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Review

The Engineering of Natural Killer Cells as an Emerging Adoptive Cancer Immunotherapy

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Cellular therapeutics is an emerging field with significant advances in the engineering of immune effector cells, which play a revolutionary role in treatment for cancer. Although most immunomodulatory strategies focus on enhancing T cells – which have proved their ability in successful cellular therapies against leukemia – this strategy may soon face competition. Through several preclinical studies, researchers have discovered new guardian immune cells called chimeric antigen receptor (CAR)-modified natural killer (NK) cells, which show cytotoxic activity against various solid tumor types. The preclinical evidence suggests that NK cells have the same cancer-homing receptors as T cells and but need to be genetically modified to recognize and kill targets. This can be achieved by introducing CARs into NK cells and cell lines. Scientists also face the challenge of properly manufacturing engineered NK cells. If successful, CAR NK cells could be safer, cheaper, easier to produce, and more widely applicable than T cells. This review focuses on recent advances in NK cell engineering and discusses how NK cells contribute to new immunotherapeutic approaches for treatment against refractory hematological malignancies.

INTRODUCTION

In the United States, approximately 5000 people die each year from bone and soft-tissue sarcomas, which are commonly called tumors (McCarthy, 2006). Although there are new chemotherapeutic drugs, radiotherapy, and innovative surgical techniques in the clinical arsenal, there is no definite cure for malignant tumors (Arruebo et al., 2011). Therefore, in an attempt to reduce death rates, potential treatment modalities are being investigated.

One of the most talked about and promising treatments is immunotherapy. The idea of immunotherapy was first developed back in 1891 when a surgical oncologist, William Coley, investigated how the body's immune system could be enhanced to attack malignant tumors. Coley injected more than 1000 of his cancer patients with heat-killed streptococcal organisms to cause erysipelas (a bacterial skin infection) to stimulate the body's "resisting powers" (McCarthy, 2006). The tumors disappeared, presumably because they were attacked by the improved immune system. Seeing that the results were positive, his approach caught on. In recent years, the most prominent immunomodulatory strategy has been using chimeric antigen receptor T-cell (CAR-T) immunotherapy, which has had striking complete remission (CR) rates as high as 90% in acute lymphoblastic leukemia (ALL).

Unfortunately, CAR-T cells have some significant limitations. One of the major hardships with the generation of an autologous CAR-T cell product is that it is derived from each patient individually, making it too difficult to scale for widespread clinical use. In fact, it takes a minimum of two to three weeks to manufacture CAR-T cells (Hay and Turtle, 2017). Therefore, for a patient in critical condition with a rapidly advancing disease, treatment with CAR-T cells would be impractical. Additionally, it is difficult to collect the required quantity of lymphocytes from patients to generate CAR-T cells. And, in the case of allogenic T cells, which are transported from a donor, they can cause graft-versus-host disease (GVHD) (Liu et al., 2017).

The newly discovered CAR NK cells are perhaps more promising than the CAR-T cells. NK cells are cytotoxic, or cell-killing, and kill their targets in a non-specific manner. This means NK cells don't have to recognize a specific antigen on viral-infected cells or cancer cells (Farag and Caligiuri, 2006: Locatelli et al., 2014). Consequently, this enhances their immunosurveillance. The NK cells decide whether to kill cells based on signals from activating and inhibitory receptors on the NK cell surface. While activating receptors 'switch on' the NK cell when recognizing cell-surface molecules expressed on cancer cells, inhibitory receptors 'switch off' the NK cell and prevent it from killing cells pos-

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sessing cognate major histocompatibility complex (MHC) I molecules (Orr and Lanier, 2010). Cancer cells and infected cells become vulnerable to NK cell killing because they often lose their MHC I. Once the decision to kill is made, cytotoxic granules are released by the NK cell, leading to lysis of the target cell (Topham and Hewitt, 2009). Unlike a CAR-T cell, a CAR NK cell does not carry the risk of GVHD, and, therefore, opens the doors for development of off-the-shelf allogeneic products that could be readily available for immediate clinical use to treat thousands of patients (Yoon et al., 2010; Moretta et al., 2011). Furthermore, since CAR NK cells can retain their full array of native receptors, they have a natural ability to identify and target cancer cells. This could reduce the risk of relapse due to a loss of CAR-targeted antigen, as noted in CAR-T treatments (Sotillo et al., 2015). This would ultimately make disease escape through downregulation of the CAR target antigen less likely.

NK BIOLOGY AND ADOPTIVE IMMUNITY

NK cells are called "natural" killers because they have the ability to kill cancer and virus-infected cells without prior sensitization, which is crucial for cancer immunotherapy. NK cells are primarily found in the blood, liver, and spleen, but can also be found in lymph nodes (Campbell et al., 2001; De Maria et al., 2011).

As described earlier, almost all NK cell functions – degranulation, cytokine release, and cytotoxicity – are governed by signals from activating receptors and inhibitory receptors. The main activating receptors include natural cytotoxicity receptors (NCRs) and C-type lectin-like activating immunoreceptors (NKG2D2), while the main inhibitory receptors include killer Ig-like receptors (KIRs) and heterodimeric C-type lectin receptors (NKG2A). Inhibitory receptors play a crucial role in ensuring that NK cells do not aberrantly activate against normal tissues, a mechanism referred to as "self-tolerance". For example, inhibitory KIRs (iKIRs) by human leukocyte antigen (HLA) class I molecules transmit an inhibitory signal to block NK cell triggering during effector responses. However, infected cells lack HLA class I molecules (a concept called "missing self"), which means NK cells will not receive any inhibitory signal (Campbell and Hasagawa, 2013). Instead, cellular stress and DNA damage increase the regulation of "stress ligands," activating NK receptors and signaling the NK cell to kill the target (Bradley et al., 1998; Campbell and Hasagawa, 2013).

There are various mechanisms of NK-mediated cytotoxicity. NK cells can directly kill tumor cells by releasing cytoplasmic granules containing perforin and granzyme, which prompt tumor cell lysis (Bradley et al., 1998; Screpanti et al., 2001). Alternatively, NK cells can express tumor necrosis factor (TNF) family members like FasL and TNF-related apoptosis-inducing ligand (TRAIL), which induce tumor cell apoptosis. Moreover, some NK cells contain the Fc receptor CD16 that induces degranulation against antibody-covered tumor cells, resulting in antibody-dependent cellular cytotoxicity (ADCC) (Farag and Caligiuri, 2006).

ADOPTIVE TRANSFER OF NK CELLS TO TARGET TUMORS

The ability of NK cells to exert rapid cytotoxicity against various hematologic malignancies such as acute myeloid leukemia (AML) (Stringaris et al., 2013), ALL (Bachanova and Miller, 2014; Rouce et al., 2016), multiple myeloma (MM) (Swift et al., 2012), as well as many solid tumors including neuroblastoma, ovarian, colon, renal cell, and gastric carcinomas (Bachanova and Miller, 2014; Gras Navarro A et al., 2015) make them perfect for use in adoptive therapy. However, different tumors have developed different evasion strategies to protect themselves from NK cells. This evasion is achieved by maintaining high surface expression of HLA molecules to become invisible to NK cells (Rouce et al., 2016) or by lacking ligands that signal through activating NK cell receptors. Because of this, scientists have soughts strategies to enhance NK cell activity, one of which includes cytokines and artificial antigen-presenting cells (APCs) with enhanced costimulatory molecules as feeder cells for in vivo expansion. After incubation with cytokines, the NK cells gain the ability to kill tumors that are usually not sensitive to NK lysis. Scientists often combine this with monoclonal antibodies (mAb) to boost ADCC (Lin et al., 2008; Kanasawa et al., 2014; Romain et al., 2014).

In recent years, different groups of scientists have explored various methods of deriving functional NK cells for immunotherapy. Adoptive transfer of expanded, activated autologous NK cells, however, has not been very effective due to the inhibition of autologous NK cells by self-HLA molecules (Locatelli et al., 2014; Gras Navarro et al., 2015). Cells from an allogeneic source, on the other hand, have proven to be more promising for therapy. For example, as seen in preclinical studies using adoptively transferred haploidentical NK cells (NK cells from a half-matched donor used to replace damaged cells), alloreactive NK cells (cells that can recognize foreign (allogeneic) MHC molecules) can help create graft-versus-leukemia/tumor (GvL/GvT) effect while not contributing to GVHD (Ruggeri et al., 2002; Olson et al., 2010). Though the allogeneic NK cells are safe in patients with hematologic and solid tumors, they were only shown to be moderately effective in clinical activity (Yoon et al., 2010).

CHALLENGES

Despite the many advantages of NK cells, there is some hesitation to utilize NK cells for CAR-modified therapy due to questions about their ability to migrate to and penetrate tumor tissues. As a result, work has largely been limited to pre-clinical trials (Nayyar et al., 2019). Scientists are also rethinking the effects of the limited in vivo persistence of the NK cells because, while it increases the safety of the treatment, it may reduce its effectiveness. Although recent stud-

ies are proving to be more successful, there have been several impediments to the successful generation of CAR NK cells for clinical use. In the past, genetic engineering of NK cells, even with viral methods, reported <10% transduction efficiency (Mehta and Rezvani, 2018). The biggest challenge in CAR NK (and CAR T) cell engineering involves identifying appropriate target antigens that are pervasively expressed by tumor cells, but not expressed by normal tissue, thus limiting on-target off-tumor effects (Rezvani et al., 2017).

STUDIES WITH CAR-MODIFIED PRIMARY NK CELLS

There are many ways to derive functional NK cells for adoptive therapy. Expanded, activated cord blood (CB), or peripheral blood (PB)-derived NK cells have their own capabilities that play an important role in gene modification. For example, expanded, activated NK cells are known for expressing many activating receptors like CD16, NKG2D, and NCRs (Bi and Tian, 2017). NK cells have also shown to reduce tumor activity in studies with hematologic malignancies, such as AML. Furthermore, *ex vivo* NK cells produce a broader spectrum of cytokines including interferon (IFN)-γ, IL-3, and granulocyte macrophage colony-stimulating factor (GM-CSF), which is thought to reduce the risk of heart and kidney problems (Rezvani et al., 2017).

Most of the preclinical studies involving NK cells concentrated on targeting anti-CD19 and CD20-CARs in B cell malignancies (Imai et al., 2005; Li et al., 2010). The infusion of CD19-CAR-T cells following lymphodepletion has shown to be very positive in cases where the patient has relapsed or refractory CD19+ malignancies. However, results have not been so positive for cases where the patient has refractory Burkitt lymphoma (BL) (Rezvani et al., 2017). Scientists then began to target CD20+ aggressive B-cell non-Hodgkin lymphoma using anti-CD20 CAR mRNA-modified expanded natural killer cells in vitro and in NSG mice. The anti-CD20-4-1BB-CD3ζ CAR was then used in the gene modification of PB NK cells from a group of healthy donors. After activation with a K-562-based feeder cell line that expressed membrane-bound IL-15 and 4-1BB ligand (K562-mbIL15-41BBL), 50%–95% of the expanded PB NK cells expressed the CAR molecules. Moreover, they also displayed an enhanced in vitro cytolytic activity against rituximab-sensitive and resistant BL cells. Therefore, this also extended the survival of the Raji-xenografted mice models (Chu et al., 2015). But, in the clinical setting, these CAR molecules would likely need to be continuously infused several times due to its short-lived nature.

A recent study, however, has claimed to have found a new way to generate CAR-CD19+ NK cells that are not short-lived. The scientists genetically modified the CB-derived NK cells using a retroviral vector (iC9/CAR.19/IL15) with the gene for CAR CD19, allowing it to redirect specificity to CD19. The retroviral vector ectopically produced IL-15, a cytokine crucial for NK cell survival and proliferation, as well as expressed inducible caspase-9 (iC9), a suicide gene, that could be pharmacologically activated to eliminate transduced cells (Di Stasi et al., 2011). All these features equipped the NK cells with the genetic modifications needed to competently kill the B cell leukemia or lymphoma cells (Liu et al., 2018).

Throughout the different studies done with NK cells, various transduction strategies (most commonly using retrovirus or lentivirus-based vectors) have produced a broad spectrum of transduction efficiencies with reports ranging from 1% to 90% (Imai et al., 2005; Li et al., 2010). Lentiviral transduction is easily the most popular form of transduction because it has multiple additional benefits compared to retrovirus. For example, lentiviral transduction allows for transduction of primary, non-activated cells since it does not need actively dividing cells like retrovirus (Rezvani et al., 2017). Nonetheless, scientists are exploring other non-viral transduction methods like electroporation, which immediately expresses the CAR molecule by introducing CAR-encoding mRNA through pores. But, due to the fact that mRNA electroporation and single lentiviral transduction usually result in lower PB and umbilical CB-derived NK cell efficiencies (<10% and <30% respectively in a study), retroviral transduction may be more appropriate for gene modification of primary and CB NK cells. One way to solve this problem would be to express the CAR in induced pluripotent stem cells (iPSCs) that mature NK cells, as will be explained in the "Alternative Sources of NK Cells" section below (Hermanson and Kaufman, 2015).

STUDIES WITH CAR-MODIFIED NK CELL LINES

Most of the studies on NK cells have focused on the role of NK cell lines in the expression of CAR molecules, the most widely studied cell line being NK-92, which is a human cell line obtained from a patient with non-Hodgkin's Lymphoma (NHL). The specialty of NK-92 cells is that they are missing all inhibitory KIRs except KIR2DL4 (Tom et al., 2015), allowing in vitro activity against tumor targets. In fact, NK-92 cells have been administered in over 40 patients with advanced cancer, but their efficacy is not sufficient even though they can be infused multiple times (Tom et al., 2015). This has caused scientists to turn to CAR modification in hopes to increase antitumor activity in the cells.

NK-92 cell lines, for various reasons, are theoretically thought to aid more positive results when genetically modified over primary NK cells. First, NK-92 is a well-established cell line that has been reproduced and expanded repeatedly using good manufacturing practice (GMP)-compliant cryopreserved master cell banks and is plentiful in number for cancer therapy. Due to its potential, many scientists have genetically modified NK-92 cells to express CARs like CD19 and CD20, targeting hematologic and solid malignancies for B cell leukemia and lymphoma, CD38 and CS-1 for multiple

myeloma, and HER-2 for epithelial cancers. Another specialty of NK-92 cells is that they can be given to patients through intratumoral injections, which gives them the ability to traffic to tumor sites and produce a vaccine-like mechanism effect. Additionally, due to the uniformity of the cell line, NK-92 cells are more consistent with CAR expression, and their average transduction efficiency was around 50% (Boissel et al. , 2009; Boissel et al., 2013).

However, despite the fact that NK-92 cells have useful features like large-scale expansion and safety, they also have disadvantages. Some of the most important drawbacks are that NK-92 cells are potentially tumorigenic (since they have to be obtained from a patient with NHL), express multiple cytogenetic abnormalities, and have latent infections with Epstein-Barr virus (EBV) (Uphoff et al., 2010). Therefore, to ensure safety, these cells are irradiated at minimum 1000 cGy before clinical use, though this reduces their in vivo proliferation, persistence, and long-term antitumor efficacy (Uphoff et al., 2010). Moreover, though NK-92 cells have the ability to be repeatedly infused, continuous infusion may result in rapid rejection and cellular immunity against the allogeneic cell line.

ALTERNATIVE SOURCES OF NK CELLS

Another source where NK cells usable for CAR expression can be extracted is from human pluripotent stem cells (HPSCs), since both human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) produce a limitless number of NK cells. Lowe et al. developed a strategy for the differentiation of NK cells from CD34+ human HPSCs isolated from cryopreserved CB, which were then modified to express CD19-CAR. They also described a platform to express other CAR molecules by using a feeder-free protocol for the generation of gene-modified NK cells from HPSCs using insulin-like growth factor 1 (Lowe et al., 2016).

CARS TARGETING ACTIVATING RECEPTORS OR OTHER NK CELL SIGNALING MOLECULES

The CAR-NK constructs that have been explained above all deal with the intracellular signaling chain CD3ζ, conferring specific cytotoxicity to surface-tumor antigens. An alternative strategy is developing CAR-NK cells that target ligands for activating NK receptors like NKG2D. The NKG2D ligands, major histocompatibility complex (MHC) class I chain-related A (MICA), MICB, and several UL-16-binding proteins (ULBPs) cover tumor and virally infected cells. This is the reason why an NKG2D CAR can identify almost all (90%) human tumor types and on immunosuppressive cells expressing NKG2D ligands, such as myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) (Chang et al., 2013). However, the ligands bring up the challenge of “on-target/off-tumor” toxicity, as they are produced during many physiological circumstances, such as inflammation. NKG2D CAR cells prompt enhanced cytotoxic activity because NKG2D cells lack signaling motifs, and the NKG2D CAR, when ligated, sends a signal via the phosphorylation of DNAX-activating protein 10 (DAP10), which in turn recruits downstream signaling effector molecules (Chang et al., 2013). In this study, the researchers wanted to see if supraphysiologic activating signals could enhance NK-mediated cytotoxicity, and co-expressed DAP10 with the NKG2D/CD3ζ CAR. They tested the activity of NK cells transduced with this CAR against multiple cell lines from various malignancies. Intriguingly, there were positive responses with all the cell lines, including osteosarcoma, prostate carcinoma, and rhabdomyosarcoma. However, this strategy resulted in the loss of activating ligands on few primary hematologic malignancies, ultimately affecting NKG2D-mediated cytotoxicity. The authors, surprisingly, did not find any correlation between the level of NKG2D ligand expression and NKG2D-DAP10-CD3ζ receptor-mediated cytotoxicity (Chang et al., 2013).

In another study, Topfer et al. wanted to identify a CAR that could activate NK cells using a different method, and incorporated DNAX-activation protein 12 (DAP12) and prostate stem cell antigen (PSCA) scFv (derived from the hybridoma 7F5) in primary NK cells and the NK cell line YTS (Topfer et al., 2015). While DAP12 is expressed in NK cells (as is in many activating receptors), the anti-PSCA-DAP12 CAR is expressed in primary NK cells as well as the YTS-NK cell line, and has the ability to lyse otherwise resistant PSCA+ HLA-B/C- and HLA-C-matched tumor cells. Interestingly though, the anti-PSCA-CD3ζ-based CAR did not enhance cytotoxicity as well as the YTS-NK CAR incorporating the DAP-12 signaling domain. This finding was crucial. To this date, it is the first to show that a single immunoreceptor tyrosine-based activation motif (ITAM)-containing DAP12-CAR has the ability to signal as effectively as a CD3ζ-based CAR containing three ITAMs. Additionally, this DAP12-signaling CAR did not need any extra costimulatory signaling molecules for in vitro activation and cytotoxicity (Topfer et al., 2015).

While CAR modification is an effective immunotherapy, engineered NK cells can also increase cell cytotoxicity by expressing cytokines. This strategy might be even more beneficial than CAR modification, as it increases NK cell persistence while also eliminating the need for a toxic in vivo cytokine supplementation. A major challenge is overcoming the risk of inducing CRS, cytokine-induced systemic toxicity, and malignant transformation in the transduced cells. Some scientists proposed the idea of temporarily introducing genes coding for IL-2 or IL-15 using short-lived expression models like mRNA electroporation to avoid toxicities.

SPECIAL CONSIDERATIONS FOR CLINICAL TRANSLATION OF CAR-NK CELL THERAPY

There have been clear positive results with both CAR-NK cells and a strong safety profile for non-genetically modified NK cells. However, there have only been two clinical trials of CAR-NK cell therapy with patients: NCT00995137 from St. Jude Children's Research Hospital and NCT01974479 from The National University Health System, Singapore. The two trials are using an identical second-generation anti-CD19 CAR with the 4-1BB costimulatory domain (anti-CD19-BB-ζ) to target refractory CD19+ ALL (Shimasaki et al., 2012). Though the Singapore trial is enrolling both children and adults, the St. Jude trial was only open to pediatric patients and is no longer accepting patients. This dose-escalation trial gives patients a single intravenous (i.v.) infusion of anti-CD19-BB-ζ NK cells at doses of 0.5 × 107 to 1 × 108 CD56+ cells/kg, and the clinical results are still being awaited.

In the last year, many more studies regarding CAR-NK cell therapy were conducted and registered on ClinicalTrials.gov. One example is PersonGen BioTherapeutics, which was given permission to administer sequential doses of third-generation (relevant scFv attached to TCRζ, CD28, and 4-1BB signaling domains) CAR-transduced NK-92 cells (on days 0, 3, and 5). The scientists in that group are focusing on targeting refractory CD7+ leukemia and lymphoma in adults (NCT02742727), CD33+ myeloid malignancies in children and adults (NCT02944162), refractory CD19+ ALL malignancies in patients undergoing hematopoietic stem cell transplantation (HSCT) (NCT02892695), and MUC1+ relapsed and refractory solid tumors (NCT02839954). Other scientists are working to determine the safety and efficacy levels of escalating doses of off-the-shelf CB-derived NK cells, which express iC9.CAR19.CD28-ζ-2A-IL-15 for relapsed or refractory B-lymphoid malignancies.

Undoubtedly, there are several challenges and questions that must be answered before CAR-NK therapy can be used to treat a larger number of patients. Although many trials have explained strategies for isolation, expansion, and transduction of NK cells, the generation of the cells is still lengthy and difficult. While retroviral constructs have greater efficacy than lentiviral constructs, they have a greater risk of contributing to the development of insertional mutagenesis, creating a regulatory hurdle. In order to avoid the risks of oncogene activation and insertional mutagenesis, several scientists have used electroporation. Unfortunately, the studies have reported a very low success rate, with transfection efficiencies as low as 10% (Boissel et al., 2009). Additionally, since CAR molecule expression usually lasts less than 7 days, it is likely to lower the long-term efficacy level of the CAR-NK cells (Zhao et al., 2010).

Another question that still needs to be studied is whether the infused allogeneic CAR-NK cells (CAR-NK cells from a donor, rather than from self) will be rejected, and if so, whether lymphodepletion will be necessary. The problem is that lymphodepleting chemotherapy will deplete other immunosuppressive cells within the tumor-like Tregs and MDSCs. This will hurt NK cell cytotoxicity and in vivo expansion. Moreover, due to some of the safety questions raised with infused CAR-modified T cells, scientists are looking into whether a suicide system (e.g., based on caspase-9 or thymidine kinase), or programmed cell death, will need to be incorporated (Zhao et al., 2010; Di Stasi et al., 2011; Zhou et al., 2015).

Despite the several preclinical trials done following CAR-NK and CAR-T cells, there are still many unanswered questions. These include whether repeated infusions could trigger immunogenicity, elicit human anti-mouse antibodies (HAMAs), or cause cellular-mediated rejection/sensitization in CAR-NK cells. The question of HAMA immunogenicity, however, has been explored in CAR-T cells, and has proven not to be a concern, likely due to the fact that many of the patients have received a single infusion of autologous cells.

Without doubt, in order to establish a safe and effective CAR-NK immunotherapy, further studies regarding the optimal vector, construct, and transduction method are needed.

CONCLUSION

We are in an exciting era in the field of cellular therapy, where many new ideas for cancer treatment are being explored. The theory of NK cell immunotherapy is one of the most promising strategies against refractory malignancies due to its high cytotoxicity. NK cells have proven to be significantly more diverse than once believed and have shown great potential in tumor control and immunosurveillance. Most importantly, NK cells hold great promise for the development of an off-the-shelf cellular product that could eliminate the need for a patient-specific diagnosis, making them readily available for immediate clinical use. Although much has been discovered, there are still numerous scientific questions and regulatory hurdles that must be addressed before NK cells can be extended to larger cohorts of patients. For example, it is very important to identify the ideal vector, signaling endodomain, and costimulatory molecule for NK cells that provides the best response and safety profile. In order to do this, different combinatorial techniques will have to be tested to improve the efficacy of tumor-specific NK cells. This will likely be done by harnessing the innate power of the NK cell, inhibiting or knocking out immune checkpoints, or by targeting the tumor microenvironment. Furthermore, additional gene editing techniques like CRISPR/Cas9 will have to be analyzed in the setting of NK cells. Targeted genome editing has shown to be more effective with CRISPR-Cas9 ribonucleoprotein (RNP) complexes using a nuclear localized signal (NLS)-tagged Cas9 when compared to Cas9 plasmid transfection. CRISPR-Cas9 RNPs substitute the need for plasmid transcription and translation, circumvent NK cell

sensitivity to DNA, and increase nuclear delivery (Riggan et al., 2020). Currently, only one clinical trial recruiting patients is exploring this strategy, although trials targeting immune checkpoints NY-ESO1 and PD-1 are in development (Poirot et al., 2015; Qasim et al., 2015; Qasim et al., 2017). Immune checkpoint inhibitors like anti-PD-1 could help control malignant melanoma (EbioMedicine, 2019). Without doubt, therapeutic strategies designed to leverage engineered NK cells will make a significant contribution to the recent paradigm shift in cancer treatment.

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