nature immunology

REVIEW ARTICLE

https://doi.org/10.1038/s41590-021-00899-0

Extracellular vesicles in immunomodulation and tumor progression

Carolyn Marar[1,2,6,7], Bartholomew Starich[1,3,7] and Denis Wirtz[1,3,4,5] ✉

Extracellular vesicles have emerged as prominent regulators of the immune response during tumor progression. EVs contain a diverse repertoire of molecular cargo that plays a critical role in immunomodulation. Here, we identify the role of EVs as mediators of communication between cancer and immune cells. This expanded role of EVs may shed light on the mechanisms behind tumor progression and provide translational diagnostic and prognostic tools for immunologists.

Cells communicate via contact-dependent and contact-independent interactions through the secretion of chemokines, cytokines, growth factors, and associated cell receptors. Over the past 30 years, the secretion of EVs has also emerged as a major mechanism for cell–cell and cell–environment interactions. These 30- to 5,000-nm lipid-membrane-bound vesicles contain functional proteins, nucleic acids, lipids, and other bioactive molecules that can modulate the behavior of recipient cells[1].

EVs are generally split into three subtypes on the basis of their mechanism of biogenesis—exosomes, shed microvesicles, and apoptotic bodies (Box 1). EV biogenesis and secretion have been well reviewed elsewhere[2–4]. In brief, exosomes are formed via the inward budding of the cell plasma membrane and the subsequent formation of multi-vesicular bodies. Conversely, shed microvesicles—also referred to as ectosomes—are produced via the outward budding of the plasma membrane. Apoptotic bodies are released during cellular apoptosis. Due to the difficulty of isolating EV subtypes, they are often labeled, according to their size, as small, medium, and large EVs (Box 1). Following the International Society for Extracellular Vesicles (ISEV) nomenclature[5], we will use the term EV for all lipid-bilayer particles secreted by cells, specifying size where appropriate. While some proteins may be shared within an EV subtype, vesicular cargo is highly dependent on the type and state of the donor cell[6]. This diversity in cargo allows EVs to modulate several important cellular processes, including cell differentiation, blood coagulation, and angiogenesis in tissue homeostasis and development[7]. Studies have demonstrated the key role that EVs play in tumor progression and antitumor immune responses[8]. Here, we review the role of EVs as mediators of communication between cancer and immune cells, as well as the potential clinical uses of EVs that arise from these interactions.

The role of extracellular vesicles in the immune cascade

Immune-derived EVs carry a variety of cargo that functions in immune activation and suppression (Fig. 1a). The role of EVs in an immune response was first described in 1996 (ref. [9]). This study observed that small EVs released by B cells not only carried major histocompatibility complex (MHC) class II molecules, but also elicited immune responses in T cells[9]. Soon after, a second study[10] described the ability of small EVs released by dendritic cells (DCs) to induce tumor suppression in vivo. In 2002, another study elaborated on this work to describe a mechanism for indirect T cell stimulation by DC small EVs[11]. These discoveries established EVs as a major mechanism of cellular communication and provided a basis for immune EV research over the past two decades.

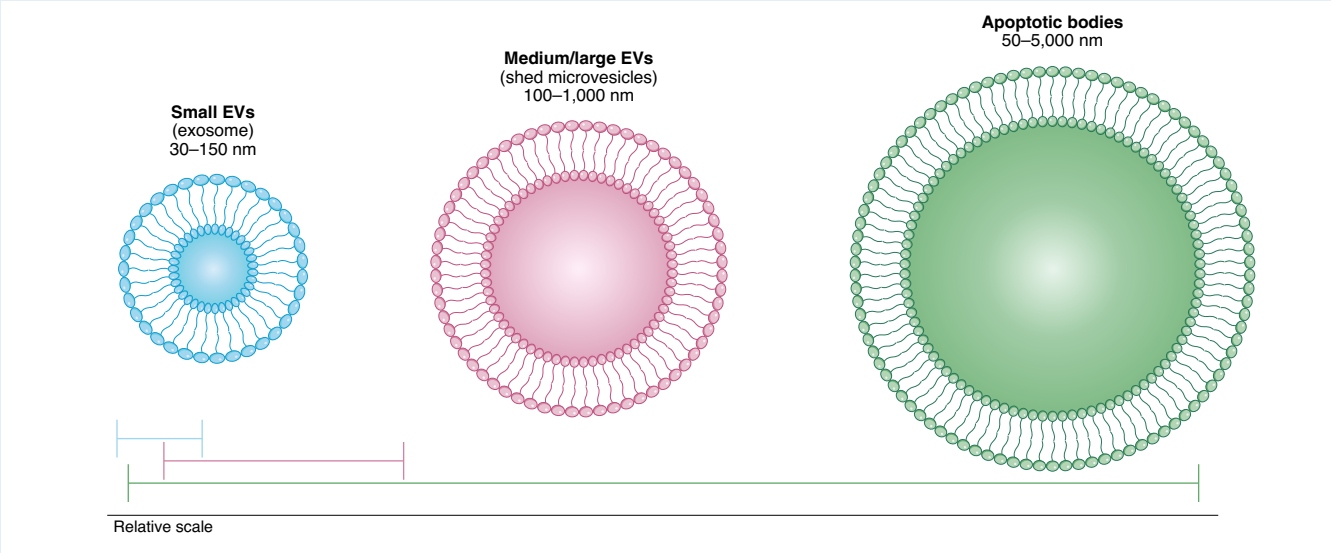
DCs are antigen-presenting cells (APCs) that orchestrate the immune response against a specific antigen. DC EVs have become a focus of the field due to their ability to stimulate antitumor immune responses. DC EVs harbor MHC I and MHC II molecules, tetraspanins, adhesion molecules, heat shock proteins, and costimulatory molecules that confer their immunomodulatory capabilities[12–19]. It is debated, however, whether EVs can carry out these functions via direct interaction with target cells[18,20,21], or if the presence of DCs is necessary[11,22–24]. Interestingly, in vitro modeling of primary mouse DCs might set a new example for the immunomodulatory capabilities of EVs. Shown to transfer tumor-antigen-loaded vesicles via tight synaptic connections, DCs highlight a potential downstream mechanism for EV antigen presentation[25]. Further in vitro modeling involving priming of DCs via antigen laden EVs might reveal that internalized EV cargo is also shared between APCs during this synaptic transfer.

Whether their effects are direct or indirect, APC-derived EVs, and specifically DC EVs, play a major role in antigen presentation and immune activation[26–28]. EVs have the remarkable ability to transfer preformed functional peptide–MHC complexes from APCs to recipient cells[29,30]. Among the MHC II molecules present on DC EVs, HLA-DQ enables DC EVs to promote T cell proliferation[23,31,32]. DC small EVs induce maturation and differentiation in several cell types, including immature DCs and monocytes[33,34]. DC EVs also stimulate interferon-γ (IFN-γ) production by naive $CD4^+$ T cells and induce differentiation into type 1 helper T (T_H1), type 2 helper T (T_H2), and regulatory T (T_{reg}) cells[23,35–38]. Interestingly, the molecular cargo of DC EVs varies on the basis of the presence of surrounding cells. Coculture assays using primary DCs derived from human monocytes reveal that, in the presence of bystander T cells, DCs secrete small EVs enriched in microRNAs (miRNAs) miR-30b, miR-146a, and miR-155 (ref. [39]). These miRNAs carried by DC EVs are functionally active in promoting further $CD8^+$ T cell activation[39].

[1]Institute for NanoBiotechnology (INBT), The Johns Hopkins University, Baltimore, MD, USA. [2]Department of Biomedical Engineering, The Johns Hopkins University, Baltimore, MD, USA. [3]Department of Chemical and Biomolecular Engineering, The Johns Hopkins University, Baltimore, MD, USA. [4]Departments of Oncology and Pathology, Sidney Kimmel Cancer Center, The Johns Hopkins University School of Medicine, Baltimore, MD, USA. [5]Johns Hopkins Physical Sciences Oncology Center, The Johns Hopkins University, Baltimore, MD, USA. [6]Present address: Biomedical Engineering Department, Boston University, Boston, MA, USA. [7]These authors contributed equally: Carolyn Marar, Bartholomew Starich. ✉e-mail: wirtz@jhu.edu

Box 1 | Common EV subtypes

EV subtypes are classically defined by size, and no specific molecular markers exist to distinguish between them. While a number of molecules tend to be enriched in EV fractions, many traditional markers (CD9, CD63, CD81, TSG101, Alix, Flotillin-1, HSC70, Actin, MHC I, and MHC II) coisolate with multiple EV subtypes, leading to confusion and misinterpretation in literature. Special consideration should be directed towards extensive characterization of EV isolations prior to publication of any work on EVs.



While T cells generally carry out immune responses, their EVs have also emerged as important immunomodulators. Small EVs from activated T cells are enriched in nucleic acids with a variety of functions. Found to contain mitochondrial DNA (mtDNA), small T-cell-derived EVs prime DCs to more efficiently respond to future attacks by a familiar antigen[40]. Acting through the cGAS–STING cytosolic DNA-sensing pathway, DNA bound to the surface of small T-cell-derived EVs confers an increased resistance to infection in recipient DCs[40]. Activated T cells secrete EVs containing specific transfer-RNA fragments, which would otherwise inhibit T cell activation[41]. Secretomic analysis of cell-culture-derived T cell EVs revealed systemic encapsulation of immunomodulatory cytokines[21]. EV-associated cytokine encapsulation by T cells further stabilize the functional ability of these cytokines in comparison to their free counterparts[21]. This stabilizing effect of EVs has been exploited in drug-delivery applications (described in detail below).

While the differences among EV subtypes are well accepted, reports suggest that heterogeneity within EV subtypes remains largely unaccounted for[23,35,42,43]. The maturity of donor DCs as well as variation in EV enrichment profiles affect naive T cell development[23,35,42]. Small EVs and EVs secreted by mature DCs preferentially induce T_H1 activation, while larger EVs induce T_H2 differentiation[23]. Similarly, T-cell-derived EVs bearing different markers—namely CD47, CD63, and MHC I—are enriched in different sets of RNAs[43]. These developments have shed light on why EV research often produces sometimes conflicting results and provide more evidence for why it is typically inaccurate to attribute any effect to a single subpopulation of EVs.

Autoimmune suppression via extracellular vesicles

The primary function of T_{reg} cells is to prevent autoimmunity by promoting self-tolerance of the immune system[44]. T_{reg} cells employ EVs to suppress the activity of other immune cells[44]. T_{reg}-derived EVs contain several miRNAs and miRNA precursors[45–50]. Uptake of EVs containing miR-142-3p and miR-150-5p by DCs induces a decrease in proinflammatory cytokine interleukin-6 (IL-6) expression and an increase in immunosuppressive cytokine IL-10 expression[45]. These miRNAs interfere with antigen processing and presentation in DCs[51], thus inhibiting immune activation (Fig. 2).

T_{reg} cells also target other T cells as a method of immune regulation. T_{reg}-derived EVs suppress the proliferation of T cells via EV-mediated transfer of miRNAs and miRNA precursors[46–50]. T_{reg}-derived EVs are enriched in miR-146a-5p, which targets and suppresses signal transducer and activator of transcription 1 (*STAT1*) and IL-1 receptor-associated kinase-like 2 (*IRAK2*) in recipient $CD4^+$ T cells to inhibit proliferation[49]. The miRNA precursor Let-7d is carried by T_{reg}-derived EVs and transferred to the target cell, where it appears to inhibit *COX2* and decrease IFN-γ secretion[48]. A specialized subset of $CD4^+CD25^-$ T_{reg} cells release EVs found to induce a T_{reg} phenotype in naive $CD4^+$ T cells (Fig. 2). These EVs, containing miR-9, miR-330, miR-503, and inducible nitric oxide synthase mRNA, suppress proliferation and induce an increase in IL-10 secretion in recipient T cells[47].

In addition to directly influencing immune-cell functions, T_{reg}-derived EVs exert secondary immunosuppression effects by transferring proteins to recipient cells. On their surface, T_{reg}-derived EVs display both the Ebi3 and p35 subunits of IL-35 in association with tetraspanin CD81 (ref. [52]). Cells that uptake these EVs exogenously express IL-35 on their surface. IL-35 interacts with the cell's own IL-35 receptors (IL-35R) to induce cell exhaustion via expression of the inhibitory receptors programmed cell death protein 1 (PD-1), T cell immunoglobulin and mucin domain-containing protein 3 (TIM3), and lymphocyte-activation gene 3 (LAG3)[53]. Other lymphocytes may also interact with IL-35 on the surface of these recipient cells, causing secondary suppression of the neighboring cells. This effect has been observed in $CD4^+$ T cells, $CD8^+$ T cells, and B cells[52] (Fig. 2).

Antigen-specific immunosuppression is exhibited by EVs derived from APCs[54,55]. APC-derived EVs express MHC II molecules and Fas ligand (FasL), which allow them to induce apoptosis

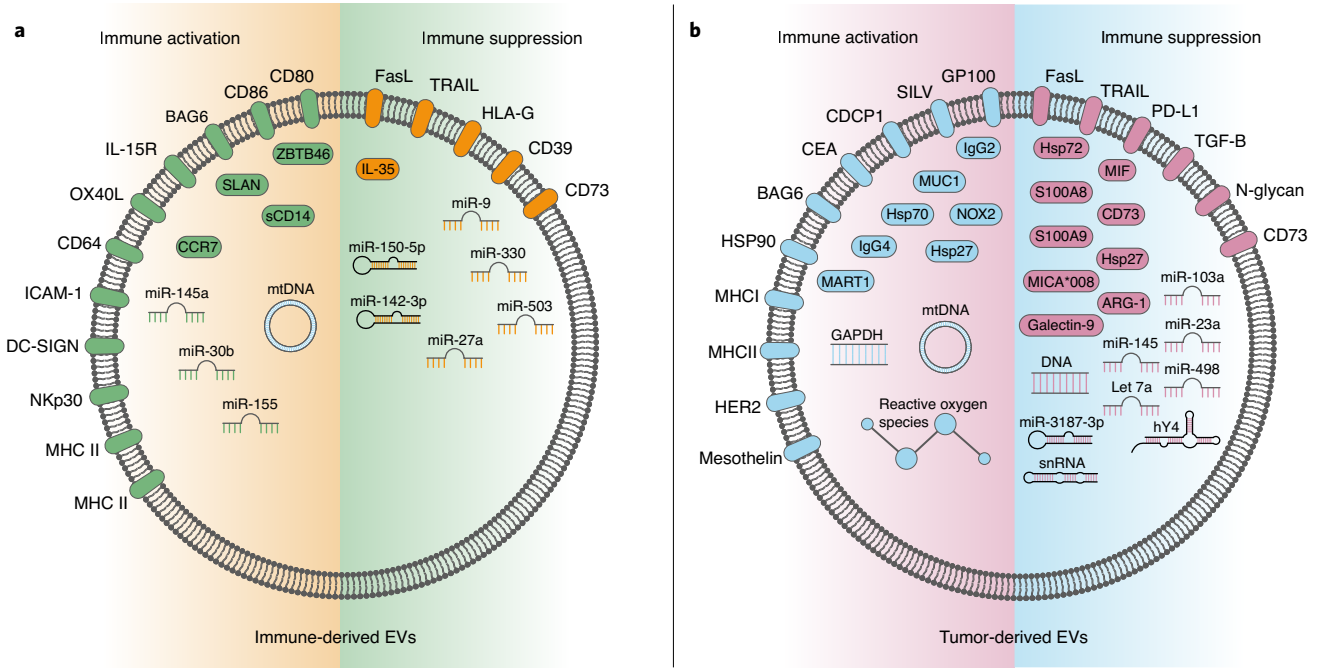


Fig. 1 | Heterogeneous cargo of EVs. Secreted from tumor and immune cells alike, EVs contain molecular cargo that facilitates immunostimulatory and immunosuppressive interactions. Trafficking transmembrane and cytosolic proteins, RNAs, and DNAs, EVs regulate autoimmune and disease responses. **a**, EVs secreted by immune cells carry cargo that directly controls peripheral immune function by either promoting a regulatory response or conditioning surrounding cells, reducing inflammation. **b**, Tumor-derived EVs express surface and cytosolic markers from their cellular progenitor, which in turn induce phenotypic changes in recipient cells.

in T cells[56]. Since this mechanism of immunosuppression is antigen-specific, the host must be immunized against the antigen for the effect to occur. Similarly, overactivated T cells undergoing activation-induced cell death secrete EVs expressing FasL, which suppresses the immune response[57].

The role of cancer-derived extracellular vesicles in immune evasion

A hallmark of cancer is tumor cells' ability to evade detection by an individual's immune system[58]. Colloquially termed 'immune escape', successful immune evasion utilizes a variety of mechanisms to stifle both adaptive and innate immune responses (Fig. 3)[8]. Immune cells experience a wide variety of inhibitory interactions from cancer cells, both via direct physical contact and through endogenous soluble factors[58–61]. As a significant driver of these interactions, cancer EVs can exhibit both immune evasion[62–65] and immunogenic properties (Fig. 1b)[66].

Early work on metastatic melanoma revealed that upregulation of the protein tyrosine kinase MET (MET) in small EVs permanently re-educates circulating bone-marrow progenitors into pro-tumorigenic mediators of premetastatic niche formation in vivo[67]. Similarly, small EVs from pancreatic cancer cells, which contain a high amount of macrophage migration inhibitory factor (MIF), induce transforming growth factor beta (TGF-β) release by Kupffer cells to remodel the extracellular matrix (ECM) in the liver[68]. More recent work has shown that cancer EVs carry an abundance of functional mRNA, non-coding RNA, and proteins thought to be crucial for interacting with both the innate and adaptive immune responses (Fig. 3)[69–71].

Highlighting the diverse role of cancer-cell EVs, RNA sequencing of small chronic lymphocytic leukemia EVs revealed the non-coding Y RNA hY4 increased programmed death-ligand 1 (PD-L1) protein expression in circulating monocytes via toll-like receptor (TLR) 7 signaling[72]. PD-1–PD-L1 interactions regulate T cell receptor (TCR) activation to prevent autoimmune response[73]. Like its tumor-cell progenitor, PD-L1 on small EVs isolated from glioblastoma and metastatic melanomas can directly activate the PD-1–PD-L1 immune checkpoint[69,71] (Fig. 3). Small EVs isolated from metastatic melanoma feature identical PD-L1 membrane topology[69] and, as such, functionally bind PD-1 (refs. [69,71]). A syngeneic mouse model of melanoma further revealed that PD-L1$^+$ EVs prevent the proliferation of PD-1$^+$CD8$^+$ T cells, and consequently the number of tumor-infiltrating lymphocytes[69]. Expressed in a concentration-dependent manner, PD-L1$^+$ EVs are upregulated by soluble IFN-γ and correlate to overall tumor burden[69,71]. The identification of EVs as vessels for PD-L1 has emerged as a prominent subclass of EV-mediated immune suppression. Exosomal PD-L1 in immune evasion has been well reviewed elsewhere[74].

In addition to functional membrane-bound PD-L1, small EVs can carry miRNAs and enzymatically active Arginase-1 (ARG1), which both directly impact T cell activation, proliferation, and cytokine release via T cell receptor (TCR) downregulation[70,75]. Transfer of miR-498 in vitro directly impacts the release of tumor necrosis factor (TNF) release in CD8$^+$ T cells in metastatic melanoma[75]. Meanwhile, miR-3187-3p can suppress CD45 membrane expression and consequent TCR activation in melanoma (Fig. 3)[75]. ARG1, found in ovarian carcinoma EVs, has emerged as a metabolic mechanism for T cell dysfunction. A catalyst for the urea cycle, ARG1-mediated depletion of L-arginine suppresses T cell immune response in vitro by downregulating the TCR complex component CD3ζ (ref. [76]). and by causing cell-cycle arrest in the G1 phase via RICTOR in the mTORC2 complex[77,78]. Isolated from human plasma and ascites, ARG1$^+$ ovarian cancer EVs actively suppress T cell proliferation, both directly and via DC antigen crosspresentation[70]. ARG1$^+$ cancer EVs have potentially far-reaching effects in T cell inhibition, as ARG1$^+$ macrophages have been observed to actively arrest T cell proliferation in the absence of TCR signaling[77].

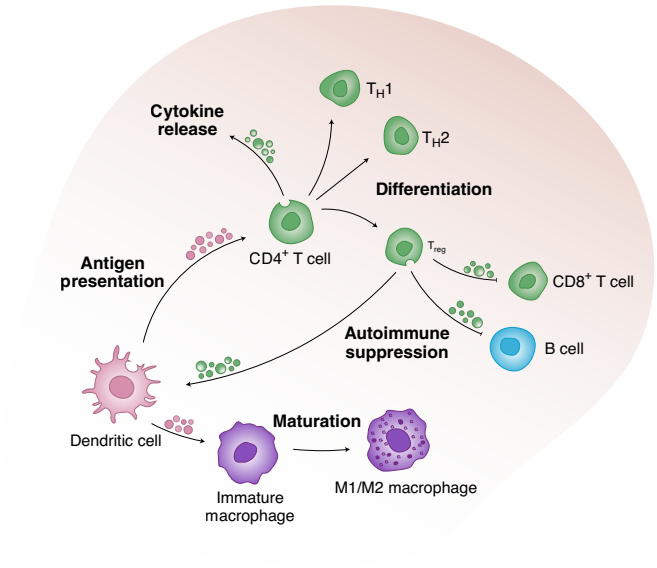


Fig. 2 | Mechanisms of EV immune regulation. EVs released by immune cells constantly balance immunoregulatory and autoimmune responses of surrounding cells in a highly interwoven network of immune responses. EVs promote cell maturation, polarization, and differentiation in T cells and mononuclear cells. Encapsulation and stabilization of EV-associated cytokines further extend the functional capacity of immune EVs by regulating cytokine release and downstream proliferation.

There are also indirect routes for EV-mediated suppression of T cells. Since $CD8^+$ T cells primarily undergo activation and proliferation via APC upregulation of MHC I, indirect activation via APCs presents an alternative avenue for EV-mediated suppression[11]. Knockdowns of G-protein Rab27a using short hairpin RNA in an in vitro irradiated prostate-cancer model revealed a predominantly immunosuppressive role for EVs during antigen crosspresentation[79]. Inducing a non-native $CD73^+$ phenotype to DCs, EVs derived from irradiated prostate cancer promote an adenosine-mediated suppression of $CD8^+$ T cell activation via impaired DC antigen presentation[79].

While EVs and their contents can drastically affect immune responses, it is important to understand where those signals originate. A significant body of evidence suggests external factors unique to the tumor microenvironment regulate the content and quantity of cancer EVs[80–83]. Consequently, environmental stresses are often reflected by EV protein and RNA expression[81]. Hypoxia, an intrinsic property of desmoplastic and late-stage cancers, promotes increased secretion of EVs often rich in immunosuppressive proteins and miRNAs[82,84–86]. A syngeneic mouse model of macrophage infiltration revealed that hypoxic small EVs isolated from B16F0 melanoma cells promote an anti-inflammatory M2-like phenotype in infiltrating macrophage cells[82]. Furthermore, small EV transfer of miR-103a and Let-7a upregulated under hypoxic conditions promote M2-like polarization in lung and melanoma cancers[80,82], the latter working through the downregulation of an insulin–AKT–mTOR signaling pathway[82]. Meanwhile, microvesicular miR-23a and TGF-β carried by hypoxia-induced EVs have implications for natural killer (NK) cell suppression via downregulation of CD107a and NKG2D in vitro (Fig. 3)[86]. Although chemical and physical cues may regulate EV content, the mechanism through which EVs systematically disseminate in tissue may also have implications for the downstream immunoregulatory capabilities of EVs.

Strikingly, EVs may acquire innate diffusive capacities within the tumor microenvironment. Physically restricted by a biocompatible nanoporous matrix, labeled EVs from mouse mesenchymal stromal cells were observed to readily diffuse through an otherwise spatially confined environment[83]. Indicative of mechanosensing properties, changes in the matrix stiffness and stress served to enhanced EV transport through the nanoporous matrix. This enhanced diffusion is also accompanied by physical deformation of the EV itself[83]. Mediated by the transport of water through aquaporins, AQP1 depletion increased the Young's modulus (that is, the stiffness) of EVs and reduced diffusivity within the porous matrix[83]. Interestingly, whether and how the physical properties of the tumor microenvironment can affect EV production, cargo, and function remain unexplored. Further research into the impact of biomechanical cues—including stromal matrix stiffness[87], topological cues[88,89], and fluid shear stresses[90,91]—on EV content and function could also elucidate why many preclinical immune therapies based on EVs fail to return clinical benefits.

The role of extracellular vesicles in the antitumor immune response

While cancer EVs can mediate tumor progression, they also play an immunogenic role in the body's response to cancer[32,92–101]. Cancer EVs carry tumor-associated antigens (TAAs), damage-associated molecular patterns (DAMPs), and other cargo, and these are taken up by immune cells and leveraged to mount an antitumor response[32,92,93,95–99] (Fig. 1). Cancer EVs deliver TAAs and peptide–MHC complexes (pMHC) for antigen presentation and tumor-specific T cell stimulation[92,94,96,98,101]. More recently, lymphoma and melanoma EVs have been found to affect antigen-processing machinery in DCs. Carrying mucin 1 (MUC1), NADPH oxidase 2 (NOX2), and reactive oxygen species (ROS), cancer EVs may alkalinize the DC phagosomal compartment via ROS production, making antigen processing within the DC more efficient[92,101]. Cancer EVs may also carry DNA fragments, including *GAPDH* gene fragments and mtDNA, which induce maturation in DCs via the cGAS–STING pathway[95,98,100]. However, the presence of dsDNA in small EVs has been disputed. Shown to coisolate with small EVs, dsDNA may not be trafficked by small EVs at all, but instead released primarily and independently through a similar endosomal mechanism[102].

Cancer EVs can also exert immune-stimulating effects directly on effector cells[103]. Melanoma EVs can increase IFN-γ secretion, promote proliferation, and increase the tumor-cytotoxic activity of NK cells[104]. Displaying an activating NKp30 ligand BAG6 on their surfaces, melanoma EVs can trigger an antitumor response in NK cells upon binding[104]. Furthermore, pancreatic and colon carcinoma EVs carrying heat-shock protein 70 (Hsp70) stimulate NK cells to secrete granzyme B, an apoptosis factor, in vitro[105]. In the long term, however, this effect is reversed with tumor EVs promoting a decrease in cytotoxic activity[106]. The ability of cancer EVs to directly stimulate effector cells is dependent upon the presence of adhesion and costimulatory molecules on their surfaces, in addition to the maturation status of the target cell[107]. The mechanisms by which cancer EVs stimulate effector cells require further study.

EVs contribute to the immune response in several other ways. For example, glioma EVs reduce the presence of T_{reg} cells at the tumor site, attenuating inherent immune suppression[108]. EVs from non-small lung cancer activate mast cells in vitro and increase TNF and CCL2 production[109]. Immune-cell-derived EVs can also directly carry out antitumor functions[110–113]. $CD8^+$ T cell EVs are cytotoxic and can directly kill tumor cells[111,112]. Furthermore, $CD8^+$ T cell EVs deplete mesenchymal tumor cells via the transfer of cytotoxic miR-298 to prevent invasion and metastasis[111,112]. DC EVs from people with hepatocellular carcinoma reduce T_{reg} cells at the tumor site[113]. Macrophages have also been found to suppress tumor immune evasion

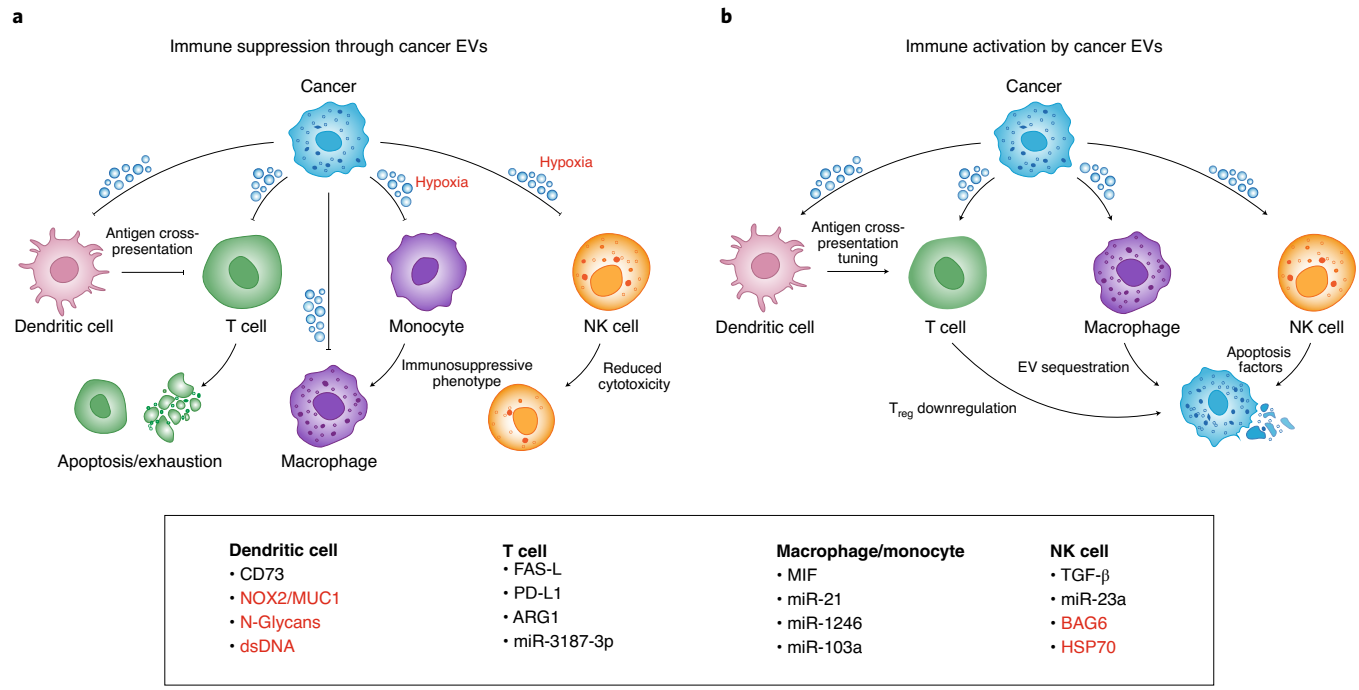


Fig. 3 | Cancer EVs directly regulate tumor progression. a, EVs released by cancer cells promote tumor progression through the suppression of adaptive and innate immune cells. Impeding effective antigen crosspresentation in DCs, EVs also contribute to T cell dysfunction through check point inhibition. Additionally, cancer EVs polarize mononuclear cells towards an immunosuppressive phenotype, while reducing cytotoxicity in NK cells. Less restricted by physical constraints, cancer EVs demonstrate far-reaching immunosuppressive effects on circulatory and distal immune cells. **b**, Cancer EVs function as vessels for tumor recognition. During a functional immune response, EVs deliver foreign antigens to dendritic cells indirectly stimulating cytotoxic T cells, while macrophages actively sequester cancer EVs from circulation, promoting cancer-cell recognition. Molecules shown in red stimulate immune activation; molecules shown in black are immunosuppressive cargo.

by taking up melanoma EVs to prevent their tumor-promoting interactions with B cells[110].

Several studies have noted discrepancies in function between soluble factors and their vesicular counterparts. Tumor antigens seem to be more efficiently taken up by immune cells when associated with EVs rather than in soluble form[92,94,98]. Additionally, vesicular Hsp70 can contribute to radiotherapy resistance in tumors[114], while immunization with non-vesicular Hsp70–peptide complex is associated with more positive effects[115,116]. These findings cast doubt on whether functions are always correctly attributed to EVs instead of soluble factors. This is especially concerning since it is difficult to segregate soluble factors from EV preparations[102]. While ideal single EV subtype models and validation techniques remain largely elusive, effects attributed to EV populations should always be extensively validated through multiple isolation and analysis techniques[35].

Clinical applications of extracellular vesicles in immunotherapy, drug delivery, and prognostics

The immunotherapeutic potential of EVs was first demonstrated in 1998 (ref. [10]). The properties (that is, antigen presentation and costimulatory molecules) that made EVs a functional communication pathway have since been widely explored, leading to the inception of a new class of EV-centered cancer immunotherapies. After more than two decades of probing EV function in immune–cancer interactions, the field has turned its focus to the engineering and targeting of EVs for cancer therapy and diagnosis. As more immunological and oncogenic characteristics of EVs come to light, new therapeutic targets and mechanisms will become available (Fig. 4).

The involvement of EVs in cancer–immune cell crosstalk presents many opportunities for cancer immunotherapy. Proposed therapies use natural and engineered EVs to enhance the existing immune response to cancer, or to inhibit their immunosuppressive functions[117,118]. Currently, there are no approved EV-based immunotherapies. However, a considerable number of clinical trials have started over the past 5 years.

EVs are ideal candidates for drug development and delivery due to their biocompatibility, stability, targeting capabilities, and scalability. EVs are enriched in adhesion and signaling molecules, which allow them to hone to target cells and stimulate uptake[62]. The presence of transmembrane CD47 permits EVs to avoid immune rejection via CD47–SIRPα 'don't eat me' signaling[119]. This immune-escape mechanism contributes to the extended circulation time of EVs in comparison with that of free drug or cell-based therapies, which are more susceptible to immune clearance. In addition to their extended half-life, EVs demonstrate greater cellular targeting and uptake than does free drug delivery[120,121].

A major advantage of EVs over cell-based therapies is their amenability to specialized and scaled-up production. Several platforms are being developed for scaled-immunoprecipitation (IP) production and purification of EVs[122,123]. Recently described methods even allow for rapid, automated collection and surface modification of EVs on a microfluidic device[124]. For internal cargo modification, several methods have been established, including sonication, electroporation, and passive diffusion[125]. Furthermore, EVs are better suited to long-term storage than are cells, experiencing limited loss of function[126,127]. Cytokine-release syndrome (CRS), however, remains a safety concern for the use of EVs in cancer immunotherapy. CRS is a potentially fatal reaction to immunotherapies that target T cells[128]. While it has not been investigated or observed in EV therapies, CRS should be taken into account during the development of EV-based cancer immunotherapies.

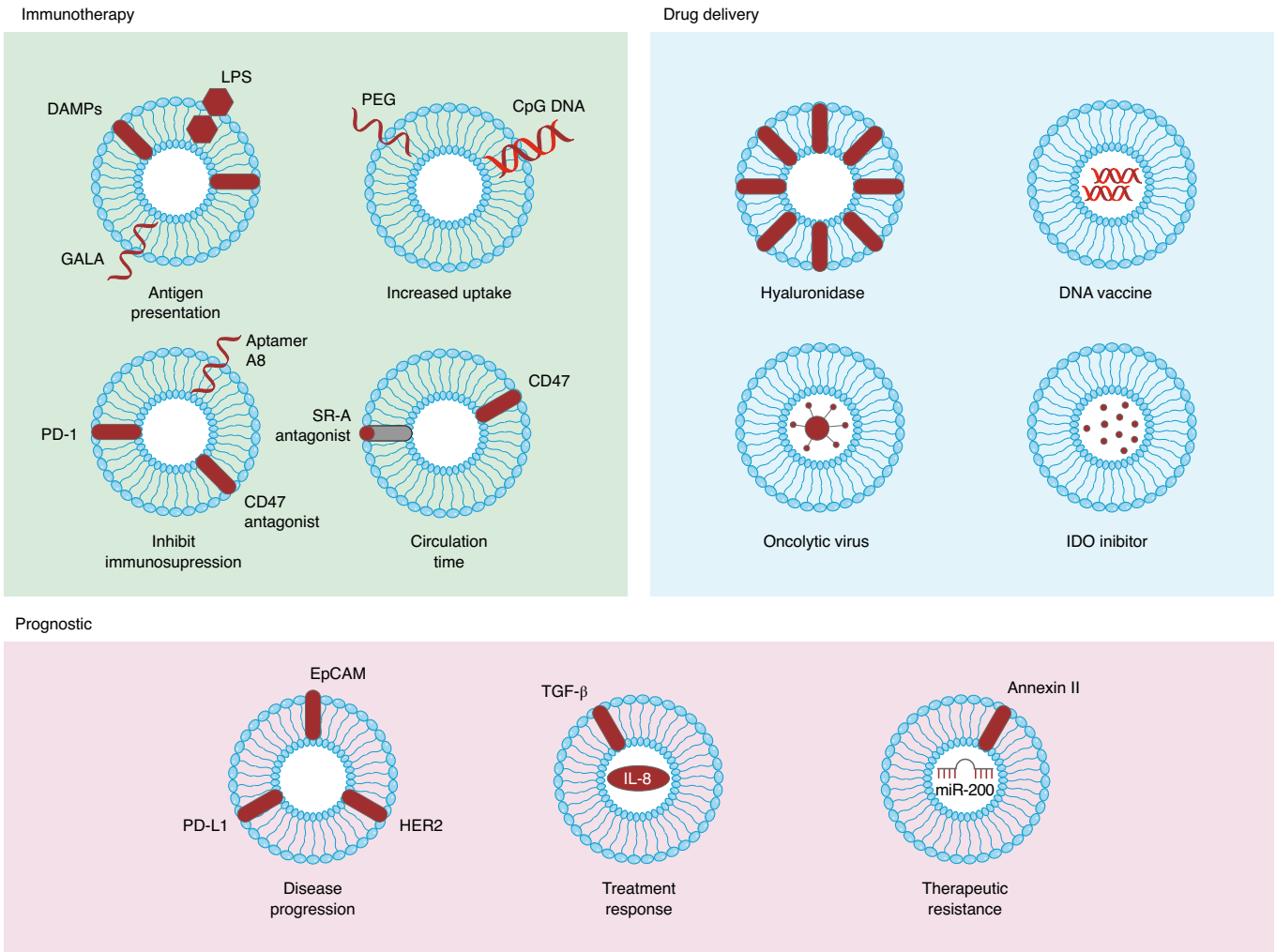


Fig. 4 | Potential clinical translations for EVs. EVs have emerged as powerful tools for cancer treatment. Synthetic and reappropriated natural EVs carry bioactive molecules that enhance the immunological roles of EVs, indicate disease prognosis, and facilitate drug delivery to the tumor site. These capacities are the focus of several ongoing clinical trials.

The presence of TAAs on cancer EVs makes DCs a major target for cancer EV-based immunotherapy[129–135]. Treatment with EVs from colorectal carcinoma, glioblastoma, myeloid leukemia, renal carcinoma, melanoma, tongue carcinoma, and lung cancer has demonstrated increased T cell activation, proliferation, tumor infiltration, and tumor cytotoxicity with simultaneous decrease in immunosuppression and tumor growth[129–136]. Proposed treatments collect, modify, and return tumor EVs to improve in vivo targeting of DCs and enhance the immune response[129,131–135,137]. Melanoma EVs have been engineered to present immunostimulatory CpG-DNA for preferential uptake by DCs[133,137]. Upregulation of IL-12 (ref. [132]) or the addition of miR-155 (ref. [129]), pH-sensitive fusogenic GALA peptide[134], or DAMPs[135] to cancer EVs enhances antigen presentation and immune activation by DCs. Myeloid leukemia EVs have also been used to condition DCs ex vivo prior to DC vaccination to improve efficacy of treatment[131] (Fig. 4).

Other EV-based therapies inhibiting tumor progression involve EV-mediated immunosuppression strategies that preserve their immunostimulatory functions[93,130,132,138]. Depletion of suppressive factors such as Siglec-9 ligands[130] or TGF-β[93,132,138] in glioblastoma, colon carcinoma, and leukemia EVs resulted in increased uptake by DCs and an enhanced antitumor immune response. Subjecting colon cancer cells to heat stress produces EVs enriched in Hsp70 that stimulate IL-6 production in DCs, increases T_H17 polarization, and decreases T_{reg} polarization, leading to an enhanced immune response[139]. Similarly, irradiated hepatoma cells secrete EVs enriched in TAAs, like CDCP1, and DAMPs, including Hsp70 and Hsp90 (ref. [96]). Another potential target to inhibit tumor EV-mediated immune suppression is myeloid-derived suppressor cells (MDSCs)[140]. Hsp70 on breast, lung, and ovarian cancer EVs was found to bind TLR2 on MDSCs, stimulating EV-mediated suppression of T cells and NK cells. Addition of the peptide aptamer A8 blocks TLR2 binding and may prevent immune suppression by MDSCs[140].

Immune-cell-derived EVs also have potential for cancer immunotherapy. DC EVs, in particular, have garnered attention for their ability to stimulate tumor-specific immune responses[141–143]. DC EVs loaded with IFN-γ enhance this effect, increasing secretion of IFN-γ and TNF by NK cells[144]. It was hypothesized that the addition of melanoma antigen recognized by T cells 1 (MART1) to these IFN-γ-enriched EVs might stimulate a tumor-specific response in NK cells, but the effect was small in humans[144]. Other DC EV modifications aim to enhance their immune-stimulating capabilities. Adding TAAs—such as melanoma-associated antigen 3 (MAGE-A3), MART1, glycoprotein 100 (gp100), or HPV16 E7—to the surfaces of DC EVs increases uptake by monocytes

Table 1 | Ongoing and completed clinical trials involving EVs at www.ClinicalTrials.gov

Clinical trial	Identifier
Prediction of Immunotherapeutic Effect of Advanced Non-small Cell Lung Cancer	NCT04427475
Clinical Research for the Consistency Analysis of PD-L1 in Cancer Tissue and Plasma Exosome (RadImm01)	NCT02890849
Clinical Research for the Consistency Analysis of PD-L1 in Lung Cancer Tissue and Plasma Exosome Before and After Radiotherapy (RadImm02)	NCT02869685
Anaplastic Thyroid Cancer and Follicular Thyroid Cancer-derived Exosomal Analysis Via Treatment of Lovastatin and Vildagliptin and Pilot Prognostic Study Via Urine Exosomal Biological Markers in Thyroid Cancer Patients	NCT02862470
Study of Molecular Mechanisms Implicated in the Pathogenesis of Melanoma. Role of Exosomes (EXOSOMES)	NCT02310451
Study of Exosomes in Monitoring Patients With Sarcoma (EXOSARC)	NCT03800121
Exosomes and Immunotherapy in Non-Hodgkin B-cell Lymphomas (ExoReBLy)	NCT03985696
Hemopurifier Plus Pembrolizumab in Head and Neck Cancer	NCT04453046
Study Investigating the Ability of Plant Exosomes to Deliver Curcumin to Normal and Colon Cancer Tissue	NCT01294072
Pilot Immunotherapy Trial for Recurrent Malignant Gliomas	NCT01550523
Antisense102: Pilot Immunotherapy for Newly Diagnosed Malignant Glioma	NCT02507583

and promotes T cell proliferation, stimulation, and cytotoxicity via DCs[124,145]. Increasing alpha-fetoprotein (AFP) expression in DC EVs also enhances tumor immunity. AFP promotes enrichment of MHC I, MHC II, and costimulatory molecules on DC EVs, which induces a change in the tumor microenvironment from immunoinhibitory to immunostimulatory by increasing T cell tumor infiltration and reducing the presence of T_{reg} cells[113]. Similarly, adding ovalbumin, lipopolysaccharides, and IFN-γ to DC EVs promotes the conversion of immunosuppressive M2 macrophages into immunostimulatory M1 macrophages, promotes antigen presentation in DCs, and directly activates T cells[146].

EVs from NK cells and macrophages have also been investigated for immunotherapy. NK cell EVs contain FasL and TNF, making them cytotoxic to cancer cells[147,148]. Vesicles generated from NK cell membranes also exhibit these properties[147]. Modifications to enhance tumor attack include priming the NK cells with IL-15. This treatment increases TNF-related apoptosis-inducing ligand (TRAIL), and expression of the activatory receptors NKp46 and NKp30 on NK cell EVs, leading to more efficient tumor targeting and cytotoxicity[149]. Macrophage EVs have been employed for antigen presentation to DCs. The addition of hyaluronic acid (HA), 3-(diethylamino) propylamine, monophosphoryl lipid A, and MUC1 trigger uptake by DCs and the release of TAA in the endocytic compartment for improved antigen presentation and T cell activation[150].

Clinical trials for extracellular-vesicle-based cancer diagnostics and therapy

Early clinical trials investigating the use of EVs in cancer immunotherapy have shown little more than that EVs are safe for human use[141–143]. This has not deterred further attempts, however, as our knowledge of EV function continues to expand. Current clinical studies aim to determine the effects of immunotherapy, among other treatments, on vesicular cargo, including PD-L1 and miRNA expression profiles (Table 1; NCT04427475, NCT02890849, NCT02869685, NCT02310451, and NCT03800121). Other clinical research is investigating the role of EVs in therapeutic resistance (Table 1; NCT02310451 and NCT03985696). EVs are hypothesized to contribute to therapeutic resistance via enrichment of immunotherapeutic targets, like CD20 and PD-L1. A possible solution, currently undergoing early feasibility phase I clinical trials, is to deplete circulating EVs via a proprietary hemopurifier device (Table 1; NCT04453046), neutralizing their immunosuppressive and therapeutic resistance effects. Another phase I clinical trial is investigating the immune modulation and anticancer effects of curcumin-loaded plant EVs (Table 1; NCT01294072). An upcoming phase II clinical trial investigating the use of an antisense oligodeoxynucleotide drug (IMV-001) against insulin-like growth factor type I has shown dependence on TAA-bearing tumor EVs to stimulate antitumor immunity. The IMV-001 drug released from an implanted biodiffusion chamber for the treatment of malignant gliomas, induces apoptotic cell death in surrounding tumor cells. IMV-001 is hypothesized to work together with these TAA-bearing tumor EVs released from apoptotic cells to stimulate antitumor immunity in the surrounding tissue (Table 1; NCT01550523). The results of this treatment strategy have been positive thus far and emphasize the downstream potential for TAA loaded EVs to promote immune response (Table 1; NCT02507583)[151,152].

Engineered and synthetic vesicles are gaining popularity for application in drug delivery, due to their stability and homing capabilities. For example, oncolytic virus is an established cancer treatment, but due to rapid immune clearance, it must be administered locally[153]. Encapsulation of the virus in cancer EVs enables systemic delivery and tumor-site homing for the targeting of cancer metastases[153]. Macrophage EVs have likewise been used to deliver the antitumor drug doxorubicin to the tumor site in vivo[150]. DNA vaccinations may even be targeted to EVs to enhance immunogenicity. An expression plasmid encoding antigen-fused CD63 was able to deliver the antigen onto EVs in vivo, eliciting a stronger antitumor response in a syngeneic lymphoma model[154].

EVs derived from the human HEK293 cell line are another common platform for cancer therapy. HEK293 EVs have been modified with signal regulatory protein alpha (SIRPα) to block CD47 at the tumor site, resulting in increased phagocytosis of tumor cells by macrophages and increased $CD8^+$ T cell tumor infiltration[118]. HEK293 EVs can also be loaded with PH20 hyaluronidase to break down high-molecular-weight HA in the tumor microenvironment. The resulting oligo-HA induces DC maturation via TLR4 activation and elicits a more potent antitumor response[155]. Vesicles made from HEK293 cell membrane expressing PD-1 were used to block tumor PD-L1 and deliver indoleamine 2,3-dioxygenase-1 inhibitor to the tumor microenvironment, resulting in reduced T_{reg} presence and improved antitumor response[156]. Efforts to decrease liver uptake of HEK293 EVs, thus increasing circulation time and tumor-site accumulation, use dextran sulfate to block scavenger receptor class A (SR-A) on the EVs, optimizing their performance[122].

Efforts to engineer synthetic vesicles attempt to recapitulate the tumor-targeting and immune-stimulating properties of EVs, via the repurposing of cell membranes, for instance. Leukocyte membranes have been collected and extruded into vesicles for the coating of synthetic microcapsules. Melanoma cell membranes were similarly used to generate poly(ethylene glycol) (PEG)ylated nanovesicles capable of inducing an antitumor immune response[117]. Synthetic multivalent antibodies retargeted exosomes (SMART-Exos) are modified with tumor antigen and CD3 to induce antigen-specific immune responses.

EV isolation remains bound by labor-intensive purification techniques. Techniques such as IP and size-exclusion chromatography (SEC) supplement the ad hoc standard ultracentrifugation in a laboratory setting. However, extensive sample preparation and incubation steps negate the practical benefits of IP and SEC techniques clinically. While advancements in microfluidic 'chip' isolations promise to bridge this laboratory–clinic divide, it reflects a time-untested methodology.

The quickest route to clinical practicality of EVs is as prognostic biomarkers. Currently limited by low throughput of isolation techniques, human-derived EVs isolated from bodily fluids—whole blood, plasma, urine, ascites, and so on—and tissue biopsy have the potential to be a critical prognostic tool for immunologists. In addition to catapulting EVs into mainstream clinical use, high-throughput EV-isolation methods for liquid and solid biopsies might further reveal systematic vs local EV function. The focus of over 100 completed and ongoing clinical trials, the EV prognostic field is rapidly expanding. EVs from samples could possibly indicate diseases progression, as well as response to therapy, allowing for more personalized treatment.

Characterization of samples has, however, correlated several immune-related vesicular biomarkers with poor prognosis. Vesicular annexin II is involved in the activation of proinflammatory signaling in macrophages leading to breast cancer metastasis. This role and the correlation of vesicular annexin II with breast cancer progression suggest it may serve as a biomarker for breast cancer prognosis (Fig. 4). Similarly, elevated serum levels of vesicular miR-200b and miR-200c have been correlated with the spread of epithelial ovarian cancer to the lymph nodes (FIGO stage III-IV).

In addition to predicting and measuring disease progression, EVs may indicate response to cancer treatment. In general, people with long-term clinical remission of acute myeloid leukemia, as well as people tested after induction of chemotherapy, have lower vesicular expression levels of TGF-β than do those tested at diagnosis. Another study in people with anaplastic astrocytoma found that decreases in vesicular IL-8 and TGF-β correspond to an increase in immune activity after antitumor vaccination. The relationship between vesicular TGF-β and cancer progression is likely due to its role in NK cell suppression. TGF-β may, therefore, serve as a prognostic biomarker for response to chemotherapy.

EVs may emerge as powerful indicators of immunotherapeutic resistance as well[69]. The monoclonal antibody drug trastuzumab is used to treat HER2$^+$ breast cancer, but not everyone responds to the treatment. HER2 and epithelial cell adhesion molecule (EpCAM) present on breast cancer EVs have been found to sequester therapeutic antibodies and interfere with tumor targeting (Fig. 4). Meanwhile circulating vesicular PD-L1 has proved to be a predictor of clinical response to the landmark immunotherapy pembrolizumab (anti-PD-1)[69]. Thus, the presence of vesicular tumor antigens may serve as indicators of immunotherapeutic resistance.

Conclusion

Burdened by low-yield and labor-intensive isolation techniques, the purity, quality, and characterization of EVs remain paramount for both preclinical and clinical research. Preclinical studies have revealed a diverse repertoire of EV functions, particularly with regards to cell maturation, antigen presentation, and immune suppression. Essential to both the antitumor and immune-regulatory responses, the emergence of EVs as immune-checkpoint vessels continues to be an exciting avenue for clinical translation. Moreover, the contribution of the physical properties of the extracellular matrix (for example, matrix stiffness and porosity) to EV secretion, cargo, and consequent immune suppression remain largely unexplored. A holistic approach to immune suppression beginning with environment origin, immune-cell migration, and consequent adaptive immunity may elucidate why and when EVs are able to suppress immune response.

Currently secondary in clinical applications, significant progress has been made in the past five years towards the use of EVs in cancer immunotherapy. Their safety, targeting capabilities, and clinical practicality make EVs an attractive candidate for drug development and delivery. Tumor and immune-cell EVs have shown significant effects on cancer and the antitumor immune response in disease models. The challenge remains, however, to harness these abilities to produce a meaningful benefit to people. For the time being, EVs are better suited to diagnostic and prognostic use than treatment. Future work should focus on developing EVs as adjuvant therapies to combat the side effects and drawbacks of established treatments.

Received: 11 March 2020; Accepted: 3 February 2021;
Published online: 22 March 2021

References

1. Yáñez-mó, M. et al. Biological properties of extracellular vesicles and their physiological functions. *J. Extracell. Vesicles* **4**, 27066 (2015).
2. Colombo, M., Raposo, G. & Théry, C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu. Rev. Cell Dev. Biol.* **30**, 255–289 (2014).
3. Kalra, H., Drummen, G. P. C. & Mathivanan, S. Focus on extracellular vesicles: Introducing the next small big thing. *Int. J. Mol. Sci.* **17**, 170 (2016).
4. Raposo, G. & Stoorvogel, W. Extracellular vesicles: exosomes, microvesicles, and friends. *J. Cell Biol.* **200**, 373–383 (2013).
5. Théry, C. et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J. Extracell. Vesicles* **7**, 1535750 (2018).
6. Niel, G. Van, Angelo, G. D. & Raposo, G. Shedding light on the cell biology of extracellular vesicles. *Nat. Rev. Mol. Cell Biol.* **19**, 213–228 (2018).
7. Xu, R. et al. Extracellular vesicles in cancer—implications for future improvements in cancer care. *Nat. Rev. Clin. Oncol.* **15**, 617–638 (2018).
8. Becker, A. et al. Extracellular vesicles in cancer: cell-to-cell mediators of metastasis. *Cancer Cell* **30**, 836–848 (2016).
9. Raposo, G. et al. B lymphocytes secrete antigen-presenting vesicles. *J. Exp. Med.* **183**, 1161–1172 (1996).
10. Zitvogel, L. et al. Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. *Nat. Med.* **4**, 594–600 (1998).
11. Théry, C. et al. Indirect activation of naïve CD4$^+$ T cells by dendritic cell-derived exosomes. *Nat. Immunol.* **3**, 1156–1162 (2002).
12. Mallegol, J. et al. T84-intestinal epithelial exosomes bear MHC class II/peptide complexes potentiating antigen presentation by dendritic cells. *Gastroenterology* **132**, 1866–1876 (2007).
13. Admyre, C. et al. Exosomes with major histocompatibility complex class II and co-stimulatory molecules are present in human BAL fluid. *Eur. Respir. J.* **22**, 578–583 (2003).
14. Utsugi-Kobukai, S., Fujimaki, H., Hotta, C., Nakazawa, M. & Minami, M. MHC class I-mediated exogenous antigen presentation by exosomes secreted from immature and mature bone marrow derived dendritic cells. *Immunol. Lett.* **89**, 125–131 (2003).
15. Clayton, A. et al. Analysis of antigen presenting cell derived exosomes, based on immuno-magnetic isolation and flow cytometry. *J. Immunol. Methods* **247**, 163–174 (2001).
16. Lynch, S. et al. Novel MHC class I structures on exosomes. *J. Immunol.* **183**, 1884–1891 (2009).
17. Bobrie, A., Colombo, M., Raposo, G. & Théry, C. Exosome secretion: molecular mechanisms and roles in immune responses. *Traffic* **12**, 1659–1668 (2011).
18. Hwang, I., Shen, X. & Sprent, J. Direct stimulation of naïve T cells by membrane vesicles from antigen-presenting cells: Distinct roles for CD54 and B7 molecules. *Proc. Natl Acad. Sci. USA* **100**, 6670–6675 (2003).
19. Alexander, M. et al. Exosome-delivered microRNAs modulate the inflammatory response to endotoxin. *Nat. Cell Biol.* **6**, 7321 (2015).
20. Admyre, C., Johansson, S. M., Paulie, S. & Gabrielsson, S. Direct exosome stimulation of peripheral human T cells detected by ELISPOT. *Eur. J. Immunol.* **36**, 1772–1781 (2006).
21. Fitzgerald, W. et al. A system of cytokines encapsulated in extracellular vesicles. *Sci. Rep.* **8**, 8973 (2018).
22. Qazi, K. R., Gehrmann, U., Jordo, E. D., Karlsson, M. C. I. & Gabrielsson, S. Antigen-loaded exosomes alone induce T_H1-type memory through a B cell–dependent mechanism. *Immunobiology* **113**, 2673–2683 (2009).
23. Tkach, M. et al. Qualitative differences in T-cell activation by dendritic cell-derived extracellular vesicle subtypes. *EMBO J.* **36**, 3012–3028 (2017).

24. Vincent-Schneider, H. Exosomes bearing HLA-DR1 molecules need dendritic cells to efficiently stimulate specific T cells. *Int. Immunol.* **14**, 713–722 (2002).
25. Ruhland, M. K. et al. Visualizing synaptic transfer of tumor antigens among dendritic cells. *Cancer Cell* **37**, 786–799.e5 (2020).
26. Alvarez-Jiménez, V. D. et al. Extracellular vesicles released from mycobacterium tuberculosis-infected neutrophils promote macrophage autophagy and decrease intracellular mycobacterial survival. *Front. Immunol.* **9**, 272 (2018).
27. Danesh, A. et al. Granulocyte-derived extracellular vesicles activate monocytes and are associated with mortality in intensive care unit patients. *Front. Immunol.* **9**, 956 (2018).
28. Xu, Y. et al. Macrophages transfer antigens to dendritic cells by releasing exosomes containing dead-cell-associated antigens partially through a ceramide-dependent pathway to enhance $CD4^+$ T-cell responses. *Immunology* **149**, 157–171 (2016).
29. Becker, P. D. et al. B lymphocytes contribute to indirect pathway T cell sensitisation via acquisition of extracellular vesicles. *Am. J. Transplant*. https://doi.org/10.1111/ajt.16088 (2020).
30. Zeng, F. & Morelli, A. E. Extracellular vesicle-mediated MHC cross-dressing in immune homeostasis, transplantation, infectious diseases, and cancer. *Semin. Immunopathol.* **40**, 477–490 (2018).
31. Huang, L. et al. Exosomes from thymic stromal lymphopoietin-activated dendritic cells promote Th2 differentiation through the OX40 ligand. *Pathobiology* **86**, 111–117 (2019).
32. Kremer, A. N. et al. Natural T-cell ligands that are created by genetic variants can be transferred between cells by extracellular vesicles. *Eur. J. Immunol.* **48**, 1621–1631 (2018).
33. Wei, G. et al. Dendritic cells derived exosomes migration to spleen and induction of inflammation are regulated by CCR7. *Sci. Rep*. **7**, 42996 (2017).
34. Schierer, S. et al. Extracellular vesicles from mature dendritic cells (DC) differentiate monocytes into immature DC. *Life Sci. Alliance* **1**, e201800093 (2018).
35. Kowal, J. et al. Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. *Proc. Natl Acad. Sci. USA* **113**, E968–E977 (2016).
36. Cui, X. et al. Thyrocyte-derived exosome-targeted dendritic cells stimulate strong $CD4^+$ T lymphocyte responses. *Mol. Cell. Endocrinol.* **506**, 110756 (2020).
37. Cai, Z. et al. Immunosuppressive exosomes from TGF-$β$1 gene-modified dendritic cells attenuate Th17-mediated inflammatory autoimmune disease by inducing regulatory T cells. *Cell Res.* **22**, 607–610 (2012).
38. Zhang, M. et al. Inhibition of MicroRNA let-7 depresses maturation and functional state of dendritic cells in response to lipopolysaccharide stimulation via targeting suppressor of cytokine signaling 1. *J. Immunol.* **187**, 1674–1683 (2011).
39. Lindenbergh, M. F. S. et al. Bystander T-cells support clonal T-cell activation by controlling the release of dendritic cell-derived immune-stimulatory extracellular vesicles. *Front. Immunol.* **10**, 448 (2019).
40. Torralba, D. et al. Priming of dendritic cells by DNA-containing extracellular vesicles from activated T cells through antigen-driven contacts. *Nat. Commun.* **9**, 2658 (2018).
41. Chiou, N. et al. Selective export into extracellular vesicles and function of tRNA fragments during T cell activation. *Cell Rep.* **25**, 3356–3370.e4 (2018).
42. Segura, E., Amigorena, S. & The, C. Mature dendritic cells secrete exosomes with strong ability to induce antigen-specific effector immune responses. *Blood Cells, Mol. Dis.* **35**, 89–93 (2005).
43. Kaur, S. et al. CD63, MHC class 1, and CD47 identify subsets of extracellular vesicles containing distinct populations of noncoding RNAs. *Sci. Rep*. **8**, 2577 (2018).
44. Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M. & Toda, M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor a-chains (CD25). *J. Immunol.* **155**, 1151–1165 (1995).
45. Tung, S. L. et al. Regulatory T cell-derived extracellular vesicles modify dendritic cell function. *Sci. Rep.* **8**, 6065 (2018).
46. Tung, S. L. et al. Regulatory T cell extracellular vesicles modify T-effector cell cytokine production and protect against human skin allograft damage. *Front. Cell Dev. Biol.* **8**, 317 (2020).
47. Aiello, S. et al. Extracellular vesicles derived from T regulatory cells suppress T cell proliferation and prolong allograft survival. *Sci. Rep*. **7**, 11518 (2017).
48. Okoye, I. S. et al. MicroRNA-containing T-regulatory-cell-derived exosomes suppress pathogenic T helper 1 cells. *Immunity* **41**, 89–103 (2014).
49. Torri, A. et al. Extracellular MicroRNA signature of human helper T cell subsets in health and autoimmunity. *J. Biol. Chem.* **292**, 2903–2915 (2017).
50. Chen, L. et al. Exosomes derived from T regulatory cells suppress $CD8^+$ cytotoxic T lymphocyte proliferation and prolong liver allograft survival. *Med. Sci. Monit.* **25**, 4877–4884 (2019).
51. Naqvi, A. R., Fordham, J. B., Ganesh, B. & Nares, S. MiR-24, miR-30b and miR-142-3p interfere with antigen processing and presentation by primary macrophages and dendritic cells. *Sci. Rep.* **6**, 1–12 (2016).
52. Sullivan, J. A. et al. Treg-cell-derived IL-35-coated extracellular vesicles promote infectious tolerance. *Cell Rep.* **30**, 1039–1051.e5 (2020).
53. Turnis, M. E. et al. Interleukin-35 limits anti-tumor immunity. *Immunity* **44**, 316–329 (2016).
54. Kim, S. H., Bianco, N. R., Shufesky, W. J., Morelli, A. E. & Robbins, P. D. MHC class II^+ exosomes in plasma suppress inflammation in an antigen-specific and fas ligand/fas-dependent manner. *J. Immunol.* **179**, 2235–2241 (2007).
55. Kim, S. H. et al. Exosomes derived from genetically modified DC expressing FasL are anti-inflammatory and immunosuppressive. *Mol. Ther.* **13**, 289–300 (2006).
56. O Reilly, L. A. et al. Membrane-bound Fas ligand only is essential for Fas-induced apoptosis. *Nature* **461**, 659–663 (2009).
57. Monleón, I. et al. Differential secretion of fas ligand- or APO2 Ligand/TNF-related apoptosis-inducing ligand-carrying microvesicles during activation-induced death of human T cells. *J. Immunol.* **167**, 6736–6744 (2001).
58. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
59. Hiratsuka, S., Watanabe, A., Aburatani, H. & Maru, Y. Tumour-mediated upregulation of chemoattractants and recruitment of myeloid cells predetermines lung metastasis. *Nat. Cell Biol.* **8**, 1369–1375 (2006).
60. Oskarsson, T. et al. Breast cancer cells produce tenascin C as a metastatic niche component to colonize the lungs. *Nat. Med.* **17**, 867–874 (2011).
61. Kano, A. Tumor cell secretion of soluble factor(s) for specific immunosuppression. *Sci. Rep.* **5**, 8913 (2015).
62. Hoshino, A. et al. Tumour exosome integrins determine organotropic metastasis. *Nature* **527**, 329 (2015).
63. Muller, L., Mitsuhashi, M., Simms, P., Gooding, W. E. & Whiteside, T. L. Tumor-derived exosomes regulate expression of immune function-related genes in human T cell subsets. *Sci. Rep.* **6**, 20254 (2016).
64. Clayton, A. & Tabi, Z. Exosomes and the MICA-NKG2D system in cancer. *Blood Cells, Mol. Dis.* **34**, 206–213 (2005).
65. Capello, M. et al. Exosomes harbor B cell targets in pancreatic adenocarcinoma and exert decoy function against complement-mediated cytotoxicity. *Nat. Commun.* **10**, 254 (2019).
66. Mahaweni, N. M., Kaijen-Lambers, M. E. H., Dekkers, J., Aerts, J. G. J. V. & Hegmans, J. P. J. J. Tumour-derived exosomes as antigen delivery carriers in dendritic cell-based immunotherapy for malignant mesothelioma. *J. Extracell. Vesicles* **2**, 22492 (2013).
67. Peinado, H. et al. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat. Med.* **18**, 883–891 (2012).
68. Costa-Silva, B. et al. Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. *Nat. Cell Biol.* **17**, 816–826 (2015).
69. Chen, G. et al. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. *Nature* **560**, 382–386 (2018).
70. Czystowska-Kuzmicz, M. et al. Small extracellular vesicles containing arginase-1 suppress T-cell responses and promote tumor growth in ovarian carcinoma. *Nat. Commun.* **10**, 3000 (2019).
71. Ricklefs, F. L. et al. Immune evasion mediated by PD-L1 on glioblastoma-derived extracellular vesicles. *Sci. Adv.* **4**, eaar2766 (2018).
72. Haderk, F. et al. Tumor-derived exosomes modulate PD-L1 expression in monocytes. *Sci. Immunol.* **2**, 28 (2017).
73. Karwacz, K. et al. PD-L1 co-stimulation contributes to ligand-induced T cell receptor down-modulation on $CD8^+$ T cells. *EMBO Mol. Med.* **3**, 581–592 (2011).
74. Daassi, D., Mahoney, K. M. & Freeman, G. J. The importance of exosomal PDL1 in tumour immune evasion. *Nat. Rev. Immunol.* **20**, 209–215 (2020).
75. Vignard, V. et al. MicroRNAs in tumor exosomes drive immune escape in melanoma. *Cancer Immunol. Res.* **8**, 255–267 (2020).
76. Rodriguez, P. C. et al. Regulation of T cell receptor CD3ζ chain expression byl-Arginine. *J. Biol. Chem.* **277**, 21123–21129 (2002).
77. Van De Velde, L. A. et al. T cells encountering myeloid cells rogrammed for amino acid-dependent immunosuppression use Rictor/mTORC2 protein for proliferative checkpoint decisions. *J. Biol. Chem.* **292**, 15–30 (2017).
78. Rodriguez, P. C., Quiceno, D. G. & Ochoa, A. C. L-arginine availability regulates T-lymphocyte cell-cycle progression. *Blood* **109**, 1568–1573 (2006).
79. Salimu, J. et al. Dominant immunosuppression of dendritic cell function by prostate-cancer-derived exosomes. *J. Extracell. Vesicles* **6**, 1368823 (2017).
80. Hsu, Y. L. et al. Hypoxic lung-cancer-derived extracellular vesicle microRNA-103a increases the oncogenic effects of macrophages by targeting PTEN. *Mol. Ther.* **26**, 568–581 (2018).
81. de Jong, O. G. et al. Cellular stress conditions are reflected in the protein and RNA content of endothelial cell-derived exosomes. *J. Extracell. Vesicles* **1**, https://doi.org/10.3402/jev.v1i0.18396 (2012).

82. Park, J. E. et al. Hypoxia-induced tumor exosomes promote M2-like macrophage polarization of infiltrating myeloid cells and microRNA-mediated metabolic shift. *Oncogene* **38**, 5158–5173 (2019).
83. Lenzini, S., Bargi, R., Chung, G. & Shin, J.-W. Matrix mechanics and water permeation regulate extracellular vesicle transport. *Nat. Nanotechnol.* **15**, 217–223 (2020).
84. Gilkes, D. M., Semenza, G. L. & Wirtz, D. Hypoxia and the extracellular matrix: drivers of tumour metastasis. *Nat. Rev. Cancer* **14**, 430–439 (2014).
85. Diaz, B. & Yuen, A. The impact of hypoxia in pancreatic cancer invasion and metastasis. *Hypoxia* **2**, 91 (2014).
86. Berchem, G. et al. Hypoxic tumor-derived microvesicles negatively regulate NK cell function by a mechanism involving TGF-β and miR23a transfer. *Oncoimmunology* **5**, e1062968 (2016).
87. Kim, D.-H. & Wirtz, D. Cytoskeletal tension induces the polarized architecture of the nucleus. *Biomaterials* **48**, 161–172 (2015).
88. Fraley, S. I., Feng, Y., Giri, A., Longmore, G. D. & Wirtz, D. Dimensional and temporal controls of three-dimensional cell migration by zyxin and binding partners. *Nat. Commun.* **3**, 719 (2012).
89. Khatau, S. B. et al. A perinuclear actin cap regulates nuclear shape. *Proc. Natl Acad. Sci.* **106**, 19017–19022 (2009).
90. Wirtz, D., Konstantopoulos, K. & Searson, P. C. The physics of cancer: The role of physical interactions and mechanical forces in metastasis. *Nat. Rev. Cancer* **11**, 512–522 (2011).
91. Follain, G. et al. Fluids and their mechanics in tumour transit: shaping metastasis. *Nat. Rev. Cancer* **20**, 107–124 (2020).
92. Dionisi, M. et al. Tumor-derived microvesicles enhance cross-processing ability of clinical grade dendritic cells. *Front. Immunol.* **9**, 2481 (2018).
93. Huang, F., Wan, J., Hu, W. & Hao, S. Enhancement of anti-leukemia immunity by leukemia-derived exosomes via downregulation of TGF-β1 expression. *Cell. Physiol. Biochem.* **44**, 240–254 (2017).
94. Rughetti, A. et al. Microvesicle cargo of tumor-associated MUC1 to dendritic cells allows cross-presentation and specific carbohydrate processing. *Cancer Immunol. Res.* **2**, 177–186 (2014).
95. Kitai, Y. et al. DNA-containing exosomes derived from cancer cells treated with topotecan activate a STING-dependent pathway and reinforce antitumor immunity. *J. Immunol.* **198**, 1649–1659 (2017).
96. Lin, W. et al. Radiation-induced small extracellular vesicles as 'carriages' promote tumor antigen release and trigger antitumor immunity. *Theranostics* **10**, 4871–4884 (2020).
97. Squadrito, M. L., Cianciaruso, C., Hansen, S. K. & De Palma, M. EVIR: chimeric receptors that enhance dendritic cell cross-dressing with tumor antigens. *Nat. Methods* **15**, 183–186 (2018).
98. Zhang, H. et al. Cell-free tumor microparticle vaccines stimulate dendritic cells via cGAS/STING signaling. *Cancer Immunol. Res.* **3**, 196–205 (2015).
99. Caruso Bavisotto, C. et al. Immunomorphological pattern of molecular chaperones in normal and pathological thyroid tissues and circulating exosomes: Potential use in clinics. *Int. J. Mol. Sci.* **20**, 4496 (2019).
100. Diamond, J. M. et al. Exosomes shuttle TREX1-sensitive IFN-stimulatory dsDNA from irradiated cancer cells to DCs. *Cancer Immunol. Res.* **6**, 910–920 (2018).
101. Ma, J. et al. Mechanisms by which dendritic cells present tumor microparticle antigens to $CD8^+$ T cells. *Cancer Immunol. Res.* **6**, 1057–1069 (2018).
102. Jeppesen, D. K. et al. Reassessment of exosome composition. *Cell* **177**, 428–445.e18 (2019).
103. Menay, F. et al. Exosomes isolated from ascites of T-cell lymphoma-bearing mice expressing surface CD24 and HSP-90 induce a tumor-specific immune response. *Front. Immunol.* **8**, 286 (2017).
104. Daßler-Plenker, J. et al. RIG-I activation induces the release of extracellular vesicles with antitumor activity. *Oncoimmunology* **5**, e1219827 (2016).
105. Gastpar, R. et al. Heat shock protein 70 surface-positive tumor exosomes stimulate migratory and cytolytic activity of natural killer cells. *Cancer Res.* **65**, 5238–5247 (2005).
106. Li, Q. et al. Bifacial effects of engineering tumour cell-derived exosomes on human natural killer cells. *Exp. Cell. Res.* **363**, 141–150 (2018).
107. Greening, D. W., Gopal, S. K., Xu, R., Simpson, R. J. & Chen, W. Exosomes and their roles in immune regulation and cancer. *Semin. Cell Developmental Biol.* **40**, 72–81 (2015).
108. Scholl, J. N. et al. Characterization and antiproliferative activity of glioma-derived extracellular vesicles. *Nanomedicine* **15**, 1001–1018 (2020).
109. Salamon, P., Mekori, Y. A. & Shefler, I. Lung cancer-derived extracellular vesicles: a possible mediator of mast cell activation in the tumor microenvironment. *Cancer Immunol. Immunother.* **69**, 373–381 (2020).
110. Pucci, F. et al. SCS macrophages suppress melanoma by restricting tumor-derived vesicle–B cell interactions. *Science* **352**, 242–246 (2016).
111. Seo, N. et al. Activated $CD8^+$ T cell extracellular vesicles prevent tumour progression by targeting of lesional mesenchymal cells. *Nat. Commun.* **9**, 435 (2018).
112. Peters, P. J. et al. Cytotoxic T lymphocyte granules are secretory lysosomes, containing both perforin and granzymes. *J. Exp. Med.* **173**, 1099–1109 (1991).
113. Lu, Z. et al. Dendritic cell-derived exosomes elicit tumor regression in autochthonous hepatocellular carcinoma mouse models. *J. Hepatol.* **67**, 739–748 (2017).
114. Ostheimer, C., Gunther, S., Bache, M., Vordermark, D. & Multhoff, G. Dynamics of heat shock protein 70 serum levels as a predictor of clinical response in non-small-cell lung cancer and correlation with the hypoxia-related marker osteopontin. *Front. Immunol.* **8**, 1305 (2017).
115. Gao, Y. et al. Enhancing the treatment effect on melanoma by heat shock protein 70-peptide complexes purified from human melanoma cell lines. *Oncol. Rep.* **36**, 1243–1250 (2016).
116. Blachere, B. N. E. et al. Heat shock protein—peptide complexes, reconstituted in. *J. Exp. Med.* **186**, 1315–1322 (1997).
117. Ochyl, L. J. et al. PEGylated tumor cell membrane vesicles as a new vaccine platform for cancer immunotherapy. *Biomaterials* **182**, 157–166 (2018).
118. Koh, E. et al. Exosome–SIRPα, a CD47 blockade increases cancer cell phagocytosis. *Biomaterials* **121**, 121–129 (2017).
119. Kamerkar, S. et al. Therapeutic targeting of oncogenic KRAS in pancreatic cancer by engineered exosomes. *Nature* **546**, 498–503 (2017).
120. Rana, S., Yue, S., Stadel, D. & Zöller, M. Toward tailored exosomes: the exosomal tetraspanin web contributes to target cell selection. *Int. J. Biochem. Cell Biol.* **44**, 1574–1584 (2012).
121. Sun, H. et al. A multifunctional liposomal nanoplatform co-delivering hydrophobic and hydrophilic doxorubicin for complete eradication of xenografted tumors. *Nanoscale* **11**, 17759–17772 (2019).
122. Watson, D. C. et al. Efficient production and enhanced tumor delivery of engineered extracellular vesicles. *Biomaterials* **105**, 195–205 (2016).
123. Whitford, W. & Guterstam, P. Exosome manufacturing status. *Future Med. Chem.* **11**, 1225–1236 (2019).
124. Zhao, Z., McGill, J., Gamero-Kubota, P. & He, M. Microfluidic on-demand engineering of exosomes towards cancer immunotherapy. *Lab Chip* **19**, 1877–1886 (2019).
125. Luan, X. et al. Engineering exosomes as refined biological nanoplatforms for drug delivery. *Acta Pharmacol. Sin.* **38**, 754–763 (2017).
126. Zhou, H. et al. Collection, storage, preservation, and normalization of human urinary exosomes for biomarker discovery. *Kidney Int.* **69**, 1471–1476 (2006).
127. Jeyaram, A. & Jay, S. M. Preservation and storage stability of extracellular vesicles for therapeutic applications. *AAPS J.* **20**, 1–7 (2018).
128. Shimabukuro-Vornhagen, A. et al. Cytokine release syndrome. *J. Immunother. Cancer* **6**, 56 (2018).
129. Asadirad, A. et al. Phenotypical and functional evaluation of dendritic cells after exosomal delivery of miRNA-155. *Life Sci.* **219**, 152–162 (2019).
130. Dusoswa, S. A. et al. Glycan modification of glioblastoma-derived extracellular vesicles enhances receptor-mediated targeting of dendritic cells. *J. Extracell. Vesicles* **8**, 1648995 (2019).
131. Gu, X., Erb, U., Büchler, M. W. & Zöller, M. Improved vaccine efficacy of tumor exosome compared to tumor lysate loaded dendritic cells in mice. *Int. J. Cancer* **136**, E74–E84 (2015).
132. Rossowska, J. et al. Antitumor potential of extracellular vesicles released by genetically modified murine colon carcinoma cells with overexpression of interleukin-12 and shRNA for TGF-β1. *Front. Immunol.* **10**, 211 (2019).
133. Matsumoto, A., Takahashi, Y., Ariizumi, R., Nishikawa, M. & Takakura, Y. Development of DNA-anchored assembly of small extracellular vesicle for efficient antigen delivery to antigen presenting cells. *Biomaterials* **225**, 119518 (2019).
134. Morishita, M., Takahashi, Y., Nishikawa, M., Ariizumi, R. & Takakura, Y. Enhanced class I tumor antigen presentation via cytosolic delivery of exosomal cargos by tumor-cell-derived exosomes displaying a pH-sensitive fusogenic peptide. *Mol. Pharm.* **14**, 4079–4086 (2017).
135. Zuo, B. et al. Alarmin-painted exosomes elicit persistent antitumor immunity in large established tumors in mice. *Nat. Commun.* **11**, 1–16 (2020).
136. Al-Samadi, A. et al. Crosstalk between tongue carcinoma cells, extracellular vesicles, and immune cells in in vitro and in vivo models. *Oncotarget* **8**, 60123–60134 (2017).
137. Morishita, M., Takahashi, Y., Matsumoto, A., Nishikawa, M. & Takakura, Y. Exosome-based tumor antigens–adjuvant co-delivery utilizing genetically engineered tumor cell-derived exosomes with immunostimulatory CpG DNA. *Biomaterials* **111**, 55–65 (2016).
138. Huang, F. et al. TGF-β1-silenced leukemia cell-derived exosomes target dendritic cells to induce potent anti-leukemic immunity in a mouse model. *Cancer Immunol. Immunother.* **66**, 1321–1331 (2017).
139. Guo, D. et al. Exosomes from heat-stressed tumour cells inhibit tumour growth by converting regulatory T cells to T_H17 cells via IL-6. *Immunology* **154**, 132–143 (2018).
140. Gobbo, J. et al. Restoring anticancer immune response by targeting tumor-derived exosomes with a HSP70 peptide aptamer. *J. Natl Cancer Inst.* **108**, 1–11 (2016).

141. Bell, B. M., Kirk, I. D., Hiltbrunner, S., Gabrielsson, S. & Bultema, J. J. Designer exosomes as next-generation cancer immunotherapy. *Nanomed. Nanotechnol. Biol. Med.* **12**, 163–169 (2016).
142. Pitt, J. M. et al. Dendritic cell-derived exosomes for cancer therapy. *J. Clin. Invest.* **126**, 1224–1232 (2016).
143. Tran, T. H., Mattheolabakis, G., Aldawsari, H. & Amiji, M. Exosomes as nanocarriers for immunotherapy of cancer and inflammatory diseases. *Clin. Immunol.* **160**, 46–58 (2015).
144. Besse, B. et al. Dendritic cell-derived exosomes as maintenance immunotherapy after first line chemotherapy in NSCLC. *Oncoimmunology* **5**, e1071008 (2016).
145. Chen, S. et al. Poly(I:C) enhanced anti-cervical cancer immunities induced by dendritic cells-derived exosomes. *Int. J. Biol. Macromol.* **113**, 1182–1187 (2018).
146. Matsumoto, A., Asuka, M., Takahashi, Y. & Takakura, Y. Antitumor immunity by small extracellular vesicles collected from activated dendritic cells through effective induction of cellular and humoral immune responses. *Biomaterials* **252**, 120112 (2020).
147. Zhu, L. et al. Novel alternatives to extracellular vesicle-based immunotherapy–exosome mimetics derived from natural killer cells. *Artif. Cells, Nanomed. Biotechnol.* **46**, S166–S179 (2018).
148. Zhu, L. et al. Exosomes derived from natural killer cells exert therapeutic effect in melanoma. *Theranostics* **7**, 2732–2745 (2017).
149. Zhu, L. et al. Enhancement of antitumor potency of extracellular vesicles derived from natural killer cells by IL-15 priming. *Biomaterials* **190–191**, 38–50 (2019).
150. Lee, H., Park, H., Noh, G. J. & Lee, E. S. pH-responsive hyaluronate-anchored extracellular vesicles to promote tumor-targeted drug delivery. *Carbohydr. Polym.* **202**, 323–333 (2018).
151. Meng, X. et al. Diagnostic and prognostic relevance of circulating exosomal miR-373, miR-200a, miR-200b and miR-200c in patients with epithelial ovarian cancer. *Adv. Exp. Med. Biol.* **924**, 3–8 (2016).
152. Maji, S. et al. Exosomal annexin II promotes angiogenesis and breast cancer metastasis. *Mol. Cancer Res.* **15**, 93–105 (2017).
153. Muller, L. *et al*. Exosomes isolated from plasma of glioma patients enrolled in a vaccination trial reflect antitumor immune activity and might predict survival. *Oncoimmunology* **4**, e1008347 (2015).
154. Ciravolo, V. et al. Potential role of HER2-overexpressing exosomes in countering trastuzumab-based therapy. *J. Cell. Physiol.* **227**, 658–667 (2012).
155. Battke, C. et al. Tumour exosomes inhibit binding of tumour-reactive antibodies to tumour cells and reduce ADCC. *Cancer Immunol. Immunother.* **60**, 639–648 (2011).
156. Cordonnier, M. et al. Tracking the evolution of circulating exosomal-PD-L1 to monitor melanoma patients. *J. Extracell. Vesicles* **9**, 1710899 (2020).

Acknowledgements

This work was supported through grants from the National Institutes of Health (T32GM008764) to C.M. and the National Cancer Institute (U54CA143868) and the National Institute on Aging (U01AG060903) to D.W. Figures designed with BioRender.com.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence should be addressed to D.W.

Peer review information Zoltan Fehervari was the primary editor(s) on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.