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Emilie Narni-Mancinelli, Eric Vivier

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Emilie Narni-Mancinelli1 ·* and Eric Vivier1•2•3

1Aix Marseille Univ, CNRS, INSERM, CIML, Marseille, France
2Innate Pharma Research Laboratories, lnnate Pharma, Marseille, France
3APHM, Hôpital de la Timone, Marseille-lmmunopôle, Marseille, France
'Correspondence: narni@ciml.univ-mrs.fr

Natural killer (NK)-based therapies against cancer are emerging, but the understanding of NK cell functions needs to be completed to optimize these treatments. ln this issue, Pan et al. (2022) show that pro-apoptotic molecules, such as BH3-mimetics, synergize with NK cells to induce mitochondria-driven apoptosis in tumor cells, thereby enhancing the efficacy of NK cell therapies.

Cancer is a major healt11 problem, and cancer t11erapies have been intensively investigated over decades. Patients can benefit from surgery, radiotl1erapy, and systemic chemotherapy depending on cancer indication. Surgery, when possible, remains the most effective treatment of localized primary tumors and associated regional lymphatics. Advanced cancers require systemic cytotoxic chemotherapy. However, the side effects of these treatments limit their application and effi-cacy. Cancer patients can also benefit from targeted therapies, such as mono-clonal antibodies (mAbs) or small mole-cules, which can either deplete tumor cells or target the tumor cell growth signal cascade.

lmmuno-oncology is revolutionizing the treatment of cancers. Recent advances have focused on generating or unleashing tumor antigen-specific cytotoxic T cell re-sponses. However, resistance to treat-ment and toxicity are often reported. Thus, despite major advances in these therapies, several cancer conditions remain true unmet medical needs and prompt the generation of a second wave of immune-oncology treatments (Demaria et al., 2019). Along this line, bringing natu-ral killer (NK) cells to the clinic is very promising for a variety of reasons. NK cells can recognize a wide array of tumor cells across all cancer types and contribute to their elimination by direct cytotoxicity and by shaping a multicellular protective immune response via their secretion of cytokines and chernokines (Demaria et al., 2019; Myers and Miller, 2021). The presence of functional NK cells correlates with better overall survival in several blood and solid cancers. Therefore, harnessing NK cells to fight cancer is being evaluated in clinic using mAbs or cell therapies. lmportantly, NK-cell treatments appear saler than T cell treat-rnents (Daher and Rezvani, 2021).

ln this issue of Cel/, Pan and colleagues sought to improve antiturnor NK cell activ-ity by making tumor cells more sensitive to NK-cell-induced death (Pan et al., 2022). There are several forms of cell death: apoptosis, necrosis, pyroptosis, and ferroptosis. lt has been known for years that NK cells induce the apoptosis of tumor cells. Two pathways can trigger apoptosis: the extrinsic pathway induced by engagement of the death receptors such as TRAIL and Fas, and the intrinsic pathway involving the mitochondria. Pan et al. discovered that the NK-cell-medi-ated killing mechanism requires the mito-chondrial-mediated apoptosis pathway within the tumor cell (Pan et al., 2022). This mechanism occurs at a low effector:-target ratio, a condition that is likely found in patients where NK cells are outnum-bered by cancer cells.

Apoptosis via mitochondria is regulated by the pro-and anti-apoptotic proteins of the BCL-2 family. Anti-apoptotic proteins (BCL-2, BCL-w, MCL-1, BFL-1, and BCL-XJ prevent apoptosis by binding and

sequestering their pro-apoptotic counterparts (PUMA, BIM, BIO, BAD, BIK, NOXA, and BMF) (Singh et al., 2019). A subset of BH3 (BCL-2 homology domain 3)-only proteins, including BIM and BIO, activate the effectors of apoptosis (BAX and BAK), which lead to mitochondrial outer membrane permea-bilization (MOMP). MOMP is recognized as the point of no return in programmed cell death and causes the release of cyto-chrome c into the cytosol, resulting in the formation of the apoptosome and, ulti-mately, activating the caspase cascade (caspases 9/7 /3). A cell that expresses just enough pro-survival proteins to contain pro-apoptotic signais is consid-ered "primed" for apoptosis. Consistent with the importance of mitochondrial apoptosis in the regulation of NK cell-mediated killing of tumor cells, Pan and coworkers showed in t11eir model that the silencing of the NK cell cytolytic mole-cule granzyme B impaired NK-cell-medi-ated tumor cell killing. Granzyme B has indeed been reported to induce mito-chondrial apoptosis through the activa-tion of BIO (Lord et al., 2003). However, the contribution of NK cell cytokines, such as IFN-y, in the induction of mito-chondrial apoptosis in cancer cells re-mains to be analyzed.

ln the search of novel antitumor thera-pies, efforts have been made to identify cytotoxic molecules that directly act on BCL-2 protein family, such as the BH3 mimetics (Singh et al., 2019). These are small molecules that disrupt the interac-tion of the BH3 domain pro-apoptotic molecules with BCL-2 proteins, thus limiting cell survival. The authors showed that NK cell treatment com-bined with low doses of BH3 mimetics synergizes to reach the threshold for apoptosis and cause cell death. The dependence on specific pro-survival BCL-2 family proteins is specific to each type of tumor cell and can be determined via a BH3 profiling assay. Of note, BH3 mimetics sensitize tumor cells for NK-cell-mediated killing but do not promote the expression of activating ligands, such as the NKG2O ligand MICNB. The induction of other ligands for other activating receptors remain to be evaluated. lmportantly, NK cell pre-activation with interleukin-2 (IL-2) makes them less sensitive to BH3 mimetics, further supporting their com-bination for effective antitumor treat-ment. ln xenograft mouse models of blood and solid cancers, BH3 mimetics administration in combination with the transfer of activated NK cells signifi-cantly improve tumor cell control and mouse survival.

Several studies have reported that in-creases in the numbers of intratumoral and circulating NK cells are associated with favorable outcomes of treatment with anti-EGFR mAb (cetuximab) and anti-CD20 mAb (obinutuzumab and ritux-imab) (Myers and Miller, 2021). lndeed, a cell that is dependent on EGFR will upre-gulate pro-apoptotic BIM when EGFR signaling is inhibited, thus lowering the threshold for apoptosis (Deng et al., 2007). Pan and coworkers showed in their report that rituximab also pushes cells to-ward apoptosis (Pan et al., 2022). There-fore, NK-cell-mediated killing of primed tumor cells may be involved in the under-lying mechanism of anti-EGFR-mAb and anti-CD20-mAb therapies.

The findings of Pan et al. could be applied in a combinatorial way to improve NK-cell-based immunotherapies(Figure 1), for example, by combining BH3 mimetics with recent therapies that aim al unleash-ing NK cells with immune checkpoint inhib-itors, such as anti-NKG2A (monalizumab), anti-TIGIT (tiragolumab and ociperlimab), and anti-LAG3 (relatlimab) mAbs (Demaria et al., 2019). Alternatively, BH3 mimetics could be combined with recent therapeu-tics designed to promote NK cell activity via NK cell engagers, which bind NK cell activating receptors (CD16 and/or natural cytotoxic receptor) and a tumor antigen (Demaria et al., 2021). The benefit of combining these therapies remains to be tested.

Harnessing the potential of apoptosis as a strategic approach to kill cancer cells is not new. Many common types of anti-cancer therapies, such as chemotherapy and radiotherapy, engage the mitochon-drial apoptosis pathway (Singh et al., 2019). The approval of the BH3 mimetic BCL-2 selective inhibitor venetoclax in several malignant therapies is a milestone in targeting the apoptotic pathway for cancer treatment. Venetoclax provides an additive effect to the anti-CD38 mAb daratumumab for

NK-cell-mediated killing of multiple myeloma cell lines (Na-kamura et al., 2021). In general, cancer cells that are sensitive to these drugs are more prone to apoptosis priming than their non-transformed counterparts, thus creating a therapeutic window for tumor response to pro-apoptotic therapy (Singh et al., 2019). However, toxicity of these pro-apoptotic agents is a burning malter limiting their efficacy. This issue could be circumvented by reducing the dose of these treatments and combining them to NK cell therapies, sa that the level of apoptotic stress induced within a cancer cell is insufficient to trigger apoptosis but enough ta lower the threshold for apoptosis, thereby sensitizing tumor cells for an efficient NK-cell-mediated killing.

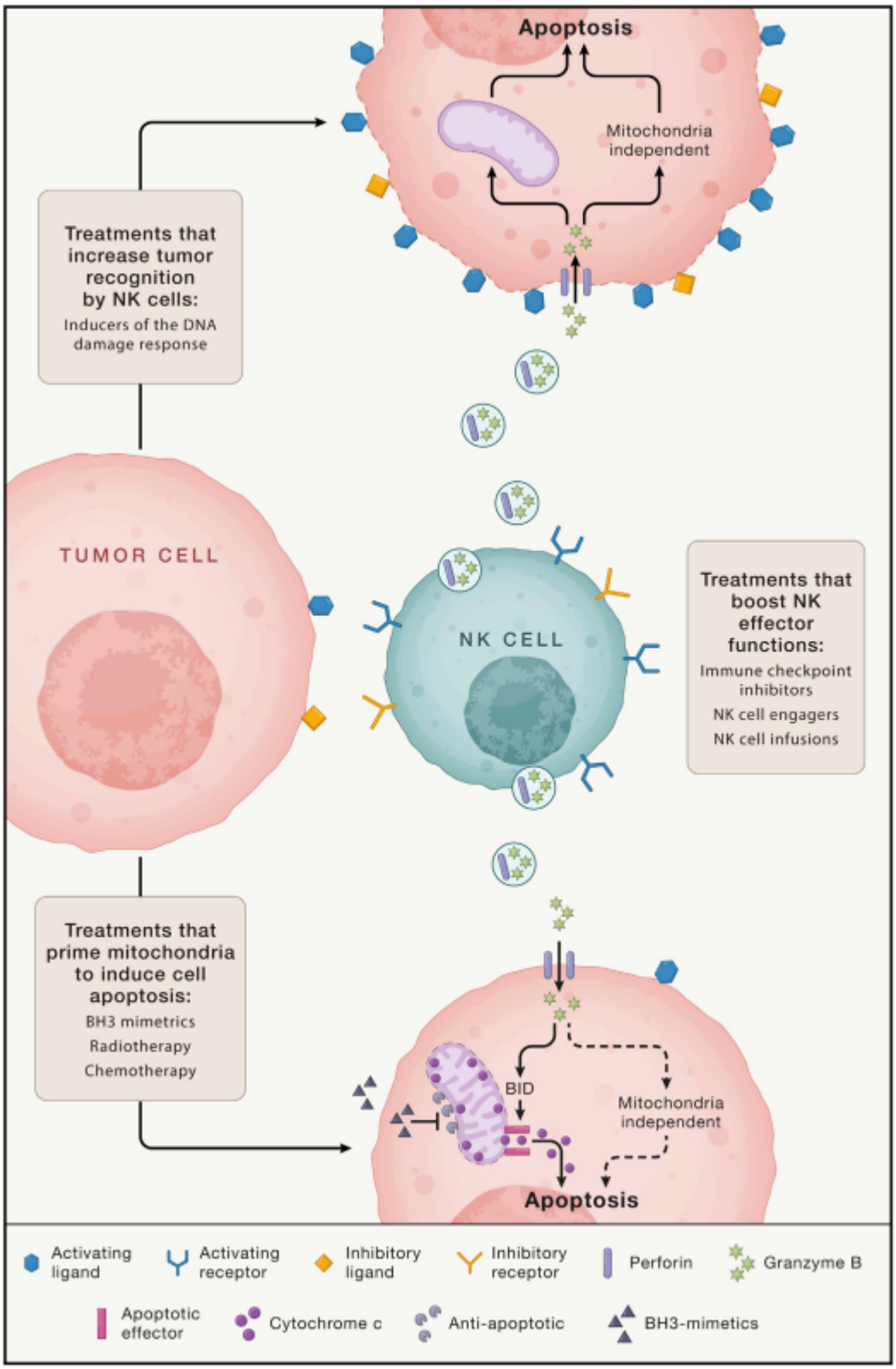

Figure 1. Sensitizing tumor cells to NK-cell-mediated killing

Drugs such as the inducers of the DNA damage response sensitize tumor cells to NK-cell-mecliated killing by increasing the amounts of ligands recognized by NK cell activating receptors (Ruscetti et al., 2018). Pro-apoptotic drugs, such as chemotherapy, radiotherapy, and BH3 mimetics, promote mitochondrial apoptosis. BH3 mimetics diffuse into tumor cells and inhibit specific anti-apoptotic BLC-2 protein family members. Activated NK cells can produce granzyme B that cleaves BIO, which activates the apoptotic ettectors. Bath events synergize and converge toward the MOMP, cytochrome c release, and programmed cell death. Treatments that sensitize tumor cells to NK-cell-mediated killing could thus improve NK-cell-based therapies, including NK cell infusions, or the boost of NK cell functions by immune check point inhibitors (ICIs) or NK cell engagers (NKCEs).

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DECLARATION OF INTERESTS

E. V. is an employee of lnnate Pharma.

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