupled receptor consisting of 7 transmembrane-spanning α-helices, appears to play a major role in stress-related immunomodulation with variable levels of expression across nearly all immune cell subtypes with up to 2000 binding sites per human leukocyte (excluding Th2 helper T cells) [1,32,33]. Most of the immunoregulatory mechanisms of β2-ARs appear to be via cyclic AMP (cAMP) signaling pathways upon activation by stressful stimuli in the form of norepinephrine directly secreted into secondary lymphoid organs by post-ganglionic sympathetic neurons [34]. However, these β2-ARs also possess cAMP-independent regulatory mechanisms including coupling to inhibitory (Gi) proteins with resultant extracellular signaling of the mitogen-activated protein kinase pathways [34]. These dynamic cellular interactions, in addition to tissue-dependent levels of β2-AR expression, allow each immune cell subtype to exhibit unique response patterns upon up- and downregulation of these receptors. For example, β2-AR activation appears to (1) decrease pro-inflammatory cytokine production by macrophages and monocytes [35], (2) reduce Th1 cytokine production by CD4 + T cells [36], (3) increase acquired mutations via suppression of p53 expression [37], (4) enhance immunosuppressive populations of regulatory T-cell (Treg) as well as myeloid-derived suppressor cells (MDSCs) [17,38,39], and (5) suppress CD8 + T cell effector function by inhibiting cytokine production and cytolytic activity through impairment of metabolic reprogramming during activation [33,40,41]. Although comprehensive reviews are available elsewhere highlighting the unique patterns of adrenergic receptor expression and function within each immune cell subtype [1,42], an imperative overarching theme is that chronic exposure to stress-related β2-AR signaling causes undesirable immunomodulatory sequelae including selective suppression of crucial effector function and cytolytic capacity of CD8 + T-cells while establishing an exhausted immunophenotype via direct and indirect mechanisms within primary and secondary lymphoid structures [33,40,41]. In addition, β2-AR signaling appears to be directly correlated with the frequency and viability of tumorigenic MDSCs within the TME [17,38]. Interestingly, the downstream effects of reducing stress-related adrenergic signaling via therapeutic β2-AR antagonists appear to reverse many of the aforementioned exhausted and suppressed mechanisms of the immune response, thus introducing a potential therapeutic means to improve endogenous immunity and enhance responses to immune-modulating therapies [43,44].

## Impact of ß-adrenergic immune modulation in cancer

Early observations of chronic stress-induced β-AR signaling involved the acknowledgement that mice maintained in universally regulated housing temperatures of 22°C appear to exhibit increased production and signaling of norepinephrine with resultant adaptive thermogenesis in order to maintain homeostatic body temperatures [41,45,46]. These β-AR signaling patterns were significantly reduced in 'non-stressed' mice housed at thermoneutral temperatures that do not require the metabolic demand of sustained heat production, as well as those exposed to therapeutic nonselective beta-adrenergic blockade (propranolol) [46,47]. Mice housed in chronically stressed (standard) temperatures were further noted to exhibit more rapid tumor growth in multiple tumor models, with associated increases in norepinephrine and β2-AR signaling within the TME, when compared to those kept in thermoneutral conditions or treated with propranolol [16,40,41,46]. Further, the addition of propranolol to anti-programmed death receptor-1 (PD-1) ICI exhibited an additive anti-tumoral benefit in breast and melanoma murine models [41,43].

There is now an abundance of additional data identifying specific tumorigenic roles of chronic stress-induced adrenergic pathways in promoting most of the well-established 'hallmarks' of cancer [16,17,19,37,40,48,49]. Although thorough reviews on the impacts of adrenergic signaling within various cancer types are available elsewhere [9,10,18,43,50–54], a few foundational observations are worth highlighting. Several studies have appreciated that β2-AR knockout mice do not exhibit the enhanced tumor growth seen in chronically stressed control mice, suggesting the critical role of this specific receptor subtype in

suppressing desirable anti-tumoral immune responses [16,46]. Further, chronic stress-related adrenergic signaling triggers a complex cascade of anti-inflammatory and immunosuppressive effects. For example, β2-AR expression on CD 8 + T cells appears to suppress their anti-tumoral effector function and cytolytic capacity by suppressing glucose uptake and metabolic reprogramming, with resultant reduction of the Th1 immune response [1,33,40,42]. In addition, increased secretion of norepinephrine and ensuing chronic β2-AR signaling appears to promote several pathways of immune escape via impairment of dendritic cell maturation as well as enhanced recruitment of immunosuppressive MDSCs and Tregs within the TME [17,41,55,56]. Numerous tumor types also utilize axonogenesis, a process of recruiting and promoting nerve growth within a designated tissue, which causes locally enhanced direct and indirect impacts of autonomic innervation within the TME [18]. This process appears to enhance various stages of tumorigenesis, including an apparent dependency for sympathetic β2-AR activation to achieve tumor growth as well as a direct correlation of increased nerve density in tumors with more aggressive characteristics [18]. Further evidence of this immunosuppressive milieu localized within the TME via enhanced axonogenesis and resultant β2-AR signaling suggests that these pathways dampen the anti-tumoral effectiveness of endogenous tumor-infiltrating lymphocytes as well as ICI agents, and has therefore become a targetable pathway of therapeutic interest [16,41,43,44].

## Impact of ß-adrenergic immune modulation in melanoma: pre-clinical evidence

To date, a wealth of pre-clinical data has been reported regarding the effects of chronic stress-related adrenergic signaling in melanoma models. Murine melanoma models exposed to chronic social or environmental stressful stimuli exhibited an appropriate increase in serum catecholamine levels but also exhibited an increased rate and severity of metastatic disease when compared to non-stressed control mice [43,55,57,58]. Although the precise mechanisms of these observations are unclear, the expression of both β1 and β2-ARs in melanoma cell lines appear to increase upon exposure to catecholamines [30]. This signaling results in activation and production of matrix metalloproteinase (MMP)-2, MMP-9, vascular endothelial growth factor (VEGF), interleukin (IL)-8, and IL-6 within the TME which results in enhanced proangiogenic (VEGF), chemotactic (IL-8) and autocrine (IL-6) properties within melanoma cells [28,30,59,60]. The β2-AR appears to be predominantly upregulated through the malignant transformation of these lesions, suggesting a possible role of this specific adrenergic subtype as a contributing mechanism of melanoma tumorigenesis and metastasis [28,61]. In addition, expression of β2-ARs on lymphatic endothelial cells within murine melanoma models appear to stimulate secretion of localized nitric oxide, resulting in lymphatic vasodilation and subsequent impairment of tumoral lymphatic drainage [62]. Further, experimental exposure of catecholamines to both primary and metastatic melanoma cells expressing these receptors appears to enhance their motility and metastatic capacity, potentially via upregulation of dermal fibroblast activation [28]. Further, human melanocytes appear to promote autocrine production of catecholamines with resultant neuroneoplastic axonogenesis in normal pigment development [63]. This process appears to be further upregulated in melanoma cell types, therefore suggesting that β2-AR signaling pathways may be self-perpetuating to promote neoangiogenesis and lymphangiogenesis within a developing TME while simultaneously contributing to a positive feedback loop with the HPA to further intensify the focal release of these tumorigenic catecholamines [63–65].

A variety of translational and clinical models have suggested that the immunosuppressive patterns of chronic stress-induced β2-AR signaling can be mitigated via reduction of stressful stimuli as well as pharmacologic blockade of these receptors [66]. For example, the aforementioned tumorigenic upregulation of VEGF, IL-6, and IL-8 pathways associated with the neuroendocrine stress response in melanoma cell lines appears to be abrogated by a non-selective inhibitor of β-AR signaling (propranolol) [28,60]. Intriguingly, additional efforts have further supported that β-AR blockade with propranolol in murine melanoma models decreases tumor growth and metastasis while simultaneously exerting an anti-tumoral effect through decreasing MDSCs and increasing CD8 + T-cell and natural killer cells within the TME [67,68]. In addition, synergistic anti-tumoral activity with standard of care anti-PD-1 ICI therapy combined with propranolol was achieved when compared to each agent as monotherapy in murine melanoma models maintained in a chronically stressed environment [41,43,69]. Interestingly, this activity with combination therapy was not observed in SCID or β2-AR knockout mice, emphasizing the need for a functional immune and sympathetic nervous system in order to mount an optimal antitumoral response [41,43]. Additional systemic therapies, including anti-CTLA4 ICI agents and novel cancer vaccines, also appear to achieve an enhanced antitumoral response in melanoma cell lines when combined with pharmacologic β2-AR blockade [70–72].

Although the β2-AR subtype appears to be consistently involved in the tumorigenic and malignant potential of melanoma cells as outlined above, the β3-AR also appears to impact melanomagenesis. Like β2-ARs, the β3-ARs are more commonly expressed by melanoma cells with advanced and/or aggressive histologic characteristics [29]. These receptors are further upregulated by environmental changes associated with advanced progression within the TME, such as hypoxia and glucose depletion, which results in localized production of pro-angiogenic

nitric oxide and promotion of immune evasion via metabolic reduction of intracellular lactate levels [29,73–75]. Further, targeted β3-AR blockade in murine melanoma models appears to reverse the aforementioned metabolic changes while simultaneously enhancing tumoral invasion by host macrophages and neutrophils along with an increased $CD8^{+}$T-cell/Treg ratio and increased expression of apoptotic markers within the TME [29,74,76]. In contrast, less supportive and conflicting data exists regarding the tumorigenic impact of the β1-AR subtype in both preclinical and clinical models [16,20,77–82]. Although intriguing, the role and clinical implications of β1- and β3-ARs remain unclear given their dynamic cross-communication between environmental factors as well as complex regulatory mechanisms between other adrenergic receptors [75,83,84]. Given the observations above in addition to the classically heterogeneous nature of the melanoma TME, additional single-cell translational multi-omics data combined with prospective clinical efforts are required to more clearly define the dynamics of all β-AR subtypes in melanomagenesis while simultaneously identifying potential pharmacologic targets within these pathways.

Lastly, additional pre-clinical exploration of β-ARs within uveal melanoma models has also provided provocative observations. Similar to cutaneous melanoma, there appears to be enhanced β2-AR expression in more aggressive histologic subtypes of uveal melanoma lesions as well as a dose-dependent antitumoral effect of non-selective β-AR blockade in reducing metastatic migration, angiogenesis, and proliferation [85]. Further, treatment naïve and irradiation-refractory uveal melanoma cell lines grown within three-dimensional spheroids that mimic the in-vivo anatomical configuration of this disease exhibited significant antitumoral responses when exposed to non-selective β-AR blockade along with an additive effect when combined with irradiation [86]. Similar to cutaneous melanoma models, further confirmatory and clinical correlations of β-AR blockade within this disease subtype are anticipated.

### Impact of ß-adrenergic immune modulation in melanoma: clinical evidence

Within the clinical setting, there are a variety of intriguing observations and ongoing trials regarding melanoma and adrenergic signaling pathways. Samples of human melanocytic cutaneous lesions have enhanced expression of β1, β2, and β3 receptors that appear to increase based on the degree of the lesion's malignant properties [28,75]. In the non-metastatic setting, a prospective single-center study utilizing off-label adjuvant propranolol (80 mg daily) for 19 patients with newly diagnosed stage IB to IIIA cutaneous melanoma exhibited an 80% risk reduction in melanoma recurrence compared to those without propranolol use (hazard ratio, 0.18; 95% CI, 0.04–0.89; $P = 0.03$) [24]. An additional study observed an enhanced anti-tumoral immune response within the TME of 212 patient's primary melanoma lesions when exposed to nonselective- β-AR blockade as well as significant clinical improvements in progression-free survival (PFS) and melanoma-specific survival in patients taking nonselective β-AR blockade for non-oncologic reasons compared to those who did not [87,88]. Some discordance has been noted regarding these observations, including two large retrospective analyses noting no statistically significant survival benefit for patients with melanoma taking any sort of β-AR blockade [82,89,90]. However, an encouraging survival benefit trend was observed in the minority of patients on nonselective compared to selective β-AR blockade agents, further supporting the role of the β2-AR subtype in regulating anti-tumoral immunity.

Although there are insufficient data to definitively accept that nonselective β-AR blockade independently achieves a survival benefit in patients with melanoma, evidence does consistently support that these agents enhance responses to ICI in the clinical setting. Multiple retrospective efforts have noted either statistically significant or strong trends of improved overall survival without an increase in the toxicity profile in patients with metastatic melanoma treated with ICI who are concurrently taking β-AR blockade when compared to patients treated with ICI who were not taking a β-blocker [20,81]. A particularly striking observation within these efforts noted that this survival benefit appears to be lost in patients only taking selective (β1) blockade regardless of the ICI agents administered, thus supporting the previously observed anti-tumoral benefits of non-selective beta-blockade in this setting [20]. A prospective effort by our group to confirm these findings included a phase I dose-escalation clinical trial combining anti-PD1 ICI (pembrolizumab) with nonselective β-AR blockade (propranolol) in patients with treatment-naïve advanced melanoma. This trial noted no dose-limiting toxicities within the 9 enrolled patients and provocative signals of both enhanced objective responses and a reduced toxicity profile compared to historical observations of pembrolizumab monotherapy [31]. In effort to validate these encouraging findings, our group is leading a multicenter phase II portion of this trial (NCT03384836) [91]. In addition, a large review of the EORTC 1325/KEYNOTE-054 trial for patients with resected stage IIIA, IIIB, and IIIC melanoma treated with adjuvant ICI (pembrolizumab) versus placebo noted that although no independent effect on recurrence-free survival was observed in the 10% (n = 99) of patients treated with ICI who also received some form of β-AR blockade within 30 days of starting the trial, there was compelling improvement in those on combination ICI plus β-AR blockade (HR = 0.34) compared to those on ICI without β-AR blockade (HR = 0.59) [92]. Although the latter observation did not reach statistical significance, it is worth noting that the strongest signal of an enhanced response with combination ICI plus β-AR

blockade was observed in a small subset (n = 19; 19%) of patients who were taking nonselective β-AR blockade compared to the majority (81%) of patients on a selective β-AR blocker, which further supports the previous observations regarding the importance of nonselective β-AR blockade (particularly targeting the β2-AR) as a means to fully optimize these therapeutically additive immunologic responses [92]. Several additional clinical trials utilizing nonselective β-AR blockade, with and without ICI, for patients with varying stages of melanoma are ongoing for which each trial's preliminary findings are highly anticipated [66].

## Conclusions and future directions

Chronic stress-induced signaling of β-ARs in melanoma tumor models appear to cultivate a complex and self-perpetuating immunosuppressive TME through multiple G-protein coupled dependent and independent mechanisms. These signals appear to result in major immunologic and metabolic derangements with subsequent promotion of tumor progression and immune evasion. Currently, however, the local and systemic implications of chronic adrenergic signaling across all receptor subtypes in relation to the immunomodulation of melanoma remains a topic of active translational and clinical investigation. The intricate and dynamic pathways of how each α and β-AR subtype modulates the human immune system, both independently as well as via cross-regulatory feedback mechanisms, remains a crucial knowledge gap that is required for optimal clinical application [83,93]. Current clinical observations suggest that these pathways appear to be driven largely in part via expression of the β2-AR subtype, and increasing pre-clinical evidence suggests the β3-AR pathways may also be involved. Antagonism of β2-ARs has now exhibited repeated evidence of restoring anti-tumoral immunogenicity as well as enhancing effectiveness of ICI therapies within both pre-clinical and clinical melanoma models [28,66,71]. Our group's aforementioned multicenter phase II clinical trial observing the benefits of nonselective β-AR when combined with ICI (pembrolizumab) in patients with advanced melanoma is actively enrolling patients, and is expected to provide a more definitive answer regarding the clinical benefit of this combination in the setting of advanced melanoma [91].

There are several important considerations regarding future exploration in this space. Given traditional nonselective β-AR blocking agents do not inhibit the β3-AR subtype, introducing β3-AR blockade with or without both β2-AR blockade and/or ICI in the clinical setting for patients with melanoma is an exciting concept anticipated to provide additional valuable insights into the key mechanisms of adrenergic signaling as they relate to the anti-tumoral immune response. Similarly, the utility of combining β-AR blockade with alternative standard of care ICI for advanced melanoma, including anti-CTLA4 and the newly approved anti-LAG3 agents, are also in need of further investigation. For example, preliminary evidence of enhanced anti-CTLA4 responses when combined with β-AR blockade has been observed in non-melanoma cancer models [44]. Further, additional efforts investigating the utility of manipulating β-AR pathways in patients with progressive and/or refractory melanoma following ICI or targeted first-line therapies is also a major topic of interest given the hypothesized ability of β-AR blockade to reinvigorate the exhausted immunophenotype within the TME and enhance anti-tumoral immunity in order to regain disease control in that setting [16]. Lastly, further investigation into alternative neuro-immunologic pathways is warranted, given preliminary evidence suggesting that sensory neurons may further modulate pathways of immunosurveillance and tumorigenesis [94,95]. Given these intriguing observations and ongoing clinical efforts, the modulation of β-AR signaling is anticipated to provide a safe and cost-effective means of optimizing endogenous immune surveillance while enhancing the anti-tumoral immune response of current standard-of-care treatments for patients with melanoma as well as many other cancer types.

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### Conflicts of interest

I.P.: consulting fees: Nektar, Nouscom, Oncorus, Regeneron, Sanofi, Iovance. stock options: Seneca Therapeutics – none of these are connected to submitted work. S.G.: Advising: AstraZeneca and Biotheranostics. For the remaining authors, there are no conflicts of interest.

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