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IOTECH IMMUNO-ONCOLOGY AND TECHNOLOGY

REVIEW

Engineering approaches for innate immune-mediated tumor microenvironment remodeling

G. I. Kane[1,2], C. F. Lusi[1,2], M. L. Brassil[1,2] & P. U. Atukorale[1,2,3]*

[1]Department of Biomedical Engineering, University of Massachusetts Amherst, Amherst; [2]University of Massachusetts Cancer Center, Worcester; [3]Division of Innate Immunity, Department of Medicine, University of Massachusetts Chan Medical School, Worcester, USA

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Cancer immunotherapy offers transformative promise particularly for the treatment of lethal cancers, since a correctly trained immune system can comprehensively orchestrate tumor clearance with no need for continued therapeutic intervention. Historically, the majority of immunotherapies have been T cell-focused and have included immune checkpoint inhibitors, chimeric antigen receptor T cells, and T-cell vaccines. Unfortunately T-cell-focused therapies have failed to achieve optimal efficacy in most solid tumors largely because of a highly immunosuppressed 'cold' or immune-excluded tumor microenvironment (TME). Recently, a rapidly growing treatment paradigm has emerged that focuses on activation of tumor-resident innate antigen-presenting cells, such as dendritic cells and macrophages, which can drive a proinflammatory immune response to remodel the TME from 'cold' or immune-excluded to 'hot'. Early strategies for TME remodeling centered on free cytokines and agonists, but these approaches have faced significant hurdles in both delivery and efficacy. Systemic toxicity from off-target inflammation is a paramount concern in these therapies. To address this critical gap, engineering approaches have provided the opportunity to add 'built-in' capabilities to cytokines, agonists, and other therapeutic agents to mediate improved delivery and efficacy. Such capabilities have included protective encapsulation to shield them from degradation, targeting to direct them with high specificity to tumors, and co-delivery strategies to harness synergistic proinflammatory pathways. Here, we review innate immune-mediated TME remodeling engineering approaches that focus on cytokines, innate immune agonists, immunogenic viruses, and cell-based methods, highlighting emerging preclinical approaches and strategies that are either being tested in clinical trials or already Food and Drug Administration approved.

Key words: antibody-drug conjugate, cell engineering, cell therapy, dendritic cells, immune-cytokines, therapeutic vaccine

SIGNIFICANCE OF INNATE IMMUNE-MEDIATED TUMOR MICROENVIRONMENT REMODELING AS A TREATMENT PARADIGM

Cancer immunotherapy offers transformative promise for the treatment of lethal cancers, since a correctly trained immune system can comprehensively orchestrate tumor clearance without any need for continued intervention. Historically, the majority of immunotherapies have been T cell-focused, including immune checkpoint inhibitors (ICIs), chimeric antigen receptor (CAR) T cells, and T-cell vaccines.[1] Currently, ICIs, such as anti-programmed cell death protein 1 (PD-1) and anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4), are clinically used to treat many solid malignancies.[2] CD8+ CAR T therapies are 'last-resort' treatments for leukemias, lymphomas, and other blood cancers in both adult and pediatric settings.[3] CD4+ CAR T cells are also being developed and tested in preclinical settings.[4] Cytotoxic CD8+ T lymphocytes (CTLs) have been chief effector cells of choice since CTLs are direct specific killers and have robust memory subsets that can be activated to protect against tumor recurrence. However, while T-cell therapies have had enormous success in blood cancers, they have failed to be effective in most solid tumors largely because of their immunosuppressed 'cold' or even immune-excluded microenvironments.[5,6]

Cancers advance by promoting subsets of pro-tumor immune cells, including M2 macrophages, myeloid-derived suppressor cells (MDSCs), and regulatory T cells (T_{regs}) that generate an immunosuppressive cytokine milieu in the tumor microenvironment (TME) that inactivates any infiltrating CTLs and shields it from antitumor immune

*Correspondence to: Prof. Prabhani U. Atukorale, 364 Plantation Street, LRB 219, Worcester, MA 01605, USA. Tel: +1 (508) 856-1562
E-mail: patukorale@umass.edu (P. U. Atukorale).

surveillance.[1,6] Besides these biological barriers, there are additional chemical and physical barriers in the TME, such as hypoxic niches, acidic pH, disordered and sometimes poor vascularization, a rigid extracellular matrix, and high interstitial tissue pressure, that further impede CTL infiltration and their sustained activation.[7,8] In recent years, a rapidly growing treatment paradigm has emerged that focuses on activation of tumor-resident innate antigen-presenting cells (APCs), such as dendritic cells (DCs) and macrophages, to drive a proinflammatory immune response that remodels the TME from 'cold' or immune-excluded to 'hot', often in a highly effective self-amplifying fashion.[5,6] Type I interferons (IFNs) especially are hallmark cytokines in effective TME remodeling.[5,6] This approach has been shown to be highly effective in promoting antitumor immunity from within the TME itself, activating and recruiting CTLs, and sustaining their activation. This review will focus on innate immune-mediated engineering strategies for TME remodeling (Figure 1).

ADVANTAGES OF ENGINEERING APPROACHES

Early strategies for TME remodeling centered on free cytokines and agonists, but these approaches have faced significant hurdles in both delivery and efficacy. In particular, systemic toxicity from off-target inflammation is a paramount concern in these therapies. To address this critical gap, engineering approaches have provided the opportunity to add 'built-in' capabilities to therapeutic agents to improve delivery and efficacy.[9-11] Such capabilities or design principles have included protective encapsulation to shield them from degradation, targeting to direct them with high specificity to tumors, and co-delivery strategies to harness synergistic immunomodulatory pathways. These approaches have included the fabrication of synthetic materials and also the modification of naturally occurring therapeutics, including viruses and whole cells. Notably, tumor APCs are target cells of choice for TME remodeling approaches, and the tumor perivascular region, where they are enriched, is readily accessible via the systemic blood circulation.[5,12,13] This effect is augmented by enhanced permeation and retention that occurs due to the 'leaky' tumor vasculature with widened gaps between endothelial cells.[14,15] This physiological makeup favors innate immune-mediated TME remodeling using nanoparticles (NPs) and other systemically delivered approaches since deposition only needs to occur in the perivascular space, in contrast to delivery of chemotherapies and other conventional treatments with tumor cells as targets that necessitate drugs to penetrate well beyond the perivascular region and well into the core of the tumor. Further, advances in powerful diagnostic techniques such as *in situ* hybridization readily enable the detection of immunomodulatory targets, further improving the design of these approaches.[16]

Method of administration can also play a critical role in delivery and efficacy. Intratumoral delivery may more readily mitigate systemic off-target toxicity issues, but this method is limited in its reliance on prior knowledge of tumor locations, which is often clinically infeasible. Even when tumor locations are known, intratumoral delivery is limited since high interstitial tumor tissue pressure can prevent dissemination to target cells and injected volumes often remain in bolus masses at the injection site.[8,17,18] Intratumoral injection in advanced tumors also carries the risk of dislodging tumor cells and inducing metastasis. By contrast, while systemic administration may require more stringent engineering design to address clearance-related toxicities, this method offers widespread deposition in primary tumor masses that are often highly vascularized and is the sole route of administration that can target even individual metastases.[8,18,19]

Here, we highlight engineering approaches that focus on delivery of cytokines, innate immune agonists, viruses, and whole cells (Figure 1) and highlight and compare emerging preclinical approaches as well as strategies that are either being tested in clinical trials or already Food and Drug Administration (FDA) approved (Table 1).

CYTOKINE APPROACHES

Cytokines are critical immune regulators and play a central role in driving an antitumor immune response. Modulating the cytokine milieu in the TME is an attractive therapy as a means to augment antitumor immunity by TME remodeling.[20,21] Unfortunately, the clinical use of cytokine therapy has been limited by serious side-effects (e.g. cytokine storm) and underwhelming efficacy, resulting in limited regulatory approval for clinical use.[22,23] However, the large number of ongoing clinical trials using cytokines as therapeutics[24] is representative of their utility and strongly warrants a critical need for approaches that incorporate engineering capabilities to cytokines to improve their safety and efficacy. Many of the therapies we discuss here have aimed to deliver proinflammatory cytokines that prime and activate both innate and adaptive immune cells, including type I IFNs, interleukin (IL)-2, IL-12, and tumor necrosis factor-α (TNF-α), while a smaller subset of therapies have focused on delivering anti-inflammatory IL-10 (Figure 2).

PEGylated cytokines

Conjugation of cytokines to hydrophilic poly(ethylene glycol) (PEG), or PEGylation, has been shown to increase blood solubility for longer circulation and improved delivery to tumors.[24-26] In early studies, PEGylated IFN-α-2b significantly prolonged relapse-free survival in melanoma patients and was approved as a post-surgical resection treatment in 2011.[27] Recent data, however, have shown that naturally occurring anti-PEG antibodies are detectable in over 70% of tested populations.[24,28,29] PEGylated IL-10 recently tested in phase I clinical trials in combination with ICIs for treatment of metastatic non-small-cell lung cancer failed to meet primary endpoints, strongly suggesting that further understanding of the confounding spatiotemporal role of PEG antibodies is needed.[30,31]

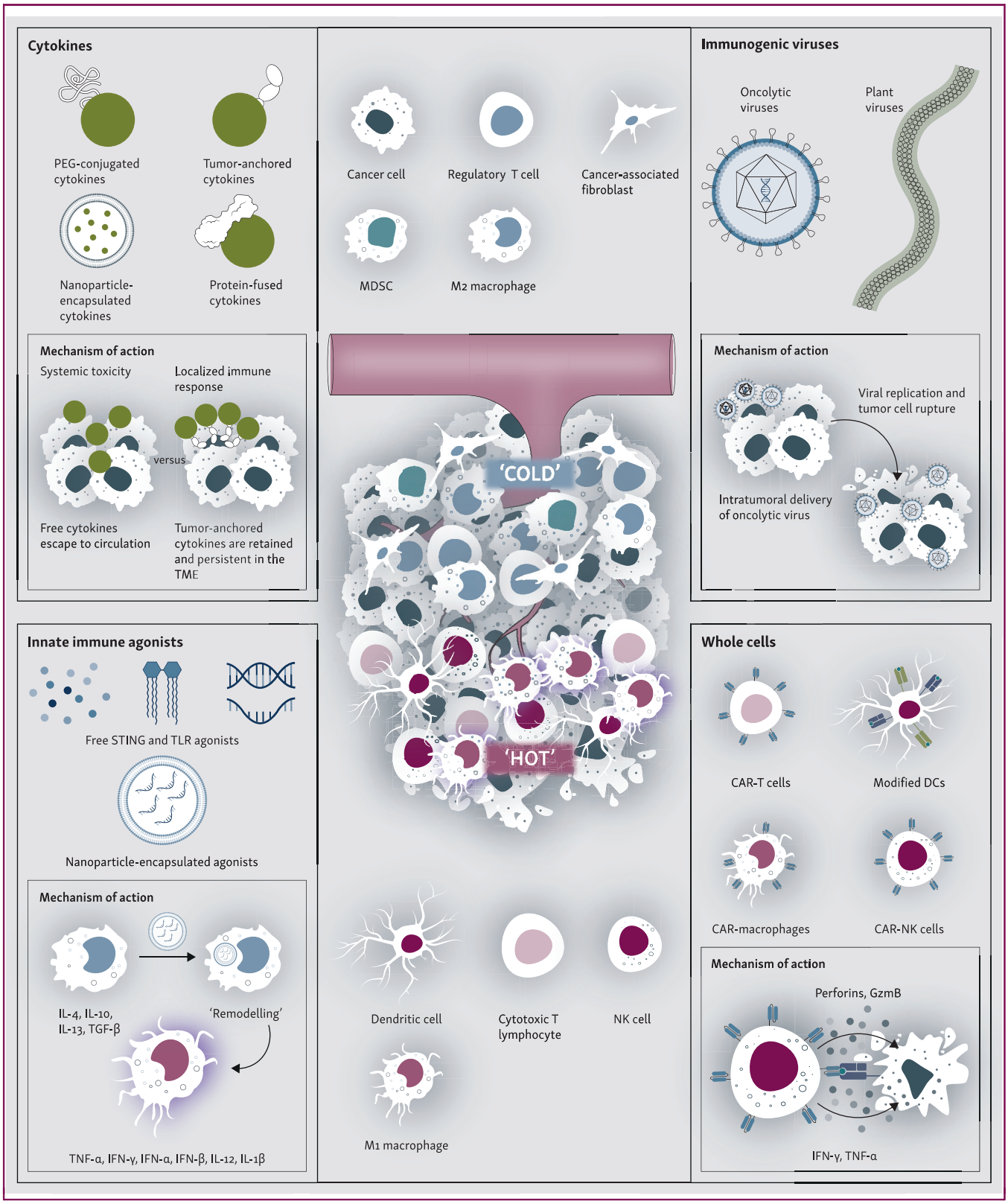

Figure 1. Summary schematic of innate immune-mediated tumor microenvironment (TME) remodeling with emphasis on the four engineering approaches that are the focus of this paper.
CAR, chimeric antigen receptor; DC, dendritic cell; IFN, interferon; IL, interleukin; MDSC, myeloid-derived suppressor cell; NK, natural killer; PEG, poly(ethylene glycol); STING, stimulator of interferon genes; TGF-β, transforming growth factor-β; TLR, Toll-like receptor; TNF-α, tumor necrosis factor-α.

Protein-fused cytokines

Chemically fusing proteins to cytokines has been shown to improve therapeutic outcomes and safety.[24,32,33] Due to their larger size, protein-fused cytokines have been shown to reduce renal excretion and decrease interstitial transport rates to improve delivery to tumors in mouse models of carcinoma.[34]

Table 1. Summary of TME remodeling innate immune engineering approaches currently being tested or in use in the clinic

Innate immune engineering approaches for reprogramming tumor microenvironment					
Therapeutic	Cancer types	Delivery route	Engineering strategy	Advantages	Challenges
Cytokines	NSCLC, melanoma, neuroblastoma, lung carcinoma, ovarian carcinoma, colorectal carcinoma, renal cell carcinoma, sarcoma, hematological malignancies	i.v., s.c., i.t.	PEGylated cytokines,[22,31,a] protein-fused cytokines,[38,39] tumor-anchored cytokines (preclinical)[58,59,61]	-Limit systemic toxicity -Extend half-life -Tailored designs improve draining to preferential sites -Reduced renal excretion	-Reliant on-target cell uptake -Inherent pleiotropism
Innate immune agonists	Colorectal carcinoma, TNBC, melanoma, glioblastoma, PDAC	i.t., i.v., s.c.	Peptide-modified agonists (preclinical),[69,70] nanoparticle-encapsulated agonists (preclinical)[19,71-74,79-81]	-Limit systemic toxicity -Extend half-life -Improve draining to preferential sites -Protection from nuclease degradation -Co-uptake of synergistic agonists	-Reliant on-target cell uptake
Immunogenic viruses	Melanoma, TNBC, ovarian cancer, gastrointestinal adenocarcinoma, epithelial cancer of head and neck, Merkel cell carcinoma	i.t., i.v., i.p.	Oncolytic viruses,[95-97,100] plant viruses (preclinical)[102,103]	-Limit systemic toxicity -Highly tumor-selective -Preferential replication in tumor cells -Empty capsids can encapsulate alternative therapeutics	-Inherent risk of mammalian virus in mammalian host (not applicable for plant viruses)
Whole cells	Hematological malignancies, TNBC, neuroblastoma, prostate cancer, ovarian cancer	i.v.	CAR-NK cells (preclinical),[117] CAR-Macs,[128] DC vaccines[130]	-Do not rely on cellular uptake of therapeutic -Inherently proinflammatory when activated	-Manufacturing and regulatory hurdles[112] -Reliant on cellular trafficking/ tumor infiltration -On-target off-tumor effects

CAR, chimeric antigen receptor; DC, dendritic cell; i.p., intraperitoneal; i.t., intratumoral; i.v., intravenous; NK, natural killer; NSCLC, non-small-cell lung cancer; PDAC, pancreatic ductal adenocarcinoma; s.c., subcutaneous; TNBC, triple-negative breast cancer.
[a]Trial failed to meet primary endpoints.

Albumin, in particular, has unique properties when fused with cytokines since it enables recycling through FcRn receptors and does not require frequent dosing.[35] Albumin-fused IL-2 exhibited prolonged serum half-life and preferential draining to spleen, liver, and lymph nodes in a canine model of systemic histiocytic sarcoma.[36] Recently, albumin—IL-2 fusions have been used effectively in combination immunotherapies for treatment of metastatic melanoma mouse models.[37] Conjugation of antibodies to cytokines to formulate immuno-cytokines has also been shown to confer high targeting specificity to tumors. Many immuno-cytokines are currently being tested in clinical trials as treatment for neuroblastoma, melanoma, lung cancer, ovarian cancer, and colorectal cancer.[38,39] Antibodies have also been conjugated to biologically active or cytotoxic agents to form antibody-drug conjugates (ADCs), which have been highly successful in the clinic in multiple types of solid tumor settings.[40-43] ADCs have been used widely to target tumor cells and promote their apoptosis, while sparing healthy cells, which also have a pivotal secondary effect in releasing neoantigens from dying tumor cells that can be more readily presented by APCs as part of TME remodeling approaches. Engineering design parameters, such as PEGylation, glycosylation, control of antibody-drug ratio, charge, the use of stable or releasable linkers, and pharmacokinetics, can be readily tuned to control ADC stability in circulation and drug delivery specificity and release.[44-46]

Nanoparticle-encapsulated cytokines

Lipid-based NPs (LNPs) have been widely used to encapsulate cytokines due to their design versatility as 'smart' targeting systems for efficient tumor delivery.[47,48] In early studies, sodium dodecyl sulfate NPs were used to non-covalently complex IL-2 and were effective in preventing lung metastasis in renal cell carcinoma due to micro-aggregate formation in the metastatic TME following systemic injection.[22,49,50] More recently, liposomal NPs incorporating both IL-2-Fc and anti-CD137 on their surfaces were shown to elicit effective antitumor responses with minimal toxicity following systemic delivery in a mouse model of metastatic melanoma.[51] LNPs have also been used to deliver messenger RNA encoding for cytokines, which is reviewed extensively elsewhere.[52]

NPs have also been used to create depots of encapsulated cytokines in the TME following intratumoral injection, often with slow and controllable release kinetics with minimal leakage and systemic toxicity compared to free cytokines. IL-2 and anti-CD137 were incorporated on to liposomal NP surfaces to generate both local and systemic antitumor immunity and prevent lethal toxicity following intratumoral injection in mouse models of melanoma.[53] Recently, layer-by-layer synthesis approaches were used to coat LNPs with IL-12 for prolonged binding to tumor cell surfaces and, following intratumoral delivery, this strategy showed increased efficacy in mouse models of ovarian and colon cancer.[54,55]

Notably, as TME conditions often play a crucial role in the delivery of nanocarrier (or microcarrier) systems, the tailored design of these carriers to be responsive to these conditions has become an area of intense focus. By controlling characteristics such as size, shape, nanomaterial,

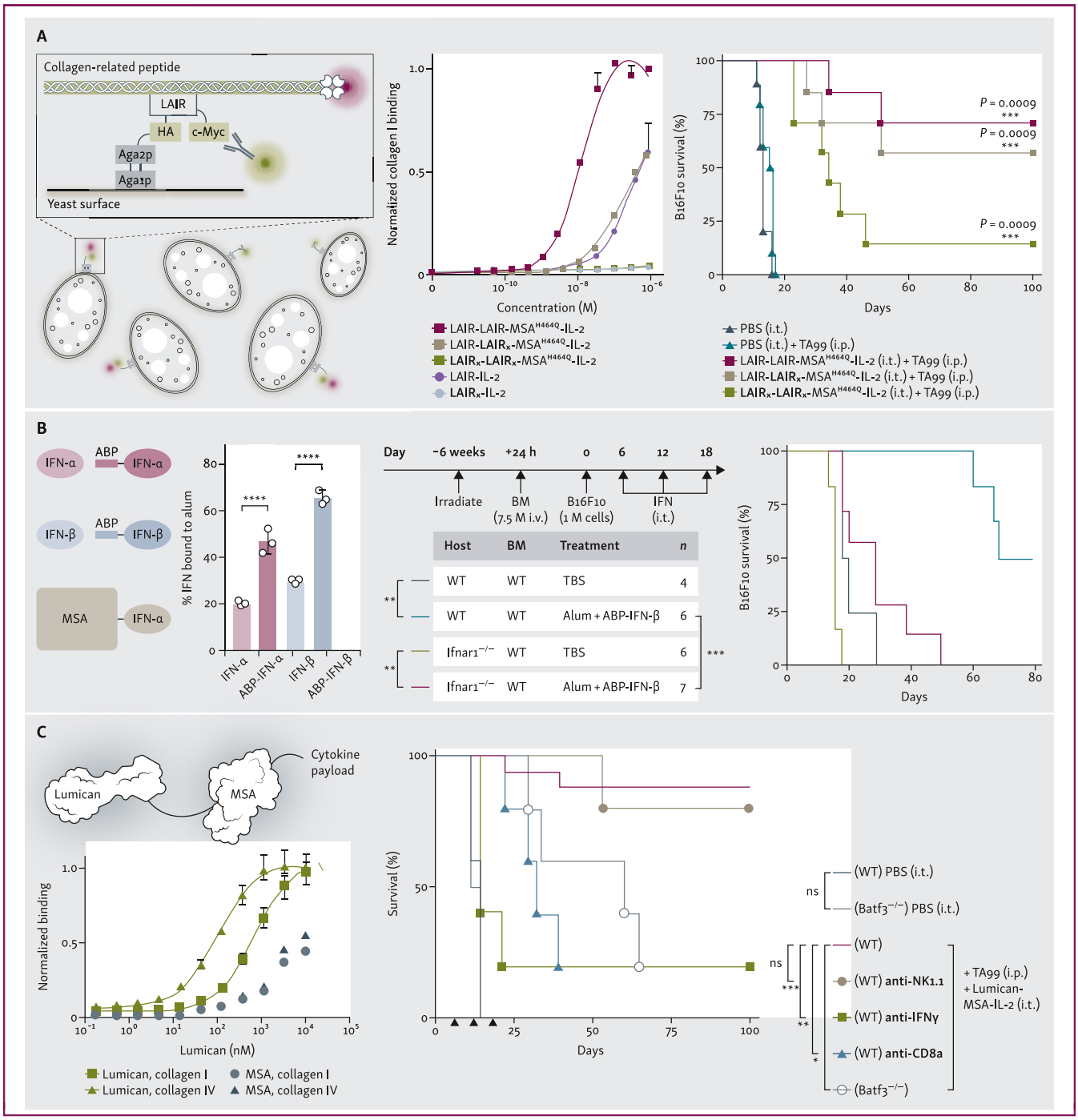

Figure 2. Engineered cytokines.
(A) Collagen-binding interleukin (IL)-2 construct via yeast surface display enhances efficacy of TA99 therapy in B16F10 melanoma model.[58] (B) Alum-anchored type I interferons (IFNs) achieve high cure rates in B16F10 tumors.[60] (C) Lumican—cytokine fusions prolong local retention and combine with tumor-targeting antibody for curative responses in B16F10 tumors.[61] ABP, alum-binding peptide; BM, bone marrow; i.p., intraperitoneal; i.t., intratumoral; IL, interleukin; LAIR, leukocyte-associated immunoglobulin-like receptor-1; MSA, mouse serum albumin; PBS, phosphate-buffered saline; TBS, Tris-buffered saline; WT, wild type.

and surface chemistry, 'smart' NPs have been engineered over the last decades to drive the delivery of cytokine and other cargo payloads to target sites with reduced or minimal dosing frequency while mitigating off-target side-effects often associated with free cytokine therapies.[14,56]

Tumor-anchored cytokines

Engineering methods to chemically anchor cytokines in the TME have been used as a highly effective approach to mitigate dose-limiting toxicities from systemic leakage. The majority of these treatments are delivered by intratumoral injection. Aluminum hydroxide, or alum, approved by the FDA as a vaccine adjuvant since the 1930s, has been used to anchor cytokines in tumors via binding to an alum-binding peptide (ABP) motif displayed on the cytokines. ABP is highly phosphorylated and exhibits tight bonding to alum through ligand exchange reactions with surface hydroxyls. Rod-shaped alum nanocrystals aggregate to form

long-lasting physical depots, as shown in a mouse melanoma model (Figure 2A).[57,58] Following intratumoral injection, ABP-IL-12 was shown to be stably retained in the TME in a fibrosarcoma mouse model, generating not only curative responses in the majority of mice but also immunological memory.[59] ABP-IFN-α and ABP-IFN-β, both of which are type I IFNs, have also been found to have significant therapeutic efficacy compared to free cytokines in colon carcinoma and melanoma mouse models (Figure 2B).[60]

Collagen and fibronectin, highly abundant TME proteins, have also been used as anchoring targets for cytokines that are linked to binding motifs. Notably this strategy is strongly dependent on intratumoral injection as similar matrix attachment sites in the liver and kidney can also capture these cytokine formulations if injected systemically. Both IL-2 and IL-12 have been anchored to collagen, effectively localizing in areas dense with effector T cells and combining effectively with a tumor-targeting antibody, ICIs, or CAR T-cell therapy in melanoma and triple-negative breast cancer (TNBC) mouse models (Figure 2C).[58,61] Fibronectin-anchored IL-12 and TNF-α produced responses in the majority of non-injected lesions in melanoma patients, suggesting modulation of both local and systemic immunity.[62]

INNATE IMMUNE AGONIST APPROACHES

While many cytokine therapies focus on delivery of TME remodeling cytokines that directly aim to activate and sustain CTLs in the TME, there is a fast-growing treatment paradigm that focuses on the delivery of innate immune agonists to activate innate APCs and even natural killer (NK) cells.[63,64] Notably, many of these innate agonists bind to pattern recognition receptors (PRRs) and have been FDA approved for use as vaccine adjuvants, enabling them to be readily translated for clinical testing in TME remodeling approaches (Figure 3).

Free and peptide-modified agonists

Agonists of the Toll-like receptor (TLR) and stimulator of interferon genes (STING) pathways have been widely used to activate APCs in the TME. In their free form, they must be injected intratumorally to avoid systemic off-target toxicity and ensure delivery.[65-68] Unmethylated cytosine-phosphate-guanine (CpG) motifs are TLR9 agonists and have been delivered intratumorally in combination with anti-OX40 in metastatic mouse models of colorectal carcinoma and TNBC as neoadjuvant immunotherapy and followed by surgical resection to remove uncleared cells in the TME.[69] In such neoadjuvant treatment scenarios, CpG not only drives APC activation and TME remodeling to promote a CTL response and protective memory, but CpG-mediated tumor clearance also significantly reduces the size of tumors and decreases the likelihood of regrowth after surgical resection.[70] Very recently, a polyspecific integrin-binding peptide was covalently attached to CpG and injected systemically in mouse models of TNBC to achieve effective tumor targeting, APC activation, and TME remodeling.[18]

Nanoparticle-encapsulated agonists

Due to their small size compared to cytokines, innate immune agonists can be readily encapsulated within NPs that can be used as 'smart' delivery systems to augment immune activation in the TME and mitigate systemic toxicity.[14,47] Encapsulation within nanocarriers also protects many nucleic acid-based agonists of the TLR and STING pathways since they can otherwise be readily degraded by nucleases.[70] CpG encapsulated in liposomal NPs and delivered via direct intratumoral injection showed significant immune activation in metastatic melanoma mouse models.[71] Recently, significant attention has been focused on designing effective NP-based delivery strategies for STING agonists, which are known to drive potent type I IFN expression by APCs and other cells in the TME with potential for powerful TME remodeling responses.[72-74] Various nanomaterials have been used to formulate STING agonist-encapsulated NPs, including polymeric- and lipid-based compositions.[70,75-78] In efforts to deliver STING agonists to their cytosolic receptor, polymeric NPs with specific endosome-rupturing capabilities have been developed and have shown significant TME remodeling efficacy in melanoma, glioblastoma, and TNBC mouse models following both systemic and intratumoral delivery (Figure 3A).[71,79-81]

Very recently, the versatility of synthesis of LNPs has been exploited to co-encapsulate multiple synergistic innate immune agonists for systemic delivery to tumors in efforts to promote the robust production of type I IFNs by TME APCs and TME remodeling.[19] This engineering strategy uniquely harnessed both STING and TLR4 pathways, which share common downstream effectors, such as the interferon regulatory factor 3 transcription factor (Figure 3B). Using LNP synthesis strategies, which enabled co-encapsulation of both hydrophobic and hydrophilic agonists on the same nanocarrier, these dual-agonist NPs promoted the synergistic production of TME type I IFNs and the activation of local otherwise 'exhausted' CTLs for clearance in mouse models of TNBC and pancreatic cancer.[19,82] Due to their small size and PEGylated surface coating, these dual-agonist NPs were safely delivered in the systemic blood circulation, which, in turn, enabled widespread deposition in the TME perivascular regions of highly vascularized tumors that are rich in target APCs. Notably, the synergistic type I IFN production in this strategy enabled a significant reduction in overall systemic dose, resulting in minimal and only transient hepatotoxicity. In metastatic mouse models of TNBC, a significant reduction in metastatic burden was also demonstrated following systemic delivery of dual-agonist NPs, highlighting the central importance of systemic delivery for the treatment of metastatic disease.[19,83] When used as neoadjuvant immunotherapy, dual-agonist NPs mediated tumor-free curative results following surgery with protective immunological memory in metastatic melanoma and TNBC models.[83]

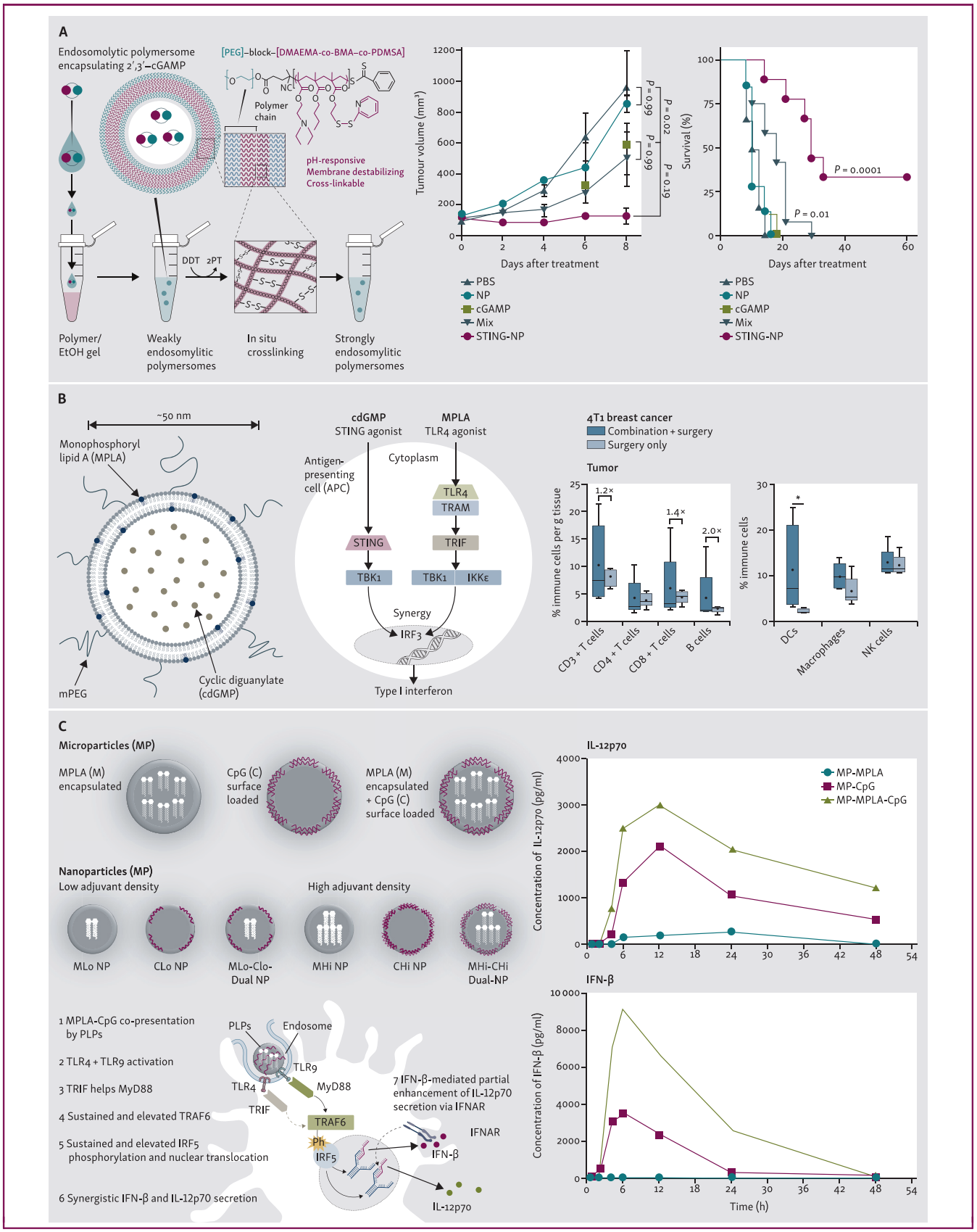

Figure 3. Engineered innate immune agonists.

(A) Stimulator of interferon genes (STING)-activating nanoparticles (NPs) enhance therapeutic efficacy of cyclic guanosine monophosphate—adenosine monophosphate (cGAMP), inhibit tumor growth, and prolong survival.[79] (B) Synergistic dual-agonist lipid NPs delivered systemically drive tumor microenvironment (TME) reprograming in triple-negative breast cancer (TNBC). $^{*}P < 0.05$.[83] (C) Pathogen-like particle (PLP)-based delivery of synergistic innate immune agonists drives robust interleukin (IL)-12p70 and interferon (IFN)-β responses in bone marrow-derived antigen-presenting cells (APCs).[85] CpG, cytosine-phosphate-guanine; DC, dendritic cell; DTT, dithiothreitol; mPEG, methoxy-terminated poly(ethylene glycol); MP, microparticles; MPLA, monophosphoryl lipid A; NK, natural killer; PBS, phosphate-buffered saline; 2PT, 2-pyridinethione; TLR4, Toll-like receptor 4.

Polymeric NPs have also been used to co-encapsulate dual- and tri-agonist combinations of TLR2, -4, -7, -9, and STING agonists.[84] Co-delivery of rational combinations via a nanocarrier has been shown to increase both the depth and breadth of the cytokine response, driving the robust production of type I IFNs as well as other proinflammatory cytokines, such as IL-12p70, IL-10, and IFN-γ. When taken together and applied in a tumor treatment setting, these efforts can enhance antigen cross-presentation, CTL priming, and NK cell activity (Figure 3C).[85]

IMMUNOGENIC VIRUS APPROACHES

Certain subsets of viruses have been exploited for TME remodeling therapies due to their immunogenic and tumor-selective properties. Some classes of viruses have been extensively studied and, therefore, very well-characterized over the last decades, enabling them to be de-risked and readily adapted for clinical translation.[86] Emerging classes of viruses, by contrast, are still being tested in preclinical tumor models to establish their mechanistic basis of action. Fully characterizing any potential off-target effects and undesirable pathogenicity are critical parameters for clinical translation of these novel viruses (Figure 4).[87]

Oncolytic viruses

Oncolytic viruses (OVs) have been shown to be effective as targeted therapy since they preferentially replicate in tumor cells, allowing specifically for elimination only of cancer cells and mitigating off-target toxicities that are common in untargeted nonspecific therapies. Both targeted oncolysis and innate antiviral immunity lead to tumor cell lysis, the release of tumor antigens, and subsequent uptake and cross-presentation by APCs, resulting in a powerful TME remodeling response.[88]

The use of OVs for cancer therapy dates back to the treatment of bone carcinoma in the 1890s with *Streptococcus pyogenes*.[89-92] Until recently, however, limitations of viral engineering often translated to limited efficacy or adverse side-effects.[93] Recent advances in recombinant biotechnology and controlled modification of viruses have enabled the production of viral therapies that are less pathogenic and more effective.[93,94] In 2015, talimogene laherparepvec (T-VEC) became the first OV cancer therapy approved by the FDA for the treatment of advanced unresectable melanoma.[95]

T-VEC is a genetically modified herpes simplex type 1 virus. This genetic modification includes deletion of the herpes virus gene ICP34.5 to decrease pathogenicity and insertion of two copies of the granulocyte—macrophage colony-stimulating factor (GM-CSF) gene to increase immunogenicity. Upon administration, T-VEC preferentially replicates within tumor cells, leading to direct tumor cell lysis and release of tumor antigens. The GM-CSF modification promotes recruitment and maturation of APCs which present these tumor antigens to T cells in the lymph nodes to stimulate a tumor-specific CD8+ T-cell response. A randomized phase III clinical trial of T-VEC for advanced-stage melanoma showed a promising safety profile and prolonged overall survival, leading to FDA approval in 2015.[96] Further studies have shown that T-VEC can be used in combination with other immunotherapies such as anti-PD-1 to enhance treatment response rates (Figure 4A).[97-100]

Though OV therapies show clinical potential, they can be limited by the inherent risks of a mammalian virus applied to a mammalian host. Current and future research on OV therapies aims to address the potential for off-target virulence and toxicity and find methods to mitigate or utilize innate antiviral immune responses that can otherwise inhibit OV efficacy, allowing for systemic or repeat administration.[86,98,101]

Plant viruses

Plant virus-based therapies have recently been shown to be highly immunogenic and can include whole virions (plant viral nanoparticles, PVNPs) or empty plant virus capsids (virus-like particles, VLPs) that can encapsulate alternative therapeutics. In contrast to OVs, plant viruses are not infectious to mammalian hosts, and can thereby circumvent some of the challenges of OV therapy.[87] Plant viruses have foreign surface antigens that can be recognized by innate PRRs and are readily phagocytosed by APCs. A number of plant viruses and VLPs have shown efficacy in cancer therapy and TME remodeling, including cowpea mosaic virus (CPMV)[102,103] (Figure 4B), papaya mosaic virus,[104] potato virus[105] (Figure 4C), and tobacco mosaic virus.[102,106] Applications of PVNPs and plant VLPs in cancer immunotherapy have been reviewed elsewhere.[87,107]

CPMV is one of the most thoroughly studied VNPs used in cancer therapy. CPMV can target tumor endothelial cells by binding to vimentin on the surfaces of these cells.[108] CPMV also has an icosahedral conformation that makes it well-suited for surface conjugation and drug encapsulation.[109] *In situ* vaccination with CPMV has been shown to elicit a potent antitumor immune response.[102,103] Intratumoral administration promotes stimulation and recruitment of monocytes and NK cells leading to adaptive immune responses through APC recruitment of CD4+ and CD8+ T cells and effector memory cells. In mouse models of melanoma, *in situ* vaccination with CPMV was shown to slow tumor growth and extend overall survival.[102] CPMV has also been shown to combine effectively with anti-CD47 by promoting macrophage polarization from M2 to M1 for enhanced antitumor immunity (Figure 4).[103] While plant viruses are an appealing platform for TME remodeling and cancer immunotherapy, their clinical applications are limited, and further research and development of manufacturing facilities will be required for clinical translation.[87,110]

CELL-BASED APPROACHES

Cell-based therapies that focus on the development of CARs for innate immune cells, such as NK cells and macrophages, have recently gained attention not only for their effector responses but also for their significant potential for TME

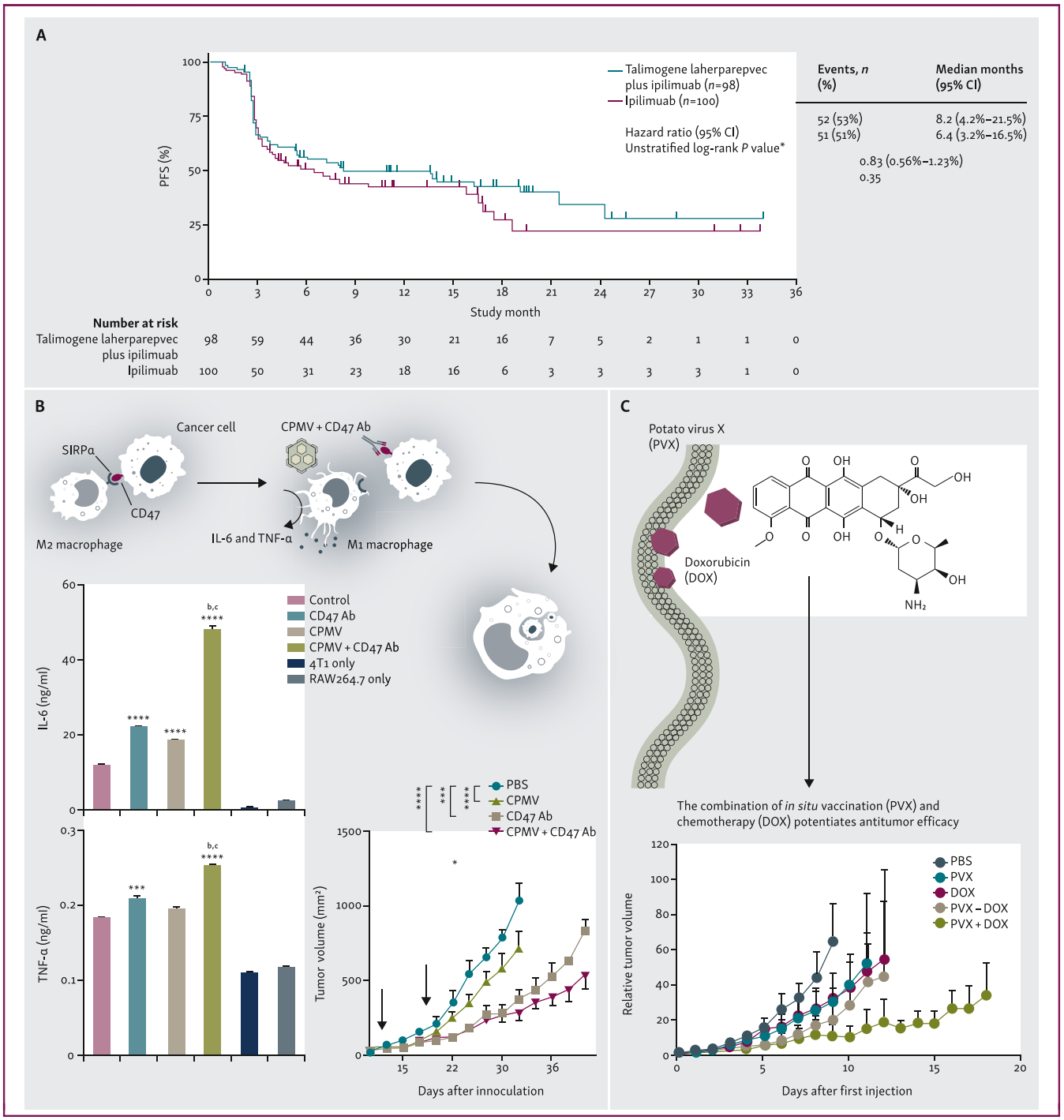

Figure 4. Engineered immunogenic viruses.
(A) Talimogene laherparepvec (T-VEC) combines with checkpoint blockade for improved therapeutic responses in melanoma patients.[97] (B) Cowpea mosaic virus (CPMV) nanoparticles (NPs) combine with CD47 blockade for enhanced efficacy in triple-negative breast cancer (TNBC).[103] (C) *In situ* vaccination with plant virus nanoparticle (NP) combines with chemotherapy to potentiate antitumor responses.[105] Ab, antibody; CI, confidence interval; IL, interleukin; TNF-α, tumor necrosis factor-α

remodeling due to their inherent proinflammatory role when activated. Besides CAR cell-based therapies, DCs have been tapped as cell-based therapies that harness their professional antigen-presenting function in cancer vaccination approaches.[111] Cell-based therapies face significant manufacturing and regulatory challenges that must be addressed before their wide-scale clinical application.[112] Additionally, these therapies are limited in that they often rely on cellular trafficking and tumor infiltration and CAR cells have been known to exhibit on-target off-tumor effects resulting in systemic inflammation (Figure 5).[113]

CAR-NK cells

NK cells are especially ideal candidates for CAR therapy since, like CTLs, they are direct killers of tumor cells, but unlike CTLs, they can promote a powerful proinflammatory cytokine response and TME remodeling for self-amplifying

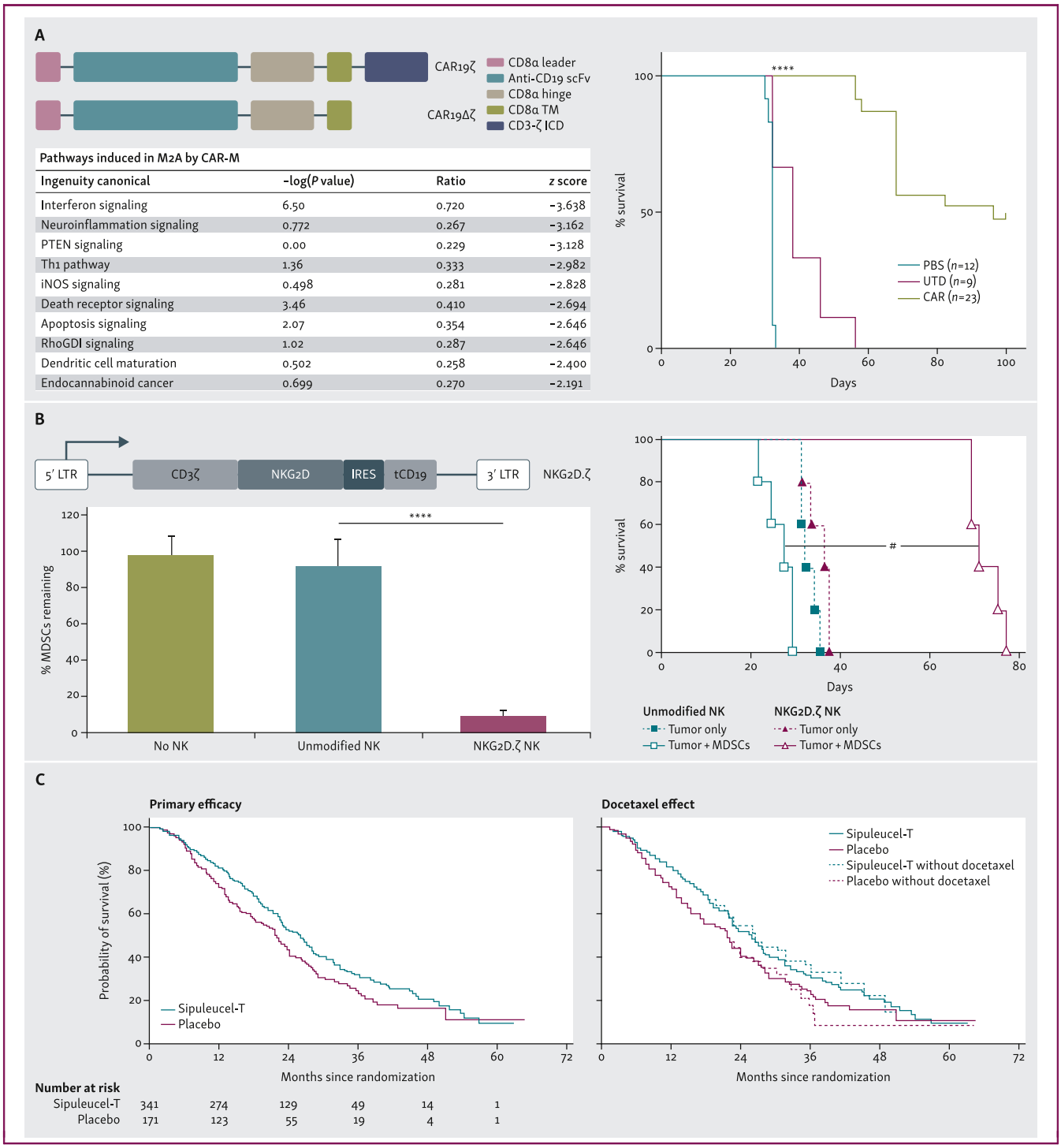

Figure 5. Engineered innate immune cells.
(A) Anti-human epidermal growth factor receptor 2 (HER2) chimeric antigen receptor (CAR) macrophages induce genes consistent with M1 phenotype and drive enhanced survival in an ovarian cancer model.[125] (B) CAR-natural killer (NK) cells containing activating NKG2D receptor eliminate myeloid-derived suppressor cells (MDSCs) and prolong survival in a neuroblastoma model.[117] (C) Sipuleucel-T (Provenge) demonstrates 4-month prolonged median survival in phase III clinical trial.[130] PBS, phosphate-buffered saline; UTD, untransduced.

and long-lasting antitumor immunity.[114] The 4-1BB or CD28 CAR first used in T cells was shown to also be an effective CAR for NK cells in the treatment of T-cell malignancies.[115] Further, the addition of 2B4 or CD244, a well-characterized NK-specific costimulatory domain, to this CAR showed many improved features compared to the original construct, including improved cytotoxicity, rapid proliferation, augmented cytokine release, and decreased apoptosis.[115] Epidermal growth factor receptor (EGFR) CAR-NK cells have been shown to be highly effective in the treatment of TNBC.[116] TNBC cells are known to up-regulate EGFR significantly as these cancers advance and EGFR CAR-NK cells have been shown to trigger specific lysis of TNBC cells both *in vitro* and in mouse models. In follow-up studies with

patient-derived xenograft mouse models of breast cancer, EGFR CAR-NK cell treatment mediated significant improvement in survival and delay in tumor growth and CAR-NK cells were found in the tumor core region for 7 days, strongly suggesting that they were driving long-term TME remodeling.[116] In proof-of-concept studies, NK cells were engineered with a CAR receptor consisting of the activating NKG2D receptor fused to the cytotoxic ζ-chain of the T-cell receptor (TCR) (Figure 5B).[117] These CAR-NK cells secreted proinflammatory cytokines in response to MDSCs in the TME and significantly improved infiltration and antitumor activity of subsequently infused CAR T cells, suggesting that the combination of CAR-NK and CAR T cells may be very promising potential therapies. Clinical studies have appeared to corroborate these findings since CAR-NK cells generated from pediatric neuroblastoma patients were shown to be capable of killing autologous MDSCs, which are known to suppress CAR T function in the TME.[118,119]

CAR macrophages

Since macrophages are central regulators and effectors of innate immunity and highly capable of phagocytosis, cytotoxicity, proinflammatory cytokine secretion, and antigen presentation, they have potential to be powerful effectors as the focus of cell-based therapies.[120] Several macrophage-based therapies geared toward polarizing existing M2 macrophages in the TME toward the immunostimulatory M1 phenotype are currently in clinical development, but are confounded since tumor-associated macrophages express both activating and inhibitory Fc receptors.[121,122] CAR macrophages are unique CAR candidates because they are professional APCs that play a key role in promoting adaptive antitumor immune responses and also because CAR macrophages, by definition, bear the M1 phenotype that is a potent driver of TME remodeling. Due to their relative lack of plasticity compared to non-transduced macrophages, CAR macrophages have the potential to strongly address the conundrum faced by many tumor macrophage polarization strategies that attempt to switch macrophage phenotypes from M2 to M1.[123-125]

Initial clinical attempts at adoptive transfer of autologous macrophages into solid tumors demonstrated that infusion of cells was safe and feasible, but these efforts have crucially failed to show significant antitumor effects.[126,127] In 2020, the first CAR macrophage therapy was developed and tested as a systemically delivered antitumor therapy (Figure 5A).[125] Human epidermal growth factor receptor 2 (HER2) CAR macrophages were systemically administered in multiple human solid tumor xenograft mouse models. Despite eventual cancer progression, a single CAR macrophage infusion extended overall survival and led to tumor regression in the majority of treated mice. Additionally, transduction of macrophages with the Ad5f36 vector induced several IFN-associated genes consistent with an M1 phenotype, which was confirmed to be maintained for at least 40 days post-infusion, representing long-term TME remodeling. CAR macrophages are being currently tested in clinical trials.[128] CAR macrophages have also been developed from induced pluripotent stem cells and have been shown to be capable of antigen-specific expression and secretion of cytokines as well as polarization toward an M1 phenotype and enhanced antitumor activity in mouse models of ovarian cancer.[129]

DC therapy

DCs have been shown to be highly skilled and communicative sensors of microbes, and are linked tightly to their environment through a plethora of molecular sensors. The goal of DC therapies is ultimately to induce tumor-specific effector CTLs, which orchestrate tumor clearance. To achieve this, DCs can be stimulated or engineered with tumor-specific antigens either *ex vivo* (using DCs derived from patients with an adjuvant) or *in vivo* (by inducing uptake of the antigen). Sipuleucel-T is an *ex vivo* approach using autologous DCs stimulated with a fusion protein composed of prostate cancer antigen linked to GM-CSF. Activated DCs are injected back to the patient intravenously. Sipuleucel-T was approved by the FDA in 2011 for treatment of metastatic castration-resistant prostate cancer, but has demonstrated only a 4-month prolonged median survival in phase III trials (Figure 5C).[130] High cost and lack of identification of optimal biomarkers are among the limiting factors in application of this therapy.[131] Despite some initial success, further development and testing of DC vaccines have produced limited results in the clinic. Although these DC vaccines are capable of generating strong immunological responses, they have failed to show true clinical benefit. In hindsight, it is appreciated that approaches must be engineered to break down both local and systemic tumor immunosuppressive barriers to enable DC-activated CTLs to infiltrate tumors.

CONCLUSIONS

Taken together, engineering approaches provide critical design capabilities that address the critical delivery and efficacy gap faced by current TME remodeling strategies. Key limitations exist for each of the strategies we discuss here, including serious off-target effects for cytokine and innate immune approaches, unpredictable pathogenicity for immunogenic viruses that is as-yet uncharacterized, and manufacturing and regulatory challenges for cell-based approaches. Future directions will need to center extensively on rational engineering approaches for the design of novel delivery approaches for cytokines, innate immune agonists, immunogenic viruses, and whole cells to help overcome these limitations and increase their translational potential and clinical efficacy.

DISCLOSURE

The authors have declared no conflicts of interest.

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