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Review

In situ programming of unconventional T cells

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Unconventional T cells (UTCs), including γδ T cells, mucosal-associated invariant T cells, and natural killer T cells, recognize conserved nonpeptide antigens through semi-invariant T cell receptors in an human leukocyte antigen-independent manner, making them attractive candidates for broadly applicable immunotherapies. However, their clinical translation remains limited by poor spatiotemporal control of activation, functional exhaustion, and insufficient tissue homing *in vivo*, particularly in solid tumors. Biomaterial-based delivery systems provide a promising strategy to overcome these barriers by enabling localized and sustained delivery of UTC ligands, cytokines, and nucleic acids within diseased tissues. Through precise *in situ* programming of UTC responses, these platforms may enhance therapeutic efficacy and safety. This review summarizes current biomaterial-based strategies and discusses emerging opportunities for *in situ* UTC programming in human cancers.

Highlights

Unconventional T cells recognize tumors independently of classical major histocompatibility complex molecules, making them attractive for broadly applicable cancer immunotherapy.

Biomaterial platforms convert short-lived soluble ligands into localized, sustained signals that enhance unconventional T cell activation while limiting systemic toxicity and functional exhaustion.

Lipid-, polymer-, and hybrid-based delivery systems program unconventional T cells through the codelivery of agonists, cytokines, antigens, or nucleic acids.

Recent studies show that both dendritic cell-targeted vaccination and direct activation of unconventional T cells amplify antitumor immunity through coordinated innate and adaptive immune responses.

Tissue-directed biomaterial strategies are emerging to improve unconventional T cell trafficking, persistence, and therapeutic efficacy in solid tumors.

Unique MHC-independent recognition of conserved antigens enables *in situ* programming of unconventional T cells

Adoptive cellular immunotherapy has transformed modern oncology through the clinical success of chimeric antigen receptor (CAR)-T cells and engineered T cell receptor (TCR)-based therapies. However, these approaches primarily rely on conventional αβ T cells, whose antigen recognition is strictly dependent on peptide presentation by highly polymorphic major histocompatibility complex (MHC) molecules [1–4]. This MHC restriction imposes several major therapeutic limitations. First, the polymorphic nature of human leukocyte antigen (HLA) molecules creates significant barriers for **allogeneic** (see Glossary) applications, as donor-derived αβ T cells can recognize recipient tissues as foreign and induce **graft-versus-host disease (GvHD)** [5–7]. Consequently, all currently FDA-approved **CAR-T cell** products require individualized **autologous manufacturing**, which is associated with high production costs, prolonged manufacturing timelines, and inconsistent product quality in heavily pretreated patients. Second, tumor immune escape frequently occurs through transcriptional downregulation or genetic loss of MHC molecules or β_2 microglobulin within the tumor microenvironment (TME), rendering malignant cells invisible to conventional T cell surveillance [8–10]. Together, these limitations have driven increasing interest in alternative immune cell populations capable of overcoming MHC dependence.

Unconventional T cells (UTCs), including γδ T cells, invariant natural killer T (iNKT) cells, and mucosal-associated invariant T (MAIT) cells, represent a distinct class of lymphocytes that bridge innate and adaptive immunity [11–16]. Unlike conventional αβ T cells, UTCs recognize highly conserved, nonpeptide antigens through invariant or semi-invariant TCRs in an MHC-independent manner. Human iNKT cells express a restricted invariant TCR—typically Vα24-Jα18 TCRα chain paired with Vβ11 TCRβ chain—that recognizes endogenous and exogenous glycolipid antigens presented by the monomorphic MHC class I-like molecule cluster of differentiation 1d (CD1d) [17,18]. MAIT cells similarly express semi-invariant TCRs that recognize microbial riboflavin metabolite derivatives, including 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU), presented by the nonpolymorphic major histocompatibility complex, class I-related (MR1) molecule [19]. In contrast, the dominant

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human gamma delta T cell receptor variant 9 delta 2 (Vγ9Vδ2) γδ T cell subset recognizes phosphoantigens, such as microbial (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) or tumor-associated **isopentenyl pyrophosphate (IPP)**, through **butyrophilin** family proteins butyrophilin subfamily 2 member a1 (BTN2A1) and butyrophilin subfamily 3 member a1 (BTN3A1) without requiring classical antigen presentation [20–23].

Beyond their allogeneic compatibility [24–27], UTCs possess several biological features that are highly advantageous for cancer immunotherapy. UTCs rapidly produce inflammatory cytokines, cytotoxic granules, and chemokines upon activation, enabling immediate effector responses against infected or transformed cells. Importantly, the ligands recognized by UTCs are frequently induced by cellular stress, such as upregulation of stress-associated ligands on transformed or damaged cells; microbial metabolism, including microbially derived vitamin B metabolites presented by MR1 to MAIT cells and phosphoantigens produced through microbial isoprenoid biosynthesis that are recognized by Vγ9Vδ2 T cells; or oncogenic dysregulation, in which tumor-associated metabolic reprogramming generates activating ligands [28–30]. For example, dysregulated **mevalonate** metabolism in tumor cells leads to the intracellular accumulation of IPP, thereby sensitizing malignant cells to Vγ9Vδ2 T cell recognition [31,32]. Similarly, iNKT and MAIT cells can coordinate broader immune responses through interactions with **dendritic cells (DCs)**, **macrophages**, NK cells, and conventional T cells. These innate-like properties enable UTCs not only to directly eliminate tumor cells but also to reshape the immunosuppressive TME and amplify endogenous antitumor immunity.

Despite these advantages, clinical translation of UTC-targeted therapies has been limited by the inability to precisely control activation *in vivo*. Early strategies relying on systemic administration of soluble UTC agonists, including **α-galactosylceramide (α-GalCer)**, phosphoantigen-inducing **aminobisphosphonates**, and synthetic MAIT agonists, have generally demonstrated limited therapeutic efficacy [33–36]. A major challenge is the lack of spatiotemporal control over ligand exposure. Systemic delivery often results in rapid ligand clearance, poor tissue retention, and insufficient accumulation within tumors or lymphoid tissues [19,37,38]. Conversely, excessive systemic activation can induce cytokine-mediated toxicity, cellular exhaustion, or long-term **anergy**, particularly in iNKT cells [39–41]. Furthermore, effective UTC activation frequently requires coordinated antigen presentation by antigen-presenting cells (APCs), including DCs and macrophages, through CD1d, MR1, or butyrophilin-dependent pathways. These requirements highlight the need for localized and sustained delivery strategies capable of precisely orchestrating UTC activation within diseased tissues.

Biomaterial-based delivery systems have emerged as a promising approach to address these challenges and enable *in situ* programming of UTCs. Engineered nanoparticles, liposomes, hydrogels, and polymeric matrices can provide controlled release of UTC ligands, cytokines, nucleic acids, and co-stimulatory signals while simultaneously targeting APCs or the TME [42,43]. By regulating ligand pharmacokinetics and spatial distribution, biomaterials can sustain UTC activation while minimizing systemic toxicity and functional exhaustion. In addition, these systems allow combinatorial delivery of cytokines, mRNA, and immunomodulatory molecules to enhance UTC expansion, persistence, and antitumor function. In this review, we summarize emerging biomaterial platforms for localized UTC activation and highlight recent case studies involving γδ T cells, iNKT cells, and MAIT cells. Finally, we discuss future opportunities for integrating biomaterials, synthetic immunology, and UTC engineering to develop next-generation universal immunotherapies.

Biomaterials for *in situ* programming of UTCs

Design principles

The central rationale for biomaterial-enabled *in situ* programming of UTCs is to convert short-lived, systemically dispersed immune stimuli into localized, sustained, and instructive signals for

Glossary

α-Galactosylceramide (α-GalCer): potent synthetic glycolipid antigen presented by CD1d that specifically activates iNKT cells and induces rapid cytokine production.
Alendronate: nitrogen-containing bisphosphonate that inhibits the mevalonate pathway, causing intracellular phosphoantigen accumulation, which activates Vγ9Vδ2 T cells through BTN3A1-dependent sensing.
Allogeneic: treatment made from cells donated by a healthy person rather than from the patient's own cells.
Aminobisphosphonates: class of bone-targeting drugs, such as ZA, that inhibit the mevalonate pathway and induce intracellular phosphoantigen accumulation, thereby activating Vγ9Vδ2 γδ T cells.
Anergy: hyporesponsive functional state in which immune cells remain alive but fail to respond effectively to antigenic stimulation following excessive or inappropriate activation.
Autologous manufacturing: process of making a personalized cell therapy using a patient's own immune cells.
Bispecific T-cell engager (BiTE): dual-targeting antibody construct that simultaneously binds a tumor-associated antigen and T cell markers, such as CD3, to redirect and activate T cell-mediated cytotoxicity against cancer cells.
Butyrophilins: a family of immunoregulatory cell-surface proteins, including BTN2A1 and BTN3A1, that mediate phosphoantigen sensing and activation of γδ T cells.
CAR-T cells: immune cells that have been genetically modified to recognize and destroy cancer cells more effectively.
Dendritic cells (DCs): specialized immune cells that help activate the body's immune system by showing it what to attack, such as cancer cells or infections.
Graft-versus-host disease (GvHD): potentially severe immune complication in which donor immune cells attack healthy recipient tissues after allogeneic transplantation or cell therapy.
Isopentenyl pyrophosphate (IPP): intermediate metabolite of the mevalonate pathway that accumulates in stressed or transformed cells and serves as an endogenous phosphoantigen recognized by Vγ9Vδ2 γδ T cells.

tissue-resident or recruited UTCs (Figure 1). Unlike adoptive cell therapy, in which cells are expanded, genetically modified, and phenotypically shaped *ex vivo*, *in situ* programming depends on the ability of a material platform to organize immune cues directly within the host. For UTCs, this requirement is particularly stringent because their activation is determined not by ligand availability alone, but by the coordinated presentation of conserved antigens, inflammatory cytokines, co-stimulatory signals, and tissue-contextual cues by APCs or tumor-associated compartments [13,44]. Therefore, the design of biomaterials for UTC programming should be guided by three interrelated principles: controlling release kinetics, enabling multi-cargo codelivery, and directing payloads to the appropriate cellular and anatomical targets [45].

Controlled release kinetics are a foundational design parameter for biomaterial-enabled UTC programming. Many canonical UTC agonists, including phosphoantigens for Vγ9Vδ2 T cells [22], α-GalCer for iNKT cells [46], and MR1 ligands or precursors for MAIT cells [47], have limited *in vivo* persistence when administered as soluble agents. Rapid systemic dilution, enzymatic degradation, hepatic and renal clearance, and nonspecific distribution can all reduce the probability that these ligands will reach APCs in a form compatible with productive antigen presentation. Biomaterials, including hydrogels and other tissue-adhesive interfaces, have demonstrated the ability to establish local depots or durable adhesive niches that prolong therapeutic residence at target tissues [48,49]. For UTC programming, such retention may convert transient agonist exposure into sustained APC presentation while reducing peak systemic concentrations. This control is important because excessive bolus stimulation can induce counterproductive outcomes, including cytokine toxicity, activation-induced cell death, or functional anergy, whereas insufficient exposure may fail to expand or polarize the desired effector population. For example, α-GalCer-adjuvanted vaccine delivery through biomaterial-based microdevices shows how spatially restricted release can support mucosal immune activation while avoiding purely soluble administration of lipid agonists [50].

Beyond release kinetics, effective UTC programming also depends on coordinated codelivery of complementary immunological cues. TCR ligands can define subset-selective activation through CD1d-, MR1-, or BTN-dependent presentation, whereas cytokines such as interleukin-2 (IL-2), IL-12, and IL-15 can shape proliferation, cytotoxicity, survival, and effector polarization. IL-2 may support expansion but also risks nonspecific lymphocyte activation [51]; IL-12 can promote type 1 inflammatory programming and enhance interferon gamma (IFN-γ) production [52]; and IL-15 is attractive for sustaining cytotoxic lymphocytes without the same degree of terminal differentiation associated with repeated strong stimulation [53–55]. Biomaterials make it possible to adjust the stoichiometry, spatial proximity, and release order of these signals. For instance, a platform may rapidly release a UTC ligand to promote APC loading or intracellular ligand accumulation, followed by slower cytokine release to sustain UTC expansion and function. Alternatively, cytokines can be immobilized or encapsulated to restrict activity to a tumor or lymphoid niche [56].

Taken together, biomaterials for *in situ* UTC programming should be viewed as programmable immune niches. Their purpose is to choreograph when, where, and in which combinations UTC-relevant signals are encountered (Figure 1 and Box 1). By integrating controlled release, multi-signal codelivery, and selective targeting, these platforms can provide spatially localized, temporally sustained, and coordinated immune cues while mitigating the pharmacokinetic and toxicological limitations of soluble agonists. This framework also provides a basis for comparing lipid-based systems, polymeric carriers, and hybrid platforms, which differ in cargo loading, release control, endosomal escape, tissue retention, and tissue tropism.

Lipopolyplex: hybrid nucleic acid delivery system composed of lipids and polymer–nucleic acid complexes designed to enhance cellular uptake, stability, and intracellular delivery of therapeutic cargoes such as mRNA and DNA.
Macrophages: immune cells that engulf and remove harmful microbes, damaged cells, and cellular debris while also helping coordinate immune responses.
Mevalonate: key intermediate in the metabolic pathway responsible for cholesterol and isoprenoid biosynthesis, generating phosphoantigens recognized by Vγ9Vδ2 T cells.
Polyethyleneimine: cationic polymer widely used for nucleic acid delivery due to its high buffering capacity and its ability to facilitate cellular uptake and endosomal escape of therapeutic cargo.
Viscoelasticity: material property describing the combination of elastic, solid-like behavior and viscous, fluid-like deformation in response to mechanical stress over time.
Zoledronate acid (ZA): potent nitrogen-containing bisphosphonate that promotes phosphoantigen accumulation via mevalonate pathway inhibition, thereby stimulating Vγ9Vδ2 T cell activation and antitumor immune responses.

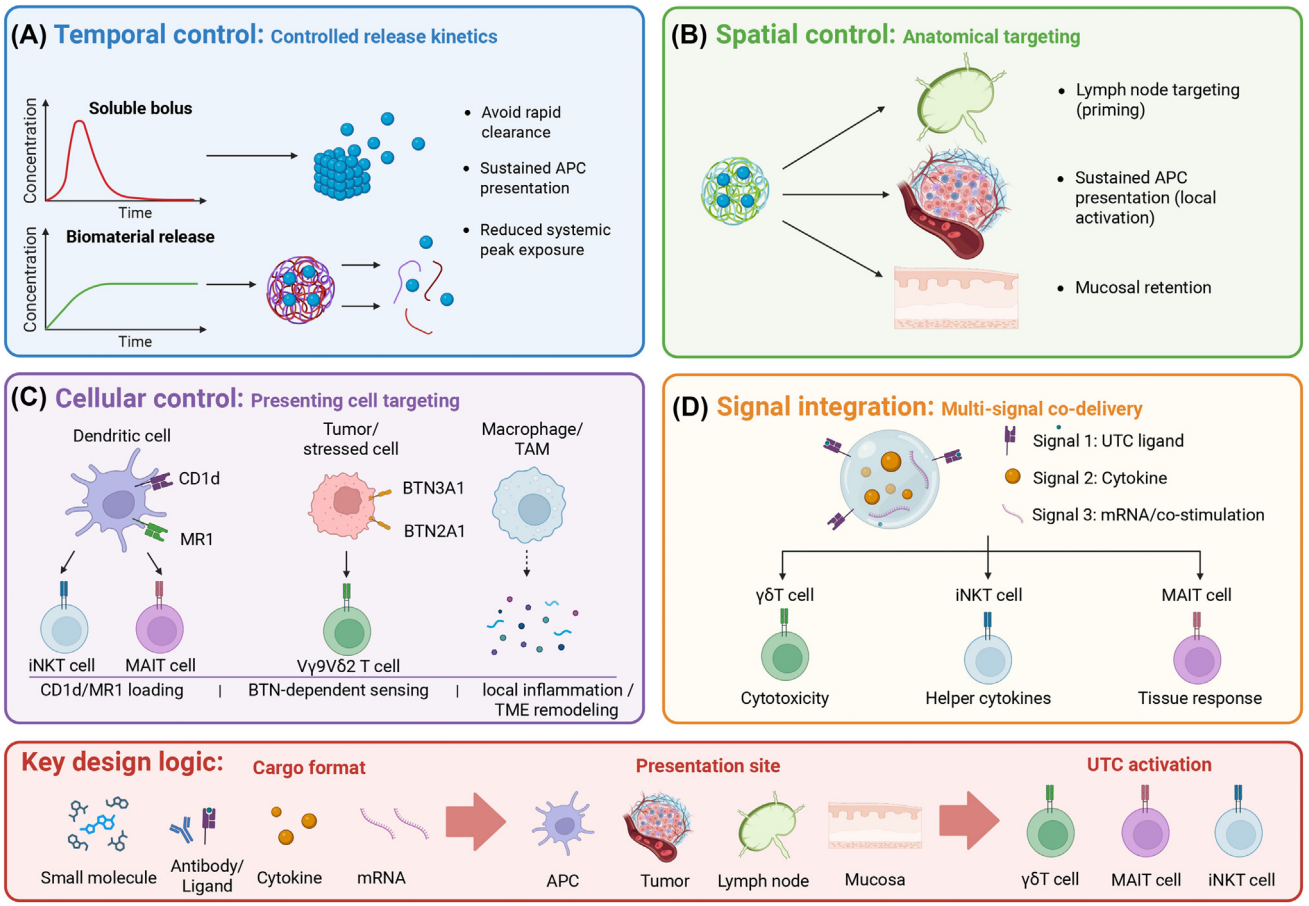

Figure 1. Design principles for biomaterial-enabled *in situ* programming of UTCs. Biomaterials can coordinate UTC activation by controlling the timing, location, presentation compartment, and combination of delivered immune cues. (A) Controlled release prolongs local exposure to short-lived UTC agonists, sustains APC presentation, and reduces peak systemic exposure compared with soluble bolus administration. (B) Anatomical targeting directs cargoes to lymph nodes, tumors, or mucosal tissues to support priming, local activation, or engagement of tissue-resident UTCs. (C) Cellular targeting aligns cargo delivery with UTC-specific recognition pathways, including CD1d- and MR1-dependent activation of NKT and MAIT cells by dendritic cells, BTN2A1/BTN3A1-dependent sensing by Vγ9Vδ2 T cells, and macrophage-mediated modulation of local inflammation. (D) Multi-signal codelivery integrates UTC ligands, cytokines, and nucleic acid or co-stimulatory cues to promote cytotoxic, helper, and tissue-resident UTC responses. The bottom schematic summarizes the design logic linking cargo format, presentation site, and UTC activation. APC: antigen-presenting cell; BTN2A1: butyrophilin subfamily 2 member A1; BTN3A1: butyrophilin subfamily 3 member A1; CD1d: cluster of differentiation 1d; iNKT: invariant natural killer T; MAIT: mucosal-associated invariant T; MR1: major histocompatibility complex class I-related gene protein; TAM: tumor-associated macrophage; UTCs: unconventional T cells; Vγ9Vδ2: gamma delta T cell receptor variant 9 delta 2.

Lipid-based systems

Lipid-based delivery systems are among the most clinically advanced biomaterial platforms for immune modulation and therefore represent an attractive strategy for *in situ* programming of UTCs (Figure 2A) [63]. Liposomes and lipid nanoparticles (LNPs) can encapsulate or associate with structurally diverse cargoes, including hydrophobic lipid antigens, hydrophilic small molecules, proteins, and nucleic acids.

Liposomes provide a useful platform for UTC ligand delivery because their aqueous core and lipid bilayer allow simultaneous loading of hydrophilic and hydrophobic cargoes [64]. For example, aminobisphosphonates, such as **alendronate** and **zoledronate**, can be encapsulated within liposomes to enhance intracellular accumulation in APCs or tumor cells [65]. These drugs inhibit

Box 1. Advanced targeting strategies and nucleic acid-enabled immune programming for *in situ* UTC activation

Nucleic acid cargo expands UTC programming by enabling local APC reprogramming. Messenger RNA can encode tumor antigens, cytokines, co-stimulatory ligands, chemokines, or synthetic immunomodulators, thereby coupling antigen presentation with UTC-mediated adjuvanticity. A nanovaccine co-loaded with mRNA and α-GalCer, for example, can engage conventional T cells through an mRNA-encoded antigen while simultaneously activating NKT cells through CD1d-mediated lipid presentation, thereby linking antigen-specific adaptive immunity with UTC-driven helper and inflammatory functions [57]. This principle is beginning to extend to other UTC subsets, as illustrated by mRNA nanovaccines incorporating DC and MAIT cell activators [58]. Similar codelivery logic could be explored for γδ T cell programming by pairing phosphoantigen-generating cues with co-stimulatory or cytokine-encoding mRNAs. Codelivery of these cargoes in the same particle, APC, or tissue niche promotes signal integration and coordinated immune programming.

Targeting is equally important because UTC ligands often require specialized presentation by APCs or disease-associated tissue compartments such as tumor-associated macrophages. Effective targeting requires coordination of anatomical, cellular, and molecular design features. Particle size can be tuned to favor lymphatic trafficking; surface ligands, such as mannose, can enhance uptake by APCs [59]; and stimuli-responsive or bioorthogonally cleavable linkers can restrict cargo release to defined biochemical or externally triggered microenvironments [60]. The anatomical target should match the intended immunological outcome: lymph-node-targeted delivery may be preferable for priming and expansion, whereas tumor-localized delivery may be better suited for overcoming immune suppression, recruiting effector cells, and amplifying local cytotoxicity.

The TME is a particularly important target because it contains suppressive myeloid cells, abnormal vasculature, hypoxia, metabolic stress, and inhibitory cytokines. Biomaterials can be engineered not only to deliver activating signals but also to remodel this local ecology. For example, particles can codeliver UTC ligands with agents that repolarize macrophages, block checkpoint pathways, normalize extracellular matrix barriers, or induce immunogenic tumor cell death. Such systems may allow UTCs to serve as both direct effectors and immune amplifiers, a concept supported by evidence that γδ T cells can mediate antitumor immunity even in HLA-I-defective tumors [61]. However, this strategy requires careful calibration. UTCs are highly responsive cells, and uncontrolled activation in suppressive or inflammatory tissues may cause exhaustion, bystander damage, or systemic cytokine release. Thus, safety should be built into material design through localized administration, biodegradable carriers, tunable degradation rates, and, where possible, tissue- or cell-specific release mechanisms [62].

the mevalonate pathway and promote endogenous phosphoantigen accumulation, thereby activating Vγ9Vδ2 T cells through the BTN2A1/BTN3A1 axis [66,67]. Importantly, this mechanism illustrates that many biomaterial strategies do not directly stimulate UTCs in isolation. Instead, they program accessory cells or diseased tissues to become more effective UTC activators. Compared with soluble agonists, liposomal delivery can improve circulation time, reduce systemic exposure, and enhance uptake by phagocytic APCs, which may help localize γδ T cell activation and reduce systemic inflammatory toxicity [68].

LNPs have further expanded the potential of lipid-based immunoengineering by enabling efficient *in vivo* delivery of mRNA and other nucleic acids, an approach that has recently demonstrated clinical feasibility for CAR gene delivery to conventional T cells [63]. In the context of UTC programming, LNPs can deliver mRNA encoding cytokines, co-stimulatory ligands, antigen-presenting molecules, or other immunomodulatory factors that reshape the local immune niche. LNPs may also be used for receptor engineering, including transient delivery of CAR-encoding mRNA to generate CAR-UTC responses *in situ* [69]. However, this application should be viewed as an emerging and technically challenging direction rather than an established strategy. Efficient *in vivo* CAR-UTC programming would require selective delivery to rare UTC populations, sufficient transfection efficiency, controlled receptor expression, and minimal off-target gene delivery to hepatocytes, myeloid cells, or conventional T cells.

Despite these advantages, lipid-based systems also have important limitations [70]. Many LNP formulations show liver-biased biodistribution, which may be advantageous for hepatic malignancies or infections but less suitable for tumors in other tissues without additional targeting strategies. Lipid particles can also trigger innate immune sensing, complement activation, or

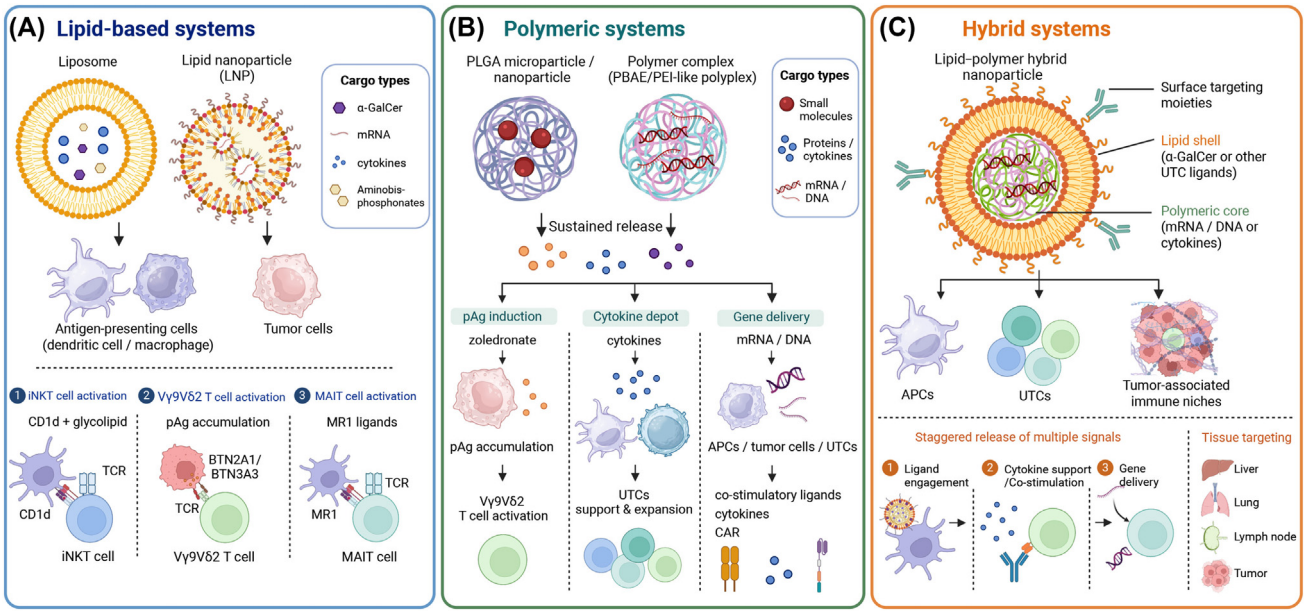

Figure 2. Biomaterial platforms for *in situ* programming of UTCs. (A) Lipid-based systems, including liposomes and LNPs, enable delivery of hydrophobic and hydrophilic cargo such as α-GalCer, aminobisphosphonates, proteins, and nucleic acids. These systems promote UTC activation indirectly through APCs or tumor cells, including CD1d-mediated activation of iNKT cells, BTN2A1/BTN3A1-dependent activation of Vγ9Vδ2 T cells, and MR1-mediated activation of MAIT cells. LNPs also support mRNA delivery for cytokine expression or transient CAR engineering. (B) Polymeric systems, including PLGA particles and cationic polymer complexes, provide sustained release of small molecules, cytokines, and nucleic acids to enhance UTC activation, expansion, and effector function. Polymeric platforms may also support *in situ* CAR-UTC programming through gene delivery. (C) Hybrid lipid–polymer systems integrate controlled release, ligand presentation, and tissue targeting to coordinate UTC activation with cytokine support, gene delivery, and local immune modulation. α-GalCer: α-galactosylceramide; APCs: antigen-presenting cells; BTN2A1: butyrophilin subfamily 2 member A1; BTN3A1: butyrophilin subfamily 3 member A1; CAR: chimeric antigen receptor; CD1d: cluster of differentiation 1d; iNKT: invariant natural killer T; LNPs: lipid nanoparticles; MAIT: mucosal-associated invariant T; MR1: major histocompatibility complex class I-related gene protein; pAg: phosphoantigen; PBAE: poly(beta-amino ester); PEI: polyethylenimine; PLGA: poly(lactic-co-glycolic acid); TCR: T cell receptor; UTCs: unconventional T cells; Vγ9Vδ2: gamma delta T cell receptor variant 9 delta 2.

inflammatory toxicity depending on their composition [71]. In addition, lipid systems may have limited control over long-term release kinetics compared with polymeric depots [72]. Therefore, future lipid-based UTC programming platforms should be designed with careful attention to tissue tropism, cargo selection, ligand presentation, and the cellular target responsible for initiating UTC activation.

Polymeric systems

Polymeric biomaterials offer complementary advantages over lipid-based systems, particularly in sustained release, tunable degradation, and structural flexibility [73]. For *in situ* UTC programming, polymeric systems can be broadly divided into platforms for small-molecule drug delivery, protein or cytokine delivery, and gene delivery (Figure 2B and Box 2).

Biodegradable polymers such as poly(lactic-*co*-glycolic acid) (PLGA) are widely used for controlled release of small molecules and proteins. In the context of γδ T cell programming, PLGA-based nanoparticles or microparticles can encapsulate aminobisphosphonates such as zoledronate acid (ZA) [85]. These drugs do not function as classical extracellular TCR ligands. Instead, they act intracellularly in APCs or tumor cells to induce phosphoantigen accumulation, which is subsequently sensed by Vγ9Vδ2 T cells through BTN-mediated recognition [85]. Polymeric encapsulation may improve local retention, reduce systemic toxicity, and provide sustained stimulation compared with soluble

Box 2. Polymeric gene delivery strategies for *in situ* CAR engineering of UTCs

In addition to drug and protein delivery, cationic polymers have emerged as versatile platforms for gene transfer and transient receptor engineering. Materials such as poly(β-amino esters) and **polyethyleneimine** derivatives can complex with mRNA, plasmid DNA, or siRNA through electrostatic interactions and promote endosomal escape through proton-buffering or membrane-disruptive mechanisms [74]. For UTC programming, polymeric gene delivery could be used in several mechanistically distinct ways. Delivery to APCs could induce local expression of cytokines, co-stimulatory ligands, or antigen-presenting machinery. Delivery to tumor cells could increase local immune stimulation or susceptibility to UTC-mediated killing [75]. Delivery to UTCs themselves could support transient receptor engineering, including CAR expression, or enhance survival, trafficking, and effector function.

Importantly, recent clinical studies have demonstrated the feasibility of *in vivo* CAR gene delivery using targeted viral vectors and RNA LNPs, validating *in situ* immune cell engineering as a therapeutic strategy [76–82]. Similar approaches could potentially be adapted for *in situ* CAR-UTC generation by delivering CAR-encoding mRNA or DNA directly to endogenous UTCs or to adoptively transferred UTCs after infusion. This approach could combine the innate-like effector function, tissue surveillance, and reduced alloreactivity of UTCs with the antigen specificity of CAR engineering. Although *in vivo* CAR engineering of UTCs has not yet been demonstrated, the underlying delivery technologies are highly modular and could, in principle, be retargeted to UTC-specific surface receptors. However, successful CAR-UTC programming would require precise control over cellular tropism, transfection efficiency, receptor expression duration, tonic signaling, and inflammatory toxicity. Because UTCs are relatively rare compared with conventional T cells and myeloid cells, achieving selective *in vivo* gene delivery remains a major barrier.

Polymeric platforms also have material-specific limitations. Cationic polymers may cause membrane damage, cytotoxicity, or nonspecific inflammation if not carefully optimized. PLGA and related systems can exhibit burst release, incomplete cargo release, or variable degradation depending on the polymer composition and formulation conditions. Hydrophobic UTC ligands may also be difficult to incorporate into certain polymeric matrices without compromising release or bioactivity. Therefore, polymeric systems are most promising when their degradation kinetics, cargo compatibility, and target-cell uptake are matched to the biology of the intended UTC subset. Future studies could further leverage the mechanical properties of polymeric nanoparticles and microparticles, including stiffness, **viscoelasticity**, and deformability, to optimize UTC activation by modulating particle–cell interactions, uptake, antigen presentation, and downstream immune signaling [83,84].

bisphosphonate administration [86–88]. These features are particularly important because excessive or poorly controlled γδ T cell activation can lead to inflammatory toxicity, whereas insufficient delivery may fail to generate meaningful antitumor immunity [89].

Polymeric systems are also attractive for cytokine and protein delivery. UTC activation often requires more than TCR stimulation alone; cytokines such as IL-2, IL-12, IL-15, and IL-18 can strongly influence expansion, cytotoxicity, tissue residency, and memory-like function [90,91]. Polymer-based depots can localize cytokines to tumors, lymphoid tissues, or mucosal sites, thereby increasing local immune potency while reducing systemic cytokine-associated toxicity. This approach may be especially useful for iNKT and MAIT cells, which can rapidly produce cytokines and amplify broader innate and adaptive immune responses after activation. However, because UTCs are sensitive to both overstimulation and anergy, cytokine release kinetics must be carefully controlled rather than simply maximized [37].

Hybrid systems

Hybrid biomaterial systems combine the strengths of lipid-based and polymeric platforms and may be especially powerful for UTC programming, where effective immune activation often requires coordinated delivery of multiple signals (Figure 2C) [92]. A major limitation of single-material systems is that one platform may not optimally deliver all required cargoes. For example, lipid antigens may be best incorporated into lipid membranes, whereas hydrophilic drugs, proteins, or nucleic acids may require separate compartments or release profiles. Hybrid systems can address this issue by integrating lipid and polymer components into one multifunctional delivery platform [93].

Lipid–polymer hybrid nanoparticles typically contain a polymeric core and a lipid shell, combining the structural stability and tunable release properties of polymeric nanoparticles with the

biocompatibility and membrane-interactive features of lipid systems [92]. The polymeric core can provide mechanical stability and sustained release of small molecules, proteins, or nucleic acids, while the lipid shell can improve biocompatibility, enhance membrane interaction, and enable incorporation of lipid antigens. This architecture is well aligned with the needs of UTC programming.

Hybrid systems may also support gene delivery-based UTC programming by integrating receptor engineering with controlled immune activation [94]. Cell-specific targeting can be further enhanced by displaying ligands, antibodies, or other receptor-binding moieties that have been successfully used to direct gene vectors toward DCs, macrophages, cancer-associated fibroblasts, and other cells within the TME [95–98]. Similar targeting strategies could be adapted for selective delivery to UTC subsets. In this design, gene delivery is not used as a standalone intervention but is coordinated with ligand presentation and local immune stimulation, allowing engineered antigen recognition to be coupled with the innate-like effector functions, tissue residency, and rapid cytokine responses that distinguish UTCs from conventional T cells [99].

Case studies: *in situ* programming of specific UTC subsets

iNKT cells

Biomaterial-based strategies for iNKT cell activation have primarily focused on improving the delivery of α-GalCer, while the clinical efficacy of free α-GalCer has remained limited due to several pharmacological and immunological barriers. For α-GalCer to activate iNKT cells, it must first be taken up by APCs and presented on their surfaces by the protein CD1d. However, free α-GalCer is poorly delivered to APCs and rapidly disperses throughout the body after administration, limiting its effectiveness [37]. Moreover, systemic exposure to free α-GalCer induces an initial burst of cytokine production followed by prolonged functional hyporesponsiveness, or anergy, in iNKT cells, thereby limiting sustained antitumor efficacy [39].

Targeted codelivery of tumor antigens and α-GalCer to cross-presenting DCs can enhance iNKT activation and promote robust antitumor immunity through efficient cytotoxic T lymphocyte (CTL) priming. Li and colleagues developed a PLGA nanovaccine encapsulating ovalbumin (OVA) and PBS-44, an α-GalCer analog, which induced stronger and more sustained iNKT activation than soluble formulations, together with enhanced OVA-specific T cell responses and IFN-γ production [100]. Intranasal administration produced greater immune activation than intraperitoneal delivery, emphasizing the importance of spatially controlled mucosal delivery for effective iNKT programming [100]. These findings demonstrate that biomaterial-mediated codelivery of glycolipid agonists and antigens can overcome the poor bioavailability and rapid systemic dilution of free agonists, enabling localized *in situ* activation of endogenous iNKT cells.

To further enhance CTL-mediated immunity, Ghinnagow and colleagues engineered PLGA nanoparticles functionalized with anti-C-type lectin domain family 9 member A (CLEC9A) antibodies to target cross-presenting DCs while codelivering α-GalCer and tumor-associated antigens [101]. In murine melanoma models, nanoparticles carrying α-GalCer together with tyrosinase-related protein 2 (Trp2) or glycoprotein 100 (gp100) induced potent antigen-specific $CD8^+$ T cell responses and provided both prophylactic and therapeutic protection [101]. Humanized CLEC9A-targeted nanoparticles carrying Melan-A and α-GalCer similarly expanded tumor-specific $CD8^+$ T cells *in vitro* and activated human $CD141^+$ DCs and iNKT cells in humanized immune system (HIS)-CD8/NKT mice, resulting in strong melanoma antigen A (Melan-A)-specific CTL responses [102].

Biomaterial platforms have also incorporated antigen-encoding mRNAs to enhance *in situ* iNKT programming (Table 1). Zhang and colleagues showed that LNPs codelivering α-GalCer and human papillomavirus (HPV) E7 mRNA induced complete and durable tumor eradication in TC-

Table 1. Biomaterial-based *in situ* iNKT cell programming in cancer immunotherapy

Biomaterial platform	Cargo components	Animal model	Delivery route	Therapeutic outcome	Advantages	Refs
PLGA nanocapsule	α-GalCer analog PBS-44 and OVA	C57BL/6 mice	Intranasal inhalation or intraperitoneal injection	Induced robust iNKT cell expansion and IFN-γ production, with intranasal delivery generating stronger iNKT activation than intraperitoneal administration	Enhanced immune activation and demonstrated synergistic immunostimulatory effects of the α-GalCer analog PBS-44 in nanovaccine-mediated iNKT programming	[100]
PLGA nanocapsule functionalized with anti-CLEC9A antibodies	α-GalCer and melanoma-associated self-antigens (Trp2 and gp100)	C57BL/6 mice bearing OVA-expressing EL-4 lymphoma tumors	Intravenous injection	Induced potent prophylactic and therapeutic antitumor responses, associated with increased tumor antigen-specific $CD8^+$ T cell infiltration and elevated $CD8^+$ T cell-to-regulatory T cell ratios within tumors	Targeted delivery to $CLEC9A^+$ cross-presenting DCs enhanced iNKT-mediated antitumor immunity and improved tumor immune remodeling	[101]
PLGA nanocapsule functionalized with anti-CLEC9A antibodies	α-GalCer and Melan-A tumor antigen	Humanized $HLA\text{-}A^*0201^+$ mice reconstituted with human $CD34^+$ hematopoietic stem cells	Intravenous injection	Promoted activation and expansion of human $CD141^+$ DCs and iNKT cells, resulting in robust Melan-A-specific $CD8^+$ T cell responses	Demonstrated the capacity of human iNKT cells to license cross-priming dendritic cells *in vivo, supporting translational development of iNKT-targeted cancer vaccines*	[102]
Cationic liposome	α-GalCer and TRP2 peptide	C57BL/6 mice bearing B16-OVA melanoma tumors	Intravenous and subcutaneous administration	Therapeutic vaccination significantly delayed B16F10luc2 melanoma progression, enhanced IFN-γ production, and increased cytotoxic T cell responses compared with free peptide/α-GalCer formulations	Codelivery of α-GalCer with tumor antigen improved iNKT-mediated adjuvant activity and promoted more effective antigen-specific antitumor immunity	[103]
PLGA nanocapsule	α-GalCer analog IMM60 and the cancer-testis antigen NY-ESO-1	AAD mice bearing full-length NY-ESO-1-expressing 1C12 sarcoma tumors	Intravenous injection	Codelivery of IMM60 significantly enhanced NY-ESO-1-specific $CD4^+$ and $CD8^+$ T cell responses as well as antigen-specific antibody production *in vivo*, while exhibiting minimal systemic toxicity even at high doses	Demonstrated the feasibility of PLGA-based nanovaccines for coordinated iNKT cell activation and DC licensing, thereby promoting robust antigen-specific adaptive immuneresponses with favorable safety profiles	[104]
Self-assembled β-annulus peptide-based artificial viral capsid encapsulated within a lipid shell containing α-GalCer	HER2-derived CH401 antigenic peptide	BALB/c mice	Intraperitoneal injection	The enveloped viral replica displaying CH401 peptide and α-GalCer induced robust antigen-specific antibody responses, with inclusion of α-GalCer further enhancing IgM antibody titers compared with formulations lacking α-GalCer	Demonstrated a bottom-up vaccine engineering strategy that integrates biomimetic viral architecture with iNKT cell adjuvant activity to improve humoral immune responses and antigen presentation	[105]

(continued on next page)

Table 1. (continued)

Biomaterial platform	Cargo components	Animal model	Delivery route	Therapeutic outcome	Advantages	Refs
Liposomes (mRNA galsomes)	α-GalCer and OVA mRNA	C57BL/6 mice bearing E.G7-OVA lymphoma and B16-OVA melanoma tumors	Intravenous injection	Induced robust tumor infiltration of antigen-specific cytotoxic T cells together with strong activation of iNKT and NK cells, while enhancing responsiveness to PD-L1 checkpoint blockade in B16-OVA melanoma	Enabled controllable and multifaceted antitumor immunity through simultaneous mRNA vaccination and iNKT activation, demonstrating strong therapeutic synergy with immune checkpoint inhibition	[57]
Lipopolyplex vector incorporating α-GalCer and multivalent cationic lipids	TRP2 mRNA	C57BL/6 mice bearing B16-F10 melanoma tumors	Intravenous injection	Intravenous administration efficiently targeted DCs *in vivo* and induced significant therapeutic efficacy against aggressive B16-F10 melanoma without requiring additional cell-specific targeting ligands or antibodies	Simultaneous delivery of antigen-encoding mRNA and α-GalCer promoted potent innate and adaptive antitumor immune responses through coordinated DC activation and iNKT cell stimulation	[106]
Galsomes	α-GalCer and mRNA encoding multiple myeloma-derived immunopeptides	5T33MM multiple myeloma model in C57BL/KaLwRij mice	Intravenous injection	Prime-boost vaccination with galsomes significantly suppressed multiple myeloma progression when combined with CD40 agonist therapy, accompanied by enhanced antigen presentation, co-stimulation, and cytotoxic activation of iNKT cells and $CD8^+$ T cells	Demonstrated the therapeutic potential of mRNA-based galsome vaccines for coordinated activation of iNKT and antigen-specific $CD8^+$ T-cell responses, while highlighting the importance of combinatorial strategies to overcome immune anergy	[107]
LNPs	α-GalCer and E7 mRNA	C57BL/6J mice bearing TC-1 cervical carcinoma tumors	Intramuscular injection	Enhanced DC transfection and promoted coordinated activation of tumor-specific $CD8^+$ T cells through MHC-I presentation together with iNKT cell activation via CD1d signaling. Vaccinated animals exhibited reduced tumor burden and prolonged survival	Preferential targeting of $CD11c^+$ DCs in lymph nodes facilitated simultaneous antigen presentation and innate immune activation, enabling TME reprogramming and improved antitumor immunity	[108]
iNKT BiTE	Recombinant CD1d–HER2 scFv fusion proteins	C57BL/6J mice bearing human HER2-overexpressing MC38 colon carcinoma and B16-F10 melanoma tumors	Intravenous injection	Induced multiple iNKT effector functions, including cytokine secretion, secondary NK cell activation, DC maturation, and epitope spreading that promoted tumor-specific $CD8^+$ cytotoxic T cell responses	Covalently stabilized CD1d-based BiTEs simultaneously engaged iNKT cells and tumor antigens to elicit potent antitumor immunity without inducing iNKT cell anergy or depletion	[109]

Table 1. (continued)

Biomaterial platform	Cargo components	Animal model	Delivery route	Therapeutic outcome	Advantages	Refs
CD3/BCMA bispecific antibodies (BsAbs) combined with α-GalCer-loaded DCs	Bispecific antibody targeting CD3 and BCMA together with α-GalCer-loaded DCs	C57BL/6 mice bearing VK14451 myeloma and C57BL/KaLwRij mice bearing 5TGM1-BCMA myeloma cells	Intravenous injection	Restoration of iNKT cell activity through adoptive transfer of α-GalCer-loaded DCs significantly enhanced the therapeutic efficacy of CD3/BCMA bispecific antibody treatment in multiple myeloma models	Demonstrated that iNKT cell activation can potentiate the antitumor efficacy of CD3-engaging bispecific antibodies, highlighting a synergistic strategy to improve T cell-redirecting immunotherapies	[110]
PLGA-based *in vivo* iMRAS	Human CD1d loaded with α-GalCer and IL-15Rα/IL-15 complexes	NSG mice bearing $CD19^+$ A375 and Raji tumors	Peritumoral injection	Functioned as an *in vivo* 'charging station' that promoted recruitment, activation, expansion, and prolonged persistence of CAR-iNKT cells, resulting in enhanced tumor eradication	Localized biomaterial-mediated delivery provided sustained chemotactic and activation signals to improve CAR-iNKT cell function within solid tumors, supporting more durable and effective systemic antitumor immunity	[46]

1C12: a murine sarcoma cell line; 5T33MM: a murine multiple myeloma cell line; 5TGM1: a murine multiple myeloma cell line; AAD: a transgenic, genetically humanized mouse model; B16F10luc2: a luciferase-expressing reporter variant of the B16-F10 murine melanoma cell line; BALB/c: a mouse strain; BCMA: B cell maturation antigen; C57BL/6: a mouse strain; CH401: a HER2-derived antigenic peptide; E.G7: a murine T-cell lymphoma cell line; EL-4: a murine T-cell lymphoma cell line; IMM60: an α-GalCer analog; KaLwRij: a mouse strain; MC38: a murine colon adenocarcinoma cell line; NSG: NOD *scid* gamma; NY-ESO-1: New York esophageal squamous cell carcinoma 1; PD-L1: programmed death-ligand 1; VK14451: a murine multiple myeloma cell line.

1 cervical cancer models, whereas E7 mRNA alone produced only transient responses [108]. Similarly, Guevara and colleagues developed a **lipopolyplex** vector carrying α-GalCer and tyrosinase-related protein 2 (TRP2) mRNA that selectively transfected DCs *in vivo* and generated potent antitumor immunity against B16-F10 melanoma [106].

In addition to codelivery of α-GalCer with tumor antigens for vaccination strategies, emerging approaches have focused on directly programming iNKT cells and their engineered derivatives *in vivo*. One notable strategy involves the development of iNKT-specific **bispecific T-cell engagers (BiTEs)**. Kharkwal and colleagues engineered covalently stabilized fusion proteins composed of soluble CD1d loaded with photoreactive α-GalCer analogs and linked to tumor-targeting single-chain variable fragments (scFvs), including human epidermal growth factor receptor 2 (HER2)-specific scFvs [109]. Systemic administration of these CD1d-based BiTEs induced robust mouse iNKT cell activation, characterized by cytokine secretion, secondary activation of NK cells, DC maturation, and epitope spreading that enhanced tumor-specific $CD8^+$ cytotoxic T cell responses [109]. Unlike conventional BiTEs that rely on anti-CD3-mediated polyclonal T cell activation, iNKT BiTEs exploit the natural interaction between invariant TCRs and CD1d-presented glycolipids, thereby enabling antigen-specific activation with reduced risk of nonspecific T cell activation, cytokine toxicity, and functional exhaustion.

Additional advances have focused on localized *in vivo* programming of CAR-engineered iNKT cells using biomaterial-based platforms. Li and colleagues developed a PLGA-based biomimetic '*in vivo* charging station', termed the iNKT cell-targeted microparticle recruitment and activation system (iMRAS), designed to recruit, activate, and expand human CAR-iNKT cells directly within tumors [46]. The platform incorporates CD1d/α-GalCer complexes together with IL-15 receptor subunit alpha (IL-15Rα) and IL-15 to provide localized chemotactic and proliferative signals for adoptively transferred CAR-iNKT cells [46]. In murine models of $CD19^+$ Raji lymphoma and

$CD19^+$ A375 melanoma, peritumoral administration of iMRAS promoted rapid infiltration and expansion of CAR-iNKT cells within solid tumors, resulting in enhanced tumor clearance and prolonged cellular persistence [46]. Through sustained local immune stimulation and biomimetic antigen presentation, this strategy overcomes several limitations associated with systemic cell therapy, including poor tumor trafficking and limited persistence in solid tumors.

MAIT cells

To date, biomaterial-based approaches for the delivery of MAIT cell agonists remain relatively underexplored. One notable study developed a layered double hydroxide (LDH)-based nanoparticle platform for the intranasal codelivery of 5-OP-RU and antigen-encoding mRNA [58]. Compared with conventional LNPs, the LDH nanoformulation demonstrated prolonged retention within pulmonary tissue following administration in murine models. This enhanced local delivery resulted in significantly increased expression of the activation marker CD69 on lung-resident MAIT cells compared with free 5-OP-RU or vehicle controls [58]. Furthermore, codelivery of mRNA encoding the HPV-derived E7 antigen together with 5-OP-RU promoted the expansion of E7-specific $CD8^+$ T cells and generated both prophylactic and therapeutic antitumor efficacy in models of lung metastasis [58]. These findings suggest that nanoparticle-mediated delivery of MAIT agonists can enhance local immune activation while simultaneously augmenting antigen-specific adaptive immune responses.

More recently, Li and colleagues reported preliminary findings on an *in vivo* MAIT cell activation and rejuvenation system (MARS) for liver cancer immunotherapy [111]. Given that MAIT cells constitute a substantial proportion of hepatic T cells in hepatocellular carcinoma [112], this study designed a PLGA-based biomaterial platform that selectively accumulates in the liver and delivers the potent MAIT agonist 5-OP-RU together with IL-15Rα/IL-15 complexes [111]. *Ex vivo* studies demonstrated that MARS efficiently expanded and activated human MAIT cells, leading to enhanced cytokine (e.g., IFN-γ, tumor necrosis factor alpha, and IL-2) secretion, increased cytotoxic activity, and reduced expression of exhaustion-associated phenotypes. *In vivo*, the platform preferentially localized to the liver and induced sustained human MAIT cell activation and expansion, resulting in potent antitumor activity in human liver cancer xenograft models [111]. These findings establish biomaterial-guided *in situ* MAIT cell programming as a promising therapeutic strategy for liver malignancies.

γδ T cells

Nanoparticle-mediated delivery of aminobisphosphonates has emerged as a promising strategy for *in situ* γδ T cell programming by enhancing intracellular phosphoantigen accumulation within tumor cells. Rather than directly stimulating γδ T cells, these platforms sensitize malignant cells through inhibition of the mevalonate pathway, thereby promoting BTN2A1/BTN3A1-dependent activation of Vγ9Vδ2 T cells. Parente-Pereira and colleagues investigated PEGylated liposomal formulations encapsulating either alendronic acid or ZA to enhance γδ T cell immunotherapy in ovarian cancer models [113]. Although liposomal ZA demonstrated substantial systemic toxicity, liposomal alendronic acid exhibited a markedly improved safety profile, with an approximately 150-fold higher maximum tolerated dose. In SCID beige mice bearing established IGROV-1 ovarian tumors, both liposomal formulations sensitized tumors to adoptively transferred γδ T cells, resulting in tumor regression and prolonged survival [113]. These findings support the use of liposomal aminobisphosphonate delivery to enhance tumor-directed γδ T cell immunotherapy.

Complementary advances have been reported using polymeric nanoparticle systems. Di Mascolo and colleagues developed spherical polymeric nanoparticles (SPNs) encapsulating ZA for colorectal cancer (CRC) immunotherapy [85]. These biomimetic nanoparticles substantially

improved ZA bioavailability and stimulated Vδ2 T cell activation and proliferation at concentrations approximately 300-fold lower than those of soluble ZA, while also unexpectedly supporting robust Vδ2 T cell expansion in the absence of exogenous IL-2 [85]. Functionally, ZA–SPN-activated Vδ2 T cells derived from both healthy donors and patients with CRC efficiently eliminated tumor cell lines, multicellular spheroids, and patient-derived autologous organoids. In addition, activated Vδ2 T cells demonstrated enhanced migratory capacity toward CRC cells in microfluidic migration assays [85]. Importantly, ZA–SPNs also expanded tumor-infiltrating Vδ2 T cells isolated from CRC specimens and restored their cytotoxic activity against autologous tumor organoids.

Beyond biomaterial-mediated phosphoantigen delivery, *in situ* programming of γδ T cells can also be achieved using engineered bispecific molecules that simultaneously activate endogenous γδ T cells and redirect them toward tumor cells. For example, an anti-TRGV9/anti-CD123 bispecific antibody selectively recruits Vγ9$^+$ γδ T cells to CD123-expressing acute myeloid leukemia (AML) cells, inducing potent antileukemic activity in xenograft models without broadly activating conventional T cells [114]. Similarly, a CD1d–Vδ2 BiTE co-engages Vγ9Vδ2 T cells and type I iNKT cells through variable domain of heavy-chain antibody (VHH) domains targeting the Vδ2 TCR and CD1d, resulting in robust cytokine production and cytotoxicity against CD1d$^+$ chronic lymphocytic leukemia, multiple myeloma, and AML cells while prolonging survival in preclinical models [115]. To more closely mimic physiological γδ T cell activation, a heterodimeric BTN2A1/BTN3A1–Fc fusion protein linked to a CD19-specific scFv has been engineered to recreate the endogenous BTN2A1–BTN3A1 ligand complex recognized by the Vγ9Vδ2 TCR, thereby coupling γδ TCR activation with selective targeting of CD19$^+$ lymphoma cells and enhancing granzyme B-mediated cytotoxicity [116]. Extending this concept to solid tumors, an epidermal growth factor receptor (EGFR)–Vδ2 BiTE activated endogenous Vγ9Vδ2 T cells within both peripheral blood and tumor tissues, promoted lysis of EGFR-positive tumor cells, and inhibited tumor growth in xenograft models [117]. Collectively, these receptor-targeted platforms complement biomaterial-based strategies by enabling localized activation and redirection of endogenous γδ T cells without the need for *ex-vivo* cell manipulation, thereby expanding the toolbox for *in situ* γδ T cell programming.

Concluding remarks

Biomaterial-based *in situ* programming is emerging as a powerful alternative to conventional adoptive cell therapies for harnessing UTCs. By converting soluble, short-lived immune agonists into localized and sustained signals, biomaterial platforms can overcome major barriers to UTC translation, including poor tissue retention, uncontrolled systemic activation, functional exhaustion, and inadequate tumor trafficking (Figures 1 and 2). Across γδ T cells, iNKT cells, and MAIT cells, the central therapeutic principle is the same: biomaterials can shape when, where, and how conserved immune ligands are presented, thereby enabling precise control over subset-specific activation within tumors, lymphoid organs, or mucosal tissues. In this context, biomaterials should be viewed not simply as carriers, but as programmable immune niches that coordinate antigen presentation, cytokine support, and microenvironmental remodeling.

The future challenges and opportunities discussed in Box 3 highlight several key directions for advancing biomaterial-enabled UTC immunotherapy from proof-of-concept studies toward clinical implementation. Looking forward, successful clinical translation will require careful matching of biomaterial design to UTC biology, including subset-specific antigen-presentation pathways, tissue localization, and activation thresholds (see Outstanding questions). With continued integration of immunology, materials science, and synthetic biology, *in situ* UTC programming has the potential to establish a new generation of universal immunotherapies for cancer and beyond.

Outstanding questions

How can biomaterial systems be optimized to achieve precise spatiotemporal control of UTC activation while avoiding exhaustion, anergy, or systemic inflammatory toxicity?

What combinations of UTC ligands, cytokines, and co-stimulatory signals best support durable antitumor activity in γδ T cells, iNKT cells, and MAIT cells?

Which anatomical sites are most effective for *in situ* UTC programming: tumors, draining lymph nodes, mucosal tissues, or systemic immune compartments?

How can biomaterials be engineered to selectively target rare UTC populations while minimizing off-target uptake by hepatocytes, myeloid cells, or conventional T cells?

Can synthetic antigen-presenting platforms replace endogenous APCs for direct and controllable UTC activation *in vivo*?

What material properties, including stiffness, viscoelasticity, degradability, and particle geometry, most strongly influence UTC activation, trafficking, and persistence?

How can mRNA and gene-delivery technologies be adapted for efficient *in situ* engineering of CAR-UTCs without *ex vivo* manufacturing?

What are the optimal strategies for integrating biomaterial-guided UTC programming with checkpoint blockade, radiotherapy, chemotherapy, or oncolytic therapies?

How do tissue-specific microenvironments, such as the liver, lung, or hypoxic solid tumors, alter biomaterial distribution and UTC responsiveness?

Can UTC-targeted biomaterials simultaneously remodel suppressive myeloid populations and activate cytotoxic UTC responses within the TME?

What mechanisms govern long-term UTC memory, tissue residency, and persistence following biomaterial-mediated activation?

Box 3. Future directions and translational opportunities for *in situ* UTC programming

The most compelling future opportunities lie in multi-signal and tissue-specific programming strategies. Combining signal 1 (such as UTC ligands) with signal 2 (cytokine support) and signal 3 (co-stimulation) may better reproduce physiological immune activation and improve the durability of antitumor responses. Equally important is spatiotemporal control: platforms that tune release kinetics and direct payloads to the appropriate tissue niche may maximize efficacy while minimizing toxicity and anergy. In parallel, synthetic antigen-presenting particle systems and mRNA-based programming approaches offer exciting routes to bypass endogenous APC limitations and directly instruct UTCs or their accessory cells *in situ* [42,43,118]. These advances may ultimately enable off-the-shelf, broadly applicable UTC immunotherapies that are more scalable, adaptable, and safer than current cell-based approaches.

An equally important consideration for future development is the selection of appropriate preclinical models to evaluate UTC-targeted biomaterials. Although mouse models have been instrumental in elucidating UTC biology and testing biomaterial-based immunotherapies, important differences exist between murine and human UTC subsets in terms of abundance, TCR repertoire, ligand recognition, and tissue distribution. Humanized mouse models and adoptive transfer systems provide valuable platforms for studying human UTC function *in vivo,* but remain limited by incomplete immune reconstitution and species-specific differences in the TME [88,119,120]. Therefore, integrating complementary preclinical models, including genetically engineered mice, humanized mice, and *ex-vivo* human tissue systems, will be essential for accurately predicting the efficacy, safety, and translational potential of *in situ* UTC programming strategies [121].

Beyond optimizing individual biomaterial platforms, the next phase of the field will likely focus on integrating programmable materials with synthetic immunology to achieve context-dependent UTC activation. Rather than delivering single agonists, future systems should dynamically coordinate antigen presentation, cytokine support, receptor engineering, and immune checkpoint modulation in response to local disease cues. Critical next-step studies should establish quantitative design principles linking biomaterial properties, cargo release kinetics, and UTC activation thresholds across different tissue microenvironments. In parallel, scalable manufacturing, standardized quality control, and clinically translatable formulations will be essential for broad implementation. We envision that advances in biomaterials, RNA engineering, and *in vivo* gene delivery will ultimately enable off-the-shelf, precision immunotherapies that locally recruit, program, and sustain endogenous UTCs without the need for complex *ex-vivo* cell manufacturing.

How can hybrid biomaterial systems be standardized for scalable manufacturing, reproducibility, and regulatory translation in clinical settings?

Which biomarkers best predict successful UTC activation, therapeutic response, or toxicity following *in situ* immunoengineering?

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Declaration of interests

L.Y. is a scientific advisor to AlzChem and Amberstone Biosciences. None of the declared companies contributed to this study. The remaining authors declare no competing interests.

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