Human intratumoral therapy: Linking drug properties and tumor transport of drugs in clinical trials

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ABSTRACT

Cancer therapies aim to kill tumor cells directly or engage the immune system to fight malignancy. Checkpoint inhibitors, oncolytic viruses, cell-based immunotherapies, cytokines, and adjuvants have been applied to prompt the immune system to recognize and attack cancer cells. However, systemic exposure of cancer therapies can induce unwanted adverse events. Intratumoral administration of potent therapies utilizes small amounts of drugs, in an effort to minimize systemic exposure and off-target toxicities. Here, we discuss the properties of the tumor microenvironment and transport considerations for intratumoral drug delivery. Specifically, we consider various tumor tissue factors and physicochemical factors that can affect tumor retention after intratumoral injection. We also review approved and clinical-stage intratumoral therapies and consider how the molecular and biophysical properties (e.g. size and charge) of these therapies influence intratumoral transport (e.g. tumor retention and cellular uptake). Finally, we offer a critical review and highlight several emerging approaches to promote tumor retention and limit systemic exposure of potent intratumoral therapies.

1. Introduction

Recent clinical successes of human intratumoral (IT) therapies have stimulated a wave of new trials investigating IT therapies alone and in tandem with other immuno-oncology agents. IT delivery refers to the direct injection of a drug/formulation into a tumor. IT therapy offers unique anti-cancer benefits since direct injection bypasses systemic trafficking and tumor penetration [1], and delivering small IT doses reduces severe adverse events (AEs) associated with systemic delivery of cancer therapies [2–6]. IT administration of immunostimulants, for example, can work synergistically with checkpoint inhibitors making nonresponsive ‘cold’ tumors ‘hot’ by recruiting and activating tumor infiltrating lymphocytes [4,7,8]. Intuitively, the design of IT therapies is significantly different than that of systemic cancer medications, as these localized interventions aim for retention at the administration site or draining lymph nodes with limited systemic exposure. In this review, we highlight transport mechanisms involved in IT delivery, review recent IT clinical trials, and deduce relationships between biophysical properties of IT therapeutics, potential effects on IT transport, and clinical results. Finally, we highlight emerging strategies for promoting tumor retention of IT therapies.

2. Overview of current cancer therapies

To begin, the palette of cancer therapies is briefly reviewed prior to delving into the current landscape of IT therapies. Physicians have radiation therapy, chemotherapy, and immunotherapy interventions at their disposal. Below, we only briefly touch on radiation and chemotherapy. Then, we offer a deeper background on cancer immunotherapies due to the potential synergies with IT therapies, which is the key topic of this review.

2.1. Radiation therapy

Radiation therapy employs highly focused energy to kill or damage tumor cells [9–11]. Radiation can be used to treat tumors alone, or in combination with other cancer treatments such as chemotherapy, immunotherapy, and surgery [10–12]. For instance, radiation can be used to shrink the tumor before surgery or to eliminate residual tumor cells post-surgery. Despite efforts to minimize radiation damage to non-cancerous normal tissues, damage to normal tissues is common, leading to side effects such as fatigue, hair loss, and skin irritation.
2.2. Chemotherapy

Chemotherapies are cytotoxic, anti-cancer agents that non-selectively target quickly proliferating cells [13]. The most common route of administration is IV, because most chemotherapeutic drugs exhibit poor and variable oral bioavailability [14,15]. Systemically administered chemotherapies commonly elicit some degree of unintended side-effects from non-selective cytotoxic action [16,17].

2.3. Immunotherapy

Cancer immunotherapy stems from Coley’s seminal work and harnesses the body’s own immune mechanisms to fight cancer. Major immunotherapy classes include checkpoint inhibitors, oncolytic viruses (OVs), cell-based immunotherapies, cytokines and adjuvants [18,19]. Immune checkpoint inhibitors block checkpoint receptors to prevent suppressive immune responses, resulting in enhanced anti-tumor responses. Ipilimumab (Yervoy®), an antibody against cytotoxic T-lymphonocyte antigen-4 (CTLA-4), was the first approved checkpoint inhibitor and increased the survival of metastatic melanoma patients [20,21]. Other major checkpoint inhibitor targets include the programmed cell death protein-1 (PD-1) (e.g., Keytruda) or its ligand (PD-L1) (e.g. Imfinzi or Opdivo) [21]. However, checkpoint inhibitors are often associated with irAEs and toxicities from over-activation of T lymphocytes [22]. OVs can be engineered to selectively infect cancer cells and stimulate anti-tumor immune responses. The oncolytic herpes virus, talimogene laherparepvec (T-Vec), was the first approved OV for the treatment of advanced melanoma, but toxic side effects caused by genetic manipulation still remain a safety concern [23]. Cytokines are often combined with adjuvants and are immunomodulators that enhance the host anti-tumor immune responses. Interferon-α and interleukin-2 are two cytokines that have been approved for the treatment of several types of leukemia and melanoma [24]. Adjuvants can stimulate immune responses and are often added to vaccines to improve immunogenicity [25,26]. For instance, the adjuvant AS04 is a TLR4 agonist used in Cervarix, an approved preventive vaccine for human papillomavirus (HPV) [27].

Cellular immunotherapies have provided a range of new and targeted solutions for tumor cell killing [28]. One rapidly emerging cellular immunotherapy uses chimeric antigen receptor (CAR) T-cells. A patient’s own T-cells are extracted and transfected with CAR, that binds to tumor antigens, thus addressing T-cell killing directly to antibody recognition. Typically, CAR-T therapy requires a pre-conditioning lymphodepletion treatment prior to modified T-cell infusion to increase expansion of the modified T cells. The first CAR-T cell therapy, Kymriah® (or tisagenlecleucel), was approved in 2017 for treating B-cell precursor acute lymphoblastic leukemia that is refractory or in the second or later relapse [29]. Tisagenlecleucel is composed of an anti-CD19 scFv, a CD8-α hinge region, 4-1BB (CD137) co-stimulation domains, and a CD3ζ signaling domain [30]. It is prepared from a patient’s peripheral blood mononuclear cells by enriching for T-cells, modifying the T-cells using lentiviral gene transfer and subsequently activating them with anti-CD3/CD28 [29]. The second and only other approved CAR-T cell therapy, Yescarta® (axicabtagene ciloleucel), was approved by the FDA for use in adults with relapsed or refractory diffuse large B-cell lymphoma a few months after tisagenlecleucel [31]. Axicabtagene ciloleucel is also a CD19 directed CAR-T cell but differs from tisagenlecleucel by using CD28 hinge and CD3ζ co-stimulation domains. CAR-T cells are highly effective for hematologic cancers; but have limited efficacy for solid tumors due to challenges for in T-cell recruitment, expansion, and survival within the TME [32–34]. The first FDA-approved cancer vaccine Provenge® (sipuleucel-T) is comprised of autologous T-cells selective for prostate acid phosphatase that is expressed in 95% of prostate cancers [35,36]. The most common adverse reactions to sipuleucel-T include fever and fatigue [35]. For all cell-based therapies, insufficient cell trafficking, tumor microenvironment, inhibitory cytokines, and regulatory cells are still obstacles to more wide-spread efficacy [37].

The surge of immunotherapeutic breakthroughs illustrates the immense promise of using the immune system to fight cancer, but each example carries systemic exposure risks. Adjuvants and TLR agonists can trigger intense immune anaphylaxis resembling that of sepsis. Immune therapies such as checkpoint inhibitors, CAR-T, adoptive T cell therapies, and cancer vaccines can leave patients susceptible to off-target immune-related adverse events. These potential dangers highlight the importance of new strategies for safer and more specific cancer treatments. IT administration is a compelling approach to enhance safety.

3. Intratumoral therapy

3.1. A brief history

The first successful IT cancer therapies were administered over 100 years ago by Dr. William Coley on patients with inoperable solid tumors. Coley noticed a patient with an inoperable egg-sized sarcoma on the face was completely cured after suffering a severe infection from a failed skin graft. He proposed that by introducing a bacterial infection at the site of the patient’s tumor, an immune response against the malignancy might be generated. This intervention proved an unprecedented success, and Coley went on to treat many more patients with bacteria-derivated, heat-killed toxins. Coley’s Toxins became one of the first examples of cancer immunotherapy [38,39]. However, with the introduction of modern radiation and chemotherapy protocols, Coley’s toxins largely faded into the background and are no longer in use [40].

Even though IT therapy most literally means injection directly into tumors, it can more broadly refer to any therapy that is delivered in very close anatomical proximity to a tumor with the intention of direct uptake by tumors or tumor cells. Even under this broad definition, only three IT therapies are approved today. First used nearly 40 years ago, Bacillus Calmette-Guerin (BCG) was approved by U.S. Food and Drug Administration (FDA) in 1990 and is instilled locally into patients with bladder cancer [41]. This approach applies the same concepts laid out by Coley [42]. Imiquimod, a TLR7 agonist, was FDA-approved in 1997. Imiquimod is topically applied for genital warts and basal cell carcinomas, where it can diffuse through the skin into the superficial tumors and tissues [43]. Talimogene laherparepvec (T-Vec/Imlygic®) is used to treat melanoma and was FDA-approved in 2015. T-Vec is an oncolytic herpes virus designed to kill cancer cells and stimulate an immune response by expressing granulocyte-macrophage colony-stimulating factor (GM-CSF) [44]. Today, there are many more agents being investigated for IT delivery that exploit the immune system, including pathogen associated molecular patterns (PAMPs), monoclonal antibodies (mAbs), cytokines, small molecules, viral and gene therapies, and autologous cells [45]. Merck’s $300M acquisition of Immune Design reignited activity and interest around IT therapy. Leading up to its acquisition, Immune Design disclosed the IT immunotherapy G100 [46,47]. G100 is a stable oil-in-water emulsion containing glucopyranosyl lipid A (GLA), a potent toll-like receptor 4 (TLR4) agonist that induces activation of local dendritic cells (DCs) to elicit broad, patient-specific anti-tumor immune responses [47]. Notably, G100 exhibited abscopal effects, the shrinkage of distal (non-injected) tumors [48]. This highly promising therapy received orphan drug designation by the FDA and European Medicines Agency (EMA) for follicular non-Hodgkin’s lymphoma, further highlighting IT interventions as a compelling therapeutic approach [49]. The efficacy of G100 was even more pronounced when applied in combination with Merck’s anti-PD1 checkpoint inhibitor, Keytruda®, alongside radiation therapy [47].

3.2. Mechanism of intratumoral therapy

Tumor tissue is typified by the aberrant, unchecked proliferation of cells. The immune system usually recognizes and eliminates nascent tumors, but immunosuppressive mechanisms of tumors can allow malignant cells to proliferate undetected by the immune system. T-
regulatory cells (Tregs) are attracted to the tumor by chemokines and aid in suppressing antigen presenting cells (APCs) that may otherwise stimulate a response against tumor antigens [50]. Additionally, tumor cells can secrete anti-inflammatory and regulatory cytokines (ie TGFβ, IL-10) that facilitate cancer growth and directly prevent DC activation. Tumor cells can also limit the expression of co-stimulatory molecules (MHC II, CD80, CD86), potentially inducing anergy or senescence in infiltrating T cells [50,51]. At the other extreme, overstimulation can cause T-cell exhaustion from chronic exposure to tumor antigen [52]. Finally, tumor cells can downregulate the expression of tumor antigens over time, evading recognition by cytotoxic T-lymphocytes (CTLs).

Different mechanisms can be used to destroy the primary tumor and some can even promote clearance of distal tumors (Figure 1). Systemic or local delivery of cytotoxic agents or targeted radiotherapy can be used to damage or destroy tumor cells. Destruction of cancer cells can result in the release of tumor-derived antigens. Alternatively, immunostimulants can be used to overcome the suppressive environment within the tumor, which work by recruiting immune cells or by activating the immune system to recognize and attack cancer cells. Immune cells can be activated by immunostimulants in the presence of tumor antigen, traffic to lymph nodes, and then activate tumor antigen specific T-cells via cross-presentation. Antigen-specific T-cells may then circulate back to the tumor or to distal tumors and instigate tumor-cell killing. The activation of the innate immune response creates a pro-inflammatory microenvironment and can result in recruitment of additional immune cells to the tumor. Categories of IT cancer therapies that are reviewed in this article is listed in Table 1.

With scientific advances in oncology, cancer mortality rates have decreased by 29% between 1991 and 2017 [61]. However, over 1.7 million cancer cases and over 600,000 cancer deaths occurred in the United States in 2019. Cancer therapies face the challenge of accessing targets deep within tumor tissue, and often suffer from adverse effects. Systemically delivered therapeutics encounter countless obstacles leading to a very small fraction of the drug reaching the tumor and unwanted distribution to healthy tissues [62]. After reaching the tumor, the drug penetrates the tumor and encounters the tumor microenvironment (TME), which can be heterogeneous and differs drastically from healthy tissue. While IT administration of therapeutic agents can overcome concerns associated with systemic delivery, understanding the TME for IT therapy becomes paramount for success.

4. Tumor properties to consider for intratumoral therapies

4.1. Tumor microenvironment

The TME is heterogeneous between patients, tumor types, and often even within individual tumors. Overall, tumor tissue is typically distinct from normal tissue in that it has poorly organized vasculature with inconsistent vessel diameters and more prevalent branching [63]. Tumor cell distance from blood vessels can result in restriction of oxygen supply causing hypoxia in portions of the tumor [64]. The poorly organized vasculature and hypoxic setting creates a microenvironment with increased fluid leakage and elevated interstitial fluid pressure (IFP). Compared to the extravascular space in healthy tissue,
tumors tend to have higher extracellular matrix density lacking functional lymphatic vessels, which limits interstitial diffusion and the drainage of fluid from the tissue [65,66]. Collectively, the poorly organized vasculature and increased IFP can decrease uptake of circulating therapeutic molecules, which may cause poor prognosis [65].

Intracellular pH is similar between tumors and normal tissue, although extracellular pH can be more acidic in tumors [67]. Increased extracellular acidity and anaerobic glycolysis alters the pH gradients found in the TME versus healthy tissue [67,68]. Higher acidity may increase tumor cell invasion and metastatic potential while also aiding in evasion of immune surveillance [69,70]. For drugs that rely on passive diffusion to enter cells, the decreased extracellular pH may cause weakly basic drugs to become ionized, preventing diffusion across membranes [67,71].

4.2. Intratumoral transport

In addition to the mechanism of action for the active component, the design of IT therapy formulation requires an understanding of molecular transport within the TME after the drug/formulation is delivered. After delivery (injection) into the tumor, the drug/formulation will undergo several transport and kinetic processes (Figure 2) that will ultimately determine retention or elimination of the anti-cancer therapy (Table 2). Major molecular transport and kinetic processes within the TME include extracellular binding, cellular uptake and intracellular binding, and exfiltration from the TME.

Molecular transport through normal extracellular matrix is based on both diffusion along a concentration gradient as well as advective convection (or bulk transport of mass) along a pressure gradient [72]. However, since elevated IFP makes the bulk transport (i.e. convection) of the IT therapy negligible in the TME, transport of anti-cancer agents after IT administration are typically governed by diffusion [65,73]. Though the blood vessels represent an escape route for the therapeutic agent, the abnormal and poorly organized vasculature of the TME increases retention at the tumor cells that are distant from the vessels [74]. The absence of functional lymphatics in the TME reduces the elimination of the agent, improving IT retention [75,76]. Despite the relatively ineffective lymphatic drainage in the TME, peritumoral lymphatics are a major route for metastasis and the local loss of IT therapeutics [77]. Angiogenesis, which seeks to normalize tumor vasculature leading to increased blood flow and reduced IFP, can result in decreased retention time [78–80]. Vascular permeability can also decrease tumor retention time for small molecules, but this characteristic is purported to be insignificant for macromolecules [81,82].

Densely packed collagen fibers are characteristic of the TME and pose transport resistance, which likely results in an overall increase in retention of IT therapy, although dense collagen may also restrict access to sites within the tumor [72,83]. Fibillar collagen and high IFP contribute to a high mechanical stress in the tumor [84], which promotes diffusion over convection for the IT therapy. Cellular packing density can also affect drug diffusion; loosely packed tumor cells can improve retention at tumor by enabling fast and thorough penetration of the therapeutic agent [85]. Conversely, regions of tightly packed cells may impair the penetration of the therapeutic agent throughout the tumor. Finally, cellular uptake or binding of the therapy can occur by passive diffusion, active uptake, or other mechanisms depending on molecular properties.

Drug features such as molecular size, charge, and other properties influence intratumoral residence time (Table 2). Water soluble small molecules diffuse more easily in the TME resulting in a lower retention in the tumor [86], but increases in molecular hydrodynamic radius can reverse this effect. It is critical to balance molecular size such that a therapeutic or its carrier is small enough to diffuse through the TME while avoiding clearance through lymphatic drainage or cellular uptake [87]. Large molecules such as monoclonal antibodies, for instance, can have limited tumor retention due to endocytic clearance after IT administration [88]. Drug diffusion and retention deep inside the tumor mass is affected by binding kinetics and affinity [89]. Molecular charge may also be exploited such that the acidic extracellular pH in the tumor has a positive impact on retention. The lymphatics are a primary mechanism for clearance of SC injected mAbs, and the clearance rate is mainly dependent on the isoelectric point (PI) [90]. Hence, careful antibody design utilizing physiologically based pharmacokinetic models in the development stage could lead to new generations of antibody-based therapies with enhanced local tissue and IT retention. The many factors that influence TME transport offer unique opportunities for the retention of drugs injected IT such that the exploitation of these abnormalities can be harnessed to maximize therapeutic effects.

5. Intratumoral therapies in clinical trials

Many types of IT therapies are in clinical trials, but this review focuses on trials with posted or published data with only brief consideration of clinical trials yet to produce results. While radiation and cells can be administered locally/intratumorally [91–94], these categories are not covered in this review. Moreover, many radiation therapies are given in combination with other IT therapeutics. Clinical trials of IT therapies are highlighted in Table 3 and in the following sections, with a more complete summary available in Supplementary Table 1.

5.1. Pathogen-associated molecular patterns

Pathogen-Associated Molecular Patterns (PAMPs) are non-self molecules that activate innate immune responses. PAMPs are recognized
by pattern recognition receptors (PRRs) including toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors, RIG-I-like receptors (RLR), stimulator of interferon genes (STING) receptors, and C-type lectin receptors (CLR) \[112\]. Many PAMP candidates pose a high probability of significant adverse events (AEs) when they enter systemic circulation. While IT administration can reduce the side-effects associated with systemic administration, immunostimulatory molecules can still leak out of the tumor and into the systemic circulation to cause AEs.

Unmethylated CpG oligonucleotides are PAMPs that mimic bacterial DNA and trigger an innate immune response upon binding to TLR9 \[167,168\]. PF-3512676 is a class B, linear CpG formulated as a sodium salt with a molar mass of 8204 g/mol \[169\]. The formulation of PF-3512676 is proprietary, however, literature suggests it is un-modified, water-soluble, negatively-charged, and does not form higher order structures \[97\]. Clinical trial results are promising with IT administration in B-cell lymphoma and mycosis fungoides but interestingly a higher percentage of AEs were experienced in mycosis fungoides patients receiving the same dose \[170\]. The differences between the rate of AEs could result from the extreme heterogeneity in vasculature of tumors across different types and locations. In a phase II study with lymphoma patients, an increased dose resulted in similar efficacy but more than doubled the percentage of AEs, likely a result of increased systemic exposure \[171\]. Another presumably unmodified and soluble CpG therapy, SD-101, is a class C CpG. While the structural and formulation information is proprietary, CpG class C is known to form dimers \[172\]. Several IT trials investigating SD-101 in combination with other anticancer modalities exhibited promising abscopal effects; however, there were grade 1-2 AEs in 100% of patients that includes detriments seen in authentic pathogen infections such as sepsis, with a high incidence of grade 3 or 4 AEs and some serious AEs (SAEs) \[173–176\].

Many approaches have utilized structurally modified CpG ODNs to increase immunogenicity and stability \[7,98,177\]. IMO-2125 exploits an interesting design. Two strands of class C CpG are linked at the 3’ end consisting of an 11-mer of CpG on each flanking end to allow formation of intermolecular structure that deters intramolecular interaction \[177,178\]. Favorable potency may be retained by the exposed 5’ ends which are pertinent for CpG’s binding mechanism \[168,178\]. This variant is formulated as a sodium salt with a molecular weight (MW) of 7712 g/mol and likely forms dimers \[95,168\]. IMO-2125 has been granted fast track designation and orphan drug designation by the FDA and has shown promising results in early trials with fewer AEs than most other IT TLR agonists. Additionally, this modified CpG therapy shows increased TLR9 activation over unmodified CpG likely due to increased metabolic stability from the chemical linkage of the 3’ ends \[178\].

Another consideration for CpG-based therapies is the type of backbone. Naturally-occurring CpG has a phosphorodiester (PO) backbone; however, synthetic CpG is often made with a phosphorothioate (PT) backbone to increase stability \[179\]. The creators of MGN1703 purport that the PT backbone of CpG

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**Table 2**

Factors affecting transport of therapy out of the tumor after intratumoral injection and probable transport phenomena.

<table>
<thead>
<tr>
<th>Tumor tissue factors</th>
<th>Phenomena</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microvascular permeability</td>
<td>Decreases retention at tumor for small molecules</td>
</tr>
<tr>
<td>Abnormal vascular architecture</td>
<td>May increase or decrease retention at the tumor</td>
</tr>
<tr>
<td>Absence of lymphatics</td>
<td>Increases retention at the tumor</td>
</tr>
<tr>
<td>Interstitial fluid pressure (IFP)</td>
<td>Increased IFP increases retention time in the tumor but decreases retention close to vessels</td>
</tr>
<tr>
<td>Solid stress elevation</td>
<td>Increases retention within the tumor, but decreases retention close to vessels</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td>Decreases retention at the tumor</td>
</tr>
<tr>
<td>Physicochemical factors</td>
<td>Phenomena</td>
</tr>
<tr>
<td>Concentration gradient</td>
<td>Increases diffusion out of tumor, decreasing retention</td>
</tr>
<tr>
<td>Water solubility</td>
<td>Water soluble agents diffuse easily in the TME, decreasing retention in tumor</td>
</tr>
<tr>
<td>Extracellular pH</td>
<td>Effect on retention at tumor depends on the carcinogenic agent’s molecular properties (pI, pKa)</td>
</tr>
<tr>
<td>Fibrillar collagen</td>
<td>Dense matrix increases retention at tumor</td>
</tr>
<tr>
<td>Cellular packing density</td>
<td>Packing density may increase or decrease retention at tumor</td>
</tr>
</tbody>
</table>

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Fig. 2. Transport and kinetic processes in intratumoral injection therapies. The therapeutic agent can diffuse through the TME, enter the cell, be bound by extracellular or intracellular proteins, unbind, or leave the tumor into blood vessels, lymphatics, peripheral blood, or adjacent tissue by diffusion and advective convection. Diffusional transport of the agent back into the tumor is expected to be minimal.
Table 3
Therapy characteristics chart

<table>
<thead>
<tr>
<th>Category</th>
<th>Therapy/Alternative Names</th>
<th>Description of Active</th>
<th>Characteristics and Formulation information</th>
<th>Mechanism Engaged</th>
<th>NCTs</th>
<th>Refs</th>
</tr>
</thead>
</table>
| PAMPs    | Tisotolimod/IMO-2125     | CpG class C derivative, TLR9 agonist | - Two strands of CpG linked at the 3’ ends  
- Likely forms dimers  
- MW 7712 Da  
- Formulated as a sodium salt | Intracellular TLR binding  
Exposed 5’ ends increase potency | NCT03052205  
NCT02644967  
NCT03445533  
NCT04270864  
NCT03865082  
NCT04962839 | [95] |
| SD-101   | Cpg class C, TLR9 agonist | | - Proprietary investigational  
- CpG class C has one or more TCG elements close to or at the 5’ end of the ODN and a palindromic sequence containing multiple CpG motifs  
- CpG alone is negatively charged | Intracellular TLR binding | NCT02254772  
NCT03007732  
NCT03831295  
NCT02521870  
NCT02731742  
NCT03419011  
NCT02927964  
NCT02861474  
NCT03322384  
NCT01745354  
NCT04050085 | [96] |
| PF-3512676/Agatolimod/CpG 7909 | Cpg class B, TLR9 agonist | | - MW 76/88/212 Da  
- Class B CpG is usually linear and does not form higher order structures alone  
- CpG alone is negatively charged  
- AFM measurements of CpG class B: 1.2 x 8.7 nm  
- Assembles into higher order structures  
- AFM measurements: 1.1 x 10-17 x 25-90 nm | Intracellular TLR binding | NCT00185965  
NCT00880581  
NCT02266147 | [97] |
| CMP-001 | Cpg class A derivative with native DNA backbone (PO) | | - Dumbbell shaped  
- Approximate MW 32 kDa  
- Assembles into higher order structures  
- AFM measurements: 30 nucleotides | Intracellular TLR binding | NCT03507699  
NCT03084640  
NCT03836680  
NCT02680184  
NCT03618641  
NCT02668770 | [98-100] |
| MGN1703/Lefitolimod | Cpg derivative, native DNA backbone (PO) | | - Dumbbell shaped  
- 28 base pair double-stranded middle section flanked by two single-stranded loops containing 30 nucleotides | Intracellular TLR binding | NCT03291002 | [110,111] |
| Hiltonol/polyIC-IC | TLR3 agonist | | - Complexed with polylysine (PLL)  
- Optimal PLL MW 28 kDa but ranges 13-35 kDa  
- Formulated with carbomethylcellulose (CMC)  
- In an aqueous saline solution  
- Net positively charged | Intracellular TLR binding | NCT01242386 | [101] |
| BO-112/polyIC + polyalkylamine | TLR3 agonist | | - MW 17.5-22.6 kDa  
- Zeta potential 38 mV at pH 3.1  
- 45-85 nm particles  
- polyIC/PEI ratio between 2.5-4.5 | Intracellular TLR binding | NCT01976585  
NCT03262103  
NCT01984892  
NCT03808067 | [102,103] |
| G100/GLA-SE | GLA derivative, TLR4 agonist | | - Aqueous formulation with glucose or mannitol  
- DL-α-tocopherol, and Poloxamer 188  
- Particle size 82.7-111 nm  
- Zeta potential -17 mV | Extracellular TLR binding | NCT02035657  
NCT02180698  
NCT03742804  
NCT02501473  
NCT03915678  
NCT04058071  
NCT03982121  
NCT02387125 | [104-109] |
| CV8102 | TLR7/8 and RLR agonist | | - ssRNA - 547 nucleotides  
- Complexed with cationic peptide (Cys-Arg12-Cys) that is disulfide-crosslinked | Intracellular TLR and RLR binding | NCT03291002 | [110,111] |
<table>
<thead>
<tr>
<th>Category</th>
<th>Therapy/Alternative Names</th>
<th>Description of Active Characteristics and Formulation information</th>
<th>Mechanism Engaged</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>MK4621/RGT100 (upcoming trials formulate with JetPEI)</td>
<td>RIG-I agonist • Cyclic dinucleotide • No structural information provided • Newer formulation are complexing with JetPEI which is a linear PEI with 1-3 positive charges on the nitrogen species.</td>
<td>Intracellular TLR binding</td>
<td>NCT03739138NCT03065023</td>
<td>[112]</td>
</tr>
<tr>
<td>Motolimod/VTX-2337</td>
<td>TLR8 and NOD agonist</td>
<td>MW 458.6 g/mol • No charge</td>
<td>NCT03906526</td>
<td>[113,114]</td>
</tr>
<tr>
<td>MIW815/ADU-S100</td>
<td>STING agonist</td>
<td>Synthetic cyclic dinucleotide • Formulation unknown</td>
<td>NCT03172936NCT02675439NCT03937141</td>
<td>[115,116]</td>
</tr>
<tr>
<td>MK-1454</td>
<td>STING agonist</td>
<td>Synthetic cyclic dinucleotide</td>
<td>NCT03010176</td>
<td>[117,118]</td>
</tr>
<tr>
<td>BCG</td>
<td>Derivative of BCG bacteria • Live, attenuated BCG</td>
<td>Gram positive, rod shaped • Average length 2.36 μm, width 0.474 μm, volume 0.389 μm^3 or 0.906 μm diameter</td>
<td>Vaccine contains loosely aggregated cells often but not always.</td>
<td>NCT03928275NCT01838200</td>
</tr>
<tr>
<td>Clostridium novyi-NT</td>
<td>Derivative of clostridium bacteria</td>
<td>Gram positive, contain flagella, spore forming</td>
<td>NCT01924689</td>
<td>[121,122]</td>
</tr>
<tr>
<td>Cytokine</td>
<td>Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF)</td>
<td>White blood cell growth factor • 14-35 kDa glycoprotein • 127 amino acids • 2 nm x 3 nm x 4 nm</td>
<td>Immunostimulatory cytokine</td>
<td>NCT00600002</td>
</tr>
<tr>
<td>IL-2</td>
<td>Immune cell signaling molecule • 15.5 kDa and is comprised of 133 amino acids • 18 MIU recombinant human IL-2 (Proleukin® Chiron, Ratingen, Germany) was dissolved in 6 ml glucose (5%) prepared with albumin (0.2%) solution</td>
<td>Immunostimulatory cytokine</td>
<td>NCT03233828 NCT00204581NCT01480323NCT00600002</td>
<td>[124]</td>
</tr>
<tr>
<td>PEG-IL-2</td>
<td>Modified immune cell signaling molecule • Covalent addition of 6–7 kDa poly-ethylene glycol (PEG)</td>
<td>Immunostimulatory cytokine</td>
<td>[125]</td>
<td></td>
</tr>
<tr>
<td>IL-4(38-37)-PE38KDEL</td>
<td>Immune cell signaling molecule conjugated to a toxin</td>
<td>amino acids 38–129 of IL-4, fused via a peptide linker to amino acids 1–37, which in turn is fused to the PE38KDEL toxin • PE38KDEL is composed of amino acids 253–364 and 381–608 of PE, with KDEL (an endoplasmic reticulum retention sequence) at positions 609–612.</td>
<td>Bind to IL-4 receptors on tumors</td>
<td>NCT00797940NCT00014677</td>
</tr>
<tr>
<td>IL13-PE38QQR (IL13PE)</td>
<td>Immune cell signaling molecule conjugated to a toxin</td>
<td>IL-13 conjugated to truncated PE</td>
<td>Bind to IL-13 receptors on tumors</td>
<td>NCT00064779</td>
</tr>
<tr>
<td>Antibody/Cytokine fusion</td>
<td>Darleukin (L19-IL2) and fibromun (L19-TNFα)</td>
<td>Combination of immune cell signaling molecules • Darleukin: interleukin-2 (IL-2) is fused to a human single-chain variable fragment (scFv) that recognizes L19 • Fibromun: tumor necrosis factor (TNF) is fused to a scFv that recognizes L19</td>
<td>Immunostimulatory cytokine</td>
<td>Darleukin:NCT01253096 Daromun (Darleukin and Fibromun):NCT02076633NCT02938299NCT03567889NCT00805376NCT02798406NCT02197169NCT01956734</td>
</tr>
<tr>
<td>Oncolytic Virus</td>
<td>ONYX-015</td>
<td>Adenovirus</td>
<td>NCT01956734</td>
<td>[129]</td>
</tr>
<tr>
<td>DNX-2401</td>
<td>Adenovirus</td>
<td>E1B 55.4 kDa gene deleted</td>
<td>NCT01956734</td>
<td>[130]</td>
</tr>
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<tr>
<th>Category</th>
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<th>Refs</th>
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<tbody>
<tr>
<td>Coxsackievirus A21 (CVA21)</td>
<td>coxsackievirus</td>
<td>31 nm in diameter</td>
<td>Bind to intracellular adhesion molecule 1 (ICAM-1) and decay acceleration factor (DAF) proteins on tumor cells</td>
<td>NCT01227551 NCT00438009 NCT00215482 NCT00832559 NCT02307149 NCT02428036 NCT01017185 NCT03153085 NCT03252808 NCT02272855 NCT03259425</td>
<td>[128]</td>
<td></td>
</tr>
<tr>
<td>HF10</td>
<td>Herpes simplex virus-1 (HSV-1)</td>
<td>155 – 240 nm in diameter</td>
<td>Destroy tumor cells</td>
<td>NCT00832559 NCT02307149 NCT01017185 NCT03153085 NCT03252808 NCT02272855</td>
<td>[129,130]</td>
<td></td>
</tr>
<tr>
<td>HSV-1716</td>
<td>Herpes simplex virus</td>
<td>155 – 240 nm in diameter</td>
<td>Destroy tumor cells</td>
<td>NCT00832559 NCT02307149 NCT01017185 NCT03153085 NCT03252808 NCT02272855</td>
<td>[129,130]</td>
<td></td>
</tr>
<tr>
<td>H-1 parvovirus (H-1PV, ParvOryx)</td>
<td>parvovirus</td>
<td>155 – 240 Å in diameter</td>
<td>Destroy tumor cells</td>
<td>NCT00832559 NCT02307149 NCT01017185 NCT03153085 NCT03252808 NCT02272855</td>
<td>[130–132]</td>
<td></td>
</tr>
<tr>
<td>measles virus Edmonston-Zagreb vaccine strain</td>
<td>measles virus</td>
<td>120 – 250 nm in diameter</td>
<td>Bind to CD46 that are expressed by some cancer cell lines</td>
<td>NCT01301430</td>
<td>[133,134]</td>
<td></td>
</tr>
<tr>
<td>Pelareorep (REOLYSIN®)</td>
<td>reovirus</td>
<td>Unmodified oncolytic reovirus</td>
<td>Destroy tumor cells</td>
<td>NCT00528684 NCT02723838</td>
<td>[135,136]</td>
<td></td>
</tr>
<tr>
<td>vvID-CDSR</td>
<td>Vaccinia virus</td>
<td>Vaccinia growth factor (VGF) and thymidine kinase (TK) deleted</td>
<td>Destroy tumor cells</td>
<td>NCT00528684 NCT02723838</td>
<td>[137]</td>
<td></td>
</tr>
<tr>
<td>Oncolytic virus + Talimogene laherparepvec (T-Vec); Imlygic™</td>
<td>Type I herpes simplex virus</td>
<td>ICP34.5-deficient</td>
<td>Destroy tumor cells</td>
<td>NCT0289016 NCT02574260 NCT02014441 NCT00769704 NCT01368276 NCT00769704 NCT02756845 NCT03458117 NCT0465152 NCT03086442 NCT03064763 NCT02211311 NCT02453191 NCT0308176 NCT01740297 NCT03256344 NCT0382064 NCT02236350 NCT04068128 NCT03842943 NCT02626000 NCT02509507 NCT03747744 NCT02819843 NCT01161498 NCT01161498</td>
<td>[138,139]</td>
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<tr>
<td>Pexa-Vec (JX-594)</td>
<td>Vaccina virus</td>
<td>Wyeth strain vaccinia modified by insertion of the human GM-CSF and Lac-Z genes into the vaccinia TK gene region under control of the synthetic early-late promoter and p7·5 promoter, respectively. Virion morphology and size: Enveloped, biconcave core with two lateral bodies, brick-shaped to pleo-morphic virions, ~360x270x250 nm in size</td>
<td>Replication and hGM-CSF transgene, Destroy tumor cells</td>
<td>NCT01329809, NCT01387555, NCT01169584, NCT00554372, NCT02562755, NCT01711651, NCT02977156, NCT03294083, NCT03071094, NCT04293122</td>
<td>NCT01171651, NCT02977156, NCT03294083, NCT03071094, NCT04293122</td>
</tr>
<tr>
<td>Non-oncolytic virus + vector</td>
<td>TG1042 (Adenovirus-interferon-γ)</td>
<td>Adenovirus</td>
<td>Nonreplicating (E1 and E3 regions deleted)</td>
<td>Expresses IFN-γ</td>
<td>NCT00394693 [143]</td>
</tr>
<tr>
<td></td>
<td>TNFerade Biologic (AdGVIGR.TNF.11D)</td>
<td>Adenovirus</td>
<td>Replication-deficient adenoviral vector that expresses tumor necrosis factor-α (TNFα) under the control of a radiation-inducible Egr-1 promoter</td>
<td>Expresses TNFα</td>
<td>NCT00514673, NCT00514800 [144,145]</td>
</tr>
<tr>
<td>Viral vector</td>
<td>INXN-2001 (Ad-RTS-hIL-12) with oral activator INXN-1001 (Veledimex)</td>
<td>Adenovirus</td>
<td>Expresses human IL-12</td>
<td>Expresses human IL-12</td>
<td>NCT01397708, NCT02423902, NCT03679754, NCT02026271, NCT03330197, NCT03663777, NCT04006119</td>
</tr>
<tr>
<td>Non-oncolytic virus + vector</td>
<td>adenoviral vector expressing E. coli PNP (Ad/PNP) and IV fludarabine therapy</td>
<td>adenovirus</td>
<td>Loaded with a bacterial gene called E. coli purine nucleoside phosphorylase (PNP)</td>
<td>PNP converts fludarabine to anti-cancer agent fludarabine</td>
<td>NCT01310179 [146]</td>
</tr>
<tr>
<td></td>
<td>adenoviral vector (Adv.RSV-tk) expressing the herpes thymidine kinase gene with IV Ganciclovir (GCV)</td>
<td>adenovirus</td>
<td>Adenoviral vector allow high transgene expression and high transduction efficiency of both dividing and non-dividing cells</td>
<td>Expression of herpes simplex virus thymidine kinase (HSV-tk) allow phosphorylation of GCV, forming cytotoxic GCV-triphosphate</td>
<td>NCT0844623 [147]</td>
</tr>
<tr>
<td>Plasmid</td>
<td>IL-12 plasmid cDNA (pNGVL3-mIL12)</td>
<td>adenovirus</td>
<td>Formulated in saline</td>
<td>Expression of IL-12</td>
<td>NCT0323206, NCT01579318, NCT01440816</td>
</tr>
<tr>
<td></td>
<td>Tavokinogene Telseplasmid (tavo); plasmid IL-12</td>
<td>adenovirus</td>
<td>6215 bp</td>
<td>Expression of IL-12</td>
<td>NCT0323206, NCT01579318, NCT01440816</td>
</tr>
<tr>
<td>EGFR antisense DNA</td>
<td>BC-819 (also called DTA-H19)</td>
<td>adenovirus</td>
<td>Gene for the diphtheria toxin-A chain (DT-A) under the regulation of the 814-bp 5′ flanking region of the H19 promoter sequence</td>
<td>DTA-A expressed in tumor cells that can activate H19 promoter</td>
<td>NCT0711997 [151-153]</td>
</tr>
<tr>
<td></td>
<td>EGFR antisense DNA</td>
<td>adenovirus</td>
<td>Estimated ~9600 base pairs; pNGVL vector (also called pUMVC) is 9287 bp, human u6 promoter is 241 bp, and EGFRAS is 39 bp</td>
<td>Suppresses expression of EGFR by tumor cells</td>
<td>NCT08059841 [150]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>adenovirus</td>
<td>In complex with DC-Chol liposomes</td>
<td>Inhibits tumor proliferation/growth</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>adenovirus</td>
<td>Phosphoribosyl pyrophosphate (PRPP)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>adenovirus</td>
<td>DT-A expressed in tumor cells that can activate H19 promoter</td>
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<tr>
<td>CYL-02</td>
<td></td>
<td>Complex of plasmid DNA and linear polymers of polyethyleneimine (JetPEI 22kDa from Polyplus Transfection, Illkirch, France)</td>
<td>• N/P ratio of 8 to 10&lt;br&gt;• Estimated ~45nm&lt;br&gt;• 5% w/v glucose&lt;br&gt;• OD400 = 0.821&lt;br&gt;• Average particle size (Dynamic Light scattering) of 357 nm&lt;br&gt;• Zeta potential = +59 mV</td>
<td>Expression of DCK-UMK fusion protein, which activates the cytotoxic pro-drug gemcitabine</td>
<td>NCT01274455&lt;br&gt;NCT02806687</td>
<td>[154,155]</td>
</tr>
<tr>
<td></td>
<td>pbi-shRNA STMN1 LP</td>
<td>proprietary bi-functional shRNA (bi-shRNA) platform to execute RNA interference (RNAi)-mediated gene silencing</td>
<td>• Liposome-carrier complex&lt;br&gt;• Suspended in DSW (diluent consisting of 5% dextrose in water)&lt;br&gt;• OD400 = 0.821&lt;br&gt;• Average particle size (Dynamic Light scattering) of 357 nm&lt;br&gt;• Zeta potential = +59 mV</td>
<td>Silences stathmin-1 (STMN1)</td>
<td>NCT01505153</td>
<td>[156]</td>
</tr>
<tr>
<td>Antibody</td>
<td>Ipilimumab</td>
<td>anti-CTLA-4 Immune cell signaling molecule and checkpoint inhibiting antibody</td>
<td>• Human immunoglobulin (IgG1k) consisting of four peptide chains&lt;br&gt;• ~150 kDa&lt;br&gt;• 5 mg/mL clear colorless aqueous solution. pH 7.0&lt;br&gt;• The real size of an antibody molecule is about 10 nm</td>
<td>DCs present an inhibitory signal that binds to cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) to suppress cytotoxic T lymphocytes (CTLs). Ipilimumab binds to CTLA-4 to block the inhibitory signal and release the cytotoxic reaction of CTLs to attack cancer cells.</td>
<td>NCT01672450</td>
<td>[157,158]</td>
</tr>
<tr>
<td></td>
<td>ADC 1013</td>
<td>Anti-CD40 mAbs</td>
<td>• A human monospecific IgG1 antibody&lt;br&gt;• OD400 = 0.821&lt;br&gt;• Average particle size (Dynamic Light scattering) of 357 nm&lt;br&gt;• Zeta potential = +59 mV</td>
<td>Stimulation of CD40 on dendritic cells is intended to induce effector T-cells that attack the tumor.</td>
<td>NCT02379741</td>
<td>[159]</td>
</tr>
<tr>
<td></td>
<td>TTI4621</td>
<td>Modified antibody targeting CD47</td>
<td>• Anti-CD47 antibody binding domains conjugated to human IgG1 Fc&lt;br&gt;• OD400 = 0.821&lt;br&gt;• Average particle size (Dynamic Light scattering) of 357 nm&lt;br&gt;• Zeta potential = +59 mV</td>
<td>TTI-4621 (SIRPαFc) is an immune checkpoint inhibitor designed to bind human CD47 and block the “do not eat” signal that suppress macrophage phagocytosis, thereby enhancing phagocytosis, and antitumor activity.</td>
<td>NCT02890368</td>
<td>[160]</td>
</tr>
<tr>
<td></td>
<td>BMS 986178</td>
<td>anti-OX40 mAb</td>
<td>• A human IgG1&lt;br&gt;• OD400 = 0.821&lt;br&gt;• Average particle size (Dynamic Light scattering) of 357 nm&lt;br&gt;• Zeta potential = +59 mV</td>
<td>Anti-OX40 mAbs selectively binds to and activates OX40 to induce proliferation of T lymphocytes that attack tumor associated antigens.</td>
<td>NCT03831295</td>
<td>[161]</td>
</tr>
<tr>
<td>Small Molecule</td>
<td>INT230-6</td>
<td>Cisplatin, chemotherapeutic</td>
<td>• A formulation consisting of an amphiphilic cell penetration enhancer molecule combined with cisplatin and vinblastine.&lt;br&gt;• The penetration enhancer facilitates dispersion of the two drugs throughout injected tumors and enables increased diffusion into cancer cells.</td>
<td>INT230-6 thoroughly saturates and kills injected tumors. In addition, the drug induces an adaptive (T-cell mediated) immune response that attacks not only the injected tumor, but non-injected tumors and unseen micro-metastases.</td>
<td>NCT03058289</td>
<td>[162]</td>
</tr>
<tr>
<td></td>
<td>Cisplatin/Epinephrine injectable gel</td>
<td>Cisplatin, chemotherapeutic</td>
<td>Contains 4 mg/mL cisplatin, 0.1 mg/mL epinephrine, and bovine collagen as a protein carrier matrix</td>
<td>Intratumoral injection of cisplatin/epinephrine injectable gel achieves high concentrations of cisplatin in the tumor with very low concentrations in plasma and other tissues.</td>
<td>NCT00002659&lt;br&gt;NCT00022217</td>
<td>[163]</td>
</tr>
<tr>
<td></td>
<td>Para-toluenesulfonylamide (PTS)</td>
<td></td>
<td>C$_7$H$_9$NO$_2$S, MW = 171&lt;br&gt;• Formal charge 0</td>
<td>Significantly inhibit tumor growth by eliciting tumor necrosis</td>
<td>NCT03448146</td>
<td>[164]</td>
</tr>
<tr>
<td></td>
<td>Gemcitabine</td>
<td>chemotherapeutic</td>
<td>A nucleoside prodrug, an analog of deoxycytidine&lt;br&gt;• Water-soluble, low-molecular weight (299.66)&lt;br&gt;• 10% RR in saline</td>
<td>Gemcitabine causes cancer cell death by attaching to the end of the elongating DNA strand and inhibiting DNA synthesis</td>
<td>NCT02723838&lt;br&gt;NCT01831470</td>
<td>[165]</td>
</tr>
<tr>
<td></td>
<td>PV-10</td>
<td>Rose Bengal (RB) disodium, an xanthene dye</td>
<td>Promotes expression of hallmarks related to immunogenic cell death in colon cancer cell lines</td>
<td>Promotes expression of hallmarks related to immunogenic cell death in colon cancer cell lines</td>
<td>NCT02693067&lt;br&gt;NCT02557321</td>
<td>[166]</td>
</tr>
</tbody>
</table>
causes toxic side effects [99]. They developed a covalently-closed loop of Cpg with its native PO backbone to avoid PT-associated toxicity and enhance the stability of native PO. Similarly, CMP-001 is a Cpg class A with the native PO backbone that is modified to assemble into quadruplexes [7,168]. Clinical results for both of these compounds are pending, which may provide a new precedent for future trials employing modified and native backbones of Cpg.

Formulation with a polycationic carrier can increase intracellular PAMP delivery and potency. PAMPs utilizing intracellular receptors (like TLR9, TLR3, and RIG-I) may benefit from a cationic carrier or particulate formulation for attraction to cell surfaces and increased APC uptake, respectively. Two such TLR3 agonist candidates, BO-112 and Hiltonol (polyI:C:LC), include polyI:C formulated with polycations. In the most recent update of an IT BO-112 clinical trial, patients exhibited a modest overall response rate (ORR) and high percentage of AEs. Increased circulating immune cells and no detectable BO-112 in the blood suggests injection site retention [180]. BO-112 is an aqueous composition at pH 2.7-3.4 with glucose or mannitol in an optimal particle size range of 45-85 nm and zeta potential between 40-45 mV [102]. Optimal size of particles for APC uptake and processing is approximately 100 nm, similar to the size of viruses [109]. The molar particle size range of 45-85 nm and zeta potential between 40-45 mV blood suggests injection site retention [180]. IT Hiltonol is formulated with carbboxymethylcellulose (CMC), a hydrophilic, negatively charged material, in an aqueous saline solution. The molar ratio between nitrogen in polyethylenimine (PEI) and phosphorous in DNA (N/P ratio) for the polyE/C polyethylenimine (PEI) complex is between 2.5-4.5 and the PEI MW is between 17.5-22.6 kDa [102]. IT Hiltonol showed preliminary success in a single patient on both local and distal tumor sites; however systemic side effects or AEs were not reported [181]. Hiltonol is formulated with carbboxymethylcellulose (CMC), a hydrophilic, negatively charged material, in an aqueous saline solution. The molar ratio between PO4 groups to the ε amino group of the lysine in polyIC:LC is 1:1 which corresponds to an excess of ε amino groups, which may contribute to further complexing with CMC [101]. The polylsines ranges from 13-35 kDa, however there is no report of complexes between polylsine and polyI:C.

The RIG-I agonist, MK-4621, is a synthetic RNA oligonucleotide. An IT clinical trial was terminated due to excessively high incidence of AEs: 100% grade 1-2 AEs and 48% grade 3-4 AEs [182]. To increase retention at the injection site and mitigate the side effects, negatively charged MK-4621 was complexed with a positively charged carrier (PEI variant JetPEI) [183]. A clinical trial is planned for the complexed MK-4621.

Emulsion formulations can also improve efficacy and retention of IT therapeutics. The TLR4 agonist G100 is a glucopyranosyl Lipid A (GLA) derivative with a single phosphate group and six C14 acyl chains formulated in a squalene emulsion (also called GLA-SE) [105]. The emulsion contains the excipients squalene, egg phosphatidylcholine (PC), DL-α-tocopherol and Poloxamer 188 [106]. The particle/droplet size is 82.7-111 nm [105-108] and the zeta potential is -17 mV [109]. Because TLR4 is located on the cell surface, intracellular uptake is not required. The formulation of GLA has critical effects on TLR activation [108]. GLA-SE resulted in greater immune activation than GLA formulated as an aqueous suspension in various mouse models and a human skin explant model. One trial studying G100 resulted in a 10% CR, 40% PR, and 50% PD, with an AE incidence greater than 80% [184]. Moreover, responders demonstrated increased inflammation with infiltration of CD8+ and CD4+ T-cells following treatment.

Live attenuated bacteria have also been used in IT cancer immunotherapy. IT administered BCG resulted in no better than stable responses. The TICE BCG non-live vaccine, composed of the BCG cell wall skeleton and trehalose dimycolate, resulted in at least one injected nodule responding in 48% of melanoma and breast cancer patients [187]. IT Clostridium novyi-NT trials are in progress but too early on to draw comparisons [188]. More research is needed to evaluate transport of bacterial candidates after IT injection.

Early clinical trials suggest unmodified TLR agonists lead to a greater AE incidence than those structurally modified or formulated with a cationic carrier. TLR agonists comprised of DNA or RNA motifs are negatively charged. Since extracellular space and cell membranes are also negatively charged, IT administration of these nucleic acid materials are not conducive to retention. Net positively charged formulations may improve retention at the injection site. Such interactions could limit systemic exposure and mitigate the AEs commonly associated with PAMP immunotherapies. Further exploration of optimal physiochemical properties for retention and efficacy could greatly enhance future IT therapies incorporating PAMPs.

5.2. Cytokines

Cytokines play an important cell signaling role and are major regulators of immunity. These small proteins can activate immune responses and encourage cancer cell destruction [189,190]. Like PAMPs, to avoid systemic toxicity, cytokines should be localized at the tumor.

Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) is a growth factor that stimulates hematopoietic stem cells to differentiate into dendritic cells, granulocytes, and monocytes [191]. Recombinant human GM-CSF is a 14-35 kDa glycoprotein with 127 amino acids [117] composed of four bundles of α-helices and is non-spherical with dimensions of ca. 2x3x4 nm [118]. GM-CSF has been studied as an immunostimulatory adjuvant to induce anti-tumor immunity [192] although a recent report suggests GM-CSF may stimulate tumor growth and metastasis in certain cancers [191]. Severe AEs were reported in malignant mesothelioma patients given intraluminal infusions of 2.5-10 mg/kg/day GM-CSF, which may be a result of systemic exposure [193]. Conversely, lower dosage, daily injections of 15-50 μg or 400 μg GM-CSF given to melanoma patients produced more mild side effects, likely due to lower systemic exposure [194,195]. The lower AEs seen in these studies may have resulted from a combination of the lower dosage concentration, or a difference in the location and morphology of tumors associated with the cancer type (i.e. mesothelioma vs melanoma).

The IL-2 cytokine has also been widely explored in cancer. Human IL-2 has a MW of 15.5 kDa, is comprised of 133 amino acids [119], and has a hydrodynamic radius of ~3 nm [196]. Interestingly, IL-2 can be immunostimulatory or suppressive by activating cytotoxic effector cells or regulatory T (Treg) cells, respectively [119]. Typically, high doses of IL-2 are immunostimulatory and generate an anti-tumor immune response, while low doses of IL-2 are used for immunosuppression. The T-cell expansion that ensues in the presence of IL-2 has the potential to promote an anti-tumor response that overcomes a senescent microenvironment established by tumors [197]. Trials with IL-2 commonly reported systemic AEs [120,198,199]. Nevertheless, patients with melanoma [120,199] responded better to IT IL-2 treatment compared to patients with head and neck squamous cell carcinoma (HNSSC) [198], possibly due to higher neoantigen load in melanoma compared to HNSSC [200]. Moreover, the addition of 6-7 kDa poly-ethylene glycol (PEG) chains increases the drug’s solubility, extends half-life, and reduces off-target immunogenicity, which translated into better patient responses [121,122]. Additional studies demonstrated that PEGylation lowered the drug’s affinity for receptors on Treg cells to a greater extent than receptors on CD8+ T cells, which resulted in a more favorable CD8+ T-cell activation over Tregs [201].

The anti-tumor responses to IT IL-2 generally are limited to the injected tumor, and do not extend to untreated metastases (i.e. no abscopal effect). To address the lack of systemic immunity with intratumoral IL-2 treatment, an intratumoral ipilimumab/IL-2
combination was administered in patients with unresectable stage III/IV melanoma in a phase I study [157]. The hypothesis was that the combination treatment would effectively activate tumor infiltrating lymphocytes and promote systemic immunity. A local response was observed in 67% (8 out of 12) of the patients and an abscopal response was observed in 88.9% (8 out of 9) patients. Treatment-related AEs were of grade 1 or 2. Some of the responders had increased frequency of CD8+ T-cells expressing IFNγ, Tbet, granzyme-B and/or perforin, suggesting a systemic immune response.

Additional IT cytokine immunotherapies include IFNα, TNFα, and IL-12. Recombinant α-interferon administered IT in patients with prostate cancer showed a 30% complete response (CR) rate [202]. Tumor Necrosis Factor-α (TNFα) has been intratumorally injected while co-administering subcutaneous IFN-a2b for advanced prostate cancer as well [203]. Notably, TNFα leakage into the systemic circulation was observed 2 hours after injections and may have contributed to AEs. Recombinant human interleukin-12 (rhIL-12) tested in HNSCC was detected in the plasma 30 minutes after IT injection with a half-life of 7.2 h [204]. Such systemic exposure can be harmful to patients, as a phase II study with a similar treatment regimen for 10 HNSCC patients exhibited prevalent AEs [205].

Cytokines elicit signaling cascades by acting together with other direct signals, so cocktail approaches and combination therapies have also been attempted, but with limited success. One such cocktail approach involved a multi-cytokine solution consisting of IL-2, IL-1α, IL-1β, GM-CSF, IFNα, TNFα, TNFβ, IL-3, IL-4, IL-6, IL-8, IL-10, and macrophage inflammatory protein 1α. IT or peritumoral injection in patients with HNSCC in combination with intravenous cyclophosphamide, in patients intratumorally in patients with HNSCC in combination with intravenous cyclophosphamide, intraoral indomethacin, and oral zinc showed a 16.7% CR rate [206]. Tumors accumulated an elevated number of CD4+ T-cells and natural killer cells. Notably, this high-powered cocktail led to 8.3% of patients developing sepsis and Wegener granulomatosis, suggesting systemic exposure.

To limit the systemic cytokine exposure after IT injection, a tumor-targeting domain may be conjugated or engineered into the therapeutic. For instance, IL-4(38-37)-PE38KDEL is a chimeric protein composed of modified IL-4 and a truncated form of Pseudomonas exotoxin (PE), which can target and bind to IL-4 receptor-positive glioblastoma cells [123]. Likewise, IL13-PE38QQR (ILL3PE) is a chimeric protein of IL-13 conjugated to truncated PE and binds to IL-13 receptors on malignant glioma cells [124]. The IL-2-based immunokine (darleukin) and the TNFα-based immunokine (fibromun) further incorporate a diabody derived from the L19 antibody to introduce fibronectin binding functionality that capitalizes on overexpression in tumors [207]. With the absence of the Fc region on the diabody fragment of the antibody, the molecule does not interact with FcRn and has a more limited half-life than a full IgG molecule. Nonetheless, the smaller size allows for better penetration and distribution in the tumor. In a Phase II trial in metastatic melanoma, the combination therapy of darleukin and fibromun (called daromun) resulted in AEs that were limited to local injection site reactions and an overall response rate (ORR) of 55% [208].

Cytokines are by nature small (<70 kDa) and water-soluble, which potentially confounds their retention within the TME [209]. Several clinical approaches have sought to address these detriments, but the continued development of strategies for the IT administration of cytokines within the TME will undoubtedly optimize efficacy while minimizing AEs. These strategies should continue to seek modification strategies that do not impede receptor binding or penetration within the tumor.

5.3. Oncolytic viruses

Oncolytic viruses (OVs) (20–500 nm) kill tumor cells by cell lysis [210]. Subsequent viral replication and induction of an immunogenic response further compound their effects [211]. A variety of virus types have been designed to be oncolytic for IT therapy, including adenovirus, enterovirus, herpes simplex virus, parvovirus, measles (Rubeola) virus, reoviruses, and vaccinia virus classes [127,132,134,135,137,212–217].

OVs are typically modified or designed to improve affinity for tumor cells, while also limiting infection in healthy cells. One method to achieve tumor-selective replication is to delete viral genes that maintain viral replication in cancer cells, but inhibit viral replication in normal cells [211]. Examples include the deletion of the E1B 55-kDa gene (e.g. ONYX-015) or EIA gene in adenoviruses (e.g. DXN-2401), RLI gene deletion in herpes simplex virus (HSV) type 1 (155–240 nm in diameter) (e.g. HSV-1716) [130,132], and the deletion of viral genes encoding vaccinia growth factor (VGF) and thymidine kinase (TK) in the vaccinia virus vvDD-CDSR [137].

Viruses can also be designed to specifically target tumor cells by exploiting alterations in cell surface receptors. For instance, in addition to the E1A gene deletion, DXN-2401 has an RGD-motif engineered into the fiber H-loop [127]. This motif enhances tumor infectivity/cell entry by allowing the virus to utilize the αvβ3 and αvβ5 integrins enriched on tumor cells. The coxsackievirus a21 (CVA21) (~31 nm in diameter) can bind to intracellular adhesion molecule 1 (ICAM-1) and decay acceleration factor (DAF) proteins that are highly expressed on certain tumor cells [128]. The live-attenuated measles virus Edmonston-Zagreb vaccine strain (120-250 nm in diameter) [133] can bind to CD46 that are expressed by some cancer cell lines, making these cells a preferred target [134].

The use of OVs as a monotherapy is generally well-tolerated with mild AEs such as injection site pain and flu-like symptoms. However, they have shown varying success, where a few treatments led to some clinical responses and others to no clinical responses with limited evidence of abscopal effects. A common lack of abscopal effects by OVs may suggest poor immune activation, and that the observed tumor destruction may occur as a direct consequence of viral infection and the subsequent adaptive immune response targeting viral antigens, rather than tumor neoantigens.

5.4. Cancer gene therapy

5.4.1. Viruses

Replicative (oncolytic) and non-replicative (non-oncolytic) viruses can be used as vectors to deliver foreign DNA into cells with high gene transfer efficiency [218]. Several IT viral therapies have been designed to express factors such as GM-CSF (e.g. T-Vec, ONCOS-102, Pexa-Vec) [138,140], interferon (IFN)-γ (e.g. TG1042) [143,219], tumor necrosis factor-α (TNFα) (e.g. TNFaderate) [144,145,220], or IL-12 (e.g. Ad-RTS-hIL-12 or INXN-2001) [221]. Suicide genes have also been delivered, such as the bacterial gene called E. coli purine nucleoside phosphorylase (PNP), which converts fludarabine into the anti-cancer agent fluorodeoxuridine [146]. Further, the herpes simplex kinase thymidine kinase (HSV-TK) has been used to incorporate ganciclovir into a toxic phosphorylated compound [147,222,223].

Talimogene laherparepvec (T-Vec/Imlycè®) was approved by the FDA and EMA in 2015 for treating melanoma lesions. This modified oncolytic herpes simplex virus-1 (155-240 nm) can selectively replicate in cells and destroy infected tumor cells [44]. T-Vec is ICP34.5-deficient (similar to HSV-1716) allowing selective replication in tumor cells [224]. Of note, a comparison between intratumoral T-Vec and subcutaneous GM-CSF in patients with unresectable stage IIIb/C/IV melanoma in a phase III trial showed a higher efficacy for T-Vec compared to GM-CSF alone [225]. Specifically, compared to the GM-CSF treated patients, the T-Vec-treated patients had a higher median overall survival (OS, 23.3 months vs 18.9 months), durable response rate (DRR, 19.3% vs 1.4%) ORR (31.5% vs 6.4%), CR (16.9% vs 0.7%), partial response (PR, 14.6% vs 5.7%), and disease control rate (DCR) (76.3% vs 56.7%). Common AEs with T-Vec treatment include fatigue, chills, pyrexia, nausea, and flu-like illness. However, T-Vec-treated patients
had higher instances of grade 3 or 4 AEs compared to GM-CSF-treated patients (11.3% vs 4.7%), which include vomiting, cellulitis, dehydraton, deep vein thrombosis, and tumor pain.

TNFerade uses an interesting technique for the localized delivery of TNFα. TNFerade is a replication-deficient adenovirus type 5 that carries a transgene encoding human TNFα. However, a radiation-inducible Egr-1 promoter gene was placed upstream to the TNFα cDNA, allowing for the time and location of TNFα delivery through the use of radiation therapy. Ad-RTS-hIL-12 is an adenoviral vector that was engineered for the controlled expression of IL-12. This involves the use of the RheoSwitch Therapeutic System®, which requires the oral activator veledimex to induce IL-12 expression [221]. These inducible systems allow the temporal regulation of gene product expression.

5.4.2. Plasmids

Although less common, DNA plasmids can also be used for gene delivery. For instance, the IL-12 plasmid cDNA (pNGVL3-mIL12) was given at 50 μg with saline (0.76 mL) in patients with cutaneous or subcutaneous metastases in a phase I clinical study [148]. Since the efficacy of these therapies requires their entry into cells, the negatively charged nature of plasmid DNA would likely make it difficult for DNA to pass through the negatively charged cell membranes. Nevertheless, 45.5% of the analyzed patients had SD and 54.5% of the analyzed patients had PD. 41.7% of patients had a PR (i.e. lesion size decrease) in the treated tumor, but non-treated lesions did not decrease in size. No IL-12 or IFNγ was detected in patient serum. The treatment was well-tolerated with no toxicity higher than grade 1.

Electroporation was used to help deliver tavo, a 6215 bp plasmid that encodes for the p35 and p40 subunits of the human IL-12 protein [149,226]. Clinical responses were similar between treatment the full-length IL-12 plasmid cDNA and tavo with electroporation; however, a direct comparison cannot be made due to their difference in plasmid design, study design, and dosage regime.

Another method to improve delivery of DNA into cells is through incorporation with cationic lipids or cationic polymers, forming lipoplexes or polyplexes, respectively. The EGFR antisense DNA is a plasmid of pNGVL1-U6-EGFRAS that was prepared in complex with DC-chol liposomes [227]. The plasmid is likely about 9600 base pairs; the pNGVL vector (also called pUMVC) is 9287 bp, human U6 promoter is 241 bp, and EGFRAS is 39 bp [227–229]. Mixing BC-819 (a plasmid DNA that encodes for the A fragment of diphtheria toxin under the control of a H19 gene promoter), with PEI formed 80-90 nm polyplexes [151]. BC-819 polyplexes were given as intravesical treatments in patients with bladder tumors in a phase Ibb clinical trial. None of the 39 evaluated patients had disease stage or grade progression [230]. 64% of evaluated patients were recurrence free at 3 months, and complete tumor ablation was observed in 33.3% with no new lesions at 3 months. The recurrence-free rate was 45% at 1 year and 40% at 2 years. Other than flu-like symptoms, AEs were mostly related to the lower urinary tract. Also, CYL-02 (a plasmid that encodes for the DCK-UMK fusion protein, which phosphorylates and activates the pro-drug gemcitabine) was prepared in 5% w/v glucose with a PEI nitrogen to DNA phosphate (N/P) ratio of 8 to 10. No particle size information was provided for CYL-02; however, it is likely around 45 nm based on another reported polyplex with N/P of 8-10 that was made with JetPEI, which appears to be the same JetPEI used to make CYL-02 [155]. A phase I clinical trial of IT injections of CYL-02 in combination with gemcitabine IV infusion was conducted in pancreatic cancer patients [154]. CYL-02 DNA and the expression of therapeutic messenger RNA was detected in the tumor after one month of treatment, demonstrating the successful DNA delivery to tumors and long-term gene expression. No objective response was observed, but the treatment inhibited tumor progression in 95% of patients two months after treatment and 91% of the metastasis-free patients had no new tumor development. Further, the treatment did not prevent the progression of distant tumors as metastatic tumors progressed in 71% patients. This combination treatment was well tolerated, and the toxicity profile was similar to gemcitabine alone. The treatment was well tolerated in patients and AEs associated with CYL-02 injection were of mostly grade 1 or 2.

5.5. Monoclonal antibodies

Monoclonal antibodies (mAbs) (150 kDa, ~10-15 nm) have shown promising therapeutic efficacy as cancer treatment [158]. Immunostimulatory mAbs can target antigens expressed on the surface of tumor cells and induce cytotoxic T lymphocyte (CTL) responses, which result in tumor cell death [231]. Immune checkpoint inhibitors (ICIs) are therapeutic mAbs that target the receptors of inhibitory signaling pathways to reverse immune suppression and reactivate immune-mediated antitumor responses [232]. Immune checkpoint inhibitors, such as antibodies targeting CTLA-4 and PD-1/PD-L1, have demonstrated broad activation of tumor-specific T-cells by blocking negative-feedback mechanisms of the immune system. The most common administration route of these mAbs is systemic, however, systemic delivery of mAbs can induce immune-related AEs [233]. Therefore, IT administration of mAbs has been suggested to retain mAbs in the tumor microenvironment and reduce systemic exposure and associated inflammatory side effects [234]. Local antibody administration has shown accumulation in tumor-draining lymph nodes, which may assist in generating antitumor immunity or addressing metastases [235].

Ipilimumab, a human IgG1 that targets CTLA-4, was the first approved immune checkpoint inhibitor for advanced melanoma and has significantly improved the overall survival rate associated with this disease [236]. Systemic ipilimumab administration is commonly associated with a low response rate and life-threatening toxicities, which has prompted the exploration of IT delivery. Current clinical trials of IT administered ipilimumab are mostly in combination with therapies including myeloid DCs, anti-PD-L1 mAbs, and IL-2, and these combinations showed improved tolerability and abscopal effects [157,237,238]. In one phase I study of IT ipilimumab combined with IL-2 for advanced melanoma, T-cells were activated within the tumor and in the draining lymph nodes, indicating IT administration enhanced the local anti-tumoral responses and also induced distal effects [157].

Co-stimulatory receptors such as CD40 and OX40 are other targets in cancer therapy. Co-stimulatory receptors are mainly expressed on APCs, and when activated, the presentation of tumor antigens increases and cytokines are released to improve the activation of anti-tumor T cells [239]. The human IgG1 agonistic CD40 antibody (anti-CD40 mAb), ADC-1013, has been investigated via both IT and IV administration in advanced solid malignancies. ADC-1013 has been optimized through the use of Fragment Induced Diversity (FIND) technology to improve binding affinity [240]. This optimization made it possible to achieve high efficacy with very low doses. A phase I trial for IT administered ADC-1013 in patients with advanced solid tumors has shown safety and B-cell expansion after treatment, which could be related to the antitumor efficacy [241,242]. Clinical trials of IT anti-OX40 mAbs are limited, but some are investigating the combination of anti-OX40 mAb, BMS 986178, with TLR9 agonist SD-101 and radiation in patients with advanced solid malignancies or lymphomas [243,244].

5.6. Small molecules

Compared to proteins, small molecules typically have the advantage of high potency and improved tissue penetration. Significant systemic toxicity has been a limiting factor for small molecule drugs and thus IT formulations have been investigated.

Small molecules are commonly modified into a prodrug or formulated as emulsions or colloids to improve retention after IT injection. Several delivery systems are under clinical trials including polymer-drug conjugates, liposomal carriers, and polymeric micelles [245]. For example, one of the most extensively studied and widely utilized chemotherapy drugs is cisplatin (MW = 300 Da). HNSCC patients were given IT administrations of cisplatin/epinephrine injectable gel in a
phase III study [246]. 29% of patients had a CR or PR for at least 28 days. AEs include injection-related pain, elevated blood pressure, tachycardia, and local cytotoxic effects (e.g. inflammation, bleeding, ulceration, etc.). Two patients (1.67% of patients) experienced severe pain.

Another widely utilized small-molecule treatment is PV-10. PV-10 is a 10% saline solution of Rose Bengal disodium (MW = 1018 Da), a water-soluble xanthene dye, which has been developed as an intrale- sional formulation. A phase I trial of IT injection of PV-10 in patients with metastatic melanoma showed effective chemoablation for both injected lesions and un.injected lesions, as “bystander” responses (27% ORR for non-targeted lesions), suggesting the activation of systemic immune [247,248]. The treatment was well-tolerated; common AEs include mild or moderate pain, inflammation, and pruritus at the treatment site. In the following phase II study, the observed response rate was 51% among injected lesions, with a 26% CR rate [249].

Small molecule drugs enter tumors mainly through non-selective diffusion and passive targeting, so an ideal form of these molecules is likely nonionized to fully enable conductive diffusion. The acidic microenvironment of tumor tissue causes chemoresistance against weakly-basic drugs, which become protonated and positively charged upon entering the tumor and are less membrane permeable. The alkylation agent cisplatin (pH 3.5-5.5) and nucleotide analogue gemcitabine (pH 2.7-3.3) remain nonionized and have higher cytotoxicities at lower pH. The effect of surface charge on nanoparticles has been investigated on many nano-sized formulations as well. Positively charged particles retain in the tumor at higher concentrations compared to the surrounding tissue [250] and diffuse out at a slower rate in comparison to anionic particles [251]. This observation is due to the electrostatic interactions with negatively charged proteoglycans of the tumor vasculature. For highly charged particles like gemcitabine hydrochloride and PV-10, a disodium salt, the electrostatic interactions might be a significant limitation to their mobility within the tumor, which could affect the efficacy of IT injections.

As small molecules are largely unhindered by the transport phenomena that dictate the distribution of other classes we have discussed in this review, chemical modifications can serve to selectively impede egress from the TME. Non-specific binding of small molecules to tumor cells or the extracellular matrix components can enhance the retention within the tumor. Ligand-receptor binding also delays clearance. Polymerization and complexation of small molecules enables their retention and depot-release within the TME. Tuning the charge properties of small molecules can aid intracellular penetration of these compounds as well as retention in the tumor. IT delivery of small molecules is appealing because the lower specificity of these candidates’ mechanism can be overcome by the physical retention of their presence at the TME.

6. Emerging trends in intratumoral therapies and future directions

IT cancer therapies currently in clinical trials encompass a wide range of molecular structures and formulations. Local administration and retention of IT therapies may amplify local therapeutic efficacy and limit side-effects compared to systemic cancer therapies. Tumor transport mechanisms that limit leakage from the tumor should reduce systemic toxicities. With advances in technology and surgical procedures, such as interventional radiology, endoscopy, and laparoscopic surgery, the challenge is not the act of administering IT therapies [4,252]. Although intratumoral administration helps deliver therapeutic agents to the vicinity of the target tumor cells, challenges such as the drug diffusibility, tumor-cell uptake, and drug degradation or clearance remain. To help overcome some of these challenges, the physicochemical properties of the drug or its formulation may be modified to promote tumor retention.

Property-enhancing strategies include altering the structure of the molecule and utilizing formulation strategies. Approaches such as reducing drug solubility, increasing molecular size (e.g. PEGylation), modulating charge, or including tumor-targeting or matrix-adhesive domains can all be incorporated to sustain the therapeutic at the injected tumor. For example, since the TME is more acidic than normal tissues, increasing the isoelectric point a molecule (e.g. antibody cationization or via addition of basic amino acids) may facilitate tumor retention [253–255].

Another promising approach to extend IT residence is through the use of nanoparticles (10-200 nm). Nanoparticles can serve as drug carriers, can sustain the release of the drug, and can be formulated for targeted drug delivery [256,257]. Formulating drugs in polymeric complexes or nanoparticles can also extend the persistence of the drug at the site of administration. The highly cross-linked collagen and densely packed cells in the tumor tissue limit particle diffusion [257]. Particles larger than the extracellular matrix mesh (~40 nm, but can be variable) exhibit limited diffusion in the extracellular matrix. Furthermore, particles with size < 200nm were reported to be effective for cellular uptake [258]. For example, various particles or emulsions have also been studied as slow-release systems for injected antibodies in pre-clinical studies. Anti-CD40 has been conjugated to immunostimulatory poly(γ-glutamic acid) nanoparticles to successfully improve localization of the mAb as the nanoparticle minimized systemic cytokine release [259]. Coupling anti-CD40 to poly lactide nanoparticles, however, did not show an improvement of anti-tumor activity [260]. Other anti-CD40 formulations based on mineral oil or dextran-based microparticles have shown capacity to activate tumor-specific T-cell responses and significantly decrease AEs compared to systemic infusion [261]. However, the mineral oil-based microparticles were preferred as the dextran-based microparticles caused overly severe local inflammation. Categorically, such approaches harken back to the success of G100 by Immune Design (Table 3).

As reviewed above, OVs, gene therapy, and bacterial therapies have shown promise, suggesting particles in this size range also represent a viable approach. Persisting in the tumor space is not enough, however, the therapeutic must also access the target. Agents, such as CpG, PolyI:C, or plasmids, require uptake into cells to interact with their intracellular targets. Cationic complexes, viruses, or bacterial therapies may enhance persistence in tumor tissue and also facilitate the delivery of TLR agonists, DNA or RNA molecules into cells.

Intratumoral immune stimulation with limited systemic exposure may widen the therapeutic index for immunostimulators. Combining IT therapy with checkpoint inhibitors represents a logical synergy, but drug-drug interactions raise possible concerns, which could be avoided if IT treatment is predominantly retained in the tumor. Beyond therapeutic design, other challenges such as the administration regimen (i.e. dose, volume, frequency of injections, etc.), IT injection techniques, and the identification and selection of the cancer therapy (or combination therapies) will continue to evolve as the field progresses toward stimulating more effective immune responses in tumors.

Declaration of Competing Interest

The authors report no conflicts of interest.

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Appendix A. Supplementary data

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