Preclinical experience with cisplatin, gemcitabine, and doxorubicin in pulmonary suffusion

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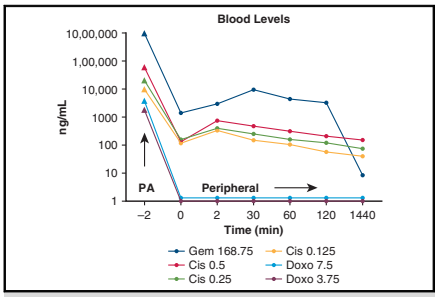
ABSTRACT

Background: Because suffusion amplifies lung chemotherapy while limiting systemic toxicity, we tested candidate drugs for treating human lung cancers and pulmonary metastases.

Methods: Immature beagle dogs underwent thoracotomy for unilateral lung suffusion of cisplatin (0.125-2 mg/kg; n = 19), doxorubicin (3.75-7.5 mg/kg; n = 7), gemcitabine (168.75 mg/kg; n = 5), or saline (n = 3). After ipsilateral lung circulation isolation and drainage, pulmonary artery chemotherapy was injected, dwelled for 30 minutes, and then aspirated. Bilateral lung biopsies and serum samples assessed delivery and leak. After lung reperfusion, animals recovered for 30 days with scheduled monitoring of vital signs, weights, and behaviors. At experiment termination, necropsy histopathologic tissue analyses assessed tolerability.

Results: All 32 animals recovered, except 1 with lung torsion and 2 with pulmonary toxicity that required early euthanasia. Serum concentrations during suffusion for cisplatin (135 ng/mL), doxorubicin (undetectable), and gemcitabine (1452 ng/mL) indicated minimal systemic leakage. Cisplatin escalations showed uniform suffusion deliveries (100% fibrosis at a 100% systemic chemotherapy dose), which was then reduced to a nondamaging 25% threshold. When the equivalent dose of doxorubicin was used, toxicity occurred, but 12.5% (2.5-fold amplification of local delivery) was well tolerated. Gemcitabine, like cisplatin, caused minimal toxicity at 25% of the systemic dose (5-fold amplification). Optimized doses caused no hematologic or metabolic derangements and necropsies showed no gross organ injury other than adhesions. Histopathology demonstrated multifocal ipsilateral lung fibrotic changes without contralateral or extrapulmonary pathology.

Conclusions: While suffusion delivery of the vesicant doxorubicin was tolerated less well than cisplatin and gemcitabine, all appear to be safe and feasible for human trials. (JTCVS Open 2025;24:484-95)



Line graph showing pulmonary artery and serum drug levels at timepoints after suffusion.

CENTRAL MESSAGE

Motivated by our early cisplatin preclinical experimentation, we completed additional experiments using doxorubicin and gemcitabine to facilitate their use in future clinical suffusion operations.

PERSPECTIVE

Non–small cell lung cancer and pulmonary metastatic disease lead to significant morbidity and mortality. Chemotherapy is a common treatment modality for these ailments, but dosages and utility are often limited by negative systemic side effects. By isolating the lung vasculature, the suffusion surgeon can amplify chemotherapy dosages delivered while increasing tolerability.

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This work was partially supported by National Cancer Institute Grant P30CA016056 involving the use of Roswell Park Comprehensive Cancer Center's Bioanalytics, Metabolomics and Pharmacokinetics (BMPK) Shared Resource.

Institutional Animal Care and Use Committee approval 1366D; approved December 27, 2017.

Read at The American Association for Thoracic Surgery International Thoracic Surgical Oncology Summit 2024, New York, New York, September 27-28, 2024.

Received for publication Dec 17, 2024; accepted for publication Dec 18, 2024; available ahead of print Feb 11, 2025.

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2666-2736

https://doi.org/10.1016/j.xjon.2024.12.012

Abbreviations and Acronyms
MRD = minimal residual disease
PA = pulmonary artery

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Lung cancer is the leading cause of cancer-related deaths worldwide.[1] In 2020, 2.2 million such cases were diagnosed (11.4% of new malignancies), and almost 1.8 million patients died (roughly 18% of all cancer mortalities).[2] The lungs are also common sites of metastases owing to their role as a vascular filter (30%-50% of extrathoracic malignancies).[3] Pulmonary metastatic disease reduces survival for sarcomas (18 months median),[4] colorectal cancers (30% at 5 years),[5] and hepatocellular carcinomas (4% at 5 years).[6] Metastasectomy for isolated lung disease may improve survival, but this has been challenged.[5] Surgery is limited by its ability to remove all tumors discernable by imaging or palpation, patient frailty, insufficient lung capacity, inflammatory responses increasing tumor growth, and (most of all) occult microscopic minimal residual disease (MRD), causing local recurrence and reduced survival.[7] We hypothesize that targeting MRD at the time of macroscopic tumor resection using regional chemotherapy at doses higher than achievable by systemic administration could improve the results of primary and secondary lung malignancy resections.

Cisplatin,[8,9] doxorubicin,[10] and gemcitabine[9,11] alone or combined with other drugs treat metastatic malignancies of surgical interest (Table 1). Regional delivery may be better for drugs like cisplatin that yield relatively low lung levels when administered systemically.[12] Local delivery should reduce systemic complications (Table 1), particularly the cardiotoxicity of doxorubicin (2% rate of congestive heart failure).[10] Reducing doxorubicin-related heart injury while increasing drug delivery is particularly attractive for chemoresistant sarcomas.[13] For other metastatic malignancies, a regional approach might improve patient tolerance, quality of life, and efficiency of identifying effective regimens.[14,15] Suffusion is a regional drug delivery strategy that capitalizes on the low metabolic activity and dual blood supply experienced by the lungs[16] to introduce relatively high drug concentrations. We demonstrated limited preclinical and human results with cisplatin suffusion in 2008.[17,18] To increase the impact of suffusion, we conducted further preclinical studies with the tabulated drugs; here we report our preclinical experience to date.

MATERIALS AND METHODS

The animals used for all experiments were immature beagles bred and commonly used for toxicology experimentation (Marshall BioResources). This canine use met Institutional Animal Care and Use Committee requirements for humane treatment of research animals. In particular, investigators complied with the 2011 Guide for the Care and Use of Laboratory Animals.[19] This study was approved by the Institutional Animal Care and Use Committee at Roswell Park Comprehensive Cancer Center (approval 1366D; approved November 27, 2017).

Suffusion Procedure

The dogs were premedicated and then underwent anesthetic induction and intubation. They were oxygenated and maintained on isoflurane through the case. Monitoring of heart rate, blood pressure, electrocardiography, and pulse oximetry was maintained throughout the procedure.

A thoracoscopic approach for component maneuvers (commonly performed in humans) was impractical and already established in a larger canine model using adult human instrumentation.[20] Therefore, experiments were simplified by performing a mini-thoracotomy and directly cannulating the pulmonary artery rather than thoracoscopic exposure and fluoroscopy-guided catheter placement. Details of the procedure have been published previously.[17,20] In brief, animals in lateral recumbency underwent left fourth interspace thoracotomy, and under direct visualization, pulmonary vein (3) and left main pulmonary artery (PA) snaring were performed, and a standard 24-gauge intravenous catheter was placed by direct puncture of the left PA for delivery of the suffusate and subsequent aspiration (Figure 1). During suffusate injection and dwell time, the ipsilateral lung was ventilated to aid drug distribution. Near completion of the dwell, approximately 1-cm^2 lung samples from the cranial, caudal, and contralateral caudal (by opening the posterior pleura) were obtained using a 30-mm blue load surgical stapler (Ethicon Endo-Surgery). The blood–drug mixture was aspirated from the PA after a 30-minute dwell, and all vascular snares were released. Wounds were closed in layers. Narcotic analgesics provided postanesthesia pain control. Animals were observed and cared for by veterinary staff. The 30-minute dwell time was chosen by our group based on our original large canine study showing that 75% of the drug remained at this time point.[20] We also believe that 30 minutes is a reasonable amount of time to add to a thoracic operation that will not be overly disruptive or cumbersome.

Experiments

Cisplatin. High-concentration experiments to test uniform, selective lung delivery began with 5 dogs (8.3-13.8 kg) that underwent suffusion with cisplatin at 2 mg/kg (n = 2; systemic dose) or 1 mg/kg (n = 2; half-systemic dose) or with saline (n = 1; control). Subsequent dose escalation experiments sought optimal preclinical dosing to avoid tissue damage. The animals (6.5-11 kg) received cisplatin at 0.125 mg/kg (n = 4), 0.25 mg/kg (n = 4), or 0.5 mg/kg (n = 4) or saline (n = 2; control).

Doxorubicin. Based on preliminary results for cisplatin, these dogs (6.14-9.26 kg) received doxorubicin at 7.5 mg/kg (n = 3) or 3.75 mg/kg (n = 4) or saline (n = 1; control).

Gemcitabine. Based on the results of the prior experiments, these dogs (8.07-9.85 kg) underwent gemcitabine suffusion with at 168.75 mg/kg (n = 5). Similar to the doxorubicin experiments, this dose was selected based on the good tolerance of the 25% systemic dose by the cisplatin-treated animals.

Pharmacokinetic Data

Cisplatin. Tissue samples were weighed, placed in 10 volumes of 0.25% Triton X-100, and processed using a tissue homogenizer (Polytron). The homogenate was analyzed for platinum using a graphite furnace

TABLE 1. Chemotherapeutic agents chosen for suffusion experimentation

Drug	Mechanism	Absorption	Susceptible malignancies	Toxicities	Dog dose (% of therapeutic systemic dose)
Cisplatin (Oxaliplatin)	Alkylating-like properties facilitate DNA adduct formation, thereby halting purine synthesis and DNA replication	Passive	Sarcoma Hepatocellular carcinoma NSCLC Breast Esophageal Ovarian Testicular Mesothelioma	Ototoxicity Nephrotoxicity Peripheral neuropathy Emesis Myelosuppression	2 mg/kg (100%) 1 mg/kg (50%) 0.5 mg/kg (25%) 0.25 mg/kg (12.5%) 0.125 mg/kg (6.25%)
Doxorubicin	Intercalates with base pairs and stabilizes topoisomerase II, preventing resealing of replicating DNA strands	Active transport	Hepatocellular Sarcoma Leukemia Breast Gastrointestinal	Hepatotoxicity Cardiotoxicity Nephrotoxicity Myelosuppression Hematologic Dermatologic	7.5 mg/kg (25%) 3.75 mg/kg (12.5%)
Gemcitabine	Complexes with deoxycytidine triphosphate and inhibits DNA synthesis	Nucleoside transport	Hepatocellular Pancreatic Bladder NSCLC Breast Ovarian Sarcoma Colorectal*	Gastrointestinal Malaise Myelosuppression Hematologic Electrolyte abnormalities	168.75 mg/kg (25%)

NSCLC, Non–small cell lung cancer. *Secondary option for pulmonary metastases with exploitable mutations.[14]

atomic absorption spectrophotometer (PerkinElmer 4100ZL). The furnace program consisted of a 2-step drying at 120 °C and 150 °C for 2 minutes, with pyrolysis at 1500 °C and atomization at 2250 °C. To avoid air leaks and stapler related-fibrosis that could be misinterpreted as drug injury, lung biopsies were not performed on the clinical dose–optimized dogs that followed the high-dose experiments.

Serum samples were diluted 10× in 0.25% Triton X-100. All samples were analyzed against a standard curve (range, 50-800 ng/mL) prepared in plasma 10× diluted with 0.25% Triton X-100. The samples were diluted as required to fall within the standard curve range. Serum samples were collected for all animals.

Doxorubicin. Beagle serum and lung tissue samples were analyzed using ultra-performance liquid chromatography with fluorescence detection for doxorubicin concentration measurements. Methods are described in detail in Table E1.

Gemcitabine. Beagle plasma and lung tissue samples were analyzed in a single analytical run using a high-pressure liquid chromatographic assay with tandem mass spectrometric detection for gemcitabine and its metabolite 2',2'-difluoro-2'-deoxyuridine. Capturing this metabolite's concentration is important because it is pharmacologically active and has a longer half-life than gemcitabine. The metabolite prolongs and potentiates the gemcitabine effects, but its longer half-life may lead to longer-lasting side effects in the case of a leak. These protocols were adapted from previous work performed by team members.[21] Detailed methods can be found in Table E1.

Infusion Dosing and Sample Collection

Peripheral blood (3 mL) was sampled (via the saphenous vein) presuffusion, 5 minutes prior to suffusion cessation, and then after treatment at 5 minutes, 30 minutes, 60 minutes, 120 minutes, and 24 hours after suffusion to assess for leak and demonstrate the delayed release of gemcitabine on reperfusion. Into 2 mL of blood, 20 μL of tetrahydrouridine was added, and the sample was spun at 2000 relative centrifugal force. Plasma was frozen at -80 °C. The following laboratory test values were also obtained preinfusion and at various time points throughout the 30-day follow-up: complete blood count, prothrombin time, fibrinogen, electrolytes (Na^+ and K^+), glucose, creatinine, total protein, albumin, bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine transaminase, lactate dehydrogenase, and amylase. In experiments other than the cisplatin dose optimization, lung biopsy specimens were taken from the left cranial, left caudal, and right lung and then snap-frozen.

Postoperative Care

Animals were supervised for any signs of distress daily. In addition, daily weights, temperature, pulse, and respiration counts were performed. On postprocedure days 7, 14, 21, and 28 a complete blood count and complete metabolic panel were drawn. At the end of the observation period, a lethal injection of potassium chloride (100 mEq) was used for euthanasia.

Necropsy and Histology

Cisplatin studies. Standard hematoxylin and eosin stains of lung and kidney tissue were examined by a pathologist for evidence of injury secondary to infusion. For the uniform selective delivery portion of the study, parenchymal injury was quantified by percent fibrosis over 10 averaged high-power fields obtained from representative areas of the lung. Vessel damage and granulomas/giant cells were quantified using a 4-grade scoring method indicating level of tissue change: mild, moderate, severe, and almost completely replaced by fibrosis. This analysis was done onsite.

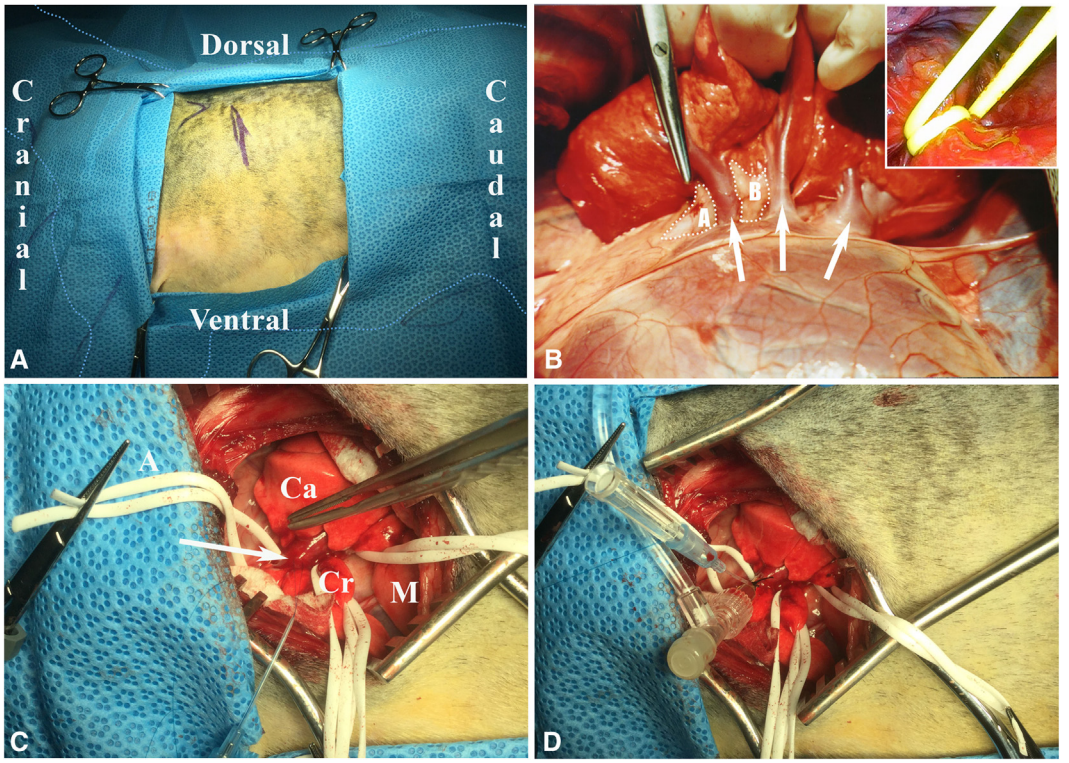

FIGURE 1. Surgical suffusion technique. A, Preclinical experimental setup showing the orientation of the surgical field and proposed thoracotomy site. The vertical line in the center of the surgical field shows the location of the thoracotomy incision caudal to an additional surgical marker notation identifying the tip of the scapula and providing an anatomic landmark. B, Closeup of dog hilar anatomy. Arrows indicate (from left to right) the cranial, middle, and caudal lobes that are encircled twice using silicone tapes. This creates a snare that when retracted occludes the vessel (as in the Inset photo from a human suffusion). A, Pulmonary artery emerging from hilum. Instrument points at cranial branch that may have had restricted flow because of its proximity to the snare. B, Bronchus. C, Posterolateral thoracotomy for suffusion. *Ca*, Caudal; *Cr*, cranial; *M*, medial. White vessel snares seen encircling pulmonary artery (A) and veins (unlabeled) and interlobar pulmonary artery is exposed and is identified with arrow at 6-0 polypropylene purse string cannulation site. D, Same anatomic arrangement seen in C with a 24G pulmonary artery catheter (PAC) inserted through a purse string suture placed in the interlobar fissure component of the vessel and partially obscuring the cranial lobe. A 22G catheter and cap with 6-0 polypropylene purse string suture are seen threaded through, acting as a Rummel tourniquet.

For the preclinical dose selection part of the study, histopathologic analysis was performed externally at Charles River Laboratories. Injury was quantified using a 5-grade scoring method to characterize the intensity and extent of tissue alterations, namely chronic inflammation and pigmented macrophage accumulation, expressed as minimal, slight, moderate, marked, or severe. Representative samples are shown in Figure 2. Tracheobronchial lymphoid tissue was examined and quantified in a similar manner. In this case, the tissue alteration analyzed was lymphoid atrophy of the paracortex.

Doxorubicin and gemcitabine studies. Thoracic cavity tissues, including lungs, were photographed following necropsy. Bilateral lung lobes and kidneys, bilateral tracheobronchial and mediastinal lymph nodes, heart, and tracheal tissues were formalin- preserved for hematoxylin and eosin histopathologic evaluation. Microscopic changes were graded (Figure 2) based on severity using the International Harmonization of Nomenclature Criteria: Nonproliferative and Proliferative Lesions of the Dog as a guide.[22]

RESULTS

Cisplatin

General observations. Dosages started at 2 mg/kg (100% systemic dose) and as toxicity was encountered, subsequent experiments were performed at 50%, 25%, 12.5%, and finally 6.25%. All 19 animals survived the surgical procedure, and 2 animals died before the 30-day endpoint. Intraoperatively, lungs receiving cisplatin were more edematous than the contralateral organ. Experiments proceeded without variations or intraoperative vascular injuries. All saline control and experimental animals that survived to the 30-day endpoint maintained normal levels of cardiac, hematologic, renal, hepatic, and pancreatic function throughout the experiment.

Recovery

Uniform delivery. Animals treated with 2 mg/kg cisplatin were ill with tachypnea, tachycardia, fever, and hemoptysis, and 1 died (on day 2; see Necropsy below). The other survived until necropsy, with symptomatic improvement starting on days 5 to 6. The cisplatin dose accordingly was reduced to 1 mg/kg with animals 3 and 4 surviving with fewer symptoms (Table 2).

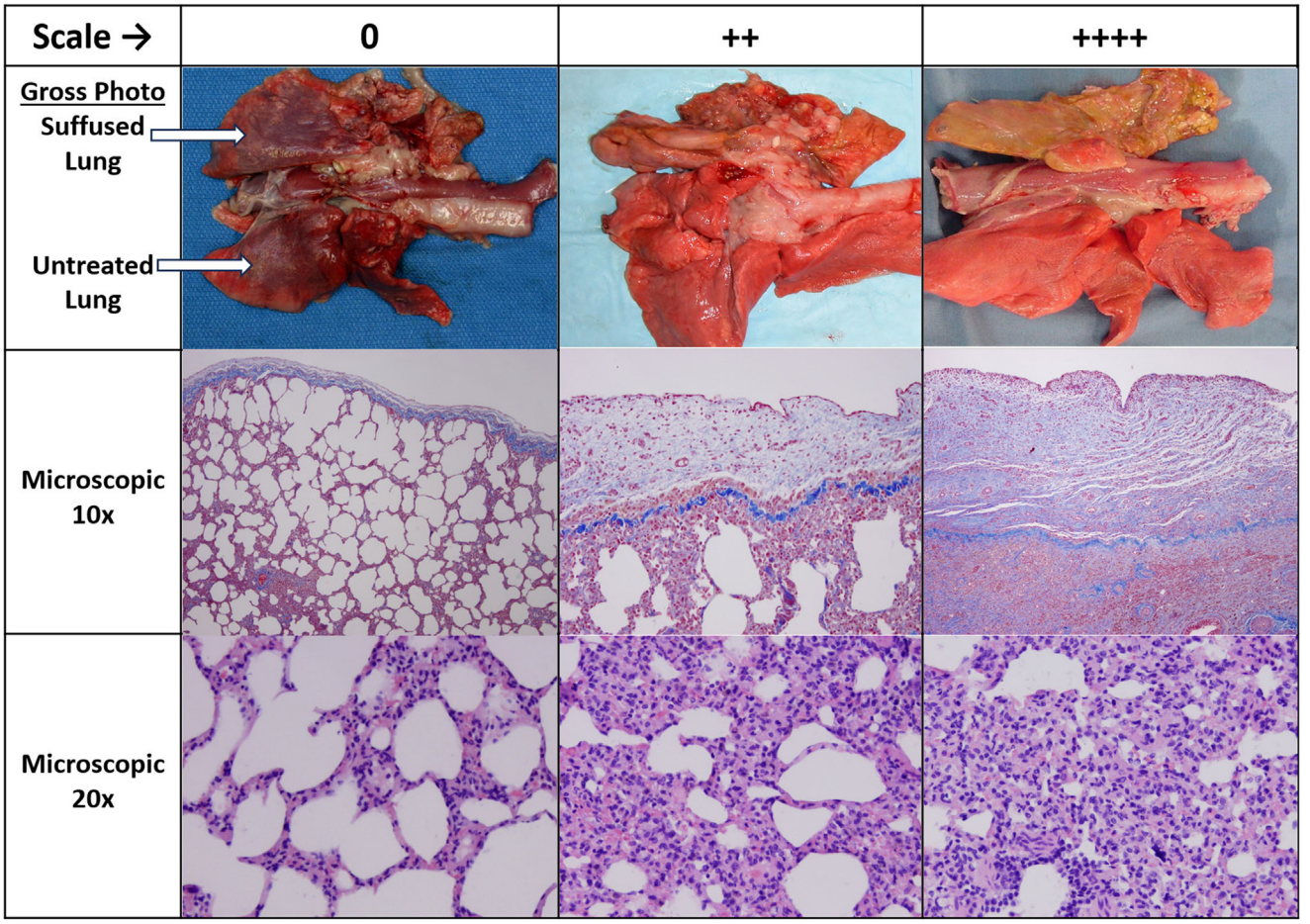

FIGURE 2. Pathologic specimens. Representative samples showing 0, ++, and ++++ fibrosis. Rows show gross lung samples, parenchymal fibrosis (Masson trichrome staining; 10× magnification), and alveolar fibrosis (hemosiderin and eosin staining; 20× magnification). As the columns progress from 0 to ++++, an increase in fibrosis can be seen in all rows. (Portion of figure *From* Demmy et al.[17])

Preclinical dose selection. Three of 4 dogs that received 0.5 mg/kg cisplatin suffusion had tachypneic, raspy, and labored breathing for 1 week postinfusion. One was euthanized on day 4 for lung hemorrhage after a suffusion where the PA catheter did not drain well and was hard to infuse. An inadvertent lung interstitium injection was suspected in this dog. The other 2 animals improved by day 10 and recovered unremarkably. Two of 4 dogs that received 0.25 mg/kg cisplatin were also tachypneic postinfusion, but both improved by day 4, considerably faster than their 0.5 mg/kg counterparts, and had an unremarkable recovery. The remaining dogs (2 recipients of 0.25 mg/kg and all 4 recipients of 0.125 mg/kg) exhibited no significant adverse events between infusion and necropsy (Table 2).

Necropsy

Uniform delivery. The 2 mg/kg animal that died prematurely had a grossly infarcted, edematous lung microscopic early fibrotic vascular adventitial change. The surviving animal had 95% lung fibrosis, effectively causing "chemical pneumonectomy" with near-complete fibrotic histologic replacement of vasculature (Figure 2). The half systemic dose animals (1 mg/kg) had a proportional response with 40% and 50% parenchymal fibrosis, respectively and moderate pulmonary vasculature fibrotic change. (Figure 2).

Preclinical dose selection. Suffused left lungs receiving 0.5 mg/kg and 0.25 mg/kg cisplatin showed fibrosis throughout. In contrast, the gross appearance of 0.125 mg/kg suffused lungs, like saline controls, had no evidence of chemically induced alteration in either lung (Figure 2). Table 1 summarizes pulmonary and lymphoid histopathologic findings for all 4 dose levels and controls. There was a general trend toward increased incidence and severity of chronic inflammation and lymphoid atrophy of the paracortex with escalating infusion dose. Accumulation of pigmented macrophages followed the same pattern, suggesting resolved small volume hemorrhagic events.

Platinum levels

Uniform dose. Figure 3 shows the mean platinum levels derived from the suffused organ, contralateral lung, systemic blood, and pulmonary artery contents. Presuffusion,

TABLE 2. Clinical results of suffusion experiments

Variable	Cisplatin					Doxorubicin		Gemcitabine
Dose, mg/kg	0.125	0.25	0.5	1	2	7.5	3.75	168.75
% Systemic	6.25	12.5	25	50	100	25	12.5	25
Number of animals	4	4	4	2	2	3	4	5
Survival, %	100	100	75	100	50	66	100	80
Cause of death (postprocedure day)	–	–	Hemoptysis (4)	–	Hemoptysis (2)	Hemoptysis (2)	–	Lung torsion (2)
Symptom days, mean	5	6	11	16	28	6	3	2
Fibrosis (gross)	+	+	++	+++	++++	+++	+	0
Lung staining (gross)	0	0	0	0	0	+++	+	0
Histologic fibrosis	+	+	+	+++	++++	++	++	+
Histologic vascular damage	+	++	++	++	+++	++	+	0
Contralateral histology	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Gross histologic results are also noted with scale: 0, none; +, trace; ++, mild; +++, moderate; ++++, severe.

cisplatin was not detected in lung tissue or serum samples. Halfway through the dwell (t = 15), mean platinum was segregated between serum (443 ng/mL) and treated lung (22,737 ng/g). Segregation persisted until reperfusion.

Preclinical dose selection. Figure 3 shows the delayed release of cisplatin on reperfusion. As expected, the relationship is dose-dependent—the rate of decrease in serum cisplatin concentration corresponds to the concentration of infusion used. Furthermore, there is evidence of a tissue saturation effect, with a much higher spike of release for the higher doses than for the lower doses (Figure 3). Direct PA draw values were 9674 ng/mL, 20,487ng/mL, and 59,976 ng/mL for animals undergoing suffusion with cisplatin at 0.125 mg/kg, 0.25 mg/kg, and 0.5 mg/kg, respectively. These values equated to 6.2, 6.6, and 9.6 amplification of the systemic drug dosage.

Doxorubicin

General observations. All 8 doxorubicin animals survived surgery, including 3 dogs at 7.5 mg/kg (25% systemic dose, extrapolated from cisplatin results, above), 4 at 3.75 mg/kg (12.5% systemic dose) and 1 control. Intraoperatively, lungs suffused with doxorubicin were more edematous than the contralateral organ. One dog had approximately 1 mL of drug spillage into the thoracic cavity, which was immediately rinsed with saline before wound closure. No other experimental protocol variances or surgical injuries occurred.

Recovery

One 7.5 mg/kg doxorubicin dog was euthanized (day 2) for lung hemorrhage. The other 2 dogs and the control dog had no postsuffusion complications, with stable vital signs and no need for interventions. All 4 recipients of 3.75 mg/kg doxorubicin survived until necropsy. One dog had coughing and hemoptysis day 6 without hemodynamic compromise or need for intervention, and the other 3 dogs survived uneventfully, with nominal behavior and vital signs until the study endpoint. All 7 survivors had normal metabolic laboratory results, with cardiac, hepatic, pancreatic, and renal function consistent with their baseline levels. Electrolyte, creatinine, and urea nitrogen levels remained at baseline. Similarly, no hematologic events, such as anemia, thrombocytopenia, or leukopenia, occurred (Table 2).

Necropsy

Table 1 summarizes the pulmonary histopathologic findings (various degrees of diffuse fibrosis like other drugs) for all dosage levels, including the control dog. Three of the 7.5 mg/kg animals had acute inflammation, classified as congestion edema, hemorrhage, and neutrophil infiltration. The 2 survivors had chronic interstitial changes, such as alveolar thickening, macrophage infiltration, and occasional hemosiderin-laden macrophages, suggestive of resolved low-level hemorrhage. There also were significant adhesions to the thoracic cavity on the suffused side. Treatment-related changes were confined to the lung, and there was no evidence of renal pathologic change for this or the lower-dose group. The 3.75 mg/kg group had intrathoracic adhesions ranging from none to moderate. Dog 3 had diffuse red discoloration suggesting vascular congestion. Dog 6 had multifocal coalescing cream-colored deposits on the suffused lung.

Doxorubicin levels

Figure 4 shows mean doxorubicin levels from suffusion and contralateral lung tissue. In the 7.5 mg/kg suffusion dogs, the mean cranial lobe level was 2517 ng/mL, and the mean caudal level was 14,293 ng/mL. For the 3.75 mg/kg dogs, these values were 375 ng/mL and 4259 mg/mL, respectively. The contralateral lung showed no doxorubicin at either dosage. Serum samples showed

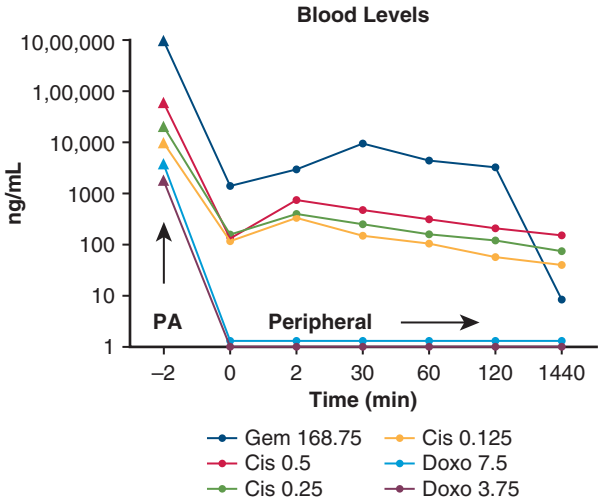

FIGURE 3. Serum drug concentrations during suffusion and in the postoperative period. Line graph showing the changes in drug concentration following the suffusion experiment and subsequent recovery period. Samples taken directly from the pulmonary artery are notated for each drug as a color corresponding triangle prior to time 0. *Gem*, Gemcitabine, *Cis*, cisplatin; *Doxo*, doxorubicin. Drug units = mg/kg animal weight.

no detectable doxorubicin leakage. Mean PA measurements were 1754 ng/mL for the low-dose group and 3840 ng/mL for the high-dose group. No other serum sample had detectable doxorubicin (Figure 3).

Gemcitabine

General observations. A dosage of 168.75 mg/kg (25% systemic) was chosen based on the foregoing experiments, and all 5 dogs received this dose and survived the procedure. As before, gemcitabine-suffused lungs demonstrated some edema but no compromised oxygenation or hemodynamic stability. No significant vascular injuries or variances in the planned procedures occurred.

Recovery

Dog 2 developed hypoxemia from lung torsion attributed to impaired thoracotomy exposure and lung biopsy, necessitating euthanasia on postoperative day 2. The other 4 animals survived to necropsy with no apparent symptoms, weight or vital sign changes. As before, serial metabolic panels demonstrated no change in organ function compared with baseline, including normal renal function tests (creatinine and blood urea nitrogen). Hemograms were normal throughout except for mild thrombocytopenia presuffusion (average, 244 K/μL; reference range, 451-525 K/μL) for unknown reasons that increased toward normal values by 30 days (average, 296 K/μL; n = 5 because of early necropsy) (Table 2).

Necropsy

One animal expired on postinfusion day 2, and necropsy showed left lung rotation with parenchymal hemorrhage

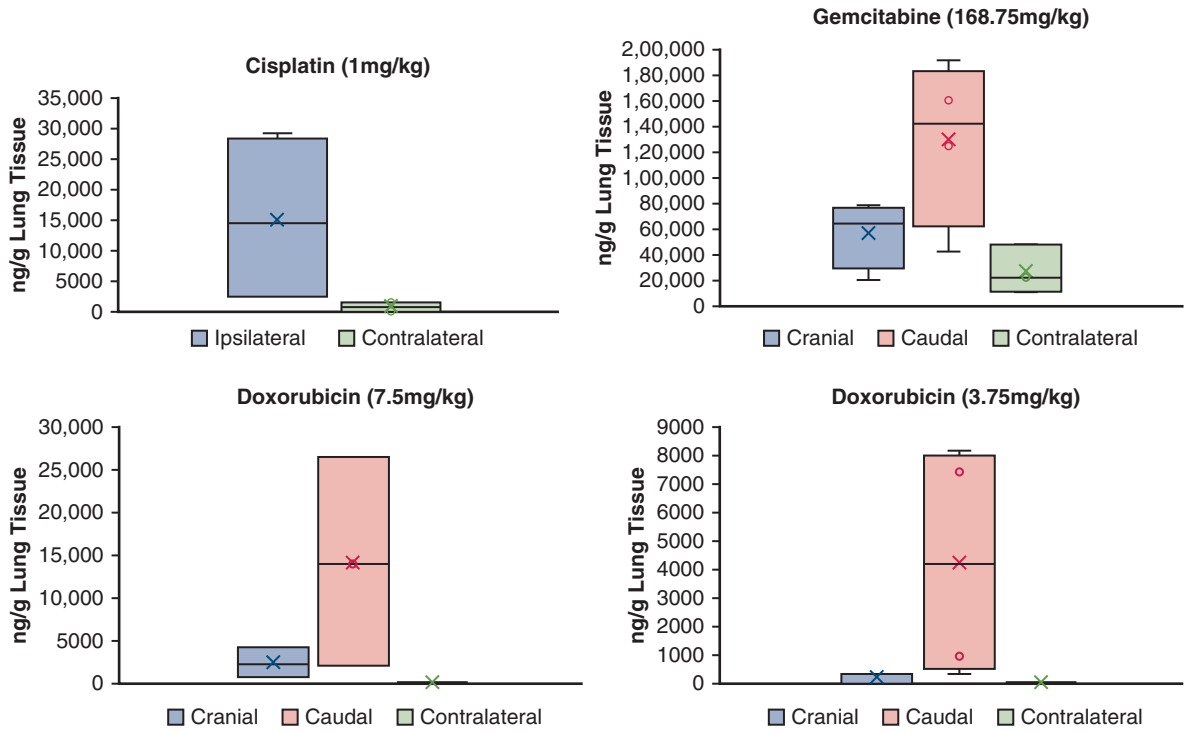

FIGURE 4. Pulmonary tissue drug concentrations. Box-and-whisker plots display lung tissue concentrations of cisplatin, gemcitabine, and doxorubicin following infusion. Concentrations are in nanograms of drug per gram of tissue.

and hemothorax. The other dogs had no gross pathologic aberrations, 3 with thoracic cavities almost free of adhesions. One animal had dense adhesions, especially between the lateral chest wall and the left lung. Otherwise, suffused and contralateral lungs appeared similar, apart from biopsy staple line locations.

Histologically, pleural fibrosis appeared in the left caudal and cranial lung lobes with alveolar walls thickened to 2-3 times normal thickness. Increased interstitial mononuclear cells and occasional perivascular infiltrates were noted. The treated lung showed scattered accumulations of macrophages with fine granular brown pigment that was likely hemosiderin. This suggests small multifocal previous hemorrhage. There were minimal lesions in the kidneys bilaterally with infrequent hyaline casts and small foci of interstitial mononuclear infiltrates. Results are summarized in Table 3.

Gemcitabine levels

Dogs displayed mean gemcitabine concentrations of 57,050 ng/g in the suffused cranial lobe and 129,400 ng/g in the caudal lobe (Figure 3). Contralateral lung samples had a mean of concentration of 27,150 ng/g. In addition to the time points depicted in Figure 3, 24-hour serum mean gemcitabine was 8.45 ng/mL. Suffusion PA levels showed a mean gemcitabine concentration of 959,200 ng/mL.

DISCUSSION

These experiments demonstrated relative safety for suffusion of cisplatin, doxorubicin, and gemcitabine in a preclinical model, warranting their use in human clinic trials. These preclinical studies alone do not display how suffusion decreases tumor burden due to the lack of thoracic pathology in the animals, although work by our group and others suggests that suffusion and similar treatment strategies invoke a local reaction in the treated areas and may decrease tumor recurrence.[18,23] While higher human doses may be possible, lung injury and fibrosis may limit doses beyond 25% full systemic for cisplatin and gemcitabine and 12.5% for doxorubicin. This still represents 5× and 2.5× local amplification, respectively, compared to intravenous delivery. To address the potential for human patients to have underlying pulmonary pathology or previous parenchymal damage from systemic chemotherapy, we opted to set a target value for dose escalation of 15% and started with 5% doses in our phase I clinical trial.[14,15] It is also worth mentioning that our maximal preclinical dosages were comparable to those reached by Cypel in their experiments.[23]

None of these experiments demonstrated systemic myelosuppression and electrolyte abnormalities, which are known toxicities of gemcitabine, cisplatin, and doxorubicin.[10] This is due in part to the relatively small doses, but our data demonstrate successful drug partitioning based on lung and serum levels. Interestingly, the caudal lung lobes uniformly had increased drug concentrations compared to the cranial lobes. This most likely was related to preferential flow, because the infusion catheter was directed caudally through the proximal PA insertion site. Retrograde flow into the cranial lobe was insufficient and possibly exacerbated by any lobar artery distortion from the proximal PA snare. In any case, tolerance of a higher relative distribution to one lobe may indicate a wider margin of safety for human use, where larger anatomy may allow more even distribution.

An additional notable finding was the undetectable level of doxorubicin, likely due to the active transport of the drug across cellular membranes.[24] This replicates findings of previous investigators using a regional canine delivery strategy.[25] Accordingly, active transport of doxorubicin may shorten the required suffusion times relative to cisplatin and gemcitabine or other passively transported drugs.

Although most animals tolerated the procedure well, there were some early deaths. A toxicity death in the cisplatin group was addressed by dosage deescalation, with subsequent groups experiencing less toxicity. A premature death due to doxorubicin toxicity also led to dosage deescalation, but this occurred at a lower relative dose (probably because of doxorubicin's vesicant properties). Finally, 1 gemcitabine animal died from a retraction-induced lung torsion that was missed because of suboptimal thoracotomy size and location that was too cranial. This did not warrant dosage modification and was prevented by optimizing incision site planning and postclosure surveillance.

Creech and colleagues[26] first described isolated lung perfusion in humans with unresectable carcinomas. Isolated canine lung perfusion was first described in 1960, and highlights of subsequent research have been reviewed previously.[27-29] The topic remains timely; thus far, the safety of isolated lung perfusion has been demonstrated in phase I[18,23,30,31] and phase II[32,33] clinical trials without operative mortality. Similar regional perfusion experiments have been conducted in pigs with oxaliplatin in which, as in our studies, 25% of systemic dosage was used.[34] Gemcitabine has been used in regional perfusion in rats in a study showing that pulmonary artery perfusion was safer than systemic administration.[35] Chemotherapeutic agents such as cisplatin, melphalan, doxorubicin, and TNF-α have been administered by methods ranging from total lung perfusion on cardiopulmonary bypass to isolated single-pass lung perfusion.[29,36] However, these modes of extracorporeal perfusion are highly invasive, risking postoperative pain, inflammatory responses to the bypass circuit, theoretical spread of malignancy, and incidental overdose by collateral leaks. Accordingly, our suffusion technique involves minimally invasive maneuvers that will be practical for frail

TABLE 3. Histologic results of suffusion experiments

Treatment and dosage	Animal	Alive at endpoint	Left (treated) lung fibrosis	Left (treated) lung vascular damage	Right (untreated) lung fibrosis	Right (untreated) lung vascular damage
Cisplatin 2 mg/kg	1	No	+	++	0	++
	2	Yes*	++++	++++	0	0
Cisplatin 1 mg/kg	3	Yes	++++	++	0	0
	4	Yes	++	+	0	0
Saline	5	Yes	0	0	0	0
	6	Yes	0	0	0	0
	7	Yes	0	0	0	0
Cisplatin 0.5 mg/kg	8	No	0	0	0	0
	9	Yes	++	+++	0	0
	10	Yes	++	+	0	+
	11	Yes	++++	+++	+	+
Cisplatin 0.25 mg/kg	12	Yes	0	0	0	+
	13	Yes	++	++	0	0
	14	Yes	++	+	0	0
	15	Yes	+++	+++	+	0
Cisplatin 0.125 mg/kg	16	Yes	0	+	0	0
	17	Yes	+++	++++	0	0
	18	Yes	++	+	0	0
	19	Yes	++	++	0	0
Doxorubicin 7.5 mg/kg	20	Yes	++	+	+	0
	21	No	++	++	++	+
	22	Yes	+++	++	+	++
Saline	23	Yes	0	0	0	0
Doxorubicin 3.75 mg/kg	24	Yes	+++	0	0	0
	25	Yes	++	+	0	0
	26	Yes	++++	+	0	0
	27	Yes	++	0	0	0
Gemcitabine 168.75 mg/kg	28	Yes	+	0	0	0
	29	No	0	++++	0	0
	30	Yes	+	0	0	0
	31	Yes	++	0	0	0
	32	Yes	++	0	0	0

0, none; +, trace; ++, mild; +++, moderate; ++++, severe. *Animal survived until the end of the observation period but was unwell.

patients who may have advanced disease, as shown in our preliminary work with cisplatin.[18]

Our group believes that multiple patient groups may benefit from suffusion treatment. Currently, we are targeting MRD in patients undergoing metastasectomy for sarcoma, colorectal carcinoma, and hepatocellular carcinoma,[15] but the long-term objective will be for any oncologic or nononcologic diagnosis for which a regional therapy might be more advantageous than systemic therapy in terms of local dosage amplification or systemic toxicity reduction. Next steps include increasing the armamentarium of drugs, particularly immune therapies. It is reasonable to expect that suffusion will have utility in treating pulmonary metastases, such as those from hepatocellular and colorectal carcinomas, and a clinical trial is ongoing.[15] Immunotherapy may reap an even bigger benefit than chemotherapeutics and lead to the abscopal effect.[37] The highly immunogenic environment of the lungs and increased contact time with the pulmonary vasculature may stimulate a stronger immune response than systemic

administration. This has impacts for non–small cell lung cancer as well as metastasis treatment. An additional advantage of intravascular delivery over bronchoscopic techniques is the ability to reach very distal aspects of the lungs and the opportunity for chemotherapy to reach and treat disease that might not yet have become apparent, such as developing lesions, small lesions, and micrometastatic disease. When the drugs are delivered through this method, the lymphatic system also benefits, leading us to postulate that it may help treat tumor cells in transit that otherwise may have an increased tumor burden. By occluding the venous drainage of the lungs, the chemotherapy can be isolated, and there is a decreased risk of washout or escape into the systemic circulation. Advanced bronchoscopic techniques allow for targeted treatment and can help with tumor resectability and treatment but do not offer these advantages. Additionally, tumors may obstruct the bronchus, making drug delivery impossible. Delivery by bronchoscopic means also may be hazardous to staff due to drug aerosolization, and some drugs are differentially more toxic to the respiratory epithelium.

Limitations of suffusion include physiologic or pathologic variations in pulmonary vasculature. Patients experiencing chronic hypoxia may be prone to increased collateralization of the pulmonary vasculature, which has the potential to lead to more systemic leakage. Even if this occurs, the proportion who would be at risk for systemic penetration would be much lower than that during typical exposure during standard of care infusion. Therefore, even in the case of increased collateralization, there should be fewer systemic effects and greater pharmacologic concentration.

An additional limitation of this work is the rigidity of the chemotherapy dwell times. These times were kept constant in an effort to standardize the experiments while trialing different chemotherapeutic agents. We also aimed to follow the protocol that was approved by our local regulatory committees for animal experimentation. Our doxorubicin experiments showed that adjusting dwell times may be beneficial, and this is a future direction for this work. We believe that much longer suffusion times are possible owing to the resiliency observed in the lungs and the lack of ischemic stress on these low metabolic need organs that remain ventilated. This would increase the level of delivery without additional drug dosing.

Although further research is needed, suffusion is a promising technique to amplify regional levels of lung chemotherapy while limiting systemic levels in the treatment of primary or metastatic pathology of the lungs. This has potential impacts as more agents are tested for both chemotherapy and immunotherapies, providing utility in malignant and benign pathology.

Webcast

You can watch a Webcast of this AATS meeting presentation by going to: https://www.aats.org/resources/preclinical-experience-with-ci-8800.

Conflict of Interest Statement

T.L.D. reported ownership of Suffusion Technologies. S.Y. reported clinical trial funding from Lumeda Inc. All other authors reported no conflicts of interest.

The *Journal* policy requires editors and reviewers to disclose conflicts of interest and to decline handling or reviewing manuscripts for which they may have a conflict of interest. The editors and reviewers of this article have no conflicts of interest.

References

1. Leiter A, Veluswamy RR, Wisnivesky JP. The global burden of lung cancer: current status and future trends. *Nat Rev Clin Oncol*. 2023;20:624-639.
2. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71:209-249.
3. Van Putte BP, Hendriks JM, Romijn S, Van Schil PE. Isolated lung perfusion for the treatment of pulmonary metastases current mini-review of work in progress. *Surg Oncol*. 2003;12:187-193.
4. Centers for Disease Control and Prevention. *US Cancer Statistics: highlights from 2017 incidence. USCS Data Brief*, 17. Centers for Disease Control and Prevention, US Department of Health and Human Services; 2020.
5. Milosevic M, Edwards J, Tsang D, et al. Pulmonary metastasectomy in colorectal cancer: updated analysis of 93 randomized patients—control survival is much better than previously assumed. *Colorectal Dis*. 2020;22:1314-1324.
6. Comacchio GM, Melan L, Zambello G, Mammana M, Rea F. Lung metastases from hepatocellular carcinoma: multidisciplinary approach—narrative review. *AME Surg J*. 2022;2:21-54.
7. Martin LW, D'Cunha J, Wang X, et al. Detection of occult micrometastases in patients with clinical stage I non–small-cell lung cancer: a prospective analysis of mature results of CALGB 9761 (Alliance). *J Clin Oncol*. 2016;34:1484-1491.
8. Chemotherapy in non–small-cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. Non–small-Cell Lung Cancer Collaborative Group. *BMJ*. 1995;311:899-909.
9. Grossi F, Gridelli C, Aita M, De Marinis F. Identifying an optimum treatment strategy for patients with advanced non–small-cell lung cancer. *Crit Rev Oncol Hematol*. 2008;67:16-26.
10. Sritharan S, Sivalingam N. A comprehensive review on time-tested anticancer drug doxorubicin. *Life Sci*. 2021;278:119527.
11. Hayashi H, Kurata T, Nakagawa K. Gemcitabine: efficacy in the treatment of advanced stage nonsquamous non–small-cell lung cancer. *Clin Med Insights Oncol*. 2011;5:177-184.
12. Stewart DJ, Molepo JM, Green RM, et al. Factors affecting platinum concentrations in human surgical tumour specimens after cisplatin. *Br J Cancer*. 1995;71:598-604.
13. Van Glabbeke M, van Oosterom AT, Oosterhuis JW, et al. Prognostic factors for the outcome of chemotherapy in advanced soft tissue sarcoma: an analysis of 2,185 patients treated with anthracycline-containing first-line regimens—a European Organization for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group Study. *J Clin Oncol*. 1999;17:150-157.

14. Cisplatin in treating patients with stage IIIB-IV non–small-cell lung cancer or lung metastasis; 2022. Accessed February 6, 2025. https://clinicaltrials.gov/study/NCT01014598
15. Pulmonary suffusion in controlling minimal residual disease in patients with sarcoma or colorectal metastases; 2024. Accessed February 6, 2025. https://clinicaltrials.gov/study/NCT03965234
16. Mallick R, Demmy T. Regional lung chemotherapy techniques. *Innovations (Phila)*. 2011;6:1-9.
17. Demmy TL. Thoracoscopic lung suffusion. *Thorac Surg Clin*. 2016;26:109-121.
18. Demmy TL, Tomaszewski G, Dy GK, et al. Thoracoscopic organ suffusion for regional lung chemotherapy (preliminary results). *Ann Thorac Surg*. 2009;88:385-390; discussion 390-391.
19. Committee for the Update of the Guide for the Care and Use of Laboratory Animals, National Research Council of the National Academies. *Guide for the Care and Use of Laboratory Animals. 8th ed.* The National Academies Press; 2011. 1-220.
20. Demmy TL, Wagner-Mann C, Allen A. Isolated lung chemotherapeutic infusions for treatment of pulmonary metastases: a pilot study. *J Biomed Sci*. 2002;9:334-338.
21. Song L, Prey JD, Xue J, et al. Pharmacokinetic measurements of IDN 5390 using electrospray ionization tandem mass spectrometry: structure characterization and quantification in dog plasma. *Rapid Commun Mass Spectrom*. 2005;19:3617-3625.
22. Woicke J, Al-Haddawi MM, Bienvenu JG, et al. International harmonization of nomenclature and diagnostic Criteria (INHAND): nonproliferative and proliferative lesions of the dog. *Toxicol Pathol*. 2021;49:5-109.
23. Cypel M. In vivo lung perfusion with oxaloplatin for patients with bilateral colorectal lung metastases: interim results of a clinical trial. Presented at International Thoracic Surgical Oncology Summit; September 28, 2024; New York, NY.
24. Dowd FJ. Pharmacokinetics: the absorption, distribution, and fate of drugs. In: Dowd FJ, Johnson BS, Mariotti AJ, eds. *Pharmacology and Therapeutics for Dentistry*. 7th ed. Mosby; 2017:15-43.
25. Minchin RF, Johnston MR, Schuller HM, Aiken MA, Boyd MR. Pulmonary toxicity of doxorubicin administered by in situ isolated lung perfusion in dogs. *Cancer*. 1988;61:1320-1325.
26. Creech O Jr, Krementz ET, Ryan RF, Winblad JN. Chemotherapy of cancer: regional perfusion utilizing an extracorporeal circuit. *Ann Surg*. 1958;148:616-632.
27. Pierpont H, Blades B. Lung perfusion with chemotherapeutic agents. *J Thorac Cardiovasc Surg*. 1960;39:159-165.
28. Grootenboers MJ, Heeren J, van Putte BP, et al. Isolated lung perfusion for pulmonary metastases, a review and work in progress. *Perfusion*. 2006;21:267-276.
29. Claes E, Wener R, Neyrinck AP, et al. Innovative invasive loco-regional techniques for the treatment of lung cancer. *Cancers (Basel)*. 2023;15:2244.
30. Burt ME, Liu D, Abolhoda A, et al. Isolated lung perfusion for patients with unresectable metastases from sarcoma: a phase I trial. *Ann Thorac Surg*. 2000;69:1542-1549.
31. Hendriks JM, Grootenboers MJ, Schramel FM, et al. Isolated lung perfusion with melphalan for resectable lung metastases: a phase I clinical trial. *Ann Thorac Surg*. 2004;78:1919-1926; discussion 1926-1927.
32. den Hengst WA, Hendriks JM, Balduyck B, et al. Phase II multicenter clinical trial of pulmonary metastasectomy and isolated lung perfusion with melphalan in patients with resectable lung metastases. *J Thorac Oncol*. 2014;9:1547-1553.
33. Beckers PAJ, Versteegh MIM, Van Brakel TJ, et al. Multicenter phase II clinical trial of isolated lung perfusion in patients with lung metastases. *Ann Thorac Surg*. 2019;108:167-174.
34. Ramadan K, Gomes B, Pipkin M, et al. A model to assess acute and delayed lung toxicity of oxaliplatin during in vivo lung perfusion. *J Thorac Cardiovasc Surg*. 2021;161:1626-1635.
35. Ward A, Prokrym K, Pass H. Isolated lung perfusion for pulmonary metastases. *Thorac Surg Clin*. 2016;26:55-67.
36. dos Santos PR, Iskender I, Machuca T, et al. Modified in vivo lung perfusion allows for prolonged perfusion without acute lung injury. *J Thorac Cardiovasc Surg*. 2014;147:774-782.
37. Brandi N, Renzulli M. The synergistic effect of interventional locoregional treatments and immunotherapy for the treatment of hepatocellular carcinoma. *Int J Mol Sci*. 2023;24:8598.

Key Words: suffusion, NSCLC, canine, minimally invasive surgery, chemotherapy, pulmonary metastasis

TABLE E1. Supplemental pharmacokinetic methods for doxorubicin and gemcitabine experiments

Doxorubicin	Gemcitabine
1. Lung tissue was homogenized in phosphate buffered saline to obtain 200 mg of tissue per 1 mL of homogenate.	1. Lung tissue was homogenized in 25% methanol with 25 μg/mL tetrahydrouridine to obtain 200 mg of tissue per 1 mL of homogenate.
2. Calibration and quality control samples were prepared in beagle serum.	2. Calibration and quality control samples were prepared in human plasma treated with tetrahydrouridine.
3. All samples were prepared using a liquid-liquid extraction procedure.	3. The calibration samples, quality control samples, plasma, and the tissue homogenate were prepared using a protein precipitation extraction procedure.
4. Following the addition of 1:1 chloroform:2-propanol to the samples, the tubes were capped, vortexed, and centrifuged.	4. Following the addition of methanol to the samples, the tubes were capped, vortexed, and centrifuged.
5. The top layer was aspirated off and the remaining fraction was evaporated under a stream of nitrogen gas.	5. Supernatant was transferred and evaporated under a stream of nitrogen gas. The residue was reconstituted and injected on the liquid chromatography tandem mass spectroscopy analytical system.
6. The residue was reconstituted and injected into the ultra-performance liquid chromatography analytical system and quantitated over a calibration range of 40.0-4800 ng/mL.	6. The analytes were quantitated over the following calibration ranges: 1.00-2000 ng/mL for gemcitabine and 5.00-10,000 ng/mL for dFdU.
7. Calibration curves were generated using analyte/internal standard area response ratios vs nominal concentrations (ng/mL) and weighted linear regressions with weighting factor 1/concentration 2.	7. Calibration curves were generated using analyte/internal standard area response ratios vs nominal concentrations (ng/mL) and weighted linear regressions with weighting factor 1/concentration 2.
8. Calibrator and quality control acceptance criteria required all acceptable concentrations to have accuracy $\leq$15% from the nominal concentrations except at the lower limit of quantitation, which was allowed $\leq$20% deviation.	8. Gemcitabine used gemcitabine-13C,15N2 as the stable labeled internal standard and dFdU used 2',2'-difluoro-2'-deoxyuridine-13C,15N2. Calibrator and quality control acceptance criteria required all acceptable concentrations to have accuracy $\leq$15% from the nominal concentrations except at the lower limit of quantitation, which was allowed $\leq$20% deviation.
9. Analyte concentrations were represented as nanogram of analyte per gram of lung tissue.	9. Analyte concentrations were represented as nanogram of analyte per gram of lung tissue.

dFdU, 2',2'-difluoro-2'-deoxyuridine.