Bronchoscopic intratumoural therapies for non-small cell lung cancer

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Bronchoscopic intratumoural injection of novel therapies holds remarkable potential for targeted treatment of non-small cell lung cancer with a reduced risk of toxicities https://bit.ly/36xraX7

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ABSTRACT The past decade has brought remarkable improvements in the treatment of non-small cell lung cancer (NSCLC) with novel therapies, such as immune checkpoint inhibitors, although response rates remain suboptimal. Direct intratumoural injection of therapeutic agents *via* bronchoscopic approaches poses the unique ability to directly target the tumour microenvironment and offers several theoretical advantages over systemic delivery including decreased toxicity. Increases in understanding of the tumour microenvironment and cancer immunology have identified many potential options for intratumoural therapy, especially combination immunotherapies. Herein, we review advances in the development of novel bronchoscopic treatments for NSCLC over the past decade with a focus on the potential of intratumoural immunotherapy alone or in combination with systemic treatments.

Introduction

Despite remarkable advances in treatment over the past decade, lung cancer remains the leading cause of cancer-related mortality worldwide [1]. The latest treatments, including antibodies to immune checkpoints such as programmed death receptor-1 (PD-1) have improved overall survival, although response rates remain suboptimal with only about 20% of patients responding in clinical trials [2, 3]. Further, immune checkpoint inhibitors are associated with immune-related adverse events (irAEs), which are occasionally serious or life threatening [4]. Intratumoural therapies, which have shown efficacy in other cancers, such as melanoma [5], have been proposed as a way to overcome resistance to checkpoint blockade and minimise side effects.

Bronchoscopy provides unique access to the airways and mediastinum and is frequently employed in the diagnosis, staging and palliation of lung cancer. Tools and techniques in bronchoscopy have advanced significantly in recent years with improvement in navigation adding to its diagnostic and therapeutic potential [6]. There has been increasing experience with endobronchial intratumoural chemotherapy (EITC) over the past decade, which supports its potential use as part of a multi-modality approach to

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malignant airway obstruction. Further, local delivery may provide the most effective route of delivery for several immunotherapies under development, such as oncolytic viruses.

In this review article, we will explore the advances in intratumoural therapies for non-small cell lung cancer (NSCLC) delivered *via* bronchoscopy, with a focus on the past decade. Although many of the therapies may be injected by both percutaneous and bronchoscopic routes, we excluded trials using only percutaneous delivery, as this is outside the scope of this review.

History of intratumoural therapies for cancer

The first successful report of intratumoural therapy for cancer was published in 1893 when William Coley, a surgeon and cancer researcher, famously described regressions of sarcoma after injecting live cultures of *Streptococcus pyogenes* directly into the tumours [7]. The mechanism underlying these regressions was not understood at the time, and Coley's work was generally shunned until after his death [8]. Only years later was he credited with the discovery of cancer immunotherapy, which has since revolutionised the treatment of malignancy.

Intratumoural injections for the treatment of cancer were revisited in the 1950s, shortly after the development of chemotherapy. For example, in 1958, investigators reported a series of patients treated with intratumoural injections of *NN'N"*-triethylene thiophosphoromide (Thio-TEPA), a phosphoramide chemotherapy agent, into tumours of patients with advanced solid tumours [9]. It was noted that Thio-TEPA was effective when given by intratumoural injection, and a higher dose could be used without side effects compared with systemic administration. Local treatment of tumours was limited to those that were accessible by percutaneous needle injection under direct visualisation. Although several investigators reported regressions of injected lesions, intratumoural chemotherapy never gained widespread acceptance [10].

The development of the flexible bronchoscope by Professor Ikeda in 1967 improved access to the airways and mediastinum [11]. In the early 1970s, a needle passed through the working channel of the flexible bronchoscope was used to inject Bacillus Calmette-Guérin (BCG) into lung cancers with endobronchial extension in an early study of immunotherapy [12]. These bronchoscopic injections were demonstrated to be safe and feasible, and necrosis of injected tumours was reported [13, 14]. A possible mechanism of immune stimulation has since been proposed: BCG activates pattern recognition receptors (especially toll-like receptors) to induce release of several inflammatory cytokines including interleukin-12 and promote adaptive immunity through the maturation of dendritic cells [15].

Since then, many different experimental agents have been delivered to lung tumours *via* transbronchial needle injection (TBNI) including chemotherapy [16–34], gene therapies [35–44], and other immune adjuvants [13, 14, 45, 46] (see table 1). These studies have established feasibility of local injection, and side effects have overall been minimal. With further understanding of the tumour microenvironment and

TABLE 1 Intratumoural therapies delivered *via* bronchoscopic injection in non-small cell lung cancer

Therapy	References
Bacillus Calmette–Guerin (BCG)[#]	[12–14]
***Nocardia rubra* cell wall cytoskeleton[#]**	[45]
OK-432	[46]
Ethanol (99.5%)	[47]
5-Fluorouracil	[17, 19]
Mitomycin	[17]
Methotrexate	[17]
Bleomycin	[17]
Mitoxantrone	[17]
Cisplatin[#]	[20, 21, 25–29, 34, 48–50]
Carboplatin	[18]
Paclitaxel	[30, 33]
Para-toluenesulfonamide	[31, 32]
Recombinant viral vector[#]	[35–44]
Gene-modified dendritic cells[#]	[51]

[#]: Used bronchosopic and percutaneous injection.

development of new immunotherapies for lung cancer, there are now more opportunities to explore TBNI in clinical practice.

Advantages of local therapies for cancer

Local injection has a number of theoretical advantages over systemic (*i.e.* intravenous) administration of cancer therapies, the most commonly used route of delivery. First, the local concentration of drug can be much higher than what may be achieved when an agent is delivered systemically. Studies have suggested that intratumoural injections of chemotherapy are able to achieve a 10- to 30-fold higher local concentration than could be achieved with systemic delivery [24, 52, 53]. These elevated concentrations may persist at the injection site for significantly longer than if the agent were delivered systemically. For example, injection of paclitaxel into the airway wall of a porcine model using a novel microcatheter produced local concentrations that were maintained above the therapeutic systemic concentration for 28 days [54]. Elevated local drug concentrations may be further prolonged using liposomal or microsphere drug formulations [24]. The ability to deliver such drug concentrations to the tumour site has clear utility for cytotoxic drugs such as chemotherapy (classically bound by the "log-kill" hypothesis), as a higher dose will kill a greater proportion of neoplastic cells [55]. The implications of the ability to achieve higher local concentrations of drugs with other mechanisms, such as immunotherapies, is not yet clear and warrants further investigation. In theory, there is also a risk of induction of immune tolerance with excessive local concentration of immunotherapeutic agents.

Because of decreased systemic concentration of the intratumourally injected agent, many side effects may be avoided [56]. As current immunotherapy regimens, particularly combination therapies, are limited by irAE, this may allow for combinations of two (or more) drugs to stimulate a systemic anti-tumour immune response [57, 58].

In addition, local injection of a drug may be able to uniquely target draining lymph nodes. Lymphatic drainage patterns of the lung have been well characterised and serve as frequent pathways for lung cancer metastasis [59]. Presumably, a higher concentration of the intratumourally injected agent may reach the draining lymph nodes than if the drug were administered systemically. If so, this may treat regional micrometastases with higher efficacy than current treatments [53]. The delivery of higher concentrations of a drug, especially an immunotherapy, to areas of T-cell priming and activation within lymph nodes may bring additional benefits [60–62, 63].

Understanding the tumour microenvironment

A tumour is composed not only of malignant cells, but also stromal cells including cancer-associated fibroblasts, vascular cells and infiltrating immune cells [64]. These cells interact with the tumour and host in several ways to create an immunosuppressive microenvironment and promote cancer growth. First, cancer-associated fibroblasts may provide a physical barrier that prevents immune cell infiltration and evasion of host responses. Additionally, cancer-associated fibroblasts, myeloid-derived suppressor cells and regulatory T cells secrete cytokines, such as transforming growth factor (TGF)-β and various interleukins which contribute to neoplastic cell growth and local immunosuppression [64]. Tumour cells often upregulate immune checkpoint molecules such as programmed death ligand-1 (PD-L1), further facilitating immune evasion [65].

By targeting molecules involved in immune evasion, such as programmed cell death protein 1 (PD-1) or its ligand, PD-L1, systemic immunotherapies have provided clinical improvement across many cancer types [3]. Many tumours, however, have primary or acquired resistance to checkpoint inhibitors [66]. Thus, combination immunotherapies have been proposed with different mechanisms of action to overcome resistance to immune checkpoint blockade and re-invigorate immunosurveillance. Because locally delivered agents in theory have fewer side effects than systemic therapies, immunotherapy combinations may be implemented intratumourally that would be intolerable systemically due to adverse effects.

Several characteristics of the immune contexture and tumour microenvironment have implications in the prognosis and treatment of cancer [65, 67]. For example, so-called "hot" tumours are infiltrated with a high number of cytotoxic T lymphocytes and are associated with an improved prognosis. "Cold" tumours, on the other hand, are characterised by a lack of immune infiltration (specifically the absence of T cells), and typically portend a worse prognosis [67]. Additionally, the presence of tertiary lymphoid structures (TLS), ectopic lymphoid-like organs located in the tumour microenvironment, confer a better prognosis in patients with NSCLC [68]. As sites of tumour antigen presentation and T and B cell activation, TLS may serve as both biomarkers and targets of therapy [69]. One of the overarching goals of immunotherapy is conversion of an immune-poor (cold) tumour microenvironment to one that is immune-infiltrated (hot). Direct intratumoural injection by bronchoscopy holds unique potential to achieve this goal based on local

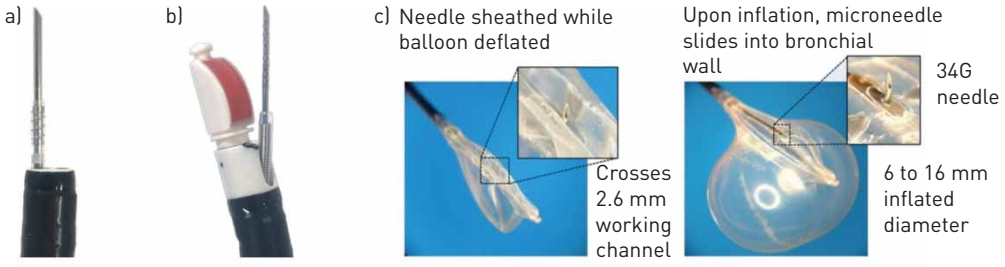

FIGURE 1 Devices used for bronchoscopic intratumoural injection. a) A conventional transbronchial needle was used for injection of several types of chemotherapy by Celikoglu *et al.* [17] among others [16, 18–25, 27, 31, 32]. b) An endobronchial ultrasound-guided transbronchial needle was used for injection of cisplatin by several investigators [26, 29, 30, 34, 48–50]. c) A novel transbronchial microcatheter (Blowfish catheter; Mercator MedSystems Inc, Emeryville, CA, USA) was used to inject paclitaxel into endobronchial lesions following standard recanalisation techniques [33]. Images in (a) and (b) are courtesy of the authors; (c) was modified from [33] with permission from publisher.

delivery of immunostimulatory agents to the tumour microenvironment and low side effect profile which may allow for novel immunotherapy combinations.

Devices for injection

Transbronchial needle

A conventional transbronchial needle has been used for decades for injection into the airways, lung parenchyma and surrounding tissues. These needles range in size from 18 to 25 gauge and fit through the working channels of flexible bronchoscopes (see figs 1 and 2). Aside from delivery of cancer therapies, transbronchial needles have been utilised for the injection of corticosteroids [71–73], anti-microbials [74–77], tranexamic acid [78], dyes [79, 80] and radioisotopes [81] for localisation of lung lesions, and tissue sealants for the closure of bronchopleural fistulae [82, 83]. The technique of TBNI has been previously reviewed [84]. A conventional transbronchial needle may be used through standard mechanisms with flexible video bronchoscopes, but TBNI could also be accomplished *via* an extended working channel of

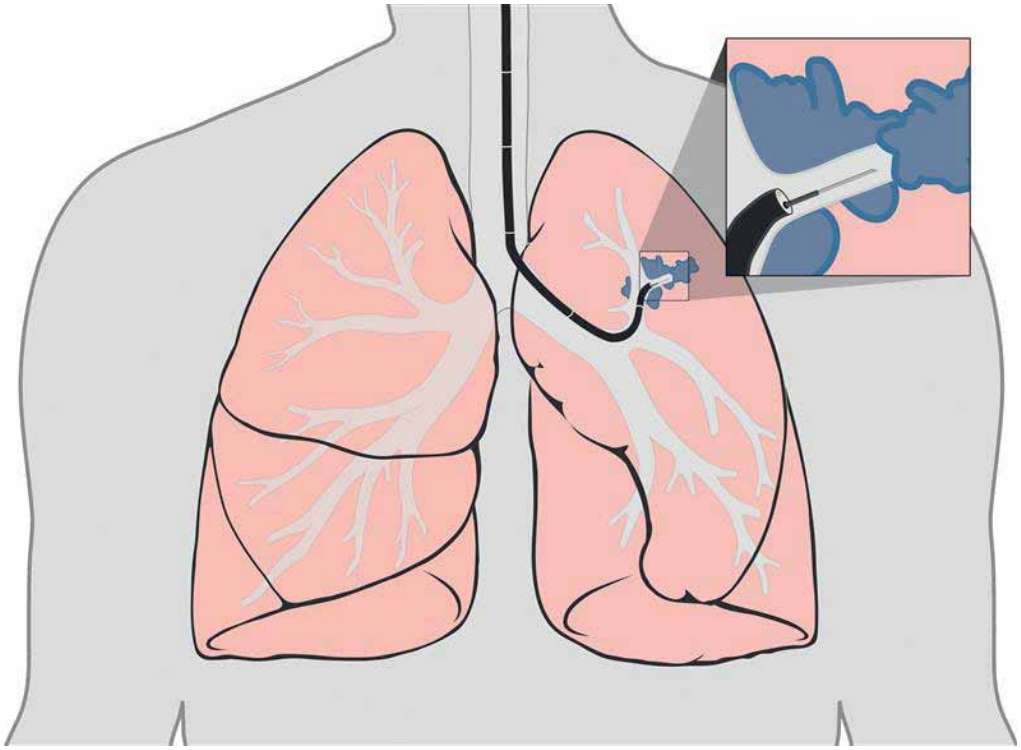

FIGURE 2 Bronchoscopic intratumoural injection using a conventional transbronchial needle. This technique has been used by several groups for endobronchial intratumoural chemotherapy (EITC) [16–25, 27, 31, 32]. Image modified from [70] under Creative Commons Attribution 2.5 license.

the bronchoscope utilising electromagnetic navigation and, in the future, through the working channel of robotic bronchoscopes. Various forms of navigation-guided tumour localisation will be necessary for the development of bronchoscopic intralesional therapies in the lung periphery.

Transbronchial needle with endobronchial ultrasound guidance

The use of convex probe endobronchial ultrasound (EBUS) to guide TBNI is a significant advance in the field. This poses the distinct advantage of direct visualisation of drug delivery (decreasing echotexture and tissue swelling seen on ultrasound images following injection) and avoidance of injection into blood vessels or other mediastinal structures [29]. EBUS TBNI was first reported in 2013 [26] and has since been described by several investigators [29, 30, 34, 48–50, 85]. EBUS TBNI in particular, facilitates improved access to target metastatic lymph nodes, as has been examined in several clinical series [26, 30] or to treat locoregional recurrence of lung cancer in a previously irradiated field [29, 48–50, 85]. EBUS TBNI is currently unable to deliver therapies to small peripheral lung nodules because the reach of the standard EBUS bronchoscope is limited by its outer diameter. A fine-needle aspiration catheter using real-time radial probe EBUS guidance to sample peripheral pulmonary lesions is currently under development and could potentially be adapted for injection [86]. A dedicated convex probe EBUS bronchoscope with a thinner diameter would also be highly beneficial for this purpose.

Transbronchial microneedle injection catheter

A novel device for endobronchial injection of chemotherapy through a transbronchial microneedle (Blowfish catheter; Mercator MedSystems Inc, Emeryville, CA, USA), was recently developed for submucosal injection into the airway wall [33, 54, 87]. The balloon-tipped catheter with retractable 34-gauge needle can inject an agent perpendicularly into the airway wall after the balloon is inflated (see fig. 1). In a recent study by Yarmus *et al.* [33], this device was used to inject paclitaxel into tumours in 19 patients with NSCLC complicated by malignant airway obstruction. After relief of airway obstruction using standard bronchoscopic techniques, multiple injections of paclitaxel (average 3.4 per patient) were performed into the bronchus circumferentially at the site of recanalisation. This technique was proven to be safe and feasible with no clinically significant adverse events due to the procedure. The improved airway patency established during the index procedure remained stable on repeat bronchoscopy after 6 weeks, and no patient required repeat intervention during the study period. This technique holds promise as part of a multi-modality strategy for relief of malignant airway obstruction, specifically to prolong the durability of response. Further investigations are necessary to evaluate the magnitude and duration of clinical effect. Although this device has only been investigated for injection of chemotherapy, the catheter could in theory be utilised to inject other therapies (including immunotherapies) into endobronchial lesions.

Recent trials in bronchoscopic intratumoural injection

Endobronchial intratumoural chemotherapy

Endobronchial intratumoural chemotherapy (EITC) has been reported by multiple investigators since 1982 [16–34]. Agents delivered by endobronchial injection include 99.5% ethanol, cisplatin, 5-fluorouracil, methotrexate, bleomycin and others (see table 1). Overall, studies have reported safety and efficacy with adverse events limited mostly to fever, myalgia, cough and minor bleeding. It is difficult to measure the effects of EITC in isolation because this technique has typically been employed in patients with advanced malignancy receiving multiple other treatments including systemic chemotherapy and radiation therapy. Commonly reported endpoints include improvement in airway patency and adverse events. For example, Celikoglu *et al.* [19] repeated bronchoscopy with EITC every week until airway patency was improved by at least 25%, which was reached in 88% of patients.

Mehta *et al.* [27] also implemented EITC in a series of 22 patients with NSCLC and malignant airway obstruction to deliver cisplatin to endobronchial lesions with large tumour bulk and rapid recurrence after ablative therapy. The investigators delivered 40 mg of cisplatin in 40 mL of 0.9% saline by 19-gauge flexible transbronchial needle. Injections were repeated weekly, up to four times, until relief of symptoms. Response, defined as at least a 50% increase in airway patency, was reported in 71% of patients. No severe treatment-related adverse effects occurred. Notably, the requirement for repeat bronchoscopy for removal of necrotic tissue and repeat injection somewhat limits the use of EITC as a complement to systemic therapy.

In a pilot trial, Hohenforst-Schmidt *et al.* [26] were first to report the utility of EBUS TBNI for the delivery of chemotherapy to lymph node metastases following a protocol involving multimodal delivery of chemotherapy in five patients with advanced NSCLC unfit for surgery, radiation and chemotherapy. They combined direct intratumoural injection (either by bronchoscopy or transthoracic needle), injection of involved lymph nodes by EBUS TBNI, and intravenous administration of platinum-doublet regimen which

was dose-reduced to 70% of the standard systemic dose. Intratumoural chemotherapy sessions were repeated weekly for 3 weeks followed by a week where chemotherapy was given only intravenously. A total of 22 sessions of intratumoural chemotherapy were performed across the cohort. For the nodal delivery, up to six different injections (each with different angulation) were performed with the goal to diffuse cisplatin throughout the nodal metastasis. If any leakage was noted, the injection was stopped and the needle position was changed. Mean dose of cisplatin was 24 mg (range 10 to 100 mg). Bleeding was reported in one patient after one intratumoural injection, but it appears to have related to the tumour itself rather than the injection and resolved after repeat injection suggesting that EITC may have a haemostatic effect. An additional patient developed mild myelosuppression from intratumoural injection of 100 mg of cisplatin (the highest reported dose delivered by TBNI), likely due to systemic dissemination. The series was able to demonstrate safety and feasibility of EBUS TBNI and laid the groundwork for future studies.

Li *et al.* [31] performed intratumoural injections of para-toulenesulfonamide (PTS), a novel cytotoxic drug with activity against several cancers, in a series of 90 patients with unresectable NSCLC complicated by malignant airway obstruction. Repeated intratumoural injections of PTS in 30% ethanol were performed two to three times weekly until the tumour was reduced by 50%. Four to six injections per session were performed into the tumour using a conventional transbronchial needle. At 30 days, there was relief of obstruction in 69% of patients by computed tomography imaging and 69% by bronchoscopy. Improvements in forced vital capacity (by 0.35 L), forced expiratory volume in 1 s (by 0.27 L) and baseline dyspnoea index were also noted. The most commonly reported adverse events included fever (4.5%), cough (12.5%), and injection site haemorrhage (10.1%). One patient developed respiratory failure due to worsening airway obstruction 3 days following PTS injection, which was attributed to the study drug, suggesting caution in its use for severe malignant airway obstruction. The side effect profile was otherwise tolerable and the study confirmed the anti-tumour effect of PTS in NSCLC. A 5-year follow up study analysing a subset of these patients with adenoid cystic carcinoma of the lung suggested similar safety and clinical benefits of intratumoural PTS [32].

Gene therapies

Over the past few decades, a number of studies evaluating endobronchial gene therapy have been performed [35–44], and have been reviewed elsewhere [88–91]. Many trials involved injection of adenoviral vector with the wild-type p53 tumour suppressor gene injected *via* bronchoscopic or percutaneous injection into tumours with absent or mutated p53. Safety and feasibility of gene transfer to lung tumours was demonstrated, although the efficiency of gene transfer was limited (*i.e.* only a fraction of tumour cells expressed the transgene). There were minimal side effects and evidence of local tumour responses at the injection sites, but no study showed an increase in overall survival. One of the limiting factors to the success of endobronchial gene therapies may have been the absence of a significant bystander effect, *i.e.* the potential for both transduced and non-transduced cells to be killed. Future studies may be designed with vectors that carry a stronger bystander effect that can generate systemic anti-tumour responses.

Immunogene therapies

Immunogene therapy has been defined as a treatment which genetically modifies human cells to generate anti-tumour immunity [92]. Dendritic cells are a commonly investigated target of immunogene therapy because they are the most potent antigen-presenting cells in humans and are essential for the generation of the adaptive immune response against cancer [93, 94].

A recent trial performed by Lee *et al.* [51] exemplifies the use of improved understanding of immunology and the tumour microenvironment for rational trial design. These investigators performed intratumoural injection of CCL21 gene-modified dendritic cells in patients with inoperable NSCLC. Autologous dendritic cells were transduced with an adenoviral vector expressing the CCL21 gene (Ad-CCL21-DC) *ex vivo,* and subsequently injected into the tumour. CCL21 was chosen as the transgene of interest for its ability to attract dendritic cells and T lymphocytes; it is localised to high endothelial venules of lymph nodes and the spleen and by binding with CCR7 guides naïve T cells to the T-cell zone [95]. In theory, by increasing CCL21 secretion at the tumour site, increasing T-cell infiltration may result in the conversion of a cold tumour microenvironment to hot. The investigators performed two injections *via* percutaneous or bronchoscopic approaches on day 0 and 7. Importantly, they also performed core needle biopsies of the tumour on day 0 and 7 to assess local immune responses. At 7 days, immunohistochemistry was able to show increased CD8+ T cell infiltration and PD-L1 expression, suggesting induction of anti-tumour immunity. Other evidence of anti-tumour immunity included detection antibody responses to tumour-associated antigens in six out of 16 patients, and by increased tumour-specific autologous T-cell interferon-γ responses and increased PD-L1 expression by PCR following vaccination. One-quarter of patients had stable disease at day 56 with median overall survival of 3.9 months. This study demonstrated

TABLE 2 Ongoing trials of intratumoural therapies for non-small cell lung cancer listed on ClinicalTrials.gov

Therapy	Phase	Site	Tumour types	Key outcome(s)	ClinicalTrials.gov ID	Title
AdV-tk (aglatimagene besadenovec) +valacyclovir	I	Intratumoural bronchoscopic injection	NSCLC	Primary: Safety Secondary: Immunologic changes, PFS, OS	NCT03131037	Intratumoural gene-mediated cytotoxic immunotherapy in patients with resectable NSCLC
CCL21 gene-modified dendritic cell with pembrolizumab	I	Bronchoscopic or CT-guided intratumoural injection	NSCLC	Primary: Maximum tolerated dose, objective response rate Secondary: Adverse events, PD-L1 expression	NCT03546361	Intratumoural administration of CCL21 gene-modified dendritic cell with intravenous pembrolizumab for advanced NSCLC
Intratumoural G100 with atezolizumab and radiotherapy	II	Intratumoural bronchoscopic injection	NSCLC Pancreatic cancer Virus-associated tumours Melanoma Bladder cancer Triple-negative breast cancer	Primary: Response by RECIST 1.1 Secondary: Adverse events, tumour immune cells, blood cytokines, lymphocytes and kynurenine	NCT03915678	Atezolizumab combined with intratumoural G100 And immunogenic radiotherapy in patients with advanced solid tumours (AGADIR)
Intratumoural ilixadencel with pembrolizumab	I/II	Intratumoural[#]	NSCLC Squamous cell carcinoma of head and neck Gastric adenocarcinoma	Primary: Adverse events, objective response rate Secondary: Objective response rate, CD8 T cell response by flow cytometry, overall survival, progression free survival	NCT03735290	Study to evaluate the safety and effectiveness of illxadencel administered into tumours in combination with checkpoint inhibitor in patients with advanced cancer (ILIAD)
Oncolytic adenovirus (CAdVEC) and T cells (HER2-AdVST)	I	Intratumoural[#]	Lung cancer Bladder cancer Squamous cell carcinoma of head and neck Cancer of the salivary gland Breast cancer Gastric cancer Oesophageal cancer Colorectal cancer Pancreatic adenocarcinoma	Primary: Maximum tolerated dose Secondary: Response by RECIST 1.1, PFS, OS, adverse events	NCT03740256	Binary oncolytic adenovirus in combination with HER2-specific chimeric antigen receptor modified cytotoxic T cells in advanced HER2 positive solid tumours (VISTA)
INT230-6	I/II	Intratumoural[#]	Lung cancer Melanoma Head and neck cancer Lymphoma Breast cancer Pancreatic cancer Liver cancer Colon cancer Glioblastoma Bile duct cancer Ovarian cancer Sarcoma Squamous cell carcinoma	Primary: Adverse events Secondary: Efficacy, pharmacokinetics and pharmacodynamics	NCT03058289	Phase 1/2 safety study of intratumourally dosed INT230-6 (IT-01)

Continued

TABLE 2 Continued

Therapy	Phase	Site	Tumour types	Key outcome(s)	ClinicalTrials.gov ID	Title
Intratumoural ABBV-927 with or without ABBV-181	I	Intratumoural[#]	NSCLC Squamous cell carcinoma of head and neck Advanced solid tumours	Primary: Maximum tolerated dose, pharmacokinetics and pharmacodynamics Secondary: Clinical benefit rate, objective response rate, PFS	NCT02988960	Study of ABBV-927 and ABBV-181, an immunotherapy, in subjects with advanced solid tumours
Clostridium novyi-NT	I	Intratumoural[#]	Advanced solid tumours	Primary: Maximum tolerated dose Secondary: Response by RECIST 1.1	NCT03435952	Pembrolizumab with intratumoural injection of Clostridium novyi-NT

NSCLC: non-small cell lung cancer; PFS: progression-free survival; OS: overall survival; RECIST: Response Evaluation Criteria In Solid Tumours. [#]: Method of intratumoural injection not specified or includes percutaneous injection.

the safety and feasibility of intratumoural Ad-CCL21-DC administration, but also showed an impressive induction of anti-tumour immunity and serves as a model for design and implementation of future tumour-directed immunotherapies.

A phase I study has paired this therapy with intravenous pembrolizumab for patients with treatment-naïve metastatic NSCLC (NCT03546361) and is currently enrolling patients (see table 2). There is a theoretic basis for synergy of these therapies, as the dendritic-cell vaccination can stimulate antigen presentation and T-cell infiltration, while immune checkpoint inhibitors may stimulate activation and proliferation of T lymphocytes and may rescue exhausted T cells. Combining these distinct mechanisms may address the multiple immunological defects to promote anti-tumour immunity: antigen presentation, tumour-specific T cell activation and proliferation, and reversal of the immunosuppressive tumour microenvironment [96].

An additional novel intratumoural immunogene therapy trial evaluates neoadjuvant delivery of a replication-deficient recombinant adenoviral vector modified to carry the herpes simplex virus (HSV) thymidine kinase gene (AdHSVtk) delivered *via* bronchoscopy or direct needle injection to patients with early-stage NSCLC prior to lung resection (NCT03131037). After intratumoural injection, patients receive 2 weeks of oral valacyclovir which is converted into a toxic metabolite in tumour cells that express HSVtk, generating a significant immunological bystander effect. Patients then undergo lung cancer resection as per standard of care. Peripheral blood samples are obtained before and after intratumoural injection and valacyclovir administration, and baseline tumour biopsies are compared with specimens from the resected lung.

Immunotherapies

Over the past decade, systemic immunotherapies, especially immune checkpoint inhibitors, have revolutionised the treatment of multiple cancer types. Many types of immunotherapy are currently under pre-clinical investigation and several of these are uniquely suited for intratumoural delivery, such as oncolytic virus therapies and potentially chimeric antigen receptor (CAR) T cells [97]. Other immunotherapies such as intratumoural cytokine-based therapies may synergise with systemic immune checkpoint inhibitors as part of a strategy to increase T-cell infiltration into tumours. To date, there have not been any clinical trials of bronchoscopic intratumoural or intranodal checkpoint inhibitor administration.

Recently, oncolytic virus therapies have been investigated as potent anti-cancer agents. Local delivery of these viruses into tumours can cause selective destruction of tumour cells. Specifically, tumour cells are more vulnerable to infection by some viruses due to deficient interferon production and the tumour suppressive microenvironment [98]. After local injection, immunogenic cell death and release of damage associated molecular patterns (DAMPs) stimulate innate immunity, while the presentation and processing of viral and tumour antigens augments adaptive immunity [99]. Because of their action on tumour-specific immunity, these agents are potentially synergistic with immune checkpoint inhibitors [98].

The first oncolytic viral therapy in humans, talimogene laherparepvec (T-VEC), was approved by the US Food and Drug Administration for melanoma in 2015 [99]. In fact, a recent study in melanoma that combined T-VEC with pembrolizumab, an immune checkpoint inhibitor, showed higher response rates

(overall response rate 62%) than either agent alone without any additional toxicities from the oncolytic virus [100]. Additionally, there were responses noted in both injected and uninjected lesions, suggesting induction of systemic anti-tumour immunity. This provides discrete evidence of converting an immunologically cold tumour to hot using combination immunotherapy with intralesional injection. Melanoma has the advantage of easy accessibility of target lesions to local injection. Oncolytic viral therapies may also be delivered *via* bronchoscopic injection and are currently being evaluated in NSCLC (table 2).

Development of an optimal immunotherapy combination will likely target multiple mechanisms. For example, one such combination may reverse tumour microenvironment immunosuppression through targeting myeloid-derived suppressor cells (MDSC) and regulatory T cells and increase cytotoxicity through T-cell activation or priming [57].

Pharmacokinetics and pharmacodynamics

Intratumoural therapy poses unique pharmacokinetic and pharmacodynamic challenges because distribution and absorption are often more variable than systemic administration. For example, the distribution of a drug within a tumour may rely on including tumour volume, interstitial pressure, concentration and lipophilicity of the injected agent, tumour vasculature and variation in injection technique [58].

To study the diffusion of an agent following intratumoural injection, Vignaud *et al.* [101] injected the tumours of 16 patients with NSCLC with methylene blue using TBNI with a 21-gauge needle just prior to lung resection. On pathologic examination of the resected specimen, the tumour was infiltrated with dye in 29±15% of the tumour volume. Dye accumulated more significantly in the stromal *versus* the tumour compartment, and some areas of the tumour were completely spared of dye infiltration. In two (13%) out of 16 patients, dye was injected into normal lung, highlighting current limitations in delivery to peripheral lung lesions (although no navigation system was used in this study). Thus, it may be difficult to reach the entirety of the tumour *via* bronchoscopic injection. This may not be necessary, however, for gene or immunotherapies if the injected agent is accompanied by a significant bystander effect.

More recently, Mori *et al.* [34] studied cisplatin pharmacodynamics following EBUS TBNI into lung tumours. They created a computational model of the tumour based on a patient's computed tomography scan and compared results with drug levels obtained by phlebotomy. Five intratumoural injections of cisplatin at a dose of 8 mg each were performed over 18 min. Peripheral blood was drawn at 5, 15, 30, 60 and 120 min following the final injection to monitor systemic drug levels. The actual measured levels in peripheral blood closely matched their computational model. Several interesting observations were noted; for example, the dose of cisplatin required using five individual injections could be three orders of magnitude less than required for a single injection. Multiple injections, therefore, should be used to achieve better distribution through the tumour if diffusion throughout the entire lesion is required. Notably, most studies have already utilised multiple injection sites to achieve this goal.

Optimal dosing of intratumourally injected agents is not known. In theory, the dose may be chosen based on the number of lesions, the volume of tumour, or weight of the patient; the dose-limiting toxicity profile (either local or systemic) may dictate the most appropriate regimen [58]. Doses of injected agents used in studies thus far have been derived empirically. For example, investigators have proposed using 2 mg of cisplatin per mL of tumour because it is a dose that showed efficacy in their clinical experience without significant toxicity [22]. Further study should evaluate optimal dosing regimens for intratumoural injection. Both drug escalation strategies (*i.e.* how to add additional drugs in combination) and the effect of repeated injections ("boosting" effect) should also be investigated [58].

Notably, traditional pharmacokinetic analyses regarding drug absorption, distribution and elimination are not relevant in intratumoural oncolytic viral therapies, as their effect is mediated by the immune response to the viral infection *in vivo*, rather than the effect of local drug accumulation [58]. Therefore, in studies of intratumoural oncolytic virus administration, researchers should focus on viral replication, shedding and clearance using various techniques. The efficacy of oncolytic virus administration should be assessed with biomarkers of immune activation (*e.g.* regulatory T cell depletion, increase in tumour-specific T cells) [58].

Safety

EBUS TBNA is a common procedure performed by pulmonologists and has a low incidence of complications [102]. Rare adverse events include mediastinitis, fistula formation, bleeding and pneumothorax. EBUS TBNI poses an additional theoretical risk of damage to nearby structures or extravasation related to the injected agent; however, this has not emerged as a significant issue in the reported experience of EITC to date. When reported in clinical series, effects from extravasation into the airway have been limited to cough even with irritant solutions such as 99.5% ethanol and cisplatin [17, 47]. Airway irritation caused by extravasation may be minimised by aggressive suctioning and careful

injection. The dead space within commonly used EBUS transbronchial needles has been measured to ensure appropriate dose and further minimise risk of extravasation [103].

There has been one published report of pericarditis associated with conventional TBNI of gene therapy [104]; this potentially could have been avoided if EBUS guidance was utilised. Immunotherapies pose a risk of systemic side effects related to the drug or vector, although studies thus far have shown mostly minor symptoms such as fever and myalgia. As with systemic immunotherapy combinations, there is a potential for an increase in irAE when multiple immunotherapies are delivered *via* bronchoscopic injection. Theoretically, with lower associated systemic drug concentrations, the incidence of adverse events with TBNI should be decreased than if the same drug combinations were delivered systemically [56, 105]. Future trials and experiences are needed for more in-depth evaluation of this hypothetical advantage.

Future directions

Local delivery of immunotherapy holds great promise to improve the outcome of patients with NSCLC. Many immunotherapy combinations have been evaluated in pre-clinical models and await translation to human testing. Ongoing studies include the use of gene-mediated cytotoxic immunotherapy (NCT03131037), gene-modified dendritic cells (NCT03546361) and adoptive cell therapy, such as chimeric antigen receptor T cells (NCT03740256), among others (see table 2). Many trials focus on combination immunotherapies to increase response rates and overcome resistance to checkpoint blockade. In addition, repurposing vaccines as local immunotherapy for cancer using intratumoural delivery has shown potential to increase immune infiltration into tumours in several pre-clinical models and may be adapted to lung cancer [106, 107].

Another attractive, but largely unexplored, form of therapy is the use of intranodal immunotherapy. Because lymph nodes are thought to be the location of T-cell priming and are essential to the formation of a systemic anti-tumour response, the tumour-draining lymph node may be the ideal site for delivery of immunotherapies [96]. Recent pre-clinical studies have suggested the importance of tumour-draining lymph nodes to response to PD-1/PD-L1 checkpoint therapy [108], and clinical studies in NSCLC have demonstrated differences in immune phenotype in tumour-draining *versus* non-draining lymph nodes [109, 110]. As only a fraction of a drug administered systemically is expected to reach the tumour-draining lymph node, direct intranodal injection may be more effective for generation of anti-tumour immunity than systemic administration [111]. Lung cancer is a favourable target for this approach as tumour-draining lymph nodes are accessible by EBUS TBNI.

An additional area of intense investigation is the use of RNA interference for cancer therapy. These therapies have been complicated by delivery issues and off-target effects [112]. A recent pre-clinical study of human lung cancer cells transplanted into mice established the feasibility of using small interfering RNA (siRNA) to eliminate residual lung cancer after incomplete microwave ablation [113]. Although further investigation is needed before this can be translated to humans, the technique showed significant potential. Specifically, by directly injecting lesions with siRNA–polymer conjugates, some of the delivery issues that have plagued trials in siRNA were minimised.

Ongoing research is required to make local delivery of immunotherapy a reality for patients with NSCLC. First, improved bronchoscopic localisation is essential to ensure appropriate delivery of therapies to peripheral lung lesions. Robotic bronchoscopy, among other technologies, will hopefully improve our ability to reach peripheral lesions. Novel immunotherapies or immunotherapy combinations provide another avenue of potential therapy. For their development, increasing knowledge in basic science remains paramount, including detailed understanding of the tumour microenvironment and mechanisms of resistance to immunotherapy [58, 114].

Conclusions

Immunotherapies, most notably immune checkpoint inhibitors, have revolutionised the treatment of multiple cancers in the past decade but are limited by suboptimal response rates and irAE. Intratumoural therapies have the potential to increase efficacy and decrease toxicity in patients with NSCLC, and the bronchoscope is apt to deliver these therapies. Recent advances in the field include endobronchial ultrasound guidance of transbronchial injections, further experience with EITC, and development of novel tools for safer and more consistent drug delivery. Development of new immunotherapy combinations, especially immune checkpoint inhibitors combined with oncolytic viral therapies, have enticing pre-clinical evidence as well as clinical evidence in other cancer types. Ongoing work continues to translate investigational therapies, such as chimeric antigen receptor T cells and siRNA into human trials.

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