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Employing Drug Delivery Strategies to Overcome Challenges using TLR7/8 Agonists for Cancer Immunotherapy

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ABSTRACT
Toll-like receptors (TLRs) are a potential target for cancer immunotherapy due to their role in the activation of the innate immune system. More specifically, TLR7/8, two structurally similar pattern recognition receptors that trigger interferon and cytokine responses, have proven to be therapeutically relevant targets for cancer in numerous preclinical and clinical studies. When triggered by an agonist, such as imiquimod or resiquimod, the TLR7/8 activation pathway induces cellular and humoral immune responses that can kill cancer cells with high specificity. Unfortunately, TLR7/8 agonists also present a number of issues that must be overcome prior to broad clinical implementation, such as poor drug solubility and systemic toxic effects. To overcome the key limitations of TLR7/8 agonists as a cancer therapy, biomaterial-based drug delivery systems have been developed. These delivery devices are highly diverse in their design and include systems that can be directly administered to the tumor, passively accumulated in relevant cancerous and lymph tissues, triggered by environmental stimuli, or actively targeted to specific physiological areas and cellular populations. In addition to improved delivery systems, recent studies have also demonstrated the potential benefits of TLR7/8 agonist co-delivery with other types of therapies, particularly checkpoint inhibitors, cancer vaccines, and chemotherapeutics, which can yield impressive anti-cancer effects. In this review, we discuss recent advances in the development of TLR7/8 agonist delivery systems and provide perspective on promising future directions.

INTRODUCTION

In 2020, over 19 million new cancer cases and 10 million cancer-related deaths occurred worldwide. By 2040, a 47% increase in global cancer burden is expected.(1,2) Despite innovation in cancer treatment, there is still much to be desired in terms of improving patient outcomes. Cancer is difficult to target due to the paucity of differences between cancer cells and healthy cells, unique molecular profiles from patient-to-patient, drug resistance, a lack of cancer epigenetic profiling, the toxicity associated with chemotherapy and radiotherapy, and the occurrence of metastases.(3–5)

The advent of cancer immunotherapy, which aims to modulate the body's innate and/or adaptive immune system to fight the disease, has provided a new tool to prolong patient survival in cases that were previously considered to be fatal.(6,7) Specifically, onco-immunotherapy is designed to stimulate several types of immune cells to initiate the body's native tumor-specific monitoring and elimination system, which is often suppressed by cancer cells.(7) Cancer immunotherapies can be divided into five subsets: checkpoint inhibitors, lymphocyte-promoting cytokines, engineered T cells, agonistic antibodies against co-stimulatory receptors, and cancer vaccines.(8)

Toll-like receptors (TLRs) are one class of pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns and damage-associated molecular patterns. TLRs are expressed in macrophages, dendritic cells (DCs), natural killer (NK) cells, and epithelial cells and can be targeted to induce cytokine production, resulting in the maturation of several immune cell populations that trigger anti-cancer responses.(9) TLRs are activated when bound to agonist molecules, which triggers an innate immune response that can be leveraged for immunotherapy.(9) TLR7 and TLR8, two structurally similar members of the TLR family expressed on the surface of the endosome, are of particular interest for cancer therapy. These receptors are known to trigger the production of type 1 interferons (IFNs) and inflammatory, cytokine-driven responses when activated via the MyD88-dependent pathway (Fig. 1).(10–13) Agonists of TLR7/8 include natural and synthetic nucleic acids and their derivatives, as well as small molecules.(13) Particularly notable agonists are imidazoquinoline-derivatives, with imiquimod being the only FDA-approved TLR7 agonist. Sold as a cream under the name Aldara, imiquimod is used to treat basal cell carcinoma, actinic keratosis, and genital warts in the United States.(14) TLR7/8 agonists can also be used to stimulate antiviral responses, to adjuvant vaccines, and to treat allergies and asthma.(13,15–19) Antagonist ligands of TLR7/8 have also shown promise for inhibiting autoimmune diseases, attenuating HIV infection, and mitigating cancer in certain tumors which overexpress TLR7/8, a relatively rare but concerning occurrence.(13,17,20–22)

Several previous studies have reviewed the potent anti-cancer effects that TLR7/8 agonists can have across a variety of tumors in preclinical and clinical trials.(23–28) In addition, many promising second-generation TLR7/8 agonists have recently entered clinical trials for non-skin cancer applications, increasing the potential of the therapy for many cancer patients in the future.(29)

Although TLR7/8 agonists show exciting potential to be effective tools in cancer immunotherapy, many hurdles remain before these therapies are appropriate for the clinic. Imiquimod and resiquimod, two of the TLR7/8 agonists most frequently used in clinical trials and preclinical studies, are poorly water soluble, which practically eliminates their potential for

oral administration.(30) Multi-dose regimens of orally administered TLR7/8 agonists have also previously been associated with fever, fatigue, headache, and hypertension in clinical studies.(31–34) Additionally, in some cases, the topical imiquimod cream has been shown to induce severe adverse effects such as erythema, hypopigmentation, and burning.(35) The immune system can also develop tolerance to TLR activation, resulting in reduced efficacy over time.(36) Still, parenteral administration remains a possibility, although poorly soluble drugs need to be administered in high volumes to reach the minimum effective concentration (MEC) and can induce potentially fatal levels of proinflammatory cytokines known as a cytokine storm.(30,37,38) This has been shown in a number of murine models and human clinical models studying the effects of TLR7/8-generated cytokines.(39–46) Additionally, the application of TLR7/8 agonists as a monotherapy has shown inconsistent and/or modest results, at best, in clinical trials, which can be attributed to differences in TLR expression from patient-to-patient, immune cell infiltration within tumors, and tumor heterogeneity.(24,47,48)

Drug delivery strategies have the potential to combat key issues that prevent the effective use of TLR7/8 agonists in cancer immunotherapy. Utilizing innovative biomaterials and drug delivery devices, it is possible to reduce the concentration of the agonist to limit toxicity while remaining above the MEC and improving upon the anti-cancer effects observed in conventional therapy. In this review, we discuss advances in the field of TLR7/8 delivery and co-delivery for cancer immunotherapy, reflecting upon the progress thus far and providing perspective on opportunities to overcome the key challenges that remain.

DRUG DELIVERY OF TLR7/8 AGONISTS

A vast variety of delivery schemes, devices, biomaterials, TLR7/8 agonists, and route of administrations have been used to treat various cancer models in preclinical studies (Table I).

Passive Delivery Systems

Passive delivery approaches for TLR7/8 agonists can be categorized into two systems: those that are directly administered to the tumor and those that passively accumulate in relevant tissues. Direct delivery systems include creams and gels administered topically or intravaginally at or near the tumor site. Alternatively, passive accumulation systems include injectables that are administered intravenously or subcutaneously, which remain in the systemic circulation and gradually accumulate at the site of the tumor. These systems generally aim to either improve drug permeation into tissue, extend the duration of action for TLR7/8 agonist therapy, or achieve a favorable concentration gradient between the tumor and rest of the body to reduce side effects.

Direct Administration to the Tumor

Nanocapsules,(49,50) nanoemulsions,(51) micelles,(52) hydrogels,(50,53) foams,(54) and microneedles(55) are all drug delivery systems that have previously been employed to enhance TLR7/8 agonist permeation, extend release, and improve therapeutic efficacy. A majority of these studies have focused on improving topical formulations to enable the delivery of agonists through the skin. In one study, Gazzi et al. combined pectin-based hydrogels with imiquimod-loaded polymeric poly(ε-caprolactone) nanocapsules to create a topical gel; the nanocapsules were able to enhance imiquimod's cytotoxic effects from just 9% as a free drug to 44% in the

delivery platform using a human melanoma cell-based assay.(50) Further, the pectin-based hydrogel component of this system also helped to enhance imiquimod permeation into skin, increase its depth of penetration, and improve adhesiveness in an *ex vivo* model. Another group showed a similar increase in cancer cell cytotoxicity by delivering nanoemulsified imiquimod intravaginally, increasing cell line death from 16% to 35% and blocking the formation of cervical cancer cell colonies.(51)

Other studies have focused solely on increasing skin penetration. One of these studies used micelles composed of methoxy-poly(ethylene glycol)-)-hexyl-substituted lactide (mPEG-hexPLA) and showed improved skin permeation of imiquimod to the viable epidermis and upper dermis, where antigen-presenting cells (APCs) reside.(52) Another used skin treatment with microneedles prior to the topical application of imiquimod to temporarily compromise the skin's barrier function, which tripled the amount of drug delivered to the stratum corneum.(55)

In addition to simply increasing skin permeability and toxicity, controlled-release drug delivery systems have also been engineered to modulate release and extend TLR7/8 agonist activity. One group sought to tune local cytotoxicity by adjusting the fabrication parameters used to create an imiquimod-loaded poly(ε-caprolactone) (IMQ-PCL) nanofibrous mesh.(56) Larger fiber diameters and higher imiquimod concentrations in the nanofiber were correlated with an increased rate of release and a significant increase in melanoma cell death *in vitro* ranging from 50% to 75%. Similarly, Dias et al. created imiquimod-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles that provided sustained drug release following topical application.(49) Compared to free imiquimod and imiquimod cream, which released 90% and 65%, respectively, of drug within 2 hours of topical administration, imiquimod-loaded PLGA nanoparticles released just 25% of their drug over the same time period. PLGA nanoparticles were also shown to improve imiquimod retention in the dermis in a mouse model. Moreover, when used in a chemopreventive activity modeling skin carcinoma in mice, these PLGA nanoparticles inhibited carcinogenesis, resulting in a significant reduction of papilloma multiplicity and size distribution, as well as reduced inflammatory infiltration.

A fish-oil-based topical bigel colloidal formulation was developed to reduce the adverse effects associated with topical imiquimod cream and to amplify anti-tumor effects.(57) The imiquimod-loaded fish oil (IMQ-FO) bigel colloid was fabricated from a combination of carbopol hydrogel and fish oil oleogel. *In vitro* epidermoid carcinoma cell viability studies revealed that IMQ-FO achieved 15% to 50% cell reduction at concentrations 100 to 250 ug/mL, proving greater efficacy than free imiquimod. Additionally, the bigel colloid also triggered an increase in anti-inflammatory cytokine interleukin-10 (IL-10) in the *in vivo* mouse skin tumor model, reducing the tumor progression via inhibition of vascular endothelial growth factor. Chronic inflammation, which has long been a concern in the use of topical imiquimod, was also decreased by the IMQ-FO formulation, as evidenced by the lower levels of TNL-α and IL-6 in serum evoked by IMQ-FO than by commercial imiquimod.

Unfortunately, topical administration may not be appropriate for deeper tumors; therefore, injectable depot systems have been developed to create a reservoir of TLR7/8 agonists within the tumor. One strategy used to create these *in situ* drug depots is to leverage the temperature change upon injection into the body.(58–62) Fakhari et al. employed this strategy to deliver MEDI9197, a lipophilic imidazoquinoline TLR7/8 agonist. The authors showed that an intratumoral injection of poloxamer 407 loaded with MEDI9197 formed a hydrogel upon

reaching body temperature and prolonged the local release of drug.(13,61) They then showed the ability to inhibit tumor growth by approximately 62.5% in 37 days using a murine model of ovalbumin (OVA)-expressing melanoma.(61) In addition, they demonstrated improved survival with 20% of animals surviving through day 37 whereas no untreated mouse lived beyond day 30. An alternative deep tumor targeting strategy was recently reported by Bahmani et al. By coating poly(lactic acid) (PLA) nanoparticles that had a resiquimod core with membranes isolated from human platelets, the authors showed the ability to create "cloaked" nanoparticles (PNP-R848) that maximized the immunotherapeutic abilities of resiquimod. In a murine colorectal tumor model, PNP-R848 treated mice demonstrated 100% survival at 56 days, whereas only 37.5% of mice treated with free R848 survived were alive at the same time point. Furthermore, 100% of PNP-R848 mice demonstrated long-term cancer immunity after two tumor re-challenges. The authors also showed that PNP-R848 treatment reduces tumor burden by approximately 2-fold in comparison to free R848 treatment in a metastatic murine triple-negative breast cancer model as well.(31)

Passive Accumulation in Therapeutically Relevant Tissues

Although direct administration is ideal for tumors accessible via topical or intravaginal routes, deeper tumors necessitate another strategy. As a result, several groups have developed delivery systems that spontaneously accumulate at the tumor site or lymph nodes to enhance the safety and efficacy of TLR7/8 agonists. However, these strategies must first overcome inherent pharmacokinetic (PK) challenges. As a result, they often leverage nanoencapsulation to improve upon an otherwise unfavorable PK profile. To improve the poor rate of encapsulation and rapid rate of release observed in polylactic acid (PLA) nanoparticles, Thauvin et al. created resiquimod (R848)-loaded poly(ethylene glycol) (PEG)-modified linear PLA-based nanoparticles.(63) By formulating PLA with both PEG and linear aliphatic chains (C8 or C20), the particles became more soluble in an aqueous environment, improved the encapsulation efficiency of hydrophobic resiquimod, and provided sustained release of resiquimod with formulations containing aliphatic chains (R848-NP-C8 and R848-NP-C20), releasing drug more slowly than when they were not used (R848-NP). Release of 31%, 53%, and 88% of payload was observed at 7 days in R848-NP-C8, R848-NP-C20, and R848-NP, respectively. When used in cell-based *in vitro* assays, particles increased the production of IL-6 by murine macrophages, with R848-NP-C20 treatment inducing the highest IL-6 concentration, followed by R848-NP-C8, R848-NP, and free R848. A phagocytic immune cell viability assay also revealed that these nanoparticles did not have a significant impact on macrophage viability.

Vinod et al. engineered an intravenously injectable system for non-small cell lung cancer (NSCLC), a disease that causes the majority of lung cancer deaths worldwide.(64) In this system, poly(2-oxazoline) (POx)-based nanomicellar resiquimod (Fig. 2a) significantly prolonged survival in a metastatic mouse model of lung adenocarcinoma.(65) Intravenously administered nanomicellar resiquimod prolonged survival to 57 days, compared to 15 days for the saline control and 22 days for intraperitoneal anti-PD-1 therapy (Fig. 2b), which may have been a consequence of dendritic cell activation and $CD8^+$ T cell proliferation (Fig. 2c,d). Nanomicellar resiquimod also reduced body weight loss, suggesting improved overall health.

Other injectable delivery systems have been engineered to accumulate in the lymph nodes rather than the primary tumor due to the high population of immune cells. Kim et al.

encapsulated 522, a potent dual TLR7/8 agonist, and its S-configured stereoisomer, 528, in PLGA nanoparticles and showed that subcutaneous injections of these particles stimulated $CD8^+$ T cells, increasing prophylactic and therapeutic efficacy in murine models of melanoma, bladder, and renal cell carcinoma.(66)

Stimulus-Triggered Delivery Systems

In contrast to passive delivery systems, stimulus-triggered systems can be controlled by introducing and removing stimuli or leveraging specific characteristics of the tumor microenvironment (TME) to achieve spatiotemporal control over TLR7/8 agonist activity.(67,68) These so-called "smart" systems can be designed to be responsive to a number of exogenous stimuli (e.g. light, ultrasound, and electrical fields) and/or endogenous stimuli (e.g. pH, heat, and enzyme concentration); controlled stimuli reduce the need for repeated administration, achieving high local drug concentrations while reducing systemic drug levels to mitigate off-target toxicity(69).

pH-Triggered Delivery Systems

Several TLR7/8 agonist delivery systems have utilized the mild acidity of the TME caused by hypoxia and anaerobic respiration to preferentially target tumors.(58,59,70–74) These pH-responsive systems are composed of acid- or base-labile moieties which undergo proton transfer in response to pH change, resulting in a conformational change within the system that initiates drug release.(69) pH-sensitive polymers can be incorporated together to create specific avenues of drug delivery when stimulated.(67) As previously mentioned, Kim et al. developed a new class of PLGA TLR7/8 agonist nanocarriers that demonstrated improved $CD8^+$ T cell activation and therapeutic benefits in several preclinical cancer models.(66) However, relatively low drug loading efficiency and desire to specifically target endosomes—where TLR7/8 are present—led the group to explore the addition of a pH-trigger. Using a double emulsion/solvent evaporation method, the authors co-encapsulated sodium bicarbonate with TLR7/8 small molecule agonist 522 in PLGA nanoparticles.(75) Sodium bicarbonate was added due to the compound's known generation of CO_2in acidic environments, which researchers hypothesized would physically disrupt the polymeric nanoparticle matrix and provide immediate release of 522 within the tumor. The use of this pH-sensitive moiety to create these gas generating nanoparticles (GGNPs) improved drug loading by over 33% in comparison to PLGA nanoparticles without sodium bicarbonate. *In vitro*, pH-sensitive particles exhibited a burst-release profile that increased by as much as 67% under environmental conditions simulating TME or endosomal pH levels. GGNPs delivered with solubilized OVA increased the population of OVA-specific $CD8^+$ T cells by over 70% in comparison to OVA co-delivered with PLGA nanoparticles lacking sodium bicarbonate in a naive mouse model. Further, when animals were subjected to a B16F10-OVA tumor challenge, OVA and GGNP co-delivery significantly inhibited tumor growth. 30 days after tumor inoculation, 80% of mice receiving OVA and GGNPs were alive compared to 0% in the group receiving OVA and nanoparticles without sodium bicarbonate.(75) More recent studies of this drug delivery device have confirmed that GGNPs display enhanced *in vivo* cytotoxicity and prolonged activation of NK cells in comparison to a soluble subcutaneous injection of 522.(76)

Other groups have also employed pH-sensitive nanocarriers to deliver TLR7/8 agonists to the

endosome. A zeolitic imidazolate framework-8 (ZIF-8) was used to provide pH-responsive dissolution properties for imiquimod delivery in the acidic endosomal environment; formulated with imiquimod, the immunomodulator 1-methyl-D-tryptophan (1-MT), and mannan, the particles were administered intravenously and improved the development of both $CD4^+$ and $CD8^+$T cells in a murine model of melanoma.(77) Interestingly, strong therapeutic effects were observed in both primary and abscopal tumor models, which prolonged animal life by 25% and reduced tumor burden significantly compared to no treatment.

Thermally-Triggered Delivery Systems

Thermally-triggered drug delivery devices provide another potential mechanism by which tumors can be targeted. These systems respond to changes in local temperature by exhibiting a volume phase transition that causes an abrupt change in the solvation state of the polymer, resulting in drug delivery.(68,70) Thermally sensitive TLR7/8 agonists drug delivery systems can be triggered by exogenous stimuli, such as ultrasound and photoradiation.(78,79)

Jia et al. reported a system that incorporated resiquimod and indocyanine green (ICG) with CpG oligodinucleotides, itself a TLR9 agonist, to create self-assembling nanoparticles which are embedded within a PDLLA-PEG-PDLLA (PLEL) hydrogel. When injected into the body, the gel goes through a sol-gel transition triggered by physiological temperature, and when an 808 nm laser is introduced to further heat up the tumor site to 45°C, the hydrogel returns back to the solution phase while releasing nanoparticles that are taken up by DCs on-demand.(80) When tested *in vivo* in a postoperative murine breast cancer model, the group treated with resiquimod-loaded nanoparticles irradiated with near-infrared light showed 100% survival and were 80% recurrence-free 28 days after surgery, while at the same time point in the untreated group, all mice died.

Another group reported a photothermal-triggered system consisting of an organic semiconducting polymer nanoparticle core, resiquimod, and a thermally-labile lipid coating ($SPN_{II}R$) (Fig. 3a).(81) When the tumor site was locally heated with a 1064 nm laser, the lipid coating disassembled and released resiquimod, inducing immunogenic cell death and increasing the percentage of mature DCs in tumor-draining lymph nodes by a minimum of 10% relative to all other treatment groups. In a murine breast cancer model, the irradiated resiquimod-loaded $SPN_{II}R$ treatment reduced the size of primary and distant tumors by 30-fold and 6-fold, respectively, compared to tumors in mice receiving the same nanoparticles without laser activation (Fig. 3c,d). This effect also inhibited the formation of metastatic nodules in the lungs, which were 29-fold lower than in the particle-only treatment group (Fig. 3b). Finally, the authors also observed significantly increased concentrations of $CD3^+$ $CD4^+$ and $CD3^+$ $CD8^+$ T cells in distant tumors and higher serum concentrations of IFNγ, TNF-α and IL-6.

Other Stimulus-Triggered Systems

Although most stimuli-responsive systems for TLR7/8 agonist delivery have focused on pH and thermal triggers, a limited number of studies have taken advantage of endogenous reducing environments in cancerous cells.(82) In addition, several groups have explored systems that are responsive to multiple stimuli to increase the degree of control over TLR7/8 agonist release.(58,59,83)

Active Delivery Systems

Since TLR7/8 agonists exert their beneficial effects by evoking an innate immune response, actively targeting these molecules to specific tissues and cell populations, including the lymph nodes, tumor-associated macrophages, and dendritic cells, is thought to improve efficacy and reduce potentially damaging side effects.(84) As a result, active TLR7/8 agonist delivery systems have been developed to exploit physiological processes and molecular pathways that can preferentially route agonists to the sites where they will be most therapeutically desirable.

Delivery to Lymph Nodes

The lymph nodes (LNs) are a potentially high-value target for TLR7/8 agonist delivery. A sizable fraction of the body's immune cells reside in LNs, including antigen-presenting cells, which are known to play a pivotal role in cancer immunotherapy through the generation of antitumor immunity, polarization of $CD4^+$ T cells and activation of $CD8^+$ T cells.(85,86) TLR7/8 agonist devices that directly target the lymph nodes can vary greatly in their structure and composition, but these devices are almost exclusively engineered to fall within the 10-100nm size range that is necessary for efficient transport through the lymphatics. Although a variety of different structural designs have been used for LN targeted delivery—including plant-virus based protein coats(87), synthetic polymer matrices(88–90), and peptide-based co-TLR adjuvants(91)—many systems have employed amphiphilic constructs to entrap agonists with opposing polarity to prevent leakage while in the lymphatics.(83,92,93) However, once the device is within lymph nodes, amphiphilic nanostructures containing TLR7/8 agonist-loaded particles passively accumulate, bind to dendritic cells, and disintegrate to release their payload. These actions create an immunostimulatory effect, resulting in the upregulation of cellular and humoral immune responses that improve animal survival in lymphatic and colon cancer models.

One group reported using amphiphilic diblock copolymer poly(2-ethly-2-oxazoline-poly(D,L-lactide) (PEOz-PLA) combined with carboxyl-terminated Pluronic F127 to create mixed micelles (carboxylated-NPs) for the co-delivery of antigen OVA and a TLR7 agonist, CL264, to LN-resident DCs.(94) The authors found that carboxylation of the nanoparticles improves LN targeting by 16% compared to free OVA and CL264. These micelles also resulted in more than twice the population of $CD4^+IFN\gamma^+$ and $CD8^+IFN\gamma^+$in the mouse spleen, indicating an elevated antigen-specific response. In an OVA-expressing mouse model of T cell lymphoma, carboxylated NPs containing CL264 that were co-delivered with OVA inhibited tumor growth and prolonged survival to approximately 47 days for 60% of the treatment group. In comparison, mice treated with free drug and OVA all died by approximately day 33.

Delivery to Tumor-Associated Macrophages

Macrophages are a major component of tumors and can make up as much as 50% of a tumor's mass.(95) Macrophages accumulate in cancer tissues by proliferating from tissue resident precursors or by trafficking from bone-marrow-derived precursors.(96) Once present, these tumor-associated macrophages (TAMs) are, unfortunately, known to adopt a cancer-supportive M2 phenotype, providing minimal activity against cancer cells, supporting an immunosuppressive TME, and promoting angiogenesis, tumor growth, and metastasis.(96,97) A number of studies have also correlated the concentration of TAMs in a variety of tumors with

elevated tumor grades and shorter survival in humans.(96) However, when TAMs are converted from an immunosuppressive phenotype to a pro-inflammatory phenotype, they instead produce cytokines such as IL-12, NO synthase, and TNF-α, potentially prolonging survival both in mice and humans.(96–98) As a result, a TAM repolarization scheme to increase the ratio of M1 (tumor inhibitory) to M2 macrophages in tumors is of great interest.(96,97,99,100)
TLR7/8 agonist delivery systems have been created using a variety of platforms including small molecule-drug conjugation, small molecule-peptide conjugation, liposomes, metal-organic frameworks, and sugar-based polymer strategies in order to support macrophage repolarization.(101–105) One interesting approach loaded resiquimod into lysine-crosslinked succinyl β-cyclodextrin nanoparticles (CDNPs) to specifically target TAMs while increasing drug loading (Fig. 4a) .(106) Using an *in vivo* colon adenocarcinoma model modified with plasmid H2B-mApple in MerTK$^{GFP/+}$reporter mice, the authors showed that 70% of administered CDNPs were accumulated in macrophages and approximately 35% of the macrophages were tumor-associated—double the percentage of CDNP^{+} macrophages found in the next most common tissue (liver) (Fig. 4b). Moreover, in p40-IRES-eYFP IL-12 reporter mice, CDNP-R848 treatment significantly increased pro-inflammatory IL-12 expression compared to mice treated with R848 alone (Fig. 4c). When tested in a murine model of colon adenocarcinoma, CDNP-R848 treatment improved survival by 50% and significantly inhibited tumor burden in comparison to free drug (Fig. 4d,e).

Delivery to Dendritic Cells

Dendritic cells are a class of professional APCs that process and present antigens to T cells. These cells are connected to their local environment through a variety of molecular sensors that detect foreign entities in the body. Migratory DCs, located throughout the body, can capture non-self antigens and migrate to the LNs where they interact with T cells to initiate an adaptive immune response.(107–109) Additionally, certain types of DCs permanently reside in LNs, and these populations are also activated by antigens traveling extracellularly to the LN through the lymphatics.(107,110,111) However, tumors can prevent antigen presentation to the LNs by upregulating IL-10 and TGF-β to preferentially cause monocytes to differentiate into M2 TAMs instead of pro-inflammatory M1 TAMs. Additionally, tumors can also interfere with tissue-resident DC maturation, which can negatively influence the maturation of migratory DCs to yield DC-variants that create a favorable TME.(112) In cancer therapy, the activation of TLR7/8 induces the production of type I IFN and pro-inflammatory cytokines, which can induce DC maturation and potentially reverse DC inhibition, an essential process in the activation of T cells against tumors.(113)
TLR7/8 drug delivery systems intended to modulate DC behavior are typically engineered to target specific receptors found on DCs—most often, mannose receptors, dendritic cell immunoreceptors (DCIRs), and endocytic receptors.(77,90,94,114,115) Additionally, DC behavior can be modulated *ex vivo* prior to administration for cancer immunotherapy.(116) One group highlighted the utility of DC-targeted TLR7/8 cancer immunotherapy systems by incorporating a PCL-PEG-PCL copolymer with cationic lipid 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), mannose, OVA, TLR4 agonist monophosphoryl lipid A (MPLA), and imiquimod into a nanoparticle delivery system (IMNPs).(90) Researchers then demonstrated that the addition of the mannose-receptor targeting moiety increased nanoparticle

accumulation in the LNs of mice by 20% 50 hours after administration. Furthermore, these particles were retained over 120 hours longer than free OVA and TLR agonists. Notably, seven days after administration, MHC I and MHC II DC surface markers were found to be increased by more than 20% in the inguinal LN in comparison to OVA-IMNPs without mannose.

Other Active Delivery Systems

Although lymph nodes, TAMs, and DCs are currently the primary targets in actively targeted drug delivery systems for TLR7/8 agonist therapy for cancer, monocytic myeloid-derived suppressor cells (Mo-MDSCs) and programmed cell death protein-1 (PD-1) expressing $CD8^+$ T cells have also been shown to be viable targets for cancer immunotherapy through TLR7/8 agonists.(117,118) These systems are relatively unexplored at present, but represent potentially promising strategies for future innovation in targeted TLR7/8 agonists delivery.

CO-DELIVERY OF TLR7/8 AGONISTS

Co-Delivery with Immune Checkpoint Inhibitors

Immune checkpoint inhibitors have shown great promise in the clinic for the treatment of melanoma, lung cancer, renal cell carcinoma, Hodgkin's lymphoma, and other forms of cancer.(119) However, these therapies often exhibit inconsistent therapeutic efficacy across patients with the same disease. The underlying cause of varying patient outcomes has yet to be fully elucidated, but most hypotheses include factors such as tumor mutational burden,(120) the gut microbiome,(121) as well as the quality and quantity of immune cells infiltrating the tumor microenvironment.(122) Since TLR agonists have shown the ability to increase the number of tumor infiltrating cytotoxic T lymphocytes(65,73,118) and induce an M2-to-M1 transition in TAMs(106) there is exciting potential for synergy between delivery of TLR7/8 agonists and checkpoint inhibitors.(89)

Systemic administration of TLR7/8 agonists for the treatment of metastases remains challenging due to the generation of a systemic and potentially damaging proinflammatory response. Interestingly, intratumoral injections of a TLR7 agonist linked to silica nanoshells (NS-TLR7a) were able to prevent the generation of a deleterious systemic response (Fig. 5a).(123) In this study, over 50% of nanoparticles were retained in the tumor 72 hours after injection acting as a slow-release depot. Nanoparticle retention served to limit accumulation in the liver and spleen to less than 2% of the injected dose and contributed to the influx of $CD3^+$ cells into the tumor (Fig. 5b). Further, using a two-tumor colon cancer model, an intratumoral injection of NS-TLR7a and an intraperitoneal injection of anti-PD-1 and CTLA-4 resulted in a survival rate of 60% over a period of 80 days, whereas treatment with only anti-PD-1, and CTLA-4 showed 0% survival at day 80 (Fig. 5c).(124)

Co-Delivery with Cancer Vaccines

Cancer vaccines, which aim to train the immune system to detect cancer-specific neoantigens, have achieved promising results in recent clinical trials.(125,126) Priming the immune system to neoantigens can be difficult due to the immunosuppressive properties of cancer. In addition, the inherent lack of immunogenicity of some neoantigens limits their potential use as targets.(127) TLR7/8 agonists have been shown to activate NF-κB-mediated proinflammatory cytokine and chemokine expression, which can enhance the immune response to cancer-

specific antigens. Several strategies to enhance antigen immunogenicity involving TLR7/8 agonists include direct linkage between TLR7/8 agonist and antigen(128–130), enhancing trafficking to draining lymph nodes,(94) and delivery of mRNA which serves as a TLR7 adjuvant and encodes for an antigen.(131) These systems can be used as either ~~in one of two ways a~~s prophylactics or therapeutics.

The prevention of cancer with vaccines is ideal for known cancerous targets, including those found in hepatitis B virus and human papillomavirus. Using OVA as a model antigen, Kim et al. created microneedles (MN) composed of a Pluronic F127-poly(ethylene glycol) blend and loaded with resiquimod and OVA with the goal of targeting resident DCs that are abundant in skin.(132) Once administered the microneedles dissolve and spontaneously form Pluronic F127 nanomicelles containing resiquimod and OVA (Fig. 6). This administration route reduced cytotoxic IL-6 secretion levels 10-fold compared to subcutaneous injection. OVA MN alone did not protect mice when challenged with an OVA-expressing lymphocyte cancer. However, MN administration delivering OVA and resiquimod generated twice as many $CD8^+$ T cells, resulting in 100% survival 86 days after the challenge.(133) Many other drug delivery devices of TLR7/8 agonists and antigens have also demonstrated the induction of an enhanced immune response ~~when compared to monotherapy alone i~~n comparison to monotherapy. (66,115,116,134)

Cancer vaccines can also be used therapeutically, however, incorporating a variety of antigens with varying physicochemical properties is challenging.(128) An alternative method consists of generating cancer specific antigens *in situ* using near-infrared photothermal therapy.(77,78,80,81) Chen et al. utilized this strategy by incorporating resiquimod into photothermal sensitive nanoparticles. After injection into colon carcinoma tumors, nanoparticles were exposed to near-infrared light causing thermal ablation of tumor cells and release of cancer specific antigens into the extracellular environment. Concurrent release of resiquimod assisted in priming APCs to eradicate cancer in 9 of 21 mice after 120 days (Fig. 6). The 9 surviving mice were re-challenged and no mice grew tumors demonstrating a robust immune response. In contrast, no mice treated with either monotherapy survived past 60 days.(135)

Co-Delivery with Cancer Vaccines and Checkpoint Inhibitors

Combining cancer vaccines, checkpoint inhibitors, and TLR7/8 agonists is another appealing strategy, given the potential synergy between these therapies as well as their individual success. This triple therapy has shown superior(90,91,128,136) or equivalent(137) results to duo or monotherapies when used to treat cancer.

Co-Delivery of TLR7/8 Agonists and Other Drugs

In addition to co-delivery with checkpoint inhibitors and vaccine antigens, TLR7/8 agonists have been delivered alongside conventional chemotherapeutics(72,82,105,138), vasculature disrupting agents(139), antibodies(76,101,102), and tyrosine kinase inhibitors(137) to improve cancer survival. Combined delivery of chemotherapeutics and immunotherapeutics is of particular interest. By creating a chemo-immunotherapeutic scheme in which cancer-killing and immunostimulatory components are co-delivered, it is possible to leverage the best parts of each monotherapy to synergistically combat cancer.(140,141)

Ringgaard et al. reported a particularly interesting chemo-immunotherapeutic TLR7/8 agonist

delivery system in which oxaliplatin (a chemotherapeutic) was delivered within a PCL8-U75 liposome alongside free resiquimod.(117) In a murine model of colon carcinoma, PCL8-U75 liposomes loaded with oxaliplatin and co-delivered with free resiquimod extended survival by approximately 37.5% in comparison to mice treated only with the chemotherapeutic. Interestingly, 45% of mice in this group were complete responders to the treatment.
Another group reported creating a triblock bottlebrush copolymer of polylactic acid (PLA), polyethylene glycol (PEG), and poly(N-isopropylacrylamide) (PNIPAM) that self-assembles into uniform micelles at room temperature and forms a multicompartment hydrogel depot at the site of injection immediately after administration due to the temperature increase when injected into the body(62). These researchers showed that small molecules, including resiquimod and paclitaxel (PTX, a chemotherapeutic), could be loaded into the copolymer while in the micellar phase, and demonstrated that this delivery system improved the tumor eradication rate in a murine colon carcinoma model by a factor of 8 in comparison of free resiquimod and PTX. Additionally, mice treated with this hydrogel exhibited significantly decreased levels of MCP-1, IL-2, and IL-6 in circulation in comparison to free resiquimod and PTX, indicating lower systemic inflammation with the hydrogel controlled release system.

PERSPECTIVES ON TLR7/8 AGONISTS

TLR7/8 agonists have demonstrated utility in the clinical treatment of basal cell carcinoma and continue to be explored for the treatment of other cancers in both clinical and preclinical trials. Unfortunately, imiquimod and resiquimod, the most commonly used TLR7/8 agonists, have key limitations such as low water solubility and the potential to cause high off-target toxicity, which need to be overcome before these drugs can be more broadly implemented. Biomaterial-based drug delivery devices and advanced drug formulations have demonstrated the ability to overcome both solubility and off-target toxicity issues to achieve superior therapeutic outcomes. These systems take a variety of forms ranging from topically administered drug carriers that enhance tissue penetration to light-responsive nanoparticles that release TLR7/8 agonists at the site of the tumor. Several systems have already demonstrated the ability to inhibit tumor growth and meaningfully prolong survival in preclinical studies and therefore have exciting potential for clinical translation; however, others will require further development before becoming appropriate for clinical trials.
TLR7/8 agonist delivery systems that are responsive to near-infrared light have demonstrated therapeutic benefits in several cancer models, but are ultimately limited to targeting shallow tissues (<4 mm deep) due to light attenuation.(142) To make this technology appropriate for deeper tissues, it may be possible to modify the delivery system to respond to other stimuli. Focused ultrasound has demonstrated the ability to target deeper tissues, including inaccessible tumors, such as those in the brain.(143) Therefore, ultrasound-responsive platforms may be able to leverage both the high degree of spatial positioning previously demonstrated with the therapeutic benefits derived from TLR7/8 agonist therapy.(144,145)
Another potentially exciting area that could be explored would be the delivery of TLR7/8 agonists to other immune cells. Although TAMs and DCs have been the primary cellular targets of actively targeted TLR7/8 agonist therapies to date, delivery to NK cells, which are naturally cytotoxic, or even B cells could provide synergistic effects. There is strong evidence that both of these cell types become activated when treated with imiquimod, yet there do not appear to be

any TLR7/8 agonist delivery systems that specifically target these cell populations presently.(146–148)

The co-delivery of TLR7/8 agonists with other types of therapeutics has shown potential for increasing the effectiveness of cancer immunotherapy, but there is ample opportunity to further optimize these systems. Since different therapeutics initiate anti-cancer effects through different signaling pathways, Leary et al. suggested that combining multiple therapies that trigger different signaling cascades could have interfering or inhibitory effects on one another.(149) Therefore, the rational design of these therapies should be customized to tumor-specific considerations, including cancer type, heterogeneity, aberrant signaling, and mutational burden. In the future, bioinformatics and systems biology may be able to predict synergies to select the most effective combination of therapeutics for a particular patient and tumor.(149–151)

Modifying the TLR7/8 agonist also offers further opportunity for improving therapeutic efficacy while reducing undesirable toxicity. Although imiquimod and resiquimod are widely used TLR7/8 agonists in cancer immunotherapy because they are well-characterized and well-documented, newer compounds created using medicinal chemistry approaches may ultimately possess more favorable properties. One imidazoquinoline-derivative, 852A, has been found to be both more potent than imiquimod and more selective for TLR7.(9,152,153) Additionally, this compound exhibits 40-fold higher water solubility than imiquimod. The incorporation of 852A or other improved agonists within drug delivery devices provides promise for enhanced TLR7/8 cancer immunotherapy in the future.

While it is clear that immunotherapies will serve as key tools in the fight against cancer due to their ability to achieve robust and durable responses, expanding the population of patients that can benefit from these approaches remains a critical challenge. Using alternative TLR7/8 agonists, enhancing targeting capabilities, and exploring synergistic co-delivery of other drugs all represent potential advances that, in combination, may overcome current challenges.

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CONFLICT OF INTEREST STATEMENT

None of the authors have any financial or other conflicts of interest with this work.

AUTHOR CONTRIBUTIONS

All authors contributed in designing the study. D.V., S.Q. and T.P.G. researched the topic and drafted the article. D.V. and K.J.M. revised the version to be published. K.J.M. gave final approval of the version to be published.

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Figure 1: The MyD88 signaling pathway of TLR7/8.

Figure 2: (a) TEM of resiquimod POx micelles (PM). **(b)** Survival study in murine metastatic lung adenocarcinoma. Resiquimod PM extended survival from 15 days to 57 days (n=13 mice). **(c)** Fluorescence activated cell sorting (FACS) graph of CD3+ and CD8+ cell population treated by saline control (left) and resiquimod PM (right). **(d)** Cytotoxic killer T cells quantification. Resiquimod PM doubled the number of CD45+/CD3+/CD8+. * indicates p<0.05. Reprinted/adapted from Vinod N, Hwang D, Azam SH, Swearingen AEDV, Wayne E, Fussell SC, et al. High-capacity poly(2-oxazoline) formulation of TLR 7/8 agonist extends survival in a chemo-insensitive, metastatic model of lung adenocarcinoma. Sci Adv. 2020 Jun 1;6(25):eaba5542. © The Authors, some rights reserved; exclusive licensee AAAS. Distributed under a Creative Commons Attribution NonCommercial License 4.0 (CC BY-NC) http://creativecommons.org/licenses/by-nc/4.0/"

Figure 3: **(a)** $SPN_{II}R$ nanoparticles **(b)** Number of metastatic tumor lung nodules found in mice 21 days after treatment (n=5 mice). $SPN_{II}R$ + laser treatment significantly reduced the number of nodules in comparison to other groups (c,d) Relative tumor volume in a primary **(c)** and distant **(d)** breast cancer mouse model. $SPN_{II}R$ + laser group shows significant tumor growth inhibition in comparison to other groups. *** indicates p<0.001, ** indicates p<0.01. Li J, Yu X, Jiang Y, He S, Zhang Y, Luo Y, et al.: Second Near-Infrared Photothermal Semiconducting Polymer Nanoadjuvant for Enhanced Cancer Immunotherapy. Advanced Materials. 2020. Volume 33. Page 2003458. Copyright Wiley-VCH GmbH. Reproduced with permission.

Figure 4: (a) Cyclodextrin-nanoparticle (CDNPs) synthesis and composition. **(b)** CDNP+ macrophages were found to selectively accumulate within macrophages of tumor tissue in mice (n=1). **(c)** CDNP-R848 treated mice were found to have significantly higher levels of IL-12 expression in comparison to free R848 (resiquimod) and CDNPs (n=250 cells across 3 fields of view per condition. * indicates p<0.05, **** indicates p<0.0001 **(d)** CDNP-R848 mice in a mouse MC38 tumor model were found to inhibit tumor growth significantly more than other treatment groups (n=12 tumors). ** indicates p= 0.0017, **** indicates p<0.0001 **(e)** CDNP-R848 mice showed 100% survival at 14 days (n=6 mice). ** indicates p=0.005. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Nature Biomedical Engineering, TLR7/8-agonist-loaded nanoparticles promote the polarization of tumour-associated macrophages to enhance cancer immunotherapy, Rodell CB, Ahmed MS, Garris CS, Pittet MJ, Weissleder R. Copyright 2018.

Figure 5: (a) TLR7a silica nanoshell nanoparticle system. **(b)** Immunohistochemistry of $CD3^+$ cells in tumors injected with soluble TLR7a of NS-TLR7a. **(c)** survival curve of mice (n = 8-10) intratumorally injected with vehicle (PBS), injected intraperitoneally with checkpoint inhibitors (a-PD-1 + a-CTLA-4) three times per week, or injected intraperitoneally with checkpoint inhibitors (a-PD-1 + a-CTLA-4) three times per week and of NS-TLR7a injected intratumorally every other day. ** indicates p <0.01. Reprinted (adapted) with permission from Huang CH, Mendez N, Echeagaray OH, Weeks J, Wang J, Vallez CN, et al. Conjugation of a Small-Molecule TLR7 Agonist to Silica Nanoshells Enhances Adjuvant Activity. ACS Appl Mater Interfaces. 2019;11(30):26637–47. Copyright 2019 American Chemical Society. Huang C, Mendez N, Echeagaray OH, Weeks J, Wang J, Yao S, et al.: Immunostimulatory TLR7 Agonist-Nanoparticles Together with Checkpoint Blockade for Effective Cancer Immunotherapy. Advanced Therapeutics. 2020. Volume 3. Page 1900200. Copyright Wiley-VCH GmbH. Reproduced with permission.

Figure 6: Figures from Kim et al. showing a schematic of microneedles dissolving to form self-generating nanomicelles of resiquimod (green), OVA (yellow), and F127 (red and blue). Below is a survival curve of mice (n = 5) prophylactically treated with microneedle patches on days 0, 14, and 21 followed by injection of cancer cells on day 28. Figures from Chen et al. showing the components of their drug delivery system containing polyaniline (PANI), glycol-chitosan (GCS), and Resiquimod (R848), as well as self-assembling nanoparticles. Below is a survival curve. Mice (n = 10-21) were inoculated with tumor cells on day 0 then injected and/or treated with photothermal therapy on days 14, 21, 28. * indicates p<0.05, **** indicates p<0.0001. Reprinted (adapted) with permission from Kim SY, Kim S, Kim JE, Lee SN, Shin IW, Shin HS, et al. Lyophilizable and Multifaceted Toll-like Receptor 7/8 Agonist-Loaded Nanoemulsion for the Reprogramming of Tumor Microenvironments and Enhanced Cancer

Immunotherapy. ACS Nano. 2019 Nov;13(11):12671–86.. Copyright (2019) American Chemical Society. Reprinted from Biomaterials, Vol. 230, Po-Ming Chen, Wen-Yu Pan, Cheng-Yu Wu, Ching-Yen Yeh, Chiranjeevi Korupalli, Po-Kai Luo, Chun-JuChou, Wei-Tso Chia, Hsing-Wen Sung, Modulation of tumor microenvironment using a TLR-7/8 agonist-loaded nanoparticle system that exerts low-temperature hyperthermia and immunotherapy for in situ cancer vaccination, Page No. 119629, Copyright (2020), with permission from Elsevier.

Delivery Scheme	Device Type	Biomaterial(s) Used	TLR7/8 Agonist	Route of Administration	Cancer Model(s)	Reference
Direct administration	Membrane-functionalized polymeric nanoparticle	PLA[a]	Resiquimod	Intratumoral	Colorectal, Breast	32
Direct administration	Polymeric nanoparticle	PLGA[b]	Imiquimod	Topical	Non-Melanoma	49
Direct administration	Hydrogel, polymeric nanoparticle	Pectin, PCL[c], sorbitan monostearate	Imiquimod	Topical	Melanoma	50
Direct administration	Nanoemulsion	Sorbitan monostearate, copaiba oil	Imiquimod	Intravaginal	Cervical	51
Direct administration	Hydrogel	mPEG-hexPLA[d]	Imiquimod	Topical	N/A	52
Direct administration	Hydrogel	Nucleotides	GU-rich RNA/DNA[e]	N/A	Colon	53
Direct administration	Foam	DMSO[f], oleic acid, PEG[g]	TMX-202	Topical	Non-Melanoma	54
Direct administration	Microneedles	Dermapen®	Imiquimod	Topical	N/A	55
Direct administration	Nanofibrous mesh	PCL[c]	Imiquimod	N/A	Melanoma	56
Direct administration	Bigel colloidal system	Carbopol hydrogel, fish oil oleogel	Imiquimod	Topical	Non-Melanoma	57
Direct administration	Hydrogel, liposome	Lipids, Pluronic F127	Imiquimod	*In situ*	Breast	59
Direct administration	Hydrogel	Poloxamer 407	MEDI9197	Intratumoral	Melanoma	61
Direct administration, co-delivered with chemotherapeutic	Hydrogel	PEG[g], PLA[a], PNIPAM[h]	Resiquimod	Intratumoral	Colon	62
Passive accumulation	Polymeric nanoparticle	PLA[a]	Resiquimod	N/A	N/A	63
Passive accumulation, co-delivered with checkpoint inhibitor	Polymeric nanoparticle	Poly(2-oxazoline)	Resiquimod	Intratumoral	Lung	65
Passive accumulation, co-delivered with cancer vaccine	Polymeric nanoparticle	PLGA[b]	522, 528	Subcutaneous	Melanoma, Bladder, Renal	66
pH-triggered, co-delivered with chemotherapeutic	Polymeric nanoparticle	Poly(L-histidine), hyaluronic acid	Resiquimod	Intravenous	Breast	72
pH-triggered, co-delivered with checkpoint inhibitor	Polymeric nanoparticle	mTEGMA[i], PFPMA[j]	IMDQnano[k]	Peritumoral	Breast	73
pH-triggered	Micelle	Fatty acid-ionic liquids, vitamin E TPGS[l]	Imiquimod	Topical	Melanoma	74
pH-triggered	Polymeric nanoparticle	PLGA[b], sodium bicarbonate	522	Subcutaneously	Melanoma	75

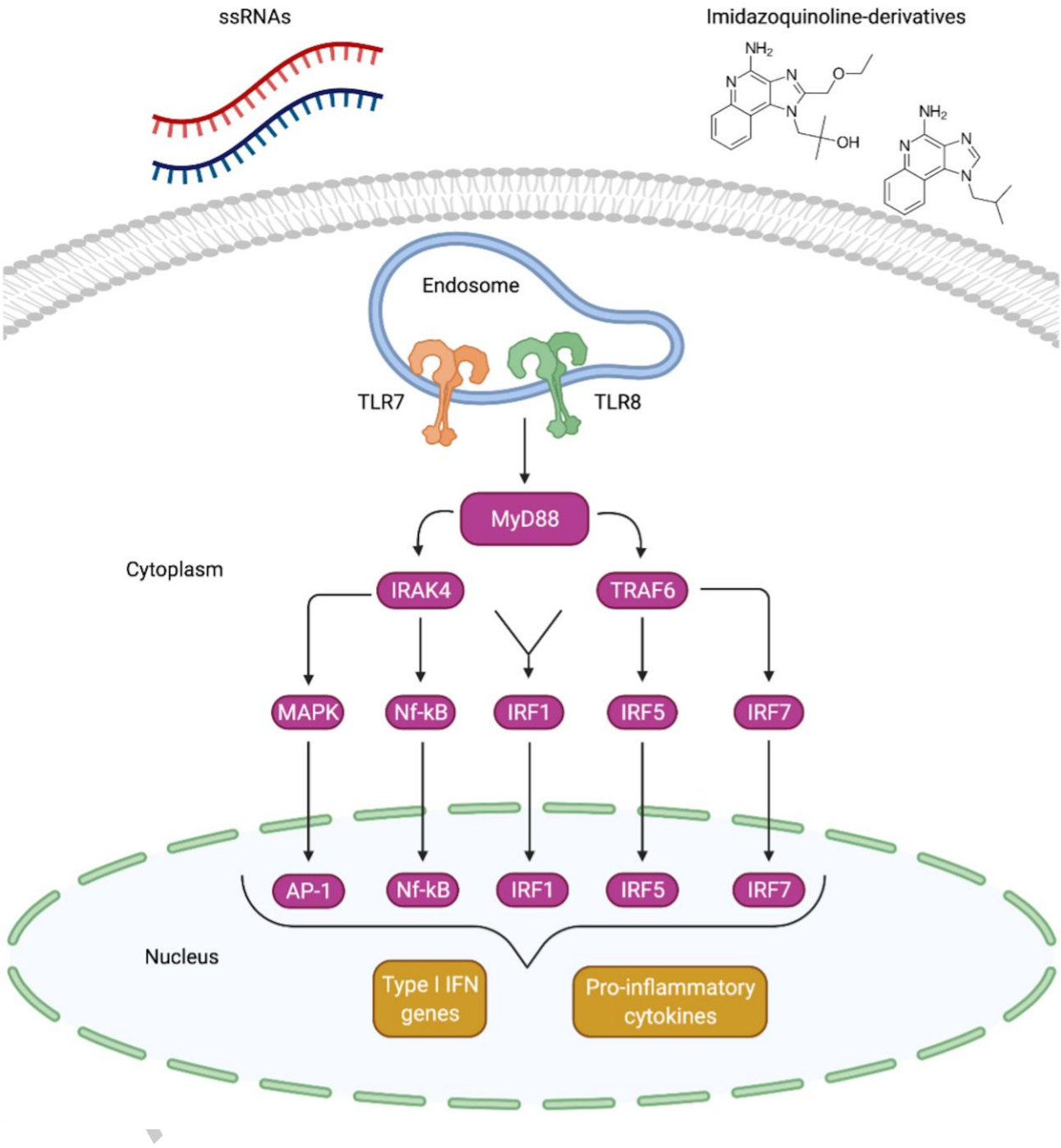
pH-triggered, co-delivered with antibodies	Polymeric nanoparticle	PLGA[b], sodium bicarbonate	522	Subcutaneously	Breast, Lung	76
pH-triggered, DC[m] targeted, with photothermal therapy	Metal-organic framework nanoparticle	Zeolitic imidazolate framework-8, IR-820, 1-MT[n]	Imiquimod	Intravenous	Melanoma	77
Thermally-triggered, with photothermal therapy	Polymeric nanoparticle	Poly(dopamine), PEG[g], carbon dots	Resiquimod	Intravenous	Breast	78
Thermally-triggered	Liposome	Iron sulfate, lipids	Resiquimod	Intratumoral	Breast	79
Thermally-triggered, with photothermal therapy	Hydrogel, polymeric nanoparticle	Indocyanine green, nucleotides, PDLLA-PEG-PDLLA[o]	Resiquimod	Subcutaneous	Breast	80
Thermally-triggered, with photothermal therapy	Lipid-functionalized polymeric nanoparticle	Lipids, PEG[g], semiconducting polymer	Resiquimod	Intravenous	Breast, Lung	81
Redox-triggered, co-delivered with chemotherapeutic	Prodrug	N/A	SZU101	Intratumoral	Breast	82
pH-triggered, enzyme-triggered, LN[p] targeted	Micelle	Poly(esteracetal) block copolymers	Adifectin	N/A	N/A	83
LN[p] targeted	Plant virus nanoparticle	Viral coat proteins	ssRNA	N/A	Leukemia	87
LN[p] targeted	Polymeric nanoparticle	mPEG-PLA[q], PLGA[b]	Resiquimod	Subcutaneously	N/A	88
LN[p] targeted, co-delivered with checkpoint inhibitor	Mannose-functionalized polymeric nanoparticle	PEG-PLA[r], mannose	Alkynated TLR7/8 agonist[s]	Intraperitoneal	Colon	89
LN[p] targeted, DC[m] targeted, co-delivered with checkpoint inhibitor and cancer vaccine	Mannose-functionalized lipid-polymer nanoparticle	PCL-PEG-PCL[t], lipids, mannose	Imiquimod	Subcutaneous	Lymphoma	90
LN[p] targeted, co-delivered with checkpoint inhibitor and cancer vaccine	Peptide-functionalized polymeric nanoparticle	Peptides, PEG-PLA[r]	Resiquimod	Subcutaneous	Colorectal	91
LN[p] targeted	Gold nanoparticle	Gold, 1-octanethiol, 11-mercaptoundecane sulfonate	Resiquimod	Subcutaneous	Colon	92
LN[p] targeted	Polymer-lipid amphiphile	Albumin, cholesterol, PABTC[u]	IMDQ[v]	Subcutaneous	N/A	93
LN[p] targeted, DC[m] targeted, co-delivered with cancer vaccine	Carboxylated Micelle	PEOz-PLA[w], PluronicF123	CL264	Subcutaneous	Lymphoma	94
TAM[x] targeted, co-delivered with	Metal organic framework	Hafnium-porphyrin conjugate	Imiquimod	Intratumoral	Colorectal	101

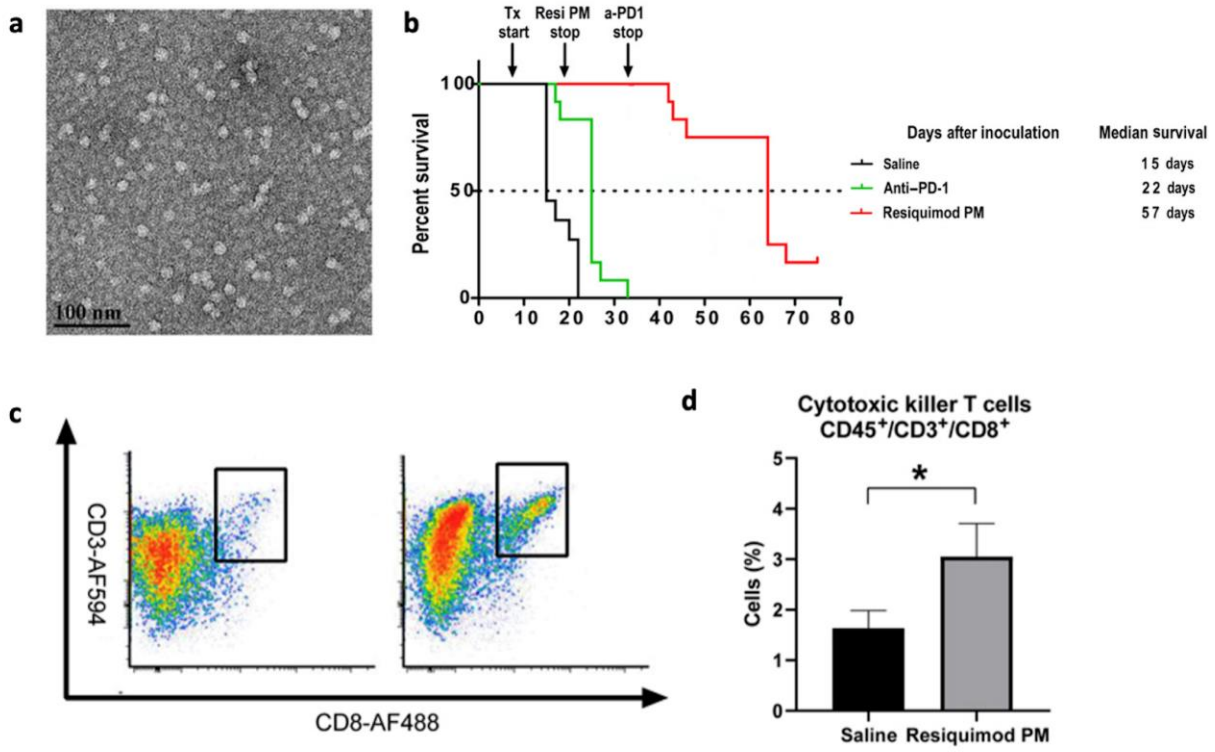
antibodies						
TAM[x] targeted, co-delivered with antibodies	Liposome	Lipids	Resiquimod	Intravenous	Colorectal	102
TAM[x] targeted	Magnetic polymer-based nanoparticle	Magnetic polymers, Fe_3O_4, lipopolysaccharide-treated macrophage membranes	Imiquimod	Intratumoral	Breast	103
TAM[x] targeted	Cyclodextrin nanoparticle	β-cyclodextrin, adamantane	Adamantane-modified resiquimod	Intravenous	Colon	104
TAM[x] targeted, co-delivered with chemotherapeutic	Peptide-functionalized polymeric nanoparticle	Lignin, peptides, maleimide-functionalized PEG[g]	Resiquimod	Intraperitoneal	Breast	105
TAM[x] targeted, co-delivered with checkpoint inhibitor	Cyclodextrin nanoparticle	Succinyl-β-cyclodextrin, lysine	Resiquimod	Intravenous	Colon, Melanoma	106
DC[m] targeted	Antibody-functionalized liposome	Lipids, monoclonal antibodies	TMX-202	N/A	N/A	114
DC[m] targeted, co-delivered with cancer vaccine	Mannose-functionalized gold nanoparticle	α-mannose, gold	1V209	Intradermal	N/A	115
DC[m] targeted, co-delivered with cancer vaccine	Polymeric nanoparticle, cell therapy	PLGA[b], indocyanine green, siRNA	Imiquimod	Subcutaneous	Lymphoma	116
monocytic myeloid-derived suppressor cell targeted, co-delivered with chemotherapeutic	Peptide-based liposome	Lipids, peptides, Cu^{64}	Resiquimod	Intravenous	Colon	117
PD-1 expressing CD8+T cells targeted, co-delivered with checkpoint inhibitor	Antibody-functionalized polymeric nanoparticle	Antibodies, maleimide-functionalized PEGylated PLGA[b]	Resiquimod	Intravenous	Colon, Melanoma	118
Co-delivered with checkpoint inhibitor	Silica nanoparticle	Silica	1V209	Intratumoral	Colorectal	124
Co-delivered with cancer vaccine and checkpoint inhibitor	Peptide-based nanoparticle	Peptides	Various	Intravenous, subcutaneous	Melanoma, colon, lung	128
Co-delivered with cancer vaccine	Peptide conjugate	Peptides	SZU101	Intraperitoneal	Breast	129
Co-delivered with cancer vaccine	Cell therapy	decitabine treated acute myeloid leukemia cells	SZU106	Subcutaneous	Lymphoma	130
Co-delivered with cancer vaccine	Lipid-polymer nanoparticle	PLGA[b], lipids	Gardiquimod	Intravenous	Melanoma	131
Co-delivered with cancer vaccine	Microneedles	Pluronic F127, PEG[g]	Resiquimod	Transdermal	Lymphoma	133

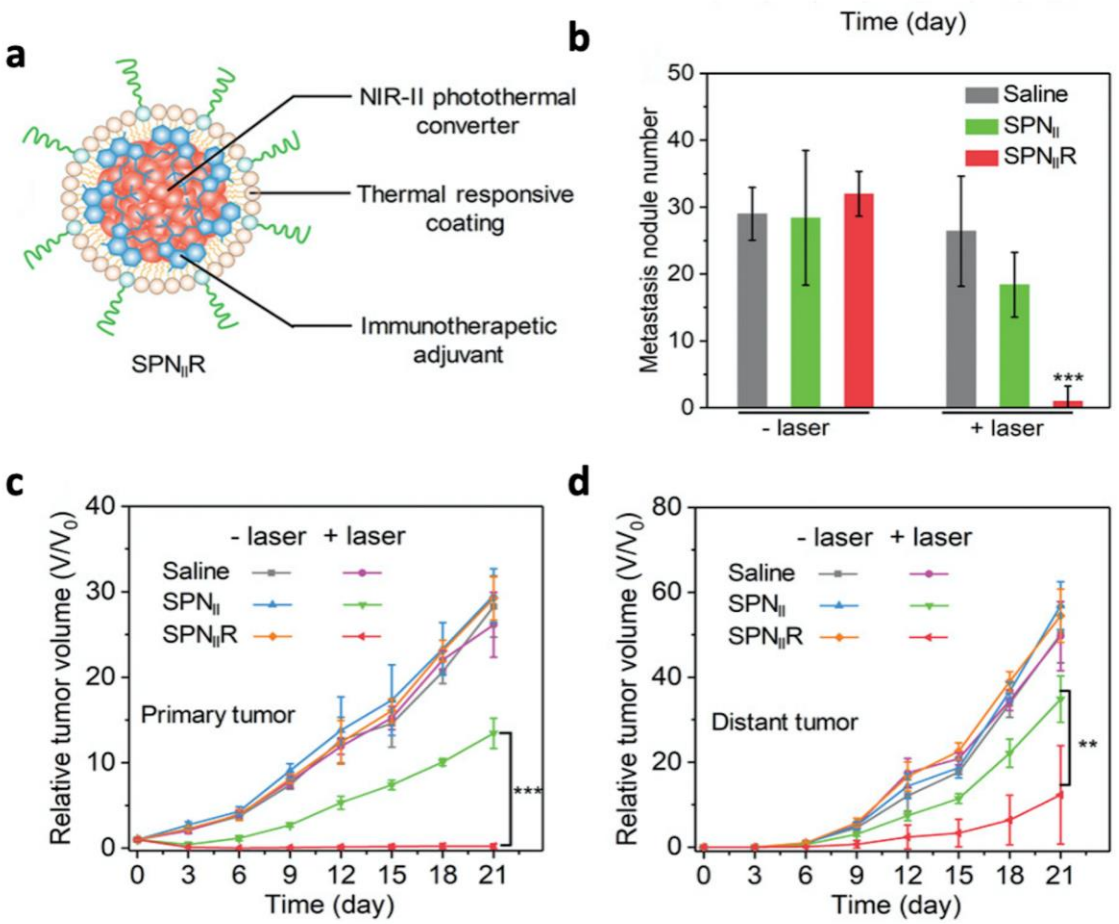
Co-delivered with cancer vaccine	Nanodispersion	L-195	Resiquimod	Transcutaneous	Lymphoma	134
Co-delivered with cancer vaccine, with photothermal therapy	Polymeric nanoparticle	Glycol-chitosan, poly(aniline)	Resiquimod	Intratumoral	Colon	135
Co-delivered with cancer vaccine and checkpoint inhibitor	Nanoemulsion	Span 85, Tween 80, Squalene, oleic acid	Resiquimod	Intratumoral	Melanoma	136
Co-delivered with cancer vaccine, checkpoint inhibitor, and tyrosine kinase inhibitor	Nanoparticle (unspecified)	N/A	522	Subcutaneous	Bladder, melanoma	137
Co-delivered with chemotherapeutic	Peptide conjugate	Peptides	T7	Subcutaneous, intraperitoneal	Breast	138
Co-delivered with vasculature disrupting agent	Polymeric nanoparticle	PLGA[b]	Gardiquimod	Intratumoral	Melanoma	139

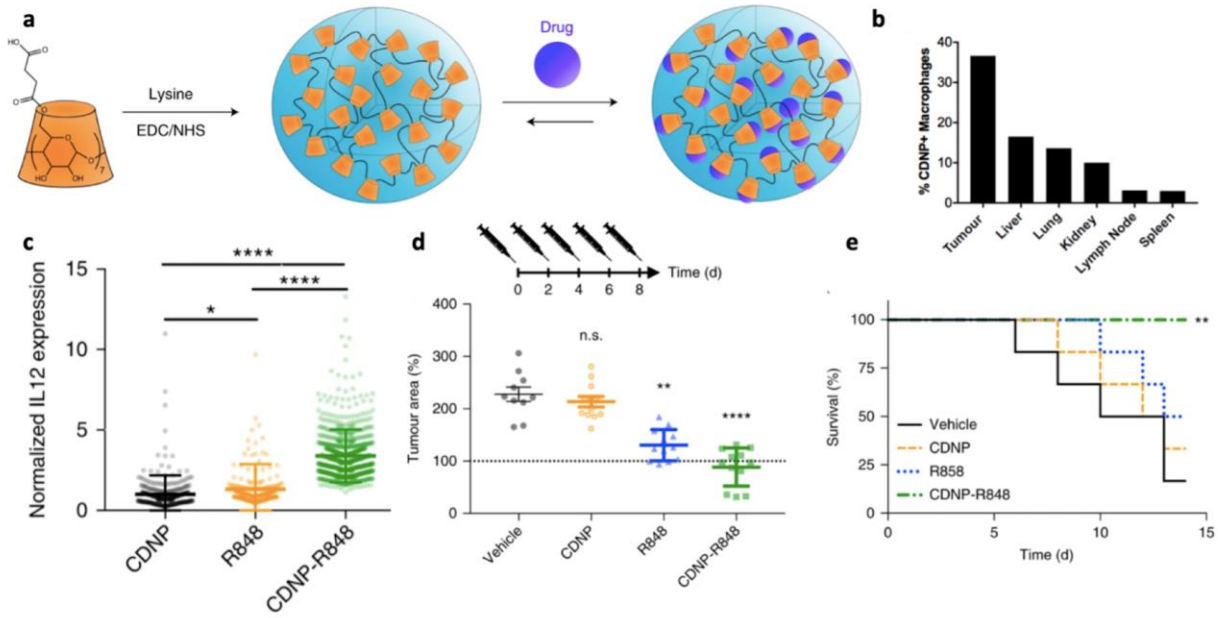
[a] Poly(lactic acid)
[b] Poly(lactic-*co*-glycolic acid)
[c] Poly(ε-caprolactone)
[d] Methoxy-poly(ethylene glycol)-)-hexyl-substituted lactide
[e] Guanosine and uridine-rich ribonucleic acid/deoxyribonucleic acid
[f] Dimethyl sulfoxide
[g] Poly(ethylene glycol)
[h] Poly(*N*-isopropylacrylamide)
[i] Methoxytriethyleneglycol methacrylate
[j] Pentafluorophenyl methacrylate
[k] 1-(4-(aminomethyl)benzyl)-2-butyl-1H-imidazo[4,5-c]quinolin-4-amine
[l] D-α-tocopheryl polyethylene glycol succinate
[m] Dendritic cell
[n] 1-methyl-d-tryptophan
[o] Poly(D,L-lactide)-b-poly(ethylene glycol)-b-poly(D,L-lactide)
[p] Lymph node
[q] Methoxypoly(ethylene glycol)-b-poly(DL-lactic acid)
[r] Poly(ethylene glycol)-b-poly(lactic acid)
[s] (1-(4-(aminomethyl)benzyl)-2-(ethoxymethyl)-1H-imidazo[4,5-c]quinolin-4-amine)
[t] Poly(ε-caprolactone)-b-poly(ethylene glycol)-b- Poly(ε-caprolactone)
[u] 2-propanoic acid butyl trithiocarbonate
[v] 1-{[4-(aminomethyl)phenyl]methyl}-2-butyl-1H-imidazo[4,5-c]quinolin-4-amine
[w] poly(2-ethyl-2-oxazoline)-b-poly(d,l-lactide)
[x] Tumor-associated macrophage

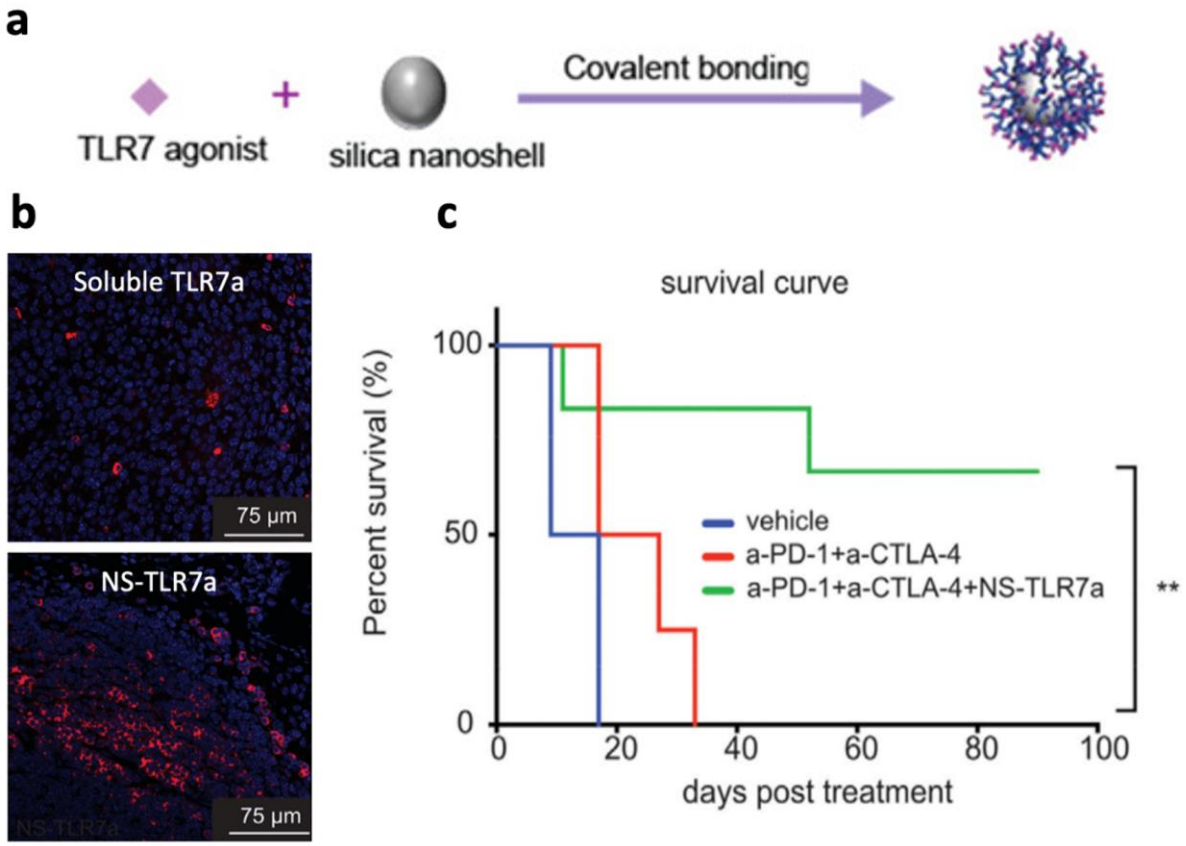
Table I: Recent TLR7/8 agonist drug delivery devices.











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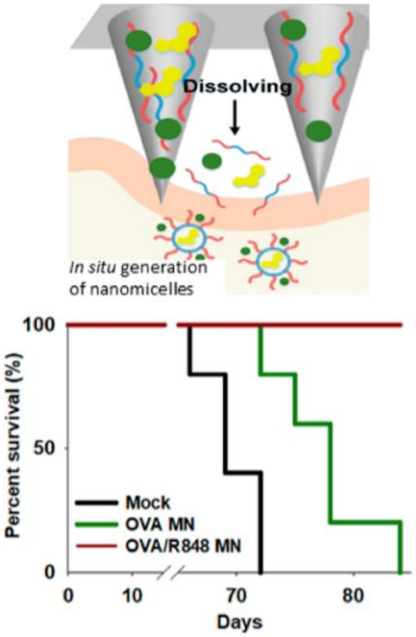

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