A model to assess acute and delayed lung toxicity of oxaliplatin during in vivo lung perfusion

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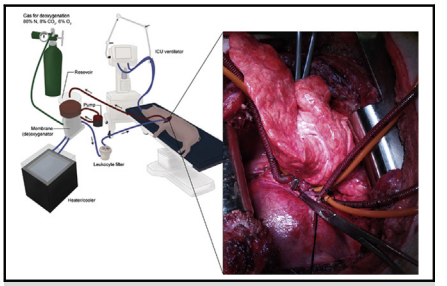
ABSTRACT

Objectives: To determine the dose-limiting toxicity of oxaliplatin chemotherapy delivered by in vivo lung perfusion (IVLP). To allow assessment of subacute toxicities, we aimed to develop a 72-hour porcine IVLP survival model.

Methods: In total, 12 Yorkshire male pigs were used. Left lung IVLP was performed for 3 hours. At 72 hours postoperatively, computed tomography imaging of the lungs was performed before the pigs were killed. Lung physiology, airway dynamics, gross appearance, and histology were assessed before and during IVLP, at reperfusion, and when the pigs were euthanized. An accelerated titration dose-escalation study design was employed whereby oxaliplatin doses were sequentially doubled provided no clinically significant toxicity was observed, defined as an arterial partial pressure of oxygen to fraction of inspired oxygen ratio <300 mm Hg or severe acute lung injury on biopsy.

Results: After an initial training phase, no mortality or adverse events related to the procedure were observed. There was no lung injury observed at the time of IVLP for any case. At sacrifice, clinically significant lung injury was observed at 80 mg/L oxaliplatin, with an arterial partial pressure of oxygen to fraction of inspired oxygen ratio of 112 mm Hg. Mild and subclinical lung injury was observed at 40 mg/L, with this dose being repeated to confirm safety.

Conclusions: A stable and reproducible porcine 3-day IVLP survival model was established that will allow toxicity assessment of agents delivered by IVLP. Oxaliplatin delivered by IVLP showed delayed-onset toxicity that was not apparent at the time of reperfusion, with a maximal-tolerated dose of 40 mg/L. This information will inform initiation of a clinical trial examining IVLP delivery of oxaliplatin at our institution. (J Thorac Cardiovasc Surg 2021;161:1626-35)



Overview of in vivo lung perfusion with schematic (*left*) and cannula placement (*right*).

CENTRAL MESSAGE

A 72-hour porcine isolated in vivo lung perfusion model to deliver oxaliplatin was developed for determination of subacute dose-limiting toxicity and may be used for safety assessment of other agents.

PERSPECTIVE

A 72-hour porcine in vivo lung perfusion model was developed to determine the dose-limiting toxicity of oxaliplatin chemotherapy delivered by isolated lung perfusion. Oxaliplatin delivered by IVLP showed a delayed-onset homogenous pattern of lung toxicity that was not apparent at the time of the initial procedure. This study will inform initiation of a clinical trial.

See Commentaries on pages 1636 and 1637.

The lung is among the most common sites of metastatic spread, with pulmonary metastases occurring in approximately 15% to 20% of patients with colorectal carcinoma (CRC).[1] Standard-of-care treatment regimens include complete metastasectomy, when possible, and adjuvant systemic chemotherapy. Of these patients, only a select proportion (5%-15%) have historically undergone surgical resection, yet even so, 5-year disease-free survival remains relatively poor, between 37% and 50%.[1-4] Among the predictors of improved survival are complete resection,

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Abbreviations and Acronyms

CRC = colorectal carcinoma
CT = computed tomography
EVLP = ex vivo lung perfusion
FiO_2 = fraction of inspired oxygen
IV = intravenously
IVLP = in vivo lung perfusion
P/F = arterial partial pressure of oxygen to fraction of inspired oxygen
SPME = solid-phase microextraction

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the number of pulmonary lesions, and the disease-free interval.[4]

Unfortunately, many patients will develop recurrent disease in the lungs even with complete resection, with pulmonary recurrence reported as at least 39%, likely as a result of undetected micrometastatic disease present at the time of initial operation.[4] Since standard chemotherapy regimens, such as FOLFOX (leucovorin, 5-fluorouracil, oxaliplatin), carry significant dose-limiting systemic toxicities, doses used may be inadequate to eradicate micrometastatic disease. Several factors may contribute to this phenomenon, including ineffectiveness of this regimen toward tumor cells, development of drug resistance, and inability to achieve effective drug concentrations within the lung and tumor. Since chemotherapy alone would be unlikely to eliminate or control gross disease, a strategy that can specifically target residual micrometastatic disease in the lung in isolation after pulmonary metastasectomy would greatly decrease the morbidity of multiple surgeries and improve survival in this patient population.

Accordingly, our group has previously developed a specific technique of isolated In vivo lung perfusion (IVLP) to provide a safe platform for selective therapeutic intervention.[5,6] This technique involves cannulation of the unilateral pulmonary artery and pulmonary veins, and central clamping proximal, to allow isolation of pulmonary vasculature. The cannulas are connected to an external circuit, where preservation solution is circulated to maintain the lung without causing injury. IVLP uses the perfusion principles of the ex vivo lung perfusion (EVLP) technique developed for assessment and treatment of injured donor lungs for transplantation.[7] Based on these principles, IVLP uses the identical circuit as EVLP as well as the same guidelines for perfusion pressures and mechanical ventilation. Thus, we developed an optimized IVLP protocol in which 4 hours of isolated lung perfusion was performed without any significant acute deleterious effects, during a 4-hour reperfusion phase.[5]

We accordingly used the IVLP platform to administer sarcoma-based chemotherapy, specifically doxorubicin, and demonstrated that high doses can be safely given without lung or systemic toxicity.[6] Currently, we are conducting a phase 1-2 clinical trial at our institution of IVLP delivery of doxorubicin with concurrent metastasectomy for patients with fully resectable disease, with 3 patients having undergone surgery to date.

The primary objective of this study was to determine the dose-limiting toxicity of oxaliplatin chemotherapy delivered by IVLP. Given early observations with IVLP clinically to deliver high-dose chemotherapy, we have observed that the full degree of lung toxicity from chemotherapy can take up to 72 hours to fully develop (M. Cypel. S. Keshavjee, T. Waddell, M. Pipkin, unpublished data, 2018-2020). As a result, we aimed to assess the safety of oxaliplatin using a dose-escalation design in a preclinical porcine IVLP 3-day survival study.

METHODS

Animals

Yorkshire male pigs weighing 35 to 40 kg were used. All animals received humane care in compliance with Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care of Laboratory Animals. The study protocol was approved by the Animal Care Research Committee at the Toronto General Hospital Research Institute.

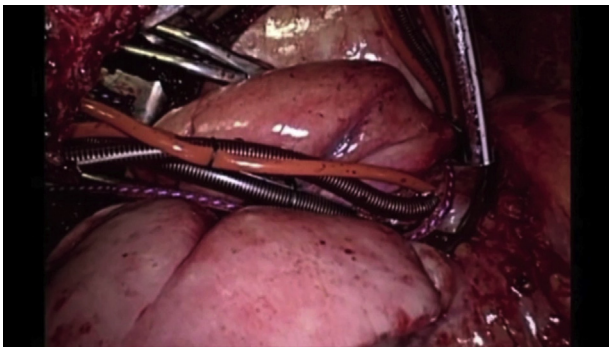
Experimental Strategy

Pigs underwent a 3-hour left lung IVLP procedure and were subsequently recovered and treated for 72 hours. Thereafter, pigs underwent a final procedure where endpoint assessments were made, before being killed by exsanguination under anesthesia.

The study used an accelerated dose-escalation study design, whereby the dose of chemotherapy was doubled with each sequential case, if no drug-related toxicity was observed during the previous case.[8] Toxicity was defined as clinically significant impairment of lung function with an arterial partial pressure of oxygen to fraction of inspired oxygen (FiO_2) (ie, P/F) ratio <300 mm Hg and/or signs of severe acute lung injury on histology. When a toxic dose was identified, the previously tolerated dose was repeated to confirm safety, and thus identify the maximal tolerated dose.

Oxaliplatin (Pfizer, Kirkland, Canada) was administered to the perfusion circuit reservoir directly as a bolus after full perfusion flow was established. The chemotherapy dose was calculated based on achieving a target concentration in the perfusion circuit. These target concentrations were based on standard doses of oxaliplatin used in clinical regimens, specifically 85 to 130 mg/m^2. The total drug amount for 85 mg/m^2, if given to the perfusion circuit, would give a concentration of approximately 160 mg/L, assuming an average size adult. Accordingly, this dose was planned as the maximum target dose. The starting dose was planned as 10 mg/L, sequentially doubling to reach the maximum dose of 160 mg/L as tolerated.

A detailed description of the IVLP procedure, perfusion circuit, priming solution, and protective perfusion/ventilation strategy has been previously



VIDEO 1. Abbreviated demonstration of a human in vivo lung perfusion procedure demonstrating the key steps of the procedure, as well as the relevant anatomy and technique. Video available at: https://www.jtcvs.org/article/S0022-5223(20)30635-8/fulltext.

described (Video 1).[5] To summarize, after induction of general anesthesia, ventilation was maintained at pressure control of 15 cm H_2O, positive end-expiratory pressure of 5 cm H_2O, FiO_2 of 50%, with a respiratory rate of 15 to 20 breaths/min, titrating to an end-tidal CO_2 below 60 mm Hg. After left thoracotomy, the left pulmonary artery, left pulmonary veins, and left atrial cuff were completely dissected. The left main bronchus was also encircled and snared to limit bronchial circulation communication into the IVLP circuit. After administration of 5000 IU of heparin, the left pulmonary artery and left atrial cuff were subsequently clamped, and the left pulmonary artery and left upper and lower pulmonary veins were cannulated with right-angle cannulas. The cannulas were connected to the IVLP circuit and the perfusion initiated at 37°C. Pulmonary artery and left atrial pressures were monitored by pressure lines inserted within each cannula, titrating to a left atrial pressure of 4 to 6 mm Hg. The perfusion circuit, its components, and position are demonstrated in Figure 1. After 3 hours of IVLP, an anterograde washout was performed with 1 liter of low-potassium dextran solution at 30 cm H_2O. The cannulas were removed, and the vessels were repaired to allow reperfusion. After chest tube insertion, the thoracotomy was closed, and the pig recovered. In the postoperative period, the pig received ceftriaxone 1 g intravenously (IV) every 12 hours, famotidine 40 mg IV every 12 hours, ciprofloxacin 400 mg IV every 12 hours, and enoxaparin 30 mg subcutaneously, daily. Slow-release buprenorphine was given intraoperatively with breakthrough doses given postoperatively as needed.

Physiologic Assessment

Lung function was assessed at baseline, hourly during IVLP, at reperfusion, and at 72 hours. These assessments were performed on pressure control ventilation as described previously, with the FiO_2 increased to 100% starting 5 minutes before measurements only during the assessment. Final assessments at 72 hours were done with the right lung hilum clamped to determine isolated left lung function. Gas exchange was evaluated through arterial blood gas. Airway dynamics were assessed via measurement of dynamic and static compliance. During IVLP, pulmonary vascular resistance of the left lung was measured hourly indirectly by measurement of pulmonary artery and left atrial pressures.

Histologic Assessment

Lung tissue biopsies were collected from the periphery of the upper lobe at baseline and after IVLP. At 72 hours, lung biopsies were taken from both upper and lower lobes. Tissue samples were fixed in 10% phosphate-buffered formalin, embedded in paraffin, and sectioned. Resulting slides were stained in hematoxylin and eosin and assessed under light microscopy using a pathologic acute lung injury assessment system as previously described.[9]

Oxaliplatin Measurement

Oxaliplatin levels were assessed using solid-phase microextraction (SPME), a novel minimally invasive chemical biopsy approach.[10,11] The technique involves insertion of a microprobe the size of an acupuncture needle (0.2-mm diameter) into tissue to a depth of its entire 8mm coating, followed by a 20-minute extraction period for the compound of interest, in this case oxaliplatin (Figure 2, *A*). The fibers were then rinsed in water manually for 5 seconds, wiped with a Kimwipe, and immediately snap frozen in dry ice. Subsequent recovery of analytes was performed by desorption in an organic solvent, followed by analysis using high-performance liquid chromatography coupled to tandem mass spectrometry.

Oxaliplatin levels were measured in the perfusate, plasma, and treated lung tissue at baseline, hourly during IVLP, and at reperfusion. For tissue measurements, 2 SPME fibers were inserted in 2 different regions of the lung at each time point, one in the upper lobe and one in the lingula (Figure 2, *B*). For perfusate and blood measurements, samples underwent

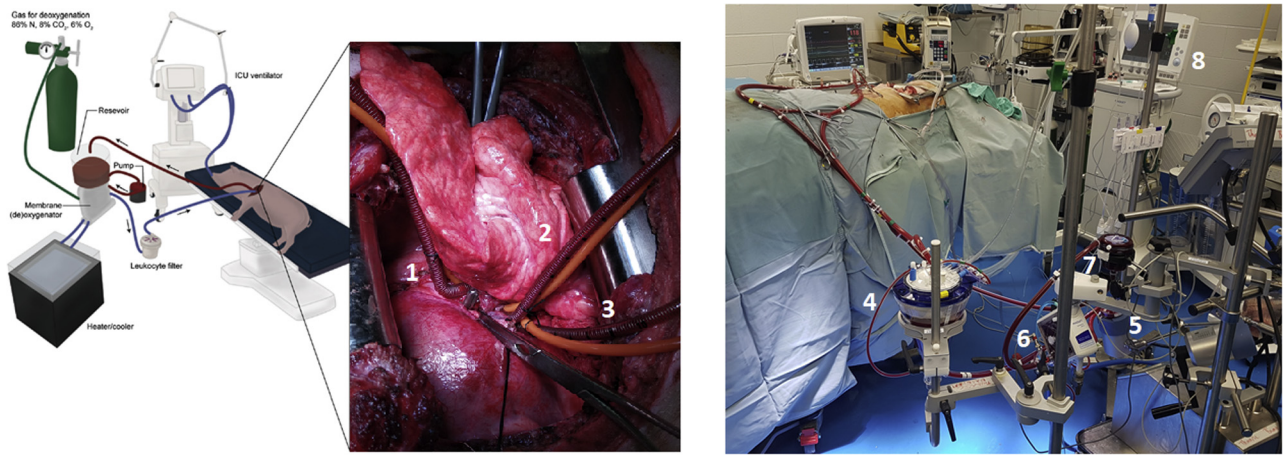


FIGURE 1. IVLP system. Inflow is achieved through the left pulmonary artery cannula (1) and outflow via the upper (2) and lower left pulmonary veins (3). Perfusate returns to the hard-shell reservoir (4), where it is directed by a centrifugal pump (5) through the membrane gas exchanger (6) to deoxygenate the perfusate and provide CO_2 for inflow. Perfusate passes through the leukocyte filter before returning to the left lung. A standard intensive care ventilator is used for both lungs. *ICU*, Intensive care unit.

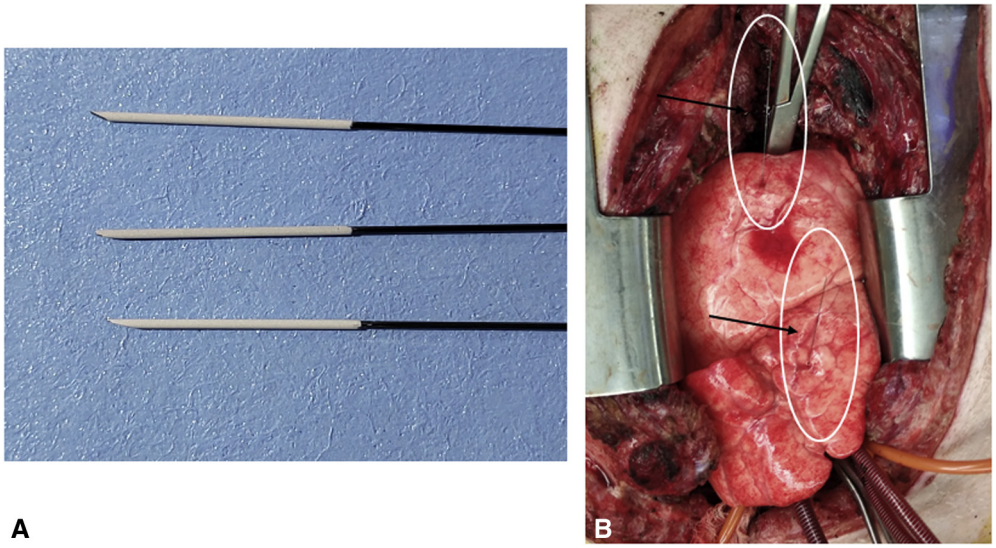


FIGURE 2. A, SPME microprobe. The fiber is 0.2-mm thick with an 8-mm long coated section for analyte extraction. B, SPME microprobe (*arrows*) inserted in lung tissue during in vivo lung perfusion for extraction of oxaliplatin concentration.

centrifugation for 2 minutes at 15,000*g* to separate any cellular components, before the insertion of SPME fibers in the resultant supernatant for analyte extraction.

Statistical Analysis

Results were analyzed using GraphPad Prism 7 (GraphPad Software, Inc, La Jolla, Calif).

RESULTS

Development of a Porcine IVLP 72-Hour Survival Model

In total, 12 pigs were used. There was an initial learning curve of 7 cases, during which 2 cases reached the 72-hour endpoint. Reasons for early termination included inability to wean from anesthesia due to hemodynamic compromise and lung injury caused by inadequate venous drainage during IVLP secondary to improper cannulation technique and placement. After the training phase, improved perioperative care, improved cannulation technique, and shorter operative times contributed to stability of the model with no further adverse events related to the surgical procedure.

Lung Function During IVLP

Lung function remained stable in the treated left lung in all cases (n = 7) for the duration of the 3-hour IVLP. Gas exchange function was preserved with a P/F ratio greater than 450 mm Hg at the end of IVLP in all cases (Figure 3, *A*). Pulmonary vascular resistance and compliance of the lung showed no significant decrease from baseline in all cases (Figure 3, *B-D*).

Lung Physiologic Assessment After IVLP

Systemic gas exchange function was stable throughout the procedure, as evidenced by a systemic P/F ratio greater than 350 mm Hg 1 hour after reperfusion in all cases (Figure 4, *A*). At 72 hours with the right lung hilum clamped, the P/F ratio was preserved or returned to baseline function in cases with doses up to 40 mg/L. However, the case at 80 mg/L showed significant impairment with a P/F ratio of 112 mm Hg. This case was characterized by a homogenous pattern of delayed lung injury affecting the entire lung, observed at 72 hours, that was not present at the time of reperfusion. Specifically, there was impairment of the tidal volume as shown by a decrease in dynamic and static compliance (Figure 4, *B* and *C*). The lung also showed gross changes including contraction and discoloration throughout both lobes as compared with cases at lower oxaliplatin doses (Figure 5, *A*). Computed tomography (CT) imaging demonstrated a global pattern of consolidation, especially in the lower lobe, that was not seen in other doses (Figure 5, *B*).

Histologic Characteristics

Histology at reperfusion showed only mild signs of acute lung injury including mild airspace hemorrhage and white blood cell infiltration (Figure 6). At 72 hours, doses up to 40 mg/L showed a slight progression of lung injury as evidenced by focal fibrin deposits and white blood cell infiltration. However, the 80 mg/L case showed significant progression at 72 hours with substantial fibrin deposits, alveolar hemorrhage, and moderate white blood cell infiltration (Figure 6).

Oxaliplatin Levels

The concentration of oxaliplatin in perfusate during IVLP peaked at 1 hour at the higher doses, showing a subsequent decline over time (Figure 7, *A*). Oxaliplatin concentration in tissue peaked at 2 hours of IVLP in most cases and

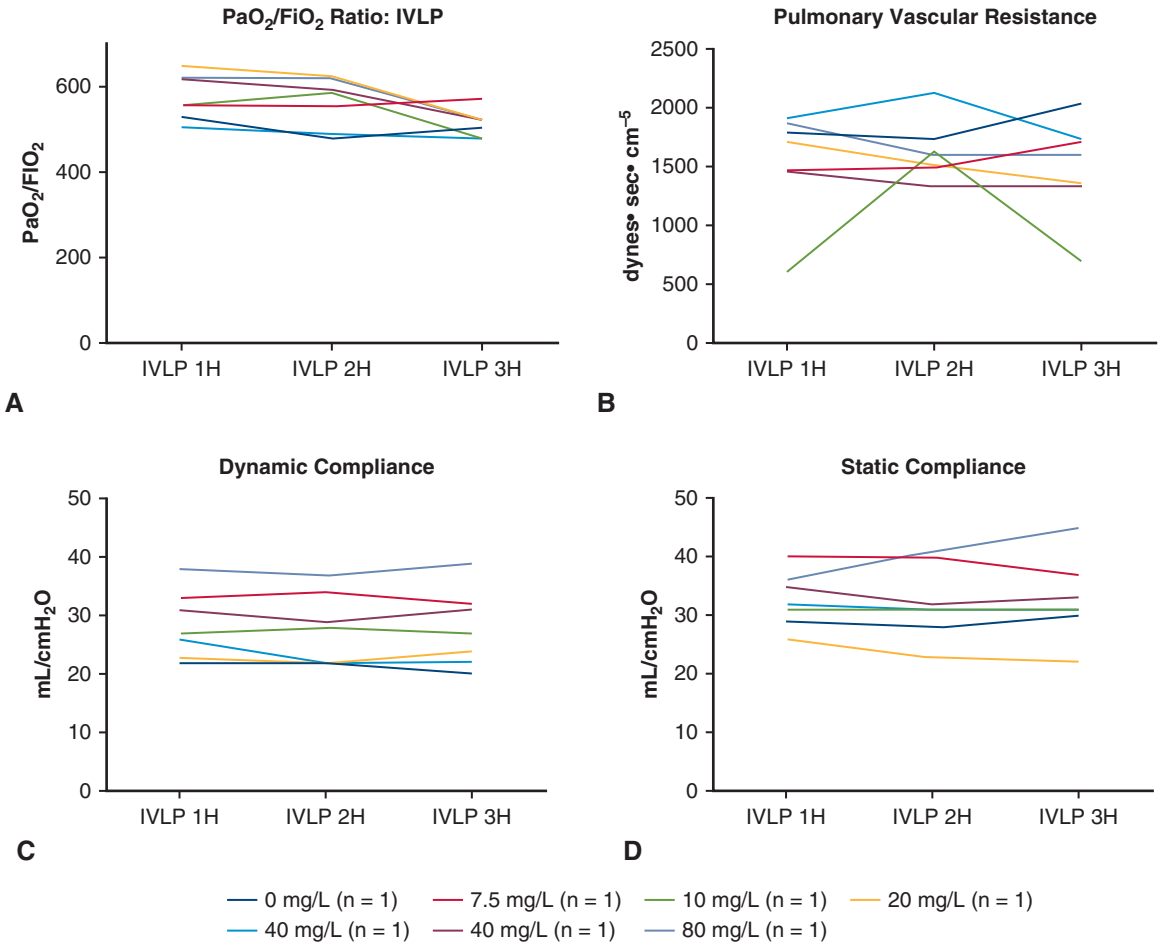


FIGURE 3. Lung functional assessment during IVLP. The treated left lung showed stability in the ratio of PaO_2/FiO_2 (A), pulmonary vascular resistance (B), dynamic compliance (C), and static compliance (D) throughout perfusion in all study doses. Lung function was stable during IVLP for all doses examined. *PaO_2/FiO_2*, Arterial partial pressure of oxygen to fraction of inspired oxygen; *IVLP*, in vivo lung perfusion.

showed a rapid decline at reperfusion (Figure 7, *B*). For the 40 mg/L cases, peak concentrations were 11.9 μg/g and 11.2 μg/g and areas under the curve were 24.5 μg/g and 26.2 μg/g. For the 80 mg/L case, the peak concentration was 22.6 μg/g and the area under the curve was 55.3 μg/g. In both perfusate and tissue, oxaliplatin concentrations showed a dose-dependent relationship. Oxaliplatin was not detected in plasma samples throughout IVLP and at reperfusion, demonstrating effective isolation from the systemic circulation.

DISCUSSION

This study demonstrates the safety of using IVLP, a method of isolated lung perfusion, to deliver high dose oxaliplatin to the lung while avoiding systemic toxicity. Through the establishment of a 72-hour porcine IVLP survival model, it was possible to assess subacute lung toxicity from the chemotherapy. Accordingly, the dose-limiting pulmonary toxicity of oxaliplatin was demonstrated at 80 mg/L, with the previous dose of 40 mg/L being repeated to confirm its safety (Figure 8). While 40 mg/L is approximately 25% of the typical systemic dose, since the pulmonary blood volume is about 5% of total blood volume, this equates to an approximate 5-fold increase in exposure to the lung given the volume of distribution.

Of note, the lung injury observed in this study was not apparent at the reperfusion phase, with preservation of lung function during the IVLP procedure apparent at all doses. The subsequent development of a significant lung injury pattern at 72 hours in the 80 mg/L case demonstrates a delayed-onset chemotherapy-induced lung toxicity. This was evidenced by significant impairment in P/F ratio, decrease in lung compliance, signs of significant histologic lung injury, global consolidation on CT imaging, and contraction and discoloration on gross examination. Cases at 40 mg/L showed a subclinical lung injury characterized by minor histologic and gross changes, and limited consolidation on CT, without impact on gas exchange. Findings of a delayed injury are consistent with observations from our clinical trial involving the use of IVLP to deliver

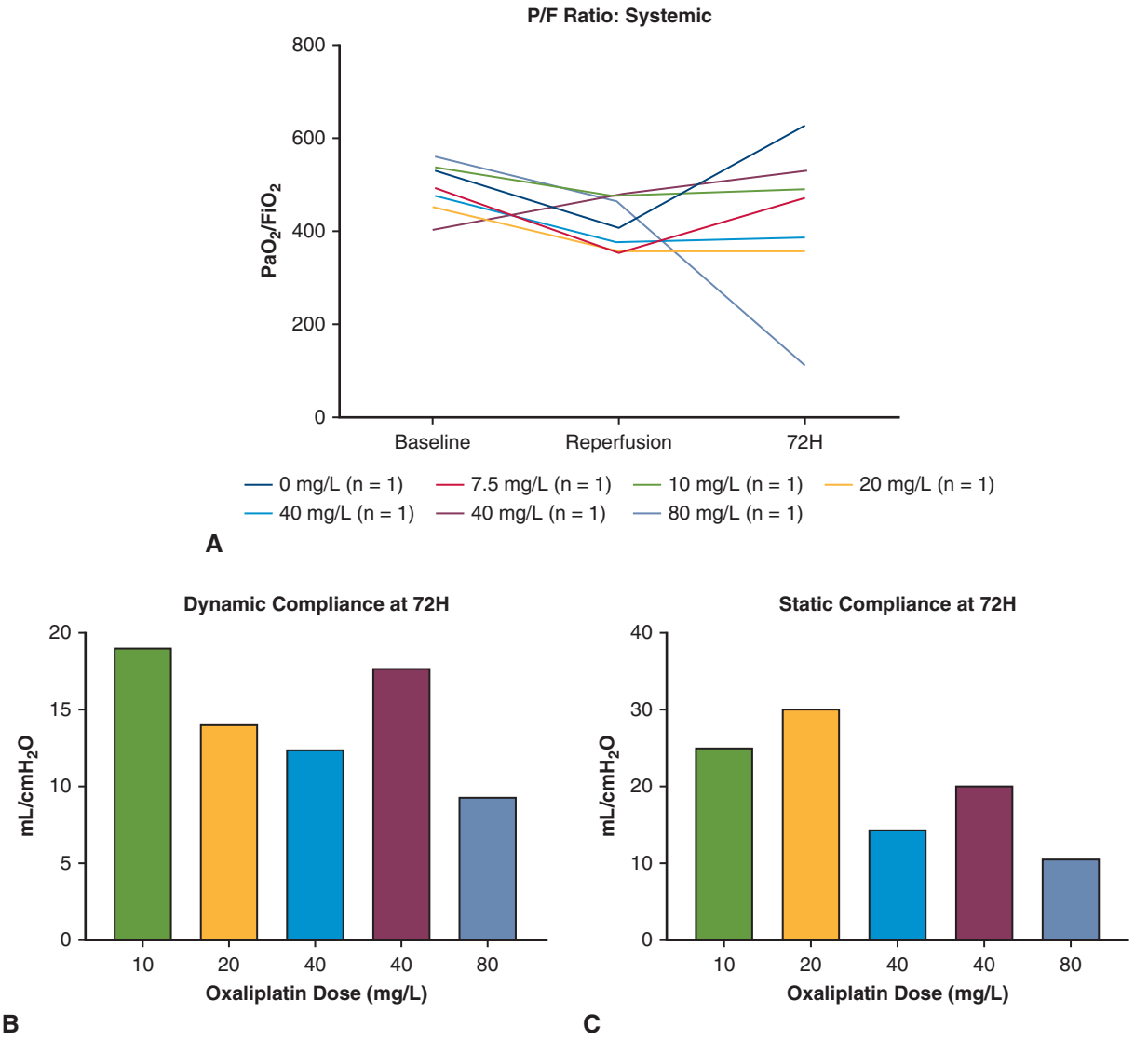


FIGURE 4. Lung physiologic assessment over the 72-hour experimental phase. Doses up to 40 mg/L showed preserved lung function at 72 hours including stability of the P/F ratio (A), and dynamic (B), and static compliance (n = 1 per *bar*) (C). For 80 mg/L, there was impaired lung function, including a P/F ratio of 112 and dynamic and static compliance of 10 mL/cmH_2O. *PaO_2/FiO_2*, Arterial partial pressure of oxygen to fraction of inspired oxygen.

doxorubicin in patients with sarcoma lung metastases, where mild subacute lung injury has been noted at 48 to 72 hours that was not initially present. Therefore, this highlights the importance of observing over a 72-hour survival period and thus the necessity of developing an IVLP survival model that covers this time frame.

In this study, we successfully established a 72-hour IVLP porcine survival model. This greatly differs from our previous publications using sarcoma-based chemotherapy, in which only a few hours' observation period was employed. Many of the challenges encountered during the training phase involved optimizing perioperative care. However, maintaining a stable perfusion by adhering to lung perfusion principles is essential, since small unrecognized errors can result in significant lung injury. The major pitfall, differing from EVLP, involves ensuring adequate outflow from upper and lower pulmonary veins as small changes in cannula position and anatomical variants can lead to outflow obstruction. The development of a stable porcine IVLP survival model will allow future safety assessment of acute and intermediate lung toxicity of other therapies that may be amenable to IVLP delivery.

The known pharmacokinetics of oxaliplatin during intravenous administration are that within 1 hour, the vast majority is rapidly bound to plasma proteins including albumin or bound within erythrocytes, whereas as much as 50% of total platinum is excreted in urine.[12,13] In the context of IVLP, the absence of urinary excretion and erythrocytes will affect these properties. Our findings showed that oxaliplatin concentrations followed a dose-dependent relationship in both

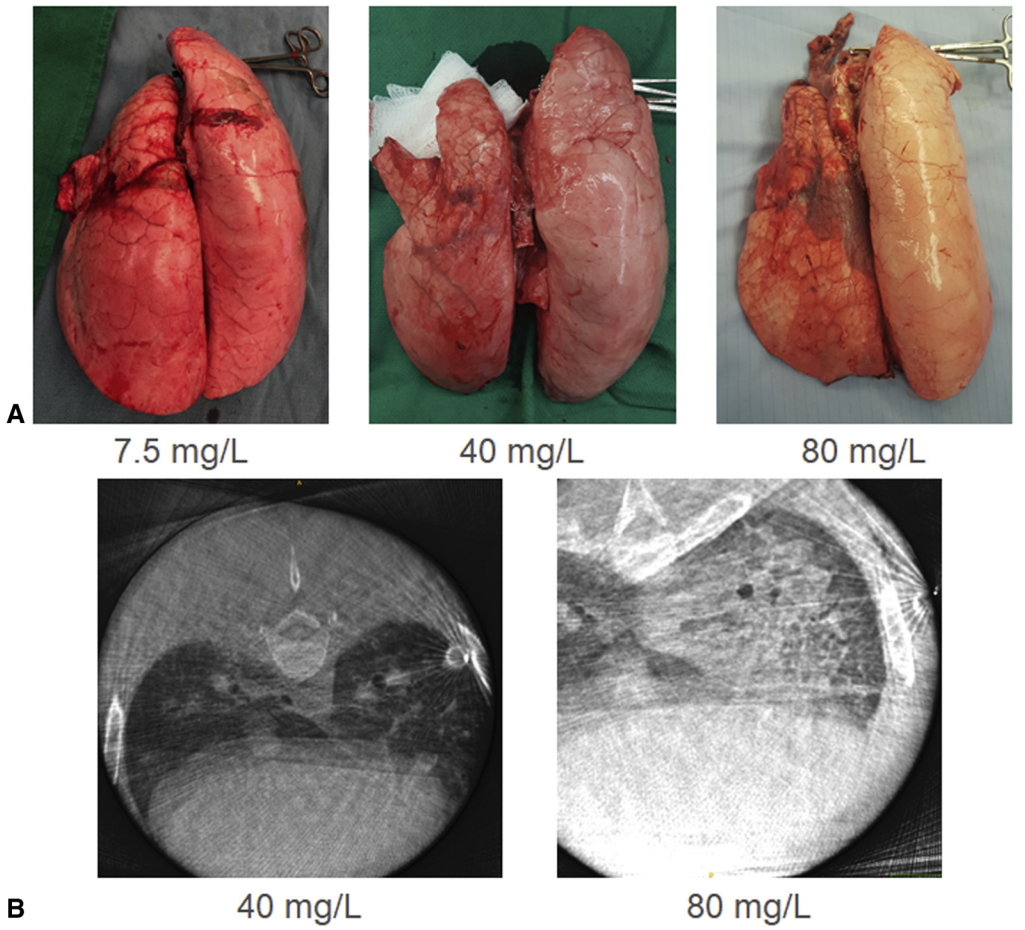


FIGURE 5. A, Gross appearance of the lung showed contraction and discoloration of the treated lung with a dose-dependent relationship. B, Computed tomography imaging showed significant diffuse consolidation at 80 mg/L, that was not seen at lower doses.

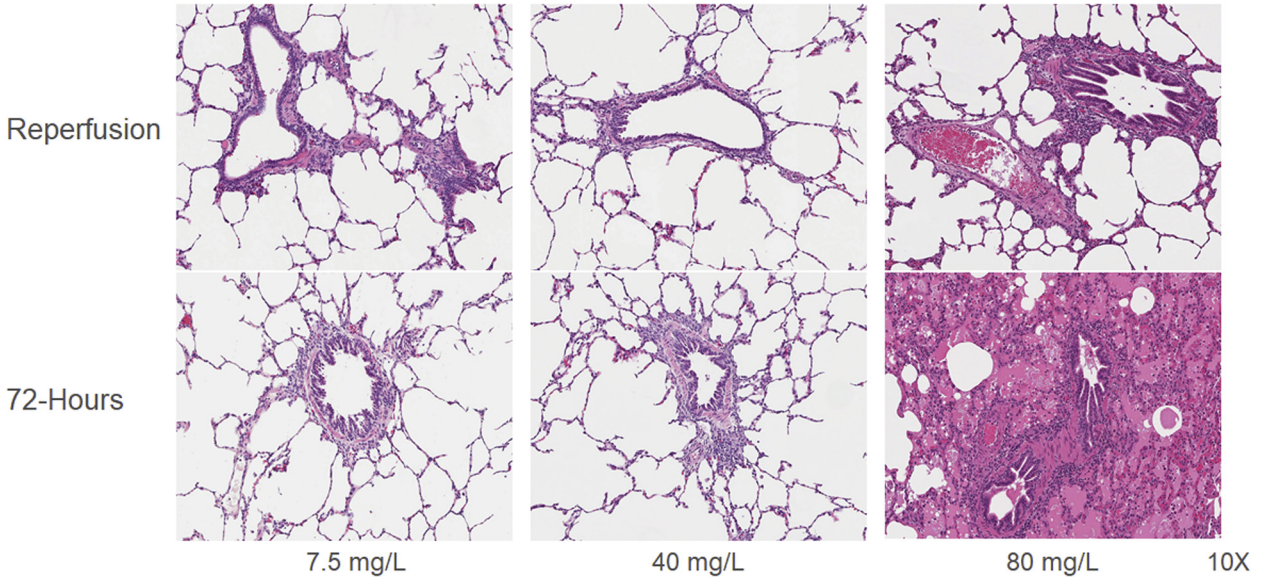


FIGURE 6. Histology showed mild alveolar hemorrhage and white blood cell infiltration at reperfusion. This mild injury pattern was stable at 72 hours at oxaliplatin doses up to 40 mg/L. However, at 80 mg/L, progression to a more severe injury pattern was apparent, characterized by significant fibrin deposition and moderate white blood cell infiltration.

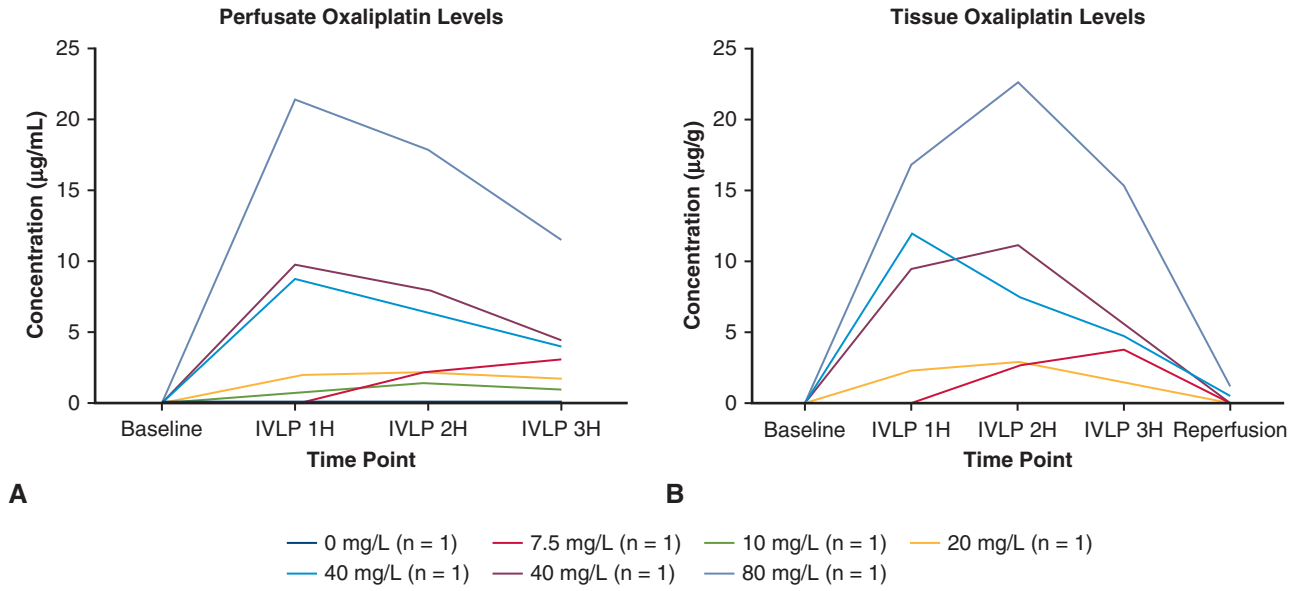


FIGURE 7. Oxaliplatin levels during IVLP. A, Perfusate concentrations of oxaliplatin showed a dose-dependent increase and peaked at 1 hour of IVLP. B, Tissue oxaliplatin concentrations also showed a dose-dependent relationship but peaked at 2 hours of IVLP, thereafter rapidly declining and approaching zero at reperfusion. *IVLP*, In vivo lung perfusion.

perfusate and tissue. In both compartments, oxaliplatin concentrations peaked quickly and remained significant during the entire perfusion, with tissue levels then approaching zero at reperfusion. Tissue oxaliplatin levels up to 12 μg/g were observed to be safe as seen in the cases at 40 mg/L. Although tissue levels may vary slightly between dependent and nondependent areas of the lung, these differences are comparable with typical perfusion variations in the lung during normal physiologic circumstances. Peak perfusate concentrations measured were approximately one quarter of those administered at 1 hour of IVLP, for example, 21.5 mg/L in the 80 mg/L case. Although diffusion of

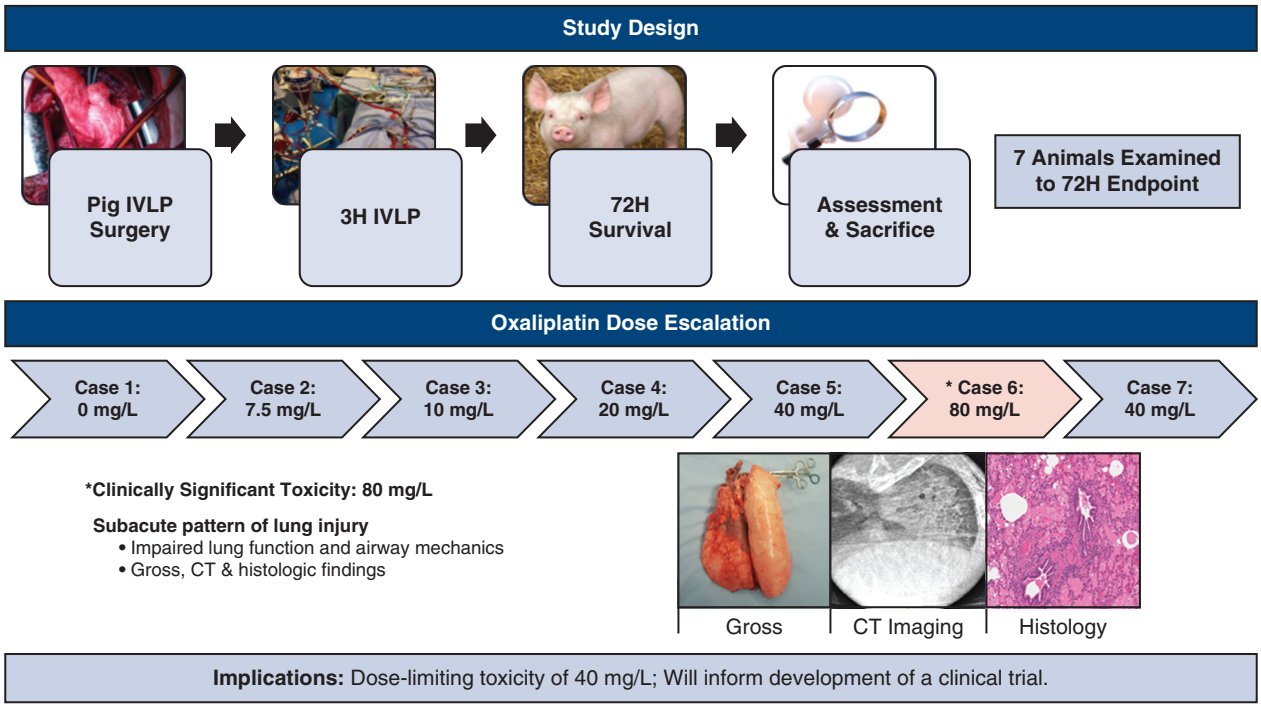


FIGURE 8. *Top*, Overview of the IVLP study design and experimental timeline. *Bottom*, Cases performed with depiction of the dose of oxaliplatin administered in each case. Case 6 with 80 mg/L oxaliplatin demonstrated a subacute pattern of lung injury. A dose-limiting toxicity of oxaliplatin delivered by IVLP of 40 mg/L was identified which will inform development of a clinical trial. *IVLP*, In vivo lung perfusion; *CT*, computed tomography.

oxaliplatin into the lung contributes to this observation, these findings are also consistent with the knowledge that oxaliplatin undergoes rapid nonenzymatic biotransformation in vivo, and thus will be cleared quickly even in an isolated IVLP platform.[14] Oxaliplatin was not detected in plasma samples throughout the IVLP procedure, thus confirming the effective separation of the IVLP and systemic circulations.

Oxaliplatin levels were measured by SPME. This exciting and novel chemical biopsy technique uses a microprobe inserted in tissue to extract the analyte of interest. Since it does not necessitate removing tissue samples, it allows for frequent repeated measurements that would not be feasible by conventional biopsy. Moreover, SPME has the potential to be directly coupled to a mass spectrometer, thereby allowing real-time results to be obtained within seconds.[15,16] This would allow titration of chemotherapy doses to a target tissue concentration, as well as gauging the evenness of perfusion and drug distribution in the lung.

Most studies examining isolated lung perfusion techniques have focused on treating sarcoma lung metastases. This is an attractive disease entity to target since sarcoma commonly presents with isolated lung metastases, occurs in a relatively young population, and few effective treatment options are available.[6,17-20] Despite this emphasis, CRC is also an attractive target. Treatment of isolated pulmonary metastases in patients with CRC has become a recognized practice with evidence of survival benefit.[2-4] Furthermore, CRC is highly prevalent and thus the subpopulation that may benefit from isolated lung perfusion is significant. Previous work has examined other chemotherapeutic options for CRC. Most notably, a multicenter phase II clinical trial has recently been reported examining the use of isolated lung perfusion to deliver melphalan chemotherapy during 30 minutes with concurrent metastasectomy for treatment of both CRC and sarcoma pulmonary metastases.[20] In a cohort of 57 patients with CRC, the study reported a 5-year disease-free survival of 26% and pulmonary progression-free rate of 44%. Importantly, they demonstrated no mortality and limited morbidity related to the procedure and showing the safety and feasibility of their technique. However, the recurrence rate in their cohort is comparable with current less-invasive standard practices where 5-year disease free survival is 37% to 40%, and so clinical benefit remains unclear. The authors note that further study of isolated lung perfusion with more effective chemotherapeutic agents is warranted. Oxaliplatin is an attractive agent to examine since it comprises one of the main cytotoxic agents used in standard chemotherapy regimens for CRC.[21] Future study of our protocol in a clinical context will allow examination of the efficacy of this agent in isolated lung perfusion.

Pulmonary artery perfusion techniques have also been explored with CRC-based chemotherapy. A phase I clinical trial using a technique of pulmonary suffusion to deliver cisplatin has been described. This technique uses a combined thoracoscopic approach for pulmonary vein occlusion, and fluoroscopic approach for balloon occlusion of the pulmonary artery and cisplatin instillation. Results showed safety of the technique as well as reduction in tumor volume.[22] Of note, this study also demonstrated a maximally tolerable dose of cisplatin at 5 times the normal exposure from typical systemic doses. More recently, a method of selective pulmonary artery perfusion was studied in a porcine model to deliver gemcitabine and carboplatin using a fully endovascular approach. This study showed improved pulmonary concentrations of carboplatin and gemcitabine compared with IV administration; however, it does not prevent systemic exposure to these agents.[23] These techniques have the advantage of a minimally invasive approach and thus reduced morbidity; however, full isolated circulation, with its benefits, is not achieved. Combining the less invasive nature of these techniques with isolated lung perfusion may produce a new method that merges the advantages of both approaches.

The main limitation of this study was the low sample size resulting from the accelerated titration design. Since this design only necessitates one case per dose, provided no drug-related adverse effects are observed, robust statistical analyses are not possible. It is difficult to draw overarching conclusions regarding the findings since they are subject to significant case-to-case variability. Moreover, the change from 40 mg/L to 80 mg/L is large, and it is possible a more exact dose-limiting toxicity may have been found had additional dose levels been examined. However, the purpose of this design is to provide a rapid estimate of the dose-limiting toxicity and guide design of a phase I dose escalation clinical trial. Given that human dose toxicities may differ from those in pigs, an estimate of the maximally tolerated dose is appropriate in this context to initiate a safety clinical trial.

An additional important limitation of this study is that examination of drug toxicities was limited by the subacute time frame examined. Oxaliplatin-induced pulmonary fibrosis has been previously reported in the literature with most cases occurring after several cycles and during active treatment.[24] However, rare exceptions involving delayed presentations even after one cycle have been reported.[25] In the setting of acute toxicity, IVLP can potentially re-circulate harmful factors, thus amplifying deleterious effects; however, there was no evidence of early lung injury even in the greatest-dose case. Since oxaliplatin concentrations in the lung reached 5 times typical systemic concentrations for the maximally tolerated dose, it is not clear if this would predispose to a greater likelihood of developing pulmonary fibrosis that was not captured within the 3-day time frame of this study. Due to complexity and resource requirements, it was not possible to maintain animals alive

for several weeks or months to assess potentially rare chronic effects.

CONCLUSIONS

This study has determined the dose-limiting toxicity of oxaliplatin delivered by an isolated IVLP technique over a 72-hour period in a porcine IVLP survival model. These findings will guide the initiation of a clinical trial protocol at our institution, examining the use of IVLP delivery of oxaliplatin with concurrent metastasectomy in patients with CRC lung metastases. As safety and feasibility of this technique continues to be demonstrated, further study is warranted exploring the use of IVLP with other treatment modalities that can benefit from this isolated treatment platform, such as targeted treatments, photodynamic therapy, and gene therapy.

Webcast

You can watch a Webcast of this AATS meeting presentation by going to: https://aats.blob.core.windows.net/media/ITSOS19/Resized%20Vidoes/new%20oct%2029/ITSOS2019_092819_In%20Vivo%20Isolated%20Lung%20Perfusion%20(IVLP)%20for%20Delivery%20of%20High%20Dose%20Chemotherapy%20for%20the%20Treatment%20of%20Colorectal%20Lung%20Metastases_Khaled%20Ramadan.mp4.

Conflict of Interest Statement

Dr Cypel, Keshavjee, and Waddell are shareholders of XOR labs Toronto. All other authors have nothing to disclose with regard to commercial support.

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