Cancer immunology and melanoma immunotherapy*

Rudinei Diogo Marques Linck[1]
Rômulo Leopoldo de Paula Costa[1]
Bernardo Garicochea[1]

DOI: http://dx.doi.org/10.1590/abd1806-4841.201756511

Abstract: The stimulation of the immune system, in order to generate an attack against cancer cells, similarly to that which occurs in infectious disease, has long been matter of interest in oncology; however, only limited success has been achieved, with different treatment strategies tested in recent years. The development of new immune checkpoint inhibitors is currently changing this scenario, and immunotherapy is becoming a real choice among traditional cytotoxic treatments to fight cancer. Recent reports have shown efficacy and safety with the use of pembrolizumab, nivolumab, and ipilimumab for the treatment of different neoplasms, especially melanoma. In this article, we propose a review of the mechanisms of action involved in cancer immunology, the response evaluation of immunotherapies, and its toxicity profile, as well as a summary of the main clinical trials that led to the adoption of these new drugs for melanoma treatment.
Keywords: Immunotherapy; Melanoma; Neoplasms

INTRODUCTION

The importance of the immune system in fighting cancer has been studied since the 19th century, when, in 1891, the American surgeon William Coley described his experiment with the intratumoral inoculation of *Streptococcus pyogenes* and *Serratia marcescens,* expecting to reproduce a rare spontaneous sarcoma remission case observed after the patient had had erysipelas.[1] The subject continued to raise interest within the scientific community. However, despite the rare exceptions, such as the case of intravesical treatment of a superficial bladder neoplasm with BCG, for a long period of time, the complex nature of the immune system action mechanisms limited the development of other effective therapies for clinical use.[2] This scenario more recently has been revolutionized, especially after the approval for the clinical use of immune *checkpoint* inhibitors in melanomas and other tumor types.

The neoplastic cells' acquisition of the capability to evade the immune system – as well as their ability to subvert it to their advantage – is one of the "milestones" for the development of neoplasms.[3] Therefore, it is acknowledged that cancer is capable of "editing" the immune system, and the neoplastic cells need to acquire the capability of "escaping" the immune system in order to develop, given that the immune system would be capable of "eliminating" these sick cells. This theory also suggests that there is a "balance" between the forces that lead to the disease's elimination and those that lead to acquiring the immune system's evasion ability. This intermediate period would at least partially explain the mechanism by which some types of neoplasms may remain stable in their growth over long periods of time, or even the mechanism that leads to late recurrences after adjuvant treatments, when micrometastases remain clinically dormant for several years.[4]

The immune system consists of two different cell types and by cells at different maturation phases in a complex interaction in which communication is performed by means of stimuli sent with the secretion of cytokines, and by the activation of membrane receptors in the contact between the cells. The immune system is subdivided into the innate immune system and the adaptive immune system, and their main difference is that the adaptive immune system is capable of specifically identifying a given aggressor (or antigen) and of maintaining this identification memory for a quick immune response in case of new exposure to the same agent. The innate immune system, however, has common abilities among the different organisms, and it is considered our first line of defense. Both the innate and the adaptive systems are involved in fighting cancer, and the different cell types play specific roles.

Immune system cells and immunological synapse

The innate immune system cells (dendritic cells, macrophages, and *natural killer* [NK] cells) are capable of identifying certain molecular patterns present in microorganisms – or in some neo-

Received on 21.09.2016.
Approved by the Advisory Board and accepted for publication on 11.02.2017.
* Work performed at the Sírio-Libanês Hospital Oncology Center, São Paulo, SP, Brazil.
Financial support: none.
Conflict of interest: none.

[1] Sírio-Libanês Hospital Oncology Center – São Paulo (SP), Brazil.

plastic cells – to differentiate them from healthy cells and, therefore, trigger the direct elimination of these aggressors by innate system cells, or by the recruitment and activation of the adaptive immune system cells. The communication between the innate and the adaptive system takes place by means of the antigen presenting cells (APC) (dendritic cells, macrophages, and B-lymphocytes), which, by identifying a foreign molecular pattern of the organism, activate the T-lymphocyte *helper* (TH or T CD4+ lymphocyte) during what is called the initiation phase. This activation is triggered by the presentation of a foreign antigen processed by the APC along with the class II MHC molecule (MHC II) to the T-cell receptor (TCR) of T CD4+ lymphocytes. However, the stimulus generated by the simple contact of the antigen connected to the MHC II molecules with TCR is incapable of generating the activation of the initiation phase, since this activation is regulated by co-stimulatory signals (connection of B7 and the CD28 receptor of the TH lymphocyte), as well as by co-inhibitory signals (connection between B7 and the CTLA-4 receptor; or between the PD-1 [PD-L1/PD-L2] binder and the PD-1, also present in the TH lymphocytes), which optimize or inhibit this activation, and are called immune *checkpoints*.

From the adaptive immune system, the activated TH lymphocytes acquire the function to regulate both the cell immune response performed by cytotoxic T lymphocytes (CTL) and macrophages (by TH1 lymphocyte stimulus), and the humoral response with the production of antibodies by B lymphocytes (mediated by TH2 lymphocytes). The CTL (or CD8+ T lymphocytes) are effector cells in the cell immune system capable of inducing cytotoxicity upon identifying foreign antigens presented by class I MHC molecules (MHC I), normally expressed in all nucleated cells in the organism. Neoplastic cells that did not lose their ability to express MHC I molecules may present, to the CTL, the neo-antigens generated from proteins expressed by mutated genes in the carcinogenesis process (because they are proteins whose structure had been altered, they will be identified by the immune system as being foreign) and, thus, trigger the cytotoxicity process against cancer. For this reason, the higher the number of mutations (or "mutational load") in a given tumor, the greater the potential benefit with immunological therapies.[5] Similarly, the greater CD8+ T lymphocyte infiltration in tumors seems to be associated with the best clinical outcomes, given that they may represent an exacerbated cytotoxicity process against cancer.[6] The identification process of antigens linked to MHC I molecules by CTL, with the consequent cytotoxicity induction, is called the cell immune system effector phase, which, similar to the initiation phase, is regulated by other molecules' stimuli on immune *checkpoints*. The main inhibition stimulus in the effector phase is generated in the connection between PD-L1 and the PD-1 molecule of the CLT. Therefore, to avoid the antitumoral response, some neoplasms are capable of super-expressing PD-L1. The NK cells are part of the innate immune system (they do not require activation by TH lymphocytes) and are capable of triggering a cytotoxic effect on cells that lost the MHC 1 molecule expression, another strategy used by tumors in the attempt to "escape" the immune system.

In addition to performing the function of antigen presenting cells, B-lymphocytes are the essential cells for humoral adaptive immunity, as well as being responsible for producing antibodies. Although cell immunity plays an apparently key role in generating an effective antitumoral response, humoral immunity acts by different mechanisms and highly important in the fight against cancer. Unlike the TCR, which are only capable of identifying peptide antigens processed by means of MHC molecules, the antibodies identify a variety of intact antigens, such as proteins in their native conformations, polysaccharides and nucleic acid, if present in the cell surface or even if soluble in plasma and in the extracellular matrix. The connection between the antibody and its specific antigen is capable of triggering the cascade activation of the complement's serum proteins, leading to the complement mediated cytotoxicity (CMC) process; of activating the antibody-dependent cell mediated cytotoxicity (ADCC), by stimulating phagocytosis by macrophages, neutrophils or NK cells; and of mediating antitumoral effects by interfering in the cell membrane receptor function, activating or blocking its signaling pathway.

Some cell types play the role of regulating the immune system and, therefore, can be co-opted to work as antitumoral response evasion mechanisms. The T regulatory lymphocytes (Treg) are a group of cells specialized in preserving the immune system's tolerance and in avoiding autoimmune reactions, capable of suppressing the expansion of effector cells against self-antigens. However, because most of the antigens expressed by the neoplastic cells are considered inherent to the organism, this system acts as a repressor of the antitumoral immune response. The interference in the secretion of certain cytotoxins in the tumoral microenvironment for the recruitment of cells capable of suppressing the immune system - such as the infiltration of myeloid-derived suppressed cells (MDSC) that secrete T cell inhibitor cytotoxins - may also be used by neoplastic cells to evade the immune system. Unlike the tumoral infiltration by effector macrophages with cytotoxic ability called M1 (M1 infiltration is associated with the increase in survival rate), the recruitment by cytotoxin secreting macrophage neoplastic cells that promote angiogenesis and limit the TH1 lymphocyte activity, also called M2 macrophages, is related to worse clinical outcomes.[7,8]

THERAPEUTIC TARGETS

Several therapeutic strategies with different approaches are being proposed in the attempt to stimulate the immunological response in fighting cancer. Some of these therapies have proven to be effective and are currently incorporated as options for clinical use. Several other therapies are in the development phase, some of which have quite promising initial results. However, toxicity is one of the main barriers faced in the development of new immunotherapies, which often becomes the limiting fact for implementing new drugs in clinical practice. Examples of this are the strategies designed to activate the immune system by the systemic administration of cytotoxins. The use of interleukin-2 (IL-2) in high doses or alpha-interferon have demonstrated that both provide certain benefits in treating melanoma and carcinoma of renal cells. However, the toxicity generated with the stimulus of a non-specific immunological response against the tumor prevents its use in an unrestricted manner.[9,10]

The manipulation of T lymphocytes in order to make them reactive to specific antigens, and therefore stimulate the antitumoral response, is among the promising immunotherapy strategies. The

CAR (chimeric antigen receptor) T cells are lymphocytes from the patients themselves, genetically modified in *ex vivo* manipulation in order to express a membrane receptor capable of activating the cell response only with the identification of specific antigens, with no need for presentation by MHC molecules. Although it is still in its initial development phase for treatment of solid tumors, the CAR T cells have shown significant benefits in clinical studies in hematologic neoplasms.[11,12] Another therapeutic strategy by T lymphocyte manipulation has been developed with *in vitro* expansion of tumor infiltrating leukocytes (TIL) extracted from fresh tumor tissue samples. The TIL have the ability to recognize tumoral antigens, but they are inhibited by the tumoral microenvironment; therefore, the reinfusion is performed after having applied a chemotherapy or radiotherapy regimen for Treg lymphocyte depletion and other inhibitor cells for *in vivo* expansion of the TIL. Some studies in advanced melanoma and other solid tumors have demonstrated this strategy's benefits.[13]

Oncolytic viruses developed to cause preferential infection of neoplastic cells may promote the reduction of the immunological tolerance of cancer by "signaling" these cells to the APC. Moreover, genetically programmed viruses may serve as vectors to produce immunomodulatory cytotoxins in the tumoral microenvironment. The intratumoral TVEC (an attenuated modified herpes simplex virus form to express granulocyte-macrophage colony-stimulating factor [GM-CSF]) stimulates the antigen presentation by dendritic cells and thus appears to increase the long-lasting response rate in melanoma treatment.[14] The stimulation of the adaptive immune response against tumoral antigens by means of vaccines has been explored in different strategies. However, the only currently approved therapy for oncological treatment to use this resource is sipuleucel-T, in which the patient's own dendritic cells are stimulated *ex vivo* with an antigen consisting of the combination of prostatic acid phosphatase (PAP) and GM-CSF. Therefore, it is possible to accelerate the maturation of the dendritic cells and stimulate the presentation of antigens by the APC after their reinfusion in the patient. The use of sipuleucel-T exhibited benefits in global survival in the treatment of patients with castration resistant prostate carcinoma.[15]

IMMUNE CHECKPOINT INHIBITORS IN MELANOMA

However, the most recent and significant advances in immunological cancer therapy, especially for melanoma, have been reached with the development of anti-CTLA-4 (ipilimumab) and anti-PD-1 (pembrolizumab and nivolumab) antibodies, immune *checkpoints* blockers used to restore or increase the antitumoral immune response.

Anti-PD-1 antibodies

Pembrolizumab is one of the most studied anti-PD-1 monoclonal antibodies in metastatic melanoma, and has been evaluated both in monotherapy and in combination with anti-CTLA-4 block. In a phase I study, 655 patients – among which one third had previously been treated with chemotherapy or immunotherapy – were allocated in four cohorts, using pembrolizumab at different dosages (10 mg/kg every two weeks; 10 mg/kg every three weeks or 2 mg/kg every two weeks). The objective response rate (ORR) was 33%, with a progression-free survival rate (PFS) at 12, 24, and 36 months of 35%, 28%, and 21%, respectively. In the end, the overall survival rate (OS) at 12, 24, and 36 months was 73%, 50%, and 40%. There was no ORR difference between those who had previously been exposed to anti-CTLA-4 block and those who had not, nor between wild or mutated V600 tumors. The absence of PD-L1 tumoral expression has not proven to be a beneficial absence marker with pembrolizumab. The most common adverse effects were fatigue, pruritus, exanthem, diarrhea, and arthralgia. Nevertheless, only 14% of the patients exhibited grade 3 or 4 adverse effects.[16-18]

In the phase II controlled study KEYNOTE-002, 540 patients were randomized after progression with ipilimumab to receive pembrolizumab (2 mg/kg or 10mg/kg every three weeks) or chemotherapy at the researcher's discretion (carboplatin with paclitaxel or monotherapy with paclitaxel, dacarbazine, or temozolomide). The primary outcome was reached with a statistically significant difference in PFS in six months (34%, 38%, and 16% in the sub-groups treated with pembrolizumab 2 mg/kg, 10 mg/kg or chemotherapy). The ORR rates were 21%, 26%, and 4%, respectively. The toxicity profile was similar to that observed in previous studies, with grade 3 to 5 adverse effects, in 11% to 14% of the patients treated with pembrolizumab, and 26% of patients treated with chemotherapy.[19]

Pembrolizumab was also compared to ipilimumab, a treatment considered to be standard at the time, in the phase III study KEYNOTE-006. The study randomized 834 patients between pembrolizumab (10 mg/kg every three or two weeks, continued for two years) and ipilimumab 3 mg/kg every three weeks for four doses. Both primary outcomes (progression-free survival and overall survival) reached a statistically significant benefit, comparing the use of pembrolizumab at different doses and chemotherapy. Therefore, the PFS in 12 months was 39% and 38% *versus* 19%, while in 24 months, it was 31% and 28% *versus* 14% (*hazard ratio* [HR] 0.68 and 0.68, respectively, for pembrolizumab every two or three weeks *versus* chemotherapy), and OS in a year was 74% and 68% *versus* 59% and, in two years, was 55% and 55% *versus* 43% (HR 0.61 and 0.69 for both comparisons). In addition, grade 3 to 5 adverse events were less frequent on the arms with the use of pembrolizumab (13% and 10% *versus* 20% in patients treated with chemotherapy).[20]

Nivolumab is a second anti-PD-1 monoclonal antibody, developed to inhibit immune checkpoints, which has proven to be useful in advanced melanoma treatment. In an initial phase I/II study, 107 patients were exposed to different nivolumab doses between 0.1 and 10 mg/kg every two weeks for up to 96 weeks. The median OS was 17 months with a global response of 32% of the patients. The OS rates in one, two, three, four, and five years were 63%, 48%, 42%, 35%, and 34%, respectively, which led to the development of the CheckMate 066 study.[21] In this phase III study, 418 patients with metastatic melanoma and wild BRAF, and who had not received prior treatment were included for the randomization between nivolumab 3 mg/kg and dacarbazine 1,000 mg/m², both every three weeks. The OS in a year was 73% *versus* 42% (HR 0.42; CI 99.8% 0.25-0.73), with an ORR rate of 40% *versus* 14% and PFS of 5.1 *versus* 2.2 months, favoring treatment with nivolumab.[22] The CheckMate 037 study was designed for patients after failure in the previous treatments (including anti-CTLA-4 and BRAF inhibitors),

and randomized 405 patients at 2:1 between nivolumab and chemotherapy (dacarbazinae or carboplatin with paclitaxel). The data were published after the interim analysis, with an OS increase over one year from 42% to 79% (HR 0.42; IC 99.8% 0.25-0.73) and an ORR rate from 32% to 47% in favor of the experimental arm. [23]

Anti-CTLA-4 antibody

Ipilimumab is an anti-CTLA-4 monoclonal antibody and was the first immune checkpoint inhibitor to be approved for clinical use. However, with the advent of anti-PD-1 therapies, ipilimumab has been losing its spotlight position due to its unfavorable toxicity profile and lower antitumoral activity. In two phase III studies, ipilimumab revealed a significant OS increase, which was associated with a benefit plateau after three years of treatment.[24-26]

The first published study randomized 676 patients after failure in the previous treatment to received ipilimumab monotherapy, ipilimumab associated with gp100 vaccine, or gp100 in monotherapy. A statistically significant benefit was reported in the sub-groups treated with ipilimumab: the OS rates in 24 months were 24%, 22%, and 14%, with ORR rates of 10.9%, 5.7%, and 1.5%, respectively.[24] The second study randomized 502 patients who had never received prior treatment into two groups: dacarbazine with ipilimumab and dacarbazine with placebo. The results were favorable to the ipilimumab arm, the median OS was 11.2 *versus* 9.1 months, with OS in a year of 47% *versus* 36% and OS in five years of 18% *versus* 9%. Although only a minority of patients has presented a complete response (CR), such responses appear to have been long-lasting in many of them.[25,26] Ipilimumab was studied at different doses. However, the approved dose for clinical use was based on the phase III study, with 3 mg/kg every three weeks for four doses.[24]

Ipilimumab was also approved in the USA as an adjuvant treatment of high-risk stage III melanoma, based on the results of a study that randomized 951 patients between placebo and ipilimumab at 10 mg/kg for four doses every three weeks, continuing with monthly applications for up to three years. This study revealed significant benefits in the relapse-free survival, with a relapse median of 26 *versus* 17 months, and a three-year relapse rate of 46.5% versus 34.8%. However, the treatment was associated with an expressive toxicity profile with 90% of the patients presenting immune-related adverse effects, including 42% of degree 3 to 4 events, in addition to five deaths related to the drug.[27] Data on this strategy's benefit in global survival, benefit equivalence with an ipilimumab dose reduction to 3 mg/kg (as is the case of the approved dose for advanced disease treatment) or the direct comparison with adjuvant therapy, with high interferon doses being actively researched.

Combination therapy and sequential treatment

The anti-PD-1 and anti-CTLA-4 block combination exhibit an increase in the tumoral activity. However, this strategy expressively increased the toxicity associated with the treatment.[28] In addition; data on the benefits to overall survival with this approach have not yet been established. Therefore, phase III definitive results are required to determine if the combination may become the new treatment standard.

The most solid results obtained so far, for a combined block in advanced melanoma, were obtained in the double-blind phase III CheckMate 067, in which 945 patients who had not received prior treatment were randomized to receive nivolumab at 1 mg/kg associated with ipilimumab 3 mg/kg every three weeks for four doses, followed by nivolumab 3 mg/kg every two weeks; nivolumab 3 mg/kg every two weeks, or ipilimumab 3 mg/kg every three weeks for four doses. Partial results, with a median follow-up of 21 months, have been published, and the PFS median for the combination of monodrug nivolumab and monodrug ipilimumab was 11.5, 6.9, and 2.9 months, with a response rate of 58%, 44%, and 19%, and a degree 3 or 4 toxicity of 55%, 16%, and 27%, respectively.[29]

Data on sequential blockage with anti-CTLA-4 and anti-PD-1 were obtained with a phase II study involving 140 patients, in which the induction scheme with nivolumab, 3 mg/kg every two weeks during six applications, followed by ipilimumab 3 mg/kg every three weeks for four cycles, or the reverse sequence, were compared. After this induction scheme, both the cohorts received nivolumab at 3 mg/kg every two weeks up to the onset of disease progression. The frequency of grade 3 to 5 adverse events was higher in the nivolumab-ipilimumab group, compared to ipilimumab-nivolumab (50% *versus* 43%). With a median follow-up of 18.6 months, the response rate was higher in the nivolumab-ipilimumab sequence, 41% versus 20%.[30] Apparently, this strategy does not seem to be less toxic or more powerful than the combined scheme.

FACTORS PREDICTING RESPONSE

The new immunotherapies have brought important advances in the treatment of patients with melanoma or other neoplasms in advanced stages. However, these treatments were associated with potentially severe side effects, with a high financial cost, and not all patients will present antitumoral response with clinical benefits. Therefore, an issue to be resolved is the selection of patients with a higher chance of obtaining gains with these new therapies.

The most extensively explored response biomarker candidate for immune *checkpoint* inhibitor is the PD-L1 immuno-histochemical expression. As previously mentioned, the interaction between PD-L1 and the PD-1 membrane receptor – present in T-lymphocytes and in other immune system cells – is responsible for limiting both the initiation phase and the effector phase of the immune response, given that PD-L1 is constitutively expressed in the APC and in the healthy cells of the organism. Therefore, the PD-1/PD-L1 complex is part of the normal immunological tolerance process for inhibiting autoimmunity, but may also be involved in the cancer immunological resistance when there is PD-L1 hyperexpression by the neoplastic cells. However, the interaction between the tumor and the immune system also involves other mechanisms that are not fully understood, and the absence of TIL, observed in some tumors, seems to be a reflection of this process, because it represents a tumor with worse prognosis and better resistance to the immunological attack.[31]

Several methodological issues are involved in the PD-L1 analysis as a biomarker, from the choice of tissue to be evaluated or the antibody used in marking the definition of positivity crite-

ria. The lack of PD-L1 expression appeared to predict the absence of response in a preliminary phase I study, which evaluated the anti-PD-1 treatment with nivolumab in different types of neoplasm.[32] However, subsequent studies were not capable of determining an expression level from which the patients no longer present benefits with immune *checkpoints* inhibitors.[23,33] Other possible response biomarkers are also being studied, as is the case of the tumoral load quantification or the DNA repair enzyme deficiency (for generating genetic instability and increasing the tumoral load); however, neither of these tests has presented definite results thus far. These tests should not be used to exclude potential candidates to undergo immunotherapies.

ASSESSMENT OF IMMUNOTHERAPY RESPONSE

The action mechanism of the chemotherapy agents is the direct cytotoxicity to neoplastic cells, and the treatment response may be measured by the tumoral volume reduction within a few weeks after its administration. However, the antitumoral effect of immunological therapies includes more sophisticated cell death induction mechanisms, and it involves the recruitment of different cells in the immune system. These cells infiltrated in the tumoral microenvironment are directly or indirectly responsible for the cytotoxicity effect. In addition, these cells may acquire the ability to perpetuate the antitumoral response, even after exposure to immunotherapy has been discontinued. Thus, the criteria used to evaluate the response of cytotoxic agents – such as RECIST – may not be capable of correctly interpreting the benefit generated with *checkpoint* inhibitors or other immunological agents.[34,35]

Some response patterns generated with immunotherapy may be expressively different from those observed with cytotoxic agents. Therefore, some patients may present significant clinical benefits without meeting objective response criteria, and they may stabilize the disease for long periods. An expressive tumoral regression – including complete response – may be reached from a slow, but progressive, reduction of the neoplasm. This improvement may be maintained even after the treatment has been discontinued. In addition, in certa*in situ*ations, a temporary and initial increase in the disease – even with the appearance of new lesions – may be observed before the response to the treatment is established. This "pseudoprogression" may be explained by the worsening of the disease prior to the start of drug action. It may also be caused by the infiltration of inflammatory cells in the tumoral tissue, accompanied by edema or not, which makes lesions more easily identifiable in image tests. In this scenario, it is important to avoid early therapy interruption. However, a rapid progression scenario or the presence of clinical deterioration normally indicates a primary resistance to treatment.[34]

The immune response criteria were proposed in the attempt to standardize the interpretation of image tests after the new immunological treatments.[34] Thus, the concept of pseudoprogression was considered to be a form of response, and the measurement of lesions that appeared after the start of treatment – which, according to RECIST or to the World Health Organization criteria, define the disease progression – is now added to the measurement of the target lesions in calculating the "tumoral load". According to the associated immune response criteria, the increase of at least 25% in the tumoral load defines disease progression; the reduction of 50% or more is considered a partial response; an intermediate variation in the tumoral load is classified as a stable disease; and the complete resolution of all lesions is understood as the complete response.[34-36]

CONCLUSION

The different types of treatment that act based on immune system modulation to fight cancer are not a totally new concept. However, the development of strategies capable of generating a more specific response against neoplastic cells, with lower toxicity to the organism, ensure these new strategies earn a growing importance among the different oncological treatment options. A more detailed understanding of the specificities of new immunotherapies is important for all medical specialties involved in melanoma treatment, as these drugs have their unique action mechanisms, with unusual response patterns and toxicity profiles, when compared to traditional cytotoxic drugs. The selection of patients that are candidates to the treatment should be a responsibility shared by all participants in this multidisciplinary team. ❑

REFERENCES

1. Colley WB. The treatment of malignant tumors by repeated inoculations of erysipelas: with a report of ten original cases. 1893. Clin Orthop Relat Res. 1991;262:3-11.
2. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature. 2011;480:480-9.
3. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. Cell. 2011;144:646-74.
4. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science. 2011;331:1565-70.
5. Campesato LF, Barroso-Sousa R, Jimenez L, Correa BR, Sabbaga J, Hoff PM, et al. Comprehensive cancer-gene panels can be used to estimate mutational load and predict clinical benefit to PD-1 blockade in clinical practice. Oncotarget. 2015;6:34221-7.
6. Mahmoud SM, Paish EC, Powe DG, Macmillan RD, Grainge MJ, Lee AH, et al. Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol. 2011;29:1949-55.
7. Ohri CM, Shikotra A, Green RH, Waller DA, Bradding P.. Macrophages within NSCLC tumour islets are predominantly of a cytotoxic M1 phenotype associated with extended survival. Eur Respir J. 2009;33:118-26.
8. Steidl C, Lee T, Shah SP, Farinha P, Han G, Nayar T, et al. Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. N Engl J Med. 2010;362:875-85.
9. Rosenberg SA, Yang JC, Topalian SL, Schwartzentruber DJ, Weber JS, Parkinson DR, et al. Treatment of 283 consecutive patients with metastatic melanoma or renal cell cancer using high-dose bolus interleukin 2. JAMA. 1994;271:907-13.
10. Mocellin S, Pasquali S, Rossi CR, Nitti D.. Interferon alpha adjuvant therapy in patients with high-risk melanoma: a systematic review and meta-analysis. J Natl Cancer Inst. 2010;102:493-501.
11. Brentjens RJ, Davila ML, Riviere I, Park J, Wang X, Cowell LG, et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Sci Transl Med. Sci Transl Med. 2013;5:177ra38.
12. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med. 2013;368:1509-18.
13. Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res. 2011;17:4550-7
14. Andtbacka RH, Kaufman HL, Collichio F, Amatruda T, Senzer N, Chesney J, et al. Talimogene Laherparepvec Improves Durable Response Rate in Patients With Advanced Melanoma. J Clin Oncol. 2015;33:2780-8.
15. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363:411-22.
16. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med. 2013;369:134-44.
17. Robert C, Ribas A, Wolchok JD, Hodi FS, Hamid O, Kefford R, et al. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. Lancet. 2014;384:1109-17.
18. Ribas A, Hamid O, Daud A, Hodi FS, Wolchok JD, Kefford R et al. Association of Pembrolizumab With Tumor Response and Survival Among Patients With Advanced Melanoma. JAMA. 2016;315:1600-9.
19. Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, Robert C, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. Lancet Oncol. 2015;16:908-18.
20. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. N Engl J Med. 2015;372:2521-32.
21. Topalian SL, Sznol M, McDermott DF, Kluger HM, Carvajal RD, Sharfman WH, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. J Clin Oncol. 2014;32:1020-30.
22. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med. 2015;372:320-30.
23. Weber JS, D'Angelo SP, Minor D, Hodi FS, Gutzmer R, Neyns B, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. Lancet Oncol. 2015;16:375-84.
24. 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;363:711-23.
25. Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011 Jun 30;364:2517-26.
26. Maio M, Grob JJ, Aamdal S, Bondarenko I, Robert C, Thomas L, et al. Five-year survival rates for treatment-naive patients with advanced melanoma who received ipilimumab plus dacarbazine in a phase III trial. J Clin Oncol. 2015;33:1191-6.
27. Eggermont AM, Chiarion-Sileni V, Grob JJ, Dummer R, Wolchok JD, Schmidt H, et al. Adjuvant ipilimumab versus placebo after complete resection of high-risk stage III melanoma (EORTC 18071): a randomised, double-blind, phase 3 trial. Lancet Oncol. 2015;16:522-30.
28. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N Engl J Med. 2015;373:23-34.
29. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob JJ, Cowey CL, et al. Updated results from a phase III trial of nivolumab (NIVO) combined with ipilimumab (IPI) in treatment-naive patients (pts) with advanced melanoma (MEL) (CheckMate 067). American Society of Clinical Oncology Annual Meeting. Chicago; 2016. J Clin Oncol. 2016;34:9505-9505.
30. Weber JS, Gibney G, Sullivan RJ, Sosman JA, Slingluff CL Jr, Lawrence DP, et al. Sequential administration of nivolumab and ipilimumab with a planned switch in patients with advanced melanoma (CheckMate 064): an open-label, randomised, phase 2 trial. Lancet Oncol. 2016;17:943-55.
31. Sznol M, Chen L. Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer. Clin Cancer Res. 2013 Mar 1;19:1021-34.
32. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366:2443-54
33. Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WE, Poddubskaya E, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. N Engl J Med. 2015;373:123-35.
34. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbé C, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res. 2009;15:7412-20.
35. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45:228-47.
36. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer. 1981;47:207-14.

Mailing address:
Rudinei Diogo Marques Linck
Rua Dona Adma Jafet, 91
Bela Vista
01308-050 São Paulo, SP
Brazil
E-mail: rudineilinck@hotmail.com

How to cite this article: Linck RD, Costa RL de P, Garicochea B. Cancer immunology and immunotherapy of melanoma. An Bras Dermatol. 2017;92(6): 830-5.