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EXPERT REVIEW

Potential Application of Exosomes in Vaccine Development and Delivery

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ABSTRACT Exosomes are cell-derived components composed of proteins, lipid, genetic information, cytokines, and growth factors. They play a vital role in immune modulation, cell-cell communication, and response to inflammation. Immune modulation has downstream effects on the regeneration of damaged tissue, promoting survival and repair of damaged resident cells, and promoting the tumor microenvironment *via* growth factors, antigens, and signaling molecules. On top of carrying biological messengers like mRNAs, miRNAs, fragmented DNA, disease antigens, and proteins, exosomes modulate internal cell environments that promote downstream cell signaling pathways to facilitate different disease progression and induce anti-tumoral effects. In this review, we have summarized how vaccines modulate our immune response in the context of cancer and infectious diseases and the potential of exosomes as vaccine delivery vehicles. Both pre-clinical and clinical studies show that exosomes play a decisive role in processes like angiogenesis, prognosis, tumor growth metastasis, stromal cell activation, intercellular communication, maintaining cellular and systematic homeostasis, and antigen-specific T- and B cell responses. This critical review summarizes the advancement of exosome based vaccine development and delivery, and this comprehensive review can be used as a valuable reference for the broader delivery science community.

KEY WORDS cancer immunotherapy · exosomes · vaccine development · infectious disease · immune system

ABBREVIATIONS

APP	Amyloid precursor protein
AKT	Protein kinase B
ARDS	Acute respiratory distress syndrome
APCs	Antigen-presenting cells
BACE	β-site APP cleaving enzyme
BCG	Bacillus Calmette–Guérin
CAF	Cancer-associated fibroblast
CARs-	Chimeric antigen receptors
CE	Cholesterol
CD	Cluster of Differentiation
circRNA	Circulating RNA
COPD	Chronic obstructive pulmonary disease
CTL	Cytotoxic T Lymphocyte
DCs	Dendritic cells
DAMPs	Damage-associated Molecular Patterns
EMT	Epithelial-mesenchymal transition
EPI	Expanded Program on Immunization
EGFR	Epidermal growth factor receptor
ESCRT	Endosomal sorting complexes required for transport
ESAT-6	Early secretory antigenic target-6
FGF	Fibroblast growth factor

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GP	Glycoprotein
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
hACER2	Human Angiotensin-Converting Enzyme Receptor II
HSP	Heat shock proteins
ICTV	International Committee on Taxonomy of Viruses
IgD	Immunoglobulin D
IL-7	Interleukin-7
ILV	Intraluminal vesicles
INF-□	Induce natural interferon-□
LCMV	Lymphocytic choriomeningitis
LMP1	Latent membrane protein 1
IPF	Idiopathic pulmonary fibrosis
MCH I	Major histocompatibility complex class I
MDSC	Myeloid-derived suppressor cell
MERS	Middle East Respiratory Syndrome
miRNAs	Micro ribonucleic acids
MMP	Matrix metalloproteinase
MVB	Microvesicles
mTORC	Mammalian target of rapamycin complex
NPs	Nano particles
nSMase	Neutral sphingomyelinase
NK cells	Natural Killer cells
NSCLC	Non small cell lung cancer
PAMPs	Pathogen-Associated Molecular Patterns
PLGA	Poly(lactic-co-glycolic acid)
PS	Phosphatidylinositol
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
RBD	Receptor-binding domain
RdRp	RNA-dependent RNA polymerase enzyme
ROS	Reactive oxygen species
SARS-CoV2	Severe Acute Respiratory Syndrome Coronavirus 2
siRNAs	Small interference RNAs
SM	Sphingomyelin
STAT3	Signal transducer and activator of transcription 3
TAG	Triacylglycerol
TCR	T-cells receptor
TEX	Tumor exosome
TGF- β	Transforming growth factor beta
TLR	Toll like receptor
TNF-ϒ	Tumor necrosis factor-ϒ
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

INTRODUCTION

Vaccination, also known as immunization, traditionally delivers a weakened or inactive form of a pathogen into the human body to induce antibodies and T cell response that protects the individual against infectious disease (1). A vaccine can also be used after infection as a therapeutic agent. Traditionally, there are two types of vaccine- one that contains live attenuated, and the other has inactivated pathogens. A live attenuated vaccine is produce by modifying a disease-producing bacterium or virus in the laboratory (2). These vaccines can replicate and induce immunity but usually do not cause illness (3). An inactivated vaccine is based on either whole viruses or bacteria or fractions of these pathogens. Fractional vaccines are either polysaccharide-based or protein-based. Most polysaccharide-based vaccines are composed of pure polysaccharides cell wall that are derived from bacteria (4). Conjugated polysaccharide vaccines contain a polysaccharide that is chemically linked to a protein, making the polysaccharide a more potent vaccine (5). The protein-based vaccines include subunit or subvirion products and toxoids (inactivated bacterial toxin) (6).

mRNA-based vaccines have been studied for Zika, flu, HIV, cytomegalovirus (CMV), and recently the FDA authorized COVID-19 vaccines from Pfizer-BioNTech and Moderna for emergency use. The surge of diseases like severe acute respiratory syndrome coronavirus (SARS), Middle East respiratory syndrome (MERS), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Ebola, Zika, HIV, all types of cancers, hepatitis, and tuberculosis, to name a few, have driven the growth of the vaccine market over the past 40 years (7). The North American vaccine market is projected to generate 24 billion USD by 2024 (8).

The ongoing vaccine program's efforts have successfully eradicated infectious diseases like polio in the USA and smallpox worldwide (9). This success led the World Health Organization (WHO) to introduce the Expanded Program on Immunization (EPI) in 1974 (10). EPI led to the foundation of promising public health interventions and comprehensive vaccine programs (11). Although progress is impressive, in 2014, nearly 19 million children had not yet received the three doses of the diphtheria-tetanus-pertussis (DTP) vaccine that are required for adequate protection (12). There is room for improvement in global vaccination coverage rates in most developing countries (13). .In contrast, most developed countries have a high child vaccination rate, indicating vaccination remains a widely accepted public health measure in the modern

world (14). However, vaccine hesitancy remains a barrier to maximizing our vaccination rates.

Given that COVID-19 has triggered severe economic loss and enormous human casualties, we urgently need to develop new vaccine strategies and understand how vaccines contain the virus's spread (15). New vaccine technologies have emerged recently, including DNA, mRNA (16), and live vectors (17). In addition, we have gained insight into the epidemiological profiles of many vaccine-preventive diseases. Together, these have significantly changed the objectives and the target of today's immunization strategies (18).

Cell-derived exosomes have emerged as a novel platform for vaccine delivery with high demand in the vaccine research field (19). Exosomes contribute to cell-cell communication and contain active molecules such as lipids, proteins, carbohydrates, and nucleic acids (16, 17). Recent studies confirmed crucial roles for exosomes in both physiological and pathophysiological processes, including antigenic presentation (20), pathogen immune surveillance (21), intercellular signaling (22), alternative secretion of protein and RNA (23), and infectious agent shuttling (22, 24). However, further investigation is required to elucidate the exact biological functions of exosomes. A recent study confirms that exosomes act as key players in viral pathogenesis (25, 26). Exosomes can transfer active molecules between cells and modify target cells (27). Li *et al.* (28) demonstrated that exosomes derived from nonpermissive liver nonparenchymal cells (LNPCs) could induce antiviral activity *via* releasing interferon-α (IFN-α) against hepatitis B virus (HBV). Exosome proteomic and lipidomic analysis also demonstrated that exosomes carry various biomacromolecules, including proteins, lipids, full-length viral RNAs, and regulatory RNAs (e.g., miRNAs and small interfering RNAs) (16, 29). Cheng *et al.* (30) found that exosomes derived from macrophages treated with *mycobacterium tuberculosis* induce antigen-specific IFN-γ and interleukin 2 (IL-2)-expressing $CD4^+$ and $CD8^+$ T cells. This exosome vaccine application can cause a similar T helper cell type 1 (Th1) immune response but a more limited Th2 response than the Bacillus Calmette–Guérin (BCG)-vaccine, providing better protective immunity.

Exosomes have also been shown to modulate cancer progression. In the case of tumor-derived exosomes (TEX), exosome cargo carries a pro-EMT (epithelial-mesenchymal transition) program, including hypoxia-inducible factor 1 alpha (HIF1α), β-catenin, caveolin-1, and transforming growth factor-beta (TGF-β) that enhance migratory capability and invasion of recipient cells (31). Thus, TDEs contribute to premetastatic niche formation and stromal remodeling. TDEs also carry endogenous tumor antigens and induce antitumor immunity *via* transferring tumor antigens to antigen-presenting cells (APCs) like dendritic cells (DCs) (32, 33). Therefore, TDEs could potentially be applied as a cancer vaccine to induce tumor antigen-specific immune responses without purification of tumor antigens (34). Additional evidence by Koyama *et al.* indicates that exosomes derived from tumor cells that are genetically modified to express early secretory antigenic target-6 (ESAT-6) from *Mycobacterium tuberculosis* act as a potential cancer vaccine (35).

In chronic infectious lymphocytic choriomeningitis (LCMV) disease, $CD8^+$ cytotoxic T lymphocyte (CTL) exhaustion is a critical factor that limits the removal of virus-infected cells (36). Previous studies found that exosome-mediated T cell-based vaccines counteract T cell anergy and convert $CD8^+$ CTL exhaustion in chronic infection *via* CD40L signaling through the mammalian target of rapamycin (mTOR) complex 1 pathway (37). This strategy could significantly improve host defense by restoring the CTL response and facilitating virus elimination to resolve chronic infectious diseases.

In this review, we discussed the potential of exosomes as a vaccine development and delivery method. We also discuss current vaccine development challenges and how exosomes are suitable for mitigating or advancing the vaccine research field. We also discuss the role of exosomes in cancer, infectious and respiratory diseases, and how DC and mesenchymal stem cell exosomes modulate the immune response and tumor metastasis.

MECHANISMS OF THE HUMAN IMMUNE SYSTEM

A pathogen can spread through water supply contamination, air inhalation, physical contact, or the exchange of body fluids like sexual intercourse or blood transfusion (38). The mechanism of pattern recognition of these pathogens (toxic, microbial, virus, and bacteria) can be separated into two general categories: 1) response encoded by germline genes of the host, and 2) response encoded by somatic rearrangements to assemble antigen-binding molecules (39). Our immune system has two major arms: the innate immune and the adaptive immune system (Fig. 1) (40). The innate immune system provides an immediate first-line defense, and adaptive immunity represents a more specialized protective immune mechanism. Both systems work in a dynamic interplay (40). The regular routes for a pathogenic organism to gain entry to the body are *via* our nose when we breathe or *via* our mouth while we eat food and drink water, else through the skin by injection or insect

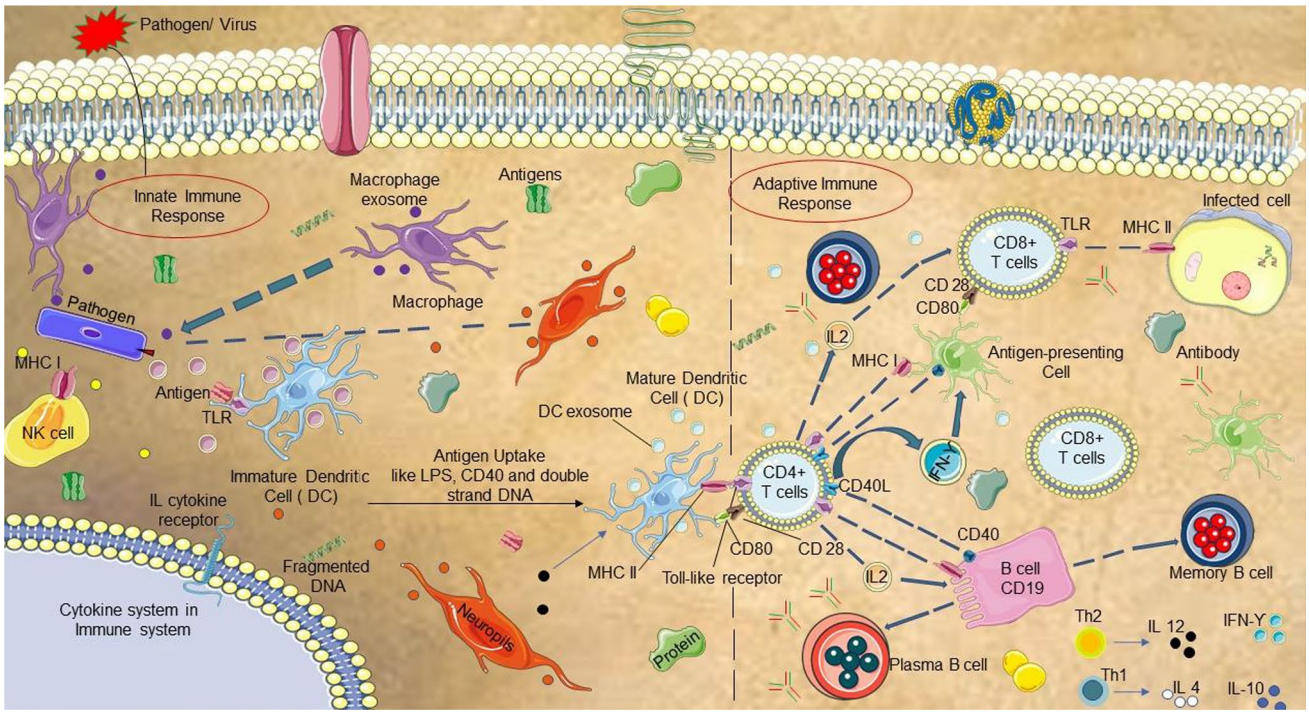

Fig. 1 The immune system is our first line of defense. The figure is inspired by the studies done in the immunology field (51–54). The figure illustrates how innate and adaptive immune systems crosstalk each other against harmful chemicals or pathogens. When our body is invaded by any pathogen, our innate immune system responds to it first. Infected cells call for help, and macrophages, neutrophils, and NK cells induce the pathogen suppression cycle. Downstream signaling pathways activate our adaptive immune system. Antigen-presenting cells, like DCs carry pathogen antigen and present both helper T cells like Th1 and Th2 and cytotoxic T cells $CD4^+$ and $CD8^+$ cells. Then B cells produce an antibody with the help of $CD4^+$ cells (55). B cells preserve or carry the memory of specific antigen via memory B cells with the help of T cells. And our blood serum carries antibodies to protect our body from future invasion by the same pathogen.

bites (41). For example, a pathogen that we breathe into our nose and throat has to survive many chemical and physical assaults, including mucus, cilia on cells that line our airways, and predatory cells called phagocytes (42). All these defenses are designed to prevent viruses or pathogens from getting into blood circulation or our body cells. Even if the first-line defense mechanism does not work, our body still has plenty of other protections. Viruses must borrow cellular components required for their replication, and our innate defenses act against those cell-invading viruses (43). When a pathogen enters into a cell, it is quickly recognized as a foreign body (44). Infected cells also send signaling molecules to the neighboring cells for precaution and signaling our innate immune system (40). Cells also prevent the virus from being replicated inside it *via* the innate immune system. When all these defenses fail, the infected cell induces an apoptotic signal cascade. By doing so, cells can remove the resources required by the virus and inhibit it from transmitting to neighboring/distant cells (40). Our innate immune system is encoded in our genes, modeling physical and chemical barriers and activating chemical signals and pathogen-eating cells (45). While the innate immune response activates, it also enables more specialized lipids, cytokines, chemokines, and signaling cascade (46). The adaptive immune system develops as people are exposed to pathogens and other potentially harmful substances (46). If the immune defense is acquired, adaptive responses have a distinctive characteristic that the innate immune systems lack (47). Immunological memory is remarkable because this is how our body remembers or informs the cells to help eliminate the pathogen in the first infection, and to train our body to recognize it more quickly if we become infected again (48). When our body becomes infected by a pathogen, our adaptive immune system recognizes specific antigens encoded by that pathogen via creating immunological memory (49). Epitope fragments, a foreign body known as antigen, are found on pathogen surfaces, and bind with the receptor of immune cells (49). Antigen-presenting cells (DCs, and macrophage), which are part of the innate immune system, have the responsibility of surveying for antigens and if found carrying them to lymph nodes (50). In lymph nodes they present these antigens to T cells activating an adaptive

immune response. The key players for recognizing foreign substances are called T lymphocytes and B lymphocytes originates from the bone marrow (50) (Figs. 2 and 3).

T or B cells start destroying the pathogen from which the epitope was obtained or killing cells that the pathogens are identified (60). Some other types of T cells regulate other immune cells and prevent the killing of healthy cells. Both T and B cell types can be recruited to the site of infection by chemical signals that cascade during the early stages of the disease by innate immune responses (61). Our adaptive immune response clears a pathogen by killing infected cells and the pathogen circulating in the blood with the help of T and B-cells (60). The most advanced feature of the adaptive immune response is that they can remember the epitope of each pathogen that attacks our body and can respond more rapidly in the future; if that specific pathogen or its strains infects us. Immunological memory is crucial in preventing us from getting infected by a pathogen always present in the population (62). Therefore, we are protected from viruses like measles or chickenpox if we were infected in our earlier life. In some cases, we are protected for life, but in other cases, the T and B cells that provide the long-term memory either do not develop well or do not seem to live as long (63). For example, many upper respiratory tract diseases caused by viral infections do not confer good long-term memory (64). In such cases, vaccines act in part as a proxy for the primary infection so that you obtain memory cells without getting infected. We understand vaccine development is a significant success for immunization from the above discussion, especially against infections, cancer, and immune diseases (65).

IMPORTANCE AND CHALLENGES OF VACCINE DEVELOPMENT

Prevention and treatment are both relative terms in vaccine development. Prevention has two facets, including avoiding contact with an infectious agent and abrogating further spread. Efficient prevention depends on a solid understanding of the dynamics of transmission of contagious disease. Treatment has fundamentally different goals, including improving patient quality of life, reducing the spread of infection, and curing the infection. In the 18th and 19th centuries, smallpox was one of the major killers of the human species (66). In the eighteenth century, variolation (inoculation of a scratch wound with material from a smallpox pustule) became common to protect people from the deadly effects of natural smallpox disease (67). Then famous English physician Edward Jenner first observed a strain of the cowpox virus circulating, which caused a mild infection in humans and gave immunity against smallpox (68). Cowpox inoculation protected people from smallpox and displayed lesser side effects than variolation (68). From that idea, the term vaccine evolved, which allows immunization to stimulate protective immunity (68).

According to a 1998 report from the CDC, there was >95% decline in the eight most common infections in the US due to the introduction of vaccines (69). However, incremental changes due to the seasonal prevalence of infectious or respiratory diseases

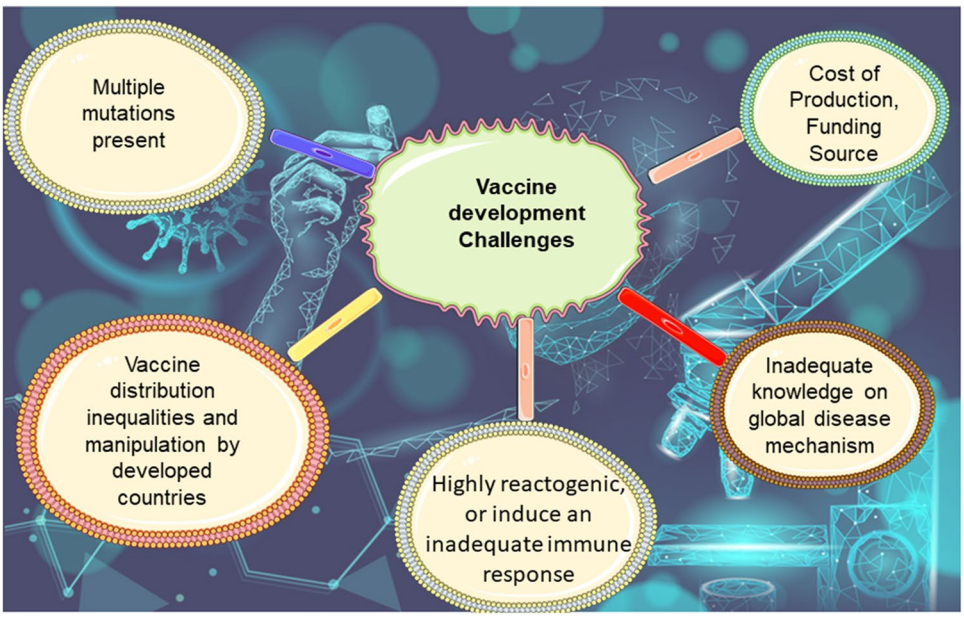

Fig. 2 We summarized some challenges for vaccine development across the globe. The most critical factors that hinder the vaccine development industry are illustrated. Many vaccines fail due to excessive immune response or inadequate immune response in the human body, and some deadly diseases have different pathology across the world. Some pathogens include multiple strains and continuously bring new mutations across the globe. Due to time limitations, some vaccine studies are suspended due to the decline of funding support. We also found that some vaccine studies fail due to a suboptimal clinical trial design.

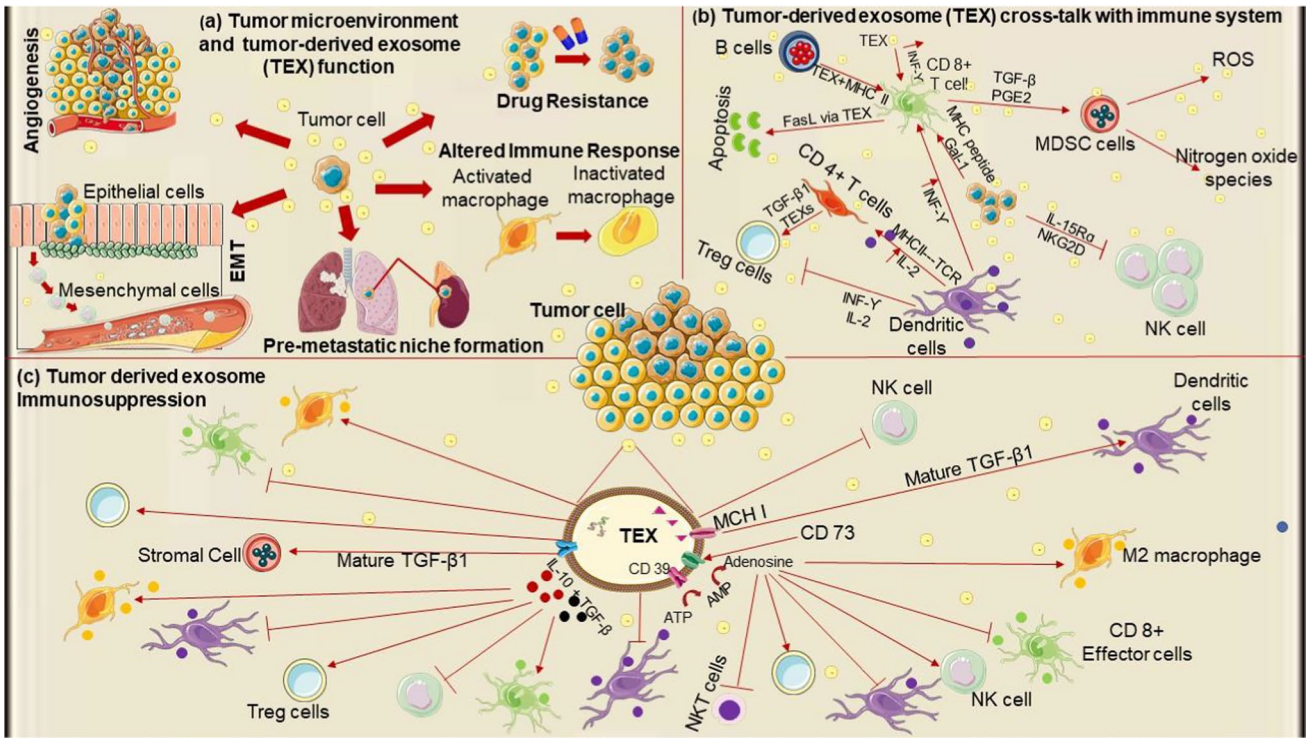

Fig. 3 Tumor microenvironment is a very dynamic and complex structure. In Fig. 3, we are inspired by recent studies on how tumors escape the immune system, tumor-derived exosome (TEX) role in immune modulation, and finally, how tumor exosomes have both excitatory and excitatory tumors inhibitory mechanisms towards our immune modulation (56–59). (**a**). Most tumor cells escape immune networks ***via*** proliferating angiogenesis, EMT, altered immune cell activation state, and drug resistance. TEX plays a crucial role in this dynamic tumor microenvironment. (**b**). both types of immune cells interact with tumor-derived exosomes and have activated downstream signaling pathways. TEX inhibits NK cell function but activates CD 8+ T cell immune activity ***via*** MHC I receptor. TEX also plays a role in CD 4+ T cell activation by the antigen-presenting DC ***via*** conjugating the TCR-MHC II receptor. CD 8+ cells induce ROS and NOS signaling in MDSC with the influence of TEX. (**c**) In figure c, we summarized how TEX modulates the immune cell's activation state and function depending on the type of receptor interaction and cytokines presence. For example, TEX inhibits NK cell activity, but other TEX activity activates NK cell activity via adenosine signaling pathways. Similarly, TEX's effector T cell activity inhibits, but IL-10 and mature TGF- β activate effector T cell's function. Figure 3 demonstrates how TEX interacts with our immune system in every step and guides us DC or mesenchymal stem cell-derived exosome could utilize as vaccine delivery and platform in the future.

often make vaccine development more challenging. Vaccines confer protection through a variety of mechanisms, which extend beyond protecting the immunized individual from infection. Firstly, many vaccines (such as the influenza vaccine) effectively prevent severe disease, despite conferring only moderate protection against infection (reference: https://www.cdc.gov/flu/vaccines-work/vaccineeffect.htm). Secondly, vaccines given to the infant's mother will transiently protect a breastfed child, even though they will not develop their own immune protection (70). Thirdly, vaccinated individuals are less likely to transmit the disease to others. Thus, the impact of global vaccination on mortality rate reduction is muchmore significant than its effect on reducing the number of cases among vaccinated people (71). The Global Alliance for vaccine and immunization organization estimates that immunity to *Hemophilus* influenza type B has averted nearly 700,000 future deaths (72). The WHO estimates that one in five children would have died before age five due to vaccine-preventable diseases (73). Thus, vaccine development has an immeasurable positive potential impact on health worldwide. The Coalition for Epidemic Preparedness Innovation (CEPI), an international nongovernmental organization funded by the Wellcome Trust, the European Commission, eight countries (Australia, Belgium, Canada, Ethiopia, Germany, Japan, Norway, and the United Kingdom), and the Gates Foundation support the development of vaccines against a prioritized list of infectious diseases (including COVID-19, Lassa fever, MERS, Nipah, Ebola, Rift Valley fever and Chikungunya). CEPI supports platform technologies with the potential to accelerate vaccine development in response to outbreaks of infectious disease, proposing to release a product for clinical trials within 16 weeks of the identification of an antigen and demonstrating effectiveness in large-scale manufacturing and suitable elimination of typical unpredicted immune response across pathogens (74).

Despite a better understanding of the immune response to infectious diseases and cancer immunotherapy, many vaccine candidates fail due to a high degree of reactogenicity, creating safety issues, or failure of the candidate vaccine to elicit an appropriate immune response for protection. Although animal models like mice, guinea pigs, and rabbits may exhibit protective immunity after vaccination, immunodominant antigen recognition may differ in these models compared to humans (75). Immune cells like DCs and macrophages are activated by pattern recognition receptors, such as TLRs, and activate multiple downstream pathways to influence the adaptive immune response; this process shows some variation between humans and rodents (76, 77). Rodents tolerate much higher doses of endotoxin (100,000-fold) compared to humans (78). The balance of circulating leukocytes and neutrophils shows significant differences in rodents *vs.* humans, with humans showing a higher proportion of neutrophils (76). In terms of adaptive immunity, there are some minor differences in antibody sub-classes between humans and rodents (e.g., IgA1 & IgA2 in human serum *versus* IgA in murine serum) (79). There are many other differences between the immune system of humans and rodents, too numerous (76). Besides, physiological differences between humans and animals can lead to disparity when well-controlled animal studies advance human trials (75, 80). Despite these differences, the rodent response to vaccines is broadly like that of humans, and mouse and rat models have proved to be a critical tool in the early vaccine development pipeline (81). The use of additional animal models, including rabbits, guinea pigs, ferrets, and non-human primates, may generate additional confidence in the vaccine's performance before proceeding to clinical trials in humans (81).

Over the last decade, scientific advancements have allowed for identifying vaccine antigens for new emerging viruses and pathogens. Most of the time, antibody responses against surface molecule markers determine the vaccine's efficiency for preventing infection (82). For example, in a bacterial infection, the antibody which binds with a surface antigen undergoes opsonization and complement-mediated lysis for phagocytosis (83). Thus, vaccine targeting surface antigens need to be strain-specific and have a multivariant formulation, which is mutated frequently (84, 85). A new generation of vaccine adjuvants has emerged via interaction with pathogen-associated molecular patterns (PAMPs) like bacterial DNA fragments and cell wall components, including lipopolysaccharide and peptidoglycan, which activate pattern-recognition receptors (PRRs), including Toll-like receptors (TLR), NOD-like receptors, and C-type lectin receptors (86, 87).

However, conventional vaccine development may not be feasible during a pandemic or epidemic situation. The unpredictable nature of emerging pathogens poses a significant problem in this context. For example, in a new influenza pandemic, a known pathogen mutates and adapts to a new host environment with unpredictable outcomes for its immunization. The same is true for SARS-CoV-2, which required expedited, concurrent vaccine safety and efficacy trials. In the current COVID-19 pandemic situation, multiple virus variants have been identified worldwide (88). Coronavirus can also be spread through the air as tiny aerosols. In addition to bigger droplets, coughing and sneezing produce microscopic particles called aerosols, which linger in the air for prolonged periods. A person can become infected from a distance of more than two meters in this case. During an epidemic situation like the current COVID-19 pandemic, the timeline to development of a new vaccine candidate can be reduced using next-generation sequencing by identifying antigens, comparatively in short period of time.

In contrast to the typical duration of vaccine development, which takes many years, the FDA approved 3 COVID-19 vaccines for emergency use within one year of the WHO declaration of the COVID-19 pandemic, and one of these vaccines recently received full approval. The mRNA vaccines for COVID-19 were developed more quickly than any other vaccine in history. The first one is from Pfizer/BioNTech mRNA vaccine (*BNT162b2*) with 95% efficacy in neutralizing COVID-19 symptoms in 44,000 participants (89). The second vaccination, known as Moderna mRNA-1273 vaccine, was found 94.1% effective in a study conducted in 30,000 subjects (90). The third is from the Janssen COVID-19 vaccine Ad26.COV2.S with 66.3% effective trial and trial participant number 44325 (91). None of the vaccines trial or participants receiving a single or double dose of vaccines has reported any severe side effects associated with the vaccine. Recently, the vaccine of Janssen company was temporarily paused due to 6 observations of rare blood clots in vaccine recipients. However, after an investigation by the FDA and CDC approved a single booster dose of the Janssen (Johnson and Johnson) COVID-19 Vaccine for individuals 18 years and older on October 20, 2021. Respective safety monitoring boards have been established for each vaccine, and FDA and other specialist panels have regularly evaluated safety data.

As of October 2021, in total, 190,793,100 persons have been fully vaccinated, accounting for 58% of the population. Vaccine hesitancy remains a significant barrier to fully vaccinating our population. Additionally, we await authorization for children under 12 to

receive these vaccines. On a global scale, however, some developing countries have limited access to vaccines. Another factor that limits the distribution is the cost of production. Using current technologies, the cost of establishing facilities containing the necessary equipment ranges from approximately 600 to 1200 million dollars (92). A further challenge is the specific methods and techniques used, which often are insufficient to support global vaccination (71). Inadequate access to vaccines can cause two million deaths each year, and two-thirds of this number are children under five years old (93). Currently, 93% of the 260,000 annual deaths from cervical cancer and over 99% of the 440,000 yearly deaths from rotavirus-associated diarrhea occur outside the 60 wealthiest countries (94). Thus, scientific, ethical, and financial challenges against vaccine development are considerable, and we must continue to break through these barriers to fight against future epidemics.

KEY STRATEGIES OF VACCINE DEVELOPMENT

Vaccine development against infectious, autoimmune, respiratory diseases, and cancer immunotherapy have changed human history by reducing mortality (95). Two prime examples include eradicating polio in the USA and the near eradication of smallpox globally (96). To date, all vaccine development strategies reduce disease progression by achieving active immunity via the targeted population's adaptive immune system and vaccine effectiveness (97).

We are bringing the SARS-CoV-2 vaccine development strategies as an example. In December 2019, a severe illness causing pneumonia and death was first reported in Wuhan, Hubei, China. After that, this illness spread across 200 countries, causing 244 M people to be infected and 4.9 M deaths to date (98). These numbers are increasing every day until we find a complete cure for this deadly disease. The WHO announced "coronavirus disease 2019 (COVID-19)" as its official name (99). Viral genome analysis reveals its phylogenetic similarity with SARS-CoV; thus, the International Committee on Taxonomy of Viruses (ICTV) designated it "SARS-CoV-2" (100). During this initial stage of the COVID-19 outbreak, some potential antiviral drugs, steroids, and monoclonal antibodies are administered to patients depending on the variation of symptoms. Some examples include favipiravir (T-705) (101, 102), remdesivir (Veklury)(GS-5734) (103–105), chloroquine/Hydroxychloroquine (106, 107), lopinavir, dexamethasone, bamlanivimab (LY-CoV555), Casirivimab and imdevimab (REGN-COV2), and ritonavir (108, 109). All these drugs, antibodies, and antibiotics are currently under clinical trials or administered to patients with FDA approval. But after getting data from patients' critical conditions and side-effect of some drugs in COVID-19 patients, FDA has revised their authorization (110). For example, Lopinavir/ritonavir, and darunavir/cobicistat haven't demonstrated clinical benefit in patients with COVID-19 (111). Adverse effect for lopinavir/ritonavir includes Nausea, vomiting, diarrhea (typical), QTc prolongation, and hepatotoxicity. FDA approves Remdesivir approves Remdesivir to administer to hospitalized adult and pediatric patients (aged ≥12 years and weighing ≥40 kg). Due to severe side-effect both hydroxychloroquine and chloroquine, the FDA revoked both emergency use in June 2020. In a pandemic situation like COVID-19, vaccine development could only control the situation. For vaccine development, precise recognition between viral surface protein and host receptor is an important target and will reveal cross-species transmission and host tropism (112). The SARS-CoV-2 Spike (S) protein binds to ACE2, allowing the virus to infect human cells (112, 113). The S1 subunit of the S protein contains a receptor-binding domain (RBD), and the S2 subunit is necessary for membrane fusion between host cells and viruses (15, 114). Clover Biopharmaceuticals tests a recombinant subunit vaccine consisting of the trimeric S protein (S-Trimer) SARS-CoV-2 (115). GSK and Clover Biopharmaceuticals announced a partnership to improve immune response by introducing GSK's adjuvant system to S-Trimer. DNA vaccines directly injected plasmids encoding the antigens, applied with prophylactic vaccines and therapeutic vaccines (116). The DNA platform uses adjuvant to enhance the immune responses and electroporation to deliver plasmids (117). INOVIO Pharmaceuticals and Beijing Advaccine Biotechnology partnered to develop a DNA vaccine (INO-4800) against COVID-19 and start pre-clinical trials (118). The mRNA technique is another advanced vaccine platform that can treat infectious diseases and cancers. mRNA-based vaccines contain mRNAs encoding the antigens and are translated into the host cellular mechanism via vaccination (119, 120). mRNA vaccines have an advantage over traditional vaccines, including improvement of the immune response, absence of genomic integration, rapid development, and production of multimeric antigens (120). Moderna, Inc. has completed clinical trials in collaboration with the National Institute of Allergy and Infectious Diseases (NIAID) on the mRNA vaccine (mRNA-1273) encoding viral spike (S) protein of SARS-CoV-2 (121). Genexine Inc. is developing a COVID-9 vaccine based on the Hyleukin-7 platform, which enhances the immune

responses by fusion of interleukin-7 (IL-7) to hyFc, and is designed to hybridize IgD and IgG4 for long-acting effects of Fc fusion proteins (122). Most vaccines available are based on inactivating/killing, live attenuated, or weakening technologies. Scientists now tune current vaccine technologies via isolated protein, subunit, split product, peptide, DNA tether, mRNA, and live vector technologies (71, 123–125). Together with the vaccine methods mentioned above and some hurdles to overcome, we can enhance vaccine development for diseases like SARS, MERS, COVID-19, infectious diseases, and cancer metastasis.

The development of the smallpox vaccine was a revolutionary achievement in the scientific world, and we will explain vaccine development strategies using the smallpox vaccine as an example. A more substantial variola DNA virus can cause a smallpox infection (126). Several important observations made during the smallpox outbreak helped to rationalize vaccine development. First, an individual who recovers from smallpox was resistant to the subsequent disease. Second, Inoculated into a scratch wound (variolation)may prevent further infection (127). The observations conclude that our defense system is versatile at recognizing and invading foreign bodies (128). While designing a vaccine, the immune defense system could be primed with a miniature, weakened version of the pathogen. In the past, health care professionals from Asia used variolation methods using inoculation of a small amount of dried or fresh smallpox materials from the nose or skin. Data suggest that the inoculation method was exceptionally effective (128)

Before the variolation concept was present in Asia, England physician Edward Jenner proved the first vaccine concept. According to the journal Baylor University Medical Proceedings 2005 report, Edward in 1996 vaccinated a child named James Phipps with pus taken from a cowpox pustule (128). However, this treatment concluded that a milkmaid infected with cowpox did not get infected with smallpox. Jenner's treatment went down in history as he first introduced vaccination for infectious diseases. These observations above led to an intense vaccination campaign conducted between 1960 and 1970 to help eradicate smallpox from the USA (127). The pathogen collected for vaccine application was prepared by inactivating or killing the virus by overheating (129). For example, the hepatitis A vaccine is also an inactive virus. When the virus is inactivated, scientists sometimes reverse or rearrange the structures. Some vaccines allow the pathogen to replicate, which helps with further recognition by T-memory cells (129). Another exciting field uses a vaccine carrying a subunit of pathogen structure to guide the vaccine to identify critical molecules of virus or bacterial structure (52). The hepatitis B virus vaccine is a prime example of this approach. Due to infection or vaccines, individuals produce antibodies in the immunization process, which results in direct protection against subsequent infection (130).

The incorporation of nanoparticles as a vaccine delivery vehicle can enhance vaccine efficiency by improving blood circulation half-life and reducing immunogenicity (131). Surface contact, encapsulation, and surface adsorption, related to vaccine design, are the foundations of this nanoparticle coating (132). Antigen adsorption depends on the surface hydrophobicity of nanoparticles (131), whereas encapsulation depends on both the physical and chemical interaction of vaccine carriers and nanoparticles (133). For example, the H1N1 antigen of influenza conjugate with chitosan NP and *Yersinia pestis* F1-antigen coated gold NPs (AuNPs) demonstrate a higher antibody level and cytokine response than unconjugated vaccines (134). Also, the studies showed that nanoparticle-delivered vaccines offer better antigen presentation and delivery (135). In the Moderna mRNA Covid-19 vaccine, four different fatty molecules have formed a protective capsule around the RNA, ensuring safer delivery and preventing degradation (136). We will discuss some factors that need to be considered while preparing nanoparticles for vaccine delivery.

Better reproduction and effectiveness can be achieved by taking advantage of the effects of physicochemical properties. Although the physiological and morphological properties are the critical parameters for vaccine stability and delivery, a challenge is the scale-up of the production of particles with uniform size and vaccines carrying nanoparticles are also required to be under a particular dimension (137). A study by Wendorf *et al.* showed that 100–110 nm PLG nanoparticles carrying antigens from Neisseria meningitides type B (MenB) results in higher efficiency in targeting and comparable immune response compared with naïve vaccine antigens (137). Another study by Benne *et al.* reviewed nanoparticles' size, shape, and rigidity loaded with vaccine tether or antigens, how these factors enhance T cell immune response, and how nanoparticles provide a roadmap to rational delivery of efficient vaccine (138). The surface charge affects the distribution and cellular uptake of particles. The surface charge plays a critical role in determining the type and amount of particle coronas. Given that the cell membrane is negatively charged, the antigen delivery of particles is influenced by the particles with a different charge (positive) (139). Although the positively charged particle should be attracted to the negatively charged

cell membrane, there have been discrepancies in the correlation between particle charge and cell interaction. Cationic liposome-regulated immune responses rely on surface charge density (140). Negatively charged liposomes can act as an adjuvant to promote cell-mediated responses (141). Particle charges on the APCs responses compared with three kinds of nanoparticles (NP); negatively charged N-NP, neutrally charged M-NP, and positively charged P-NP (141), excluding the surface charge, uniform size, and physicochemical properties were assured in the study. The amount of cellular uptake correlated with the surface charge of the particle (142). Cationic NPs cause more reliable mitochondrial and lysosomal damage and disruption of plasma membrane integrity than negative NPs (142). Surface charge and shape can be implemented into one single particle with the concept of component control. Cell membrane potential varies from −40 to −70 mV depending on its physiological condition. Nanoparticle coating and the process will differ depending on the type of vaccine delivery and the target site. The shape is an essential property of natural bio-particles. Viruses and bacteria have qualities that determine their infection efficacy and replication. The investigation for shape effect has been lagged the size and charge of the particles (133). The shape has become an important parameter to consider when designing micro and nanoscale particles. Non-spherical forms of particles have limited material choices and complicated techniques. Though the method for preparing non- spherical shaped particles are complicated, worm-shaped particles have prolonged blood circulation that enhanced organ accumulation (143). Nanospheres and nanorods can be seen to interplay with different types of cells. Rod-shaped particles have the advantage of being quickly internalized by cells. Particle internalization can be a complicated process, but micropinocytosis and phagocytosis were the predominant methods that mediate uptake of these non-spherical particles (144). Particle shape regulated intracellular distribution as well as the cytokine profile response. Altering the shape or charge of a single particle can achieve a specific function, carrier, or targeting stimulation (145). Hydrophobicity plays an essential role in the interaction between APCs and vaccine particles (146). Cell membranes are comprised of a lipid bilayer, which means that they have both a hydrophilic exterior and interior membrane which interact with water molecules. Particles that are prepared with hydrophobic and high molecular weight polymers tend to be more effective in cell interaction (147). To understand the interaction relationship, PLA, PLGA, and PEG-b-PLA were compared; in the results, the hydrophobicity gradually increased, indicating a decrease in macrophage internalization (148). The particles compared above were similar in size but different in hydrophobicity. The comparison of the particles shows that the higher the hydrophobicity, the greater the interaction between particles and cell membranes. Not only does the hydrophobicity increase the exchange, but it promotes the internalization of particles and thereby facilitates vaccine delivery (149). Nanoparticles have chemical functional groups that come from the original component of particle material. The surface modification also modulates the performances of particulate adjuvants. Antibodies have been used to target DCs since DCs are moving targets; the strategy was a practical use of a tumor vaccine (150). When using adjuvants in a vaccine, the particles can induce an effective adaptive immune response and increase survival rates in mice (151). Particle adjuvants can pave the way for vaccines with their promising effects (151). Nanoparticles can be used to prolong immune efficacy, longevity and provide a design concept that can maximize efficiency and decrease any adverse effects. The design of the nanoparticle mediated vehicle for vaccine can be seen to tailor to the high antigen payload, and the potentially enhance effectiveness (133, 152).

So, an effective vaccine induces an immune response, replaces immune potentiators, and primes the immune response against associated antigens. And nanoparticles as a vaccine vehicle can result in higher therapeutic intervention and a more robust immune response. Additionally, surface modification of NPs allows targeting of specific cell types and systematic transport (153). A recent study reveals targeting antigen to DCs is a powerful and novel strategy for vaccination. Of the main types of APC (B cell, macrophages, and DCS), the DCs are the most vigorous and are responsible for initiating all antigen-specific immune responses (152, 153). Additionally, these types of vaccine strategies efficiently extend nanoparticle-trafficking time in vessels and enhance transport.

WHY EXOSOME IS BETTER CANDIDATE FOR VACCINE DEVELOPMENT AND DELIVERY

Exosomes Composition and its Role in the Immune Response and Multiple Diseases Signaling Pathway

Almost all living organisms, including viruses and bacteria, shed exosomes into the extracellular environment (154). Proteomic analysis of the biological fluid reveals that exosomes contain numerous immune

response molecules on the surface and carry biological messengers like proteins, chaperons, mRNA, and DNA fragments (155) (156). Lipids, proteins, nucleic acid-like signal transducer, membrane trafficking, T cell stimulation, and anti-apoptosis molecules found on the exosome surface also have some immune-modulatory effects (157, 158). Additionally, a new study reported that the exosomes are present in the lymph as well. One needs to remember; immune cells receive signals from exosomes of other immune cells, microbes, tumors, and non-immune cells (159). Exosome release from immune cells via Fc gamma receptor, cytokine receptors, TCR, and BCR, helps to raise cytosolic calcium ion concentration.

Exosome biogenesis begins when the endosomal membrane transforms to generate intraluminal vesicles (ILVs) in the lumen of the organelles via- first, with the maturation of the early endosomes to microvesicles (MVB), and second, late endosomes to exosome (160). For transportation, most studied endosomal pathways are associated with endosomal complex (ESCRT-0, ESCRT-I, ESCRT-II, ESCRT-III) and AAA ATPase Vps4 associated complex (161, 162). One study has shown that the depletion of ESCRT-0 protein Hrs and ESCRT-1 protein STAM1 reduces exosomal secretion (163). ESCRT-0 proteins like Hrs, Vsp27/ STAM 1, 2 binds with a ubiquitin-protein programmed for degradation, executing a sorting of MVB in the first steps (164). ESCRT -I (TSG101, Vps28, MVB12a) and ESCRT-II (Vps22,25 and 36) promote the budding process and start the enzymatic de-ubiquitous cargo protein before the formation of MVBs in the intracellular compartment and ILVs (165) (156). On the contrary, knockdown of ESCRT-III and associated protein-such as VSP4B, VTA1, and ALIX resulted in increased exosome secretion (163). Many studies have shown lipids like sphingomyelin (SM), glycosphingolipids, cholesterol, and phosphatidylinositol (PS) (166, 167). Exosome surface contains a high level of triacylglycerol (TAG), cholesterol (CE), cardiolipin, a lipid found within the mitochondrial membrane (168), and TAG and CE were found in the lipid droplet core (166). Several lipids and lipid metabolism enzymes have shown a critical role in the release and formation of exosomes. Neutral sphingomyelinase (nSMase) contributes to forming cone-shaped ceramide, which is essential for exosome secretion (163, 169). Both cell-specific and ubiquitous proteins selectively express exosomes from their native cells. They also include cytosolic proteins like tubulin, actin, flotillin, membrane transport protein like annexin, and RAB proteins (170). They (exosome) also contain signal transduction protein-like protein kinase, heterotrimeric G protein. Even metabolic enzymes like pyruvate and lipid kinase, peroxidase is also present on the exosome surface. Western blot analysis of Exosome reveals less expression of tubulin and actin as these cytoskeleton proteins have a higher expression on the cell surface. Exosomes also carry heat shock proteins like HSP20, HSP27, HSP70, and HSP90 (171), involved in antigen presentation, loading, and binding antigen peptides onto MHC molecules, maturation of DCs, and translocation of NF-κβ into the nucleus through CD91 (156, 172). Interestingly, T cell stimulators like MCH I, MCH II, and CD81 are also available on exosome surfaces, originating from antigen-presenting cells (173). These T stimulatory molecules play a critical role in antigen-specific interaction between B and T cells. DC-derived exosomes carry T cell co-stimulatory molecules CD81 and carry T cell receptors on their surface to activate T-cells (174). Monocytes also release pro-inflammatory cytokines in the presence of soluble HSP70 via CD14 signaling pathways, Nk-cells target HSP70 for cytosolic attack (175). Even high expression HSP70 within tumor cells can kill NK cells compared to the lower expressed HSP70 tumor cells (176). HSP70 participates in inhibiting key effort signaling lings associated with apoptosis and autophagy. Thus in tumors, abnormal expression of HSP70 may participate in resistance to chemotherapeutics and oncogenesis (177). Flotillin is another exosomal protein marker found on the surface in most healthy and cancer cells T-cells express Flotillin-1 & 2, in the absence of added chemokines, forming a large preassemble platform in lymphocyte cell lines (178). Flotillin's C-terminal interactions lead to the heterodimer of FLOT1 and FLOT2, both in naïve and activated T cells (179). Flotillin has been implicated in various cellular functions, including cell-cell adhesion, regulation of G protein coupled receptor signaling, endocytosis, and modulation of the actomyosin cytoskeleton of leukocytes (180, 181). Tetraspanin is another abundant protein family that is available on the exosome surface. Tetraspanin, such as CD9, CD63, CD81, and CD82, interacts with many protein types, including integrin's and MHC class proteins, indicating they involve in large molecular complex organizations and membrane subdomains (182). Among them, CD63 and CD81 are localized to lipid raft in the plasma membrane, and this process is called palmitoylation (183). Tetraspanin is also associated with integrins of exosomes and cell surfaces. Rana *et al.* has demonstrated that preferential interaction between Tspan8 with α4 and β8 integrin chain and colonies of tetraspanin and integrin proteins strongly influence targeting cell selection *in vitro* and *in vivo* (184). In the integrin family, exosome contains a series of transmembrane protein including immunoglobulin-family

members (185) such as A33 antigen on enterocytes (186) and P-selectin on platelets, intercellular adhesion molecule 1 (ICAM1)/CD54 on B cells (187)), α- and β-chains of integrins (such as αM on DCs, β2 on DCs (188) and T cells, and α4β1 on reticulocytes), cell surface peptidases (189) (such as dipeptidyl peptidase IV/ CD26 on enterocytes and aminopeptidase N/CD13 on mastocytes). Moreover, scientists have been able to identify multiple Rab GTPase such as Rab27a, Rab27b, Rab2, Rab7, Rab11, Rab35, etc., using RNAi screening that promotes exosome secretion in a wide range of cell lines (190).

Exosome release from viral cells also carries viral miRNA, proteins, and even entire virion modulates adjacent cells and impacts immune recognition by the virus. Hepatitis C virus (HCV) membrane of the Flaviviridae, exosome isolated from HCV infected human blood, contains viral RNA co-localize with CD81 and transmit viral RNA to uninfected cells. Exosomes from HCV-infected cells induce natural interferon (INF)-□ response to neighboring DC cells via viral RNA. In contrast, natural circulating HCV infection delivers viral RNA to cells, but with the help of viral NS3/4 protease, it downregulates TLR and RLR signaling (191). These exosomes from HCV-infected cells protect uninfected cells during infection by transmitting viral RNA, not viral protein, which antagonizes the innate immune system. Circulating exosomes that contain HCV virion may possess a pro-viral role, which spreads the disease in the presence of neutralizing antibody (192). The major oncoprotein of EVB (a gamma herpes virus) and latent membrane protein 1 (LMP1) were identified in exosomes isolated from EVB infected cells (193). B lymphocytes transformation required LMP1 proteins. Exosome from B-cells contains LMP1 that inhibits NK-cells cytotoxicity and T cell proliferation (193). Also, exosomes deliver EGFR, PI3CA, and LMP1 induced growth-stimulating signaling pathways in recipient cells and activate the ERK1, PI3 kinase target, and Akt (194). For specific protein expression, we can find out on the ExoCarta website (http://exocarta.org/gene_summary?gene_id=11461). ExoCarta database has around 64,246 exosomal protein, mRNA, miRNA, and lipids entries, including both published and unpublished work. This database contains valuable information describing the characterization, biomarker screening, targeted drug delivery, and vaccine development studies.

This section will discuss studies related to exosome and their immune response during health and disease conditions. Neutrophil-derived exosomes have both anti-inflammatory and pro-inflammatory effects (195). Bacteria can interact with neuropil-derived exosomes, and surprisingly, neutrophil exosomes can bind to bacteria and reduce bacterial growth. NK cell-derived exosomes carry NK cell markers FasL and perforin, depending on cellular homeostasis and physiological condition (196). Antigen-presenting monocytes and macrophages produce abundant exosomes, which plays a vital role in antigen presentation and affects myeloid cell differentiation and proliferation (197). It has been reported that infected macrophage-derived exosomes can transfer pyroptotic caspase-1 (198). Thus caspase-1 can initiate the pyroptotic cycle in recipient cells due to infection. Mast cell-derived exosomes carry functional RNA and can trigger DC maturation. Also, mast cell exosomes induce T and B cell proliferation, contain multiple immune-modulatory proteins, and play a role in antigen delivery of immune cells (198).

DC-derived exosomes actively prime T cells and contribute to antigen presentation. Both mature DC and B cells are required to activate naïve T cells. DCs act as both innate and adaptive immune cells (199). Depending on the type of DC and its activation status, the exosome population changes its surface composition and payload. Mature DCs exosomes are enriched with MHC class II, ICAM-1, B7.2, and depleted in MFG-E8 have a stronger ability to induce an antigen-specific immune response the immature DCs derived exosome (200). Exosomes also modulate different immune cells; for example, mast cell-derived exosomes can cause degranulation and induce T cell proliferation (201). Interestingly, T cell-derived exosomes were found in extracellular spaces and immune synapses (between antigen-presenting cells and T cells). During antigen presentation, B cell-derived exosomes first interact with antigens and modulate cytokine secretion and T-cell activation (201, 202). A distinctive feature of B cell-derived exosomes is that they carry immunoglobulin, which delivers native antigens to neighboring cells. Sometimes, immune cell-derived exosomes carry different pathogen-associated dangerous messages like PAMPs and DAMPs (203). A recent study also shows that immune-derived exosomes carry cytokines like TNF, TGF-β, IL-1α, and IL-1 β. On the contrary, infected cell-derived exosomes can carry viral and bacterial particles (22, 204). These infected cell-derived exosomes can accelerate the acute inflammatory process by recruiting a significant player to regulate inflammation (205). For example, bacterial component fMLP can induce neutrophilic exosomes containing leukotriene B4 and activate neutrophils. This leukotriene B4 is necessary for neutrophil recruitment at the site of inflammation (206). When acute inflammation begins, pro-inflammatory cytokines, for instance IL-1 are predominantly found in exosomes of infected cells (206). Further

evidence shows that other pro-inflammatory cytokines like TNF and its complementary regulatory protein are also released via exosomes (207).

Exosomes also play a critical role in the chronic inflammatory process. For example, DC-derived exosomes perpetuate Th2 cells' responses and behave as antigen-presenting molecules (208). Mast and B cell-derived exosomes drive Th2 responses and promote the Th2 environment. Microphyte-derived exosomes contain LT and recruiting granulocytes, functional inflammatory enzymes, and synthesis leukotriene (209). T cells-derived exosomes stimulate the release of pro-inflammatory cytokines like IFN-γ from Th1 cells (209). IFN-γ is crucial for immunity against tumor and intercellular pathogens. Because IFN-γ is predominantly produced by NK and NKT cells and produced by Th1 CD4 and CD8 cytotoxic T lymphocyte (CTL) effector T cells. Airway epithelium cell-derived exosomes increase the release of cytokines like interleukin 13 (IL-13) from Th2 cells (210). IL-13 is a critical regulator of IgG synthesis, mucus hypersecretion, goblet cell hyperplasia, and fibrosis (210, 211). Exosomes also play a role in auto-immune diseases like type-1 diabetes, hepatitis B, multiple sclerosis, and inflammatory bowel diseases. These exosomes membrane proteins and lipids are essential when we study disease-specific biomarkers and models for vaccine development. Rather than working on a single cell, we can research exosomes derived from cells that provide us with a plethora of information regarding disease conditions.

Role of Exosome Biomarker in Vaccine Delivery

In recent years finding biomarkers for detecting suitable disease target and vaccine delivery have grown exponentially. Biomarker study is based on a biological source like urine, blood, plasma, serum, and body fluid exosome analysis. Exosome contains disease pathogens, antigens, fragmented DNA, miRNA, non-coding RNA, lncRNA, and circRNA are fascinating potential biomarkers (212, 213). Biomarker analysis also applies to tumor diagnosis, metastasis prediction, evaluation of prognosis, and treatment effectiveness. For instance, the level of miR-21, miR150, miR-223, and miR-1229 in colon cancer patients' serum exosomes was significantly higher than healthy control (214). Therefore, the exosome could be used for noninvasive tumor diagnosis. In lymph node metastasis, the concentration of miR-217 level in serum exosomes is considerably low (215). miR-127 acts as a tumor-suppressing agent in multiple cancer models. Thus, serum exosome can act as a biomarker and be utilized to diagnosis the metastasis state. Hoshino *et al.* has found higher expression of ITGβ4 in the exosome of lung metastasis patients *vs* liver metastasis patients (216). PD-1 and PD-L1 are major cancer immune checkpoint biomarkers and have been studied extensively. In triple-negative breast cancer patients, the tumor exosome expresses a higher level of PD-L1 (217). PD-1 level is associated with poor prognosis via exosome analysis of classical Hodgkin's lymphoma, and inhibiting PD-1 can potentially improve patients' prognoses (218). Immunological biomarkers are a critical part of cancer vaccine application. These biomarkers can utilize a potential targeted vaccine delivery via exosomes and avoid unspecific binding and immunosuppression via immune cells. Patients with glioblastoma multiform have different RNA content of serum exosomes compared with the healthy subject (219). Again, circulating exosomes collected from glioblastoma patients showed a higher EGFRvIII mRNA level, which can be considered a diagnostic tool readout (220). Along the same lines, the presence of EGFR on the exosome surface may be considered a possible marker for lung cancer (221). Proteoglycan glypican-1 (GP1) positive exosome have prevalent in the serum of pancreatic cancer patient (222). In breast cancer, exosome biomarkers like miR-130a target TGF-β genes responsible for tumorigenesis, miR-328 related to CD44, reduced cell adhesion, and enhanced cell migration (223). Exosome-mediated miR-10b suppresses the protein level of its target genes KLF4 and HOXD 10, which induce invasiveness in breast cancer cells (224). Circular exosomes and their miRNA content open a new horizon for cancer biomarkers to study both drug and vaccine targeting delivery. For example, miR-200b-5p can be used as a novel biomarker for lung cancer patients after surgical resection to analyze the risk of recurrence of small-cell lung tumors (225). Even two primary colorectal cancer and basal cell carcinoma oncogene PPP3CA and FZD, expression is primarily regulated by miR-100, miR-378a, and miR-629, are found in exosomes (226). Some miRNA plays a vital role in potential long-term pathways like miR-30a by inhibiting the epithelial/mesenchymal transition and targeting gene Snai1, which is involved in metastasis and cell invasion in cancer progression (227). The above discussion suggests that profiling mRNA and miRNA of circulating exosomes can be used as a substitute biomarker compared to biopsy profiling for asymptomatic populations. In another exosome protein profiling study, it was found that higher expression of cell adhesion molecules CD171 and tetraspanin CD151 and TSPAN8 in lung cancer patients compared to that of non-cancer control patients (228). Therefore, the higher-level detection of CD151 signifies the aggression of lung cancer. Exosomal CD151 and TSPAN8 are correlated to initiate metastasis behavior by

modulating EMC to associate molecules. CD171 related to EMT, prognosis, and metastasis are also observed in lung cancer patients (228). The Gng2 gene (one of the gamma subunits of a guanine nucleotide-binding protein) and Fox1 gene (role in regulating tissue and cell-specific gene transcription during development) are significantly upregulated in serum-derived exosome of pancreatic tumor-bearing mice compared with healthy control (229). Exosome plays an important role in cancer progression by promoting cell metastasis and intercellular communication. Exosome has also been studied for non-cancer diseases, including liver (230), lung (231), kidney (232), arteries (233), and CNS (234). Yang *et al.* has found miR-135a (repressed the expression and activity of BACE1) and miR-384 (regulates both amyloid precursor protein and β-site APP cleaving enzyme (BACE-1)) are upregulated, and miR-193b (regulator of amyloid precursor protein (APP) in the cerebrospinal fluid and the blood) are downregulated in serum exosome of Alzheimer's disease patients compared with controls (235). In the idiopathic pulmonary fibrosis study, it has been found that there is a negative correlation between the lungs' carbon monoxide/ alveolar volume diffusion capacity and the saliva-derived exosome containing miR-142-3p (236). miR-142-3p inhibits apoptosis and induced inflammation *via* down-regulation of CoX-2 in bleomycin-induced pulmonary fibrosis model. Goetzl *et al.* found an altered level of LAMP-1, cathepsin D, and HSP70 in preclinical Alzheimer's diseases years before disease onset (237). Pusic *et al.* have demonstrated that interferon ϒ-stimulated rat bone marrow DC-derived exosome contains miRNA-219 stimulate *in vivo* myelination and that can be used for multiple sclerosis diagnosis (238). ,Barutta and colleagues has showed that expression of miR-130, miR-145, miR-424, and miR-155 are significantly altered in diabetic nephropathy patients with type-1 diabetic (239). miR-130 promotes cancer cell invasion and migration *via* AKT and FAK phosphorylation by activating PTEN (240). miR-145 serves as a tumor suppressor gene in various tumor models like ovarian, breast, and colorectal cancers (241). Interestingly, type-2 diabetes is associated with an increased risk of developing cancer of the colon, liver, breast, and bladder.

Exosomes secreted by the tumor cells carry different content and critical molecules than that of exosomes secreted by healthy tissue. Exosomes are abundantly releases from cancer cells and broadly distributed throughout the body *via* systemic circulation. The exosome maintains its stability and carries the disease fragments from one location to the other. Studies have shown that exosomes possess intrinsic advantages in predicting prognosis, metastasis, and therapeutic intervention on tumors (19). Despite numerous reports of significant exosome biomarkers associated with various diseases, unfortunately, studies reported by the individual groups match poorly. Different extraction, isolation, and purification methods and handling contribute to this mismatch. For a biomarker study, it is important to check the specificity and sensitivity of the design study to assure reproducibility and consistency.

Role of Exosome in Stress Response

In recent times application of exosomes in delivery, science has been getting more attention due to their ability to mediate intercellular communication, maintaining cellular and systematic homeostasis. More importantly, exosomes may have improved biosafety compared to polymer-based drug delivery approaches (156). Cellular stress contributes to various environmental stressors like osmotic stress, oxidative stress, hypoxia, infection, and ionic radiation (242). Exosome content and its role vary between the stress conditions and the native state. These stress conditions are resulted in due to DNA damage response or stress-induced protein synthesis (243). In one study, the authors demonstrate that exposure to cyanobacteria with UVA and UVB increases bacterial exosome released in the culture medium. Stress changes the number of exosomes released and alters the molecular composition of exosomes (244). For example, eukaryotic cells trigger exosomes expressing an HSP molecule on the surface due to environmental stress. Due to endoplasmic reticulum stress, BeWo cells carry pro-inflammatory cytokines, DAMPs, HSP70, Histone H3, and HMGB1 (245). In another study, authors exposed cultured endothelial cells with hypoxia, high glucose, and TNF-alpha induce stresses (246). After microarray, immunoblotting, qPCR, and quantitative proteomic analysis, the authors confirm alteration expression in several molecules and mRNAs (246). Even stress-induced exosomes can activate different signaling cascade and apoptosis pathways in healthy cells (246). A recent report, naïve MCF-7 and TILA cells with heat-induced exosomes, resulting in apoptosis *via* DNA damage (247). Another feature of this stress exosomes is that it prolonged distant signaling among organs or tissues (248). For example, in chronic Epstein-Barr virus (EBV) infection, EVB related RNA was detected in circulating exosomes. Exosomes carry cellular waste outside of cells and maintain cellular homeostasis. Under inflammation or stress conditions, cells remove modified fragmented *via* released exosome (249). Also, exosomes do not release cargo in the blood without distant and neighboring cells (250). Therefore, blood is not infected due to disease

cell-derived exosome. Exosomes also carry an anti-coagulant protein called platelet tissue factor, released at the site of the wound.

Another critical function of exosomes is antigen presentation *via* MHC-I and-II, alerting the immune system to the presence of infectious stress (251). Additionally, stress-induced exosomes also play a vital role in activating immune cell chemotaxis. A study reported in 2010 shows that oxidatively stressed exosomes from donor cells are capable of preconditioning and protecting oxidative stress *via* modifying mRNA delivery (252). Up-regulation of HSP proteins occurs when a cell is under stress conditions. Expression of HSP within exosomes can cause protein aggregation in the recipient cells (252). Therefore, exosomes sometimes act as a trojan horse to other cells through transferring harmful molecules- including prion protein, amyloid-beta deposition to neurons, injection of the toll-like receptors, and sensitization of the adaptive immune system. These properties of exosomes carrying stress molecules can be utilized as a liquid biopsy for cancer detection.

These local tissue damages and physiological stressors increase inflammatory proteins in the blood and tissue of the animal body. Our literature review suggests that stress-evoked cytokines and chemokines can both facilitate host survival and endanger our health. Recent studies confirm that exosomes play a vital role in reducing the content of immune inhibitory mRNA and immune stimulatory damage-associated molecular patterns (DAMPs) from systemic circulation (253). Thus, circulating exosomes play a fundamental role in immune homeostasis during stress conditions and vice versa, depending on cellular conditions.

Role of Exosome in Tumor Microenvironment (TME) and Immune Crosstalk

The tumor microenvironment is a very dynamic and complex adaptive system. The intra-cellular exosomes play a role in inducing angiogenesis, tumor growth metastasis, and stromal cell activation (254). Cellular stress in healthy cells can increase exosomes release but cause stress conditions in cancer cells, mainly due to pathological changes (254). Cancer cells bring changes to the neighboring environments like nutrient deficiency, remodeling of the extracellular matrix, and hypoxia. Such changes in the tumor environment trigger changes in exosome molecular cargo (255). One of the hallmarks of cancer cells is vasculature that enhances the transportation of oxygen and nutrients (256). Tumor growth induces hypoxia and triggers the release of pro-angiogenic and anti-angiogenic cytokines like vascular endothelial, fibroblast, pericytes, and endothelial growth factors—continuous remodeling results in leaky blood vessels poor structural organization (257). Cancer cells become non-responsive to radiation and chemotherapeutics, where exosome plays a major role in the defensive mechanism (256). Chemotherapeutics reflux from the exosome through exocytosis process. For example, pancreatic cell-derived exosomes carrying tetraspanin-8 promote vessel branching (258). Tetraspanin-8 also modulates the binding and uptake of cancer exosomes by endothelial cells (56). Prostate cancer exosomes contain insulin growth factor receptor-1, CSEC tyrosine kinase, and focal adhesion kinase FAK, previously reported as angiogenesis promoter (57). Nedawi *et al.* has reported the finding that lung cancer-derived exosomes deliver mutated endothelial growth factor receptor to pulmonary endothelial cells, activated EGF receptor, and signaling through AKT and MAP kinase pathways. This AKT and MAP kinase pathway activation is misleading to VEGF secretion and endothelial cell's response to tumor progression (58). The same research group later demonstrated that exosome treatment could inhibit the angiogenesis of endothelial cells (259). Therefore, cancer cell-derived exosomes could potentially provide effective anti-angiogenic treatment (259). Lucero *et al.* demonstrated that glioblastoma cell exosomes mediated delivery of angiogenic mRNA translated into protein within the recipient cells (260). Another recent study reported that colorectal exosomes deliver angiogenic mRNA to endothelial cells and enhance the proliferation, migration, and tube-like formation (261). The study confirms the exosome capability of delivering pro−/anti-angiogenic factors mRNA in endothelial cells.

The tumor microenvironment is very complex and consists of multiple cells (detail in Fig. 3). Cancer-associated fibroblasts (CAF) undergo differentiation to myofibroblast, facilitating tumor proliferation and CAF manipulation by cancer cell-derived exosomes (262). Cancer cell-derived exosomes can promote the acquisition of CAF and stromal cells. Cancer cell-derived exosomes can also activate MAP kinase pathways *via* TGF-β (263). Luga *et al.* have shown that CD81 positive exosomes from stromal cells activated multiple signaling pathways in the breast cancer cell. This signaling promotes cancer cell motility, metastasis, and tumor growth (264). Boelen *et al.* have that stromal cell-derived exosomes transfer mRNA to breast cancer cells, resulting in the activation of RIG-1 antiviral signaling, resistant to both chemotherapy and radiotherapy (265). Therefore, this mechanism of cross-communication seems to aggravate cancer disease. Cancer cell progression also depends on extracellular matrix remodeling.

Cancer-associated fibroblast secretes both proteins and glycoproteins that regulate ECM composition (266). Matrix metalloproteinase (MMPs) enzyme plays a critical role in maintaining the ECM structure, and the tumor-holding MMPs helps them to bind with ECM and facilitate remodeling (266). Several groups have reported that circulating exosomes from cancer cells invoke changes in a specific organ and prepare a niche for metastasis (267). Along the same line, multiple studies show that the exosomes hold the immune activation property as well.

However, some exosomes have shown the potential of suppressing T cell activation *via* HSP protein and mRNA (201). Cancer exosomes significantly induce the differentiation of DCs without its antigen presentation to the myeloid cell, which produces TGF-β for T cell suppression (268). Further evidence is supported by tumor growth, causing the transition of monocyte to M2 macrophage phenotype (269). Cancer exosomes also partake in metastasis *via* organ tropism and involve the epithelium-to-mesenchymal transition of multiple cell types (269). There is also substantial evidence that exosomes can transfer various growth factors, fibroblast growth, and epidermal growth, and activate numerous signaling pathways in the recipient cells. Al Nedawi *et al.* report exosomes can transfer oncogenic protein EGFR from cancer cells. Recipient cells, after receiving EGFR activates Map-kinase, AKT, and protein kinase B signaling pathways and induce morphological remodeling and accelerate cancer growth (259). This cellular transformation is downstream of EGFRvIII, increases the expression of anti-apoptotic protein BCL-xL. EGFR containing exosomes also triggers VEGF release and activates VEGFR2, and endothelium cells promote angiogenesis (259).

The immune system attacks tumor cells via NK cells, CD8 T cell, antibodies from B cells, macrophages, and neutrophils (270). But tumor cells develop a different invading mechanism to escape immune attack (271). Natural killer cells decrease their cytotoxicity *via* TGF-β and NKG2DL and inhibit T cell activation and T cell's killing capacity *via* NK cell-derived exosomes (272). NK cells can promote the M2 phenotype over M1, which boosts immune escape and tumor growth. NKG2D ligand ULPB2 release ULPB3 containing exosomes and downregulate the NKG2D receptor by their metalloproteinase ADAM10 and 17 (273). Tumor exosomes containing Gelatin9 and LMP1 inhibit T-cells proliferation (274). Melanoma cell exosomes carry FasL, which kills/inhibit T-cells. Also, cancer exosomes provide CD39 and CD73, suppress T-cells *via* adenosine release, and monocytes differentiate into macrophages with the tumor-promoting ability (275). M1 macrophage produces IL-10 stimulates anti-tumor cytotoxic T-cells, and M2 macrophage release IL-12 and stimulate T-reg cells. Co-culture of CD45 positive leukocyte and GR1 expressing CD11B positive myeloid cells derived exosomes inhibits immune cells like T-cells, NK, macrophage, and DCs (276). Tumor cells expressing the upregulation of PDL1, TGF-β, and Arginase-1. When DCs are treated with tumor exosomes, they impair LPS mediated maturation of DCs. Again, tumor-derived exosomes (TEX) carrying HSP70 and HSP105 activates DCs and induces IL-6 releases *via* troll-like receptors 2 & 4 (TLR3 & TLR4) (277). Thus, tumor exosomes promote tumor metastasis *via* STAT3 dependent metalloproteinase-9 (278).

Circulating exosomes from tumor cells interfere with different treatments of cancers. Tumor exosomes carry tumor cell surface receptors to decoy tumor-targeting antibodies (279). For example, HER/HER2 overexpressing exosomes could counteract the effect of tumor therapy. Then exosomes bearing NKG2D ligand A & B and ULBP1 & 2 act as chemiflux against NK cells and impair NK cell function (280). TEX plays an important role in a different stage of the metastasis cascade and invasion *via* promoting angiogenesis, migration, EMT transition, induced drug resistance, and establishing a pre-metastatic niche (31, 281) (Illustrate in Fig. 3 a). Angiogenesis is a multi-step process, beginning with vascular endothelial growth factor VEGF/VEGF receptor activation to develop new vasculature for tumor growth and metastasis (282). TEX carry angiogenesis stimulatory factors like VEGF, tumor necrosis factor-α (TNF-α), fibroblast growth factor (FGF), transforming growth factor-β (TGF-β), and interleukin-8 (IL-8) (283). For example, human tumors, including pancreatic, glioblastoma, and nasopharyngeal carcinomas exosomes, induce angiogenesis both *in vivo* and *in vitro* (220). TEX also contains a high level of miR-221, syndecan-4, and glypican-1 that proliferates from endothelial cells and tubules *via* revascularization (284). When a tumor cell becomes more aggressive, it needs to migrate to a distant site. And process initially by developing a new metastatic niche by epithelial to mesenchymal transition (EMT) (285). TEX carry EMT element like EMT-inducer miRNAs, TGF-β, β-catenin, IL-6, HIF1α, and caveolin-1 or vimentin, which induce EMT transition *via* ECM degradation and tumor endothelium cell behave more invasive (286). In the process, tumor cells lose E-cadherin and cell polarity and gain twist, snail, N-cadherin, and vimentin (287). Again hypoxia-induced TEX carries miR-301a-3p to enhance the transition of macrophage to M2 phenotype due to activation of PTEN/PI3K pathway (288). These reports validate TEX plays a key role in EMT transition. Innate, acquired, and de

novo drug resistance remains a major obstacle for most therapies to achieve therapeutic intervention against cancer. Innate drug resistance achieves *via* drug efflux pumps that include P-glycoprotein and multidrug resistance protein, overexpression of ABC transporter. In case of De Novo drug resistance transiently acquired by signaling cascade in tumor microenvironment by cell-adhesion mediated drug resistance (CAM-DR) or soluble factor-mediated drug resistance (SFM-DR) (289). Cancer cells employ like increased drug metabolism and detoxification, increased drug efflux, decreased apoptosis, and decreased drug influx. Cancer cells further defend against chemotherapeutic and radiation *via* poor drug penetration and epigenetic modification (290). Exosome contact of both stromal and breast cancer cells *via* paracrine and juxtacrine signaling, for example, further downregulate chemo- and radio-therapeutic insults, according to a study by Boelens *et al* (265) The local inflammatory microenvironment is one of the factors for the formation of a pre-metastatic niche (216). The local inflammatory microenvironment can induce tumor cells to produce tumor-derived secreted factors (TDSFs), such as transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), interleukin (IL), and tumor necrosis factor-alpha (TNF-α) (291). Tumor-derived exosome carries PD-1 incorporate with PD-L1, inhibiting the proliferation of $CD8^+$ T cells. TEX also recruits tumor-associated neutrophils (TANs), tumor-associated macrophages (TAMs), and regulatory T (Treg) cells, which may inhibit antitumor immune responses (292). TEX also contains a substance that may inhibit immune cell function, NK cell dysfunction, block T cell activation, inhibit antigen-presenting cells, and enhance T cell apoptosis to block adaptive immune responses (268, 293). Another study reports that TEX carrying ARG1 and reactive oxygen species (ROS) in the pre-metastatic niche inhibits antitumor T cells and B-cells *via* TGF-βR1/ TGF-βR2 signaling pathway (294). Further, cancer-induced regulatory B (B_{reg}) cell exosomes mediate the immunosuppression and metastasis function of MDSCs by producing more ROS and NO to inhibit $CD8^+$ T cells (295). The pre-metastasis niche matrix environment comprises fibroblast, extracellular matrix (ECM), and endothelial cells. Fibroblasts produce growth and inflammatory factors and matrix metalloproteinase (MMP) and fibronectin (FN) (296). So, TEX exchanges information between cells to promote pre-metastatic niches. Multiple studies report TEX can promote tumor angiogenesis, vascularization, drug resistance, and pre-metastatic niches. And TEX biomarkers have great potential in liquid diagnostic and able to track tumor progression (297). Exosome function potent to signaling between cancer and surrounding cells consists of the tumor microenvironment. Thus, exosomes derived from both stromal and tumor cells have implications in therapy resistances and all stages of cancer progression. Due to their intrinsic nature of cell-cell communication, exosomes play an important part in TME proliferation and TME therapy resistance.

Role of Exosome in Infectious and Respiratory Diseases

Infectious disease is the leading cause of death for children and a significant burden for the health care system. When a pathogen enters the host body, they face hostile environments. The pathogen has a sense of extracellular vesicle signals and communicates with other cells (298). Pathogens use exosomes differentiation, growth control, transmission, and virulence coordination (299). There are two subtypes of exosomes, and one is for the pathogen to the pathogen and the next pathogen to host intercommunication. According to the WHO, malaria is one of the most devastating parasitic diseases (300). In the human body, the parasite has a complex life cycle. Malaria parasites invade human cells like hepatocytes, red blood cells, bone marrow, midgut endothelium, and saliva glands (301). Cerebral malaria is the most common clinical cause and cause of death in infection (302). Scientists found high numbers of exosomes from patients isolated circulating exosomes from P. Falciparum and *P. vivax* Plasmodium infection (299). In the pioneer study, the authors have shown TNF induced endothelium cell injection in healthy control invade malaria-like damage in the lung and brain REF. In 2013, two separate reviews demonstrated that *P. Falciparum* derived exosomes induce gametocyte formation in the host, is an essential step for disease transmission (303, 304). Exosomes from malaria are actively endocytic by endothelium and monocytes. Eventually, malaria parasites change vascular properties, stimulate DNA-sensing pathways *via* microRNA, and promote virulence *via* malaria exosomes (304). In 2011, malaria-infected reticulocyte exosomes were used as vaccine applications (305). These exosomes contain HLA class I and parasite antigen, and immunization promotes an effector phenotype by increasing non-exhaustive memory T-cells (305). Another dangerous disease is Chagas disease caused by *T. Cruzi*. They gain access to the human body *via* insect bites, and the mucosa membrane carries it *via* exosomes. The parasite is multiplying into amastigotes in the cell cytosol. Then they again transform into trypomastigotes to reach blood circulation *via* breaking host cell membranes. The trypomastigotes surface is composed

of glycoprotein and TC85, which can provoke the host immune response. The cellular response produces a set of immune cells and a humoral response in producing lytic antibodies, and both help control invading infection (306). Multiple studies confirm the role of exosomes in the pathogenesis of Chagas diseases (307).

Virus enveloped viruses are a class of exosomes, and these individual exosomes from cells are under the influence of viral genetics. The host cellular component is an envelope in exosomes, and the virus encodes an only specific protein and nucleic acid sequences. On the virion, the virus fuses its part into host cells (308). For example, the HIV trimmer consists of glycoprotein (GP) 120, surrounded by hydrophobic GP41. When HIV interacts with the target cell *via* receptor CD4 and CCR5, HIV drills into the host cell membrane with the help of GP41 (309), some viruses rely on host enzymatic genomic replication to transfer exosomes without actual virion required (310). Most virus exosomes carry viral components that can conceal viral presence from the immune system (274). In latent HIV infection, accessory protein negative regulatory factor (Nef) is released *via* exosomes, which conceal the presence of the latent infection. Even viral fragment carries *via* exosomes can manipulate host immune dynamics (310). Exosomes also have specific receptors that can internalize the viral component signifying exosomes as a vaccine for inducing antiviral immunity (308).

In one study of fungal exosomes, 200 proteins were identified in proteomic analysis (311). Fungal exosomes in the host body become biologically active during infection (312). In the case of bacteria, most bacteria exosomes are gram-negative. Bacterial exosomes have a variety of roles, including producing pathogenicity in the host. However, there are commercially available bacterial exosomes that can protect the host from inflammatory bowel disease (313). .The host immune system can detect bacterial exosomes *via* TLR; after the detection host, the immune system induces a pro-inflammatory reaction and recruit an innate immune system. However, bacterial exosomes has potential to damage cell membrane by destroying endothelium cell walls (313). After uptaken by cell, bacterial exosomes are detected by NADH1 that facilitates activation of antimicrobial human beta defense NF-κβ (314). Thus, bacterial exosomes are recognized by the immune system, provide multiple advantages to the parent bacterium. Those advantages of addressing acquire nutrients, removing toxic, and degrading antibodies.

Role of exosomes in various lung diseases, like COPD, and IPF, has also been studied by utilizing the disease model. COPD and IPF are both age-related lung diseases and PDF can be characterized by measuring the presence of fibrotic cell and excess extracellular matrix (315). Bronchial epithelium cells and macrophages play a critical role in exosomes mediated communication in the lung (316). The alveolar macrophage-derived exosomes can mediate cellular homeostasis in the airway and cellular differentiation *via* miR-223. Macrophage-derived exosomes can control inflammation communication with lung epithelium cells *via* releasing SOCS protein (317). Epithelium cell-derived exosomes involve modulating the innate immune system and maintaining the balance between epithelium and mesenchymal cells of the lung (317). Bourdonnay *et al.* showed (318) macrophage-derived exosomes carry SOCS-1 and SOCS-3 to epithelial cells to suppress the signal due to the activation of cytokines. Authors further show that exposure to smoking reduces both SOCS-1 & 3 expressions, thus reducing inflammation response. Kasimer *et al.* demonstrated that bronchial epithelium cell-derived exosomes carry mucins and play a specific role in interacting with inhaled substances and exosomes. Also, authors have shown MUNC1, 4 & 16 contribute endothelium-derived exosomes structure (319, 320). These findings conclude mucin acts as an innate defense for the lung. Exposure to stress conditions like smoking, infection, oxidative stress, and DNA damage can modify the structure of exosomes and enhance or diminish exosome release. Cigarette smoking causes airway remodeling: epithelium-damaged cells secrete exosomes. Endothelium cell-derived exosomes carrying miR-210, suppress autophagy resulting in airway remodeling (321). Exosomes from lung cells exposed to smoking taking cleaved CCN1 activate the secretion of MMP-1, promote changes in lung emphysema (321). Hence, exosomes exposed to smoking secrete miR-210 to promote myofibroblast differentiation explaining bacterially derived exosomes in COPD. Another study demonstrates pulmonary inflammation of neutrophils can induce by repeated inhalation of bacterially derived exosomes (322). Recently, Kim *et al.* have shown distinctive bacterial-derived exosomes profile of non-smoker, healthy smokers, and COPD patients. Bacterial exosomes can activate polymorphonuclear leukocytes, such as neutrophils, which can bind ECM *via* MAC-1 protein. This process degrades the matrix and leads to right ventricle hypertrophy and emphysema in the mice model (323). Idiopathic pulmonary fibrosis (IPF), another deadly lung disease, is also contributed by exosomes. Ochiya group found mitochondria damage of epithelium cells treatment with exosomes derived from lung fibroblast cells of IPF patients (324). They also have shown DNA damage and accelerated epithelium cell senescence in lung fibroblasts (324). Another study by Martin-Medina *et al.*

demonstrated bronchial lavage fluid exosomes contain WNT5A, induce proliferation of lung fibroblasts, and contribute to IPF (325). There are multiple studies of exosome application for IPF treatment. For example, bone marrow-derived exosomes inhibit BCL-2 protein and induce reverse fibrosis in IPF (326). Mesenchymal stem cell-derived (MSC) exosome has been considered novel therapeutic applications against multiple diseases, including fibrosis. Shentu *et al.* showed injection of human MSC exosomes in the IPF mouse model for therapeutic efficiency and showed Th-1 dependent uptake of MSC-derived exosome from blocking myofibroblast differentiation (327, 328). The above discussions validate the exosome's role in infectious and respiratory diseases and a solid platform for vaccine research.

This section has summarized the overall research update on exosome application in cancer, infectious and respiratory diseases. Exosomes carry myriad innate cells bioactive molecules like proteins, lipids,and RNAs (mRNA, microRNA, lncRNA) and deliver them to neighboring and distant cells. Thus exosome research is getting more attention for cancer immunotherapy. Most exosomes relate to infectious biology, either spread or limit infection based on targeting cells and types of pathogens. Exosomes and exosomal RNAs in diseases have promise in biomarker study enabling noninvasive diagnosis, better therapeutic targeting efficiency in treatment, and inducing an immune response in the disease model. Therefore, the exosome is an appropriate candidate for vaccine development and delivery in preclinical models.

LABORATORY RESEARCH AND CLINICAL TRIAL UPDATE ON EXOSOMAL VACCINE PLATFORM

Natural Functions of Exosomes Modulating Immunity during Infection and Disease

Extracellular vesicles can classify into three groups, exosomes (30–120 nm diameter), microvesicles (100–1000 nm), and apoptotic bodies (200–500 nm) (329). Exosome exocytosis and secretion depend on lipids or membrane trafficking molecules like Rab11, Rab27, Rab35, etc. (330, 331) Both *in vivo* and *in vitro* data confirms the exosome can facilitate internalization and promote cellular communication to all cells. There are multiple protein markers specific to cellular origin identified by numerous investigators. Exosomes also carry proteins, lipids, small non-coding, micro regulatory RNA (miRNA) molecules, and more abundant functional messenger RNAs (mRNA) (23). Exosomes are abundant, maintain a longer half-life, are stable, and capable of short or long-distance communication, and these features make them a suitable candidate for therapeutic application. The exosome is the viable delivery system for macro, micro molecules, and genetic delivery (DNA, mRNA, miRNA) *in vivo* and *in vitro* (29, 332). As an immunotherapeutic agent, exosomes derived from B cells and DCs can load with proteins (antigens) or peptides, demonstrating the ability to induce systematic antigen-specific B- and T cells response (333, 334). Exosomes from DCs also promise to initiate an immune response against tumor cells more precisely and accurately compared to cell therapy and other non-cell-based therapy (334). Specifically, mature and activated DC-derived exosomes carry MHC-I and MHC-II molecules and co-stimulatory molecules like CD40, CD80, CD86, active cytotoxic T- and natural killer (NK) cells *in vitro* and *in vivo via* potent antigen-specific T- and B cell responses (170, 335). And this stimulation required direct interaction of exosome's MHC-I and MHC-II and T cell receptors with $CD8^+$ or $CD4^+$ on T cells, respectively (336). Furthermore, DC-derived exosomes can activate innate and adaptive immune responses due to tumor cells inducing antigen-specific responses to overcome tumor-induced immunosuppression (337, 338). Another study also reports exosomes derived from mature DCs to induce a Th1 polarized by producing and secretion of IFN-γ and cytotoxic T lymphocyte proliferation responsible for the antigen-specific killing tumors *in vivo* immune response (339). Two-phase I clinical trials of DCs derived exosome therapy in melanoma and non-small cell lung cancer patients have been completed with no toxicity issue and better efficiency (339, 340). In both studies, patients with melanoma-associated antigen (MAGE) expressing late-stage/metastatic melanoma or non-small cell lung cancer (stage IIIb or IV) were given DC-derived exosomes loaded with MAGE MHC class I and class II-restricted peptides (339). New immunomodulatory drugs may reverse the tumor metastasis in advanced cancer patients; strategies to tolerate cancer inhibition are essential for cancer vaccine development, which can apply to a wide range of cancer patients. A previous study showed doxorubicin-treated cancer cells release HSP90 and HMGB1, act as TLR agonists in innate immune cells, and potentiate antitumor immunity (341, 342). Kitai *et al.* demonstrated that excess TPT inhibits innate immune activation because it promotes excessive cell death and produces insufficient immunostimulatory DNA or induces topoisomerase I–mediated nucleosome remodeling. The authors show DNA containing exosomes from E0771 cells delivered to the cytoplasm of GM-DCs to activate a STING-dependent

pathway to optimize antitumor immunity. The authors showed TPT activation and suppression could be controlled against antitumor immunity (343). Chemotherapeutic remains a primary treatment against anti-tumor via various mechanisms. It has a cytotoxic effect like pro-inflammatory cytokine secretion, T-cell activation, and myeloid cell activation and recruitment (344). Immunogenic reaction via cytotoxic agents released by tumor cells like protein B1 and DAMPs (345). Another issue is self-DNA, the critical cause of inflammatory and autoimmune diseases (346). Self-DNA via cytosol of DCs activates the interferon gene-dependent cytokine production stimulator by cytosolic DNA sensor cGAS (347). And another side effect of chemotherapeutic is a gastrointestinal syndrome. Authors in this study demonstrate that chemotherapeutics like irinotecan (CPT-11) and fluorouracil (5FU) can induce intestinal inflammation through dsDNA in HCT-116 exosomes by promoting IL-1β and IL-18 maturation in an absent in melanoma 2 (AIM2)-dependent manner inflammasome activation (348). This study also visualizes the AIM2 targeting vaccine platform due to chemotherapeutic induce inflammation against the anti-tumor effect. Infectious disease antigens are circulating in blood circulation and can capture by antigen-presenting cells (APCs). Vaccine delivers the inactive or natural form of proper antigen adjuvant, typically eliciting a potent immune response. In cancer research, field identification of tumor-associated antigens (TAA) in human cancers triggers a platform for cancer vaccine (349, 350). Some TAA targeting proteins are CEA, HER2, p53, MUC1, RAS (351). But generally, these TAA proteins are immunosuppressive environments and poorly antigenic. On top of it, most TAA is cell-specific, and most vaccines are not cell targeting (352). In the case of the cancer vaccine, it is a mechanism mainly on a state of TAA immune resistance is established. It has also been reported exosomes transfer intercellular antigen to APCs and induce immunogenicity (353). Hartman *et al.* suggested creating recombinant adenoviral vectors expressing into the extracellular domain (ECD) of human epidermal growth factor receptor 2 (HER2) or carcinoembryonic antigen (CEA) that linked to the C1C2 domain of lactadherin in addition to native unlinked ECD versions of CEA and HER2. Authors conclude higher protein expression in exosome fraction in the transgenic murine model due to surface modification of adenovirus expressing C1C2 modified CEA/ECD, and HER2/ECD. This study validate low immunogenicity of soluble TAAs in cancer patients and opens the platform for cancer vaccine via cancer biomarker analysis and improving anti-tumor immune response (354). Next, hepatocellular carcinoma (HCC) one of the most lethal malignancies worldwide due to high mortality, inadequate response to treatment, and aggressive nature. Immunotherapy based on DCs is promising, but it costly, and isolation and preparation are tedious. Also, DC has a very low half-life. Exosome derived from DC carrying protein like MHC class-I and class-II and other co-stimulatory molecules can utilize as alternate for DCs for cancer immunotherapeutic (334). Previously, Zitvogel *et al.* demonstrated that tumor antigenic peptide-pulsed DCs derived exosomes induce strong immune suppression and elicit immune response in mastocytoma and mammary carcinoma mice (337). Recent clinical study of DC derived exosome, conducted on advance melanoma patients, shows promising result (339). Lu *et al.* also shows in their paper, exosomes from HCC antigen-modified DCs could be used as cell-free vaccines for HCC and opens window for HCC immunotherapy (355). Another study by Geis-Asteggiante *et al.* demonstrate Myeloid-derived suppressor cells (MDSC) derived exosomes can induce immune suppression function via differential content of protein, lipids, mRNA, and miRNA (356). Inflammation caused by MDSC is associated by its abundance and suppression activity enhance tumor progression (357). Authors identified mechanism of MDSC cells for immune suppression via analyzing both MDSC and MDSC derived exosomes. This comprehensive study identified multiple miRNAs and mRNAs whose known or predicted function is consistent with their involvement in MDSC-mediated immune suppression.

Parasite or virus-infected cells or even the parasite itself can release exosomes to activate T cells via antigen presentation. In contrast, exosomes derived from microbial molecules that carry HIV Nef or leishmania GP63 can inhibit T cells activation or initiate apoptosis of immune effector cells like helper T-cell or effector B cells (21). Interestingly, macrophages infected by bacteria carry antigens that activate both $CD8^+$ and $CD4^+$ T cells via cross-priming. In comparison, some infected cells release exosomes carrying PAMS, DAMS, limiting macrophages to respond to INF-ϒ stimulation or stimulating macrophages to produce TNF-α (205). Roier *et al.* show how Gram-negative bacteria are forming outer membrane vesicles (OMV) emerging as OMV based vaccine vesicles. Heterologous H. influenza strains derived OMVs with thorough characterization and size distribution, the quantity of vesicle production among strains Rd. KW20, NTHi 2019-R strain, and Hib strain Eagan. The biomarker screening study of *H. influenza* OMV identifies 13 ATP-binding cassettes (ABC) transporters proteins and eight lipoproteins as potential vaccine targeting sites against *Haemophilus influenzae* infections. In contrast, important virulence factors like vaccine

candidates OMP 26 and protein D and serine protease HtrA on exosome derived from H. *influenzas* can utilize as a vaccine delivery platform (358). Pertussis is a respiratory infectious disease that has prevailed for a decade in the world (359). Many types of vaccines are available, like multi-antigen whole-cell pertussis (wP) vaccines, acellular pertussis (aP) vaccines. Zurita *et al.* has shown in their paper using pertussis outer membrane vesicle or exosomes containing multiple vaccines against lung infection with a circulating pertactin (PRN)-deficient isolate in mice. The traditional vaccine failed against these circulating clinical isolates due to the deficiency of PRN. Authors demonstrate long-lasting immunity effectively prevents infection against *Bordetella pertussis*. Authors also showed that two doses of OMV vaccine CD4 T cells with a tissue-resident memory (TRM) cell phenotype ($CD44^+$ $CD62L^{low}$ $CD69^+$ and $CD103^+$) accumulated in the lungs of mice 14 days after immunization (360). Exosomes are getting much more attention recently due to their intrinsic properties as a drug carrier and immune-modulatory mechanism. Anticoli *et al.* use engineer the Human Papilloma Virus (HPV) E7 protein with exosome. E7 protein elicited both effective and strong antigen-specific cytotoxic T lymphocyte (CTL) immunity (361). Author's injection of a DNA vector expressing HPV-E7 fused at the C-terminus of an exosome-anchoring protein name Nef^{mut} (Nef is the name of the protein, and when it is mutated or engineered, it is written as Nef^{mut}) (362). Authors also run immunogenicity studies in a broad array of viral products, including ebolavirus VP24, VP40, and NP, influenza virus NP, Crimean–Congo hemorrhagic fever NP, west nile virus NS3, and hepatitis C virus NS3. All antigen appears stable and detectable antigen-specific $CD8^+$ T cell treatment with exosome carrying Nef^{mut} tether with DNA vector HPV-E7. Authors propose this versatile CTL vaccine platform exhibit exosome carrying DNA vector-induced antigen-specific $CD8^+$ T cell response as vaccine application (363). This cytotoxic response is enough to kill antigen-expressing/peptide-loaded syngeneic cells. Genetically engineered allogeneic or autologous T cells expressing T cell receptors (TCRs) or chimeric antigen receptors (CARs) as cellular immunotherapy is promising as a new treatment method for multiple range of cancers (364). Despite T cell therapy efficiency, T cell therapies show unique toxicities like CAR-T-related encephalopathy syndrome (CRES) and cytokine release syndrome (CRS). Multiple studies also describe the benefit of human T cell-derived exosomes targeting cytotoxic T lymphocytes (CTL) in cancer immunotherapy application (367). CTL-cell-derived exosomes contain surface membrane molecules (CD3, CD8, and the TCRs) trigger tumor cell death by the interaction between proper antigen/MHC combination and the TCR. The author's data validate CAR-T cell-derived exosomes can be used as cancer-targeting agents and improve therapeutic efficacy and potential cancer vaccine platform (368). Li *et al.* also demonstrate that exosomes derived from *Toxoplasma gondii* after co-incubation can modulate the immune response in macrophage RAW264.7 cells. After *T. Gondii* exosome treatment, authors using enzyme-linked immunosorbent assay (ELISA) found higher production of IL-12, TNF-α, and IFN-γ and lower IL-10 in macrophage cells. Authors conclude *T. Gondii* exosome modulate macrophage activation from M1 to M2 *in vitro* and triggers cellular and humoral immune responses. This immune response can partially protect against acute parasite infection in mice models and stipulate exosomes may act as a potential vaccine candidate against toxoplasmosis (369). In another study, the authors have shown the donor antigen-specific regulatory T cells (T_{reg} cells) inhibit the immune inflammation in the allograft heart (369). We found exosome research applications on organ transplantation. One of the effective treatments for end-stage heart failure patients is allograft transplantation (370). An important drawback is an allographic rejection, and it is vital for the long-term survival of grafts (371). Immuno-suppressant may reduce the incidence of rejection, but long-term use can have adverse side effects like renal failure, malignancy, and infections (372). We also know that chronic cardiac allograft vasculopathy results in allographic failure via ischemia (373). This rejection is also associated with the $CD4^+$ T cell-mediated delayed-type hypersensitivity (DTH) (374). Drugs or molecules can induce immune tolerance via de novo differentiation of naive $CD4^+$ T cells into Treg cells by blocking the mTOR-dependent inhibition of foxp3 transcription (375). Integrin αvβ6 can convert the latent transforming growth factor (TGF)-β to promote Treg cells (376). The authors show the delivery of cardiovascular exosome carrying integrin αvβ6 promotes the generation of the donor antigen-specific immune tolerance. Another study also shows DCs derived exosomes promote heart allograft survival (365, 366). The authors finally validate donor-derived peripheral exosomes carried MMP1a promoted the allograft heart survival via inducing donor antigen-specific Treg to attenuate the T helper (Th)2 pattern inflammation (372, 373). With recent incidence we also found being immunosuppressant makes someone vulnerable to infectious disease like SARs-CoV-2 and cancer progression. In Fang Huang *et al.* paper on leukemia study, authors have shown TGF-β1 level in leukemia cell-derived exosomes (LEX) downregulated due to lentiviral shRNA silencing of TGF-β1 in parental leukemia

cells. Then authors incubate LEX $_{TGF\text{-}\beta 1si}$ with DC cells and promote the upregulation of surface expression of costimulatory factors and MHC class II molecules and inducing secretion of IL-12p70 and TNF-α. Further immunization with LEX $_{TGF\text{-}\beta 1si}$ compare with naïve LEX, stimulated stronger specific cytotoxic lymphocyte (CTL) response and nature killer (NK) cell cytotoxicity, and facilitated $CD4^+$ T cell proliferation and Th1 cytokine secretion. Authors successfully induce antitumor immunity by downregulating exosomal TGF-β expression. This leukemia immunotherapy holds potential exosome vaccine platforms in future. This exosome research field is very dynamic and changing drastically with demands. We have summarized current development of this exosome field in vaccine research and delivery. Exosome will increase efficiency of vaccine against multiple diseases and is a versatile platform to bring more vaccines for human race.

Clinical Updates

In clinical trials, only four vaccine studies have been conducted using exosomes, and these are in different clinical phases. These four trials are summarized in Table I. The above discussion focuses on exosomes as promising vaccine platforms for diseases like cancer, immune disorders, cardiac and diabetes, etc. Table I The first clinical trial was based on DC cell-derived exosome vaccine development to treat NSCL cancer. It is in phase 2 and holds promising data to start clinical trial phase 3 and vaccine production. The phase 2 study is complete and has data on the server. The study consisted of 41 participants (18 to 70 years) and maintenance immunotherapy in 41 advanced unresectable NSCLC patients responding or stabilized after induction chemotherapy with DOX-based treatment to improve PFS rate at four months. The study is based on aerosol inhalation of mesenchymal stem cells exosome. Authors have evaluated tumor antigen-loaded DC-derived exosomes on patients with unresponsive chemotherapeutic patients with NSC lung cancer. Second, in the list, the clinical trial is in phase 1 to assess the safety of MSC-derived exosomes in aerosol inhalation form. The clinical trial was conducted on 27 healthy volunteers and concluded by May 31, 2020. The results found from this study can be used to explore the safety and efficiency of MSC-exosomes aerosol inhalation for.

severe lung diseases (acute respiratory distress syndrome (ARDS), chronic obstructive pulmonary disease (COPD), and including severe lung infection, etc.). Scientists found significantly reduced pathological impairment and lung inflammation from different types of lung injury. Additionally, scientists found therapeutic

Table I Current Clinical Trial Update on Exosomal Vaccine Development in Different Clinical Phases

No	Vaccine Name	Drug and Nanoparticles	Disease target	Evaluation	Clinical Phase	Clinical Trial Identification Number	Reference
1	Vaccination with Tumor Antigen-loaded DC-derived exosomes (CSET 1437)	Metronomic cyclophosphamide (mCTX) and vaccine tumor antigen-loaded DC-derived exosomes.	Non-small cell lung cancer	Phase 1 completed. Phase 2 ongoing	Phase 2	NCT01159288	(337)
2	Aerosol inhalation of mesenchymal stem cells exosomes in healthy volunteers	Mesenchymal stem cells (MSCs) derived exosomes.	Healthy control, lung inflammation pathological impairment, lung injury, COPD.	Safety and tolerance of inhaled exosomal aerosol	Phase 1	NCT04313647	(377)
3	Pilot immunotherapy trial for recurrent malignant gliomas	IGF-1R/AS ODN, bio-diffusion chamber	Malignant glioma of the brain	Radiographic tumor regression	Phase 1	NCT01550523	(378, 379)
4	Pilot immunotherapy for newly diagnosed malignant glioma	Surgery with tissue harvest and implantation 20 diffusion chambers in the rectus sheath with IGF-1R/AS ODN	Malignant, glioma neoplasms	It recognizes the immune system to identify the tumor through immune surveillance from later tumor growth	Phase 1	NCT02507583	377

outcomes, bacterial killing, and macrophage phagocytosis. Phase 1 study has been completed by 4th August 2021 and the result is available publicly in online. Number 3 trial based on pilot clinical trial of immunotherapy for malignant gliomas. This phase 1 study is recruiting and is complete; the investigation is ongoing. The study targets malignant gliomas of the brain via exosome-carrying tumor antigens. Along with antisense molecules, exosomes work together to activate the immune system against the tumor (380). This combination product, therefore, acts as a slow-release antigen depot. Exosomes with antisense will initiate our immune response and activating immune system T cells that attack and eliminate the tumor (381). By training our immune system, the patient will have an immune memory of tumor antigen and protect them in the future from other tumors that carry the same antigen (382). This approach can also be potentially used for vaccine delivery for anti-tumor immunogenicity and adaptive immune system memory to prevent tumor progression. Based on the previous one, the last study also found on antisense102: Pilot immunotherapy for newly diagnosed malignant glioma. This study is phase 1 and has a total of 32 participants. This study is also targeting malignant glioma and Neoplasms. The delivery of exosomes with antisense molecules together will activate the immune system against the tumor. We have found only four clinical trials related to exosome vaccine application, but this number will go beyond a hundred if we consider exosome cancer immunotherapy.

(https://www.clinicaltrials.gov/ct2/results?recrs=&cond=Cancer&term=exosome+&cntry=&state=&city=&dist=). These data validate how exosomes could be a game-changer for vaccination and immunotherapy. Recently exosome-based product has materialized to initiate biomedical start-up, and some of the product is being evaluated through clinical trials. For instance, Lonza pharma, and Organicell Regenerative Medicine are working on exosome-based therapies. These products are in the early pre-clinical or clinical stage and already in the GMP process for their exosome therapy product from mid-2020 (383). Takara has a high purity exosome isolation kit for RNA purification for RT-qPCR and NGS analysis. Our literature review gives us a better picture of exosome capability and the future of precision or personalized medicine and vaccine application. We hope exosomes will help scientists envelop the limitation of immunotherapy.

Clinical Challenges of Exosome Translational Application

Similar to other biomedical research, exosome-based applications also suffer significantly from being translating bench to bedside. The most difficult aspects of exosome research are reproducibility, translation and scalling up from *in vitro* to *in vivo* while maintaining the consistency.

Exosomes generated by cells and collected from their supernatants were used in the majority of the early exosome research (384). Multiple conditions associated with exosome production must be optimized for validation and reproducibility. Exosomes released from tumor cells are responsible for immune suppression function (385). Exosomes produced by cancer cells that contain tumor-associated antigens and are discharged into body fluids signal the presence of a tumor. Using this property, exosomes are analysed for pancreatic cancer cell proteomes and the finding of enrichment in GPC1 proteoglycan in both pancreatic cancer cells and exosomes derived by them (glypican 1) (222). The presence of GPC1+ exosomes (crExos) in patient serum can be measured using the fluorescence-activated cell sorting (FACS) technology. It was shown that using GPC1+ exosomes can facilitate for the early diagnosis of pancreatic cancer and differentiating patients at different phases of the disease's progression (222). The ability to modulate lymphocyte activities depends on the disease activity of HNC patients, according to an investigation of exosomes found in the plasma of patients with head and neck cancer (HNC) (293). However, little is known about the reproducibility, efficiency, and reliability of the protocols routinely used to quantify exosomes in the human serum. If the generalized and ptimized protocol can be introduced and applied, the reproducibility issue likely be overcomed.

Exosome isolation and characterization techniques are evolving and adaptive. Different scientific groups establish a protocol to isolate and preserve exosomes. However, the mass production of exosomes is challenging in preclinical stage with non-human primates. Therefore, exosomes scale up with cGMP guidelines still at the early phase. However, various studies reported on the pathways of exosome biogenesis to manipulate some genes to increase exosomes production (385, 386). Generally, two major strategies are employed to increase the production of exosomes. First, oogenesis pathways that are genetically manipulated to overexpress activator genes that play a role in exosome biogenesis and downregulate the genes involved in exosome recycling pathways (387). Second, adjustment of the cell culture medium, treatment with specific therapeutics, and limiting certain physiological conditions that can force the cell to produce more exosomes (388). The potential for adjustments in cellular phenotype, during scale-up and equipment change, must be considered. As

the dynamics of exosome biogenesis are only at the beginning, alterations in any factors might alter the exosomes' production, composition, surface marker, or function. As mentioned, beyond cell-mass expansion requirements, it is essential to control pre-identified production environmental parameters such that the cell's phenotype, culture pH, CO_2 percentage, and therefore secreted exosome characteristics (389, 390).

Exosome proteomics has unquestionably made significant development in recent years. The advancement of exosome isolation methods was a key element in this phenomenon, but the advancement of improved instrumentation for proteome analysis, as well as its increased sensitivity allowed for a significant improvement in the exosome research. Despite significant advancement over the last decade, there are still a lot of unsolved gaps to be addressed. There are still no universal exosome markers, for example, that allow for precise identification of these vesicles and differentiation from other EVs. Furthermore, standard methods for tracking and characterizing exosome *in vitro* and *in vivo* studies are still unavailable. However, the subject of exosome study is still in its infancy due to the vast technical disparities between present methods for isolation and characterization. These technical obstacles must be solved to be able to use exosomes for diagnostic or prognostic monitoring of cancer and infectious disorders and to build innovative exosome-based personalized therapeutics. The International Society for Extracellular Vesicles is a worldwide society of driving extracellular vesicles, exosomes, and microvesicle analysts. With more than 2,000 all out individuals, ISEV's main goal is to progress extracellular vesicle research internationally.

With substantial efforts in exosome translation, it is crucial to understand the progress made and the persisting challenges in clinical translation. Although exosome analysis methods have tremendously evolved, the exact mechanisms of biogenesis are still unknown. Conversely, improvements in the isolation methods and purifications are needed to study the cargo contents, markers, and functions, which would shed light on the biogenesis mechanism and disease condition in return. Once such drawbacks are overcomed, new biomarkers can be identified for characterization, exosome can be used in diagnostic and drug delivery applications. Moreover, with more research on exosome biogenesis and functions, there would be significant opportunities to manipulate their composition, properties, biogenesis mechanism, and cell interactions to advance their clinical applications further. The potential use of exosomes as a diagnostic, detection and therapeutic applications are demonstrated in our earlier review (156) . In conclusion, developing efficient, reproducible, scale-up, and reliable isolation methods is urgent to further advance in this field. To fully utilize the potentials of primary research, clinical data and emerging new technologies need to be integrated, setting the foundations for clinical translation, therapeutic applications of exosome.

CONCLUSION AND FUTURE REMARK

Pandemics like SARS-CoV-2, HIV, malaria, and Ebola have raised global health awareness and directed us to prepare for future outbreaks. Cancer itself is a life-long threat to the human population. Vaccine development and understanding the pathogens of these emerging diseases and cancer will be critical to saving us in the future. New vaccine platforms such as DNA, mRNA, and live vector technologies overcome some of the limitations of traditional vaccines and will allow faster vaccine production. Each vaccine platform has both advantages and disadvantages. It is unlikely that a single technology will suffice to save humanity from all cancers and infectious diseases. We need to rethink how to make the currently available vaccines more effective—one of the emerging platforms of targeted vaccine delivery involves exosomes. Understanding the role of the exosome in immune responses to many deadly diseases and its pathogenesis will be a pivotal challenge to utilize exosomes for vaccine development. Exosome, as a vaccine delivery vehicle, emerges as a novel platform for cancers and infectious diseases. Cancer immunotherapies, including CAR-T or ICI, have transformed the thinking capability of scientists, and exosomes could potentially play a critical role in cancer immunotherapy development. Some of the vaccines are not efficient enough due to their lack of target specificity. Over the years, nanoparticles-based platforms have been investigated in vaccine delivery as a non-viral vector-based viral delivery vehicle. Out of many nanoparticles, exosomes hold unique preferences due to their intrinsic cell-cell communication and interaction with the human immune system in most pathological conditions. This review focuses on the role of exosomes in cancer immunotherapy, vaccine application for infectious and respiratory diseases. How the immune system is modified and adapted during new emerging disease conditions is still unclear, but it has been found that exosome interacts with our immune system. We believe that merging with new technologies like DNA, live vector, and mRNA delivered using an exosome-based vehicle can open a new window for vaccine research and development. We envision the personalized vaccine for specific diseases

will be available sooner rather than the letter to accelerate immunization with enhanced potency. More cost-effective vaccines will facilitate distribution to the large population of developing countries who urgently need protection from infectious diseases and cancer.

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