Local treatment of non-small-cell lung cancer (NSCLC) by dry powder inhaler

Repurposing approved compounds for improved patient outcomes

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Motivations for inhaled treatment of lung cancer

Lung cancer remains the leading cause of cancer-related death worldwide. Total deaths from lung cancer exceed that of breast, colon, prostate and pancreatic cancer combined, despite its relatively low prevalence, making up only 13% of newly-diagnosed cases [1]. This is due to its low survivability; for patients diagnosed with late stage (3+, with tumors distant from the lung) non-small cell lung cancer (NSCLC), the 5-year survival rate is only 8% [2]. Approximately 83% of lung cancer cases are NSCLC, which will be the focus of this article [1].

To combat NSCLC, a wide range of new chemical entities and novel combinations of therapies are under investigation in clinical trials. For example, the United States National Cancer Institute lists 530 active clinical trials for patients throughout the stages of NSCLC [3]. Chemotherapy, radiation, immunotherapy and targeted therapies are used in combination depending on specific patient needs. Targeted therapies, which rely on genetic sequencing of tumor mutations, enable specialized treatment for each individual. The active pharmaceutical ingredients (APIs) used for NSCLC range from small molecules (e.g., cisplatin, erlotinib, crizotinib) to monoclonal antibodies (e.g., bevacizumab, pembrolizumab) [4].

All currently approved drugs for NSCLC are delivered systemically, whether by intravenous infusion or oral administration. Systemic administration can be advantageous when cancer has spread beyond the lung into other parts of the body. However, systemic administration also exposes healthy tissues to the drug, which can lead to adverse effects. For many highly potent or cytotoxic APIs, dose-limiting toxicity can prevent a drug from realizing its full therapeutic potential. Chemotherapy is a well-known example in which the patient can only tolerate the drug for a short time before adverse effects outweigh treatment benefits. For some APIs, such as bevacizumab, systemically associated adverse effects can lead to substantial exclusion criteria for patients who might otherwise benefit from treatment [5]. When treatments are administered by intravenous (IV) infusion, repeated visits to the clinic to receive treatment can place a substantial burden on patients.

Local treatment of lung diseases by inhalation has been common for centuries and is today's standard-of-care for numerous diseases, such as chronic obstructive pulmonary disease (COPD) and asthma. In recent years, local delivery has been investigated in clinical trials for additional lung indications including pulmonary hypertension, lung infection and lung cancer. The reduced risk of systemic adverse effects and potential for reduced dose are compelling advantages of local delivery. Local treatment of lung cancer by the inhalation route is an emerging therapeutic area, particularly for early stages of the disease when the cancer is localized in the lung tissue.

This article focuses on local treatment of lung cancer by dry powder inhaler (DPI), though nebulizers have also been investigated as a delivery technology, particularly for inhaled biotherapeutics. Advantages of dry powder inhalers include their ease of use, quick administration and excellent shelf stability. Here, we outline manufacturing and formulation considerations for local treatment of lung cancer by dry powder inhaler and highlight three recent case studies.

Spray drying to facilitate local delivery to the lungs

Spray drying is a scalable manufacturing technique used to produce engineered pharmaceutical powders. It combines the formulation and particle engi-

neering needs of an inhaled product into a single process step. In spray drying, the API and excipients are co-dissolved into a volatile solvent. Liquid is pumped through an atomizer, where it is broken up into small droplets. In the drying chamber, the solvent is rapidly removed from the droplets via a heated drying gas, producing a dry powder product. To achieve the target particle size necessary for delivery to the deep lung, droplet size, solution composition and drying kinetics are important process parameters. As discussed in the case studies presented here, spray drying can be applied to a wide range of APIs, including small molecules and large biotherapeutics, such as monoclonal antibodies, oligonucleotides and other proteins.

Precedented in the literature

Inhaled chemotherapy for treatment of lung cancer is precedented in the literature and was reviewed recently by Rosière. et al. [6]. A primary concern for inhaled chemotherapy delivered by nebulizer is contamination of the surrounding environment with cytotoxic compounds. Burdensome infrastructure such as containment tents, filter apparatuses or mouth-only devices have been proposed to protect healthcare workers administering these treatments . These challenges can be largely ameliorated by the use of a dry powder inhaler, in which products can be designed to prevent exhalation of cytotoxic powders [6].

In addition to the case studies discussed in detail below, here are a few studies that demonstrate dry powder formulations for local treatment of lung cancer:

- Camptothecin, a naturally occurring topoisomerase I inhibitor, was formulated as a dry powder by spray drying and delivered to rats by inhalation. Drug levels in lung tissue were ten-fold higher for the inhaled treatment compared with the same dose delivered intravenously [7].
- Topotecan, approved for IV administration for multiple cancer types, was also employed as a spray-dried powder for inhalation. Delivery by the inhalation route substantially improved topotecan's efficacy in a rat model of NSCLC, both in local and distant tumors [8, 9].
- 5-azacytidine is a demethylation agent of interest for lung cancer treatment, which has poor exposure in lung tissue when delivered intravenously. Kuehl, et al. demonstrated that a dry powder formulation delivered by inhalation had superior pharmacokinetics (PK) in rats compared with IV administration. In addition, it reduced tumor burden in an orthotopic rat model of NSCLC [10]. Inhaled 5-azacytidine also was well-tolerated by NSCLC patients in a Phase I clinical study using a nebulized liquid formulation [11].

Case study 1: Dry powder gemcitabine with excipient-enhanced growth [12]

Gemcitabine is an approved, cytotoxic chemotherapy for the treatment of NSCLC through IV delivery. Dose-limiting toxicity is a problem for gemcitabine, so local delivery to the lung could greatly enhance treatment potential and improve patient comfort. Following previous safety and efficacy studies using a nebulized formulation, Quench Medical and Lonza are partnering to deliver this API directly to the site of action by using a spray-dried powder. This formulation is designed to improve delivery efficiency and protect care providers from this highly potent API.

The strategy for delivering gemcitabine to the target tissues in the deep lung rests upon excipient-enhanced growth (EEG) technology. Using EEG, a hygroscopic excipient is included in the spray-dried intermediate, which will swell upon exposure to moisture.

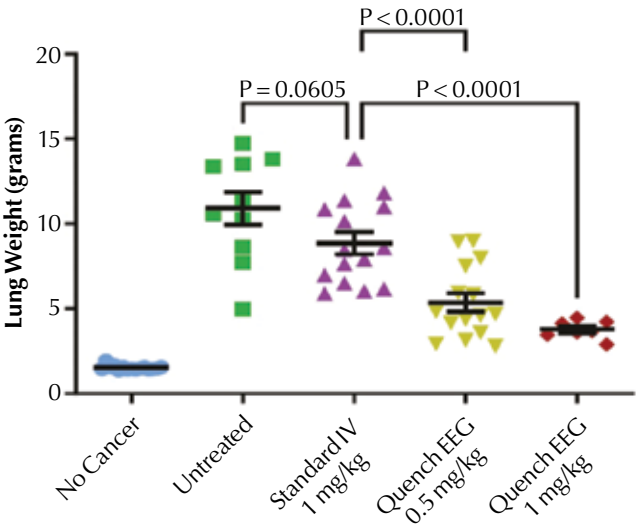
Table 1

Efficacy study design for gemcitabine. Adapted from Reference 7.

Arm	Cancer Cells	Treatment Route	Dose
Naïve Control	No	NA	NA
Sham Control	Yes	NA	NA
Clinical IV Standard	Yes	Intravenous	1 mg/kg
Inhalation - Same IV Dose	Yes	Inhalation	1 mg/kg
Inhalation - ½ IV Dose	Yes	Inhalation	0.5 mg/kg

Figure 1

Efficacy of the Quench inhaled EEG formulation with a matched dose (1 mg/kg) and half dose (0.5 mg/kg) versus IV standard-of-care (1 mg/kg). From reference 12. Reprinted with permission.



Process targets for an EEG formulation differ from typical inhalation dry powders, instead targeting particles with a distribution centered around approximately 1.5 µm. This smaller particle size allows the API to be delivered deep into the lung tissue, where the powder is exposed to the humid environment of the lung. The hygroscopic excipient absorbs water, causing particle growth. In turn, the particle growth improves particle deposition and prevents exhalation of the smallest particles. [13]

In addition to meeting the needs for delivery efficiency and reducing potential exposure for clinicians, spray drying also allows for generation of composite materials and enables the EEG platform. The key performance targets for this process are:

- Inhalation particle size, with a high fraction of particles less than 1 µm
- Low water content
- Low water exposure post-manufacturing

The spray-dried intermediate includes gemcitabine, the hygroscopic agent and a dispersion enhancer. This formulation was then tested in a pre-clinical, *in vivo* study in nude rats against the standard-of-care. The study design, a collaboration between Quench Medical and Lovelace Biomedical, is summarized in Table 1.

Rat participants not in the naïve control population were dosed with human lung adenocarcinoma A549 cancer cells, which were allowed to grow for three weeks. After that point, dosing with gemcitabine occurred once per week for four consecutive weeks. After completion of the study, lungs from the animals were measured for weight, as a proxy for disease burden. The data from the study is depicted in Figure 1.

The localized dosing of gemcitabine has a pronounced effect on lung weight. Rats without cancer have a lung weight of ~1.5 g. Lung weight above this value is attributed to the mass of cancerous tumors. Treatment by IV standard-of-care did not produce a statistically-significant reduction in lung weight ($p = 0.0605$). In contrast, the EEG half-dose treatment reduced tumor burden in the lungs significantly, compared with both untreated and IV standard-of-care groups ($p < 0.0001$). The reduction in lung weight for the EEG matched-dose group was also significant ($p < 0.0001$). This case study emphasizes that local delivery can improve efficacy in a NSCLC rat model at both matched and 50% reduced doses.

Case study 2: Inhaled bevacizumab dry powder reduces tumor burden in NSCLC rat model [14]

Bevacizumab is a monoclonal antibody vascular endothelial growth factor (VEGF) inhibitor approved for the treatment of colon cancer, NSCLC and glioblastoma. It acts in the body by disrupting angiogenesis, decreasing a tumor's ability to form new blood vessels as it grows. For late-stage NSCLC, bevacizumab is administered as an IV infusion every 3 weeks in combination with chemotherapy, and thereafter as a maintenance treatment. Due to the risk of severe bleeding adverse effects, substantial exclusion criteria prevent many patients from receiving bevacizumab. A locally delivered bevacizumab treatment could help reduce exposure of healthy tissue and, thereby, reduce adverse effects.

Compliance with repeated IV infusions can be challenging for patients, particularly during indefinite maintenance treatment. A dry powder formulation of bevacizumab could also help simplify the patient's treatment experience by enabling at-home administration with no cold chain storage requirements.

To this end, we formulated bevacizumab for dry powder inhaler, using spray drying as the particle engineering technology. The key performance attributes of this formulation were:

- Preserved bioactivity of bevacizumab to inhibit VEGF, evaluated by a cell-based assay
- Aerosol properties for delivery to the deep lung
- Physical stability of the powder at 25 °C, protected from humidity

Trehalose, a non-reducing sugar, is a common stabilizing excipient for proteins in both liquid and solid formulations. L-leucine is a surface-active amino acid often used in inhalation formulations to improve powder dispersibility. Both excipients were used in all formulations screened in this study. Initial evaluation of three active loadings showed that all three met targets for the key performance attributes and, therefore, the highest active loading was selected: a 40/40/20 bevacizumab/trehalose/L-leucine spray-dried powder by weight.

Analytical testing confirmed that the bevacizumab is undamaged by the spray-drying process: its bioactivity (anti-VEGF) was comparable to the as-received material; no aggregation or fragmentation was observed by size exclusion chromatography or dynamic light scattering; and a solution of reconstituted powder in phosphate buffer was optically transparent.

Aerosol properties were tested by Next Generation Impactor (NGI, Copley Scientific, Nottingham, UK) using a high-resistance Plastiape RS01 device (Berry Global Healthcare, Evansville, IN, US), with a fine particle fraction of 81% and a mass median aerodynamic diameter of 2.2 µm. These results were on-target for delivery to the deep lung.

The powder underwent an ICH stability study, packaged with desiccant and stored for 1 year at 5 °C and 25 °C. Other than a slight increase in the water content of the powder, all physical properties, aerosol properties and bioactivity metrics remained constant at both temperatures.

After the success of this formulation during the *in vitro* characterization, it was tested *in vivo* using an orthotopic nude rat model of NSCLC in collaboration with Lovelace Biomedical. A NSCLC human tumor cell line was intratracheally instilled into irradiated rats. After 4 weeks of tumor growth, weekly treatments were administered, and the rats' lung weight was determined at the 12-week endpoint as a measure of tumor burden. Four treatment groups were investigated, as summarized in Table 2. The standard-of-care group received injected cisplatin in combination with injected bevacizumab. Experimental group 1 received only inhaled bevacizumab, while Experimental group 2 received injected cisplatin and inhaled bevacizumab. An untreated group served as a negative control. Note that the inhaled dose was reduced 10-fold compared to the injected dose.

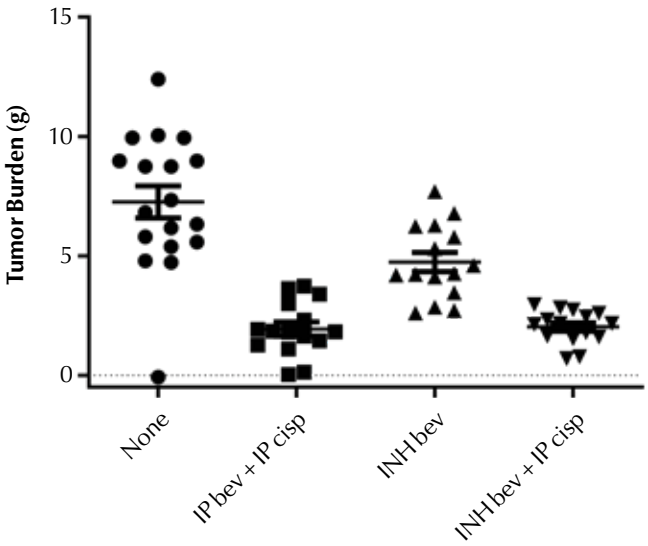
Table 2

Treatment groups for an *in vivo* study in a rat model. Adapted from Reference 14.

Treatment Group	Injected Cisplatin	Injected Bevacizumab	Inhaled Bevacizumab
Untreated	--	--	--
Standard-of-Care	3 mg/kg	15 mg/kg	--
Experimental 1	--	--	1.5 mg/kg
Experimental 2	3 mg/kg	--	1.5 mg/kg

Figure 2

Tumor burden results for an *in vivo* study in a rat model: Untreated (none), Standard-of-care (intraperitoneal (IP) bevacizumab + IP cisplatin), Experimental 1 (INH bevacizumab) and Experimental 2 (INH bevacizumab + IP cisplatin) groups are shown. From reference 14.



Results of the *in vivo* study are summarized in Figure 2. The standard-of-care, Experimental 1 and Experimental 2 groups all reduced the tumor burden significantly compared with the untreated group ($p < 0.05$). Experimental group 2 reduced tumor burden as effectively as the standard-of-care group, even with the 10-fold reduced bevacizumab dose. Experimental group 1 also demonstrated that even without cisplatin, bevacizumab delivered locally can reduce tumor burden. Together, these results demonstrated the efficacy of inhaled bevacizumab at reducing tumor burden in a rat model of NSCLC. When considered alongside 1-year physical stability at 25 °C, this formulation approach holds great promise for future treatment of lung cancer.

Case study 3: Inhalable bevacizumab/small molecule dry powder combination therapies for NSCLC [15]

As discussed in case study 2, bevacizumab is often administered in combination with other compounds, such as chemotherapies or therapies targeted to specific tumor mutations, such as programmed cell death protein 1 (PD-1) or epidermal growth factor receptor (EGFR). Treatment regimens for NSCLC are complex and full adherence can place a substantial burden on sick patients. A combination dry powder inhaler using NSCLC-relevant APIs could enable local treatment while also reducing patient treatment burden. To demonstrate this concept, three FDA-approved APIs of interest were identified for combination with bevacizumab: cisplatin, paclitaxel (both chemotherapies) and erlotinib (an EGFR inhibitor).

Manufacturing combination inhalable dry powders of a protein and a small molecule is not straightforward. Fragile molecules such as proteins are not compatible with milling micronization processes. A solution-based particle engineering technology, such as spray drying, is more appropriate. Like most proteins, bevacizumab must be in an aqueous solution to preserve its bioactivity, while paclitaxel and erlotinib are < 1 mg/mL soluble in water. Cisplatin is sparingly soluble in water but has poor chemical stability in aqueous solutions. Therefore, a common solvent could not be found to co-dissolve both APIs and excipients. Instead, we employed a simultaneous spray-drying approach, or "simul-spray," in which two dry powders are manufactured at the same time in a single spray dryer. A schematic is shown in Figure 3. In the simul-spray process, two different liquid solutions are spray dried to form two different dry powders, which are intimately mixed in the collection container.

First, mono-API dry powder formulations of each drug were developed and characterized. All contained L-leucine as an excipient to improve powder dispersibility, and cisplatin and bevacizumab also contained trehalose as a stabilizer. In this proof-

Figure 3

Schematic of a simultaneous spray-drying process. From reference 15. Reprinted with permission.

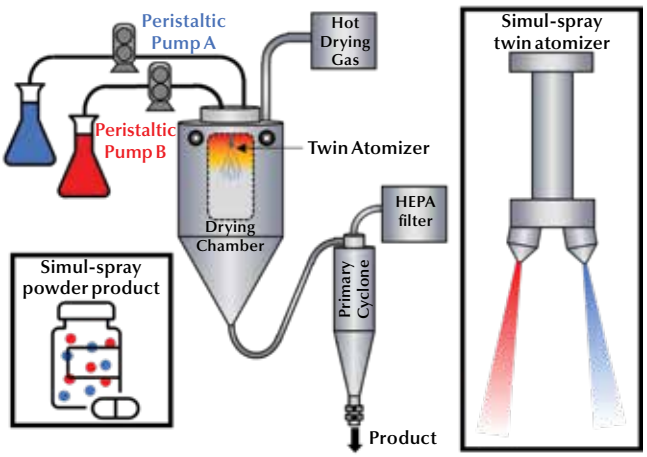


Table 3

Summary of simul-spray formulations. Adapted from reference 15.

Small Molecule Formulation (By Mass)	Bevacizumab Formulation (By Mass)	Ratios Investigated
80/20 Erlotinib/ L-leucine	40/20/20 Bevacizumab/ Trehalose/L-leucine	1:2, 1:1
80/20 Paclitaxel/ L-leucine		1:5, 1:2, 1:1, 2:1
10/70/20 Cisplatin/ Trehalose/L-leucine		2:1, 1:1, 1:2

of-concept work, nine combination formulations were manufactured, as summarized in Table 3. The bevacizumab formulation is the same one used in case study 2.

SEM images of the mono-API formulations and simul-spray formulations revealed uniformly blended powders where the two types of particles could be visually differentiated. For example, paclitaxel formulations had a spherical shape with a rough, corrugated surface, while bevacizumab formulations are collapsed spheres with a smooth surface. A simul-spray formulation of the two is shown in Figure 4.

For a combination product, it is critical that every dose have the same drug concentration of each active pharmaceutical ingredient. To this end, we measured the drug concentration in the powders for both APIs and found that all formulations were within 80-120% of the target levels. It was also critical to demonstrate the preserved anti-VEGF bioactivity of the bevacizumab in each formulation. The cell-based assay used in case study 2 could not be used due to the cytotoxic paclitaxel and cisplatin present in most of the combination formulations. Simul-spray formulations were reconstituted and incubated with VEGF to allow binding. An ELISA kit was then used to quantify the VEGF that remained unbound by the bevacizumab. VEGF levels in the presence of as-received bevacizumab were similar to those from the simul-spray formulations, thereby demonstrating the preserved bioactivity of the spray-dried powders. Even in cases where the small molecule solution was spray dried using an organic solvent (methanol for erlotinib and ethanol for paclitaxel), bevacizumab activity was preserved.

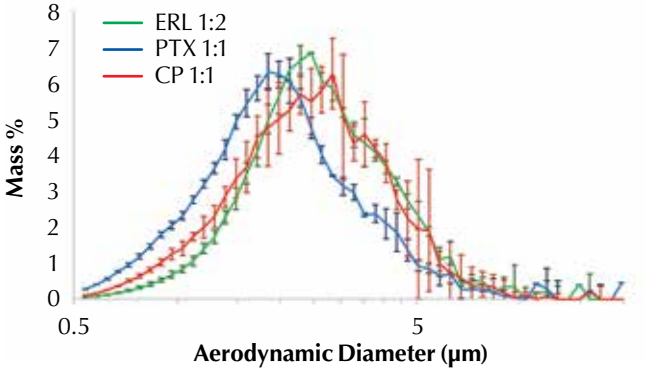
Finally, the aerosol properties of the simul-sprayed formulations were characterized using a Fast Screening Impactor (Copley Scientific, Nottingham, UK), which yields a normalized fine particle dose, and an Aerodynamic Particle Sizer (TSI, Shoreview, MN, US). Fine particle dose normalized by capsule fill mass ranged from 43-65%, with paclitaxel formulations exhibiting the highest values and erlotinib the

Figure 4

SEM images of paclitaxel mono, bevacizumab mono and bevacizumab/ paclitaxel simul-spray powders.

Figure 5

Aerodynamic particle size distribution for three representative simul-spray powders.



lowest. Selected aerodynamic particle size distributions are shown in Figure 5. In all cases, a monomodal distribution was observed, indicating that the particle size distributions of the two particle types were similar in each combination. Mass median aerodynamic diameters were 1.8-2.9 µm and geometric standard deviations were 1.6-1.7 µm. Overall, all formulations had good aerosol properties targeted for inhalation delivery to the deep lung.

Local treatment for improved patient outcomes

Local treatment of lung cancer delivered by inhalation has multiple potential advantages to improve therapeutic outcomes in patients. By avoiding systemic administration, potent compounds can be targeted to the site of the disease, reducing exposure of healthy tissue and reducing dose-limiting toxicity. When dry powder inhalers are used to facilitate pulmonary administration, there is also potential for self-administration and good shelf stability.

This article summarized recent work on dry powder formulations for lung-cancer-relevant targets, including topoisomerase 1, VEGF, EGFR and chemotherapeutic agents. Using spray drying to engineer respirable particles, the case studies demonstrated the broad applicability of this technology to small and large molecules alike. Looking forward, future clinical trials for local treatment of lung cancer by dry powder inhaler hold great promise for patients of this challenging disease.

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