

Review

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[Muhammad Shakeeb Sharif](#) , [Luca Luzzi](#) , [Michelino De Laurentiis](#) , [Antonio Giordano](#) , [Marcella Barbarino](#) *

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Review

From Sequence to Site: RNA Therapeutics and Direct Pulmonary Delivery in Lung Cancer

Muhammad Shakeeb Sharif ^{1,2}, Luca Luzzi ³, Michelino De Laurentiis ⁴, Antonio Giordano ^{2,5} and Marcella Barbarino ^{2,5,*}

¹ Department of Clinical and Translational Oncology, Scuola Superiore Meridionale Via Mezzocannone, 4 80134, Naples, Italy

² Department of Medical Biotechnology, University of Siena, 53100 Siena, Italy

³ Thoracic Surgery Unit and Lung Transplant, Siena University Hospital, 53100 Siena, Italy

⁴ Department of Breast and Thoracic Oncology, Istituto Nazionale Tumori IRCCS “Fondazione G. Pascale”, Naples, Italy

⁵ Sbarro Institute for Cancer Research and Molecular Medicine, Center for Biotechnology, College of Science and Technology, Temple University, Philadelphia, PA 19122, USA

* Correspondence: marcella.barbarino@unisi.it

Abstract

More than three decades have passed since gene therapy potential was first recognized, following the first clinical trials, marking the beginning of a new era in targeted cancer therapy. Despite its early promise, the clinical development of gene therapy progressed slowly due to major safety concerns, limited efficiency of early vectors, and stringent regulatory constraints. Recent advances in vector engineering and genome editing technologies have revitalized the field, while innovative direct drug delivery approaches represent a promising approach to enhance gene delivery in oncology. Although encouraging results have been reported across various cancer types, the application of these delivery modalities in lung cancer remains limited due to a range of clinical and translational challenges. This review provides a comprehensive overview of recent both approved and investigational RNA-based candidates, with a focus on their application in lung cancer. As emerging strategy, we discuss the potential of direct pulmonary drug delivery (DPDD) as a valuable approach to improve the precision, efficacy, and safety of gene therapy. Additionally, we analyze *in vivo* studies used to evaluate DPDD strategies, underscoring their translational relevance and the challenges that must be addressed to enable clinical implementation. By synthesizing advances from 2020 to 2025, this review outlines the current landscape and future directions for RNA therapeutics and localized delivery systems in lung cancer.

Keywords: gene therapy; RNA-based therapies; non-small cell lung cancer; direct pulmonary drug delivery; endobronchial ultrasound

1. Introduction

Gene therapy refers to the introduction, removal, or modification of genetic material within a patient’s cells to treat disease, and emerged as a revolutionary concept in 1990 when the four-year-old girl Ashanthi DeSilva received the first clinical gene therapy with the insertion of a functional adenosine deaminase gene into her T cells for the treatment of severe combined immunodeficiency [1]. Although it was not a permanent cure, it was a milestone in gene therapy demonstrating, for the first time, that genetic modification of human cells was both feasible and safe in a clinical setting. This breakthrough paved the way for subsequent developments, but it also raised major concerns, particularly regarding the possibility that genetic interventions might affect the germline, especially when the gene is introduced through viruses.

Since then, more efficient vectors have developed, evolving from early viral vector to advanced systems making gene-therapies transforming modern medicine for genetic, infectious, and oncological diseases [2], with 3 new approvals in 2024 around the globe [3–5].

In parallel, advances in biomaterials and nanotechnology, such as lipid nanoparticles (LP), N-acetylgalactosamine conjugates (GalNAc), microrobotic swarms, biodegradable polymers, lipid-based carriers, and magnetic or ultrasound-responsive nanoparticles[6,7], have expanded the precision and versatility of local drug administration. In addition to refining targeting precision and release kinetics, these advanced biomaterials, ranging from nano- to microscale platforms, contribute significantly to the stabilization of RNA therapeutics by shielding them from enzymatic degradation. This protective function helps maintain molecular integrity and biological activity throughout the delivery process. As a result, such materials play a pivotal role in enhancing the translational viability of RNA-based interventions and expanding their potential application in oncology.

As an additional driver of feasibility for gene therapy, Direct Drug Delivery (DDD) has recently emerged providing new opportunities to overcome some of the key limitations of systemic administration. By facilitating localized and controlled release of therapeutic agents at or near the disease site, DDD reduces systemic exposure, allows for lower effective doses, and helps protect RNA molecules from enzymatic degradation, thereby preserving their biological activity and enhancing therapeutic efficacy. Several technological platforms are currently under investigation or in clinical use, ranging from intratumoral injections and implantable depots to inhalable aerosols, catheter-based delivery, and image-guided minimally invasive procedures.

Within oncological indications, non-small cell lung cancer (NSCLC) constitutes a strategic target for gene therapy due to its molecular heterogeneity including mutations in EGFR, KRAS, ALK, and TP53, which complicates treatment stratification and contributes to resistance against standard chemotherapeutic and targeted agents [8]. In this context, we review the recent clinical studies with direct delivery systems for lung cancer. As no clinical trials employing direct pulmonary drug delivery (DPDD) platforms to administer RNA-based therapies have been initiated in the past five years, this section includes only conventional therapeutic agents delivered via DPDD in lung cancer.

Lastly, we review recent progress in *in vivo* models suitable for DPDD, highlighting both the therapeutic advantages and the current gaps that must be addressed to translate this approach into clinical practice.

Studies published between 2020 and 2025 were considered in order to provide a comprehensive up-to-date synthesis of the most recent developments in RNA therapeutics and localized delivery approaches for lung cancer.

2. RNA-Based and Oligonucleotide Therapies for Non-Oncological Diseases

Over the past five years, regulatory agencies have approved a growing number of nucleic acid-based therapies with multiple approvals in neuromuscular, metabolic, and rare diseases.

These therapies can be broadly categorized into antisense oligonucleotides (ASOs) which employ synthetic oligonucleotides to modulate RNA function, and RNA-based drugs which use RNA molecules themselves as active agents. In regulatory frameworks such as those of the FDA and EMA, ASOs are classified separately from RNA-based drugs like siRNA and mRNA therapeutics, due to their distinct chemical composition and mechanism of action.

ASOs have demonstrated clinical efficacy in several genetic disorders. Composed primarily of chemically modified DNA or synthetic analogs, ASOs exert their effects by binding to RNA targets to modulate splicing or induce degradation *via* RNase H. Recent ASOs approvals include Tofersen (Qalsody) in 2023 [9], targeting SOD1 mRNA for the treatment of amyotrophic lateral sclerosis (ALS) in patients with confirmed SOD1 mutations, and Viltolarsen and Casimersen, approved for Duchenne muscular dystrophy (DMD) [10,11], where they promote exon skipping to restore the dystrophin reading frame in specific genotypes.

In parallel, a growing number of small interfering RNAs (siRNAs) have gained regulatory approval, primarily for liver-targeted indications. These siRNAs, conjugated to N-

acetylgalactosamine (GalNAc) or formulated in lipid nanoparticles (LNPs), exploit endogenous RNA interference mechanisms to silence disease-driving transcripts. Therapeutic applications span hereditary amyloidosis, hepatic porphyrias, hypercholesterolemia, and primary hyperoxaluria, where siRNAs selectively downregulate genes such as TTR, ALAS1, PCSK9, HAO1, and LDHA, resulting in durable biochemical correction and improved clinical outcomes[12–17]. More recently, siRNA platforms have extended into hematologic disorders, with targeted silencing of SERPINC1 (encoding antithrombin) offering a novel strategy to restore thrombin generation in hemophilia, independent of factor replacement [18]. Approved RNA-Based and Oligonucleotide Therapies are summarized in Table 1.

Table 1. Approved RNA-Based Therapies for non-oncological diseases (2020–2025).

Drug Name (INN / brand)	RNA Type / Carrier	Molecular Target(s)	Primary Indication			Administration Route
Tofersen (Qalsody) [9]	ASO	SOD1 mRNA	ALS (SOD1 mutation)			Intrathecal injection
Viltolarsen (Viltepso) [10]	ASO (PMO)	DMD exon 53	Duchenne dystrophy	muscular		Intravenous infusion
Casimersen (Amondys 45)[11]	ASO (PMO)	DMD exon 45	Duchenne dystrophy	muscular		Intravenous infusion
Patisiran (Onpattro) [12]	siRNA/LNP	TTR mRNA	hATTR amyloidosis			Intravenous infusion
Vutrisiran (Amvuttra)[13]	siRNA/GalNAc	TTR mRNA	hATTR amyloidosis			Subcutaneous injection
Givosiran (Givlaari)[14]	siRNA/GalNAc	ALAS1 mRNA	Acute hepatic porphyria			Subcutaneous injection
Inclisiran (Leqvio) [15]	siRNA/GalNAc	PCSK9 mRNA	Hypercholesterolemia			Subcutaneous injection
Lumasiran (Oxlumo)[16]	siRNA/GalNAc	HAO1 mRNA	PH1			Subcutaneous injection
Nedosiran (Rivfloza) [17]	siRNA/GalNAc	LDHA mRNA	PH1			Subcutaneous injection
Fitusiran (Qfitlia)[18]	siRNA/GalNAc	Antithrombin mRNA	Hemophilia inhibitors	A/B	±	Subcutaneous injection

Abbr.: ASO = Antisense Oligonucleotide; PMO = Phosphorodiamidate Morpholino Oligomer; siRNA = Small Interfering RNA; LNP = Lipid Nanoparticle; GalNAc = N-Acetylgalactosamine; SOD1 mRNA = Superoxide Dismutase 1 messenger RNA; DMD = Dystrophin gene; TTR mRNA = Transthyretin messenger RNA; ALAS1

mRNA = 5-Aminolevulinate Synthase 1 messenger RNA; PCSK9 mRNA = Proprotein Convertase Subtilisin/Kexin Type 9 messenger RNA; HAO1 mRNA = Hydroxyacid Oxidase 1 messenger RNA; LDHA mRNA = Lactate Dehydrogenase A messenger RNA; ALS (SOD1 mutation) = Amyotrophic Lateral Sclerosis (Superoxide Dismutase 1 mutation); hATTR amyloidosis = Hereditary Transthyretin Amyloidosis; PH1 = Primary Hyperoxaluria Type 1.

In parallel with the expanding portfolio of approved RNA-based therapies, several siRNAs are advancing through clinical development across diverse therapeutic areas.

Three investigational siRNA, SLN360, Olpasiran, and Lepodisiran, are currently in clinical development for the reduction of cardiovascular risk associated with elevated lipoprotein(a) (Lp(a)) levels. Although developed by different pharmaceutical companies, these agents share a common molecular target (LPA mRNA) and employ liver-directed delivery strategies, such as GalNAc conjugation or long-acting formulations, to achieve sustained suppression of Lp(a) production. SLN360 has completed a Phase I trial (NCT04606602) in healthy volunteers with elevated lipoprotein(a), demonstrating up to 98% reduction in circulating levels and a favorable safety profile [19]. A Phase II trial (NCT05537571) is ongoing in patients at high cardiovascular risk, but outcomes data are not yet available. For Olpasiran (AMG 890), Phase I study (NCT03626662) reported good tolerability following a single dose, and 71-97% reduction in Lp(a) concentration persisting for several months [20]. Phase II data (NCT04270760) confirmed dose-dependent reductions of 70% to 100% in Lp(a), with durable effects lasting up to 48 weeks [21]. The compound is currently being evaluated in the Phase III trial (NCT05581303) evaluating its impact on major cardiovascular events in patients with atherosclerotic disease. For Lepodisiran (LY3819469) three clinical trials have been started over the last five years. Phase I (NCT04914546) and Phase II (NCT05565742), demonstrated good tolerability and dose-dependent, long-lasting reductions in Lp(a) levels [22] and 94% reduction in lipoprotein(a) after a single subcutaneous dose, with pharmacodynamic effects persisting for up to 18 months [23]. Lepodisiran is currently under investigation in the Phase 3 trial (NCT06292013).

Zilebesiran, a GalNAc-siRNA targeting AGT mRNA, is in development for hypertension; the KARDIA-1 Phase 2 trial (NCT04936035) demonstrated durable blood pressure reduction with quarterly dosing, supporting its potential as a long-acting antihypertensive agent [24].

Within hematology, SLN124 acts on TMPRSS6 to restore iron homeostasis, offering a therapeutic strategy for iron-loading anemias. Preliminary results from Phase I/II trial (NCT05499013) showed increased hepcidin levels and reduced transferrin saturation, with a favorable safety profile [25].

STP705, is a dual siRNA therapeutic targeting TGF- β 1 and COX-2, formulated with a proprietary Histidine-Lysine Polymer (HKP) nanoparticle system for non-viral delivery. It has been evaluated across a broad spectrum of indications, both oncologic and non-oncologic. In regenerative and aesthetic medicine, STP705 showed promising results in a Phase I trial for localized fat reduction in abdominoplasty patients (NCT05422378), with histological evidence of adipocyte remodeling and volume reduction [26]. For scar modulation, STP705 was tested in a Phase II trial for keloid recurrence prevention post-excision (NCT04844840), and in a phase I trial for wound healing and fibrosis modulation study (NCT04844879). For hypertrophic scars is still ongoing a dose-escalation phase I/II study (NCT05196373).

As a forward-looking comparator within RNA-enabled modalities, VERVE-101 represents a first-in-class base-editing therapy, delivered *via* lipid nanoparticles. It combines guide RNA and mRNA encoding an adenine base editor to inactivate the *PCSK9* gene in hepatocytes. In the phase 1b trial (NCT05398029), VERVE-101 achieved dose-dependent reductions in LDL-C and PCSK9 protein levels in patients with heterozygous familial hypercholesterolemia (HeFH), with durable effects observed up to six months [27].

Investigational RNA-based therapies for non-oncological diseases are summarized in Table 2.

Table 2. Investigational RNA-Based Therapies for Non-Oncological Diseases (2020–2025).

Drug Name	RNA Type / Technology	Molecular Target(s)	Primary Indication	Administration Route	Clinical Phase	Trial ID (NCT)
SLN360	siRNA / GalNAc	LPA mRNA	Elevated lipoprotein(a) / cardiovascular risk	Subcutaneous injection	Phase I	NCT04606602
Olpasiran (AMG 890)	siRNA / GalNAc	LPA mRNA	Cardiovascular disease	Subcutaneous injection	Phase II	NCT04270760
Olpasiran (AMG 890)	siRNA / GalNAc	LPA mRNA	Cardiovascular outcomes	Subcutaneous injection	Phase III	NCT05581303
Lepodisiran (LY3819469)	siRNA / GalNAc	LPA mRNA	Elevated LPA / cardiovascular risk	Subcutaneous injection	Phase I	NCT04914546
Lepodisiran (LY3819469)	siRNA / GalNAc	LPA mRNA	Elevated LPA / cardiovascular risk	Subcutaneous injection	Phase II	NCT05565742
Lepodisiran (LY3819469)	siRNA / GalNAc	LPA mRNA	Elevated LPA / cardiovascular risk	Subcutaneous injection	Phase III	NCT06292013
Zilebesiran	siRNA / GalNAc	AGT mRNA	Hypertension	Subcutaneous injection	Phase II	NCT04936035
SLN124	siRNA / GalNAc	TMPRSS6 mRNA	β-thalassemia / MDS	Subcutaneous injection	Phase I	NCT04718844
STP705	siRNA / HKP nanoparticle	TGF-β1 / COX-2	Keloid recurrence	Intralesional injection	Phase II	NCT04844840
STP705	siRNA / HKP nanoparticle	TGF-β1 / COX-2	Hypertrophic scars	Intralesional injection	Phase I/II	NCT05196373
STP705	siRNA / HKP nanoparticle	TGF-β1 / COX-2	Localized fat reduction	Intralesional injection	Phase I	NCT05422378
STP705	siRNA / HKP nanoparticle	TGF-β1 / COX-2	Wound healing / fibrosis modulation	Intralesional injection	Phase II	NCT04844879
VERVE-101	Base editing / LNP	PCSK9 gene	Heterozygous familial hypercholesterolemia (HeFH)	Intravenous infusion	Phase I	NCT05398029

Abbr.: siRNA = Small Interfering RNA; GalNAc = N-Acetylgalactosamine; HKP = Histidine-Lysine Polymer; TGF-β1 = Transforming Growth Factor Beta 1; COX-2 = Cyclooxygenase-2; AGT = Angiotensinogen; LpA = Lipoprotein(a); TMPRSS6 = Transmembrane Protease, Serine 6; PCSK9 = Proprotein Convertase Subtilisin/Kexin Type 9; LNP = Lipid Nanoparticle.

2. RNA-Based and Oligonucleotide Therapies in Cancer Targeted Therapy

RNA-based therapeutics are increasingly being explored in oncology, offering precision-targeted strategies for both solid tumors and hematologic malignancies. Between 2020 and 2025, four

candidates have entered clinical trials, across different cancers and with delivery routes ranging from direct intratumoral injection to systemic intravenous or subcutaneous administration.

STP705, a siRNA targeting TGF-β1 and COX-2, has been investigated in cutaneous squamous cell carcinoma in situ (isSCC) delivered intralesionally *via* HKP nanoparticles (phase I/II NCT05421013, phase IIb NCT04844983) and hepatocellular carcinoma (HCC) (NCT04676633). STP707 represents a systemic adaptation of the same dual-target RNAi strategy, formulated for intravenous administration with a modified nanoparticle system. In a completed Phase I basket trial (NCT05037149), STP707 was administered to patients with advanced solid tumors. Preliminary results showed a strong safety profile and disease stabilization in a substantial proportion of evaluable cases [28].

In parallel, Imdusiran, a GalNAc-conjugated siRNA targeting hepatitis B virus (HBV) transcripts is under investigation in hepatocellular carcinoma associated with chronic HBV infection (NCT05001022), aiming to suppress viral replication and oncogenic signaling in HBV-driven liver cancer. Additionally, a GalNAc-siRNA targeting TMPRSS6, administered subcutaneously, is being studied in hematologic disorders characterized by dysregulated iron metabolism (NCT04718844), with potential relevance to cancer-associated anemia and iron-driven inflammatory pathways. Investigational RNA-based therapies for oncological diseases are summarized in Table 3.

Table 3. Investigational RNA-based Therapies in Oncology (2020 – 2025).

Drug Name (INN / Brand)	RNA Type / Technology	Molecular Target(s)	Primary Indication	Administration Route	Clinical Phase	Trial (NCT)	ID
STP705	siRNA/HKP nanoparticles	TGF-β1, COX-2	isSCC	Intratumoral injection	Phase I/II	NCT05421013	
STP705	siRNA/HKP nanoparticles	TGF-β1, COX-2	isSCC	Intratumoral injection	Phase IIb	NCT04844983	
STP705	siRNA/HKP nanoparticles	TGF-β1, COX-2	HCC	Intratumoral injection	Phase I/II/IIb	NCT04676633	
STP707	siRNA/HKP nanoparticles	TGF-β1, COX-2	Advanced solid tumors	Intravenous infusion	Phase I	NCT05037149	
Imdusiran	GalNAc- conjugated siRNA	HBV and HBx	HBV-related HCC	Subcutaneous injection	Phase I/II	NCT05001022	
SLN124	GalNAc- conjugated siRNA	TMPRSS6	Iron- dependent malignancies	Subcutaneous injection	Phase I	NCT04718844	

Abbr.: siRNA = small interfering RNA; HKP = histidine–lysine polymer; TGF-β1 = transforming growth factor beta 1; COX-2 = cyclooxygenase-2; SCC = squamous cell carcinoma; HCC = hepatocellular carcinoma; RECIST = Response Evaluation Criteria in Solid Tumors; GalNAc = N-acetylgalactosamine; HBV = hepatitis B virus; HBx = hepatitis B virus X protein; HBsAg = hepatitis B surface antigen; TMPRSS6 = transmembrane serine protease 6.

3. Direct Pulmonary Delivery for Lung Cancer

Direct Drug Delivery (DDD) represents a paradigm shift in modern medicine aiming to enhance efficacy by administering pharmacological agents precisely at the site of disease to enable a rapid and localized drug action. By bypassing systemic circulation, locally delivered drugs often require lower minimum effective doses, thereby reducing systemic toxicity and improving therapeutic selectivity.

The lung offers unique anatomical and physiological advantages for DDD: a vast surface area, dense vascularization, a thin alveolar–capillary interface, ready accessibility and easily repeatable.

Initially established in chronic respiratory diseases such as asthma, chronic obstructive pulmonary disease and pulmonary fibrosis [29,30], Direct Pulmonary Drug Delivery (DPDD) is emerging as a promising modality now being actively explored as a therapeutic avenue in lung cancer.

Two delivery routes have demonstrated clinical applicability for DDD to the lungs in humans: inhalation therapy and endobronchial administration.

3.1. Direct Pulmonary Drug Delivery Through Inhalation

Inhalation drug delivery is one of the distinct ways of localized therapeutic modality, emerging as an optimistic approach for lung cancer therapy. Inhalation strategies include aerosolized and dry powder formulations, nebulizers, pressurized metered-dose inhalers (pMDIs), soft mist inhalers (SMIs), and emerging smart inhalers. Delivery through inhalation offers unique prospects due to its site specific and non-intrusive pulmonary administration, augmented drug retention and minimized the risk of systemic toxicity in contrast to traditional approaches of drug administration [31].

Inhalation therapy is widely used for the management of chronic conditions such as asthma, cystic fibrosis and chronic obstructive pulmonary disease (COPD), where β_2 -agonists (e.g., salbutamol), corticosteroids (e.g., budesonide), and long-acting bronchodilators (e.g., formoterol) are delivered via metered-dose inhalers or dry powder inhalers [29,32,33]. In infectious diseases, inhaled antibiotics such as tobramycin and colistin are used to manage recurrent pulmonary infections in cystic fibrosis [30]. More recently, the inhalation route has gained traction for the delivery of biologics, including interferons and monoclonal antibodies, in the context of viral respiratory infections such as SARS-CoV-2 [34]. Even if inhaled chemotherapy is not devoid of risk, adverse events reported in the literature are infrequent and predominantly attributable to administration-related factors [35].

Although preclinical studies have demonstrated encouraging results with aerosolized formulations of chemotherapeutics, biologics, and RNA-based agents, over the past five years only a small number of early-phase clinical trials have been started to study the inhalation route for cancer therapy (Table 4), reflecting the delayed progression from preclinical models to human trials.

Table 4. Inhalation-delivered chemotherapy (2020 – 2025).

Drug Name (INN / Brand)	Primary Indication	Administration Route	Clinical Phase	Trial ID (NCT)
Cisplatin	Stage IV NSCLC	Inhalation	Phase I/II	NCT06896890
Azacytidine + Platinum-Based Chemotherapy + Durvalumab (MEDI4736)	Early-Stage NSCLC	Inhalation	Phase I/II	NCT06694454
BMD006	Lung Cancer	Inhalation	Phase I	NCT06928922

Abbr.: NSCLC: non-small cell lung cancer; PD-L1: programmed death-ligand 1; TAAs: tumor-associated antigens.

3.2. Direct Pulmonary Drug Delivery Through Bronchoscope

Originally developed as a diagnostic tool for visualizing and sampling the airways, bronchoscopy has progressively evolved into a versatile platform for therapeutic intervention including airway stenting or transbronchial biopsy [36]. In non-oncologic settings, it has enabled

localized drug delivery for infections, inflammation, and airway obstruction. Building on this foundation, interventional bronchoscopy in oncology has expanded dramatically, transforming the bronchoscope into a conduit for direct tumor-targeted therapies. Clinically validated applications include photodynamic therapy and endobronchial brachytherapy, which remain among the most established bronchoscopic modalities for delivering treatment directly to malignant lesions within the central airways.

Endobronchial intratumoral therapy (EBIT) dates back to 1922, when Yankauer used a rigid bronchoscope to place radium capsules directly into a bronchogenic carcinoma, establishing the foundation for therapeutic bronchoscopy [37]. Since then, bronchoscopic techniques have evolved substantially, with the introduction of endobronchial ultrasound (EBUS) marking a pivotal advancement. In 2001, Herth and colleagues demonstrated the utility of convex probe EBUS for mediastinal lymph node sampling, significantly improving diagnostic accuracy and procedural safety [38], progressively replacing the more invasive surgical mediastinoscopy.

Beyond diagnosis, EBUS has emerged as a versatile platform particularly in centrally located or deep-seated lesions for therapeutic intervention. Examples include, endobronchial brachytherapy, microwave ablation, and thermal tumor destruction, all made possible by the development of minimally invasive techniques characterized by high efficacy, minimal invasiveness, favorable safety profiles and enhanced patient outcomes.

EBUS ability to provide real-time ultrasound guidance for transbronchial needle placement (Endobronchial Ultrasound-Guided Transbronchial Needle Injection; EBUS-TBNI) has opened new possibilities for localized drug administration, particularly in lung cancers situated deep within the pulmonary parenchyma such as NSCLC [39], with low incidence of complications [40].

Recent studies have demonstrated the feasibility of using EBUS to deliver intratumoral chemotherapy, tumor-targeted vaccines, and biologic agents directly into malignant lymph nodes or parenchymal lesions [41]. This approach enables precise deposition of therapeutic compounds, and administration of multiple agents or injections at different sites within the same tumor to optimize distribution. As such, EBUS is increasingly recognized as a minimally invasive platform for targeted therapy.

Technological advances have further expanded the capabilities of bronchoscopic drug delivery, particularly for lesions located in the peripheral lung parenchyma. Electromagnetic navigation bronchoscopy (ENB) integrates CT-derived virtual airway mapping with real-time electromagnetic tracking, enabling access to small, difficult-to-reach pulmonary nodules [42].

Building upon the principles of ENB, robotic-assisted bronchoscopy (RAB) introduces enhanced stability and precision through the use of a robotic articulated bronchoscope associated with a 3D image reconstruction for a real-time endobronchial navigation. Systems such as Monarch™, Galaxy™ and Ion™, with a superior localization accuracy, allow operators to reach narrower airways and perform complex procedures with greater control [43]. The robotic ultrathin bronchoscopy, employing optical fibers with an outer diameter ≤ 3.0 mm, providing access to distal airways up to the 12th bronchial generation. Initially developed to improve diagnostic yield in peripheral pulmonary lesions, ultrathin bronchoscopes have shown promise for targeted drug delivery and micro instillation of therapeutic agents [44]. Although limited by a narrow working channel, which restricts standard biopsy tools and large-volume lavage, this technique enables localized sampling and delivery in immunological and oncological research. As miniaturized instruments and robotic platforms continue to evolve, ultrathin bronchoscopy may become a key enabler of precision medicine in peripheral lung cancer.

Since 2020, only two new phase I clinical trials have been initiated to investigate bronchoscopic intratumoral drug delivery in LC (Table 5), highlighting the limited clinical exploration of this approach to date.

Table 5. Investigational Bronchoscopic Intratumoral Chemotherapy (2020-2025).

Drug Compound	Name /	Primary Indication	Clinical Phase	Administration Route	Trial ID /	Reference
Cisplatin		resectable NSCLC	Phase I	bronchoscopy with cone- beam CT confirmation; IT	NCT04809103	
Cisplatin		Stage IV LC	Phase I	EBUS-TBNI; IT	NCT04311762	

Abbr.: LC = lung cancer; CT = Computed Tomography; EBUS = endobronchial ultrasound; TBNI = Transbronchial Needle Injection; IT = intratumoral;.

Both studies employ EBUS-TBNI to deliver cisplatin directly into lung tumors. Preliminary feasibility data from NCT05713434, published in 2024 [45], demonstrated that a single 20 mg intratumoral dose of cisplatin was well tolerated in patients with stage IV NSCLC, with no dose-limiting toxicities and plasma platinum levels approximately 100-fold lower than those observed with intravenous administration. Although the trial was halted early due to emerging immunomodulatory data, it confirmed the procedural feasibility of a “diagnose-and-treat” paradigm. Similarly, NCT04809103 is evaluating intratumoral cisplatin as a neoadjuvant strategy in resectable early-stage NSCLC. Preliminary data from the first three patients confirmed accurate needle placement via cone-beam CT and procedural safety [46].

4. Direct Pulmonary Delivery in In Vivo Studies for Lung Cancer

The path toward clinical translation of local delivery approaches in lung cancer is grounded in robust preclinical evidence. In vivo models represent an indispensable step, as regulatory approval for the initiation of any clinical trial requires comprehensive in vivo data demonstrating not only proof-of-concept efficacy but also safety, biodistribution, and tolerability. These models are therefore essential to anticipate safety liabilities of novel drugs or delivery devices, inform risk–benefit assessment, optimize dosing strategies, and generate the mechanistic rationale required for subsequent clinical development.

Orthotopic models, which preserve the native anatomical and microenvironmental context of the lung, represent the most appropriate platform for evaluating DPDD strategies. Such models allow investigators to examine critical factors including lung-site drug dispersion, persistence, microenvironmental remodeling, and the relevant interactions with surrounding tissues, vasculature structures, and immune components. This approach offers a more physiologically relevant context for evaluating tumor response to therapy, thereby making it essential for translational relevance and preclinical validation.

Among available systems, murine models remain the most widely employed due to their low cost, ease of handling, and compatibility high-throughput experimentation. *In vivo* studies in orthotopic models have progressively investigated delivery strategies for lung cancer, particularly through inhalation and intratracheal instillation. In addition to these approaches, bronchoscopic techniques have been explored in only a limited number of preclinical studies, mostly involving rat mice and pig models for localized delivery or tumor visualization rather than direct therapeutic evaluation.

The following section explores the results from recent *in vivo* studies about DPDD therapies delivered either by inhalation and via bronchoscope for LC, focusing specifically on orthotopic mouse models and porcine models.

We have included studies testing antitumor drugs both as single agents and employing carrier-based delivery platforms.

4.1. Direct Pulmonary Drug Delivery Through Inhalation

Recent preclinical investigations have explored a diverse array of inhalable strategies for treating orthotopic lung cancer, ranging from conventional chemotherapeutics to biologics and nanocarrier-based platforms. Inhaled formulations of cisplatin and topotecan have demonstrated notable efficacy. Dry powder cisplatin (CIS-DPI), evaluated in M109 orthotopic models, induced both cytotoxic and immunogenic effects, including upregulation of Programmed Death-Ligand 1 (PD-L1) expression, suggesting a mechanistic synergy with immune checkpoint inhibition. Co-administration of CIS-DPI with anti-PD-1 therapy resulted in enhanced tumor regression and prolonged survival compared to monotherapy [47]. Similarly, inhaled topotecan at 1 mg/kg exhibited superior pharmacokinetic profiles and antitumor activity compared to intravenous delivery at 5 mg/kg, achieving complete growth arrest in EGFR- and KRAS-mutant NSCLC models and significantly reducing tumor burden across multiple xenografts. In particular, topotecan delivered via inhalation suppressed tumor burden by 88% in H358 orthotopic models, compared to a 44% reduction observed with intravenous administration. Moreover, inhalation inhibited tumor growth in both H358 and H1975 xenografts and induced significant tumor regression in A549-derived tumors [48].

In parallel, biologic and immunotherapeutic approaches have been investigated for pulmonary delivery. Aerosolized administration of hesperetin-loaded nanoparticles (HNPs) in combination with anti-CD40 immunotherapy enabled selective tumor uptake and improved survival outcomes [49]. Additionally, inhalable exosome-based platforms derived from CAR-T cells (CAR-Exos), engineered to express anti-mesothelin scFv, demonstrated immunomodulatory efficacy and good tolerability in mesothelin-positive orthotopic lung cancer models, supporting the potential of complex biologics for respiratory delivery [50].

A rapidly expanding area involves the use of nanocarrier systems to enhance therapeutic precision and exploit tumor-specific vulnerabilities. Macrophage membrane-coated nanoreactors (DHA-N@M), administered via nebulization, achieved tumor deposition levels approximately 70-fold higher than intravenous delivery. When combined with X-ray irradiation, these nanoparticles released nitric oxide (NO), which synergized with radiation-induced reactive oxygen species (ROS) to generate peroxynitrite (ONOO⁻), triggering ferroptosis and resulting in 93% tumor inhibition [51].

Other inhalable nanotherapies have targeted cancer stem cells (CSCs) through modulation of tumor-associated macrophages (TAMs), as demonstrated by iron-containing nanoparticles functionalized with dextran, which accumulated in microlesions and disrupted metabolic and redox homeostasis, culminating in CD44 upregulation and ferroptotic cell death [52]. Furthermore, up-conversion nanoparticle nanocages functionalized with VEGF-siRNA and AS1411 aptamer have shown promising results in orthotopic models, with reduced tumor proliferation, improved survival, and stable systemic tolerability [53].

4.2. Direct Pulmonary Drug Delivery Through Bronchoscope

Preclinical research has increasingly focused on bronchoscopic delivery as tools for airway-targeted drug delivery in lung cancer. Orthotopic mouse models generated through transbronchial approaches provide valuable proof-of-concept data; however, technical limitations, such as the small size of murine airways, continue to challenge reproducibility and translational relevance [54].

Nonetheless, recent advances have demonstrated the feasibility of bronchoscopic administration in rodents. Recent advances have nonetheless demonstrated the feasibility of bronchoscopic administration in rodents, enabling controlled evaluation of tumor progression and therapeutic response [55].

Porcine models complement rodent studies with superior anatomical and physiological resemblance to the human lung, including comparable airway branching, lobular structure, and immune responses. Engineered “Oncopigs” further reproduce the complexity of the tumor microenvironment, providing a relevant platform for testing localized therapeutic strategies such as viral vector delivery and CRISPR/Cas9-based gene editing[56].

Another study has evaluated alpha radiotherapy as a localized treatment modality, demonstrating the feasibility and safety of bronchoscopic implantation of Alpha DaRTs (Radium-224 fixed) in porcine lungs. These studies achieved precise deposition of radioactive sources in clusters ≤ 4 mm apart, with no systemic toxicity, no device migration, and only minimal local fibrotic or inflammatory responses. The results support the safety and precision of bronchoscopic Alpha DaRT administration and its translational potential [57].

Together, rodent and porcine models provide complementary strengths: advantages: rodents allow rapid mechanistic insight, while swine better recapitulate human pulmonary anatomy and physiology, supporting clinically relevant validation.

Overall, the available data indicate that bronchoscopic delivery represents a versatile platform for direct pulmonary drug administration, suitable not only for conventional agents but also for next-generation therapies based on gene modulation, immune targeting, and nanotechnology.

5. Discussion

Nucleic acid therapeutics has become a central strategy in biomedical innovation with RNA-based therapeutics, including siRNA, mRNA, RNAi, and antisense oligonucleotides, gaining regulatory approval across a range of non-oncologic indications. In parallel, a number of clinical trials have been initiated for both malignant and non-malignant conditions, reflecting the increasing interest in RNA-based approaches for precision medicine.

This expansion has been catalyzed by advances in nano e micro-carriers and delivery technologies, which collectively protect nucleic acid from degradation, and enhance cellular uptake and tissue specificity.

Lung cancer remains a leading cause of cancer-related mortality worldwide [58]. In patients with unresectable or recurrent disease, therapeutic precision is critical to improving outcomes. The pulmonary route offers a unique anatomical advantage for localized drug administration, and recent preclinical studies have begun to explore DPDD via inhalation, instillation, and bronchoscopic approaches. Although promising, the number of clinical trials investigating DPDD in lung cancer remains limited.

Among available modalities, bronchoscopic delivery presents distinct advantages. It bypasses patient-dependent variables such as airway geometry, respiratory dynamics, and mucociliary clearance, allowing for precise deposition of therapeutic agents. Moreover, the bronchoscope enables intratumoral injections at different sites within the same tumor to optimize distribution, and co-administration of multiple agents or other localized interventions such as thermal ablation, cryotherapy, and brachytherapy. These features position bronchoscopic DPDD as a highly suitable platform for the localized delivery of RNA-based therapeutics. Technological advances in interventional pulmonology, including electromagnetic navigation, robotic-assisted bronchoscopy, and ultrathin scopes, have expanded access to peripheral and deep-seated lesions. Moreover, these tools enable the integration of the therapeutic procedures into diagnostic workflows, enabling simultaneous visualization, sampling, and treatment.

RNA-based therapies represent a promising frontier in lung cancer treatment, particularly for non-resectable NSCLC, which is frequently driven by actionable oncogenic mutations. RNA-based targeting of oncogenic mutations benefits from well-established pharmacological backgrounds, supported by existing small-molecule inhibitors against these same drivers used in standard chemotherapy protocols. Recent experimental innovations, such as microneedle arrays fabricated from biodegradable materials, and intralesional injection of siRNA–polymer conjugates, can improve retention and have expanded the toolkit for localized delivery of RNA [59,60].

Despite all these advances, several translational challenges persist. The distal regions of the lung, including alveolar compartments, remain difficult to access and replicate in small-animal models. While orthotopic lung cancer models offer a more physiologically relevant platform for evaluating DPDD strategies, current studies predominantly focus on inhalation and instillation routes, with limited exploration of bronchoscopic delivery. To advance the clinical translation of new RNA-based therapies delivered by DPDD in lung cancer, it is essential to integrate in vivo models with ex vivo lung systems and computational modeling.

In summary, the convergence of interventional pulmonology, nanotechnology, and RNA biology offers a unique opportunity to redefine therapeutic paradigms in thoracic oncology. Realizing this potential will require sustained interdisciplinary collaboration among molecular biologists, pharmaceutical scientists, and clinicians. Through coordinated efforts, these disciplines can identify novel therapeutic targets, engineer effective delivery systems, and develop clinically viable platforms that translate molecular innovation into patient-centered benefit.

6. Conclusions

Despite the significant therapeutic potential offered by intratumoral therapy and the refinement of available techniques, its application in experimental treatments, particularly gene-based therapies, remains limited. This is especially true for diseases affecting the distal regions of the lung, such as the alveoli, where access is technically challenging and preclinical models are difficult to reproduce in vivo. These limitations hinder the accurate determination of key pharmacologic parameters, including optimal dosing, frequency of administration, and distribution kinetics, thereby slowing the clinical translation of novel therapeutic strategies. Overcoming these barriers will be essential to fully harness the promise of direct pulmonary drug delivery in next-generation respiratory medicine.

7. Methodology

Clinical Data Collection

Clinical studies published between 2020 and 2025 were identified through PubMed and Web of Science using the terms: “EBUS”, “TBNi”, “Inhalation”, “Bronchoscopy”, “Drug Delivery”, “Drug Carriers”, and “Molecular Targets”. Clinical trial records were obtained from ClinicalTrials.gov (2010–October 2020) with the following keywords: “Gene”, “Gene Therapy”, “CAR T cell”, “Chimeric Antigen Receptor”, “CRISPR”, “DC”, “Engineered”, “Lenti”, “Retro”, “Adeno”, “AAV”, “HSV”, “MicroRNA”, “miR”, “miRNA”, “siRNA”. Only English-language publications were included.

Preclinical In Vivo Studies

In vivo investigations of RNA- and non-RNA-based therapeutics delivered via direct pulmonary drug delivery were reviewed, with priority given to murine and porcine models. Search terms included “NSCLC”, “RNA-based therapies”, “non-RNA-based modalities”, “Pulmonary delivery”, “siRNA”, “Nanoparticles”, and “Orthotopic Models”. Studies were assessed for delivery route, platform, and model relevance, with particular focus on evidence of biocompatibility, translational feasibility, and molecular targeting in preclinical lung cancer models.

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Abbreviations

The following abbreviations are used in this manuscript:

DPDD	Direct Pulmonary Drug Delivery
LN	Lipo Nanoparticles
GalNAc	N-acetylgalactosamine Conjugates
ASOs	Antisense Oligonucleotides
Lp(a)	Lipoprotein(a)
DMD	Duchenne Muscular Dystrophy
PMO	Phosphorodiamidate Morpholino Oligomer
SiRNA	Small Interfering RNA
SOD1	Superoxide Dismutase 1
DMD	Dystrophin gene
TTR	Transthyretin
ALAS1	5-Aminolevulinate Synthase 1
PCSK9	Proprotein Convertase Subtilisin/Kexin Type 9
HAO1	Hydroxyacid Oxidase 1
LDHA	Lactate Dehydrogenase A
ALS	Lateral Sclerosis
SOD1	Superoxide Dismutase 1
hATTR	Hereditary Transthyretin
PH1	Primary Hyperoxaluria Type 1
HSK	Histidine-Lysine Polymer
TGF-β1	Transforming Growth Factor Beta 1
COX-2	Cyclooxygenase-2
TMPRSS6	Transmembrane Protease, Serine 6
HBV	Hepatitis B Virus
HKP	Histidine–Lysine Polymer
SCC	Squamous Cell Carcinoma
HCC	Hepatocellular Carcinoma
RECIST	Response Evaluation Criteria in Solid Tumors
HBsAg	Hepatitis B Surface Antigen
pMDIs	Pressurized Metered-Dose Inhalers
SIMs	Soft Mist Inhalers
COPD	Chronic Obstructive Pulmonary Disease
PD-L1	Programmed Death-Ligand 1
NSCLC	Non-Small Cell Lung Cancer
TAAAs	Tumor-Associated Antigens
EBIT	Endobronchial Intratumoral Therapy
EBUS	Endobronchial Ultrasound
TBNI	Transbronchial Needle Injection
ENB	Electromagnetic Navigation Bronchoscopy
RAB	Robotic-Assisted Bronchoscopy
CT	Computed Tomography
IT	Intra Tumoral
CIS-DPI	Cisplatin Dry Powder Inhaler
HNPs	Hesperetin-Loaded Nanoparticles
CSCs	Cancer Stem Cells
TAMs	Tumor-Associated Macrophages

References

1. Blaese, R.M.; Culver, K.W.; Miller, A.D.; Carter, C.S.; Fleisher, T.; Clerici, M.; Shearer, G.; Chang, L.; Chiang, Y.; Tolstoshev, P.; et al. T Lymphocyte-Directed Gene Therapy for ADA– SCID: Initial Trial Results After 4 Years. *Science* **1995**, *270*, 475–480, doi:10.1126/science.270.5235.475.

2. Wang, C.; Pan, C.; Yong, H.; Wang, F.; Bo, T.; Zhao, Y.; Ma, B.; He, W.; Li, M. Emerging Non-Viral Vectors for Gene Delivery. *Journal of Nanobiotechnology* **2023**, *21*, 272, doi:10.1186/s12951-023-02044-5.

3. Golikeri, A.; Yi, S.; Fashoyin-Aje, L. Eladocogene Exuparvovec for Aromatic L-Amino Acid Decarboxylase Deficiency. *JAMA* **2025**, *333*, 1449–1450, doi:10.1001/jama.2024.28666.
4. Keam, S.J. Afamitresgene Autoleucel: First Approval. *Mol Diagn Ther* **2024**, *28*, 861–866, doi:10.1007/s40291-024-00749-3.
5. Rasko, J.E.J.; Samelson-Jones, B.J.; George, L.A.; Giermasz, A.; Ducore, J.M.; Teitel, J.M.; McGuinn, C.E.; High, K.A.; de Jong, Y.P.; Chhabra, A.; et al. Fidanacogene Elaparvovec for Hemophilia B - A Multiyear Follow-up Study. *N Engl J Med* **2025**, *392*, 1508–1517, doi:10.1056/NEJMoa2307159.
6. Viral and Non-Viral Vectors in Gene Therapy: Current State and Clinical Perspectives - eBioMedicine Available online: [https://www.thelancet.com/journals/ebiom/article/PIIS2352-3964\(25\)00278-6/fulltext](https://www.thelancet.com/journals/ebiom/article/PIIS2352-3964(25)00278-6/fulltext) (accessed on 30 August 2025).
7. Active Microgel Particle Swarms for Intrabronchial Targeted Delivery | Science Advances Available online: <https://www.science.org/doi/10.1126/sciadv.adr3356> (accessed on 30 August 2025).
8. Ziolkowska-Suchanek, I.; Rozwadowska, N. Advancements in Gene Therapy for Non-Small Cell Lung Cancer: Current Approaches and Future Prospects. *Genes* **2025**, *16*, 569, doi:10.3390/genes16050569.
9. Blair, H.A. Tofersen: First Approval. *Drugs* **2023**, *83*, 1039–1043, doi:10.1007/s40265-023-01904-6.
10. Dhillon, S. Viltolarsen: First Approval. *Drugs* **2020**, *80*, 1027–1031, doi:10.1007/s40265-020-01339-3.
11. Shirley, M. Casimersen: First Approval. *Drugs* **2021**, *81*, 875–879, doi:10.1007/s40265-021-01512-2.
12. Hoy, S.M. Patisiran: First Global Approval. *Drugs* **2018**, *78*, 1625–1631, doi:10.1007/s40265-018-0983-6.
13. Keam, S.J. Vutrisiran: First Approval. *Drugs* **2022**, *82*, 1419–1425, doi:10.1007/s40265-022-01765-5.
14. Scott, L.J. Givosiran: First Approval. *Drugs* **2020**, *80*, 335–339, doi:10.1007/s40265-020-01269-0.
15. Lamb, Y.N. Inclisiran: First Approval. *Drugs* **2021**, *81*, 389–395, doi:10.1007/s40265-021-01473-6.
16. Scott, L.J.; Keam, S.J. Lumasiran: First Approval. *Drugs* **2021**, *81*, 277–282, doi:10.1007/s40265-020-01463-0.
17. Syed, Y.Y. Nedosiran: First Approval. *Drugs* **2023**, *83*, 1729–1733, doi:10.1007/s40265-023-01976-4.
18. Lee, A. Fitusiran: First Approval. *Drugs* **2025**, *85*, 1073–1077, doi:10.1007/s40265-025-02203-y.
19. Nissen, S.E.; Wolski, K.; Balog, C.; Swerdlow, D.I.; Scrimgeour, A.C.; Rambaran, C.; Wilson, R.J.; Boyce, M.; Ray, K.K.; Cho, L.; et al. Single Ascending Dose Study of a Short Interfering RNA Targeting Lipoprotein(a) Production in Individuals With Elevated Plasma Lipoprotein(a) Levels. *JAMA* **2022**, *327*, 1679–1687, doi:10.1001/jama.2022.5050.
20. Koren, M.J.; Moriarty, P.M.; Baum, S.J.; Neutel, J.; Hernandez-Illas, M.; Weintraub, H.S.; Florio, M.; Kassahun, H.; Melquist, S.; Varrieur, T.; et al. Preclinical Development and Phase 1 Trial of a Novel siRNA Targeting Lipoprotein(a). *Nat Med* **2022**, *28*, 96–103, doi:10.1038/s41591-021-01634-w.
21. O'Donoghue, M.L.; Rosenson, R.S.; Gencer, B.; López, J.A.G.; Lepor, N.E.; Baum, S.J.; Stout, E.; Gaudet, D.; Knusel, B.; Kuder, J.F.; et al. Small Interfering RNA to Reduce Lipoprotein(a) in Cardiovascular Disease. *N Engl J Med* **2022**, *387*, 1855–1864, doi:10.1056/NEJMoa2211023.
22. Nissen, S.E.; Linnebjerg, H.; Shen, X.; Wolski, K.; Ma, X.; Lim, S.; Michael, L.F.; Ruotolo, G.; Gribble, G.; Navar, A.M.; et al. Lepodisiran, an Extended-Duration Short Interfering RNA Targeting Lipoprotein(a): A Randomized Dose-Ascending Clinical Trial. *JAMA* **2023**, *330*, 2075–2083, doi:10.1001/jama.2023.21835.
23. Nissen, S.E.; Ni, W.; Shen, X.; Wang, Q.; Navar, A.M.; Nicholls, S.J.; Wolski, K.; Michael, L.; Haupt, A.; Krege, J.H.; et al. Lepodisiran - A Long-Duration Small Interfering RNA Targeting Lipoprotein(a). *N Engl J Med* **2025**, *392*, 1673–1683, doi:10.1056/NEJMoa2415818.
24. Bakris, G.L.; Saxena, M.; Gupta, A.; Chalhoub, F.; Lee, J.; Stiglit, D.; Makarova, N.; Goyal, N.; Guo, W.; Zappe, D.; et al. RNA Interference With Zilebesiran for Mild to Moderate Hypertension: The KARDIA-1 Randomized Clinical Trial. *JAMA* **2024**, *331*, 740–749, doi:10.1001/jama.2024.0728.
25. Kremianskaya, M.; Hoffman, R.; Chew, L.P.; Sharif, A.; Semov, B.; Kori, A.N.; Sia, H.; Grove, C.S.; Ng, A.P.; Kalro, A.; et al. Initial Results from a Phase 1/2 Study Evaluating Divesiran, a Novel Galnac Conjugated siRNA, in Patients with Polycythemia Vera (SANRECO). *Blood* **2024**, *144*, 656, doi:10.1182/blood-2024-205854.
26. Nestor, M.S.; Hetzel, J.; Awad, N.; Bhupalam, V.; Lu, P.; Molyneaux, M. A Novel Injectable Polypeptide Nanoparticle Encapsulated siRNA Targeting TGF-β1 and COX-2 for Localized Fat Reduction II: Phase I Clinical Trial. *J Cosmet Dermatol* **2025**, *24*, e16722, doi:10.1111/jocd.16722.

27. Vafai, S.B.; Gladding, P.A.; Scott, R.; Kerr, J.; Taubel, J.; Cegla, J.; Barbir, M.; Humphries, S.E.; Karsten, V.; Jensen, C.L.; et al. Safety and Pharmacodynamic Effects of VERVE-10.
28. Sirnaomics Announces Successful Phase I Clinical Study of RNAi Therapeutic STP707 for Treatment of Multiple Solid Tumors | Sirnaomics Available online: <https://www.sirnaomics.com/en/news-room/press-release/20230831sirnaomics-announces-successful-phase-i-clinical-study-of-rnai-therapeutic-stp707-for-treatment-of-multiple-solid-tumors/> (accessed on 1 September 2025).
29. Inhaled Reliever Therapies for Asthma: A Systematic Review and Meta-Analysis | Asthma | JAMA | JAMA Network Available online: <https://jamanetwork.com/journals/jama/fullarticle/2825533> (accessed on 31 August 2025).
30. Taccetti, G.; Francalanci, M.; Pizzamiglio, G.; Messori, B.; Carnovale, V.; Cimino, G.; Cipolli, M. Cystic Fibrosis: Recent Insights into Inhaled Antibiotic Treatment and Future Perspectives. *Antibiotics* **2021**, *10*, 338, doi:10.3390/antibiotics10030338.
31. Xie, L.; Xie, D.; Du, Z.; Xue, S.; Wang, K.; Yu, X.; Liu, X.; Peng, Q.; Fang, C. A Novel Therapeutic Outlook: Classification, Applications and Challenges of Inhalable Micron/Nanoparticle Drug Delivery Systems in Lung Cancer (Review). *International Journal of Oncology* **2024**, *64*, 1–16, doi:10.3892/ijo.2024.5626.
32. Axson, E.L.; Lewis, A.; Potts, J.; Pang, M.; Dickinson, S.; Vioix, H.; Quint, J.K. Inhaled Therapies for Chronic Obstructive Pulmonary Disease: A Systematic Review and Meta-Analysis. *BMJ Open* **2020**, *10*, e036455, doi:10.1136/bmjopen-2019-036455.
33. Shaibie, N.A.; Mohammad Faizal, N.D.F.; Buang, F.; Srichana, T.; Mohd Amin, M.C.I. Inhaled Biologics for Respiratory Diseases: Clinical Potential and Emerging Technologies. *Drug Deliv. and Transl. Res.* **2025**, doi:10.1007/s13346-025-01909-6.
34. Polack, F.P.; Thomas, S.J.; Kitchin, N.; Absalon, J.; Gurtman, A.; Lockhart, S.; Perez, J.L.; Pérez Marc, G.; Moreira, E.D.; Zerbini, C.; et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med* **2020**, *383*, 2603–2615, doi:10.1056/NEJMoa2034577.
35. Shaibie, N.A.; Mohammad Faizal, N.D.F.; Buang, F.; Srichana, T.; Mohd Amin, M.C.I. Inhaled Biologics for Respiratory Diseases: Clinical Potential and Emerging Technologies. *Drug Deliv. and Transl. Res.* **2025**, doi:10.1007/s13346-025-01909-6.
36. Criner, G.J.; Eberhardt, R.; Fernandez-Bussy, S.; Gompelmann, D.; Maldonado, F.; Patel, N.; Shah, P.L.; Slebos, D.-J.; Valipour, A.; Wahidi, M.M.; et al. Interventional Bronchoscopy. *Am J Respir Crit Care Med* **2020**, *202*, 29–50, doi:10.1164/rccm.201907-1292SO.
37. Polo, A.; Castro, M.; Montero, A.; Navío, P. Brachytherapy for Lung Cancer. In *Advances in Radiation Oncology in Lung Cancer*; Jeremic, B., Ed.; Springer: Berlin, Heidelberg, 2011; pp. 477–488 ISBN 978-3-642-19925-7.
38. Herth, F.; Becker, H.D.; Manegold, C.; Drings, P. Endobronchial Ultrasound (EBUS)--Assessment of a New Diagnostic Tool in Bronchoscopy for Staging of Lung Cancer. *Onkologie* **2001**, *24*, 151–154, doi:10.1159/000050303.
39. Mori, V.; DuComb, E.A.; Wagner, S.; Khan, F.; Bates, J.H.T.; Kinsey, C.M. Visualizing Intratumoral Injections in Lung Tumors by Endobronchial Ultrasound. *J Cancer* **2023**, *14*, 544–553, doi:10.7150/jca.81793.
40. Eapen, G.A.; Shah, A.M.; Lei, X.; Jimenez, C.A.; Morice, R.C.; Yarmus, L.; Filner, J.; Ray, C.; Michaud, G.; Greenhill, S.R.; et al. Complications, Consequences, and Practice Patterns of Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration: Results of the AQuIRE Registry. *Chest* **2013**, *143*, 1044–1053, doi:10.1378/chest.12-0350.
41. Marabelle, A.; Tselikas, L.; de Baere, T.; Houot, R. Intratumoral Immunotherapy: Using the Tumor as the Remedy. *Annals of Oncology* **2017**, *28*, xii33–xii43, doi:10.1093/annonc/mdx683.
42. Tsai, Y.-M.; Kuo, Y.-S.; Lin, K.-H.; Chen, Y.-Y.; Huang, T.-W. Diagnostic Performance of Electromagnetic Navigation versus Virtual Navigation Bronchoscopy-Guided Biopsy for Pulmonary Lesions in a Single Institution: Potential Role of Artificial Intelligence for Navigation Planning. *Diagnostics* **2023**, *13*, 1124, doi:10.3390/diagnostics13061124.
43. Chang, A.T.C.; Chan, J.W.Y.; Siu, I.C.H.; Liu, W.; Lau, R.W.H.; Ng, C.S.H. Robotic-Assisted Bronchoscopy-Advancing Lung Cancer Management. *Front Surg* **2025**, *12*, 1566902, doi:10.3389/fsurg.2025.1566902.

44. Miyake, K.; Shiroyama, T.; Satoh, S.; Adachi, Y.; Ohira, K.; Abe, Y.; Takata, S.; Masuhiro, K.; Naito, Y.; Hirata, H.; et al. Balloon Dilatation for Bronchoscope Delivery in a Swine Model: A Novel Technique for Ultra-Peripheral Lung Field Access and Accurate Biopsy. *Respiration* **2024**, *103*, 205–213, doi:10.1159/000536666.
45. Khan, F.B.; Gibson, P.C.; Anderson, S.; Wagner, S.; Cole, B.F.; Kaufman, P.; Kinsey, C.M. Initial Safety and Feasibility Results From a Phase 1, Diagnose-and-Treat Trial of Neoadjuvant Intratumoral Cisplatin for Stage IV NSCLC. *JTO Clinical and Research Reports* **2024**, *5*, 100634, doi:10.1016/j.jtocrr.2024.100634.
46. Mori, V.; Spivey, J.; Pierre, L.; Bates, J.H.T.; Kinsey, C.M. Intraoperative Cone-Beam CT Scan Can Inform Short-Timescale Drug Retention Following Intratumoral Delivery of Cisplatin in Non-Small Cell Lung Cancer. In Proceedings of the B110. PROBING THE LUNG: THE LATEST IN INTERVENTIONAL PULMONARY; American Thoracic Society, May 2023; pp. A4462–A4462.
47. Davenne, T.; Percier, P.; Larbanoix, L.; Moser, M.; Leo, O.; Meylan, E.; Goriely, S.; Gérard, P.; Wauthoz, N.; Laurent, S.; et al. Inhaled Dry Powder Cisplatin Increases Antitumour Response to Anti-PD1 in a Murine Lung Cancer Model. *Journal of Controlled Release* **2023**, *353*, 317–326, doi:10.1016/j.jconrel.2022.11.055.
48. Kuehl, P.J.; Yingling, C.M.; Dubose, D.; Burke, M.; Revelli, D.A.; Chen, W.; Dye, W.W.; Belinsky, S.A.; Tessema, M. Inhalation Delivery Dramatically Improves the Efficacy of Topotecan for the Treatment of Local and Distant Lung Cancer. *Drug Deliv* **2021**, *28*, 767–775, doi:10.1080/10717544.2021.1912209.
49. Yasmin-Karim, S.; Richards, G.; Fam, A.; Ogurek, A.-M.; Sridhar, S.; Makrigiorgos, G.M. Aerosol Delivery of Hesperetin-Loaded Nanoparticles and Immunotherapy Increases Survival in a Murine Lung Cancer Model. *Nanomaterials* **2025**, *15*, 586, doi:10.3390/nano15080586.
50. Zheng, W.; Zhu, T.; Tang, L.; Li, Z.; Jiang, G.; Huang, X. Inhalable CAR-T Cell-Derived Exosomes as Paclitaxel Carriers for Treating Lung Cancer. *Journal of Translational Medicine* **2023**, *21*, 383, doi:10.1186/s12967-023-04206-3.
51. Inhalable Biomimetic Polyunsaturated Fatty Acid-Based Nanoreactors for Peroxynitrite-Augmented Ferroptosis Potentiate Radiotherapy in Lung Cancer | Journal of Nanobiotechnology | Full Text Available online: <https://jnanobiotechnology.biomedcentral.com/articles/10.1186/s12951-025-03409-8> (accessed on 30 August 2025).
52. Nebulized Therapy of Early Orthotopic Lung Cancer by Iron-Based Nanoparticles: Macrophage-Regulated Ferroptosis of Cancer Stem Cells | Journal of the American Chemical Society Available online: <https://pubs.acs.org/doi/10.1021/jacs.3c08032> (accessed on 30 August 2025).
53. Dual-Targeted Lung Cancer Therapy via Inhalation Delivery of UCNP-siRNA-AS1411 Nanocages | Cancer Biology & Medicine Available online: <https://www.cancerbiomed.org/content/19/7/1047> (accessed on 30 August 2025).
54. Schelde, K.; Rosenjack, J.; Sonneborn, C.; Jafri, A.; Kavran, M.; Brumbaugh, S.; Rietsch, A.; Darrah, R.J.; Hodges, C.A.; Flask, C.A.; et al. A Minimally Invasive Bronchoscopic Approach for Direct Delivery to Murine Airways and Application to Models of Pulmonary Infection. *Lab Anim* **2023**, *57*, 611–622, doi:10.1177/00236772231175553.
55. Induction of Lung Lesions by Bronchial Administration Using Bronchoscope Technique in Mice Available online: https://www.jstage.jst.go.jp/article/tox/37/2/37_2023-0123/_article (accessed on 30 August 2025).
56. Joshi, K.; Katam, T.; Hegde, A.; Cheng, J.; Prather, R.S.; Whitworth, K.; Wells, K.; Bryan, J.N.; Hoffman, T.; Telugu, B.P.; et al. Pigs: Large Animal Preclinical Cancer Models. *World J Oncol* **2024**, *15*, 149–168, doi:10.4021/wjon.v15i2.1763.
57. Sadoughi, A.; Sarkar, A.; Novotney, C.; Chan, C.; Moreno, G.; Oren, S.; Moss, L.; Aharoni, K.; Bari, S.E.; Edery, N.; et al. Bronchoscopic Deployment and Implantation of Diffusing Alpha-Emitters Radiation Therapy into the Lung and Mediastinum for Treatment of Lung Cancer: A Pre-Clinical Safety and Feasibility Study. *Transl Lung Cancer Res* **2024**, *13*, 60–75, doi:10.21037/tlcr-23-639.
58. Zhou, J.; Xu, Y.; Liu, J.; Feng, L.; Yu, J.; Chen, D. Global Burden of Lung Cancer in 2022 and Projections to 2050: Incidence and Mortality Estimates from GLOBOCAN. *Cancer Epidemiol* **2024**, *93*, 102693, doi:10.1016/j.canep.2024.102693.

59. Nguyen, T.D.; Nguyen, T.-H.; Vo, V.T.; Nguyen, T.-Q. Panoramic Review on Polymeric Microneedle Arrays for Clinical Applications. *Biomed Microdevices* **2024**, *26*, 41, doi:10.1007/s10544-024-00724-z.
60. Kubo, T.; Seyama, T. Preclinical Development of siRNA Conjugates to Target Tumor Antigens. *Expert Opinion on Investigational Drugs* **2025**, *34*, 449–452, doi:10.1080/13543784.2025.2511181.

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