The incidence of melanoma has been increasing for several decades, and metastatic melanoma patients still have a poor prognosis. However, promising therapeutic approaches have recently been developed for the treatment of these patients. One of such strategies relies on the use of mitogen-activate protein kinase (MAPK) inhibitors targeting the BRAF/MEK/ERK pathway, which is constitutively activated in a majority of melanomas. Two potent BRAF inhibitors, vemurafenib and dabrafenib, have recently been licensed by the US Food and Drug Administration (FDA), representing a breakthrough in the clinical management of melanoma patients. These inhibitors are specific for mutated variants of BRAF, which are expressed in ~65% of melanomas.1 BRAF inhibitors induce an objective response in 70% of patients.2 However, despite rapid and spectacular clinical responses, melanoma patients on BRAF inhibitors typically progress after a median of 5–7 mo from the initiation of therapy. Multiple mechanisms have been identified that may underpin the ability of melanoma cells to become resistant to BRAF inhibitors, including the reactivation of downstream signal transducers such as MEK. In line with this notion, the FDA has recently approved the association of dabrafenib and trametinib (a MEK inhibitor) for use in melanoma patients.

A second innovative approach for the treatment of metastatic melanoma patients relies on the use of monoclonal antibodies (mAbs) targeting the key regulators of the immune checkpoints that inhibit T-cell activation, including cytotoxic T lymphocyte-associated protein 4 (CTLA4), programmed cell death 1 (PDCD1, best known as PD-1) and its major ligand, i.e., CD274 (best known as PD-L1). Anti-CTLA4 (e.g., ipilimumab) and anti-PD-1/PD-L1 (e.g., nivolumab) mAbs induce lower response rates than BRAF inhibitors, but such responses are generally durable. Of note, the survival benefits conveyed by these agents are sometimes limited by autoimmune reactions (e.g., colitis, dermatitis, hepatitis, and endocrinopathies).3,4 Combinatorial therapies based on BRAF inhibitors and immunomodulatory mAbs are currently being evaluated in clinical trials. The clinical effects of immune checkpoint blockers as well as numerous experimental arguments indicate that a tumor-specific immune response is elicited in melanoma patients. Therefore, a better understanding of the molecular and cellular mechanisms whereby antitumor immunity is established is crucial for the development of efficient immunotherapeutic strategies targeting malignant cells and/or their microenvironment (Fig. 1).

As a central component of the innate immune system, natural killer (NK) cells mediate spontaneous cytotoxic effects against tumor cells, hence representing a suitable candidate for the development of novel immunotherapeutic approaches. This is particularly true in the context of melanoma, since these cells express a large panel of ligands for activating and co-stimulatory NK-cell receptors.5

We have shown not only that NK cells infiltrate primary neoplastic
lesions in metastatic melanoma patients, but also that circulating NK cells exhibit functional alterations starting from early disease stages. These findings indicate that NK cells are involved in the pathogenesis of melanoma throughout all stages of disease. Moreover, we identified a positive correlation between the expression levels of natural cytotoxicity triggering receptor 1 (NCR1, best known as NKp46) on the surface of circulating NK cells and the duration of stage IV disease.6

We next characterized the NK cells that infiltrate diseased regional lymph nodes in metastatic melanoma patients, as this represents not only the most frequent and early site of dissemination but also an important immune system headquarter, especially for the differentiation and maturation of NK cells. We described a novel subset of CD56brightCD16+ NK cells infiltrating regional metastatic lymph nodes.7 CD56brightCD16+ NK cells are characterized by increased expression levels of various NCRs, killer cell lectin-like receptor subfamily K, member 1 (KLRK1, best known as NKG2D) and killer immunoglobulin-like receptors (KIRs) than both their CD56dimCD16+ nodal counterparts and circulating NK cells.6,8 Of note, we failed to detect CD56dimCD16+ NK cells within metastatic lymph nodes. Functionally, CD56brightCD16+ and CD56dimCD16+ nodal NK cells displayed a comparable (relatively low) degranulation potential upon exposure to K562 cells, but the former cells exhibited increased perforin levels and were able to
mediate antibody-dependent cell-mediated cytotoxicity (ADCC). Furthermore, we observed that immunoselected nodal NK cells activated with interleukin (IL)-2 and IL-15 efficiently killed metastatic melanoma cell lines independently of the percentage of cancer cells invading the lymph node. Of note, such cytokine-activated nodal NK cells killed allogeneic melanoma cells more rapidly and more efficiently than their circulating counterparts. Nodal CD56brightCD16+ NK cells may originate from the maturation of resident CD56brightCD16dim NK cells that become activated upon the infiltration of malignant cells. Alternatively, circulating CD56brightCD62L+ NK cells may become activated upon the infiltration of metastatic lymph nodes and upregulate amount of mature CD56. The presence of a significant proportion of CD16+KIT+ cells appears to favor this hypothesis. However, further experiments are required to precisely characterize these NK cells and determine whether they can be found in the lymph nodes of patients affected by other metastatic cancers (e.g., breast carcinoma).

Our data suggest that targeting nodal NK cells may constitute an attractive therapeutic option, in particular for patients in which melanoma has spread to sentinel lymph nodes, who may benefit from adjuvant NK-based treatments (Fig. 1). Several exciting possibilities are emerging to optimally activate NK cells in vivo, hence circumventing the need for adoptive transfer. These include the administration of cytokines (e.g., IL-2, IL-15) or immunocytokines (mAb-cytokine fusions) that stimulate the maturation of nodal NK cells, as well as the use of mAbs that block KIRs or killer cell lectin-like receptor subfamily C, member 1-like (KLRCl, best known as NKG2A), or trigger activating receptors (NKp46), hence boosting NK-cell cytotoxicity. A further option is provided by bispecific mAbs that simultaneously engage NK cells (through CD16 or NCR) and tumor-associated antigens to trigger ADCC at the tumor site.

Recently, it has been shown that beside exerting antineoplastic effects via cancer cell-intrinsic circuitries, kinase inhibitors may sensitize cancer cells to the attack of the immune system.9,10 We have preliminary data showing that MAPK inhibitors modulate the immunogenicity of melanoma cells and favor their efficient lysis by IL-15-activated NK cells. These findings may represent a solid argument for combining MAPK inhibitors with NK-based immunotherapy to induce long-lasting clinical responses in melanoma patients.

Disclosure of Potential Conflicts of Interest

AC declares a research contract with Roche to assess the impact of PLX4032 on melanoma cell immunogenicity.

References

1. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Wolfendin H, Garnett MJ, Bottomley W, et al. Mutations of the BRAF gene in human cancer. Nature 2002; 417:949-54; PMID:12068308; http://dx.doi.org/10.1038/nature00766
2. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Souman JA, O’Dwyer PJ, Lee RJ, Grippa JF, Nolop K, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med 2010; 363:809-19; PMID:20818844; http://dx.doi.org/10.1056/NEJMoa1002011
3. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Souman JA, Atkins MB, et al. Safety, activity, and immune correlates of anti-CD152 antibody in cancer. N Engl J Med 2012; 366:2433-43; PMID:22658127; http://dx.doi.org/10.1056/NEJMoa1206890
4. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010; 363:711-23; PMID:20529992; http://dx.doi.org/10.1056/NEJMoa1003466
5. Fregni G, Perier A, Avril MF, Caigard A. NK cells sense tumors, course of disease and treatments: Consequences for NK-based therapies. Oncoimmunology 2012; 1:38-47; PMID:22720210; http://dx.doi.org/10.4161/onci.1.1.18312
6. Fregni G, Messaoudene M, Fourmentraux-Neves E, Mazouz-Dorval S, Chanal J, Maubec E, Marinho E, Scheer-Senyarich I, Cremer I, Avril MF, et al. Phenotypic and functional characteristics of blood natural killer cells from melanoma patients at different clinical stages. PLoS One 2013; 8:e76928; PMID:24204708; http://dx.doi.org/10.1371/journal.pone.0076928
7. Messaoudene M, Fregni G, Fourmentraux-Neves E, Chanal J, Maubec E, Mazouz-Dorval S, Coutraud B, Girard A, Sastre-Garau X, Albert S, et al. Mature Natural Killer Cells Can Infiltrate Lymph Nodes Adjacent to Metastatic Melanoma. Cancer Res 2014; 74:81-92; PMID:24225017; http://dx.doi.org/10.1158/0008-5472.CAN-13-1303
8. Perier A, Fregni G, Wittebeek S, Gad S, Allard M, Gervois N, Escudier B, Azzarone B, Caigard A. Mutations of the von Hippel-Lindau gene confer increased susceptibility to natural killer cells of clear-cell renal cell carcinoma. Oncogene 2011; 30:2622-32; PMID:21258414; http://dx.doi.org/10.1038/onc.2010.638
9. Begley J, Ribas A. Targeted therapies to improve tumor immunotherapy. Clin Cancer Res 2008; 14:4385-91; PMID:18628452; http://dx.doi.org/10.1158/1078-0432.CCR-07-4804
10. Knight DA, Ngjio SF, Li M, Parmenter T, Mok S, Cass A, Haynes NM, Kintzios K, Yagita H, Koya RC, et al. Host immunity contributes to the anti-melanoma activity of BRAF inhibitors. J Clin Invest 2013; 123:1371-81; PMID:23454771; http://dx.doi.org/10.1172/JCI66236