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When unity makes strength
Combinatorial NK cell-based immunotherapies against melanoma

Meriem Messaoudene[1], Marie-Françoise Avril[1,2], and Anne Caignard[1,*]

[1]Institut Cochin, INSERM U1016; CNRS UMR 8104; Université Paris Descartes; Paris, France;
[2]APHP, University Paris Descartes; Department of Dermatology; Cochin Hospital; Paris, France

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Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; KIR, killer immunoglobulin-like receptor; MAPK, mitogen-activated protein kinase; NCR, natural cytotoxicity receptor; NK, natural killer

In metastatic melanoma patients, circulating natural killer (NK) cells display phenotypic and functional alterations that appear to correlate with the duration of stage IV disease. Moreover, specific NK cell subsets that exhibit robust tumoricidal functions upon activation by cytokines infiltrate the diseased lymph nodes of these patients. These data suggest that NK cells may be harnessed for the development of novel combinatorial immunotherapies against melanoma.

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 tramenib (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

*Correspondence to: Anne Caignard; Email: anne.caignard@inserm.fr
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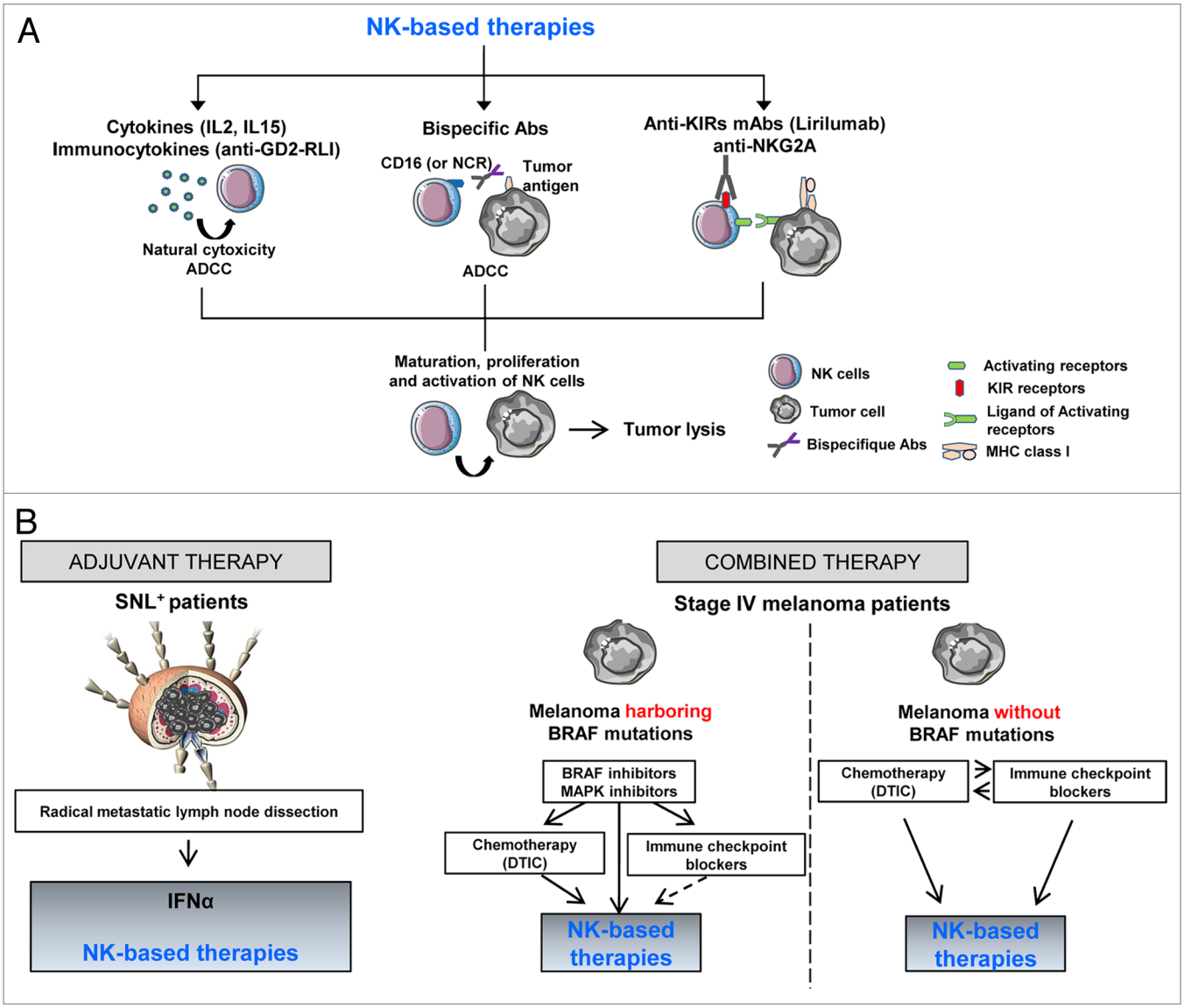

Figure 1. NK cell-based therapeutic possibilities for melanoma patients. (**A**) Different strategies for the activation of natural killer (NK) cells exerting robust antineoplastic effects against melanoma. (**B**) Possible NK cell-based immunotherapeutic approaches for melanoma patients at different stages of the disease. These strategies include the adjuvant treatment of sentinel lymph node (SLN)$^+$ patients with NK cell-based regimens as well as the use of NK cell-based approaches coupled to recently developed therapeutic paradigms in Stage IV patients. ADCC, antibody-dependent cell-mediated cytotoxicity; DTIC, dacarbazine; KIR, killer immunoglobulin-like receptor; IFNα, interferon α; IL, interleukin; mAb, monoclonal antibody; NCR, natural cytotoxicity receptor.

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, an activating receptor 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 $CD56^{bright}CD16^+$ NK cells infiltrating regional metastatic lymph nodes.[7] $CD56^{bright}CD16^+$ 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 $CD56^{bright}CD16^-$ nodal counterparts and circulating NK cells.[6,8] Of note, we failed to detect $CD56^{dim}CD16^+$ NK cells within metastatic lymph nodes. Functionally, $CD56^{bright}CD16^+$ and $CD56^{bright}CD16^-$ 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 $CD56^{bright}CD16^{+}$ NK cells may originate from the maturation of resident $CD56^{bright}CD16^{dim}$ NK cells that become activated upon the infiltration of malignant cells. Alternatively, circulating $CD56^{bright}CD62L^{+}$ NK cells may migrate to metastatic lymph nodes and upregulate CD56. The presence of a significant amount of mature $CD56^{bright}CD16^{+}NCR^{+}$ NK cells within metastatic lymph nodes coupled to a relatively low percentage 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 (KLRC1, 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.

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