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Hydrogel-enabled, local administration and combinatorial delivery of immunotherapies for cancer treatment

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Throughout the last decade, interventions to engineer the immune system called immunotherapy have revolutionized the fields of oncology and autoimmune disease. Researchers are developing platforms that enable new modes of immunotherapy and expand the current limitations by incorporating non-intravenous delivery strategies. Recent advances in the immunotherapy include the use of chemokines to direct immune cells into tumors, alternative combinatorial therapies, and oncolytic viruses. Similarly, there have been significant breakthroughs in the design and understanding of new biocompatible hydrogel-based materials for diverse biomedical applications, including large molecule drug delivery. In this review, we discuss how hydrogel platforms can enable modes of immunotherapy that are otherwise not feasible. Despite the many pre-clinical successes of hydrogels for the delivery of immunotherapies for treatment of cancer, hydrogels still face challenges in getting to the clinic and eventually approved. Herein we examine the application of hydrogels in high concentration subcutaneous, intratumoral, peritumoral, intraperitoneal, intracranial, and pulmonary delivery of immunotherapies. By analyzing the results of many pre-clinical hydrogel-enabled immunotherapy studies, we describe that local hydrogel delivery is a promising approach to increase the efficacy and decrease systemic toxicities of immunotherapies. We also discuss the application of hydrogels for synergistic combinatorial immunotherapy. Furthermore, we summarize the advancements and obstacles in local intratumoral administration and sustained release of immunotherapy-loaded hydrogels. Finally, we discuss challenges in the translational research, clinical development, and manufacturing of hydrogels which must be addressed to advance the field.

Keywords: Immunotherapy; Hydrogels; Antibody Delivery; Biomaterials; Therapeutic Window

Introduction

Immunotherapies are treatments designed to modulate the immune system to enhance the innate or adaptive immunity [1]. Immunotherapies have demonstrated high efficacy and versatility in the treatment of various diseases, including cancer, autoimmune, and infectious diseases. The origins of immunotherapy clinical trials date back to the 18th century with the deliberate infection of children with small amounts of smallpox by Dr. Charles Maitland with the aim of preventing smallpox infection after reexposure [2]. In the early stages, immunotherapies were primarily developed for infectious diseases, but in the late 19th century, researchers began studying immunotherapies in the context of cancer treatment. In 1893, William Coley injected a bacterium into patients with unresectable sarcomas and achieved more than a 10% cure rate with nearly 900 total patients [3]. This early experiment by Coley demonstrated the ability to modulate the immune system to

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detect and eliminate cancerous cells. In the 20th century, researchers began identifying tumor-associated antigens, which proved to be promising targets for immunotherapies [4]. The discovery of these tumor-associated antigens and the T-cell antigen receptor triggered the development of monoclonal antibodies for immunotherapy, which have demonstrated strong success in the treatment of cancer today. Monoclonal antibodies are commonly used as immune checkpoint inhibitors for the treatment of cancers and have received several U.S. Food and Drug Administration (FDA) approvals recently [5]. Many other approaches to cancer immunotherapy, such as cell therapies, oncolytic viruses, cytokines, and chemokines, are also currently being investigated. Cancer immunotherapy typically involves the activation and recruitment of cytotoxic T cells to kill cancer cells and is designed to act against the tumor immune system evasion mechanism [6].

The field of immunotherapy is rapidly evolving and advancing. For example, we have only begun to map the role of chemokines in immune cell infiltration into tumors, representing a promising new strategy to improve immunotherapies [7]. This directed migration of immune cells into tumors by chemokines is controlled by local concentration gradients [8], therefore, suggesting that future immunotherapy treatments based on chemokines can be feasible with local administration. A combinatorial immunotherapy treatment study by Kim et al. examined locally administrated nitric oxide donor in combination with a CTLA-4 checkpoint blockade antibody using a thermosensitive hydrogel, to achieve durable anti-tumor effects in mice [9]. Oncolytic viruses, which can be carefully engineered to have high tumor-specificity and deliver immunotherapy payloads efficiently, are also emerging as powerful immunotherapy approaches [10]. However, immunotherapies still face efficacy challenges, especially in the treatment of solid tumors [11–13]. Significant challenges in cancer immunotherapy include targeted delivery, the immunosuppressive tumor microenvironment (TME), drug escape from the tumor, the possibility of creating an immune system imbalance, and toxicities caused by the need for high doses of immunotherapies [14]. Complex tumor phenotypes, which often differ from patient to patient, also present challenges to the success of immunotherapy treatments. Solutions must bring localization, improved dosing, and adaptability to different tumor phenotypes. The use of hydrogel delivery systems is a promising approach to address many of the challenges facing immunotherapy and reduce patient burden by requiring fewer doses or ideally only one sustained-release dose [9,15,16].

Hydrogels are a type of biomaterial which consist of crosslinked networks of hydrophilic polymers [17]. The crosslinked networks of hydrogels are formed by either covalent or/and non-covalent (e.g., ionic) bonds [18,19]. Hydrogels can be classified into many different categories based on the type of polymer used, size, pore structure, and cross-linking method. Additionally, hydrogels can be designed for different modalities of administration, such as implantable scaffolds or injectable materials. Hydrogels provide important benefits for improving the safety and efficacy of cancer immunotherapies. Firstly, hydrogels can be designed as biocompatible materials with desired biodegradability which minimizes safety risks when administering immunotherapy agents. Secondly, hydrogels can be loaded with different types of payloads, ranging from small molecules to whole cells, and allow for controlled and targeted release of these payloads [9,15,20,21]. Hydrogels also offer protection to immunotherapeutic drugs from both the immune cells and enzymes which can degrade the drugs. Combinations of immunotherapeutic payloads can also be easily loaded into hydrogels, which can trigger a synergistic localized immune response. Hydrogels can be delivered using various routes, such as subcutaneous injection, intratumoral injection, surgical implantation, and systemic intravenous administration [17]. While there is significant interest and promising pre-clinical results on use of hydrogel for delivery of immunotherapies, these finding have not been translated to clinic yet. At the time of writing this paper there are no completed clinical studies involving hydrogels for immunotherapy registered in National Library of Medicine (NLM) at the U.S. Institutes of Health (NIH).

This review focuses on local (non-intravenous) and combinatorial delivery of cancer immunotherapies using hydrogels. We describe the challenges facing immunotherapies, especially in local administration to tumors. We examine efforts to utilize hydrogels for immunotherapy delivery, alternative routes of immunotherapy administration, and sustained release of immunotherapies. Nanogels, which are more closely related to nanoparticles that are typically administered intravenously, are beyond the scope of this review [22]. We place emphasis on the application of hydrogels for synergistic combinatorial immunotherapies. We discuss the recent breakthroughs in using chemokines as immunotherapies Additionally, we aim to bridge the gap between materials science and clinical immunotherapy research. Recent breakthroughs in understanding the role of chemokines, tumor antigens, sting agonists, and combinatorial therapies justify an updated review of the field. We examine the outcomes of many different cases in the literature on hydrogels and immunotherapy types as well as combinatorial immunotherapy treatments. We have tabulated summaries of use of hydrogels-based immunotherapies based on their chemistry, modes of administration and also summarized the combinatorial immunotherapies delivered with hydrogels. Finally, challenges in the clinical translation of hydrogel drug products and manufacturing considerations are discussed. The overarching goal of the review is to facilitate the improvement of hydrogel technologies with the goal of translational research and ultimately adoption by clinicians and the biopharmaceutical industry.

Immunotherapy and the motivation for local administration

Immunotherapy approaches

The immune system can recognize cancer cells as “foreign” because of the mutant proteins expressed by these cells [23,24]. However, negative regulators of immune activation can cause attenuated cytotoxic T-cell responses, leading to tumor progression. Pioneering work exploring checkpoint pathways, including cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1), led to the discovery that immune checkpoint blockade (ICB) therapy can boost the anti-tumor immune responses [24]. Since then, immunotherapies

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have revolutionized the clinical treatment of cancer. Immunotherapies can be classified into several primary categories: immune modulators, checkpoint blockade monoclonal antibodies (mAbs), cancer vaccines, cell therapies, and oncolytic viruses. Fig. 1 shows various examples of immunotherapies and their simplified mechanisms of action.

Monoclonal antibodies in the context of cancer immunotherapy are often used as ICB therapies (Fig. 1A). Immune checkpoints are pathways that allow cancer cells to escape the immune system, often by cancer cells overexpressing ligands that bind to inhibitory T cell receptors [29]. Immune checkpoint inhibitors release the brakes of the immune system by preventing inhibitory ligand binding thus promoting anti-tumor T cell activity. FDA-approved immune checkpoint inhibitors include antibodies to cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) receptors and programmed death receptor/ligand-1 (PD-1/PD-L1) [30].

Cancer vaccines (Fig. 1B) are often antigens that are expressed by cancer cells and preferably not expressed by normal cells. Upon injection, the vaccine help in training the T cells to recognize these cancer antigens and develop a cancer-specific immune response [31]. Most of the single peptide antigen-based cancer vaccines have failed during phase 3 clinical studies because of lack of efficacy [32]. These results may be explained by several factors, including tumor immune escape mechanisms, immunosuppressive tumor microenvironment, and deficient cancer vaccine formulations [33]. Only over the past decade we have observed the rise of neoantigen-based cancer vaccines which have shown encouraging results in clinical trials [34–37]. Neoantigens arise through a variety of mutational events and neoantigen-based vaccines owe their improved efficacy by being effective targets of tumor- specific immune responses [37].

Immune modulators (Fig. 1 C and D) are agents which modify or enhance the immune response toward cancer cells. Immune modulators can be further classified into four categories: cytokines, chemokines, stimulator of interferon gene (STING) agonists, and toll-like receptor (TLR) agonists. Cytokines are glycoproteins or polypeptides which provide various signals to cells, such as inflammatory or anti-inflammatory signals [26]. Cytokines can inhibit tumor cell growth directly via anti-proliferative or pro-apoptotic activity, or indirectly by stimulating immune cells [26]. Chemokines are a class of immune modulators that direct the immune cell towards targets and in the case of cancer, the infiltration of immune cells into tumors [38]. However, chemokines can have both anti-tumor and pro-tumor effects. For example, CCL22 recruits regulatory T cells (T_{reg}) cells into the tumor microenvironment, which suppresses anti-tumor immunity [38]. The third class of immune modulators, STING agonists, activate the STING pathway, which enhances anti-tumor immunity by inducing pro-inflammatory cytokines and chemokines, such as type I interferons (IFNs) [39]. STING agonists are often cyclic dinucleotides (CDNs), such as cyclic di-GMP. The use of STING agonists is often limited by their high toxicity and susceptibility to degradation, which reduces their effectiveness. Addressing these shortcomings are topics of active research and novel polymeric STING agonists have been proposed to address the high toxicity and the susceptibility to degradation [40–42]. TLR agonists activate toll-like receptors, which then induce transcription of type I IFN genes and proinflammatory cytokines. Activation of TLRs also causes T-cell activation and dendritic cell (DC) maturation. CpG oligodeoxynucleotides (ODNs), for example, activate TLR9 and have been used as immunotherapy agents in various tumor types [43].

Cell therapies for cancer involve the genetic engineering of a patient's immune cells to more effectively target and kill cancer cells (Fig. 1E) [44]. Chimeric antigen receptor T (CAR-T) cell therapies are an example of adoptive cell therapy (ACT), in which a patient's own T cells are extracted, genetically modified with an antigen-specific receptor, and then administered to the patient. Although CAR-T cell therapies have demonstrated great efficacy in the treatment of hematological cancers, majority of the trials have not shown strong responses in solid tumors, primarily due to the lack of obvious CAR target antigens in solid tumors and the immunosuppressive tumor microenvironment [45]. CAR-NK therapy is another class of cell therapy that uses natural killer (NK) cells instead of T cells to target cancer cells and has recently demonstrated some promising advantages over CAR-T cell therapy, such as reduced side effects [46]. In addition, CAR-macrophage (CAR-M) therapy is another proposed cell therapy that can present neoantigen through phagocytosis of macrophages and improve tumor microenvironment with efficacy against solid tumors [47–49]. Finally, oncolytic viruses are a type of immunotherapy that uses a genetically modified virus, such as an adenovirus, to infect and kill cancer cells. Oncolytic viruses can be intelligently designed to have high selectivity to tumor cells, restrict their replication only to cancer cells and not normal cells, and to carry therapeutic transgenes [10].

Tumors possess different immune phenotypes, which are regulated by different biological mechanisms and influence their ability to be treated effectively with immunotherapies. These phenotypes can be classified into three categories: immune-inflamed, immune-excluded, and immune-desert [50]. Fig. 1F shows some characteristic features of these immune phenotypes. Immune-inflamed tumors are characterized by having immune cells, such as helper T cells (CD4- expressing) and killer T cells (CD8- expressing), in the tumor parenchyma. This phenotype is the most responsive to checkpoint blockade therapy and allows for T cell infiltration into the tumor. Immune-excluded tumors contain immune cells in the stroma surrounding the tumor, but T cells are unable to infiltrate the tumor itself. The immune-desert phenotype is characterized by a lack of T cells in both the tumor parenchyma and stroma, presenting a non-inflamed tumor microenvironment. Immune-desert tumors very rarely respond to checkpoint blockade therapy as they possess very few tumor-specific T cells [50].

The success of single monoclonal antibody (mAb) therapies, such as anti-PD-1, in cancer treatment can be limited and many patients either fail to respond or eventually relapse. For instance, ovarian cancer has demonstrated limited success with ICB therapy (Epithelial ovarian cancer with median response rates of 10–15%). [11]. Even though high-grade serous ovarian cancer cells show increased expression of PD-1 and its ligand, which indicates potential for success of anti-PD-1 immunotherapies, less than half of patients respond to the immunotherapy [11]. This shortcoming is not limited to ovarian cancer and similar

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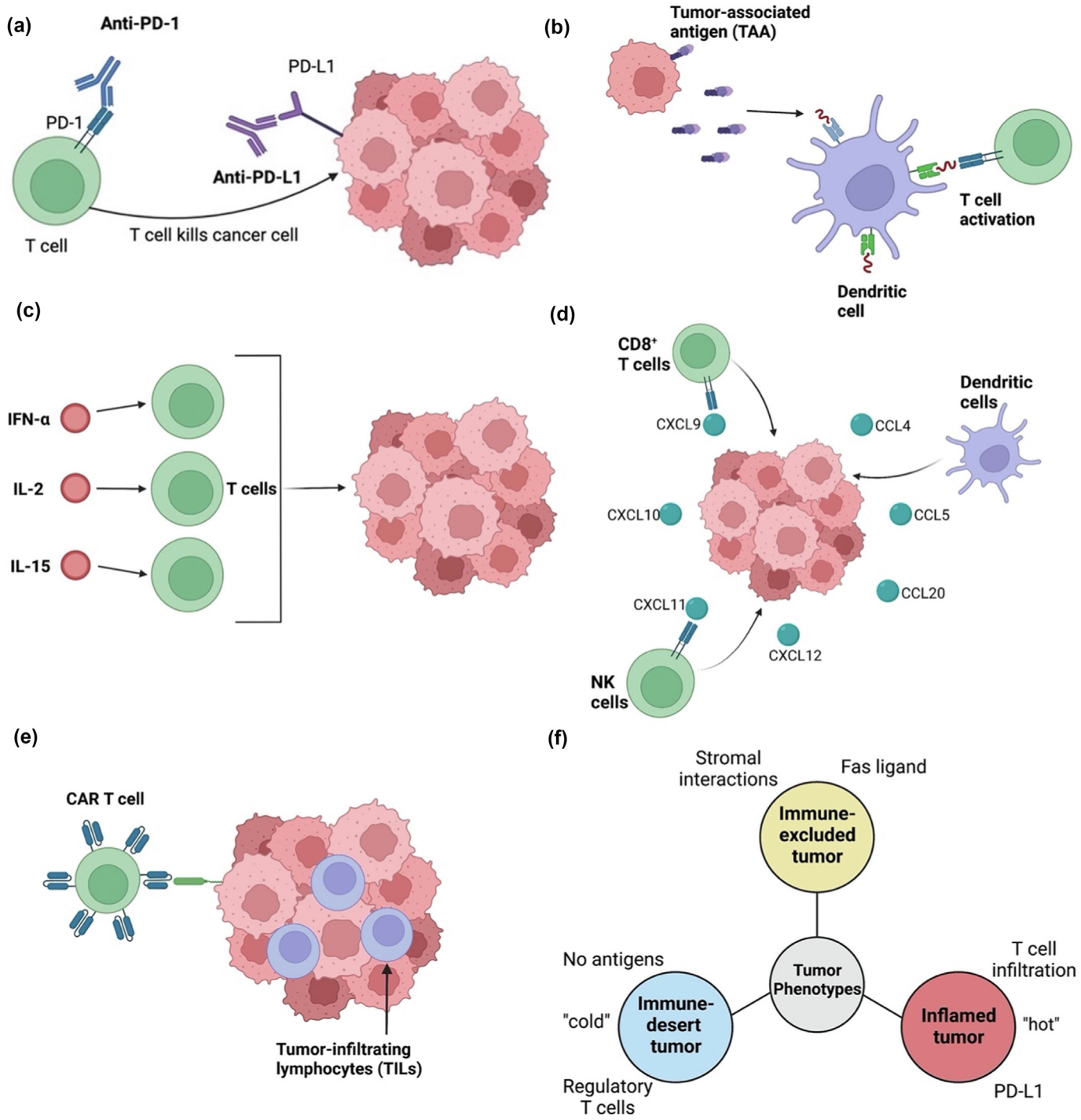

FIGURE 1

Various types of immune engineering used in cancer immunotherapies (A-E) and tumor phenotypes (F). (A) Checkpoint blockade antibodies anti-PD-1 (targeting T cells) and anti-PD-L1 (targeting cancer cells), inhibit the regulatory pathways which prevent cancer cell elimination [25]. (B) Tumor-associated antigens (TAAs) as cancer vaccines prime the immune system with cancer antigens [21]. (C) Pro-inflammatory cytokines act as a gas pedal in boosting the immune response [26]. (D) Chemokines directing migration of immune cells into the cancer tumor [8]. (E) Chimeric antigen receptor (CAR) T cells are synthetic receptors that are designed to interact with target cancer cells [27]. (F) Understanding different tumor immune phenotypes (immune-desert, immune-excluded, and inflamed) is essential to understand the immune response against cancer and the immunotherapy approach [28].

results can be observed in success of immunotherapies in treating breast [12], melanoma [51], colon [52], and pancreatic cancers [13], highlighting an opportunity to identify new combinatorial therapies and more effective modes of delivery. Perhaps one of the main reasons for the lack of efficacy of immunotherapies, which prevents clinical treatment and also FDA approval, can be attributed to the dose-limiting toxicity of combinatorial cancer treatments. [53].

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The motivation for local administration of immunotherapies

Despite the successes of immunotherapies, they still face numerous delivery challenges which significantly affect their safety and efficacy. Narrow therapeutic index and immune-related adverse events are major challenges that need to be addressed [54]. Many immunotherapies require high doses to be efficacious, and these high doses can cause immunotoxicities and autoimmunity [55]. For example interleukin-12 (IL-12), has not advanced into phase 3 clinical trials due to toxicities. Yet in recent years there has been new efforts for direct delivery to tumors using novel delivery systems including hydrogels to reduce toxicity. In this new direction, nanoscale drug delivery systems, including hydrogels, may play a key role [56,57].

Immunotherapies, such as checkpoint blockade antibodies, are often administered systemically, which increases the chances of off-target toxicities throughout the body. Since the immunotherapy dose must be low to avoid adverse events, multiple doses can be required, placing more burden on the patient. Another important challenge for immunotherapies is the complex tumor microenvironment [58]. As discussed previously, tumors display unique phenotypes which influence the effectiveness of immunotherapies. Immune-excluded and immune-desert tumors are especially challenging to treat with immunotherapies, as they have very low levels of T cell infiltration into the tumor. Therefore, novel delivery mechanisms have strong potential to improve the safety and efficacy of immunotherapies. In particular, using hydrogels as a delivery mechanism can address many of the problems facing immunotherapy and improve clinical outcomes. Hydrogels offer three important advantages to the delivery of immunotherapies: improved drug release kinetics, local targeting which avoids off-target effects, and a reduction in patient burden by requiring fewer doses. As new hydrogels are developed, the gap between materials science and cancer biology must be bridged, so that hydrogel-based delivery systems are matched to the immunotherapies' mechanisms and specific needs.

Hydrogels for the delivery of immunotherapy agents

Hydrogels, hydrated crosslinked networks of polymers, are viable candidates for a range of drug delivery applications [20,59–61]. These applications can be extended to local administration of biologics, including immunotherapy agents for cancer [62–64]. Hydrogels are formed by physical or chemical crosslinking of polymers. In the physically crosslinked hydrogels, non-covalent interactions such as ionic, electrostatic, and metal–ligand interactions form the 3D network entrapping water and other solutes [65]. Compared to physically crosslinked hydrogels, chemically crosslinked hydrogels generally have the advantage that their mechanical properties and chemical composition are more tunable [66].

Hydrogels are very tunable and can differ in many properties, such as their size, composition (e.g., natural vs. synthetic polymers), and modality of administration. Additionally, an important benefit of hydrogels is their ability to mimic biological tissues, which provides a high degree of biocompatibility. Hydrogels can be designed to range in size from micrometers to millimeters, which makes them amenable to many different routes of administration [17]. In addition to porous macrostructures, hydrogels can be designed to be non-porous and release drugs as they degrade [17]. Hydrogels can be synthesized from a variety of materials and can be broadly classified into several categories based on their composition: synthetic polymers, polysaccharides, nucleic acids, peptides, proteins, and hybrids. Examples of these hydrogel types are shown in Fig. 2. Each hydrogel type offers distinct drug delivery benefits and can improve the efficacy of the immunotherapy payload significantly.

Hydrogels can also differ in their modality of administration, as shown in Fig. 3. Some hydrogels are designed to be injectable, while others are implantable scaffolds. For injectable hydrogels, formulations include both *in situ* hydrogel formation (hydrogel forms immediately after injection) and injectable microspheres. Implantable hydrogel scaffolds can be designed to release cargo or be infiltrated by immune cells. *In situ* hydrogel formation can be triggered by physiological stimuli, such as a change in temperature or pH. Injectable hydrogel formulations are preferred for patients, as the procedure is minimally invasive compared to implantable hydrogel formulations. However, *in situ* hydrogel formulations may be more difficult to approve, as the post-administration hydrogel product might not form properly or retain complete function.

Synthetic polymer-based hydrogels (Fig. 2A), such as poly (ethylene glycol) (PEG) and poly (lactic-co-glycolic acid) (PLGA), exhibit high tunability, structural control, stability, and mechanical strength [67]. Li et al. synthesized a poly vinyl alcohol (PVA) and N^1-(4-boronobenzyl)-N^3-(4-boronophenyl)-N^1,N^1,N^3,N^3-tetramethylpropane-1,3-diaminium (TSPBA) cross-linked hydrogel scaffold to deliver anti-PD-L1 antibody and IPI-549, a PI3 kinase inhibitor, to tumor-bearing mice [81]. This therapy is administered as an intratumoral injection after inadequate microwave ablation (iMWA) treatment, and the hydrogel scaffold is responsive to reactive oxygen species (ROS) which form after iMWA. Importantly, this hydrogel immunotherapy approach inhibited tumor progression and metastasis, induced systemic immune responses, and protected against tumor rechallenge [81].

Polysaccharide-based hydrogels (Fig. 2B) come from natural sources and demonstrate favorable biological properties, such as high biocompatibility, biodegradability, and similarity to the extracellular matrix (ECM) [82–85]. Examples of polysaccharides used to synthesize hydrogels include alginate, chitosan, and cellulose [86]. Zhang and colleagues designed an injectable sodium alginate (SA) hydrogel microsystem and a SA nanogel to deliver immunotherapies post-operatively [87]. The SA hydrogel microsystem was loaded with CpG oligodeoxynucleotides (ODNs), which are toll-like-receptor (TLR) agonists. CpG ODNs activate numerous types of immune cells in the body, including NK cells, DCs, and macrophages. The SA nanogel was surface-modified with anti-PD-L1 antibodies and contained indocyanine green (ICG) for imaging. These micro/nano SA hydrogel delivery systems protected the immunotherapy payloads from degradation, improved the delivery to immune cells, and prevented tumor postoperative recurrence and metastasis [87].

Nucleic acid-based hydrogels (Fig. 2C) also demonstrate favorable biocompatibility, biodegradability, and precise control over size and shape. A distinct advantage of nucleic acid hydrogels, such as DNA hydrogels, is that crosslinking can be achieved

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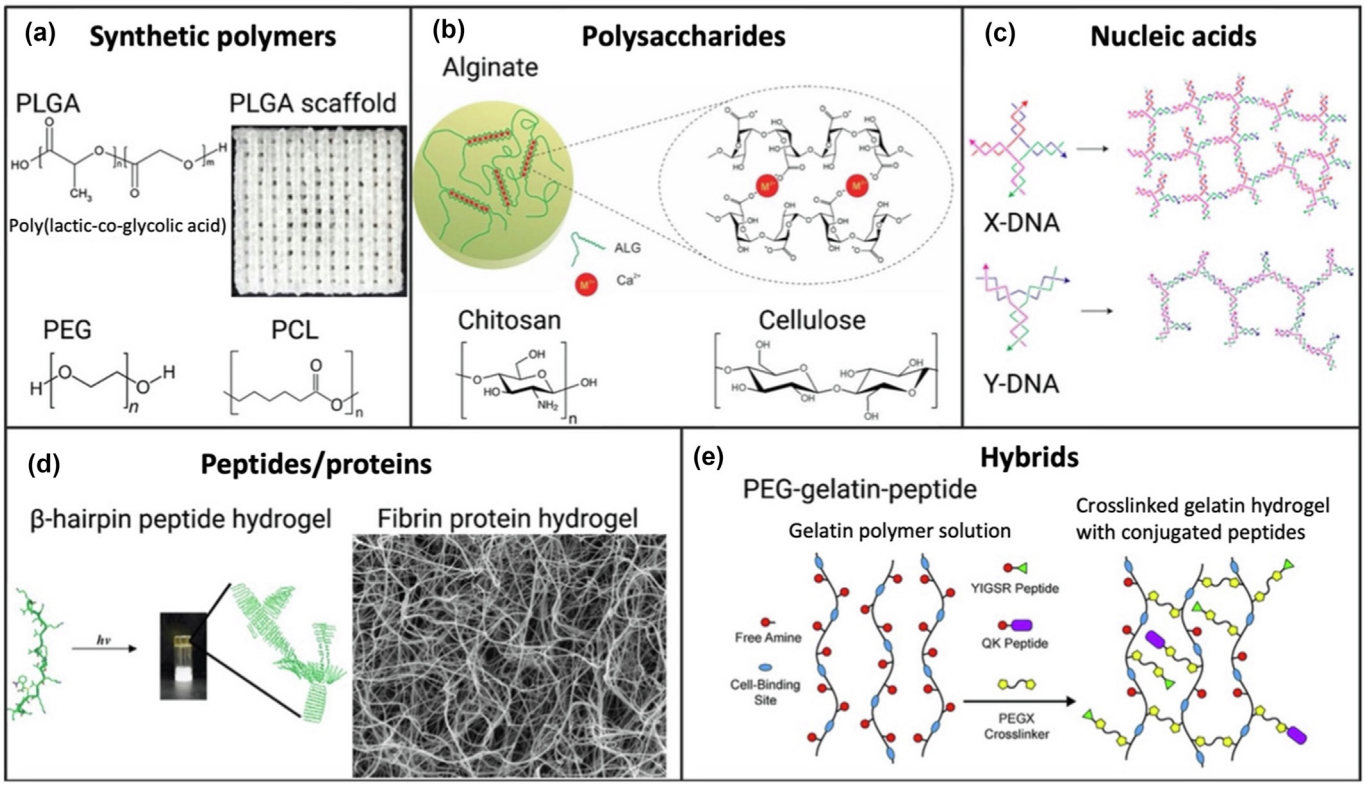

FIGURE 2

Hydrogel chemistries utilized for the local administration of immunotherapies: (A) synthetic polymers, [68,69]. (B) polysaccharides [19,69,70], (C) nucleic acids [71], (D) peptides/proteins [72–75], and (E) hybrids [76,77]. Reprints with permission from [19,71–73,77,78].

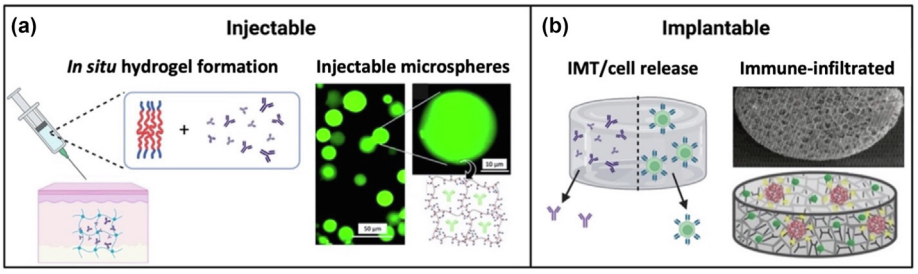

FIGURE 3

Hydrogel modalities: injectable versus implantable. (A) Injectable modality: formulations include both in situ hydrogel formation and injectable microspheres [15,18,60,79]. (B) Implantable modality: designed to either release the immunotherapy agents or be infiltrated by immune cells [80]. Reprints with permission from [79,80].

through efficient ligase-mediated reactions [71]. Wu et al. designed a CpG-based DNA hydrogel loaded with melanin and STING agonists which was administrated intratumorally to mice [88]. The melanin allowed for photothermal therapy when exposed to near infrared (NIR) illumination, which resulted in the photothermal killing of primary tumors. Additionally, this DNA hydrogel photothermal immunotherapy boosted the release of tumor antigens, activated DCs, and created a systemic immune response [88].

Peptide- and protein-based hydrogels (Fig. 2D) also offer very high biocompatibility and biodegradability and can be easily designed to respond to external stimuli, such as temperature, pH, and light [89,90]. Additionally, through advances in computation modeling, peptide and protein sequences can be intelligently designed to form hydrogels with diverse properties. Examples of peptides and proteins which have been used to assemble hydrogels include gelatin, fibrin, and melittin. Gelatin, for example, contains the cell-binding motif arginylglycylaspartic acid (RGD), which allows for facile cell encapsulation within gelatin-based hydrogels [67]. Verma et al. designed a fibrin-based hydrogel scaffold to deliver dendritic cells (DCs) to tumors in mice [91]. The hydrogel scaffold was surgically implanted after tumor resection, and it exhibited a strong anti-tumor immune response and induced immunocyte infiltration into the scaffold. In another example, Li et al. formed supramolecular nanofibrils by co-assembly of the clinically approved peptide drugs to form

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the 3D network of the hydrogel and achieved positive results in inhibiting tumor growth [74].

Hybrid hydrogels (Fig. 2E) can be synthesized by combining multiple different types of materials, which can further improve desirable delivery properties. Guerra et al. constructed a PEG diacrylate – gelatin crosslinked hydrogel to deliver M1 macrophages to tumor-bearing mice [92]. The hydrogel was injected subcutaneously directly adjacent to the tumor and the immunotherapy was effective in inducing apoptosis of hepatocellular carcinoma (HCC) cells and resulted in tumor regression. Mei et al. used the self-assembly of collagen and alginate to form a hybrid system encapsulating with photothermal and immunotherapy agents [76]. They illustrated that a shear-thinning and self-healing hydrogel can be readily constructed by self-assembly of positively charged collagen and negatively charged alginate.

Table 1 summarizes the different hydrogel types and their immunotherapy payloads which have been used in various preclinical studies. Table 2 summaries clinical studies involving hydrogels for treatment of cancer registered in National Library of Medicine (NLM) at the United States National Institutes of Health (NIH).

Although hydrogels offer promising immunotherapy delivery benefits, very few hydrogels encapsulating biomacromolecules have reached clinical studies or commercialization (none for immunotherapy treatments). [102]. There are major difficulties that can act as deterrents in the development of hydrogel delivery strategies [103]. These difficulties can be attributed to shortcomings in *materials and processes* that are used in such studies that can make translational research unfeasible or make the technology impractical from a manufacturing perspective. Even though natural polymer-based hydrogels offer distinct advantages, only synthetic polymer hydrogels have been FDA-approved thus far. This may be due to concerns in biopolymers' batch to batch variability and limited supply/manufacturing of purified pharmaceutical grade biopolymers.

Hydrogels for alternative routes of administration of immunotherapy agents

Local administration of immunotherapies can be significantly improved using hydrogels [94,100,104]. There are several challenges that can hinder translational research on local administration of immunotherapies. First, some immunotherapies, such as anti-PD-1 checkpoint blockade therapy, require large doses of the mAb for the treatment. A typical dosage for anti-PD-1 pembrolizumab contains 400 mg of the mAb. Recent clinical studies have used anti-PD-1 antibodies at even at higher concentrations [81]. Considering the limitation in the volume of a local injection, formulating the mAb at required high concentrations is a major challenge. Perhaps more importantly, mAbs and immunomodulatory cytokines are readily soluble in body fluids. As such, local bolus administration without ability to control the release will not be effective when considering the rapid escape of cytokines/mAbs from the tumor site [105]. For instance, rapid escape of cytokines can result in unwanted cytokine release syndrome (CRS) limiting the treatment options. Another challenge is the feasibility of co-formulations, as a combination of mAbs or mAbs and cytokines can even be more challenging to formulate. These challenges need to be considered in developing a technology to enable high concentration, local administration with sustained release for long-lasting anti-tumor effectiveness.

The primary routes of administration of immunotherapies with hydrogels which will be described here include subcutaneous injection, intratumoral injection, intraperitoneal injection, intracranial delivery, and pulmonary delivery (Fig. 4). The choice of the local administration route is to help improve the effectiveness of local immunotherapy primarily through enhancing the targeting ability of drugs, minimizing the degradation of therapeutic agents, overcoming physical barriers, preventing systemic side effects, and achieving local sustained release. The human body can develop anti-drug antibodies (ADA) which can be detrimental to the safety of the efficacy of immunotherapy agents [106]. A strategy to avoid ADA is to reduce the dosage by the means of local administration, which can be achieved through local hydrogel delivery, such as intratumoral injection.

The route of administration often depends on the type of tumor or tumor location in the body. Subcutaneous injections have the benefit of high patient preference compared to other parenteral methods [112]. Yin et al. performed a subcutaneous injection in melanoma-bearing mice of a graphene oxide (GO) and polyethylenimine (PEI) hydrogel [93]. This hydrogel carried an mRNA nano-vaccine encoding ovalbumin and resiquimod, a small molecule immune modifier (TLR 7/8 agonist), and exhibited a 30-day drug release profile. In high-concentration SC administration, one of the potential problems is that stabilizers in the mAb formulation can leach from the administration site quickly, leaving the unprotected mAb molecules at high concentrations alone [113]. This can potentially cause aggregation and denaturing of the mAb. The hydrogel must be formulated to stabilize the drug to ensure maximum efficacy [59,114].

Local administration via intratumoral injections of drug-loaded hydrogels can result in greater accumulation of drugs in tumors. Yao et al. delivered a multimodule DNA network hydrogel carrying PD-1 aptamers and CpG ODNs via intratumoral injection in mice [99]. This hydrogel delivery system allowed for sustained concentrations of the PD-1 checkpoint inhibitor and captured tumor-infiltrating T cells, resulting in highly localized immunotherapy efficacy. It is important to note that intratumoral injections are not always feasible, such as in the case of lung cancers, in which the risk of damaging the patient's lungs is too high. Additionally, intratumoral injections have not always shown the most effectiveness in animal studies. A study by Park et al. found that perioperative delivery of a hydrogel carrying immunotherapeutic payloads resulted in prolonged survival of tumor-bearing mice compared to intratumoral injection [107].

Intraperitoneal injections refer to injections performed into the abdominal cavity. Such method of administration can be implemented for cancers that spread surfaces of the peritoneal cavity such as ovarian, colorectal, and appendiceal cancers. Shao et al. performed an intraperitoneal injection in mice of a DNA supramolecular hydrogel (DSH) loaded with CpG ODNs, the tumor-associated antigen MUC1, and peptide P30 [97]. This DSH immunotherapy delivery system mimicked the function of a lymph node and resulted in strong recruitment and activa-

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TABLE 1

Hydrogels used for pre-clinical studies of delivery of immunotherapy payloads organized by hydrogel chemistry, summarizing modes of administration and key results of the study.

Hydrogels	Payload(s)	Features	Stage of study	Mode of administration	Results	Reference
Synthetic Polymers						
Poly vinyl alcohol (PVA) + TSPBA, ROS-responsive hydrogel	Anti-PD-L1 antibody & IPI-549 (PI3 kinase inhibitor)	ROS-responsive scaffold	*In vivo* (mouse, CT26/4T1 tumors)	IT injection	Inhibits tumor progression & metastasis; protects against tumor rechallenge	Li et al. (2022)[81].
Graphene oxide (GO) and polyethylenimine (PEI) hydrogel	mRNA nanovaccine encoding ovalbumin, resiquimod (TLR7/8 agonist)	Interface transformation to release nanoparticles	*In vivo* (mouse, B16-OVA melanoma model)	SC injection	30-day release; inhibit tumor growth & generate tumor antigen antibodies to prevent metastasis	Yin et al. (2021)[93].
Nanofiber hydrogel (betamethasone phosphate + calcium ions)	Anti-PD-L1 antibody	Hydrogel is formed from betamethasone phosphate, an anti-inflammatory steroid drug	*In vivo* (mouse, CT26 tumors)	IT injection	Systemic anti-tumor responses & effective T cell activation in abscopal tumors; reprogramming of immunosuppressive TME	Chen et al. (2020)[94].
Polysaccharides						
Sodium alginate (SA) hydrogel: SA microsystem SA nanogel	1) TLR agonists (CpG ODNs) 2) Anti-PD-L1 antibody + indocyanine green (ICG)	Anti-PD-L1 antibody attached to surface of nanogel	*In vivo* (mouse, lung metastasis 4 T1 tumor model)	1) SC injection 2) IV injection	Inhibition of tumor postoperative recurrence and metastasis	Zhang et al. (2021)[87].
Hyaluronic acid low viscosity hydrogel (LVH)	CAR T cells	CAR T cells treat glioblastoma by targeting EGFRvIII antigen	*In vivo* (mouse, U87MG human cell line)	IC injection via convection enhanced delivery (CED)	Delivery rate of CAR T cells increased by 20X by using the hydrogel; no acute toxicity to brain	Atik et al. (2018)[21].
Proteins/Peptides						
Fibrin-based hydrogel scaffold	Dendritic cells (DCs)	Prevents direct exposure of DCs to immunosuppressive tumor microenvironment	*In vivo* (mouse, cervical cancer & melanoma model)	Surgical implantation after tumor resection	Strong anti-tumor immune response locally and systemically; immunocyte infiltration into DC scaffold	Verma et al. (2016)[91].
Melittin hydrogel	Tumor cell-derived secretions (CDS) & hypochlorous acid (HOCl)	MELR hydrogel: Melittin cross-linked to $RADA_{24}$ polypeptide -superior drug loading, sustained release, cytotoxic effects -also combined with anti-PD-1	*In vivo* (mouse, B16-F10 melanoma model)	IT injection	Promoted tumor cell death, cytotoxic T lymphocyte infiltration, and augmented therapeutic effects of anti-PD-1	Zhou et al. (2022)[95].
Nap-GFFpY-OMe peptide supramolecular hydrogel	Ovalbumin (OVA)	Used both L- and D-peptides for hydrogels; D-peptide more effective	*In vivo* (C57BL/6J mouse)	SC injection	Enhanced antigen uptake, induced dendritic cell maturations, evoked germinal center formation	Wang et al. (2016)[96].
Supramolecular Nanofibrils of thymopentin and indocyanine green	Thymopentin (TP5) and indocyanine green (ICG)	Co-assembly of clinically approved drugs	*In vivo pancreatic tumor-bearing mice*	IT injection	Integration of rapid photothermal therapy and moderate immunomodulation for inhibiting tumor growth	Li et al. (2021)[74].
Nucleic Acids						
DNA supramolecular hydrogel (DSH)	CpG ODN, tumor-associated antigen MUC1, peptide P30	CpG incorporated directly into DNA hydrogel network	*In vivo* (mouse, B16-F0 cell line)	IP or SC injection	Recruitment and activation of macrophages; induce high titer antibody response	Shao et al. (2018)[97].
STINGel (Stimulator of Interferon Genes)	Cyclic dinucleotides (CDNs) - class of stimulator of	Self-assembling, multidomain peptide hydrogel fused to CDNs	*In vivo* (mouse, MOC2-E6E7 tumor	IT injection	60% of STINGel treated mice achieved complete anti-tumor response and	Leach et al.

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TABLE 1 (*CONTINUED*)

Hydrogels	Payload(s)	Features	Stage of study	Mode of administration	Results	Reference
	interferon genes (STING)	via electrostatic interactions	cells)		acquired immunity	(2018)[98].
CpG DNA hydrogel	Melanin, CpG ODN, STING agonist (c-di-GMP)	CpG forms hydrogel structure and is coated with melanin for photothermal immunotherapy	*In vivo* (mouse, CT26 cancer cell line)	IT injection	Complete ablation of primary tumor, boosted release of tumor antigens, activation of DCs; systemic immune response	Wu et al. (2021)[88].
Multimodule DNA network hydrogel	PD-1 aptamer, CpG ODN, captured T-cells	PD-1 aptamer captures infiltrating T cells and blocks immune checkpoint of T cells	*In vivo* (mouse, melanoma model)	IT injection	Efficient isolation of tumor-infiltrating T-cells; high efficacy in localized immunotherapy	Yao et al. (2021)[99].
Hybrids						
Poly (ethylene glycol)-g-chitosan hydrogel	Genetically modified T lymphocytes	Treatment of glioblastoma (most aggressive and fatal form of brain tumor)	*In vitro* (U-87 MG human cell line)	Intended for IC injection	T lymphocytes killed about 30% of glioblastoma cells	Tsao et al. (2014) [100].
Hyaluronic acid functionalized with levodopa and poly(ε-caprolactone-*co*-lactide) ester	GM-CSF & OVA-expressing plasmid	Sol-gel phase transition between room and body temperature	*In vivo* (mouse, human lung carcinoma model)	SC injection	Enhanced recruitment of DCs, macrophages, and neutrophils	Duong et al. (2020) [101].
Poly (ethylene glycol) diacrylate – gelatin crosslinked hydrogel	M1 macrophages	Gelatin modified with cysteine	*In vivo* (mouse, hepatocellular carcinoma model)	SC injection directly adjacent to tumor	Induced apoptosis in HCC cells and tumor regression	Guerra et al. (2017)[92].

SC = subcutaneous, IV = intravenous, IT = intratumoral, IP = intraperitoneal, IC = intracranial.

tion of macrophages due to the high local concentration of cytosine-phosphate-guanine. Ovarian cancer, which tends to confine itself to the peritoneal cavity, is an important target for intraperitoneal injections of immunotherapies and chemotherapies, and using drug-loaded hydrogels to treat ovarian cancer can further improve efficacy and reduce systemic adverse effects [115].

Intracranial delivery of cancer therapies is another area that is gaining significant attention [21]. Intracranial delivery is motivated by the fact that the blood–brain barrier significantly lowers the bioavailability of large molecule drugs in the brain. [116]. Additionally the fast clearance of the drugs from the brain can limit the efficacy of the treatment. [117]. Drug-laden scaffolds such as Gliadel wafers containing carmustine have been implanted for delivery of chemotherapy agents post-surgery. [118]. Atik et al. performed an intracranial injection of a hyaluronic acid (HA) low viscosity hydrogel (LVH) carrying CAR T cells to treat glioblastoma in mice [21]. Delivery was improved using a technique called convection enhanced delivery (CED), which improves drug distribution by creating a positive pressure gradient. This HA-LVH delivery system increased the rate of delivery of CAR T cells by 20 times, as compared to a saline solution carrier, and did not cause any acute brain toxicities.

Pulmonary delivery of drug-loaded hydrogels involves formulating the hydrogel so that it can be inhaled and can be used to treat lung cancers more effectively. However, there are currently no controlled- or sustained-release pulmonary drug formulations on the market, largely due to the effective airway clearance mechanisms of the lungs [119]. Hydrogels may prevent rapid airway clearance of immunotherapies, further improving their efficacy. Nikjoo et al. developed hyaluronic acid hydrogel microparticles through a combination of chemical crosslinking with either urea or glutaraldehyde followed by spray drying [111]. The spherical hydrogel microparticles had diameters ranging from 2.3 to 3.2μ m and exhibited adequate aerosolization and swelling performance. However, *in vivo* studies using these hydrogel inhalation powders loaded with drugs are needed.

Hydrogels for combinatorial therapies

Combinatorial immunotherapy has demonstrated exciting potential to improve cancer outcomes over single agent treatments. For example, pre-clinical studies show that combining the checkpoint blockade inhibitors anti-PD-1 and anti-CTLA-4 has improved anti-tumor immune responses compared to each agent alone, suggesting they have a synergistic effect [25]. Immunotherapies can also be combined with other therapies, such as chemotherapy [120]. or radiation therapy [121]. However, chemotherapy can have immune-suppressive effects, so the combination of immunotherapy and chemotherapy requires careful dosing and timing [122]. Hydrogels can be used to effectively deliver combinations of immunotherapies in a localized, targeted, and sustained manner, which can further improve synergistic effects while limiting toxicities. Combinatorial immunotherapies with the goal of reducing systematic toxicity combined with local administration strategies for achieving sustained release can be a potential solution for treating tumors. Studies have shown that intratumoral injection of combinatorial

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TABLE 2

Clinical studies involving hydrogels for treatment of cancer registered in National Library of Medicine (NLM) at the United States National Institutes of Health (NIH). The clinical trials involving use of hydrogels as spacers, tracers, or in post-treatment healing (e.g., wound healing, dermatitis treatment) for cancer patients are excluded as they are beyond the scope of this review.

Hydrogels	NCT Number	Payload(s)	Target	Features	Status	Mode of administration
Immunotherapy						
Mucoadhesive and				thermosensitive hydrogel (Poloxamer 407)		NCT04062721
GM-CSF and Mifamurtide (a TLR agonist)	Unresectable colorectal liver metastases	In situ		immunotherapy muco-adherent hydrogel	Not yet recruiting	IT injection
Chemotherapy						
TC-3 Hydrogel (UroGen)	NCT02891460 NCT01648010 NCT01803295 NCT02307487	Mitomycin-C	Invasive bladder cancer	Thermosensitive hydrogel	Completed	IVES injection
Lifepearls microspheres	NCT04595266	Irinotecan	Colorectal/liver cancer	Improved tumor penetration by avoiding proximal occlusion of the vessels.	Recruiting	Intra-arterial injection of beads (embolization)
UGN-102 hydrogel (UroGen)	NCT05243550 NCT05136898 NCT03558503 NCT04688931	Mitomycin-C	Bladder cancer	Thermally responsive gel	Active, not recruiting	IVES injection
Polyvinyl alcohol polymer hydrogel beads	NCT02470533	Doxorubicin	Hepatocellular carcinoma	Hydrogel for slow release	Unknown	Intra-arterial injection of beads (embolization)
Embozene TANDEM microspheres.	NCT04803019	Doxorubicin	Hepatocellular carcinoma	TANDEM embozene microspheres. non-resorbable, microspheres. coated with an inorganic perfluorate polymer.	Recruiting	Intra-arterial injection of beads (embolization)
Hormone Therapy						
Supprelin LA	NCT01394263 NCT01697384	Histrelin hydrogel Goserelin	Prostate cancer	Non-biodegradable, diffusion-controlled, hydrogel for slow release.	Completed	Subdermal implant

IT = intratumoral, IVES: intravesical.

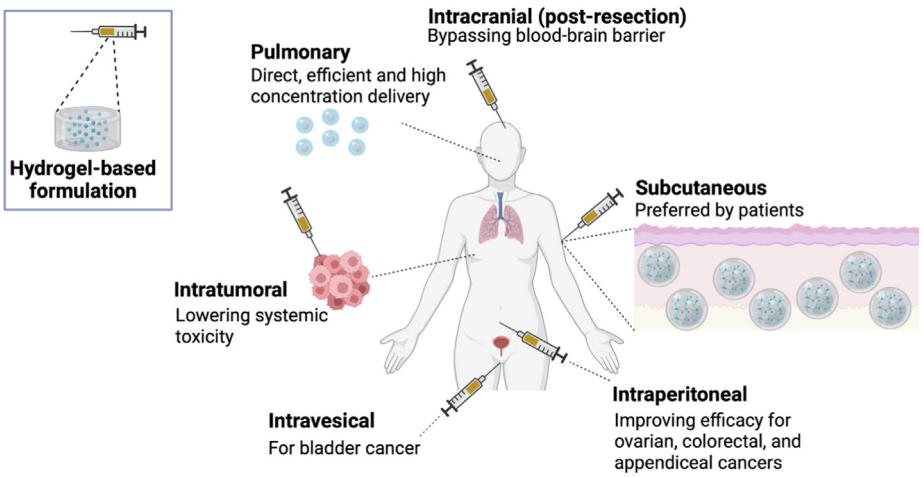

FIGURE 4

Local (non-intravenous) routes of administration of immunotherapy agents with hydrogels [107–109]. Hydrogel-based formulation have been studied for administration of immunotherapies for intratumoral or peritumoral [107,108], intraperitoneal [107], subcutaneous [110], intracranial [109], intravesical, and pulmonary delivery [111]. Hydrogels are amenable to various routes of administration and can be adapted to the tumor type/location.

therapies can help in treatment of large tumors otherwise untreatable using non-combinatorial intravenously administrated treatments [104]. Fig. 5 illustrates examples of delivery strategies for combinatorial immunotherapies using hydrogels.

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Combination of proinflammatory cytokines such as interleukin-2 (IL-2), IL-12, and IL-15 with other immunotherapy agents can be a powerful strategy to improve the efficacy of immunotherapies [50]. Systemic IL-2 therapy, despite receiving FDA approval, can cause severe adverse effects limiting its clinical use. Similarly, systemic IL-12 therapy has not advanced into phase 3 clinical trials because of its narrow therapeutic window. Delivery of these proinflammatory cytokines with hydrogels can reduce these adverse effects and maximize efficacy. Wu et al. delivered IL-15 and cisplatin, a chemotherapy drug, within a PEG-poly(γ-ethyl-l-glutamate) diblock copolymer hydrogel to treat melanoma-bearing mice [120]. The hydrogel was administered via peritumoral injection and resulted in tumor cell cycle arrest, proliferation of $CD8^+$ T cells, and reduced systemic toxicity. Combining proinflammatory cytokines with checkpoint blockade inhibitors has also demonstrated promising results. A pre-clinical study in a mouse metastatic colon carcinoma model found that combining IL-15 with antibodies against both PD-L1 and CTLA-4 significantly increased anti-tumor activity over IL-15 alone [125]. It is noteworthy very few studies have investigated the delivery of cytokines and checkpoint blockade inhibitors together in hydrogels. This combination of cytokines and checkpoint blockade inhibitors co-delivered with hydrogels should be explored, as local and sustained delivery of these immunotherapy agents together may demonstrate high efficacy.

Checkpoint blockade inhibitors have been combined with other agents using hydrogels as a delivery platform. Kim et al. delivered monoclonal antibodies to CTLA-4 combined with S-nitrosoglutathione (GSNO), a nitric oxide donor, packaged within a Pluronic® F127-gelatin hydrogel in a mouse melanoma model [9]. Interestingly, at high concentrations nitric oxide has anticancer properties, including pro-apoptotic and anti-angiogenic signaling. The hydrogel and payloads were administered via subcutaneous injection and resulted in DC expansion and activation and significantly slowed the tumor progression in both primary and secondary tumors. The combination of anti-CTLA-4 monoclonal antibody and GSNO demonstrated significant anti-tumor effects, while monotherapy with either agent showed minimal efficacy. Liu and colleagues delivered a synthetic ^{D}PPA-1 peptide, a checkpoint blockade inhibitor that binds to PD-L1 on tumor cells, and doxorubicin, a chemotherapy, to tumor-bearing mice using a hyaluronic acid-based supramolecular hydrogel [63]. The thermoresponsive hydrogel was administered via intratumoral injection and this synergistic chemo-immunotherapy approach had strong anti-tumor effects. Sustained release of doxorubicin induced immunogenic cell death (ICD) of cancer cells and the ^{D}PPA-1 peptides activated T-cell mediated immune responses. Additionally, the synthetic ^{D}-PPA-1 peptides possess several advantages over traditional checkpoint blockade inhibitors, such as lower manufacturing costs, less immunogenicity, and higher stability.

Table 3 describes a variety of combinatorial immunotherapies delivered with hydrogels. The only hydrogel-immunotherapy approach for cancer currently in clinical trials (phase 1) is Poloxamer 407, a mucoadhesive thermosensitive hydrogel, delivering GM-CSF and mifamurtide, an immunomodulator that activates monocytes and macrophages [16]. This treatment is indicated for unresectable colorectal liver metastases, and the hydrogel is administered via intratumoral injection after radiofrequency ablation (RFA).

Other immune modulators, such as STING agonists and TLR agonists, have also been effectively used in combinatorial immunotherapies and delivered with hydrogels. Wang and colleagues delivered cyclic di-AMP (a STING agonist) and the chemotherapy camptothecin using a self-assembling amphiphilic hydrogel [128]. The hydrogel was synthesized by chemically conjugating the hydrophilic peptide iRGD to the hydrophobic camptothecin, and it spontaneously assembles into supramolecular nanotubes in aqueous solution. The drug camptothecin induces cell death by causing DNA damage, which leads to the release of fragmented DNA into the cytosol and subsequent stimulation of the STING pathway resulting in synergistic immune activation [129]. The hydrogel and chemo-immunotherapy combination was administrated intratumorally and resulted in significant tumor regression, 100% animal survival, and long-term immunological memory and surveillance in mice. Smith et al. designed an alginate porous scaffold to deliver STING agonists in combination with CAR T cells [124]. The scaffold was surgically implanted into mice and resulted in synergistic activation of host APCs, increased T cell activation, and systemic anti-tumor immune activity.

Sustained release and localization of immunotherapy agents using hydrogels

Sustained-release therapies have demonstrated strong advantages over single-dose therapies [15,105,130]. Tam et al. addressed a fundamental question on the effects of a sustained dose on the immune response and compared it to a single dose [130]. They investigated the immune response to model HIV subunit vaccines and found that an exponentially increasing dosing regimen significantly increased antibody production relative to constant dosing and a bolus vaccination. In fact, exponentially increasing dosing led to greater than 10-fold higher antibody levels compared to bolus vaccination [130]. These findings can be applied to the administration of immunotherapies and the kinetics of hydrogel release. Hydrogels offer a distinct advantage in that they can be locally administrated and designed to release drugs over an extended period, which can potentially prevent dose-related toxicities and increases effectiveness. Localization of immunotherapies with hydrogels can broaden their therapeutic index (Fig. 6), making the drugs safer and more effective. The sustained release kinetics achieved by hydrogels can help in maintaining the drug concentrations within the therapeutic level over time, thus enhancing its effectiveness [131]. Additionally, sustained-release hydrogel formulations can eliminate the need for multiple immunotherapy dosing, which reduces the burden on patients. Hydrogel structure and cross-linking can be easily tuned and modified to achieve desired drug release kinetics. Hydrogels also act as barriers to prevent rapid degradation of labile immunotherapeutic molecules, which increases their residence time and effectiveness.

Pre-clinical studies have indicated that sustained release of immunotherapies can help in preventing tumor recurrence and metastases [107]. Park et al. developed a stable hyaluronic acid hydrogel scaffold to deliver various immunotherapies to tumor-

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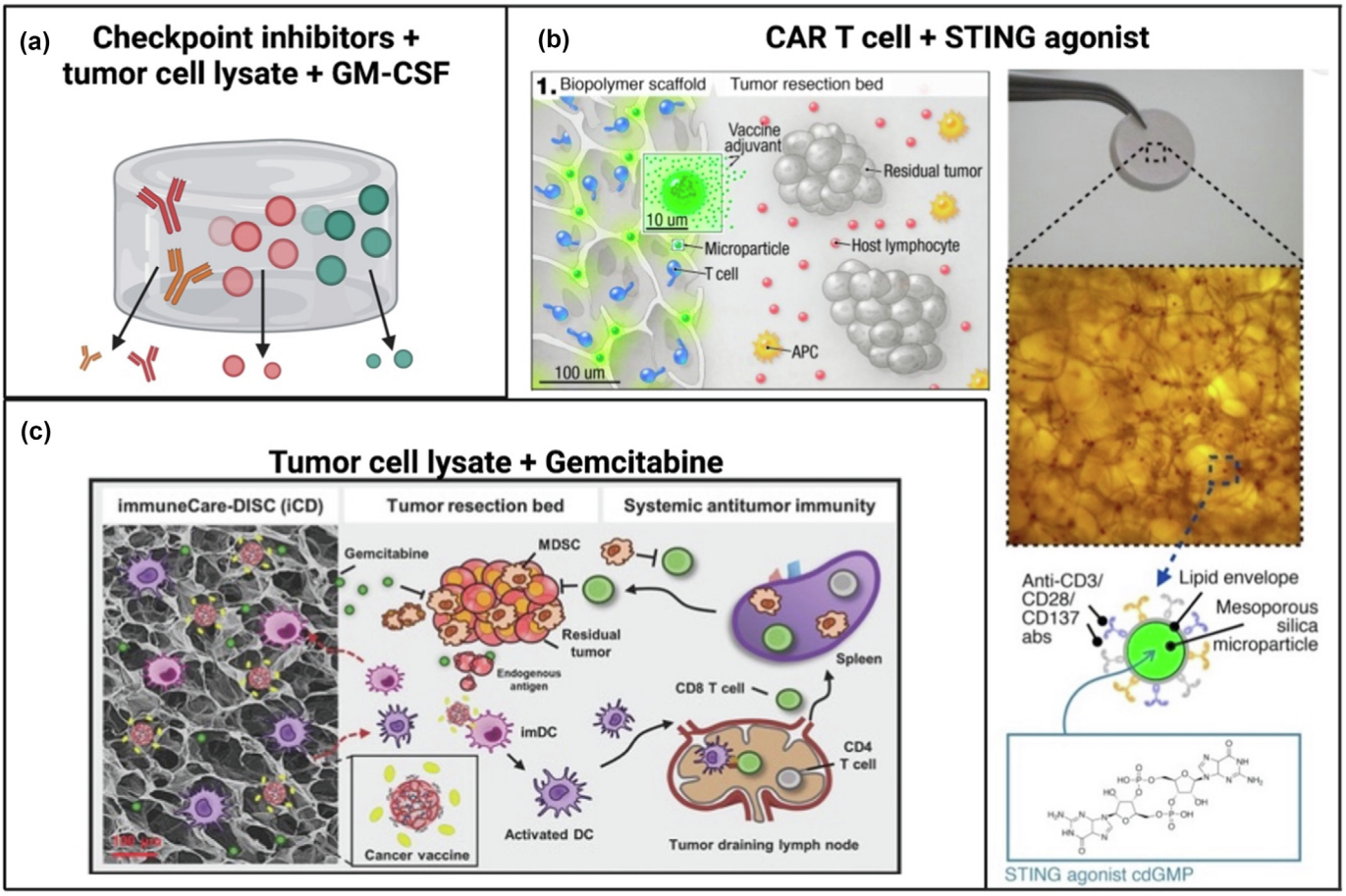

FIGURE 5

Combinatorial immunotherapies delivered with hydrogels. (A) Cocktail of immune checkpoint blockade inhibitors + tumor cell lysate + GM-CSF delivered using an injectable peptide hydrogel, resulting in superior immunotherapy effects over monotherapy [123]. (B) CAR T cell + STING agonist delivered using alginate porous scaffolds, resulting in an enhanced immune response and systemic anti-tumor immunity [124]. (C) ImmuneCare-DISC (iCD) delivery of tumor cell lysate + gemcitabine, resulting in systemic anti-tumor immunity and prevention of tumor recurrence [80]. Reprints with permission from [80,124].

bearing mice, which significantly extended the drug release [107]. The hydrogel scaffold began degrading at 5 weeks post-administration and was completely resorbed by 12 weeks [107]. Importantly, this study found that extended local release of immunotherapies using hydrogels increased the number of anti-tumor immune cells, eliminates metastases, and prevented tumor recurrence.

Challenges still exist with the local administration of immunotherapies using hydrogels. Local injection does not guarantee local retention [105]. Smaller therapeutics like cytokines which are administered intratumorally can rapidly disperse systematically driven by high intratumoral pressure [134]. In addition, activated immune cells and the cytokines and chemokines they secrete can also disseminate and exert a systemic response, which can be very dangerous to the patient [135]. Successful efforts have been made to prevent this rapid leakage of small immunotherapeutics from the tumor, such as fusing cytokines to lumican, a collagen-binding protein [105]. This strategy is based on the abundance of collagen in many tumors. A similar strategy could be used for hydrogel delivery, in which collagen-binding proteins are bound to the surface of the hydrogel.

Challenges in translational Research, clinical Studies, and manufacturing of Hydrogel-Based immunotherapies

Despite the many pre-clinical successes of hydrogels for the delivery of immunotherapies for cancer, hydrogels still face challenges in getting to the clinic and eventually approval. First, since hydrogel-enabled drug delivery is a relatively new advancement, years of research are required to ensure the safety of hydrogel materials *in vivo*. Challenges in translating hydrogels include design, GMP (good manufacturing practice) manufacturing, and regulatory approvals [136].

In most cases, the FDA considers hydrogels as a device to deliver the therapeutics. In some cases, the FDA classifies some hydrogels as biologics, such as ECM-derived hydrogels because they are derived from animal tissue, so it is required to get regulatory approval for not only the immunotherapy but also the hydrogel itself [137]. The regulatory cost vary significantly depending on the type of approval. For instance, while the average cost of approval for a device is $50 million, approval costs of a biologic is $800 million [138]. Furthermore, drug-laden hydrogels are considered a combination product by the FDA, requiring an estimated approval time of 7–10 years [136].

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TABLE 3

Combinatorial immunotherapies delivered with hydrogels. Categories include cytokines, checkpoint blockade inhibitors, STING/TLR agonists, and tumor antigens combined with other cancer therapies to achieve synergistic effects.

Payloads	Hydrogel	Features	Stage of study	Results	Mode of administration	Reference
GM-CSF + mifamurtide	Mucoadhesive thermosensitive hydrogel (Poloxamer 407)	Immunotherapy administered after radiofrequency ablation (RFA); indicated for unresectable colorectal liver metastases	Pre-clinical, Phase 1 clinical trials	No results in humans; study not yet recruiting	IT injection	Lemdani et al. (2019) [16]
GM-CSF + tumor cell lysate + CpG ODN	PLG (poly lactide-co-glycolide) implantable scaffold	Immune checkpoint antibodies (anti-PD-1and anti-CTLA-4) administered systemically in combination with hydrogel	*In vivo* (mouse, B16 tumor model)	B16 (melanoma) tumor regression and 75% survival rates	SC injection	Ali et al. (2016)[110]
IL-15 + chemotherapy (cisplatin)	PEG-poly(γ-ethyl-l-glutamate) diblock copolymer hydrogel	Thermosensitive polypeptide hydrogel	*In vivo* (mouse, B16F0-RFP melanoma cell line)	Tumor cell cycle arrest; recruitment/ proliferation of $CD8^+$ T cells/ NK cells; reduced systemic toxicity	Peritumoral injection	Wu et al. (2017)[120]
Anti-PD-1		antibody + DCs + tumor antigens	RADA16 peptide-based self-assembling nanofibrous hydrogel	Peptide spontaneously assembles into nanofibers in aqueous solution	*In vivo* (mouse, EG7-OVA tumor cell line)	Improved DC viability and duration time at tumor; proliferation and infiltration of intratumoral $CD8^+$ T cells
SC injection	Yang et a. (2018)[126]					
Anti-PD-1 + anti-angiogenic (celecoxib)	Alginate hydrogel	Celecoxib inhibits angiogenesis in tumors	*In vivo* (mouse)	Increased $CD4^+$ and $CD8^+$ T cells, reduced Tregs and MDSCs in TME	SC injection	Li et al. (2015)[127]
Anti-CTLA-4 + S-nitrosoglutathione (GSNO, a nitric oxide donor)	Pluronic® F127 + gelatin hydrogel	At high concentrations, NO has anticancer properties: pro-apoptotic signaling, anti-angiogenesis	*In vivo* (mouse, B16F10-OVA melanoma cell line)	DC expansion and activation, expansion of CTLA-4-expressing immune cells; synergistic anti-tumor effects	SC injection	Kim et al. (2022)[9]
^{D}PPA-1		peptide + chemotherapy (doxorubicin)	Hyaluronic acid-based supramolecular hydrogel	^{D}PPA-1 peptide has high binding affinity to PD-L1 on tumor cells	*In vivo* (mouse and rabbit, CT26 tumor cells)	Direct killing of tumor cells by DOX and improved T-cell-mediated immune response by ^{D}PPA-1 peptide
IT injection	Liu et al. (2021)[63]					
STING agonist (cyclic di-AMP) + chemotherapy (camptothecin)	Self-assembling drug amphiphile hydrogel (supramolecular nanotubes)	Peptide-drug conjugate: iRGD binds to NRP1, highly expressed in tumors	In vivo (mouse, CT26, 4 T1, GL-261 cell lines)	Tumor regression and increased animal survival (100%); long-term immunological memory	IT injection	Wang et al. (2020) [128]
STING agonist + CAR T cells	Alginate porous scaffolds	Scaffolds had migration-promoting macromolecules (e.g., a collagen-mimetic peptide) and stimulatory antibodies on microparticles	*In vivo* (mouse)	Synergistic activation of host APCs, improved T cell activation; systemic anti-tumor immunity	Surgical implantation	Smith et al. (2017) [124]
CpG ODN + Iodine-131 (^{131}I) (radioisotope	Sodium alginate hydrogel	Rapid *in situ* gelation of hydrogel; systemic administration of anti-CTLA-4	*In vivo* (mouse, 4 T1 tumor	100% tumor elimination, no evident toxicities to animals	IT injection	Chao et al. (2018) [121]

(continued on next page)

TABLE 3 (*CONTINUED*)

Payloads	Hydrogel	Features	Stage of study	Results	Mode of administration	Reference
therapy) + catalase			cells)			
Tumor cell lysate + chemotherapy (gemcitabine)	Collagen + hyaluronic acid (HA), implantable	immuneCare DISC (iCD) scaffold	*In vivo* (mouse)	Prevention of tumor recurrence and metastasis	Post-surgical implantation	Phuengkam et al. (2018) [80]

Many biomaterials have not yet demonstrated safety in clinical trials, so this creates a barrier to innovation and adoption by biopharmaceutical companies. However, efforts have been made to identify biomaterials that mitigate immune responses, such as the study conducted by Vegas et al. which screened a combinatorial hydrogel library for materials that mitigated immune responses in primates [139]. Another challenge is the lack of collaboration between clinicians and the engineers or materials scientists designing hydrogels. By facilitating collaboration between these groups, the unmet clinical need can be identified, and the hydrogel can be designed in such a way that it is compatible with the type of cancer, immunotherapy drug, and optimal drug release kinetics. As more hydrogels enter the clinic, clinicians may be more willing to experiment with unconventional hydrogel delivery systems. The possibility or feasibility of intratumoral injection with hydrogels is another concern, as this procedure has not yet been performed in humans and could be difficult depending on the tumor location.

For translation of hydrogel-based immunotherapy treatments from bench to clinic, perhaps the two most important topics that need to take center stage are the chemistry, manufacturing, and controls (CMC) management, and good manufacturing practices in scaled-up processes for hydrogel manufacturing. Methods of manufacturing hydrogels are not compatible with current GMP practices in the biologics industry which can discourage private industry investment. There should be great emphasis on developing methods for hydrogel synthesis that can be adaptable to large-scale processing with a focus on robustness, safety, and compatibility with current GMP facilities. Additionally developing platform-based technologies that can be adapted to multiple drug delivery scenarios can help in overcoming this hurdle.

In the case of material selection, one of the most common FDA-approved polymeric materials studied for biomacromolecule drug delivery is poly(lactic-co-glycolic acid). Nevertheless, it has been shown that these polymer networks can cause protein aggregation and denaturation which can take place during encapsulation or storage. [140]. This can be attributed to the harsh conditions for encapsulation and particle formation that can promote protein destabilization. Polyethylene glycol (PEG) based hydrogels are another type of material that have been widely studied for biomacromolecule drug delivery [141]. However, hydrogels made with PEG are highly permeable, making long-term (weeks) sustained release (necessary for eliminating solid tumors) very difficult to achieve. Similarly, at high concentrations, mAb molecules encapsulated in such permeable hydrogel networks at physiological temperature have limited colloidal stability, which can cause further aggregation [142]. Perhaps even more importantly, the processes used for encapsulating biologics are not fully compatible with labile nature of these molecules and are not compatible with clinical research. To form these hydrogels, chemical reactions (involving chemical reagents or UV light) take place in the presence of active immunotherapy biomacromolecules which can generate physical or chemical damage or impurities Such impurities and be a major concern in clinical studies.

As discussed previously, another challenge of immunotherapy delivery is drug leakage or escape from the tumor site, which might not adequately be addressed through hydrogel delivery

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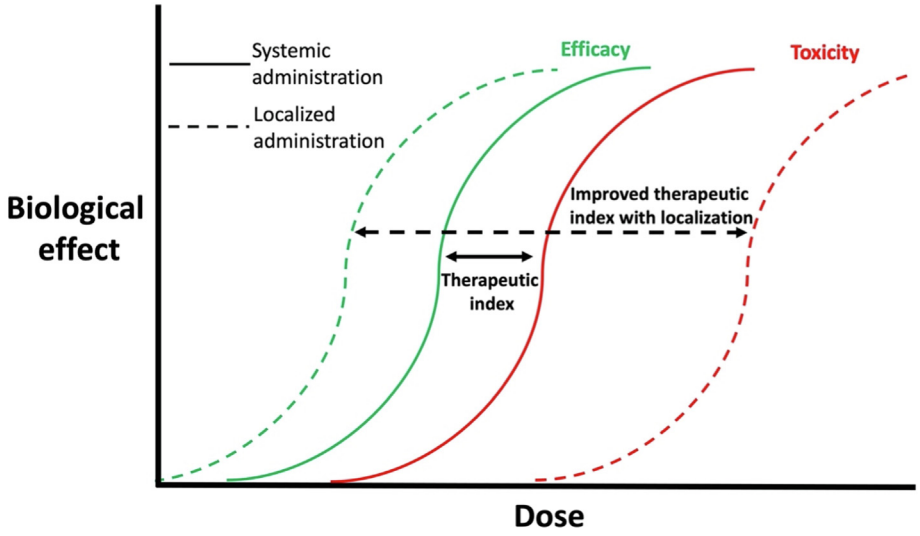

FIGURE 6

Effect of drug localization on widening the therapeutic index [132]. Widening the therapeutic index is an important challenge in immunotherapy [54,133]. Local administration can increase the concentration in the desired tissue (hence enhance efficacy) while lowering the systemic concentration (hence lowering the systemic toxicity).

systems. More pre-clinical studies are needed to assess the ability of hydrogels to maintain drugs within tumors at therapeutic levels with minimal systemic escape. Finally, the quality and consistency of hydrogel manufacturing methods are a significant challenge. Since hydrogels for immunotherapy delivery are currently in the pre-clinical stage, they are synthesized in small batches and are often not compatible with large-scale manufacturing. The heterogeneity of natural polymer-based hydrogels can pose a problem for manufacturing consistency and consistent properties, such as drug release and stability [136]. New hydrogel fabrication strategies must be developed, and these hydrogels must demonstrate high batch-to-batch consistency to ensure safety and eventual regulatory approval. Additionally, the reliability and consistency of *in situ*-forming hydrogels must be carefully assessed upon administration to humans.

Concluding remarks and future perspective

Immunotherapies have demonstrated the ability to modulate the immune system to treat a variety of cancer types. However, immunotherapies currently face several challenges, including the need for high doses (which can cause toxicities) and a limited effectiveness in solid tumors. Hydrogel-based delivery platforms are strongly suited for the delivery of immunotherapies, as they allow for the localization and sustained release of drugs, minimizing systemic toxicities and maximizing effectiveness. Hydrogels can be synthesized from a variety of different natural, synthetic, and hybrid materials and can be modified to have many desirable drug delivery properties.

In pre-clinical studies, hydrogel delivery systems have demonstrated higher efficacy than immunotherapy agents delivered alone, while also preventing systemic toxicities. Hydrogels are amenable to various routes of administration, and local or intratumoral delivery approaches have demonstrated high efficacy in pre-clinical mouse models with many different tumor types. Combination immunotherapies delivered locally with hydrogels often have synergistic effects and can result in greater efficacy than monotherapy. Sustained delivery through hydrogels broadens the therapeutic index of drugs, increasing efficacy and decreasing toxicities.

Through continued research and development, hydrogels for the delivery of immunotherapies for cancer possess promising future prospects. Pre-clinical immunotherapy studies often use multiple doses, which can be especially burdensome for patients. High concentration, sustained release hydrogel delivery platforms are effective approaches to reduce patient burden by requiring fewer doses. These hydrogel platforms may make the delivery of narrow therapeutic index immunotherapies, such as IL-12, possible. The trend toward combinatorial immunotherapy treatments can be assisted through hydrogel delivery platforms, as they have demonstrated minimal toxicities and increased efficacy through localization and sustained release.

Although combinations of checkpoint blockade antibodies and cytokines are being tested in clinical trials with promising results, there is a lack of research using hydrogels to co-deliver combinations of these immunotherapies. Several Phase I/II clinical trials are currently investigating the use of the cytokines IL-2, IL-10, IL-12, IL-15, IL-21, or IFN-α in combination with PD-1/PD-L1 checkpoint blockade to treat various cancer types, such as renal cell carcinoma, metastatic solid tumors, and non-small cell lung cancer [143]. The combinatorial delivery of checkpoint blockade inhibitors and cytokines using hydrogels could be a very fruitful area of future research. The combination and local administration of chemokines to direct the immune response towards cancerous tumors with other immunotherapy agents must be explored, as this research is currently lacking. Continued pre-clinical screening and testing of new hydrogel materials to assess their immunogenicity will enable more advanced hydrogel materials to reach the clinic sooner.

Finally, as hydrogel manufacturing methods improve and scale, hydrogel delivery platforms will be more readily adopted

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by clinicians and the biopharmaceutical industry. A successful product development roadmap for delivery of immunotherapy agents using hydrogels should place emphasis on the technology's versatility, safety/efficacy, and manufacturability. The time and cost of the approval process for combination products such as drug-laden hydrogels, are the most important barriers for introducing such technologies for immunotherapy to the clinic. As such, a feasible strategy to overcome these problems would be to pursue hydrogel-based immunotherapies as second-generation products for already approved immunotherapies. Such strategy can entice the biopharmaceutical industry for larger investments as part of life product cycle management strategy while bringing real benefits (improved efficacy, lower toxicity, and improved patient preference) to the patients.

Data availability

No data was used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] X. Li et al., Cell. Mol. Immunol. 18 (6) (2021) 1607–1609.
[2] J. Eno, J. Adv. Pract. Oncol. 8 (7) (2017) 747–753.
[3] W.B. Coley, Am. J. Med. Sci. 1827-1924 105 (6) (1893) 487.
[4] C.R. Parish, Immunol. Cell Biol. 81 (2) (2003) 106–113, https://doi.org/10.1046/j.0818-9641.2003.01151.x.
[5] D.G. Leach, S. Young, J.D. Hartgerink, Acta Biomater. 88 (2019) 15–31, https://doi.org/10.1016/j.actbio.2019.02.016.
[6] J.A. Joyce, D.T. Fearon, Science 348 (6230) (2015) 74–80.
[7] F. Märkl et al., Trends Cancer (2022), https://doi.org/10.1016/j.trecan.2022.04.001.
[8] K. Kohli, V.G. Pillarisetty, T.S. Kim, Cancer Gene Ther. 1–12 (2021).
[9] J. Kim et al., Nat. Commun. 13 (1) (2022) 1479, https://doi.org/10.1038/s41467-022-29121-x.
[10] O. Hemminki, J.M. dos Santos, A. Hemminki, J Hematol Oncol 13 (1) (2020) 84, https://doi.org/10.1186/s13045-020-00922-1.
[11] I. Palaia et al., OncoTargets Ther. 13 (2020) 6109.
[12] Y. Tokumaru, D. Joyce, K. Takabe, Surgery 167 (3) (2020) 628–630.
[13] N. Pu, W. Lou, J. Yu, J. Pancreatol. 2 (01) (2019) 6–10.
[14] T.L. Whiteside et al., Clin. Cancer Res. 22 (8) (2016) 1845–1855.
[15] J. Kim, D.M. Francis, S.N. Thomas, Nanomaterials 11 (2) (2021) 471.
[16] K. Lemdani et al., Int. J. Pharm. 567 (2019), https://doi.org/10.1016/j.ijpharm.2019.06.012 118421.
[17] J. Li, D.J. Mooney, Nat. Rev. Mater. 1 (12) (2016) 16071, https://doi.org/10.1038/natrevmats.2016.71.
[18] A. Erfani et al., J. Appl. Polym. Sci. 137 (40) (2020) 49550.
[19] F. Abasalizadeh et al., J. Biol. Eng. 14 (1) (2020) 8, https://doi.org/10.1186/s13036-020-0227-7.
[20] M.E. Wechsler et al., Biomed. Microdevices 21 (2) (2019) 1–15.
[21] A.F. Atik et al., J. Clin. Neurosci. Off. J. Neurosurg. Soc. Australas. 56 (2018) 163–168, https://doi.org/10.1016/j.jocn.2018.06.005.
[22] X. Ma et al., Chem. Soc. Rev. (2022).
[23] A. Diefenbach, D.H. Raulet, Immunol. Rev. 188 (1) (2002) 9–21.
[24] M.M. Gubin et al., Nature 515 (7528) (2014) 577–581.
[25] Z.N. Willsmore et al., Eur. J. Immunol. 51 (3) (2021) 544–556.
[26] P. Berraondo et al., Br. J. Cancer 120 (1) (2019) 6–15, https://doi.org/10.1038/s41416-018-0328-y.
[27] J.W. Fischer, N. Bhattarai, Front. Immunol. 12 (2021).
[28] A. Echarti et al., Cancers 11 (9) (2019) 1398.
[29] R. Franzin et al., Front. Immunol. 11 (2020).
[30] K.M. Hargadon, C.E. Johnson, C.J. Williams, Int. Immunopharmacol. 62 (2018) 29–39, https://doi.org/10.1016/j.intimp.2018.06.001.
[31] Y. Igarashi, T. Sasada, J Immunol. Res. 2020 (2020).
[32] L. Buonaguro et al., Clin. Vaccine Immunol. 18 (1) (2011) 23–34.
[33] Y. Hailemichael et al., *Nat. Med.* 19 (4) (2013) 465–472.
[34] P.A. Ott et al., Nature 547 (7662) (2017) 217–221.
[35] D.B. Keskin et al., Nature 565 (7738) (2019) 234–239.
[36] B.M. Carreno et al., Science 348 (6236) (2015) 803–808.
[37] E. Blass, P.A. Ott, Nat. Rev. Clin. Oncol. 18 (4) (2021) 215–229.
[38] N. Nagarsheth, M.S. Wicha, W. Zou, Nat. Rev. Immunol. 17 (9) (2017) 559–572, https://doi.org/10.1038/nri.2017.49.
[39] T. Su et al., Theranostics 9 (25) (2019) 7759–7771, https://doi.org/10.7150/thno.37574.
[40] J. Zhao et al., Adv. Mater. 34 (10) (2022) 2109254.
[41] M. Luo et al., Nat. Nanotechnol. 12 (7) (2017) 648–654.
[42] S. Li et al., Nat. Biomed. Eng. 5 (5) (2021) 455–466.
[43] S. Adams, Immunotherapy 1 (6) (2009) 949–964, https://doi.org/10.2217/imt.09.70.
[44] S. Guedan, M. Ruella, C.H. June, Annu. Rev. Immunol. 37 (1) (2019) 145–171, https://doi.org/10.1146/annurev-immunol-042718-041407.
[45] A.N. Renrick, Z.T. Dunbar, A. Shanker, Immunotherapy 11 (1) (2019) 15–20.
[46] N. Albinger, J. Hartmann, E. Ullrich, Gene Ther. 28 (9) (2021) 513–527, https://doi.org/10.1038/s41434-021-00246-w.
[47] M. Klichinsky et al., Nat. Biotechnol. 38 (8) (2020) 947–953.
[48] M. Kang et al., Adv. Mater. 33 (43) (2021) 2103258.
[49] C. Chen et al., Sci. Transl. Med. 14 (656) (2022) eabn1128.
[50] D.S. Chen, I. Mellman, Nature 541 (7637) (2017) 321–330.
[51] R.R. Munhoz, M.A. Postow, Cancer J. Sudbury Mass 24 (1) (2018) 7.
[52] H. Ruan et al., Mol. Carcinog. 59 (7) (2020) 783–793.
[53] R. Baluna, E.S. Vitetta, Immunopharmacology 37 (2–3) (1997) 117–132.
[54] I. Puzanov et al., J. Immunother. Cancer 5 (1) (2017) 1–28.
[55] C.H. June, J.T. Warshauer, J.A. Bluestone, Nat. Med. 23 (5) (2017) 540–547, https://doi.org/10.1038/nm.4321.
[56] A.L. Jenner et al., Appl. Sci. 10 (8) (2020) 2872.
[57] I. Lai et al., J. Immunother. Cancer 6 (1) (2018) 1–11.
[58] M. Binnewies et al., Nat. Med. 24 (5) (2018) 541–550, https://doi.org/10.1038/s41591-018-0014-x.
[59] A. Erfani et al., Biomacromolecules 21 (7) (2020) 2557–2573.
[60] J.M. Schieferstein et al., Adv. Ther. 4 (4) (2021) 2000216.
[61] D. Ding et al., ACS Appl. Bio Mater. 1 (3) (2018) 561–571.
[62] A. Bak, M. Ashford, D.J. Brayden, Adv. Drug Deliv. Rev. 136 (2018) 2–27.
[63] M. Liu et al., ACS Appl. Mater. Interfaces 13 (29) (2021) 33874–33884.
[64] X. Ren et al., Acta Biomater. 124 (2021) 179–190.
[65] R. Dong et al., Biomater. Sci. 3 (7) (2015) 937–954.
[66] H. Nasution et al., Gels 8 (9) (2022) 568.
[67] Q. Chai, Y. Jiao, X. Yu, Gels Basel Switz. 3 (1) (2017) 6, https://doi.org/10.3390/gels3010006.
[68] P. Gentile et al., Int. J. Mol. Sci. 15 (3) (2014) 3640–3659, https://doi.org/10.3390/ijms15033640.
[69] P. Zarrintaj, in: A.R. Ajitha, S. Thomas (Eds.), *Compatibilization of Polymer Blends*, Elsevier, 2020, pp. 511–537, https://doi.org/10.1016/B978-0-12-816006-0.00018-9.
[70] S.M.F. Kabir et al., Prog. Biomater. 7 (3) (2018) 153–174, https://doi.org/10.1007/s40204-018-0095-0.
[71] S.H. Um et al., Nat. Mater. 5 (10) (2006) 797–801, https://doi.org/10.1038/nmat1741.
[72] P.A. Janmey, J.P. Winer, J.W. Weisel, J. R. Soc. Interface 6 (30) (2009) 1–10, https://doi.org/10.1098/rsif.2008.0327.
[73] L.A. Haines et al., J. Am. Chem. Soc. 127 (48) (2005) 17025–17029, https://doi.org/10.1021/ja054719o.
[74] S. Li et al., Adv. Mater. 33 (21) (2021) 2100595.
[75] Q. Zou et al., J. Controlled Release 319 (2020) 344–351.
[76] E. Mei et al., Colloids Surf. Physicochem. Eng. Asp. 577 (2019) 570–575.
[77] J. Su et al., Biomaterials 201 (2019) 99–112.
[78] C. Shuai et al., Int. J. Adv. Manuf. Technol. 69 (1) (2013) 51–57.
[79] A. Erfani et al., Soft Matter 17 (21) (2021) 5349–5361.
[80] H. Phuengkham et al., Adv. Mater. 30 (18) (2018) 1706719.
[81] S. Li et al., Adv. Sci. 2104619 (2022).
[82] E.F.S. Vieira et al., Biomacromolecules 9 (4) (2008) 1195–1199, https://doi.org/10.1021/bm7011983.
[83] Y. Wang, D.J. Irvine, Biomaterials 32 (21) (2011) 4903–4913, https://doi.org/10.1016/j.biomaterials.2011.03.027.
[84] K. Ganguly et al., Drug Deliv. Res. Asia Pac. Reg. 193 (2014) 162–173, https://doi.org/10.1016/j.jconrel.2014.05.014.
[85] R. Sun et al., Int. J. Pharm. 613 (2022), https://doi.org/10.1016/j.ijpharm.2021.121390 121390.
[86] H.B. Eral et al., Cryst. Growth Des. 14 (4) (2014) 2073–2082.

Please cite this article in press as: A. Erfani et al., Materials Today, (2023), https://doi.org/10.1016/j.mattod.2023.03.006

[87] Y. Zhang et al., ACS Biomater. Sci. Eng. 7 (12) (2021) 5717–5726, https://doi.org/10.1021/acsbiomaterials.1c01216.
[88] Y. Wu et al., J. Controlled Release 330 (2021) 540–553, https://doi.org/10.1016/j.jconrel.2020.12.040.
[89] P. Katyal, F. Mahmoudinobar, J.K. Montclare, Membr. Eng. Des. 63 (2020) 97–105, https://doi.org/10.1016/j.sbi.2020.04.007.
[90] R. Chang, X. Yan, Small Struct. 1 (2) (2020) 2000068.
[91] V. Verma et al., Oncotarget 7 (26) (2016) 39894–39906, https://doi.org/10.18632/oncotarget.9529.
[92] A.D. Guerra et al., Theranostics 7 (15) (2017) 3732–3744, https://doi.org/10.7150/thno.20251.
[93] Y. Yin et al., Nano Lett. 21 (5) (2021) 2224–2231.
[94] M. Chen et al., Nano Lett. 20 (9) (2020) 6763–6773.
[95] Y. Zhou et al., Bioact. Mater. 9 (2022) 541–553.
[96] H. Wang et al., Adv. Funct. Mater. 26 (11) (2016) 1822–1829.
[97] Y. Shao et al., ACS Appl. Mater. Interfaces 10 (11) (2018) 9310–9314, https://doi.org/10.1021/acsami.8b00312.
[98] D.G. Leach et al., Biomaterials 163 (2018) 67–75, https://doi.org/10.1016/j.biomaterials.2018.01.035.
[99] C. Yao et al., J. Am. Chem. Soc. 143 (46) (2021) 19330–19340, https://doi.org/10.1021/jacs.1c07036.
[100] C.-T. Tsao et al., Biomacromolecules 15 (7) (2014) 2656–2662.
[101] H.T.T. Duong et al., Biomaterials 230 (2020) 119599.
[102] E. Caló, V.V. Khutoryanskiy, Eur. Polym. J. 65 (2015) 252–267.
[103] Z. Hamrang, N.J. Rattray, A. Pluen, Trends Biotechnol. 31 (8) (2013) 448–458.
[104] M. Dai et al., Clin. Cancer Res. 21 (5) (2015) 1127–1138.
[105] N. Momin et al., Sci. Transl. Med. 11 (498) (2019) eaaw2614.
[106] K.D. Ratanji et al., J. Immunotoxicol. 11 (2) (2014) 99–109.
[107] C.G. Park et al., Sci. Transl. Med. 10 (433), eaar1916 (2018).
[108] N.M. Muñoz et al., J. Immunother. Cancer 9 (2021) 2.
[109] J. Zhang et al., Nat. Nanotechnol. 16 (5) (2021) 538–548.
[110] O.A. Ali et al., Cancer Immunol. Res. 4 (2) (2016) 95–100, https://doi.org/10.1158/2326-6066.CIR-14-0126.
[111] D. Nikjoo et al., Pharmaceutics 13 (11) (2021) 1878.
[112] P.M. Overton et al., Patient Prefer. Adherence (2021) 811–834.
[113] J. Schuster et al., J. Pharm. Sci. 110 (6) (2021) 2386–2394, https://doi.org/10.1016/j.xphs.2021.03.007.
[114] A. Erfani et al., Adv. Healthc. Mater. (2023) 2202370.
[115] K. Jaaback, N. Johnson, T.A. Lawrie CD005340–CD005340, Cochrane Database Syst. Rev. No. 11 (2011), https://doi.org/10.1002/14651858.CD005340.pub3.
[116] I. St-Amour et al., J. Cereb. Blood Flow Metab. 33 (12) (2013) 1983–1992.
[117] M. Dostalek et al., Clin. Pharmacokinet. 52 (2) (2013) 83–124.
[118] R. Nishikawa et al., Neurol Med. Chir. (Tokyo) (2021). oa. 2021–0024.
[119] Z. Liang et al., Drug Discov. Today 20 (3) (2015) 380–389, https://doi.org/10.1016/j.drudis.2014.09.020.
[120] X. Wu et al., J. Controlled Release 255 (2017) 81–93.
[121] Y. Chao et al., Nat. Biomed. Eng. 2 (8) (2018) 611–621.
[122] C.G. Drake, Ann. Oncol. Off. J. Eur. Soc, Med. Oncol. 23 (Suppl 8) (2012) viii41–viii46, https://doi.org/10.1093/annonc/mds262.
[123] H. Song et al., Theranostics 9 (8) (2019) 2299–2314, https://doi.org/10.7150/thno.30577.
[124] T.T. Smith et al., J. Clin. Invest. 127 (6) (2017) 2176–2191, https://doi.org/10.1172/JCI87624.
[125] P. Yu et al., Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 16 (24) (2010) 6019–6028, https://doi.org/10.1158/1078-0432.CCR-10-1966.
[126] P. Yang et al., Nano Lett. 18 (7) (2018) 4377–4385, https://doi.org/10.1021/acs.nanolett.8b01406.
[127] Y. Li et al., Oncoimmunology 5 (2) (2015) e1074374–e, https://doi.org/10.1080/2162402X.2015.1074374.
[128] F. Wang et al., Nat. Biomed. Eng. 4 (11) (2020) 1090–1101, https://doi.org/10.1038/s41551-020-0597-7.
[129] J. Ahn, P. Ruiz, G.N. Barber, J. Immunol. 193 (9) (2014) 4634–4642.
[130] H.H. Tam et al., Proc. Natl. Acad. Sci. 113 (43) (2016) E6639–E6648.
[131] T. Thambi, Y. Li, D.S. Lee, J. Controlled Release 267 (2017) 57–66.
[132] C. Li et al., Acta Pharm. Sin. B 9 (6) (2019) 1145–1162, https://doi.org/10.1016/j.apsb.2019.08.003.
[133] A. Singh, S. Dees, I.S. Grewal, Br. J. Cancer 124 (6) (2021) 1037–1048.
[134] C.M. Van Herpen et al., Clin. Cancer Res. 9 (8) (2003) 2950–2956.
[135] E.I. Buchbinder et al., J. Immunother. Cancer 7 (1) (2019) 1–7.
[136] A. Mandal et al., Bioeng. Transl. Med. 5 (2) (2020) e10158.
[137] K. Xue et al., Adv. Ther. 2 (1) (2019) 1800088.
[138] E. Hunziker et al., Tissue Eng. 12 (12) (2006) 3341–3364.
[139] A.J. Vegas et al., Nat. Biotechnol. 34 (3) (2016) 345–352.
[140] M. van de Weert, W.E. Hennink, W. Jiskoot, Pharm. Res. 17 (10) (2000) 1159–1167.
[141] A.L. Liu, A.J. García, Ann. Biomed. Eng. 44 (6) (2016) 1946–1958.
[142] W. Jiskoot et al., J. Pharm. Sci. 101 (3) (2012) 946–954.
[143] R.K.S.G. Mohammad et al., J. Cell. Physiol. 235 (7–8) (2020) 5449–5460.

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