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Ultrasound-triggered Immunotherapy for Cancer Treatment: An Update

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Abstract: Over the past few decades, immunotherapy has emerged as a promising therapeutic approach to treat some types of cancer. Moreover, antibody-based cancer therapies can trigger apoptosis and cell growth inhibition to induce immune cell destruction of target cells through antibody-dependent cellular cytotoxicity (ADCC). Nevertheless, immunotherapeutic efficiency is often restricted due to deficient delivery or low accumulation of therapeutic molecules at the tumor site. The development of pegylated liposomes with monoclonal antibodies conjugated to their surfaces (immunoliposomes) and triggered with ultrasound can effectively improve drug accessibility by enhancing cell membrane permeability and drug release. This review summarizes existing traditional cancer treatments and their limitations, emphasizing the recent advancements in ultrasound-triggered immunotherapy

Keywords: Ultrasound, immunotherapy, liposome, microbubbles, cancer, cell growth.

1. INTRODUCTION

Cancer is the second leading cause of death globally; it is caused by environmental and genetic factors, including radiation, bacterial infection, and genetic abnormalities [1]. Cancer can be further categorized into (1) carcinoma, (2) sarcoma, (3) lymphoma, (4) leukemia, and (5) myeloma; Table (**1**) details the different types of cancer [2, 3].

Traditional cancer therapies include radiation therapy, chemotherapy, and surgery. Surgery is the oldest form of cancer treatment; it is used to prevent, diagnose, stage, and treat cancer. Currently, the surgical treatment of cancer is paired with chemotherapy and radiation therapy to decrease the chance of recurrence. Chemotherapy utilizes toxic drugs to kill abnormal cells; the main drawback of chemotherapy is that it is nonspecific, causing damage to healthy cells as well as cancerous ones. Another challenge is multidrug resistance (MDR) to chemotherapeutic drugs [4-6]. MDR can develop against every anticancer drug and can occur due to several mechanisms, including decreased drug uptake, increased drug efflux, activation of DNA repair mechanisms, and evasion of drug-induced apoptosis [7]. Moreover, the non-selectivity of chemotherapy can cause hepatotoxicity, nephrotoxicity, and cardiotoxicity.

Radiotherapy is another method of cancer treatment, developed in 1896 by Emil Grubbe [8]. It treats cancer by degrading DNA and inhibiting cell division and is often used in combination with other treatments such as surgery. Unfortunately, radiation has proven ineffective in the treatment of metastatic cancer. Also, radiotherapy is associated with radiation-related complications to the heart and lungs and damages healthy cells, stimulating the formation of new tumors [9, 10].

Although traditional means of treatment can effectively treat early-stage cancers, new treatments are needed to offset the limitations of the existing methods, such as the inability to control recurrence and metastasis. For instance, chemotherapy has been improved using biologically targeted therapies [11]. Modern hormonal and targeted therapies interfere with cancer cell proliferation by blocking certain processes and targeting specific receptors and antigens expressed on these abnormal cells [12]. Recently, immunotherapy has witnessed dramatic progress and has shown impressive results in terms of the overall survival of patients with advanced-stage cancers [13]. Immunotherapies, specifically monoclonal antibodies (mAbs) [14] and chimeric antigen-specific receptor-transfected T-cells (CAR-T cells), target tumor cells by manipulating immune regulation [15, 16]. However, this form of immunotherapy is limited by over specificity, resulting in an effective treatment in 49-72% of patients with metastatic cancers [17, 18]. Another type of immunotherapy uses checkpoint inhibitors (CI), such as anti-PD-1 antibodies (aPD-1), to release the "natural brakes" of the immune system so that T-cells can recognize and attack tumors. CI drugs have been successful in clinical use [19]. Despite some success with aPD-1 based therapies, many immunotherapies fail to deliver sufficient concentrations of therapeutic molecules to the tumor site [20].

Nano immunotherapy utilizes functionalizable, nanosized chemical structures to address the limitations of immunotherapy and enhance the efficacy of immune-based therapies [21]. Immunoliposomes are liposomes encapsulating therapeutic drugs and functionalized with antibodies. The encapsulation of anticancer drugs inside liposomes protects them from degradation. The circulation time can be increased by coating liposomes with polyethylene glycol (PEG), which prevents the opsonization and uptake of the liposomes by the reticuloendothelial system (RES) [22-25]. Immunoliposomes are commonly functionalized with mAbs or antibody fragments, which actively target overexpressed antigens on the surfaces of cancer cells [26, 27]. Immunoliposomes constitute a promising form of cancer therapy, having undergone clinical trials but have yet to receive clinical approval and reach the market. The different cancer treatment methods discussed so far are summarized in Table (**2**).

Table 1. Types of Cancers.

Cancer Types	Description
Carcinoma	Cancer found in epithelial tissues, lining organs, glands, and body structures
Sarcoma	Tumors of connective tissues including cartilage, fat, muscle, tendon, and bone
Lymphoma	Abnormal cells originating from the nodes or glands of the lymphatic system or brain and breast organs
Leukemia	Bone marrow cancer, which inhibits the production of red and white blood cells and platelets
Myeloma	Malignant cells which grow in the plasma cells of the bone marrow and can form bone tumors

Table 2. Overview of Cancer Treatments.

Cancer Therapy	Advantages	Disadvantages
Radiation Therapy	− Inhibits cancer cell growth using radiation	− Radiation-related complications due to non-specificity − Can cause the formation of new cancers
Chemotherapy	− Kills abnormal cells using toxic drugs	− Damages healthy cells − Cells can become resistant to anti-neoplastic drugs
Surgery	− Removes abnormal cells	− Cancer must be caught early − Best used in conjunction with radiation and/or chemotherapy
Hormonal/Targeted Therapy	− Directly discriminates cancerous cells	− Targeted molecules may be expressed on healthy tissues
Immunotherapy	− Manipulates the body's immune system, reducing damage to healthy cells	− Can be over specific and misses malignant cells
Immunoliposomes	− Easily modified to target cancer cells − Release can be controlled − Circulates well in the body − Can be made echogenic	− Targeted antigens may be expressed on healthy tissues

The efficacy of antibody-targeted liposomes in increasing the concentration of therapeutic drugs at the tumor site relies on the liposome's drug release rate at the target site. Immunoliposomes need to have temporal and spatial control to ensure uninhibited circulation in the bloodstream, accumulation at the target site, and specific release in the presence of stimuli. There are several stimuli-based nanoparticles covered in recent reviews [28]. Many studies reinforced the use of focused ultrasound (FUS), not just as a way to improve the effectiveness of immunotherapeutic drug delivery but also to enhance the efficacy of immunotherapy by amplifying the natural anticancer immune response [29, 30]. FUS agitates the tumor, generating tumor fragments and tumor-associated antigens, which leads to stimulating the immune response towards the tumor cells [31-33]. Liposomes and immunoliposomes are easily modified to be echogenic and induce the release of anticancer drugs upon exposure to ultrasound (US). This article reviews the use of antibody-modified liposomes and nanoparticles to treat cancer and discusses the potential and limitations of ultrasound-triggered (US triggered) immunoliposomes.

2. ANTI-BODY TARGETED IMMUNO-CARRIERS FOR CANCER THERAPY

Immunotherapy has established a significant clinical scheme for cancer treatment. Consequently, the number of approved immunotherapy drugs continues to increase. However, they still suffer from certain limitations such as autoimmune complexity and the enhancement of nonspecific irritation [34]. To overcome these limitations, it is essential to improve their targeting specificity and reduce their off-target effects.

The delivery of drugs and genes to cancer cells is hampered by various obstacles, including tumor vessels, the interstitium, and the cell membrane. Some of these obstacles are summarized in Table (**3**). Drug delivery vehicles control the location and rate at which a drug is released, consequently controlling the therapeutic agent infusion rate and required concentrations of the different therapeutic agents at the diseased site. Drug delivery carriers not only improve the safety and efficacy of drugs, but also allow for the administration of new therapies, previously considered highly toxic to be delivered *via* conventional ways. This led to the development of different drug delivery systems, such as mechanical pumps (implants), polymer matrices (micro-particulates), externally applied transdermal patches, and drug delivery vehicles. Each drug delivery system has advantages

and disadvantages; for example, implants are invasive as they require surgical administration and removal, while microparticles are considered too large for drug targeting and intravenous administration [35].

Table 3. Types of obstacles hindering ingmmunotherapy.

Types of Cellular Barriers	Description
Cell membrane delivery barrier	Cancer immunotherapy effectiveness is limited by the efficiency of drug delivery to cells. Tumors have irregular and disorderly blood vessels with avascular spaces of diverse sizes, rendering their vessels leaky [41, 42]. Therapeutic drugs targeting the brain must overcome the blood-brain barrier (BBB),, composed of tight junctions of endothelial cells, limit ing the delivery of anticancer therapies from the blood to the brain [43].
Enhanced interstitial fluid pressure	Breast, colorectal tumors, and metastatic melanoma have increased interstitial fluid pressure (IFP) [44]. The mechanical stress induced by tumor cell proliferation [45] results from stromal cells and the extracellular matrix (ECM) [46] compressing blood vessels and raising IFP as well as, limiting therapeutic and lymphocyte access to the tumor site. Hyperpermeable vessels and the lack of functional lymphatic vessels in tumors emphasize the elevated IFP.
Vascular normalization of tumor environment	Normalizing vascular structure in tumors can improve the delivery of anticancer drugs and tumor-infiltrating lymphocytes [48, 49]. An advanced-stage epithelial ovarian cancer mouse model treated with 3TSR, showed enhanced tumor blood perfusion, indicating potential normalization of tumor vasculature. The treatment, consequently, allowed greater intratumoral permeation of immune cells, including macrophages, natural killer (NK) cells, cytotoxic T-cells, and T-helper cells, compared to the untreated mice. [40]. Additionally, modifying the physical or chemical properties of drugs, such as molecular weight, shape, and charge, can enhance their delivery to tumor tissues and cellular uptake [50].
Modulation of the tumor-microenvironment	As indicated by increased T-cell numbers in tumor tissue slices treated with collagenase, human lung tumors in fibronectin-rich environments have a lower abundance of T-cells than in loose fibronectin areas, despite decreased stromal collagen content [51]. Additionally, CAR-redirected T lymphocytes (CAR-T-cells), designed to express heparanase and degrade heparan sulfate polymeric molecules (ECM proteins), have heightened tumor permeation potential and antitumor effects [52].

The field of nanomedicine utilizes nanoparticles to improve selectivity, extended activity, and control drug release as well as the cellular uptake of drugs. The most significant advantage of this technology is its ability to cross physiological barriers, overcome drug resistance, and significantly reduce chemotherapeutic side effects [23, 24]. Moreover, nanoparticles can extravasate at the tumor site due to their small size making them suitable delivery systems for intracellular targeting and gene delivery [36]. Nano-based therapeutics is a rapidly evolving field characterized by the continuous improvement in the biocompatibility and shelf-life of the developed nanocarriers. There is a wide variety of nanocarriers, with sizes ranging between 10-800 nm. Nanocarriers include nanocrystals (quantum dots), nanosuspensions, nanotubes, nanowires, micelles, liposomes, metal-organic frameworks (MOFs), ceramic nanoparticles, dendrimers, solid-lipid nanoparticles, and hydrogel nanoparticles [37]. Micelles and liposomes are the most widely researched nanocarriers [38, 39]. Micelles are colloidal elements ranging in size between 5-100 nm, consisting of a hydrophobic core shaped by Vander Waals interactions and surrounded by a hydrophilic casing. However, due to their small size, micelles have a limited capacity. Additionally, micelles can only encapsulate hydrophobic drugs [40].

3. ULTRASOUND AS A TRIGGER FOR NANOCARRIER MEDIATED DRUG RELEASE

Triggering mechanisms are employed to control the therapeutic agent's release from nanocarriers and facilitate drug penetration into the tumor [53]. Triggering mechanisms reduce the unwanted side effects and circumvent the induction of drug resistance due to long accumulation times. However, triggering methods involving quick release can damage healthy cells, whereas a slow drug release will stop drugs from reaching cytotoxic therapeutic concentrations. Thus, a mechanism to control the release spatially and temporally is needed.

(US) refers to mechanical waves with frequencies above the human hearing ability (>20 kHz) [54]. US waves are cyclic sinusoidal acoustic waves with high pressure phases (compression) at the upper peaks and low pressure phases (refraction) at the lower peaks. US waves propagate through a medium by transferring energy through the oscillation of particles; thus, they propagate faster in solids than in fluids. US attenuation occurs as the waves travel through the medium, as energy is lost either by absorption or being transformed into other energy forms [55]. The biological effects of US can be divided into thermal and mechanical effects, *i.e.*, cavitation [32]. Acoustic cavitation effects have the propensity to enhance the immune response towards cancer because US enhances the delivery of genes and antigens to abnormal cells, which activates the anticancer immune response [29, 56].

High-intensity focused ultrasound (HIFU) is a non-invasive cancer therapy that utilizes non-ionizing US waves to heat tissues [57]. HIFU employs a variety of mechanisms to

augment blood or lymph flow or to disrupt tumor tissues [58]. The operating frequency of HIFU presents a trade-off between image resolution and penetration depth; because at higher frequencies, the image resolution is improved; however, there is less penetration depth, making high-frequency HIFU suitable for superficial applications. In contrast, low-frequency HIFU has poorer resolution but greater penetration depths, rendering low-frequency HIFU transducers useful for general abdominopelvic uses [59]. The benefit of HIFU treatments lies in their ability to be applied within a short period, at a reasonable cost, and with little to no threat of infections [58]. The fundamental challenge with HIFU-triggered drug delivery is to design suitable biologically active carriers that will respond to US and accumulate at the tumor site. US exposure releases energy and heat causing coagulative necrosis when applied to cancerous cells [58].

Using US in combination with nanocarriers was shown to increase the penetration of several therapeutic agents into tumors, such as chemotherapeutic drugs and genes [54], [60]. A suitable strategy for US-mediated delivery should be able to (1) pass through biological barriers including the vascular endothelium, interstitium, cell membrane, or blood-tumor barrier (BTB), and (2) improve the diffusion of therapeutic components in interstitial tissue [61-63]. US is widely used as a mechanism to trigger release due to its low cost, safety, and ability to be focused on specific areas. Moreover, US has a synergetic effect with chemotherapeutic agents making it an ideal triggering mechanism [54].

As mentioned earlier, US-triggered release is due to two different processes, namely thermal and mechanical effects. Thermal release of therapeutic agents from nanocarriers occurs when US is applied, for a specific period (a few seconds to half an hour), at moderate intensities and pressures, inducing higher temperatures (42-43°C). Temperature-sensitive liposomes (TSL) contain the drug inside the intermediate phase to reduce toxicity. At elevated temperatures, the lipid shell content is modified, undergoing a liquid-crystalline phase transition, which releases the drug [64-66]. Studies have shown that these agents can release approximately 80% of their contents after 15 minutes of hyperthermia at 43°C [67]. However, the crucial disadvantage of USinduced hyperthermia is the need for prolonged treatment sessions, thus increasing the risk of hyperthermic side effects. Mechanical effects of US result from acoustic wave propagation and pressure variations. Acoustic cavitation, in which gas bubbles are formed in liquids due to pressure changes, depends on the intensity of US and only occurs at a specific threshold. At low-pressure amplitudes, the gas bubbles exhibit stable oscillation as they contract and expand (*i.e.*, stable cavitation). Inertial cavitation, on the other hand, results from high-pressure amplitudes causing gas-bubbles to collapse. The bubbles increase rapidly in size until they reach their resonant size, where they collapse. The gas-bubble collapse results in high pressures and temperatures, which produce sonic jets of fluid and shock waves causing transient pore formation on the cellular membranes (sonoporation effect). Finally, new small bubbles are formed and reinitiate the cycle. Stable and inertial cavitation occur simultaneously and follow each other. Another mechanical effect is acoustic streaming, which is a direct result of US wave propagation through the medium. In acoustic streaming, particles move in the direction of the flow, resulting in micro-streaming, bulk-streaming, or both. Bulk-streaming is considered a powerful mechanism, which facilitates the delivery and distribution of drugs [68]. US-oriented drug-delivery is especially relevant to the development of liposomes as general drug delivery carriers [69].

4. ULTRASOUND-MEDIATED TUMOR IMMUNOTHERAPY

Tumor immunotherapy is a promising cancer treatment approach since it is designed to target tumor cells more specifically/preferentially compared to conventional treatments. Immunotherapy involves stimulating the body's immune system against cancer by introducing tumor vaccines, mAbs, cytokines, or immune cells. Immunotherapies can be classified into active or passive immunotherapy. Active immunotherapies rely on stimulating the immune system to eliminate malignant cells, while passive immunotherapies employ cytokines, mAbs, and immune cells to act directly on the tumor cells. Regardless of the type of immunotherapy used, the treatment needs to be delivered intravenously, which has some drawbacks that reduce the efficiency of delivering these drugs. US and microbubble-targeted delivery (UMTD) technology has made some progress in tumor immunotherapy. In the following sections, we briefly discuss the applications of UMTD technology in cancer immunotherapy.

4.1. Dendritic Cell-based Vaccines

US can amplify the delivery of vaccines (Ags, peptides, proteins, pDNA, or mRNA) enclosed in dendritic cells (DC), a type of antigen-presenting cell (APC), into tumor cells and APCs [70, 71]. An *in vivo* study reported a 500-800 fold increase in gene expression in APCs, when mannose-modified gene carrier bubble lipoplexes (Man-PEG_{2000}) were treated with US or transfected by mRNA-lipoplexes, and displayed tumor regression in addition to long-term antigen-specific immunological memory [72-74]. Furthermore, prophylactic immunization with BL/US-treated DCs provided a four-fold decrease in the frequency of melanoma lung metastases [75].

4.2. Regulatory T-cells

Regulatory T-cells (T_{regs}) are a target for immunotherapies because of their role in subduing the immune response against cancer by suppressing auto-reactive immune cells [76]. Specifically, short interfering RNA (siRNA) can be used to knock down the expression of T_{reg} target genes and limit the immune protective tumor activity of T_{regs}. It was previously demonstrated that combining US and SonoVue microbubbles on CD4(+)CD25(+)T_{regs} affected T_{regs} proliferation. Moreover, the optimal T_{reg} transfection rate was obtained using 10% microbubbles and US exposure for150/180s under a mechanical index (MI) of 1.4 [77]. Sonication with US with no microbubbles was also found to decrease T_{reg} proliferation

However, a prolonged exposure time is needed. Cell viability and T_{reg} proliferation decrease optimally with a 10% concentration of cavitation microbubbles, which deliver siRNA of Forkhead box P3 (FoxP3) into T_{regs} [78].

4.3. Natural Killer Cell Therapy

Natural killer (NK) cells are naturally occurring antitumor immune cells, whose killing efficiency proportionally increases with the ratio of effector to target cells [79]. Autologous NK cells can be activated and expanded *ex vivo* and adoptively transferred back to patients to target tumors. Studies that achieved some success using NK cells therapy suggest that the efficacy of this treatment is dependent on NK cells' discrimination of tumors [80]. US is applied to enhance the ability of NK cells and NK-92 (human NK cell line) to overcome the constraints imposed by the BBB [81].

5. ULTRASOUND-MEDIATED CYTOKINE-BASED TUMOR IMMUNOTHERAPY

US-mediated cytokine-based tumor immunotherapy utilizes US to induce cavitation and the subsequent sonoporation effect on cellular membranes, allowing extracellular plasmid DNA of cytokines, such as the clinically approved IFN-beta and IL-2, to induce a local anticancer immune response [82]. A study revealed that cationic liposomal IFNβ gene therapy combined with US was able to produce antitumor effects *in vitro*. Additionally, the survival of mice with a metastatic hepatic tumor of Colon26 cells was significantly extended by cationic liposomal IFNβ gene therapy alone. However, sonoporation further increased the survival rate and inhibited the mice's tumor growth rate [83]. Moreover, IL-12 gene delivery using US-mediated liposomes could inhibit tumor growth *via* significant migration of CD8(+) T cells in mice [84]. Another study exhibited increased local expression of cytokines following the combination of DOTMA, a transfection complex containing IL-12 plasmid (15 μg), and US (1.5W/cm^2, 5 min) to treat SCCVII murine tumors [82, 85]. Another study has shown the promising potentials of the sonoporation-mediated Interleukin-27 (IL-27) gene delivery [86]. In another study, the delivery of IFN-beta pDNA to murine subcutaneous ovarian carcinoma (OV-HM) model enhanced the local IL-12 production and stimulated the migration of CD8+ T-cells to the tumor site upon intratumoral injection and treatment with US and bubble liposomes (1 MHz, 0.7 W/cm^2, 1 min) [84, 87]. Unfortunately, intratumoral injection is relatively invasive and difficult to translate to deeply located tumors; therefore, US-mediated delivery of cytokine genes has better potential at increasing local cytokine expression and suppressing tumor growth.

6. ULTRASOUND-MEDIATED IMMUNOTHERAPY

6.1. Antitumor Antibodies

Antibodies are naturally produced by B-cells in response to antigen presentation by helper T-cells. Antigen-antibody binding through an epitope is the fundamental theory of immunogenicity. Antibody-based cancer therapies are a promising chemotherapeutic method [14, 88] and are particularly promising due to their specificity, high efficacy, favorable pharmacokinetics, and optimized manufacturing, [89]. The FDA has approved several mAbs for cancer treatment [90]. The ability to provoke an immune response depends on the immunogen size, chemical composition, conformation, and its foreign capacity [27]. An example of an FDA approved mAbs treatment against cancer is Trastuzumab.

- **Trastuzumab**: also known as Herceptin, is a humanized IgG(1) kappa mAb (molecular weight of 145.5 kDa) with a high and specific affinity towards the HER2 receptors overexpressed in breast tumor cells [53]. Trastuzumab can prevent HER2 hetero-dimerization and stop cell signaling related to tumor development; it is considered a HER2 receptor antagonist [91]. Trastuzumab was shown to reduce the risk of cancer recurrence when used in adjuvant therapy and was also shown to augment the effects of chemotherapy [50, 92-94]. It is commonly used in combination with Paclitaxel, Docetaxel, Navelbine, Gemcitabine, and Capecitabine in Antibody-drug Conjugates (ADCs) [95, 96].
- **Pertuzumab:** also known as Perjeta, can destroy HER2-positive breast carcinoma *via* binding to the HER2 receptor and hindering the cancer cells' capability to accept growth signals [91, 97].
- **Ado-trastuzumab emtansine:** referred to as Kadcyla, is an arrangement of Herceptin and emtansine that delivers emtansine to HER2+cells in a targeted method, which further binds to HER2 receptors on the tumor cells and transports emtansine directly to the tumor [23, 54, 91].
- **Rituximab**: an anti-CD20 mAb, is used as a conjugate in nanoparticles to target lymphoma tumors overexpressing CD20 receptors. CD20 receptors do not internalize their anti- CD20 mAbs in contrast to CD19 receptors [98].

However, limitations to mAbs include expensive production, immunogenicity, and limited conjugation density due to their large size [99]. Also, mouse-derived antibodies were shown to induce some allergy-like reactions when used in humans, prompting the need for chimeric, or humanized murine-derived antibodies, or full human mAbs [100]. Chimeric mAbs are considered less compatible with humans than humanized ones. The variable fragment of chimeric mAbs is derived from a murine source, while the constant region is from a human source. Contrary to humanized mAbs where only the complementary determining regions of the variable regions (CDRs) are from a murine source. Fully human mAbs are developed using phage-display technologies [101]. However, further modifications to mAbs are needed for conjugation purposes.

Sites for chemical binding in antibodies, and proteins, in general, include thiol groups (sulfhydryl groups) found in the cysteine residue of the protein, amine groups located in the lysine residue, and carbohydrates [102]. Typically, sulfhydryl bonds in proteins are found in their reduced version as disulfide bonds (in cysteine), which first need to be activated into a free thiol group for the conjugation to be successful [103]. These modifications are known to affect the antigen-antibody binding sites except for the carbohydrate modification. For disulfide modification at low pH, damage control can be achieved [104].

So far, mAb therapies have shown little success against solid tumors due to the irregular physiologies of the tumor microenvironment [105], and the relatively large size of mAbs, which leads to reduced vascular permeability inside tissues [106]. Moreover, the repeated delivery of antibodies in high doses needed to reach therapeutic concentrations increases the costs and can exacerbate the treatment's negative side effects [99]. The nonspecific interactions between the antibodies and cells or ECMs are also responsible for ineffective immunotherapy. To achieve success in immunotherapy, the modification of antibodies and a deeper understanding of the different physiological factors that enable enhanced vascular absorbency are needed [107-109].

6.2. Ultrasound-triggered Immunoliposomes

Liposomes are small, spherically shaped vesicles with a diameter ranging between 20 and 1000 nm. Liposomes are composed of a phospholipid membrane bilayer, where the hydrophilic tails are directed inwards while the hydrophilic heads are directed outwards towards the aqueous environment [40, 110]. Liposomes are the most widely used nanocarriers in drug delivery. They can selectively target tumor cells by utilizing the enhanced permeability and retention (EPR) effect when they range in size between 12.5 and 200 nm. Chemotherapeutic drugs commonly loaded into liposomes include doxorubicin (Dox), annamycin, daunorubicin, vincristine, cisplatin derivatives, paclitaxel, 5-fluorouracil derivatives, camptothecin derivatives, and retinoids [111]. Liposomes can also be used to entrap various types of other molecules, including vaccines, plasmid DNA, peptides, hormones, antisense oligonucleotides or ribozymes, antibodies, nutraceuticals, and cosmetics [112, 113]. As mentioned earlier, PEG renders the liposomal carriers sterically stable and less immunogenic, which increases their blood circulation time and protects them from degradation in the plasma [114].

Liposomes having mAbs or antibody fragments conjugated to their surfaces are called ‘immune-liposomes’ or ‘immunoliposomes’ [114]. Highly investigated targets for antibodies include VEGFR, EGFR, HER2, transferrin receptors, and the prostate-specific membrane antigen (PSMA) [115]. HER2 receptors can internalize their ligands resulting in the endocytosis of the antibody-mediated nanoparticles. A known humanized mAb targeting HER2 is trastuzumab, which is an FDA approved cancer medicine. Nanoparticles, coupled with trastuzumab have been intensively investigated in the treatment of HER2 positive breast cancer [97, 116]. In 2018, Amal [117] demonstrated the high efficiency of LFUS in triggering drug release from both the conventional and trastuzumab-conjugated pegylated liposomes.

Stimuli-responsive immunoliposomes are designed to accumulate in the targeted tissue and release their contents in response to a particular stimulus. This trigger can be either intrinsic, such as a change in pH, temperature, or enzyme concentration, or external such as light, magnetic or electric fields, and US [118]. Liposomes can be modified to be more sensitive to US. Echogenic liposomes are rendered sensitive to US by entrapping emulsions into their core and releasing their contents when US is applied. A study investigated the US-mediated delivery of anti-smooth muscle cell actin antibody to vascular smooth muscle cells (VSMCs) *in vitro* using echogenic immunoliposomes (ELIP) as a vector [119]. Bevacizumab, an anti-angiogenic antibody to vascular endothelial growth factor (VEGF-A), was loaded into echogenic liposomes (BEV-ELIP) and was exposed to colorDoppler US at three acoustic pressures for 3.5 min during treatment at physiologic temperature and fluid pressure using *ex vivo* carotid arteries [120]. Another study showed that US-enhanced bevacizumab release from echogenic liposomes could result in atheroma progression *in vitro* [121]. Moreover, liposomes encapsulating nanoemulsions (eliposomes), utilize the ability of nano-emulsions to change from the liquid phase to the gas phase in response to US, thus leading to the immediate disruption of the nanoparticle and the subsequent drug release. In another study, calcein release from liposomes was reported to be higher at LFUS than at high-frequency ultrasound (HFUS) [122]. The effect of liposomal membrane structure on drug release following the exposure to LFUS was studied in Dox-loaded liposomes under LFUS effects; the results showed 30% higher release from
DOPE-based liposomes compared to DSPE-based liposomes [123]. Moreover, PEGylated liposomes similarly exhibited a 10-fold increase in permeabilization upon exposure to LFUS.

6.3. Ultrasound-mediated Microbubbles Conjugated Antibody Therapy

Another approach to improve the transport and accumulation of antibodies at tumor sites is through US-triggered microbubbles therapy. In 2016, a research group demonstrated that membrane disruption using FUS combined with antibody therapy could inhibit the growth of breast cancer brain metastasis [124]. Another study showed that cetuximab, a mAb, can bind to the EGFR antigen in the extracellular domain of tumor cells, and the addition of US can enhance the drug delivery to tumors [125]. A study reported that US, in conjunction with microbubbles, may enhance the effect of cetuximab cytotoxicity in HNSCC by repressing tumor growth, which was confirmed

Table 4. Ultrasound triggered different immunotherapeutic approach in cancer.

	Material	Cell Line	Animal Model	Immunological Agent	US Parameters	Outcome	Ref.
Immunoliposomes	Liposome (DSPC, DSPE-PEG)	-	Murine ovarian carcinoma (OV-HM cells)	Cytokine (IL-12 corded pDNA)	1MHz	1 MHz US with novel US-sensitive liposomes, which contain the US imaging (gas perfluoro propane), resulted in a significant migration of CD8(+) T cells in the mice.	[84]
	Liposome	Murine colon adenocarcinoma cell line Colo 26	Xeenograft mice model inoculated with Colo26	Cytokine (IFNbeta)	1 MHz, 0.5 W/cm^2, 20% duty cycle, 30 s; in vivo:1 MHz, 2 W/cm^2, 50% duty cycle, 10 min;	Suppressed tumor growth and increased survival	[83]
	Liposome	6–8-week-old female SCCVII tumor -bearing mice	squamous carcinoma cells	Cytokine (IL-12)	1 MHz, 0.7 W/cm^2, 60s	US treatment produced significantly higher levels of IL-12 in the tumor that further inhibited tumor growth.	[85]
	Liposome	HER2-positive breast cancer	-	Trastuzumab	20kHz, 7.46-17.31mW/cm^2	Enhanced release rates	[117]
	Echogenic immunoliposomes (ELIP)	-	Vascular smooth muscle cells (VSMCs)	Anti-smooth muscle cell actin antibody	1 MHz, 0.23 ± 0.05 MPa, 2 min	Increased site-specific calcein uptake	[119]
	Echogenic immunoliposomes (ELIP)	-	Ex vivo carotid arteries	bevacizumab	1.75 MHz frequency and 10 cycle pulse duration	Enhanced bevacizumab penetration, was observed in arteries treated with BEVELIP and combinations of color-Doppler US, especially in those arteries exhibiting extensive atheromatous lesions and neointima.	[120]
	Echogenic immunoliposomes (ELIP)	Human umbilical vein endothelial cell (HUVEC) cultures	-	bevacizumab	6 MHz color Doppler ultrasound (MI = 0.4) for 5 min.	BEV-ELIP retained its VEGF-binding activity in a liposomal formulation and that clinical Doppler US could significantly increase that activity, both by releasing free BEV and by enhancing the surface exposure of the immunoreactive antibody.	[121]
	Microbubble	Murine prostate cancer cell line TRAMP-C1 and TRAMP-C2		pORF-mIL-27	1 MHz, 50% duty cycle, 45 V, 2 Hz, 2 min, 1 W/cm^2, acoustic pressure of 0.12 MPa	Reduction of Prostate tumor	[86]
	Liposome Sonovue ® microbubble	MDA-MB-468 and MCF7 cells	MDA-MB-468 xenograft mice model	EGFR	1.1 MHz, 2 MPa, 3000 cycles, 1.2 sec pulse period, 2.5 min total exposure	Enhanced drug delivery to tumors	[128]
	Man-PEG$_{2000}$ bubble lipoplexes	CD8-OVA1.3 cells, EL4 cells, E.G7-OVA		pDNA expresses OVA	2.062 MHz, duty cycle 50%; burst rate, 10 Hz; 4.0 W/cm^2, 20 s	Suppressed tumor growth	[72]
	Man-PEG$_{2000}$ bubble lipoplexes	B16BL6 melanoma cells		pDNA expresses gp100 and TRP-2	1.045 MHz; duty, 50%; burst rate, 10 Hz; 1.0 W/cm^2; 2 min	Increased CTLs prevented metastasis and relapsed melanoma	[73]
	microbubble	B16/BL6 melanoma cell line	A mouse model with lung metastasis	Dendritic cell Vaccine (Melanoma-derived antigen)	2 MHz, 2 W/cm^2, 10% duty, 3x10 seconds	A decrease in the frequency of melanoma lung metastasis	[75]
	mRNA-lipoplex loaded microbubbles		DC from bone marrow of C57Bl/6 mice	mRNA	1 MHz, 2 W/cm^2, 50% duty cycle, 30 s	DC maturation	[74]
	mRNA-loaded microbubbles	Mouse melanoma cell line MO4 and T cell lymphoma E.G7-OVA	Mice with OVA tumors	antigen mRNA and TriMix mRNA	1 MHz, 2 W/cm^2, negative peak pressure 800 kPa, 20% duty cycle (2 m on, 8 m off), 30 s total insonation time per OptiCell ™	Increased CD8 + T cells, which suppressed tumor growth and increased survival	[130]
	microbubbles	Tregs from patients with HCC		Foxp3-miRNA/shRNA	2.5 MHz, MI of 1.4, 150/181 s	Decreased ratio of Tregs/CD4+ T cells suppressed tumor growth	[78]
	SonoVue ® microbubble	Hepatocellular carcinoma		CCD4+CD25+ regulatory T cells (Tregs)	1.4 MI, 150 or 180 sec	Increased Treg proliferation	[77]
	Definity®	Human HER2-expressing MDA-MB-231 breast tumor cells		NK-92	551.5 kHz FUS, range 0.32–0.35 Mpa	HER2-specific NK92 cells accumulating in tumors suppressed tumor growth and increased survival	[81]
	microbubbles	Human colorectal adenocarcinoma cells (LS174T)		NK cells	510 kHz transducer,0.50 MPa peak acoustic pressure	NK cell accumulation in tumors increased water content and edema	[80]
	Emtansine (DM1) microbubbles	MDAMB-361 cells		Trastuzumab and Pertuzumab	FUS of 10-ms burst sonication was applied having1 Hz repetition frequency, 60 s duration). Acoustic powers between 0.40 and 0.70 W were used.	BBB disruption using focused US in combination with antibody therapy can inhibit the growth of breast cancer brain metastasis	[124]
	microbubble	Human head and neck squamous carcinoma cell lines	6-week old xenograft mice inoculated with BT474	Cetuximab	1.0 MHz, MI: 0.5, a pulse repetition period of 5s, 20% duty cycle, 5 min	Suppressed tumor growth and increased survival.	[126]
	microbubbles	HER2/neu-positive human breast cancer cells BT474	6-week old xenograft mice inoculated with BT474	Trastuzumab	PRF:1 Hz, 1% DC, PNP:0.69 MPa, sonications were 60 s in duration and consisted of 10 m bursts	Suppressed tumor growth and increased survival	[127]
	microbubbles	Murine colorectal cancer cell line CT26		Anti PD-1 antibody	1 MHz transducer, 50 0.1-ms-long pulses spaced 1 m apart, 20 s intervals, duration of 2 min Peak negative pressures 1.65 Mpa	Suppressed tumor growth and increased survival, increase in the antitumor effects of CI therapy	[129]
	microbubbles	U14 and Hela cervical cancer cell line	SPF-level BALB/c female mice	Anti-PD-L1 mAb	1 MHz, pulse repetition frequency of 1 kHz, SATP intensity of 1 W/cm^2, duty cycle of 50%, duration of 90s, probe diameter of 10 mm.	The growth of the tumor was significantly slower in the combined treatment group compared to the group treated with either drug or microbubbles	[131]

by a regression in tumor size *in vivo* [126]. The authors suggested that US-mediated antibody delivery is, therefore, able to enhance cetuximab dispersion by reducing barriers in the vasculature. Similarly, trastuzumab [127], in conjunction with FUS and microbubbles, was shown by MRI to cross the BBB to treat brain metastases. ^{111}In-EGF-LP-Dox showed specificity for and cytotoxicity towards EGFR-overexpressing cancer cells. Delivery to tumors was enhanced by the use of US-mediated cavitation of Sonovue® microbubbles, indicating that this approach has the potential to deliver cytotoxic levels of therapeutic radionuclides to solid tumors [128]. These findings indicate that US-mediated antibody delivery allows an efficient delivery of different antibodies by overcoming vascular barriers or the BBB and thus, amplifying the concentration of therapeutics at the tumor site. The accumulation and binding of antibodies at the tumor site induce specific antitumor immune responses by blocking inhibitory signals of immune cells and governing cells to eliminate abnormal cells. However, further studies are needed to determine the specific immune responses mediated by US-triggered antibody therapy.

6.4. Enhancing Checkpoint Inhibitor Therapy with Ultrasound Stimulated Microbubbles

CI immunotherapy utilizes antibodies, such as anti-PD-1 antibodies (aPD-1), to prevent the checkpoint-mediated inhibition of T-cell responses toward abnormal cells [8, 9]. Unfortunately, only a minority of the patients treated with CI immunotherapy exhibited significant antitumor immune responses [129]. The observed ineffectiveness is attributed to the immunosuppressed tumor microenvironments in clinical settings. A study investigated coupling aPD-1 with microbubbles triggered by low-intensity US has shown that the combination therapy was able to amplify immune-tumor responses and increase survival rate compared to the independent treatments [129]. Table **4**() presents a summary of some relevant studies using US-triggered immuno-carriers for cancer therapy.

CONCLUSION

The future of cancer immunotherapy relies on developing effective methods to deliver immunotherapeutic agents to diseased cells. US-mediated liposome or microbubble treatments can help overcome biological barriers, thereby offering an alternative approach to present therapies. The use of mAbs is a significant advancement in the treatment of cancer. There are growing efforts to enhance the production of tumor-targeted antibodies and to understand the detailed immunogenic responses as well as their delivery portfolio. However, this field is still in its infancy as many of the fundamental mechanisms of these bioprocesses are not fully understood. In addition, the ultrasonic delivery of immuno-liposomes has been limited to *in vitro* experiments. Recently, emerging *in vivo* data have shown promising potentials of this therapeutic platform to reaching clinical trials. Other factors, such as the genetic background, type of cancer, age, drug mechanism, US parameters (frequency, power density, pulse duration), as well as safe delivery protocols, should be considered.

AUTHOR CONTRIBUTION

All authors contributed to writing and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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CONFLICT OF INTEREST

The authors have no conflicts of interest, financial or otherwise.

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REFERENCES

[1] Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer statistics, 2020. *CA Cancer J. Clin.,* **2020**, *70*(1), 7-30. http://dx.doi.org/10.3322/caac.21590 PMID: 31912902

[2] Zhang, P.W.; Chen, L.; Huang, T.; Zhang, N.; Kong, X.Y.; Cai, Y.D. Classifying ten types of major cancers based on reverse phase protein array profiles. *PLoS One,* **2015**, *10*(3), e0123147. http://dx.doi.org/10.1371/journal.pone.0123147 PMID: 25822500

[3] Blackadar, C. B. Historical review of the causes of cancer. *World Journal of Clinical Oncology.,* **2016**, *7*(1), 54-86. http://dx.doi.org/10.5306/wjco.v7.i1.54

[4] Kibria, G.; Hatakeyama, H.; Harashima, H. Cancer multidrug resistance: mechanisms involved and strategies for circumvention using a drug delivery system. *Arch. Pharm. Res.,* **2014**, *37*(1), 4-15. http://dx.doi.org/10.1007/s12272-013-0276-2 PMID: 24272889

[5] Center, E.M. *Multi-Drug Resistance and Chemotherapy; Overcoming Standard Cancer Treatment Failures.,* https://www.envita.com/cancer/multi-drug-resistance-and-chemotherapy-overcomingstandard-cancer-treatment-failures

[6] Alfarouk, K. O. Resistance to cancer chemotherapy: Failure in drug response from ADME to P-gp. *Cancer Cell International.,* **2015**, *15*(1), 71. http://dx.doi.org/10.1186/s12935-015-0221-1

[7] Gillet, J.P.; Gottesman, M.M. Mechanisms of multidrug resistance in cancer. *Methods Mol. Biol.,* **2010**, *596*, 47-76. http://dx.doi.org/10.1007/978-1-60761-416-6_4 PMID: 19949920

[8] Baskar, R.; Lee, K.A.; Yeo, R.; Yeoh, K.W. Cancer and radiation therapy: current advances and future

directions. *Int. J. Med. Sci.,* **2012**, *9*(3), 193-199. http://dx.doi.org/10.7150/ijms.3635 PMID: 22408567

[9] Brownlee, Z.; Garg, R.; Listo, M.; Zavitsanos, P.; Wazer, D. E.; Huber, K. E. Late complications of radiation therapy for breast cancer: Evolution in techniques and risk over time. *Gland Surgery.,* 2009*7*(4), 371-378. http://dx.doi.org/10.21037/gs.2018.01.05

[10] Kim, B. M. Therapeutic implications for overcoming radiation resistance in cancer therapy. *International Journal of Molecular Sciences.,* **2015**, *16*(11), 26880-26913. http://dx.doi.org/10.3390/ijms161125991

[11] Schirrmacher, V. From chemotherapy to biological therapy: A review of novel concepts to reduce the side effects of systemic cancer treatment (Review). *Int. J. Oncol.,* **2019**, *54*(2), 407-419. http://dx.doi.org/10.3892/ijo.2018.4661 PMID: 30570109

[12] Messerschmidt, J.L.; Prendergast, G.C.; Messerschmidt, G.L. How Cancers Escape Immune Destruction and Mechanisms of Action for the New Significantly Active Immune Therapies: Helping Nonimmunologists Decipher Recent Advances. *Oncologist,* **2016**, *21*(2), 233-243. http://dx.doi.org/10.1634/theoncologist.2015-0282 PMID: 26834161

[13] Chen, D. S.; Mellman, I. Oncology meets immunology: The cancer-immunity cycle. *Immunity.,* **2013**, *39*(1), 1-10. http://dx.doi.org/10.1016/j.immuni.2013.07.012

[14] Scott, A.M.; Allison, J.P.; Wolchok, J.D. Monoclonal antibodies in cancer therapy. *Cancer Immun.,* **2012**, *12*, 14. PMID: 22896759

[15] Dotti, G.; Gottschalk, S.; Savoldo, B.; Brenner, M.K. Design and development of therapies using chimeric antigen receptor-expressing T cells. *Immunol. Rev.,* **2014**, *257*(1), 107-126. http://dx.doi.org/10.1111/imr.12131 PMID: 24329793

[16] Smith, A.J.; Oertle, J.; Warren, D.; Prato, D. Chimeric antigen receptor (CAR) T cell therapy for malignant cancers: Summary and perspective. *J. Cell. Immunother.,* **2016**, *2*(2), 59-68.

http://dx.doi.org/10.1016/j.jocit.2016.08.001

[17] Han, X.; Wang, Y.; Han, W-D. Chimeric antigen receptor modified T-cells for cancer treatment. *Chronic Dis Transl Med,* **2018**, *4*(4), 225-243. http://dx.doi.org/10.1016/j.cdtm.2018.08.002 PMID: 30603741

[18] Jena, B.; Dotti, G.; Cooper, L. J. N. Redirecting T-cell specificity by introducing a tumor-specific chimeric antigen receptor. *Blood.,* **2010**, *116*(7), 1035-1044. http://dx.doi.org/10.1182/blood-2010-01-043737

[19] Wu, X. Application of PD-1 Blockade in Cancer Immunotherapy *Computational and Structural Biotechnology Journal.,* **2019**, *17*, 661-674. http://dx.doi.org/10.1016/j.csbj.2019.03.006

[20] Yang, Y. Cancer immunotherapy: harnessing the immune system to battle cancer. *J. Clin. Invest.,* **2015**, *125*(9), 3335-3337. http://dx.doi.org/10.1172/JCI83871 PMID: 26325031

[21] Yan, S.; Zhao, P.; Yu, T.; Gu, N. Current applications and future prospects of nanotechnology in cancer immunotherapy. *Cancer Biology and Medicine.,* **16**, *16*(3), 486-497.

[22] Milling, L.; Zhang, Y.; Irvine, D. J. Delivering safer immunotherapies for cancer. *Advanced Drug Delivery Reviews.,* **2017**, *114*, 79-101. http://dx.doi.org/10.1016/j.addr.2017.05.011

[23] Li, Z.; Tan, S.; Li, S.; Shen, Q.; Wang, K. Cancer drug delivery in the nano era: An overview and perspectives (Review). *Oncology Reports.,* **2017**, *38*(2), 611-624. http://dx.doi.org/10.3892/or.2017.5718

[24] Dadwal, A.; Baldi, A.; Kumar Narang, R. Nanoparticles as carriers for drug delivery in cancer. *Artif. Cells, Nanomedicine, Biotechnol.,* **2018**, *46*(sup2), 295-305. http://dx.doi.org/10.1080/21691401.2018.1457039

[25] Nisini, R.; Poerio, N.; Mariotti, S.; De Santis, F.; Fraziano, M. The multirole of liposomes in therapy and prevention of infectious diseases. *Frontiers in Immunology.,* **2018**, *9* http://dx.doi.org/10.3389/fimmu.2018.00155

[26] Zhao, M. Antibody-Targeted Immunocarriers for Cancer Treatment*Smart Drug Delivery System*; InTech., **2016**, pp. 139-158. http://dx.doi.org/10.5772/61288

[27] Julien, D. C.; Behnke, S.; Wang, G.; Murdoch, G. K.; Hill, R. A. Utilization of monoclonal antibody-targeted nanomaterials in the treatment of cancer. *mAbs.,* **2011**, *3*(5), 467-478. http://dx.doi.org/10.4161/mabs.3.5.16089

[28] Jhaveri, A.; Deshpande, P.; Torchilin, V. Stimuli-sensitive nanopreparations for combination cancer therapy. *Journal of Controlled Release.,* **2014**, *190*, 352-370. http://dx.doi.org/10.1016/j.jconrel.2014.05.002

[29] Unga, J.; Hashida, M. Ultrasound induced cancer immunotherapy. *Advanced Drug Delivery Reviews.,* **2014**, *72*, 144-153. http://dx.doi.org/10.1016/j.addr.2014.03.004

[30] Shi, G.; Zhong, M.; Ye, F.; Zhang, X. Low-frequency HIFU induced cancer immunotherapy: tempting challenges and potential opportunities. *Cancer Biology and Medicine.,* **2019**, *16*(4), 714-728.

[31] Quaia, E.; D'Onofrio, M.; Palumbo, A.; Rossi, S.; Bruni, S.; Cova, M. Comparison of contrast-enhanced ultrasonography versus baseline ultrasound and contrast-enhanced computed tomography in metastatic disease of the liver: Diagnostic performance and confidence *European Radiology.,* **2066**, *16*(7), 1599-1609.

[32] Khokhlova, V.A.; Bailey, M.R.; Reed, J.A.; Cunitz, B.W.; Kaczkowski, P.J.; Crum, L.A. Effects of nonlinear propagation, [49] cavitation, and boiling in lesion formation by high intensity focused ultrasound in a gel phantom. *J. Acoust. Soc. Am.,* **2006**, *119*(3), 1834-1848. http://dx.doi.org/10.1121/1.2161440 PMID: 16583923

[33] Shanks, P.; Curran, M.; Fletcher, P.; Thompson, R. The effectiveness of therapeutic ultrasound for musculoskeletal conditions of the lower limb: A literature review. *Foot,* **2010**, *20*(4), 133-139. http://dx.doi.org/10.1016/j.foot.2010.09.006 PMID: 20961748

[34] June, C. H.; Warshauer, J. T.; Bluestone, J. A. Is autoimmunity the Achilles' heel of cancer immunotherapy? *Nature Medicine.,* **2017**, *23*(5), 540-547. http://dx.doi.org/10.1038/nm.4321

[35] Langer, R. New methods of drug delivery. *Science,* **1990**, *249*(4976), 1527-1533. http://dx.doi.org/10.1126/science.2218494 PMID: 2218494

[36] Patra, J.K.; Das, G.; Fraceto, L.F.; Campos, E.V.R.; RodriguezTorres, M.D.P.; Acosta-Torres, L.S.; Diaz-Torres, L.A.; Grillo, R.; Swamy, M.K.; Sharma, S.; Habtemariam, S.; Shin, H.S. Nano based drug delivery systems: recent developments and future prospects. *J. Nanobiotechnology,* **2018**, *16*(1), 71. http://dx.doi.org/10.1186/s12951-018-0392-8 PMID: 30231877

[37] Lombardo, D.; Kiselev, M.A.; Caccamo, M.T. Smart Nanoparticles for Drug Delivery Application: Development of Versatile Nanocarrier Platforms in Biotechnology and Nanomedicine. *J. Nanomater.,* **2019**, *2019* http://dx.doi.org/10.1155/2019/3702518

[38] Amreddy, N. Recent Advances in Nanoparticle-Based Cancer Drug and Gene Delivery*Advances in Cancer Research*; Academic Press Inc., **2018**, 137, pp. 115-170. http://dx.doi.org/10.1016/bs.acr.2017.11.003

[39] Mitra, A.K.; Agrahari, V.; Mandal, A.; Cholkar, K.; Natarajan, C.; Shah, S.; Joseph, M.; Trinh, H.M.; Vaishya, R.; Yang, X.; Hao, Y.; Khurana, V.; Pal, D. Novel delivery approaches for cancer therapeutics. *J. Control. Release,* **2015**, *219*, 248-268. http://dx.doi.org/10.1016/j.jconrel.2015.09.067 PMID: 26456750

[40] Sharma, A.; Jain, N.; Sareen, R. Nanocarriers for diagnosis and targeting of breast cancer. *BioMed Res. Int.,* **2013**, *2013*, 960821. http://dx.doi.org/10.1155/2013/960821 PMID: 23865076

[41] Castermans, K.; Griffioen, A. W. Tumor blood vessels, a difficult hurdle for infiltrating leukocytes. *Biochimica et Biophysica Acta Reviews on Cancer.,* **2007**, *1776*(2), 160-174.

[42] Minchinton, A. I.; Tannock, I. F. Drug penetration in solid tumours. *Nature Reviews Cancer.,* **2006**, *6*(8), 583-592. http://dx.doi.org/10.1038/nrc1893

[43] Daneman, R.; Prat, A. The blood-brain barrier. *Cold Spring Harb. Perspect. Biol.,* **2015**, *7*(1), a020412. http://dx.doi.org/10.1101/cshperspect.a020412 PMID: 25561720

[44] Gutmann, R.; Leunig, M.; Feyh, J.; Goetz, A.E.; Messmer, K.; Kastenbauer, E.; Jain, R.K. Interstitial hypertension in head and neck tumors in patients: correlation with tumor size. *Cancer Res.,* **1992**, *52*(7), 1993-1995. PMID: 1551128

[45] Padera, T.P.; Stoll, B.R.; Tooredman, J.B.; Capen, D.; di Tomaso, E.; Jain, R.K. Pathology: cancer cells compress intratumour vessels. *Nature,* **2004**, *427*(6976), 695. http://dx.doi.org/10.1038/427695a PMID: 14973470

[46] Kjær, M. Role of Extracellular Matrix in Adaptation of Tendon and Skeletal Muscle to Mechanical Loading. *Physiological Reviews.,* **2004**, *84*(2), 649-698. http://dx.doi.org/10.1152/physrev.00031.2003

[47] Matuszewska, K.; Santry, L.A.; van Vloten, J.P.; AuYeung, A.W.K.; Major, P.P.; Lawler, J.; Wootton, S.K.; Bridle, B.W.; Petrik, J. Combining vascular normalization with an oncolytic virus enhances immunotherapy in a preclinical model of advanced-stage ovarian cancer. *Clin. Cancer Res.,* **2019**, *25*(5), 1624-1638. http://dx.doi.org/10.1158/1078-0432.CCR-18-0220 PMID: 30206160

[48] Goel, S.; Wong, A.H.K.; Jain, R.K. Vascular normalization as a therapeutic strategy for malignant and nonmalignant disease. *Cold Spring Harb. Perspect. Med.,* **2012**, *2*(3), a006486. http://dx.doi.org/10.1101/cshperspect.a006486 PMID: 22393532

[49] Mattheolabakis, G.; Mikelis, C. M. Nanoparticle Delivery and Tumor Vascular Normalization: The Chicken or The Egg? *Frontiers in Oncology.,* **2019**, *9*, 1227. http://dx.doi.org/10.3389/fonc.2019.01227

[50] Yameen, B.; Il Choi, W.; Vilos, C.; Swami, A.; Shi, J.; Farokhzad, O. C. Insight into nanoparticle cellular uptake and intracellular targeting. *Journal of Controlled Release.,* **2014**, *190*, 485-499. http://dx.doi.org/10.1016/j.jconrel.2014.06.038

[51] Salmon, H.; Franciszkiewicz, K.; Damotte, D.; Dieu-Nosjean, M.C.; Validire, P.; Trautmann, A.; Mami-Chouaib, F.; Donnadieu, E. Matrix architecture defines the preferential localization and migration of T cells into the stroma of human lung tumors. *J. Clin. Invest.,* **2012**, *122*(3), 899-910. http://dx.doi.org/10.1172/JCI45817 PMID: 22293174

[52] Caruana, I.; Savoldo, B.; Hoyos, V.; Weber, G.; Liu, H.; Kim, E.S.; Ittmann, M.M.; Marchetti, D.; Dotti, G. Heparanase promotes tumor infiltration and antitumor activity of CAR-redirected T lymphocytes. *Nat. Med.,* **2015**, *21*(5), 524-529. http://dx.doi.org/10.1038/nm.3833 PMID: 25849134

[53] Shin, D.H.; Koo, M.J.; Kim, J.S.; Kim, J.S. Herceptin-conjugated temperature-sensitive immunoliposomes encapsulating gemcitabine for breast cancer. *Arch. Pharm. Res.,* **2016**, *39*(3), 350-358. http://dx.doi.org/10.1007/s12272-016-0707-y PMID: 26781980

[54] Martins, A.M.; Ahmed, S.E.; Vitor, R.F.; Husseini, G.A. Ultrasonic Drug Delivery Using Micelles and Liposomes.*Handbook of Ultrasonics and Sonochemistry*; Springer Singapore: Singapore, **2016**, pp. 1-35.

[55] Moussa, H.G.; Martins, A.M.; Husseini, G.A. Review on triggered liposomal drug delivery with a focus on ultrasound. *Curr. Cancer Drug Targets,* **2015**, *15*(4), 282-313. http://dx.doi.org/10.2174/1568009615666150311100610 PMID: 25760762

[56] Masuzaki, R.; Shiina, S.; Tateishi, R.; Yoshida, H.; Goto, E.; Sugioka, Y.; Kondo, Y.; Goto, T.; Ikeda, H.; Omata, M.; Koike, K. Utility of contrast-enhanced ultrasonography with Sonazoid in radiofrequency ablation for hepatocellular carcinoma. *J. Gastroenterol. Hepatol.,* **2011**, *26*(4), 759-764. http://dx.doi.org/10.1111/j.1440-1746.2010.06559.x PMID: 21054516

[57] Elhelf, I. A. S.; Albahar, H.; Shah, U.; Oto, A.; Cressman, E.; Almekkawy, M. High intensity focused ultrasound: The fundamentals, clinical applications and research trends *Diagnostic and Interventional Imaging.,* **2018**, *99*(6), 349-359. http://dx.doi.org/10.1016/j.diii.2018.03.001

[58] Kennedy, J.E. High-intensity focused ultrasound in the treatment of solid tumours. *Nat. Rev. Cancer,* **2005**, *5*(4), 321-327. http://dx.doi.org/10.1038/nrc1591 PMID: 15776004

[59] Monn, M.F.; Chandra, K.F.; Koch, M.O. High-Intensity Focused Ultrasound.*Prostate Cancer*; Academic Press, **2016**, pp. 551-562. http://dx.doi.org/10.1016/B978-0-12-800077-9.00059-1

[60] Xu, W.; Zhang, X.; Hu, X.; Zhiyi, C.; Huang, P. Translational Prospects of ultrasound-mediated tumor immunotherapy: Preclinical advances and safety considerations *Cancer Letters.,* **2019**, *460*, 86-95. http://dx.doi.org/10.1016/j.canlet.2019.06.017

[61] Burgess, A.; Shah, K.; Hough, O.; Hynynen, K. Focused ultrasound-mediated drug delivery through the blood-brain barrier *Expert Review of Neurotherapeutics.,* **2015**, *15*(5), 477-491. http://dx.doi.org/10.1586/14737175.2015.1028369

[62] Oberli, M. A.; Schoellhammer, C. M.; Langer, R.; Blankschtein, D. Ultrasound-enhanced transdermal delivery: Recent advances and future challenges *Therapeutic Delivery., 5*(7), 843-857. http://dx.doi.org/10.4155/tde.14.32

[63] Miller, D. L.; Smith, N. B.; Bailey, M. R.; Czarnota, G. J.; Hynynen, K.; Makin, I. R. S. Overview of therapeutic ultrasound applications and safety considerations *Journal of Ultrasound in Medicine.,* **2012**, *31*(4), 623-634. http://dx.doi.org/10.7863/jum.2012.31.4.623

[64] Schroeder, A.; Kost, J.; Barenholz, Y. Ultrasound, liposomes, and drug delivery: principles for using ultrasound to control the release of drugs from liposomes. *Chem. Phys. Lipids,* **2009**, *162*(1-2), 1-16. http://dx.doi.org/10.1016/j.chemphyslip.2009.08.003 PMID: 19703435

[65] Ta, T.; Porter, T.M. Thermosensitive liposomes for localized delivery and triggered release of chemotherapy. *J. Control. Release,* **2013**, *169*(1-2), 112-125. http://dx.doi.org/10.1016/j.jconrel.2013.03.036 PMID: 23583706

[66] Barenholz, Y. Doxil® - The first FDA-approved nano-drug: Lessons learned *Journal of Controlled Release.,* **2012**, *160*(2), 117-134. http://dx.doi.org/10.1016/j.jconrel.2012.03.020

[67] Mannaris, C.; Efthymiou, E.; Meyre, M.E.; Averkiou, M.A. *in vitro* localized release of thermosensitive liposomes with ultrasound-induced hyperthermia. *Ultrasound Med. Biol.,* **2013**, *39*(11), 2011-2020. http://dx.doi.org/10.1016/j.ultrasmedbio.2013.06.001 PMID: 23972488

[68] Elkhodiry, M.A.; Momah, C.C.; Suwaidi, S.R.; Gadalla, D.; Martins, A.M.; Vitor, R.F.; Husseini, G.A. Synergistic Nanomedicine: Passive, Active, and Ultrasound-Triggered Drug Delivery in Cancer Treatment. *J. Nanosci. Nanotechnol.,* **2016**, *16*(1), 1-18. http://dx.doi.org/10.1166/jnn.2016.11124 PMID: 27398430

[69] Weinstein, J.N.; Magin, R.L.; Yatvin, M.B.; Zaharko, D.S. Liposomes and local hyperthermia: selective delivery of methotrexate to heated tumors. *Science,* **1979**, *204*(4389), 188-191. http://dx.doi.org/10.1126/science.432641 PMID: 432641

[70] Reardon, D. A.; Mitchell, D. A. The development of dendritic cell vaccine-based immunotherapies for glioblastoma *Seminars in Immunopathology.,* **2017**, *39*(2), 225-239. http://dx.doi.org/10.1007/s00281-016-0616-7

[71] Garg, A.D.; Vandenberk, L.; Koks, C.; Verschuere, T.; Boon, L.; Van Gool, S.W.; Agostinis, P. Dendritic cell vaccines based on immunogenic cell death elicit danger signals and T cell-driven rejection of high-grade glioma. *Sci. Transl. Med.,* **2016**, *8*(328), 328ra27. http://dx.doi.org/10.1126/scitranslmed.aae0105 PMID: 26936504 Cintolo, J. A.; Datta, J.; Mathew, S. J.; Czerniecki, B. J. Dendritic cell-based vaccines: Barriers and opportunities *Future Oncology.,*

[72] **2012**, *8*(10)1273 http://dx.doi.org/10.2217/fon.12.125

[73] Un, K.; Kawakami, S.; Suzuki, R.; Maruyama, K.; Yamashita, F.; Hashida, M. Suppression of melanoma growth and metastasis by DNA vaccination using an ultrasound-responsive and mannosemodified gene carrier. *Mol. Pharm.,* **2011**, *8*(2), 543-554. http://dx.doi.org/10.1021/mp100369n PMID: 21250746

[74] De Temmerman, M.L.; Dewitte, H.; Vandenbroucke, R.E.; Lucas, B.; Libert, C.; Demeester, J.; De Smedt, S.C.; Lentacker, I.; Rejman, J. mRNA-Lipoplex loaded microbubble contrast agents for ultrasound-assisted transfection of dendritic cells. *Biomaterials,* **2011**, *32*(34), 9128-9135. http://dx.doi.org/10.1016/j.biomaterials.2011.08.024 PMID: 21868088

[75] Oda, Y.; Suzuki, R.; Otake, S.; Nishiie, N.; Hirata, K.; Koshima, R.; Nomura, T.; Utoguchi, N.; Kudo, N.; Tachibana, K.; Maruyama, K. Prophylactic immunization with Bubble liposomes and ultrasound-treated dendritic cells provided a four-fold decrease in the frequency of melanoma lung metastasis. *J. Control. Release,* **2012**, *160*(2), 362-366. http://dx.doi.org/10.1016/j.jconrel.2011.12.003 PMID: 22192573 Stockis, J.; Liénart, S.; Colau, D.; Collignon, A.; Nishimura, S.L.; Sheppard, D.; Coulie, P.G.; Lucas, S. Blocking

[76] immunosuppression by human Tregs *in vivo* with antibodies targeting integrin αVβ8. *Proc. Natl. Acad. Sci. USA,* **2017**, *114*(47), E10161E10168. http://dx.doi.org/10.1073/pnas.1710680114 PMID: 29109269 Shi, C.; Zhang, Y.; Yang, H.; Dong, T.; Chen, Y.; Xu, Y.; Yang,

[77] X. Combined effect of ultrasound/SonoVue microbubble on CD4(+)CD25(+) regulatory T cells viability and optimized parameters for its transfection. *Ultrasonics,* **2015**, *62*, 97-102. http://dx.doi.org/10.1016/j.ultras.2015.05.006 PMID: 26048174 Shi, C.; Zhang, Y.; Yang, H.; Dong, T.; Chen, Y.; Xu, Y.; Yang,

[78] X.; Liu, P. Ultrasound-targeted microbubble destruction-mediated Foxp3 knockdown may suppress the tumor growth of HCC mice by relieving immunosuppressive Tregs function. *Exp. Ther. Med.,* **2018**, *15*(1), 31-38. http://dx.doi.org/10.3892/etm.2017.5421 PMID: 29387180

[79] Larsen, S.K.; Gao, Y.; Basse, P.H. NK cells in the tumor microenvironment. *Crit. Rev. Oncog.,* **2014**, *19*(1-2), 91-105. http://dx.doi.org/10.1615/CritRevOncog.2014011142 PMID: 24941376

[80] Sta Maria, N.S.; Barnes, S.R.; Weist, M.R.; Colcher, D.; Raubitschek, A.A.; Jacobs, R.E. Low dose focused ultrasound induces enhanced tumor accumulation of natural killer cells. *PLoS One,* **2015**, *10*(11), e0142767. http://dx.doi.org/10.1371/journal.pone.0142767 PMID: 26556731

[81] Alkins, R.; Burgess, A.; Kerbel, R.; Wels, W. S.; Hynynen, K. Early treatment of HER2-amplified brain tumors with targeted NK-92

cells and focused ultrasound improves survival http://dx.doi.org/10.1093/neuonc/nov318

[82] Miller, D. L.; Pislaru, S. V.; Greenleaf, J. F. Sonoporation: Mechanical DNA delivery by ultrasonic cavitation *Somatic Cell and Molecular Genetics.,* **2002**, *27*(1-6), 115-134.

[83] Hayashi, S.; Mizuno, M.; Yoshida, J.; Nakao, A. Effect of sonoporation on cationic liposome-mediated IFNbeta gene therapy for metastatic hepatic tumors of murine colon cancer. *Cancer Gene Ther.,* **2009**, *16*(8), 638-643. http://dx.doi.org/10.1038/cgt.2008.1 PMID: 19498458

[84] Suzuki, R.; Namai, E.; Oda, Y.; Nishiie, N.; Otake, S.; Koshima, R.; Hirata, K.; Taira, Y.; Utoguchi, N.; Negishi, Y.; Nakagawa, S.; Maruyama, K. Cancer gene therapy by IL-12 gene delivery using liposomal bubbles and tumoral ultrasound exposure. *J. Control. Release,* **2010**, *142*(2), 245-250. http://dx.doi.org/10.1016/j.jconrel.2009.10.027 PMID: 19883708

[85] Anwer, K.; Kao, G.; Proctor, B.; Anscombe, I.; Florack, V.; Earls, R.; Wilson, E.; McCreery, T.; Unger, E.; Rolland, A.; Sullivan, S.M. Ultrasound enhancement of cationic lipid-mediated gene transfer to primary tumors following systemic administration. *Gene Ther.,* **2000**, *7*(21), 1833-1839. http://dx.doi.org/10.1038/sj.gt.3301302 PMID: 11110415

[86] Zolochevska, O.; Xia, X.; Williams, B.J.; Ramsay, A.; Li, S.; Figueiredo, M.L. Sonoporation delivery of interleukin-27 gene therapy efficiently reduces prostate tumor cell growth *in vivo*. *Hum. Gene Ther.,* **2011**, *22*(12), 1537-1550. http://dx.doi.org/10.1089/hum.2011.076 PMID: 21801027

[87] Sakakima, Y.; Hayashi, S.; Yagi, Y.; Hayakawa, A.; Tachibana, K.; Nakao, A. Gene therapy for hepatocellular carcinoma using sonoporation enhanced by contrast agents. *Cancer Gene Ther.,* **2005**, *12*(11), 884-889. http://dx.doi.org/10.1038/sj.cgt.7700850 PMID: 15891773

[88] Allen, T.M. Ligand-targeted therapeutics in anticancer therapy. *Nat. Rev. Cancer,* **2002**, *2*(10), 750-763. http://dx.doi.org/10.1038/nrc903 PMID: 12360278

[89] Brodsky, F.M. *Monoclonal antibodies*; Access Sci, **2014**.

[90] Liu, J.K.H. The history of monoclonal antibody development Progress, remaining challenges and future innovations. *Ann. Med. Surg. (Lond.),* **2014**, *3*(4), 113-116. http://dx.doi.org/10.1016/j.amsu.2014.09.001 PMID: 25568796

[91] Yu, S. Development and clinical application of anti-HER2 monoclonal and bispecific antibodies for cancer treatment *Experimental Hematology and Oncology.,* **2017**, *6*(1), 31. http://dx.doi.org/10.1186/s40164-017-0091-4

[92] Casals, E.; Gusta, M. F.; Cobaleda-Siles, M.; Garcia-Sanz, A.; Puntes, V. F. Cancer resistance to treatment and antiresistance tools offered by multimodal multifunctional nanoparticles *Cancer Nanotechnology.,* **2017**, *8*(1) http://dx.doi.org/10.1186/s12645-017-0030-4

[93] Sercombe, L.; Veerati, T.; Moheimani, F.; Wu, S. Y.; Sood, A. K.; Hua, S. Advances and challenges of liposome assisted drug delivery *Frontiers in Pharmacology.,* **2015**, *6* http://dx.doi.org/10.3389/fphar.2015.00286

[94] Benvenuto, M.; Focaccetti, C.; Izzi, V.; Masuelli, L.; Modesti, A.; Bei, R. Tumor antigens heterogeneity and immune response-targeting neoantigens in breast cancer. *Semin. Cancer Biol.,* **2019**, (Nov), S1044-579X(19)30299-8.

[99] active targeting by nanoparticles: a comprehensive review of literature *Journal of Cancer Research and Clinical Oncology.,* **2015**, *141*(5), 769-784. http://dx.doi.org/10.1007/s00432-014-1767-3

[100] Chames, P.; Van Regenmortel, M.; Weiss, E.; Baty, D. Therapeutic antibodies: Successes, limitations and hopes for the future *British Journal of Pharmacology.,* **2009**, *157*(2), 220-233. http://dx.doi.org/10.1111/j.1476-5381.2009.00190.x

[101] Getts, D. R.; Getts, M. T.; McCarthy, D. P.; Chastain, E. M. L.; Miller, S. D. Have we overestimated the benefit of human(ized) antibodies? *mAbs.,* **2010**, *2*(6), 682-694. http://dx.doi.org/10.4161/mabs.2.6.13601

[102] Harding, F.A.; Stickler, M.M.; Razo, J.; DuBridge, R.B. The immunogenicity of humanized and fully human antibodies: residual immunogenicity resides in the CDR regions. *MAbs,* **2010**, *2*(3), 256-265. http://dx.doi.org/10.4161/mabs.2.3.11641 PMID: 20400861

[103] Hamblin, T.J. Modifications of monoclonal antibody for immunotherapy. *Transfus. Sci.,* **1989**, *10*(1), 27-37. http://dx.doi.org/10.1016/0955-3886(89)90006-4

[104] Chung, W.K.; Russell, B.; Yang, Y.; Handlogten, M.; Hudak, S.; Cao, M.; Wang, J.; Robbins, D.; Ahuja, S.; Zhu, M. Effects of antibody disulfide bond reduction on purification process performance and final drug substance stability. *Biotechnol. Bioeng.,* **2017**, *114*(6), 1264-1274. http://dx.doi.org/10.1002/bit.26265 PMID: 28186329

[105] Robotham, A.C.; Kelly, J.F. Detection and quantification of free sulfhydryls in monoclonal antibodies using maleimide labeling and mass spectrometry. *MAbs,* **2019**, *11*(4), 757-766. http://dx.doi.org/10.1080/19420862.2019.1595307 PMID: 30894096

[106] Koppe, M.J.; Postema, E.J.; Aarts, F.; Oyen, W.J.G.; Bleichrodt, R.P.; Boerman, O.C. Antibody-guided radiation therapy of cancer. *Cancer Metastasis Rev.,* **2005**, *24*(4), 539-567. http://dx.doi.org/10.1007/s10555-005-6195-z PMID: 16408161 Jain, M.; Venkatraman, G.; Batra, S.K. Optimization of radioimmunotherapy of solid tumors: biological impediments and their modulation. *Clin. Cancer Res.,* **2007**, *13*(5), 1374-1382. http://dx.doi.org/10.1158/1078-0432.CCR-06-2436 PMID: 17309914

[107] Netti, P.A.; Hamberg, L.M.; Babich, J.W.; Kierstead, D.; Graham, W.; Hunter, G.J.; Wolf, G.L.; Fischman, A.; Boucher, Y.; Jain, R.K. Enhancement of fluid filtration across tumor vessels: implication for delivery of macromolecules. *Proc. Natl. Acad. Sci. USA,* **1999**, *96*(6), 3137-3142. http://dx.doi.org/10.1073/pnas.96.6.3137 PMID: 10077650 Kinuya, S.; Yokoyama, K.; Michigishi, T.; Tonami, N. Optimization of radioimmunotherapy interactions with hyperthermia. *Int. J. Hyperthermia,* **2004**, *20*(2), 190-200. http://dx.doi.org/10.1080/02656730310001611044 PMID: 15195513

[108] Hauck, M.L.; Dewhirst, M.W.; Bigner, D.D.; Zalutsky, M.R. Local hyperthermia improves uptake of a chimeric monoclonal antibody in a subcutaneous xenograft model. *Clin. Cancer Res.,* **1997**, *3*(1), 63-70. PMID: 9815539

[109] Simões, S. Cationic liposomes for gene delivery *Expert Opinion on Drug Delivery.,* **2005**, *2*(2), 237-254. http://dx.doi.org/10.1517/17425247.2.2.237

[110]

[95] http://dx.doi.org/10.1016/j.semcancer.2019.10.023 PMID: 31698088

[96] Ojima, I.; Lichtenthal, B.; Lee, S.; Wang, C.; Wang, X. Taxane anticancer agents: A patent perspective *Expert Opinion on Therapeutic Patents.,* **2016**, *26*(1), 1-20. http://dx.doi.org/10.1517/13543776.2016.1111872

[97] Wolska-Washer, A.; Robak, T. Safety and Tolerability of Antibody-Drug Conjugates in Cancer *Drug Safety.,* **2019**, *42*(2), 295-314. [113] http://dx.doi.org/10.1007/s40264-018-0775-7

[98] Capala, J.; Bouchelouche, K. Molecular imaging of HER2-positive breast cancer: A step toward an individualized 'image and treat' strategy *Current Opinion in Oncology.,* **2010**, *22*(6), [114] 559-566. http://dx.doi.org/10.1097/CCO.0b013e32833f8c3a

Bazak, R.; Houri, M.; El Achy, S.; Kamel, S.; Refaat, T. Cancer [115]

active targeting by nanoparticles: a comprehensive review of literature. *J. Cancer Res. Clin. Oncol.,* **2015**, *141*(5), 769-784. [124] http://dx.doi.org/10.1007/s00432-014-1767-3 PMID: 25005786

[116] Rong, L. Trastuzumab-modified DM1-loaded nanoparticles for HER2 $^{+}$ breast cancer treatment: an *in vitro* and *in vivo* study. *Artif. Cells Nanomed. Biotechnol.,* **2017**, (Oct), 1-11. http://dx.doi.org/10.1080/21691401.2017.1391821 PMID:[125] 29069935

[117] Ahmed, A.E.E. *Ultrasoud Triggered Release of Trastuzumab-conjugated Immunoliposomes Targeting Breast Cancer*; American University of Sharjah, **2018**.

[118] Ahmed, S. E.; Martins, A. M.; Husseini, G. A. The use of ultrasound to release chemotherapeutic drugs from micelles and liposomes *ournal of Drug Targeting.,* **2015**, *23*(1), 16-42. [126] http://dx.doi.org/10.3109/1061186X.2014.954119

[119] Laing, S.T.; Kim, H.; Kopechek, J.A.; Parikh, D.; Huang, S.; Klegerman, M.E.; Holland, C.K.; McPherson, D.D. Ultrasound-mediated delivery of echogenic immunoliposomes to porcine vascular smooth muscle cells *in vivo*. *J. Liposome Res.,* **2010**, *20*(2), 160-167. [127] http://dx.doi.org/10.3109/08982100903218918 PMID: 19842795

[120] Sutton, J.T.; Haworth, K.J.; Shanmukhappa, S.K.; Moody, M.R.; Klegerman, M.E.; Griffin, J.K.; Patton, D.M.; McPherson, D.D.; Holland, C.K. Delivery of bevacizumab to atheromatous porcine carotid tissue using echogenic liposomes. *Drug Deliv.,* **2016**, *23*(9), 3594-3605. [128] http://dx.doi.org/10.1080/10717544.2016.1212441 PMID: 27689451

[121] Klegerman, M.E.; Naji, A.K.; Haworth, K.J.; Zou, Y.; Golunski, E.; Peng, T.; Britton, G.L.; Huang, S.L.; Holland, C.K.; McPherson, D.D. Ultrasound-enhanced bevacizumab release from echogenic liposomes for inhibition of atheroma progression. *J. Liposome Res.,* **2016**, *26*(1), 47-56. [129] http://dx.doi.org/10.3109/08982104.2015.1029494 PMID: 25865025

[122] Afadzi, M.; Davies, Cde.L.; Hansen, Y.H.; Johansen, T.; Standal, O.K.; Hansen, R.; Måsøy, S.E.; Nilssen, E.A.; Angelsen, B. Effect of ultrasound parameters on the release of liposomal calcein. *Ultrasound Med. Biol.,* **2012**, *38*(3), 476-486. [130] http://dx.doi.org/10.1016/j.ultrasmedbio.2011.11.017 PMID: 22264409

[123] Evjen, T.J.; Nilssen, E.A.; Barnert, S.; Schubert, R.; Brandl, M.; Fossheim, S.L. Ultrasound-mediated destabilization and drug release from liposomes comprising dioleoylphosphatidylethanolamine. *Eur. J. Pharm. Sci.,* **2011**, *42*(4), 380-386. [131] http://dx.doi.org/10.1016/j.ejps.2011.01.002 PMID: 21238586 Kobus, T.; Zervantonakis, I.K.; Zhang, Y.; McDannold, N.J. Growth inhibition in a brain metastasis model by antibody delivery using focused ultrasound-mediated blood-brain barrier disruption. *J. Control. Release,* **2016**, *238*, 281-288. http://dx.doi.org/10.1016/j.jconrel.2016.08.001 PMID: 27496633 Sha, H.; Zou, Z.; Xin, K.; Bian, X.; Cai, X.; Lu, W.; Chen, J.; Chen, G.; Huang, L.; Blair, A.M.; Cao, P.; Liu, B. Tumor-penetrating peptide fused EGFR single-domain antibody enhances cancer drug penetration into 3D multicellular spheroids and facilitates effective gastric cancer therapy. *J. Control. Release,* **2015**, *200*, 188-200. http://dx.doi.org/10.1016/j.jconrel.2014.12.039 PMID: 25553823 Heath, C.H.; Sorace, A.; Knowles, J.; Rosenthal, E.; Hoyt, K. Microbubble therapy enhances anti-tumor properties of cisplatin and cetuximab *in vitro* and *in vivo*. *Otolaryngol. Head Neck Surg.,* **2012**, *146*(6), 938-945. http://dx.doi.org/10.1177/0194599812436648 PMID: 22323435 Park, E.J.; Zhang, Y.Z.; Vykhodtseva, N.; McDannold, N. Ultrasound-mediated blood-brain/blood-tumor barrier disruption improves outcomes with trastuzumab in a breast cancer brain metastasis model. *J. Control. Release,* **2012**, *163*(3), 277-284. http://dx.doi.org/10.1016/j.jconrel.2012.09.007 PMID: 23000189 Thomas, E.; Menon, J.U.; Owen, J.; Skaripa-Koukelli, I.; Wallington, S.; Gray, M.; Mannaris, C.; Kersemans, V.; Allen, D.; Kinchesh, P.; Smart, S.; Carlisle, R.; Vallis, K.A. Ultrasound-mediated cavitation enhances the delivery of an EGFR-targeting liposomal formulation designed for chemo-radionuclide therapy. *Theranostics,* **2019**, *9*(19), 5595-5609. http://dx.doi.org/10.7150/thno.34669 PMID: 31534505 Bulner, S.; Prodeus, A.; Gariepy, J.; Hynynen, K.; Goertz, D.E. Enhancing Checkpoint Inhibitor Therapy with Ultrasound Stimulated Microbubbles. *Ultrasound Med. Biol.,* **2019**, *45*(2), 500-512. http://dx.doi.org/10.1016/j.ultrasmedbio.2018.10.002 PMID: 30447880 Dewitte, H.; Van Lint, S.; Heirman, C.; Thielemans, K.; De Smedt, S.C.; Breckpot, K.; Lentacker, I. The potential of antigen and TriMix sonoporation using mRNA-loaded microbubbles for ultrasound-triggered cancer immunotherapy. *J. Control. Release,* **2014**, *194*(1), 28-36. http://dx.doi.org/10.1016/j.jconrel.2014.08.011 PMID: 25151979 Ma, Y.; Han, J.; Jiang, J.; Zheng, Z.; Tan, Y.; Liu, C.; Zhao, Y. Ultrasound targeting of microbubble-bound anti PD-L1 mAb to enhance anti-tumor effect of cisplatin in cervical cancer xenografts treatment. *Life Sci.,* **2020**, *262*, 118565. http://dx.doi.org/10.1016/j.lfs.2020.118565 PMID: 33038371

Medina, O.P.; Zhu, Y.; Kairemo, K. Targeted liposomal drug delivery in cancer. *Curr. Pharm. Des.,* **2004**, *10*(24), 2981-2989. http://dx.doi.org/10.2174/1381612043383467 PMID: 15379663 Ulrich, A. S. Biophysical aspects of using liposomes as delivery vehicles *Bioscience Reports.,* **2002**, *22*(2), 129-150. http://dx.doi.org/10.1023/A:1020178304031 Schwendener, R. A. Liposomes as vaccine delivery systems: A review of the recent advances *Therapeutic Advances in Vaccines.,* **2014**, *2*(6), 159-182. http://dx.doi.org/10.1177/2051013614541440 Torchilin, V. P. Recent advances with liposomes as pharmaceutical carriers *Nature Reviews Drug Discovery.,* **2005**, *4*(2), 145-160. http://dx.doi.org/10.1038/nrd1632

Bazak, R.; Houri, M.; El Achy, S.; Kamel, S.; Refaat, T. Cancer