Intratumoral Immunotherapy for Early-stage Solid Tumors

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ABSTRACT

The unprecedented benefits of immunotherapy in advanced malignancies have resulted in increased interests in exploiting immune stimulatory agents in earlier-stage solid tumors in the neoadjuvant setting. However, systemic delivery of immunotherapies may cause severe immune-related side-effects and hamper the development of combination treatments. Intratumoral delivery of neoadjuvant immunotherapy provides a promising strategy in harnessing the power of immunotherapy while minimizing off-target toxicities. The direct injection of immune stimulating agents into the tumor primes the local tumor-specific immunity to generate a systemic, durable clinical response. Intratumoral immunotherapy is a highly active area of investigation resulting in a plethora of agents, for example, immune receptor agonists, non-oncolytic and oncolytic viral therapies, being tested in preclinical and clinical settings. Currently, more than 20 neoadjuvant clinical trials exploring distinct intratumoral immune stimulatory agents and their combinations are ongoing. Practical considerations, including appropriate timing and optimal local delivery of immune stimulatory agents play an important role in safety and efficacy of this approach. Here, we discuss promising approaches in drug delivery technologies and opportunity for combining intratumoral immunotherapy with other cancer treatments and summarize the recent preclinical and clinical evidences that highlighted its promise as a part of routine oncologic care.

Introduction

Significant advances in the field of immunotherapy over the past decade have provided an alternative to conventional treatments, which in many advanced solid tumors involve chemotherapy and/or radiotherapy, and associated toxicities. Strong clinical benefits of immunotherapy have been observed for patients receiving immunotherapy in second-line therapy or even in the adjuvant setting (defined as treatment after primary surgical resection or radiation; refs. 1–3). This has generated interest in the adoption of cancer immunotherapies before local treatments as a neoadjuvant modality (4). Intratumoral immunotherapy, the direct inoculation of immune-stimulating agents into the tumor itself, has a number of features that makes it particularly useful in the neoadjuvant setting. In this approach, immune-stimulating agents are injected directly into the tumor site, thus avoiding off-target toxicities and adverse effects that can accompany global immune stimulation. Whereas toxicity of systemic immunotherapy has been shown to be dose related (5), local delivery of a high concentration immunotherapy agent generally translates to overall lower systemic dosages and lower systemic exposure due to limited diffusion of the agent (6). In addition, when compared with systemic administration, local administration requires a much lower dose of the agents to induce a local and systemic antitumor response. Intratumoral delivery can thus avoid problems with dose-limiting toxicity or allow for the use of combinations of agents that have poor systemic safety profiles (6).

Furthermore, direct injection at the tumor site ensures access to tumor infiltrating T cells already in the tumor microenvironment and potentially enriched for tumor antigen recognition. Recent studies have noted intratumor differences in T-cell density and clonality, possibly due to differences in neoantigens in different tumor regions (7). As a result, local immunotherapy may elicit an immune response where systemic administration of immunotherapy is not efficacious by leveraging the rich pool of antigens within the tumor to provide better priming of polyclonal antitumor response (8).

In this review, we will address practical considerations for intratumoral immunotherapy and summarize recent preclinical and clinical studies using neoadjuvant intratumoral immunotherapy. Finally, we will synthesize findings from clinical literature to provide suggestions for combining cancer surgery with intratumoral therapy to improve outcomes for patients with resectable solid tumors that have a high risk of distant recurrence.

Considerations for Clinical Utilization

The goal of intratumoral immunotherapy is to prime immune cells locally to generate a systemic antitumor effect. By using the tumor as its own vaccine, this approach allows for the generation of antitumor immunity against multiple cancer cell antigens without having to pre-identify those antigens. As such, intratumoral immunotherapy can potentially elicit a polyclonal antitumor immune response against multiple concurrent targets and provide a broad attack against tumor heterogeneity (**Fig. 1**).

Furthermore, intratumoral injections allow for the delivery of high concentrations of drugs at the tumor site while keeping total dose and systemic drug exposure low. However, there are several

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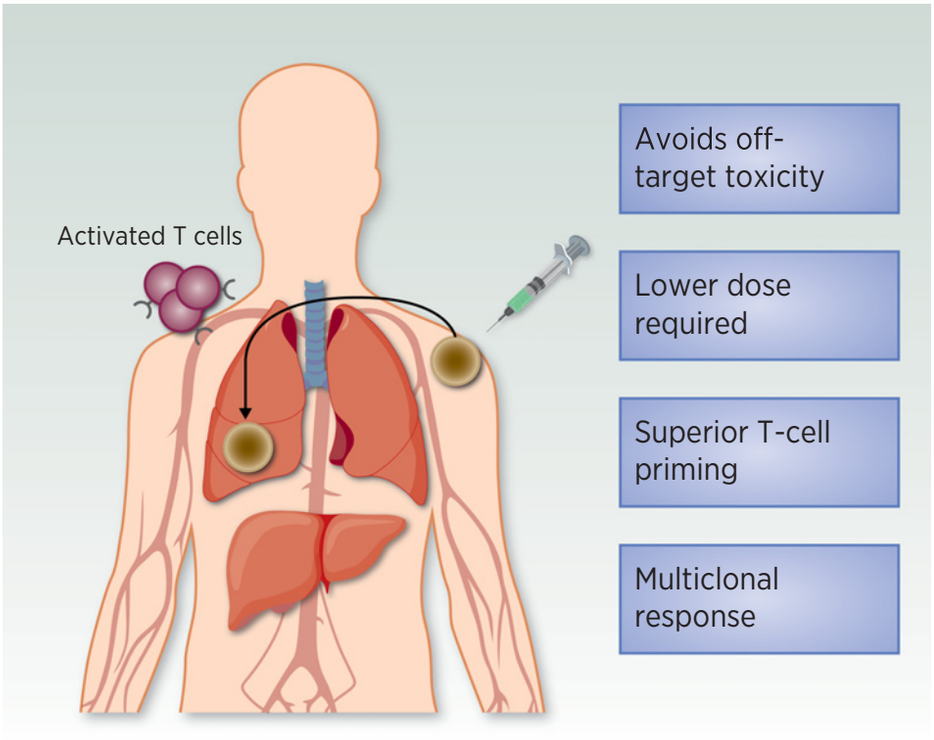

Figure 1.
Intratumoral immunotherapy initiates systemic antitumor response. Intratumoral delivery of immunotherapy involves the direct injection of immune-stimulating agents into the tumor. This primes tumor-infiltrating T cells, which then circulate systemically to generate a global antitumor response. Compared with systemic immunotherapy, this approach avoids off-target toxicities, requires a much lower dose of immune-stimulating agents, uses the antigen-rich tumor microenvironment to provide superior T-cell priming and allows for the generation of a polyclonal T-cell response.

clinical factors that must be taken into consideration when using local immunotherapy. These include timing and scheduling of therapy, choice of agents used, and opportunity for combination with other therapies.

Timing of Therapy: Earlier the Better?

Traditional chemotherapeutic or radiotherapy before surgery, termed neoadjuvant therapy, has demonstrated improved patient outcomes across a spectrum of solid tumors (9–11). The potential of neoadjuvant immunotherapy, however, remains under clinical evaluation and its utility in this setting is unclear (**Fig. 2**).

Several groups have postulated that early immunotherapy in the neoadjuvant setting can potentially improve treatment options for a large number of patients with resectable solid tumors (4, 12, 13). There are several mechanisms by which this would be possible. First, upfront treatment can cause a local therapeutic effect to reduce primary tumor burden, minimizing the extent and morbidity of surgery. For some patients with locally advanced and borderline resectable tumors, neoadjuvant immunotherapy can shrink the primary tumor and even make an unresectable tumor resectable. Second, earlier treatment can reduce recurrence in patients by targeting systemic micro-metastatic disease at time of resection. Third, upfront immunotherapy allows for faster determination of initial therapeutic success through the evaluation of the resected tumor specimen to determine efficacy of treatment on primary tumor tissue and guide subsequent clinical decision-making. Finally, neoadjuvant immunotherapy capitalizes on the presence of primary tumor as a rich source of antigens for T-cell priming (14, 15). The intact tumor can thereby act as an "*in situ*" vaccine, thereby inducing a broader and more potent antitumor immune response. This particular benefit can be magnified through the use of intratumoral immune stimulation to target tumor-infiltrating lymphocytes. Therefore, by creating an earlier therapeutic window, decreasing risk of relapse, and providing an opportunity for tailored treatment planning, neoadjuvant treatment can improve the therapeutic power of immunotherapy. Indeed, a number of preclinical studies have recently demonstrated that administering immunotherapy before definitive surgical resection produces significant clinical benefits (15–18).

A direct comparison of neoadjuvant to adjuvant immunotherapy that included T regulatory cell (Treg) depletion, anti-CD25, anti–PD-1, and anti-CD137 in a murine model of metastatic triple-negative breast cancer found that neoadjuvant treatment was more efficacious than adjuvant treatment, and resulted in more long-term survivors (15). A subsequent study using a transgenic murine model of pancreatic adenocarcinoma found that neoadjuvant treatment with a PD-1 antagonist improved survival whereas adjuvant treatment was ineffective. Furthermore, the study determined that a combined maneuver using neoadjuvant PD-1 blockade with gemcitabine along with adjuvant inhibition of CD96 and gemcitabine facilitated long-term remission and long-term survival, whereas other treatment groups not using neoadjuvant therapy succumbed to recurrent disease (17). This study highlights the increased therapeutic power of a neoadjuvant approach as well as the increased benefit from combining immunomodulating approaches with traditional chemotherapy.

In addition, using an orthotopic murine model of triple-negative breast cancer, our laboratory has found that upfront intratumoral administration of a combination of CpG and agonistic antibody anti-OX40 (aOX40) together with resection offered the best local and distant disease control and survival compared with cohorts receiving immunotherapy or resection alone. When long-term survivors from the neoadjuvant immunotherapy cohort were re-challenged with the

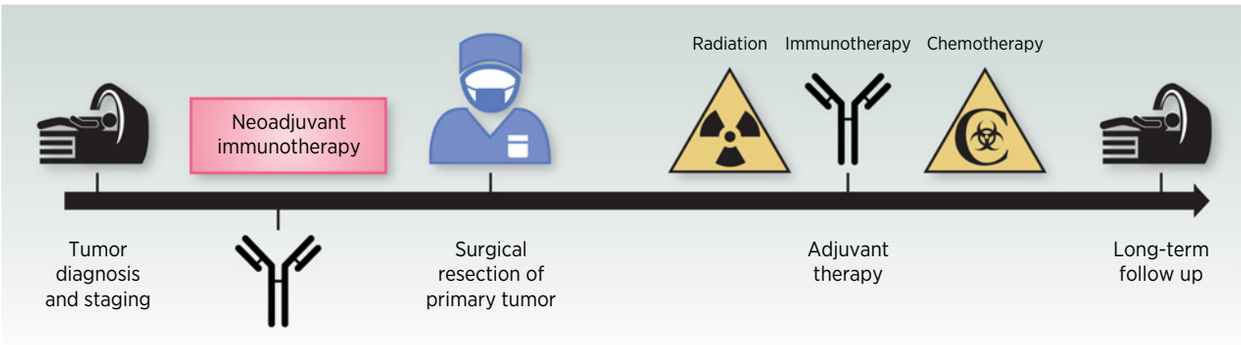

Figure 2.

Possible intervention timeline for neoadjuvant immunotherapy. Neoadjuvant therapy is the therapy given before the primary cancer treatment, usually surgery, with the goal of enhancing the outcome of the primary treatment modality. Traditionally chemoradiation has been used but there is now increasing interest in incorporating immunotherapy into the neoadjuvant setting.

same tumor, all cancer cells were rejected, while challenge of these survivors with an unrelated tumor was unimpeded, confirming the ability of neoadjuvant intratumoral CpG/aOX40 to confer specific immunological memory (unpublished results).

Intratumoral Therapies under Development

A number of high-profile neoadjuvant immunotherapy trials are currently underway (14, 19–22). At the time of this writing, there are 130 clinical studies listed on the Clinical Trials.gov website, studying a diverse array of immune modulating therapies in the neoadjuvant setting for treatment of solid tumors. However, clinical investigation into neoadjuvant intratumoral immunotherapy is still relatively new (**Table 1**).

Out of the 130 recently completed and ongoing neoadjuvant immunotherapy trials, only 24 use intratumoral immunotherapy (**Fig. 3**). Of the completed trials, all use *ex situ* dendritic cell vaccines (autologous vaccines generated *ex vivo on a customized basis*; NCT01347034, NCT00499083, NCT00365872). One of the three completed studies examined combination radiotherapy and intratumoral dendritic cell injection in patients with intermediate or high-grade soft tissue sarcomas and found enhanced T-lymphocyte response in 5/14 patients in the experimental arm versus 2/6 in the control arm (NCT01347034). Another study, also in patients with intermediate or high-grade soft tissue sarcoma, recorded a T-cell response in 10/18 individuals who received intratumoral dendritic cell vaccine with some correlation to clinical response (23).

Although these studies demonstrate promising preliminary results, a cell-based vaccine is both labor and resource intensive. The most desirable intratumoral approach would be agnostic to tumor type, capable of eliciting an effective local and systemic antitumor against, and compatible with large-scale production. An *in situ* immunization by which intratumoral administration of immune stimulating agents elicit a T-cell response is most likely to meet the above criteria. This could include intratumoral gene therapy, antibodies or small-molecule immune modulators.

Intratumoral gene therapy

Intratumoral electroporation of the IL-12 gene has been shown to be safe and effective in clinical trials in the metastatic disease setting, demonstrating systemic antitumor effects. As a monotherapy for melanoma, electroporation-mediated transfection of a plasmid encoding human IL-12 yielded a 33% overall response rate, with 50% of patients showing regression of untreated lesions (24, 25). This therapeutic modality is currently under study as a monotherapy in patients with triple-negative breast cancer (NCT02531425) and can be readily translated to the neoadjuvant setting.

Studies are also underway to examine the utility of intratumoral mRNA in solid tumors. Neoadjuvant intratumoral administration of mRNA encoding costimulatory molecule CD70, CD40 ligand, and Toll-like receptor 4 (TLR 4), collectively referred to as TriMix mRNA, is currently being investigated in patients in the neoadjuvant setting with early-stage breast cancer (NCT03788083).

Immunostimulatory antibodies

There are a number of theoretical advantages to local administration of immunostimulatory antibodies. First, the most relevant target binding sites are on the surface of tumor infiltrating lymphocytes. Second, the route of administration circumvents any issues with tissue penetration of systemically administered antibodies, and allows targeting of lymphoid tissue downstream from the injected tumor. As such there is significant interest in investigating the utility of intratumoral antibodies in solid tumors, with several clinical trials looking at OX40 agonistic antibody (NCT03831295), bispecific antibody MDX-447 (NCT00005813) and anti–PD-1 and anti–CTLA-4 antibodies (NCT03058289) among a diverse array of solid tumors. However, at this current time, there are no clinical trials assessing neoadjuvant administration of immunostimulatory antibodies.

Small-molecule immune modulators

Other candidate intratumoral therapies include small-molecule immune modulators. The intratumorally administered STING agonist ADU-S100 has demonstrated antitumor activity in PD-1–naive advanced triple-negative breast cancer and PD-1–refractory melanoma when administered in conjunction with checkpoint inhibitor spartalizumab (26). It is also being studied in patients with head and neck cancer in conjunction with the checkpoint inhibitor pembrolizumab (NCT03937141).

Intratumoral administration of TLR agonists are also being studied in preclinical (8) and clinical settings (27, 28). In a recently published phase II study, using perioperative local administration of CpG

Table 1. Recent and ongoing clinical trials using neoadjuvant intratumoral immunotherapy.

	Intratumoral agent	Combination	Tumor histotype	Clinical phase	Trial ID	Status
PAMPs and analogs	Poly-ICLC (Hiltonol)		Prostate cancer	I	NCT03262103	Recruiting
	TLR7 agonist (Imiquimod)		Melanoma	III	NCT01720407	Active, not recruiting
	TLR9 agonist (CMP-001)	Anti–PD-1 (Nivolumab)	Melanoma Lymph node cancer	II	NCT03618641	Recruiting
	TLR8 agonist (VTX-2337)	Anti–PD-1 (Tislelizumab)	Head and neck cancer	I	NCT03906526	Not yet recruiting
Gene therapy	L19IL2/L19TNF (Daromun)		Melanoma	III	NCT03567889	Recruiting
	L19IL2/L19TNF		Melanoma	III	NCT02938299	Unknown
	IL2 plasmid (Leuvectin)		Prostate cancer	II	NCT00004050	Terminated
	CD70/CD40/OX40L mRNA (TriMix)		Breast cancer	I	NCT03788083	Recruiting
Cell therapy	Dendritic cells	Chemotherapy Aromatase inhibitor Estrogen receptor modulator	Breast cancer	II	NCT00499083	Completed
	E7 TCR T cells		HPV associated Head and neck cancer	II	NCT04044950	Not yet recruiting
	Dendritic cells	Radiotherapy	Soft tissue sarcoma	II	NCT00365872	Completed
	Dendritic cells	Radiotherapy	Soft tissue sarcoma	II	NCT01347034	Completed
Viro-therapy	JX-594 (Oncolytic virus)		Colorectal carcinoma	II	NCT01329809	Terminated
	T-VEC (Oncolytic virus)		Melanoma	II	NCT02211131	Active, not recruiting
	MVA-BN-Brachyury	Anti–PD-L1 (Atezolizumab) Rilimogene galvacirepvec (PROSTVAC)	Prostate adenocarcinoma	II	NCT04020094	Not yet recruiting
	GMCI (Adenovirus)	Radiotherapy Chemotherapy	Pancreatic adenocarcinoma	II	NCT02446093	Recruiting
	T-VEC (Oncolytic virus)	Anti–PD-L1 (Atezolizumab)	Breast cancer	I	NCT03802604	Recruiting
	T-VEC (Oncolytic virus)	Chemotherapy	Breast cancer	I/II	NCT02779855	Recruiting
	T-VEC (Oncolytic virus)	Anti–PD-1 (Pembrolizumab)	Melanoma	II	NCT03842943	Not yet recruiting
	T-VEC (Oncolytic virus)	BRAF Inhibitor MEK Inhibitor	Melanoma	II	NCT03972046	Recruiting
	T-VEC (Oncolytic virus)	Radiotherapy	Soft tissue sarcoma	I/II	NCT02453191	Active, not recruiting
	T-VEC (Oncolytic virus)	Chemotherapy Radiotherapy	Rectal cancer	I	NCT03300544	Recruiting
	HF10 (Oncolytic virus)	Anti–PD-1 (Nivolumab)	Melanoma	II	NCT03259425	Active, not recruiting
	OrienX010 (Oncolytic virus)	Anti–PD-1 (Treprizumab)	Melanoma	I	NCT04197882	Recruiting

oligodeoxynucleotides, a TLR9 agonist, into the resection cavity in patients with newly diagnosed glioblastoma, followed by standard-of-care therapy following resection found no difference in survival between patients who received CpG and patients who did not, though the therapy was well tolerated (28). Intratumoral administration of TLR7/8 agonist NKTR-262 is currently being studied in patients with locally advanced or metastatic solid tumors (NCT03435640). Preliminary results from the phase I/II REVEAL trial, recently reported at ASCO 2019, noted that out of 11 patients with evaluable disease, 2 patients had partial response, 3 had stable disease and 6 had progressive disease, resulting in a disease control rate of 45.5% (29). Overall, these combinations have thus far been well-tolerated with no dose-limiting toxicities and could also be readily transferred to the neoadjuvant setting.

Combination Strategies

As reflected in the growing number of clinical trials using chemotherapy and radiotherapy in conjunction with immunotherapy, combinations of immunotherapies with other therapies may be essential for achieving an effective antitumor response and improving patient outcomes (30–32). Although it is not fully understood how immune-modulating agents should best be combined synergistically, it is an actively growing field of preclinical and clinical investigation (33, 34). And as previously mentioned, one of the benefits of local delivery of immune-stimulatory agents is that by preventing significant systemic toxicity and off-target effects, it allows for safer administration of combinatory immunotherapy regimens. To date, most of the clinical trials combining intratumoral immunotherapy with other treatment modalities have been tested in patients with advanced disease.

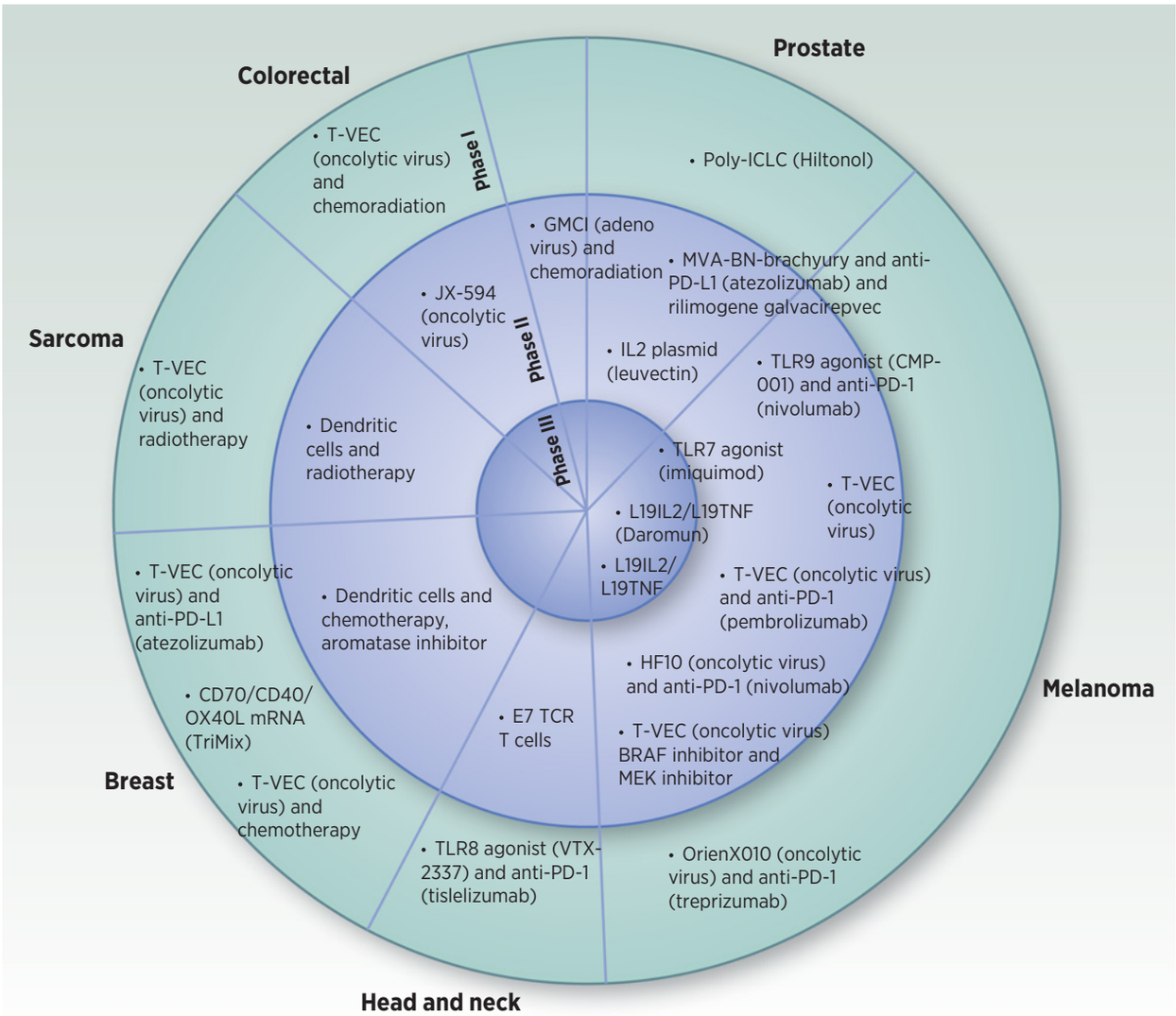

Figure 3.

Neoadjuvant intratumoral immunotherapy trials. There are 24 recent and ongoing clinical trials using neoadjuvant intratumoral immunotherapy across a diverse spectrum of solid tumors. The trial phase, immune-stimulating agents used and solid tumor type for those clinical trials is summarized in the figure.

However, these combination strategies may be even more effective in the neoadjuvant setting.

Combination intratumoral immunotherapy with checkpoint inhibitors

Several high-profile clinical trials have successfully used systemic immune checkpoint inhibitors in the neoadjuvant setting (14, 19, 22). Combining checkpoint therapy with local application of cytokines, oncolytic virus, or pathogen-associated molecular pattern to augment and maintain the local and systemic antitumor immune response is a rational next step and has demonstrated promise in preclinical and settings (35–37). For instance, in preclinical studies, intratumoral delivery of TLR 7 and TLR 9 in combination with PD-1 blockage promoted M1 polarization of tumor-associated macrophages and cytotoxic T-cell and NK activity (38–40). Furthermore, another preclinical study using a triple-negative breast cancer model demonstrated that neoadjuvant oncolytic virotherapy sensitizes the tumor to immune checkpoint therapy (16). A phase II trial investigating the combination of local administration of HF10, an oncolytic virus, and ipilimumab demonstrated an overall response rate of 41% in patients with unresectable metastatic melanoma (41). Because of these encouraging results, HF in combination with nivolumab is currently being tested in a neoadjuvant phase I trial in patients with resectable melanoma (NCT03259425).

To date, clinical trials combining intratumoral agents with checkpoint inhibitors are preliminary and mainly in patients with advanced disease. However, clinical studies of intratumoral immunotherapy in conjunction with systemic checkpoint inhibitors in the neoadjuvant

setting are ongoing and will shed light on the feasibility and efficacy of this approach in patients with earlier-stage disease.

Combining intratumoral immunotherapy with radiotherapy

It is increasingly evident that radiotherapy, in addition to causing growth arrest and cell death, may also prime antitumor immunity and synergize with immunotherapy (42–45). This synergistic effect of immunotherapy and radiotherapy is dependent on three variables: the individual radiotherapy dose, the number, and the timing of radiotherapy treatments. These are critical determinants in successful generation of an antitumor immune response (46, 47). For example, at certain dosages, radiotherapy not only eliminates tumor cells via DNA-damage and programmed cell death, but also elicits tumor-specific immune responses by tumor antigen release and activation of effector cells (43, 44, 46, 48).

So far, most ongoing clinical neoadjuvant trials combine local delivery of immune stimulatory agents with standard radiotherapy (NCT00365872, NCT01347034, NCT02446093). These conventional radiation regimens are primarily designed to eliminate tumor cells but might not be optimal for partnering with immunotherapy. Preclinical data have shown that a single dose of 20 Gy, in contrast with 3 fractions of 8 Gy, resulted in insufficient dendritic cell recruitment and lack of T-cell activation. When combined with immunotherapy, this single dose of 20 Gy failed to elicit local and systemic antitumor response (47). In this instance, a lower dose radiotherapy treatment, repeated over time, was more effective in eliciting antitumor response than a more conventional, higher, single dose of radiotherapy, illustrating the correlation between radiotherapy dosage and its immune modulating effects.

In addition, studies have shown that optimization of radiotherapy dose–time fractionation can increase dendritic cell recruitment and enhance antitumor response (49). A neoadjuvant phase I/II trial combining localized fractionated radiotherapy geared toward stimulating antitumor immune response with intratumoral administration of dendritic cells demonstrated the feasibility and efficacy of this approach in patients with high-risk soft tissue sarcoma (23, 50). Although preclinical and clinical data from this combination approach are encouraging, optimal radiotherapy regimens require further delineation and unanswered questions regarding dose, size, and fraction of radiotherapy remain.

Combining intratumoral immunotherapy with chemotherapy

The main rationale for using neoadjuvant chemotherapy is its capacity to eliminate tumor cells, reduce tumor size and facilitate surgery (51–54). In addition, neoadjuvant chemotherapy can also target micro-metastatic disease and test chemo-responsiveness (15, 55, 56). Although traditionally this modality has been considered to be immunosuppressive, this may be because cytotoxic chemotherapeutic agents were originally used in amounts that are close to the maximum-tolerated dose (MTD) in phase II and III clinical trials, which commonly causes myelo- and lymphopenia (57). Chemotherapy is now typically given at doses that are significantly lower than MTD, and have been shown to allow for the elicitation of normal immune responses by vaccines against the influenza virus, demonstrating that chemotherapy is compatible with initiation of an immune response (58, 59). Moreover, accumulating evidence over the past several years suggest that certain chemotherapeutic agents can actually modulate tumor immunity and strengthen antitumor effects when combined with local delivery of immune stimulatory agents (60–62).

For instance, low-dose cyclophosphamide depletes T regulatory cells while increasing effector T-cell function and gemcitabine enhances cross-priming of CD8 T cells and reduces MDSC infiltration (63–65). Furthermore, preclinical data show that application of low-dose cyclophosphamide before local therapy with TLR 7 and radiotherapy was able to enhance antitumor effect in a murine breast cancer model (66). And, another preclinical study found that intratumoral oncolytic peptide LTX-315 displayed a strong additive antitumoral effect when used in conjunction with standard-of-care doxorubicin (67).

Because its ability to both eliminate tumor cells and trigger immune response, chemotherapy is a promising combination partner with immunotherapy. However, the optimal integration of these two therapeutic regimens should seek to minimize antagonistic interactions.

Challenges to Intratumoral Therapy

Existing clinical data using intratumoral immunotherapy are preliminary and mainly in patients with advanced or refractory disease. To fully elucidate the potential of neoadjuvant intratumoral immunotherapy and make this therapeutic approach available to patients with early-stage cancer, a number of practical considerations must first be addressed.

The ideal dose and schedule for intratumoral immunotherapy have yet to be established and is likely to vary according to safety profile of the immune stimulating agent and the combination of agents (68). Although systemic immunotherapy is generally dosed by patient weight, intratumoral immunotherapy can be dosed according to size of a specific lesion (e.g., fixed dose/tumor volume), to overall tumor burden or to patient weight. Which dosing algorithm is most appropriate can also vary based on the agent or combination of agents used according to whether the treatment regimen is more likely to cause a local reaction or systemic toxicity. Thus, the use of biomarkers and radiologic assessments is critical in Phase I clinical trials using intratumoral immunotherapy for determining the optimal doses and schedules.

Accessibility of tumor lesions is another major challenge to intratumoral therapy, especially if repeated injections are needed to trigger an effective systemic immune response. Most intratumoral clinical studies have been performed in easily accessible lesions such as breast or skin cancer. However, due to increased availability of interventional radiologic, endoscopic, and laparoscopic procedures, most if not all lesions can now be accessed with or without the assistance of imaging modalities such as ultrasound, CT, etc (68). At the European Society for Medical Oncology (ESMO)-sponsored meeting for human intratumoral immunotherapy (HIT-IT) in 2018, experts proposed that intralesional therapy be considered for any tumor where either the primary lesion or its metastases are accessible (68). Therefore, the challenge in intratumoral therapy now lies not in initial accessibility, but in optimization of drug delivery technologies to enhance intratumoral delivery and reduce repeat injections (6, 69). Ways in which repeat dosing might be avoided include: Development of better methodologies to confine therapeutics to target cells, block therapeutic diffusion and improve drug uptake by target cells (70–72).

Recent preclinical and clinical data demonstrate that intratumoral injection of immunostimulatory agents followed by electroporation is a safe and effective method to enhance drug uptake in tumor cells (73, 74). Electroporation pulses, applied locally with electrodes, lead to permeabilization of the cell membrane and facilitate transduction of applied agents in the target cells. Clinical Phase I and II studies using electroporation have shown an improved systemic therapeutic effect in patients with advanced melanoma and triple-negative breast cancer compared with cytokine delivery alone (74, 75).

Another promising approach to promote tumor accumulation and reduce systemic effects is the utilization of nanoparticles (76–78). Nanoparticles serve as drug carriers through organ barriers or cell membranes. Because only a small portion of cells to be transduced to achieve an effective antitumor response, nanoparticles may be particularly effective when used in conjunction with intratumoral therapies (79–81). Reports of nanoparticle-based delivery, for example, delivery of PD-L1 trap fusion protein in a colon cancer model, illustrated the feasibility and efficacy of this approach (82). In addition, nanoparticles may be used to deliver multiple pro-immune agents simultaneously. Preclinical data of co-delivery, for example, IL-2 and TGF-β inhibitor or anti-CD137 and CpC, eliminated injected tumors and induced effective systemic antitumor responses (83, 84). Finally, nanoparticles can be designed to target a specific immune cell subpopulation, for example, $CD8^+$ T cells, to localize immunotherapeutic reagents and reduce off-target effects (85).

Conclusion

Intratumoral delivery of immunotherapy has multiple advantages that make it especially appealing in the neoadjuvant setting. However, to realize the full potential of neoadjuvant intratumoral immunotherapy, more clinical trials are needed to explore local administration in patients with early-stage solid tumors. For this to happen, preclinical results first need to be translated into the clinical setting. As mentioned above, one of the main difficulties of local intratumoral administration is the requirement for invasive procedures for administrating treatment to solid tumors that are not always readily accessible. However, new pharmaceutical formulations of immunotherapy agents incorporating advanced strategies of encapsulation and delivery are decreasing the procedural requirements for intratumoral immunotherapy and making it increasingly amenable to clinical translation (78, 86, 87).

Separately, a growing number of immunotherapies are being approved by the FDA and European Medicines Agency for clinical use. However, only a handful of immune-modulating agents have been studied in the intratumoral setting even though such localized testing in most cases presents less risk than systemic applications and offer increased opportunity to explore immunotherapy in a combined clinical setting. Where clinical trials study the efficacy systemic administration of specific immune-modulating agents, corresponding trials should be run in the intratumoral setting for comparison.

As increased options to incorporate immunotherapy in the clinic become available to clinicians, these studies would provide timely and clinically important information on how immunotherapy may optimally be combined with surgical resection. The results of such studies could then be used to inform establishment of neoadjuvant immunotherapy regimens for patients with early-stage and locally advanced solid tumors.

Disclosure of Potential Conflicts of Interest

R. Levy is a paid consultant for Five Prime, Quadriga, GigaGen, Teneobio, Sutro, Checkmate, Nurix, Dragonfly, Abpro, Apexigen, Viracta, Forty Seven, Spotlight, XCella, Immunocore, and Walking Fish, reports receiving commercial research grants from Bristol-Myers Squib and Janssen, and is an unpaid consultant/advisory board member for the Leukemia and Lymphoma Society and the Margaret Early Trust. No potential conflicts of interest were disclosed by the other authors.

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References

1. Eggermont AMM, Blank CU, Mandala M, Long GV, Atkinson V, Dalle S, et al. Adjuvant pembrolizumab versus placebo in resected stage III melanoma. N Engl J Med 2018;378:1789–801.
2. Allen EMV, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science 2015;350:207–11.
3. Eggermont AMM, Chiarion-Sileni V, Grob J-J, Dummer R, Wolchok JD, Schmidt H, et al. Prolonged survival in stage III melanoma with ipilimumab adjuvant therapy. N Engl J Med 2016;375:1845–55.
4. O'Donnell JS, Hoefsmit EP, Smyth MJ, Blank CU, Teng MWL. The promise of neoadjuvant immunotherapy and surgery for cancer treatment. Clin Cancer Res 2019;25:5743–51.
5. Ascierto PA, Del Vecchio M, Robert C, Mackiewicz A, Chiarion-Sileni V, Arance A, et al. Ipilimumab 10 mg/kg versus ipilimumab 3 mg/kg in patients with unresectable or metastatic melanoma: a randomised, double-blind, multicentre, phase 3 trial. Lancet Oncol 2017;18:611–22.
6. Marabelle A, Tselikas L, de Baere T, Houot R. Intratumoral immunotherapy: using the tumor as the remedy. Ann Oncol 2017;28:xii33–43.
7. Reuben A, Gittelman R, Gao J, Zhang J, Yusko EC, Wu C-J, et al. TCR repertoire intratumor heterogeneity in localized lung adenocarcinomas: an association with predicted neoantigen heterogeneity and postsurgical recurrence. Cancer Discov 2017;7:1088–97.
8. Sagiv-Barfi I, Czerwinski DK, Levy S, Alam IS, Mayer AT, Gambhir SS, et al. Eradication of spontaneous malignancy by local immunotherapy. Sci Transl Med 2018;10; pii: eaan4488.
9. NSCLC Meta-analysis Collaborative Group. Preoperative chemotherapy for non–small cell lung cancer: a systematic review and meta-analysis of individual participant data. Lancet 2014;383:1561–71.
10. Cats A, Jansen EPM, van Grieken NCT, Sikorska K, Lind P, Nordsmark M, et al. Chemotherapy versus chemoradiotherapy after surgery and preoperative chemotherapy for resectable gastric cancer (CRITICS): an international, open-label, randomised phase 3 trial. Lancet Oncol 2018;19:616–28.
11. Ilson D, Lordick F. Definitive or neoadjuvant chemoradiotherapy for squamous cell oesophageal cancer? Lancet Oncol 2018;19:1285–6.
12. Neoptolemos JP, Kleeff J, Michl P, Costello E, Greenhalf W, Palmer DH. Therapeutic developments in pancreatic cancer: current and future perspectives. Nat Rev Gastroenterol Hepatol 2018;15:333–48.
13. Mota Reyes C, Teller S, Muckenhuber A, Konukiewitz B, Safak O, Weichert W, et al. Neoadjuvant therapy remodels the pancreatic cancer microenvironment via depletion of pro-tumorigenic immune cells. Clin Cancer Res 2020;26:220–31.
14. Forde PM, Chaft JE, Smith KN, Anagnostou V, Cottrell TR, Hellmann MD, et al. Neoadjuvant PD-1 blockade in resectable lung cancer. N Engl J Med 2018;378:1976–86.
15. Liu J, Blake SJ, Yong MCR, Harjunpää H, Ngiow SF, Takeda K, et al. Improved efficacy of neoadjuvant compared to adjuvant immunotherapy to eradicate metastatic disease. Cancer Discov 2016;6:1382–99.
16. Bourgeois-Daigneault M-C, Roy DG, Aitken AS, El Sayes N, Martin NT, Varette O, et al. Neoadjuvant oncolytic virotherapy before surgery sensitizes triple-negative breast cancer to immune checkpoint therapy. Sci Transl Med 2018;10. pii: eaao1641.

17. Brooks J, Fleischmann-Mundt B, Woller N, Niemann J, Ribback S, Peters K, et al. Perioperative, spatiotemporally coordinated activation of T and NK cells prevents recurrence of pancreatic cancer. Cancer Res 2018;78:475–88.
18. Kwiatkowski DJ, Rusch VW, Chaft JE, Johnson BE, Nicholas A, Wistuba II, et al. Neoadjuvant atezolizumab in resectable non–small cell lung cancer (NSCLC): interim analysis and biomarker data from a multicenter study (LCMC3). J Clin Oncol 2019;37:8503.
19. Amaria RN, Reddy SM, Tawbi HA, Davies MA, Ross MI, Glitza IC, et al. Neoadjuvant immune checkpoint blockade in high-risk resectable melanoma. Nat Med 2018;24:1649–54.
20. Blank CU, Rozeman EA, Fanchi LF, Sikorska K, van de Wiel B, Kvistborg P, et al. Neoadjuvant versus adjuvant ipilimumab plus nivolumab in macroscopic stage III melanoma. Nat Med 2018;24:1655.
21. Schalper KA, Rodriguez-Ruiz ME, Diez-Valle R, López-Janeiro A, Porciuncula A, Idoate MA, et al. Neoadjuvant nivolumab modifies the tumor immune microenvironment in resectable glioblastoma. Nat Med 2019;25:470–6.
22. Huang AC, Orlowski RJ, Xu X, Mick R, George SM, Yan PK, et al. A single dose of neoadjuvant PD-1 blockade predicts clinical outcomes in resectable melanoma. Nat Med 2019;25:454–61.
23. Raj S, Bui MM, Springett G, Conley A, Lavilla-Alonso S, Zhao X, et al. Long-term clinical responses of neoadjuvant dendritic cell infusions and radiation in soft tissue sarcoma. Sarcoma 2015;2015:614736.
24. Daud AI, DeConti RC, Andrews S, Urbas P, Riker AI, Sondak VK, et al. Phase I trial of interleukin-12 plasmid electroporation in patients with metastatic melanoma. J Clin Oncol 2008;26:5896–903.
25. Daud A, Algazi AP, Ashworth MT, Fong L, Lewis J, Chan SE, et al. Systemic antitumor effect and clinical response in a phase 2 trial of intratumoral electroporation of plasmid interleukin-12 in patients with advanced melanoma. J Clin Oncol 2014;32:9025.
26. Meric-Bernstam F, Sandhu SK, Hamid O, Spreafico A, Kasper S, Dummer R, et al. Phase Ib study of MIW815 (ADU-S100) in combination with spartalizumab (PDR001) in patients (pts) with advanced/metastatic solid tumors or lymphomas. J Clin Oncol 2019;37:2507.
27. Frank MJ, Reagan PM, Bartlett NL, Gordon LI, Friedberg JW, Czerwinski DK, et al. In situ vaccination with a TLR 9 agonist and local low dose radiation induces systemic responses in untreated indolent lymphoma. Cancer Discov 2018;8:1258–69.
28. Ursu R, Carpentier A, Metellus P, Lubrano V, Laigle-Donadey F, Capelle L, et al. Intracerebral injection of CpG oligonucleotide for patients with de novo glioblastoma—a phase II multicentric, randomised study. Eur J Cancer 2017;73:30–7.
29. Diab A, Marcondes M, Kotzin B, Tagliaferri MA, Hoch U, Li Y, et al. Phase Ib: preliminary clinical activity and immune activation for NKTR-262 [TLR 7/8 agonist] plus NKTR-214 [CD122-biased agonist] in patients (pts) with locally advanced or metastatic solid tumors (REVEAL Phase Ib/II Trial). J Clin Oncol 2019;37:26.
30. Gandhi L, Rodriguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al. Pembrolizumab plus chemotherapy in metastatic non–small cell lung cancer. N Engl J Med 2018;378:2078–92.
31. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med 2018;379:2108–21.
32. Tawbi HA, Forsyth PA, Algazi A, Hamid O, Hodi FS, Moschos SJ, et al. Combined nivolumab and ipilimumab in melanoma metastatic to the brain. N Engl J Med 2018;379:722–30.
33. Schmid P, Cortes J, Bergh JCS, Pusztai L, Denkert C, Verma S, et al. KEYNOTE-522: phase III study of pembrolizumab (pembro) + chemotherapy (chemo) vs. placebo + chemo as neoadjuvant therapy followed by pembro vs. placebo as adjuvant therapy for triple-negative breast cancer (TNBC). J Clin Oncol 2018;36:TPS602.
34. Ready N, Hellmann MD, Awad MM, Otterson GA, Gutierrez M, Gainor JF, et al. First-line nivolumab plus ipilimumab in advanced non–small cell lung cancer (CheckMate 568): outcomes by programmed death ligand 1 and tumor mutational burden as biomarkers. J Clin Oncol 2019;37:992–1000.
35. Ribas A, Dummer R, Puzanov I, VanderWalde A, Andtbacka RHI, Michielin O, et al. Oncolytic virotherapy promotes intratumoral T cell infiltration and improves anti–PD-1 immunotherapy. Cell 2017;170:1109–19.
36. Gadkaree SK, Fu J, Sen R, Korrer MJ, Allen C, Kim YJ. Induction of tumor regression by intratumoral STING agonists combined with anti-programmed death-L1 blocking antibody in a preclinical squamous cell carcinoma model. Head Neck 2017;39:1086–94.
37. Senior M. Checkpoint inhibitors go viral. Nat Biotechnol 2019;37:12–7.
38. Sato-Kaneko F, Yao S, Ahmadi A, Zhang SS, Hosoya T, Kaneda MM, et al. Combination immunotherapy with TLR agonists and checkpoint inhibitors suppresses head and neck cancer. JCI Insight 2017;2.pii: 93397.
39. Puzanov I, Milhem MM, Minor D, Hamid O, Li A, Chen L, et al. Talimogene laherparepvec in combination with ipilimumab in previously untreated, unresectable stage IIIB-IV melanoma. J Clin Oncol 2016;34:2619–26.
40. Mullins SR, Vasilakos JP, Deschler K, Grigsby I, Gillis P, John J, et al. Intratumoral immunotherapy with TLR7/8 agonist MEDI9197 modulates the tumor microenvironment leading to enhanced activity when combined with other immunotherapies. J Immunother Cancer 2019;7:244.
41. Andtbacka RH, Ross MI, Agarwala SS, Taylor MH, Vetto JT, Neves RI, et al. Final results of a phase II multicenter trial of HF10, a replication-competent HSV-1 oncolytic virus, and ipilimumab combination treatment in patients with stage IIIB-IV unresectable or metastatic melanoma. J Clin Oncol 2017;35:15_suppl, 9510.
42. Demaria S, Golden EB, Formenti SC. Role of local radiation therapy in cancer immunotherapy. JAMA Oncol 2015;1:1325–32.
43. Golden EB, Chhabra A, Chachoua A, Adams S, Donach M, Fenton-Kerimian M, et al. Local radiotherapy and granulocyte-macrophage colony-stimulating factor to generate abscopal responses in patients with metastatic solid tumours: a proof-of-principle trial. Lancet Oncol 2015;16:795–803.
44. Twyman-Saint Victor C, Rech AJ, Maity A, Rengan R, Pauken KE, Stelekati E, et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. Nature 2015;520:373–7.
45. Formenti SC, Rudqvist NP, Golden E, Cooper B, Wennerberg E, Lhuillier C, et al. Radiotherapy induces responses of lung cancer to CTLA-4 blockade. Nat Med 2018;24:1845–51.
46. Kang J, Demaria S, Formenti S. Current clinical trials testing the combination of immunotherapy with radiotherapy. J Immunother Cancer 2016;4:51.
47. Vanpouille-Box C, Alard A, Aryankalayil MJ, Sarfraz Y, Diamond JM, Schneider RJ, et al. DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity. Nat Commun 2017;8:15618.
48. Reits EA, Hodge JW, Herberts CA, Groothuis TA, Chakraborty M, Wansley EK, et al. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. J Exp Med 2006;203:1259–71.
49. Nikitina EY, Gabrilovich DI. Combination of gamma-irradiation and dendritic cell administration induces a potent antitumor response in tumor-bearing mice: approach to treatment of advanced stage cancer. Int J Cancer 2001;94:825–33.
50. Finkelstein SE, Iclozan C, Bui MM, Cotter MJ, Ramakrishnan R, Ahmed J, et al. Combination of external beam radiotherapy (EBRT) with intratumoral injection of dendritic cells as neo-adjuvant treatment of high-risk soft tissue sarcoma patients. Int J Radiat Oncol Biol Phys 2012;82:924–32.
51. Gianni L, Baselga J, Eiermann W, Porta VG, Semiglazov V, Lluch A, et al. Phase III trial evaluating the addition of paclitaxel to doxorubicin followed by cyclophosphamide, methotrexate, and fluorouracil, as adjuvant or primary systemic therapy: European Cooperative Trial in Operable Breast Cancer. J Clin Oncol 2009;27:2474–81.
52. Thomas M, Rube C, Hoffknecht P, Macha HN, Freitag L, Linder A, et al. Effect of preoperative chemoradiation in addition to preoperative chemotherapy: a randomised trial in stage III non-small-cell lung cancer. Lancet Oncol 2008;9:636–48.
53. Girard TA, Axelrod BN, Wilkins LK. Comparison of WAIS-III short forms for measuring index and full-scale scores. Assessment 2010;17:400–5.
54. Wang M, Hou L, Chen M, Zhou Y, Liang Y, Wang S, et al. Neoadjuvant chemotherapy creates surgery opportunities for inoperable locally advanced breast cancer. Sci Rep 2017;7:44673.
55. Cortazar P, Geyer CE Jr. Pathological complete response in neoadjuvant treatment of breast cancer. Ann Surg Oncol 2015;22:1441–6.
56. von Minckwitz G, Blohmer JU, Costa SD, Denkert C, Eidtmann H, Eiermann W, et al. Response-guided neoadjuvant chemotherapy for breast cancer. J Clin Oncol 2013;31:3623–30.
57. Galluzzi L, Buqué A, Kepp O, Zitvogel L, Kroemer G. Immunological effects of conventional chemotherapy and targeted anticancer agents. Cancer Cell 2015;28:690–714.
58. Wumkes ML, van der Velden AMT, Los M, Leys MBL, Beeker A, Nijziel MR, et al. Serum antibody response to influenza virus vaccination during chemotherapy treatment in adult patients with solid tumours. Vaccine 2013;31:6177–84.

59. Blanchette PS, Chung H, Pritchard KI, Earle CC, Campitelli MA, Buchan SA, et al. Influenza vaccine effectiveness among patients with cancer: a population-based study using health administrative and laboratory testing data from Ontario, Canada. J Clin Oncol 2019;37:2795–804.
60. Abu Eid R, Razavi GS, Mkrtichyan M, Janik J, Khleif SN. Old-school chemotherapy in immunotherapeutic combination in cancer, a low-cost drug repurposed. Cancer Immunol Res 2016;4:377–82.
61. Alizadeh D, Trad M, Hanke NT, Larmonier CB, Janikashvili N, Bonnotte B, et al. Doxorubicin eliminates myeloid-derived suppressor cells and enhances the efficacy of adoptive T-cell transfer in breast cancer. Cancer Res 2014;74:104–18.
62. Sistigu A, Yamazaki T, Vacchelli E, Chaba K, Enot DP, Adam J, et al. Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. Nat Med 2014;20:1301–9.
63. Motoyoshi Y, Kaminoda K, Saitoh O, Hamasaki K, Nakao K, Ishii N, et al. Different mechanisms for anti-tumor effects of low- and high-dose cyclophosphamide. Oncol Rep 2006;16:141–6.
64. Nowak AK, Robinson BWS, Lake RA. Synergy between chemotherapy and immunotherapy in the treatment of established murine solid tumors. Cancer Res 2003;63:4490–6.
65. Suzuki E, Kapoor V, Jassar AS, Kaiser LR, Albelda SM. Gemcitabine selectively eliminates splenic Gr-1^{+}/CD11b^{+} myeloid suppressor cells in tumor-bearing animals and enhances antitumor immune activity. Clin Cancer Res 2005;11:6713–21.
66. Dewan MZ, Vanpouille-Box C, Kawashima N, DiNapoli S, Babb JS, Formenti SC, et al. Synergy of topical toll-like receptor 7 agonist with radiation and low-dose cyclophosphamide in a mouse model of cutaneous breast cancer. Clin Cancer Res 2012;18:6668–78.
67. Camilio KA, Wang MY, Mauseth B, Waagene S, Kvalheim G, Rekdal O, et al. Combining the oncolytic peptide LTX-315 with doxorubicin demonstrates therapeutic potential in a triple-negative breast cancer model. Breast Cancer Res 2019;21:9.
68. Marabelle A, Andtbacka R, Harrington K, Melero I, Leidner R, de Baere T, et al. Starting the fight in the tumor: expert recommendations for the development of human intratumoral immunotherapy (HIT-IT). Ann Oncol 2018;29:2163–74.
69. Aznar MA, Tinari N, Rullán AJ, Sánchez-Paulete AR, Rodriguez-Ruiz ME, Melero I. Intratumoral delivery of immunotherapy—act locally, think globally. J Immunol 2017;198:31–9.
70. Moyer TJ, Zmolek AC, Irvine DJ. Beyond antigens and adjuvants: formulating future vaccines. J Clin Invest 2016;126:799–808.
71. Milling L, Zhang Y, Irvine DJ. Delivering safer immunotherapies for cancer. Adv Drug Deliv Rev 2017;114:79–101.
72. Gause KT, Wheatley AK, Cui J, Yan Y, Kent SJ, Caruso F. Immunological principles guiding the rational design of particles for vaccine delivery. ACS Nano 2017;11:54–68.
73. Mukhopadhyay A, Wright J, Shirley S, Canton DA, Burkart C, Connolly RJ, et al. Characterization of abscopal effects of intratumoral electroporation-mediated IL-12 gene therapy. Gene Ther 2019;26:1–15.
74. Canton DA, Shirley S, Wright J, Connolly R, Burkart C, Mukhopadhyay A, et al. Melanoma treatment with intratumoral electroporation of tavokinogene telseplasmid (pIL-12, tavokinogene telseplasmid). Immunotherapy 2017;9:1309–21.
75. Abstract P2-09-11: Intratumoral tavokinogene telseplasmid and electroporation in pre-treated inoperable locally advanced or recurrent triple-negative breast cancer. Cancer Res [cited 2019 Sep 9]. Available from: https://cancerres.aacrjournals.org/content/79/4_Supplement/P2-09-11
76. Riley RS, June CH, Langer R, Mitchell MJ. Delivery technologies for cancer immunotherapy. Nat Rev Drug Discov 2019;18:175–96.
77. Goldberg MS. Immunoengineering: how nanotechnology can enhance cancer immunotherapy. Cell 2015;161:201–4.
78. Yoon HY, Selvan ST, Yang Y, Kim MJ, Yi DK, Kwon IC, et al. Engineering nanoparticle strategies for effective cancer immunotherapy. Biomaterials 2018;178:597–607.
79. Davis ME, Zuckerman JE, Choi CHJ, Seligson D, Tolcher A, Alabi CA, et al. Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. Nature 2010;464:1067–70.
80. Yin H, Kanasty RL, Eltoukhy AA, Vegas AJ, Dorkin JR, Anderson DG. Non-viral vectors for gene-based therapy. Nat Rev Genet 2014;15:541–55.
81. Ge Z, Chen Q, Osada K, Liu X, Tockary TA, Uchida S, et al. Targeted gene delivery by polyplex micelles with crowded PEG palisade and cRGD moiety for systemic treatment of pancreatic tumors. Biomaterials 2014;35:3416–26.
82. Song W, Shen L, Wang Y, Liu Q, Goodwin TJ, Li J, et al. Synergistic and low adverse effect cancer immunotherapy by immunogenic chemotherapy and locally expressed PD-L1 trap. Nat Commun 2018;9:2237.
83. Park J, Wrzesinski SH, Stern E, Look M, Criscione J, Ragheb R, et al. Combination delivery of TGF-β inhibitor and IL-2 by nanoscale liposomal polymeric gels enhances tumour immunotherapy. Nat Mater 2012;11:895–905.
84. Kwong B, Gai SA, Elkhader J, Wittrup KD, Irvine DJ. Localized immunotherapy via liposome-anchored anti-CD137 + IL-2 prevents lethal toxicity and elicits local and systemic antitumor immunity. Cancer Res 2013;73:1547–58.
85. Schmid D, Park CG, Hartl CA, Subedi N, Cartwright AN, Puerto RB, et al. T-cell targeting nanoparticles focus delivery of immunotherapy to improve antitumor immunity. Nat Commun 2017;8:1747.
86. Haabeth OAW, Blake TR, McKinlay CJ, Tveita AA, Sallets A, Waymouth RM, et al. Local delivery of Ox40l, Cd80, and Cd86 mRNA kindles global anticancer immunity. Cancer Res 2019;79:1624–34.
87. Goins B, Phillips WT, Bao A. Strategies for improving the intratumoral distribution of liposomal drugs in cancer therapy. Expert Opin Drug Deliv 2016;13:873–89.