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**Corresponding Author:** In-San Kim

**Authors:** Yeonsun Hong<sup>1</sup>, In-San Kim<sup>1,2,\*</sup>

**Institution:** <sup>1</sup>Biomedical Research Institute, Korea Institute of Science and Technology (KIST), Seoul 02792, Republic of Korea,

<sup>2</sup>KU-KIST Graduate School of Converging Science and Technology, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul 02841, Republic of Korea,

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**Author's name:** Yeonsun Hong<sup>1</sup>, In-San Kim<sup>1,2\*</sup>

**Affiliation:**

<sup>1</sup>Biomedical Research Institute, Korea Institute of Science and Technology (KIST), Seoul 02792, Republic of Korea

<sup>2</sup>KU-KIST Graduate School of Converging Science and Technology, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul 02841, Republic of Korea

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**Corresponding Author's Information:** iskim14@kist.re.kr

## ABSTRACT

Adoptive cell transfer (ACT), a form of cell-based immunotherapy that eliminates cancer by restoring and strengthening the body's immune system, has revolutionized cancer treatment. ACT entails intravenous transfer of either tumor-resident or peripheral blood-modified immune cells into cancer patients to mediate anti-tumor response. Although these immune cells control and eradicate cancer via enhanced cytotoxicity against specific tumor antigens, several side effects have been frequently reported in clinical trials. Recently, exosomes, potential cell-free therapeutics, have emerged as an alternative to cell-based immunotherapies, **due to their higher stability under same storage condition, lower risk of GvHD and CRS, and higher resistance to immunosuppressive tumor microenvironment**. Exosomes, which are nano-sized lipid vesicles, are secreted by living cells, including immune cells. Exosomes contain proteins, lipids, and nucleic acids, and the functional role of each exosome is determined by the specific cargo

derived from parental cells. Exosomes derived from cytotoxic effectors including T cells and NK cells exert anti-tumor effects via proteins such as granzyme B and FasL. In this mini-review, we describe the current understanding of the ACT and immune cell-derived exosomes and discuss the limitations of ACT and the opportunities for immune cell-derived exosomes as immune therapies.

## INTRODUCTION

Cancer cells functionally design their microenvironment through the secretion of various factors such as cytokines and chemokines to maintain their proliferation and survival (1). In this process, immune cells are reprogrammed to undergo a dramatic phenotypic change toward a pro-tumor profile, contributing to immune escape. Traditional cancer therapies, such as radiotherapy and chemotherapy, which target the tumor cells, initially induce positive responses in most patients, but is associated with frequent relapses and resistance (2, 3). Thus, immunotherapy, which utilizes the body's immune system to induce anti-tumor effects, is emerging as a useful tool (4-6).

The main goal of cancer immunotherapy is to boost and restore the anti-tumor immune response to eliminate cancer cells (7). Cancer immunotherapy includes adoptive cell transfer (ACT), checkpoint blockade, and anti-cancer vaccines (8). ACT utilizes T lymphocytes isolated from tumor tissues or genetically manipulated to recognize the specific antigen. The use of other immune cell types, such as natural killer (NK) cells, is also currently being studied. ACT of tumor-specific immune cells has proven clinical success in cancer treatment (9, 10). However, the challenges include autologous administration, auto-immune responses, off-tumor toxicity, and severe side effects such as cytokine release syndrome (CRS) (11).

Exosome-based cell-free therapy is emerging as a potential treatment to address these limitations of cell-based therapy. Exosomes are small endosomal derived, nano-sized lipid

bilayer extracellular vesicles that carry a cargo of proteins, lipids, and nucleic acids (12, 13). Exosomes serve as important messengers that deliver functional cargo derived from parent cells to target cells and adjust the physiological or pathological processes of the target cells (14). NK cell-derived exosomes harboring FasL and NKG2D can mediate anti-tumor response (15). Also, dendritic cell (DC)-derived exosomes can induce an adaptive immune response by activating CD4<sup>+</sup> T cells or CD8<sup>+</sup> T cells via peptide-MHC complex (16).

Recently, several preclinical studies have been conducted to verify the anti-tumor effect of exosomes as immune therapeutics (17-19). Since exosomes are biocompatible with low cytotoxicity and immunogenicity, they can be utilized as carriers of biomarkers, vaccines, drugs, and therapeutics (19-22). In this review, we provide an inclusive overview of ACT and exosomes. Also, we discuss the challenges of ACT and the therapeutic potential of immune cell-derived exosomes in cancer immunotherapy.

## **1. ACT for cancer immunotherapy**

ACT is a form of cell-based immunotherapy that uses immune cells to eliminate cancer (10, 23). ACT utilizes immune cells collected from patients selected or genetically engineered to express specific T-cell receptors (TCR) or chimeric antigen receptors (CAR). A sufficient number of immune cells expanded *ex vivo*, are infused into the patient. In this respect, ACT has multiple advantages over other forms of cancer immunotherapy that rely on the *in vivo* development of sufficient numbers of anti-tumor immune cells (24). As shown in Figure 1, ACT can be classified into four categories based on immune cell type and mechanism: tumor-infiltrating lymphocyte (TIL) therapy, engineered TCR therapy, CAR-T cell therapy and NK cell therapy.

### **1.1. The types of ACT**

### ***TIL Therapy***

TILs invading tumor tissues represent a heterogeneous population. TILs are primarily composed of T cells carrying a TCR capable of recognizing tumor-specific antigens and cytotoxic effects against tumors. TILs are emerging as important biomarkers for predicting the treatment outcome and efficacy. In the original TIL protocol, after isolating TILs from the tumor mass, a population of T cells with the desired TCR specificity can be selected and expanded in the presence of IL-2. These TILs are adaptively transferred to cancer patients via a lymphodepletion regimen.

Since TIL therapy was first administered to patients with metastatic melanoma in 1988 (25), it has been shown to be effective in many cancers, including melanoma, colon cancer, cholangiocarcinoma, and lung cancer (26). Currently, the clinical trials with autologous TIL (formally called LN-145) involve advanced cervical cancer (NCT03108495), metastatic non-small cell lung cancer (NSCLC) (NCT04614103), and triple-negative breast cancer (TBNC) (NCT04111510). Trials are also ongoing for combination therapy with TIL and chemotherapy (NCT03467516) or cytokines (NCT01740557).

### ***Engineered TCR Therapy***

Despite its clinical success, TIL therapy has limited availability and production of therapeutic T cells for a larger group of patients. T cells genetically engineered to express TCR and CAR have been proposed as an effective alternative (27). TCR therapy entails the use of TCR-introduced T cells that can be linked to tumor antigens by extending the TIL therapy protocol. TCR is an  $\alpha/\beta$  heterodimer composed of a constant region, which anchors into the T cell membrane, and a variable region, which recognizes and binds to the antigen-MHC complex.

In 2006, Morgan *et al.* reported the first successful clinical trials using autologous T cells with a TCR that was HLA-A2 restricted, and specific for the MART-1 antigen (28). A persistent

clinical response was detected in 2 of 17 patients with refractory metastatic melanoma. In a follow-up study, treatment of patients with T cells expressing highly reactive TCRs against MART-1 resulted in tumor regression among 30% of the cases (6 of 20) (29). Since then, Robbins PF *et al.* reported objective clinical responses of 60% and 45%, in synovial cell sarcoma and melanoma patients, respectively, in a study using a TCR that recognizes the NY-ESO-1 antigen (30, 31). Currently, a clinical trial of NY-ESO-1-specific T cells in combination with chemotherapy including melphalan, for ovarian cancer, is underway (NCT03691376).

### ***CAR-T Cell Therapy***

Similar to TCR therapy, CAR-T therapy involves patient-derived T cells engineered to express chimeric antigen receptors (CARs) on the cell surface. CAR is a synthetic structure containing single-chain variable fragments (scFv) of a monoclonal antibody as the ligand-binding extracellular domain, a CD3 $\zeta$  chain as the intracellular signaling domain and/or a co-stimulatory domain, mainly CD28 and 4-1BB (32, 33). Since CAR-T cells directly recognize surface antigens, but not the antigen presented by MHC, CAR-T cells can detect and attack cancer cells, unlike T cells that fail to recognize cancer cells lacking MHC class in an evasion mechanism. CAR-T cells in contact with cancer surface antigen proliferate and eliminate cancer cells via the release of effector molecules such as IFN- $\gamma$  and granzyme B.

Most clinical studies have reported remarkable response rate against hematological neoplasms, such as acute lymphoblastic leukemia (ALL) and diffuse large B-cell lymphoma (DLBCL), suggesting the therapeutic potential of CD19-CAR-T therapy (34, 35). Since the first CAR-T therapies, Kymriah<sup>®</sup> (tisagenlecleucel) suspension for intravenous infusion for B-cell ALL was approved by the FDA in 2017, Yescarta<sup>®</sup> (axicabtageneclisoleucel), Tecartus<sup>™</sup> (brexucabtageneautoleucel), Abecma<sup>™</sup> (idecabtagenevicleucel), and Breyanzi<sup>®</sup> (lisocabtagenemaraleucel) were approved for lymphoma or myeloma (36).

### ***NK Cell Therapy***

NK cells, the innate immune cells, play an essential role in cancer immune surveillance (37). NK cells can quickly recognize and eliminate cancer cells without HLA matching or prior sensitization. NK cells are capable of killing target cells via a cytotoxic mechanism similar to that of CD8<sup>+</sup> cytotoxic T cells. Activated NK cells also mediate the innate and the adaptive immune system by releasing various inflammatory factors to recruit and activate other immune cells such as T cells and DCs. In addition, the NK cells are a significant factor predicting cancer prognosis (38).

Early approaches to NK cell therapy used fresh NK cells isolated from the patient's peripheral blood mononuclear cells (PBMCs) or whole blood (39). Because the number of NK cells in peripheral or cord blood is relatively low (10-15% of all circulating lymphocytes), the use of NK cells for ACT required an *ex vivo* expansion mechanism to yield sufficient numbers of NK cells with high purity and potency. This challenge has recently been overcome via the differentiation of NK cells from pluripotent stem cells (PSCs), as well as the generation of NK-92 cell lines amenable to genetic manipulation for the recognition of specific tumor antigens (40). The use of antigen-presenting cells (APCs) as feeder cells in combination with CD137L-IL21 also enabled the production of a large number of activated NK cells. This success has enabled many clinical trials for NK cell-based cancer immunotherapy (41).

In addition, CAR-NK cells with improved anti-tumor activity than conventional NK cells have been developed using the basic structural framework of CAR designed for CAR-T cells (42). Many clinical trials are ongoing to evaluate the safety and efficacy of tumor-targeted CAR-NK cells. In the first clinical trial of CD33-CAR NK-92 cells in patients with relapsed and refractory acute myelogenous leukemia, there were no serious side effects shown when injected at doses of up to 5 billion cells per patient (NCT02944162) (43). In an ongoing CD19-targeted

CAR-NK treatment clinical trial in patients with relapsed or refractory CD19<sup>+</sup> cancer, about 73% of patients (8 of 11) manifested objective responses to treatment without major toxic effects (NCT03056339). Clinical trials of ROBO1 CAR-NK cells in solid tumors expressing ROBO1, including pancreatic cancer, are also ongoing (NCT03940820, and NCT03941457).

## **1.2. Challenges of ACT therapy**

The remarkable success of ACT therapy is undeniable, but there are still many challenges to overcome. The ACT protocol involves the deletion of pre-lymphocytes and the infusion of live immune cells. Infused immune cells can cause graft-versus-host disease (GvHD) by T cells that are not completely removed before treatment (44). Storage of expanded immune cells and reduction of their cytotoxicity and survival by freeze-thaw mechanisms should also be considered. The most common challenge is associated with toxicity including CRS induced by the immune cells used in ACT (45).

### ***On-target off-tumor toxicity***

Immune cells for ACT have been selected or genetically engineered to recognize tumor-specific antigens. However, the immune cells that target tumor antigens can also recognize healthy cells expressing the same antigen, causing “on-target off-tumor toxicity”. For example, treatment of MART-1 specific or gp-100 specific T cells for melanoma exhibited toxicity in normal tissue including skin and ear in the presence of melanocytes (29). In patients with colorectal cancer treated with carcinoembryonic antigen (CEA)-specific TCR therapy, severe inflammatory colitis occurred due to CEA reactivity expressed in the normal colon epithelium (46). Similarly, the treatment for B cell malignancies by CD19 CAR-T cell induced B cell depletion and hypogammaglobulinemia (47). It has been reported that low levels of HER2 expression in normal lung tissue resulted in fatal lung toxicity in patients with metastatic colon cancer



exposed to HER2 CAR-T therapy (48).

### ***CRS***

The most common toxicity induced by ACT involves CRS, a severe form called a cytokine storm (49). In immunotherapy, activated immune cells eliminate target tumors by releasing cytotoxic molecules including cytokines. CRS is mainly observed in CAR-T therapy due to the activation of the large number of T cells injected and antigen recognition (50-52). CRS typically occurs within a few days following immune cell infusion and is associated with cell proliferation *in vivo* and a marked increase in the level of serum cytokines such as IFN- $\gamma$ , TNF- $\alpha$ , and IL-6 (53, 54). CRS is accompanied by symptoms such as fever, hypertension and hypoxemia, which can range from mild or moderate to life-threatening manifestations. A patient with metastatic colon cancer showed elevated levels of the serum cytokines including IFN- $\gamma$ , TNF- $\alpha$ , GM-CSF, IL-6 and IL-10, after HER2 CAR-T treatment, eventually leading to death (48). In some patients (4 of 8) with B cell malignancies, excessive levels of serum cytokines (IFN- $\gamma$  and TNF- $\alpha$ ) were observed after CD19 CAR-T cell infusion (47).

### ***Immune escape***

Another challenge faced by ACT is that the tumor microenvironment (TME) depletes the anti-tumor function of the infused immune cells or interferes with their migration and penetration into solid tumors (55, 56). TME, composed of blood vessels, immune cells, extracellular matrix (ECM), and cancer cells, provides a milieu for tumor proliferation and progression. As infused T cells experience continued antigen stimulation and are exposed to immunosuppressive factors in TME, the T cells may be exhausted due to the loss of their effector function and up-regulation of inhibitory receptors such as PD-1 and Tim-3 (57). Moreover, the regulatory T cells or immunosuppressive modulators including prostaglandin E2 (PGE2), IL-6 and TGF- $\beta$  can

suppress the cytotoxicity of infused NK cells (58-60). Solid tumors also prevent cytotoxic immune cells from migrating into or invading the tumor via secretion of chemokines or the formation of biological barriers such as ECM (61). Therefore, ACT for hematologic cancers has been effective, but the efficacy for solid cancers has room for improvement.

## 2. Immune cell-derived exosomes for cancer immunotherapy

Despite its outstanding performance, ACT is limited by the direct use of immune cells. As mentioned above, several toxicities, including CRS and off-target effects, are triggered by uncontrolled immune cells *in vivo*. Moreover, current ACT strategies are limited in that they are costly and time-consuming to produce, preserve, and transport clinical-grade immune cells suitable for direct therapeutic use. Recent studies highlight the therapeutic potential and effectiveness of immune cell-derived exosomes as a cell-free immunotherapy.

### 2.1 Overview of exosomes

Exosomes are nano-sized membrane vesicles (30-150 nm) derived from various cell types including immune cells, tumor cells and mesenchymal stem cells (MSCs) (62, 63). Exosomes originate from endosomal pathway (62-64). Fusion of the multivesicular bodies (MVBs) generated by the inward budding of the late endosome with the cell membrane releases the intraluminal vesicles within into the extracellular space as exosomes. Exosomes are composed of proteins, nucleic acids, amino acids, metabolites and lipids (65). Exosomes are generally made up of many proteins involved in the biogenesis and function of exosomes including proteins associated with MVB biogenesis (Alix and TSG101), heat-shock protein (Hsp70) and the tetraspanins (CD9, CD63, CD81, and CD82) used as exosome markers. Exosomes also contain adhesion molecules such as ICAM-1 and integrins for cellular internalization (66). In addition, the exosomes contain an abundance of cholesterol, phosphatidylcholine, and

diglycerides in the lipid rafts (67).

Although exosomes were initially considered as vesicles released to eliminate unnecessary contents, exosomes are involved in intracellular communication and represent a key factor regulating cellular function, especially the immune system (68, 69). Released exosomes can be present in diverse biological fluids such as milk, urine, and saliva, and delivered to target cells via blood and other body fluids (13). Also, exosomes are continuously released by donor cells, but their release is also controlled by cellular conditions, regulating the body's physiological responses (13).

## **2.2. Immune cell-derived exosomes**

One of the earliest reported physiological targets of exosome-mediated cell-to-cell communication is the immune system. In late 1990s, Raposo *et al.* demonstrated that exosomes secreted from B lymphocytes play a role in antigen presentation by inducing an antigen-specific CD4<sup>+</sup> T cell response via a peptide-MHC class II complex on the surface (70). Subsequently, a variety of studies have reported the characteristics of exosomes derived from immune cells and their role in immune system. Immune cell-derived exosomes represent the functional properties of parental immune cells. APC-derived exosomes stimulate CD4<sup>+</sup> and CD8<sup>+</sup> T cells via antigen-MHC complex expressed on their membrane (16).

Immune cell-derived exosomes express cell-specific marker proteins such as MHC class I and II and co-stimulatory molecules on APC-derived exosomes, CD56 on NK-derived exosomes and TCR/CD3 complex on T cell-derived exosomes (71). In addition, the tetraspanin family is abundant in immune cell-derived exosomes (72). These proteins regulate the immune response by interacting with MHC molecules or cell adhesion molecules including LFA-1 and ICAM-1 or by regulating the clustering of MHC complexes.

Clinical studies of exosomes derived from dendritic cells pulsed with tumor-specific peptides,

as cancer vaccines, suggest the immunotherapeutic potential of immune cell-derived exosomes (73). Here, the anti-tumor functions of T cell-derived exosome and NK-derived exosome serving as an alternative to ACT are discussed in the following sections.

### ***T cell-derived exosomes***

T cell-derived exosomes are produced only after T cells are activated (74). It was found that the interaction between tetraspanins, ceramides, and myelin and lymphocyte protein (MAL) proteins is important for the biogenesis of exosome by T cells. In particular, MAL protein, a tetraspanning membrane protein that is partly expressed in T cells, is involved in fusion of MVBs with the cell membrane (75). T cell-derived exosomes strongly harbor TCR/CD3 complex and contain miRNAs and cytotoxic molecules including IFN- $\gamma$  and granzyme B. Exosomes originating from activated human CD3<sup>+</sup> T cells, along with IL-2, induced the proliferation of resting CD3<sup>+</sup> T cells and enhanced the level of cytokines and chemokines in the CD3<sup>+</sup> T cells (76). Li *et al.* confirmed that exosomes generated from cytotoxic T lymphocyte (CTL) stimulated with IL-12 contain enriched exosomal proteins such as Alix, CD9, CD81 and Tsg101 and CTL-associated proteins including granzyme B, STAT3, and STAT5B (77). Although these exosomes did not mediate memory CTL formation, they activated naïve CD8<sup>+</sup> T cells regardless of antigen, and reinforced the activation of CTLs under mild antigen stimulation. In addition, upon formation of immune synapse, miRNA-loaded exosomes were unidirectionally transferred from T cells to APCs (78). These results indicate that T cell-derived exosomes control the immune response.

Recently, Fu *et al.* reported that exosomes generated from CAR-T cell recognizing human EGFR and HER2 secreted cytotoxic effectors including granzyme B and perforin resulting in cytolytic activity and anti-tumor effects in xenograft models (79). In addition, CAR-T cell-derived exosomes injected into tumor-bearing mice did not express PD-1 on their membranes

and did not induce CRS unlike CAR-T cells. Yang *et al.* demonstrated that exosomes derived from CAR-T cells targeting mesothelin, one of the antigens for breast cancer treatment, effectively inhibited cancer growth in TBNC animal models without apparent side effects via expression of perforin and granzyme B (80).

### ***NK cell-derived exosomes***

NK cells can kill abnormal cells such as cancer and stimulate adaptive immune response via secretion of pro-inflammatory cytokines and chemokines (37). Similar to parental cells, NK cell-derived exosomes express NK marker CD56 and receptors such as NKG2D that bind to ligands with restricted expression in malignant cells, and contain cytolytic molecules such as FasL, perforin and granzymes (15). In an early study of the NK cell-derived exosome, Lugini *et al.* reported that exosomes purified from NK cells expressed not only NK cell marker CD56, but also FasL and perforin, and showed cytolytic activity only in hematological cancer cells such as Jurkat and K562 cell lines (81). Later, it was demonstrated that exosomes isolated on a large scale from activated NK cells exerted cytotoxic activity against several cancer types including ALL and neuroblastoma via caspase-mediated pathway (82). These studies suggest that the activation of NK cells releases potent exosomes. Zhu *et al.* observed that NK cell-derived exosomes (NK-92 Exo) induced apoptosis in melanoma, but not normal cells and inhibited tumor growth in xenografts bearing melanoma cells (83). In addition, exosomes isolated from NK cells previously exposed to neuroblastoma (NB) cells carried NK cell receptors such as CD56 and NKG2D and exhibited anti-tumor effects against NB tumors *in vitro* and *in vivo* (84). NK cell-derived exosomes also induce cancer cell apoptosis via DNAX accessory molecule-1 (DNAM1) expressed on the surface (85). These results support the therapeutic potential of NK-derived exosome against cancer. Besides proteins including FasL and perforin, miRNA contained in NK-derived exosome is also involved in anti-tumor activity.

Neviani *et al.* confirmed that exosomes derived from activated NK cells containing the tumor suppressor miR-186 displayed cytotoxicity against the MYCN-amplified NB cell line and **restrained** TGF- $\beta$  dependent immune escape (86).

***Other immune cell-derived exosomes: DC-derived exosomes and macrophage-derived exosomes***

In addition to exosomes derived from cytotoxic effectors that potentially kill tumor cells, exosomes derived from DCs or macrophages could serve as cancer vaccines. **Exosomes isolated from tumor peptide-pulsed DCs promote tumor-specific T cell priming by delivering MHC-restricted peptide loaded on the exosome surface to T cells or inducing expression of MHC/peptide complexes in DCs (16). Thus, like DC vaccines, DC-derived exosomes (DEXs) therapy can lead to tumor growth inhibition and tumor regression by inducing the patient's adaptive immune system to specific tumor antigens.**

As a representative APC, DCs play an important role in mediating innate and adaptive immunity by recognizing, processing, and presenting antigens to T cells (87). Likewise, it has been demonstrated that DEXs can induce T cell priming by directly or indirectly presenting the MHC-antigen complex to T cells, and can also amplify T cell activation via co-stimulatory molecules such as CD86 and CD80 expressed on the surface (16). In addition, DEXs can facilitate activation and proliferation of NK cells via IL-15Ra ligand and NKG2D ligand (88). It was confirmed that TNF superfamily ligands (TNFSFLs) expressed on the DEX surface induce apoptosis in cancer cells and activate NK cells (89). **Moreover, compared to DC-based vaccines, DEX immunotherapy has more resistance to tumor immunosuppression, higher bioavailability and biostability, with higher yields and lower costs (16).** Based on these effects, DEXs have been used in several clinical trials investigating NSCLC (90, 91), metastatic melanoma (92) and colorectal cancer (93).

Among macrophage subtypes, M1-like macrophages can promote T cell-mediated immune responses by releasing cytokines such as IFN- $\gamma$ , TNF- $\alpha$ , IL-12 and IL-6 or antigen presentation via phagocytosis. M1-like macrophage-derived exosomes, which retain immunostimulatory properties of parental cells can accelerate anti-cancer effects by releasing pro-inflammatory cytokines including IL-6, IL-12 and iNOS (94, 95). M1-like macrophage-derived exosomes containing anti-cancer drugs such as paclitaxel and cisplatin can enhance anti-cancer activity by inducing cancer cell apoptosis, increasing drug sensitivity and circumventing drug resistance mechanisms (95-98).

### **2.3. Opportunities and challenges of immune cell-derived exosomes in immune therapies**

Recent studies have evaluated the role of exosomes derived from immune cells such as NK cells and T cells in immune modulation and their efficacy in preclinical studies. These results suggest that immune cell-derived exosomes display numerous functions suitable for clinical application. Furthermore, their advantages relate to storage and transplantation.

Stable storage of exosomes is an important issue in the transport and clinical application of exosomes. The storage techniques currently studied include cryopreservation, freeze-drying and spray drying. Cryopreservation (-80°C frozen storage) is a complete method for the stable storage of exosomes (99). The characteristics, function and efficacy of DEXs stored for a long time at -80°C were not affected by freezing and thawing (73, 90, 100). Further, the large number of exosomes isolated from NK cells expanded *ex vivo* were stable when stored at -80°C for at least 12 months, and their cytotoxic effect was maintained (82). These results suggest that exosomes can be stably stored long term via cryopreservation.

Exosomes may be less toxic compared with cell-based therapies (ACT) that elicit serious immune responses such as GvHD or CRS. Morse *et al.* reported the treatment of patients with advanced NSCLC using DEXs without serious toxicity or autoimmune reactions, and no

serious organ damage due to the vaccine occurred (90). In addition, Fu *et al.* reported that CAR-T cell-derived exosome did not induce changes in serum cytokine levels and body weight in mice, unlike CAR-T cells (79).

Despite the long-term storage of exosomes with their efficacy and properties intact, and low toxicity in ongoing clinical trials, currently no specific regulatory guidelines are available for clinical applications such as standardization, optimization and quality control. Given the heterogeneity and complexity of exosomes and most conventional laboratory-scale methods used for exosome isolation, it is difficult to isolate large volumes of exosomes with clinical grade-quality and purity (101). Recently, tangential flow filtration combined with a chromatographic method has been attempted to mass-produce high-quality exosomes that comply with good manufacturing practice (102-104). In addition, it has been proposed to use well-characterized cell lines instead of primary cells to ensure the uniformity and stability of exosomes (104).

## CONCLUSION AND PERSPECTIVES

ACT entails the use of patient-derived immune cells such as T cells and NK cells that recognize tumor-specific antigens to eliminate cancers. Immune cells with unique cytotoxic effects show improved tumor recognition, continuous activation, and potent tumor killing capabilities through genetic engineering. ACT-based cancer immunotherapy has yielded promising results in clinical trials. Despite the encouraging results, many challenges remain. *In vivo* expansion and cytokine release of the infused immune cells induce adverse effects such as CRS and autoimmune responses. Also, the efficacy of ACT in solid cancers is limited due to poor cell migration and penetration into the tumor site, and immunosuppressive TME.

Exosomes derived from immune cells offer sufficient therapeutic potential as substitutes for



ACT. Immune cell-derived exosomes exert immune-regulatory effects due to biofunctional cargo such as proteins and nucleic acids derived from their parental cells. Here, we highlight that exosomes isolated from cytotoxic effectors including T cells and NK cells exhibit anti-tumor effects identical to parental cells mediated via cytotoxic molecules such as FasL, IFN- $\gamma$  and perforin (Figure 2). Further, the preclinical results provide evidence suggesting that these exosomes have low toxicity compared with ACT (79, 80), which reinforces the exosome therapeutic potential.

Recent interest in exosomes worldwide has spurred research and production of exosomes for therapeutic purposes. The current state of exosome therapy is similar to early years of cell-based therapy limited by poor understanding of effective cell therapy, which hindered the large-scale development and manufacture of specialized cells for treatment based on clinical studies. Further studies are needed to explore the possibility of immune cell-derived exosomes as immunotherapeutic agents. However, the results of existing preclinical studies demonstrate the potential of immune cell-derived exosomes as immunotherapeutics. Accordingly, exosomes derived from immune cells represent potential immunotherapeutic alternatives to ACT.

## ACKNOWLEDGMENTS

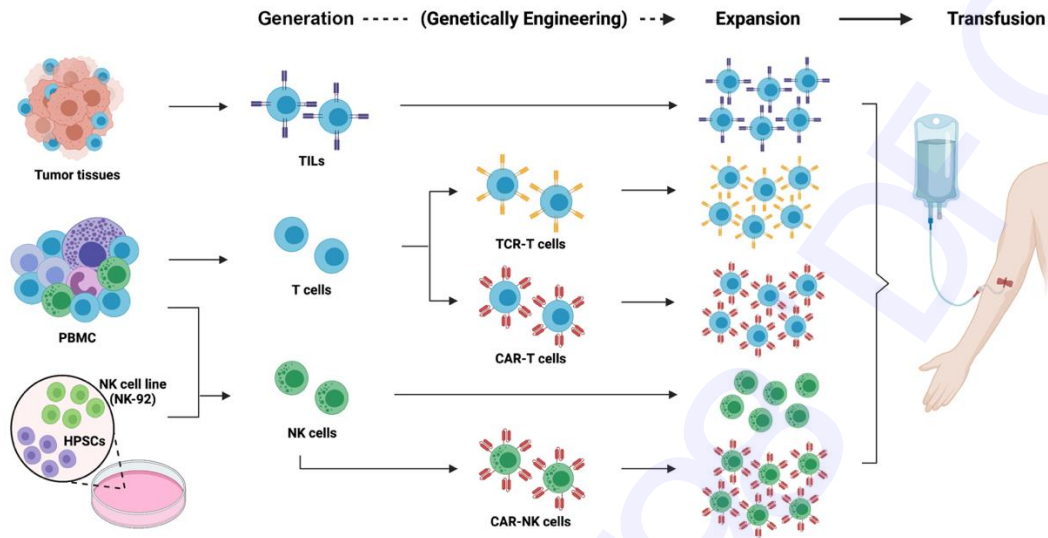
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## CONFLICTS OF INTEREST

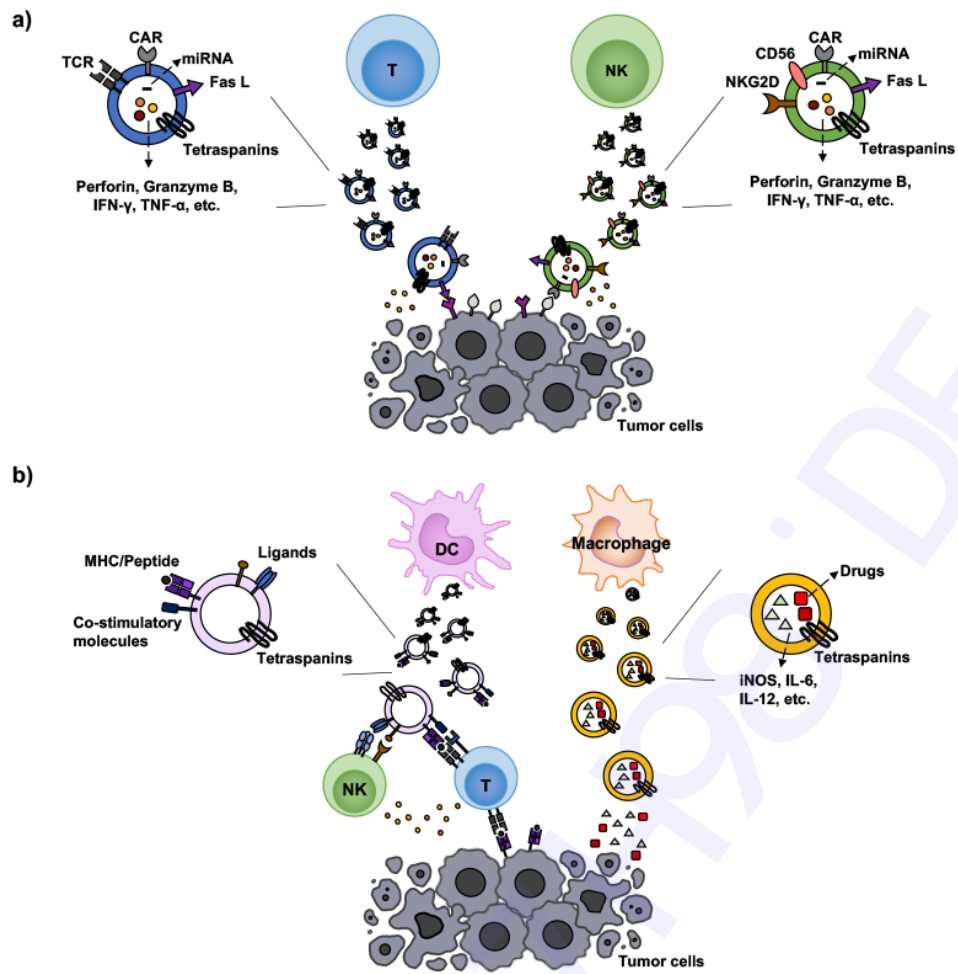
The authors declare no conflict of interest.

## FIGURE LEGENDS

**Figure 1. Schematic diagram of adoptive cell therapy (ACT) process using T cells and NK cells.**



**Figure 2. Exosomes derived from immune cells.** (a) T cell or NK cell-derived exosomes that express FasL or NKG2D and contain cytotoxic molecules such as perforin, Granzyme B, IFN- $\gamma$ , and TNF- $\alpha$  induce cancer cell death and inhibit cancer cell growth. (b) DC-derived exosomes induce the activation of T cells and NK cells through the expression of some ligands such as NKG2D ligand, co-stimulatory molecules, and MHC/antigen complex. Macrophage-derived exosomes lead to cancer cell death by releasing pro-inflammatory cytokines such as iNOS, IL-6 and IL-12 or anti-cancer drugs.



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