Abstract. Aim: The goal of this study was to investigate the therapeutic potential of combining radiation therapy and cytotoxic RNase, ranpirnase (ONCONASE®; ONC), in human lung tumor models in vitro and in vivo. As translational implications, the non-invasive monitoring response to individual therapy with ONC was also investigated to determine the underlying therapeutic mechanisms. Materials and Methods: A clonogenic survival assay was used to measure the effect of ONC and radiation on A549 human non-small cell lung carcinoma (NSCLC) cells. H&E staining, TUNEL staining and caspase-3-antibody labeling were used for in vivo analysis of apoptosis. A growth-delay assay was applied to detect the therapeutic potential of ONC as a radiation sensitizer in vivo. ONC-induced changes in blood flow and biochemical metabolites were measured by various non-invasive dynamic contrast enhanced magnetic resonance imaging (DCE MRI), non-localized 1H magnetic resonance spectroscopy (MRS), and near-infrared spectroscopy (NIRS) methods. Results: ONC at 5-10 ìg/ml sensitized the radiation response of A549 tumor cells in vitro. Remarkable increases in ONC-induced apoptosis in vivo were observed in caspase-3 antibody labeling and TUNEL staining assays. ONC significantly increased the radiation-induced tumor growth delay of A549 tumors. It was observed, when using a DCE MRI method, that there were significant increases in Ktrans values at the rim of tumor regions at 1.5 h post-injection of ONC. When using non-localized 1H MRS, an ~20% decrease in lactate levels with ONC was found. Conclusion: ONC may be a new and promising drug in the treatment of NSCLC as a radiation therapy enhancer.

The physiological barriers of anti-cancer drug delivery are due to the high interstitial fluid pressure or hypertension in solid tumors (1-2). One of the approaches to overcome the physiological barriers is the usage of relatively nontoxic pharmacological compounds, such as nicotinamide and pentoxifylline (3-5). Previously, Lee et al. reported that the cytotoxic RNase, ranpirnase (tradename, ONCONASE®; ONC, extracted from oocytes of Rana pipiens), significantly reduced tumor hypertension in several human tumor xenografts and murine isografts (6). Rybak et al. in pioneering research dealt with cellular drug resistance by natural and engineered cytotoxic ribonucleases (RNases) as an alternative to the standard DNA-damaging chemotherapeutic agents (7-10). ONC is internalized by endocytosis and released into the cytosol of the tumor cells, where it selectively degrades tRNA beyond repair (11), which causes inhibition of protein synthesis, blocks cell cycle proliferation and induces apoptosis. Thus, ONC could cause cell death by tRNA damage and is a new and attractive cancer treatment modality (11-12).

Varying fractions of viable cells in solid tumors are hypoxic due to inadequate blood flow. Therefore, minimization of hypoxic protection is a topic of interest in radiation therapy. Here, first whether ONC could enhance the radiation response of A549 human NSCLC in vitro and in vivo was studied using a clonogenic assay and growth-delay assay. Then, ONC-induced apoptosis in vivo was investigated using three techniques (hematoxylin and eosin/ H&E staining, caspase-3 antibody labeling and TUNEL staining assays), as a follow-up to our previous reports which showed that ONC significantly induced apoptosis in A549 tumor cells in vitro (13). ONC was known to decrease tumor oxygen consumption rate in A549 tumors (14). Since O2 status in tumors depends on O2 consumption and O2 availability, this decrease led to an improvement in tumor oxygenation in tumors. The ONC-induced changes in O2 availability were studied by measuring tumor blood flow

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Key Words: Lung cancer, ranpirnase, ONCONASE®, radiation sensitization, dynamic contrast-enhanced MRI, NMR spectroscopy, near-infrared spectroscopy.
(TBF) with non-invasive techniques of dynamic contrast-enhanced magnetic resonance imaging (DCE MRI) and near-infrared spectroscopy (NIRS). Using non-localized $^1$H magnetic resonance spectroscopy (MRS) in vivo spectra, acquired by a SelMQC pulse sequence, we tested whether transiently improved ONC-induced TBF could efficiently remove acidic tumor metabolites such as lactate in tumors. This study aims to identify the therapeutic mechanisms and applicability of ONC as a new radiation enhancer.

**Materials and Methods**

**Tumors.** Frozen A549 human cell lines were purchased from the American Type Culture Collection (Rockville, MD, USA), thawed, cultured and grown in vitro. Cells were maintained at 37°C for the duration of the experiments. The cells were grown in RPMI-1640 medium supplemented with 15% fetal bovine serum (FBS), 25 mM HEPES buffer, 5 mM L-glutamine and antibiotics (15% FBS-RPMI-1640).

Preparation of ONC. ONC was supplied by the Alfacell Corporation (Bloomfield, NJ, USA). Original stock solutions of ONC at 5 mg/ml were made in sterile distilled water and frozen at -20°C until needed. ONC was thawed and diluted to appropriate concentrations prior to the experiments.

Clonogenic radiation survival assay for in vitro response to ONC. Appropriate cell numbers (from 100 to 1x10^5) of A549 tumor cells were plated into 6-well plates (or T25 cell culture flasks). The cells were then incubated at 37°C for 5 h for cell attachment and incubated with ONC at 0-10 μg/ml for 24 h, then exposed to X-irradiation by putting them inside a Cs irradiator. After irradiation, the 6-well plates were rinsed twice with RPMI-1640 medium to remove the ONC from the media and fresh media containing 15% FBS-RPMI-1640 was added. Clonogenic assays were then performed as described in previous studies (15). After adding 7 ml of 15% FBS-RPMI-1640 to 6-well plates, cells were allowed to grow for 8-10 days. Cultures were fixed with 99.5% isopropyl alcohol, stained with 1% crystal violet and counted. Colonies with more than 50 cells were scored as positive. The sensitization enhancement ratio (SER) was calculated as the ratio of X-irradiation doses in the presence and absence of ONC. In this study, X-irradiation dose in the absence of ONC at SF0.1 (surviving fraction at 0.1) was derived from the non-ONC-treated survival curve, and X-irradiation dose in the presence of ONC at SF0.1 was derived from the ONC-treated survival curve.

Animals bearing tumors. Animal care was in compliance with all rules as set by the University of Pennsylvania for the care and use of laboratory animals, with standards equivalent to the UK CCCR guidelines for the welfare of animals in experimental neoplasia. Eight- to ten-week-old female athymic NCR-nu/nu nude mice (purchased from the NCI, Bethesda, MD, USA) bearing human tumor xenografts of A549 human NSCLC cells were utilized. Viable cells, 2x10^6, suspended in 50 μl of RPMI-1640 medium, were injected subcutaneously into the right thighs of mice (body weight ~ 23-25 g). Experiments were carried out when the tumor volume was between 200 and 400 mm^3 (~4 weeks after tumor implantation). Tumor volumes were calculated using the formula $V_2 = 0.4 \times AB^2$, where A & B are the longer and shorter diameters of the tumor, respectively (15).

For the in vivo ONC treatment, ONC was dissolved in sterile 0.9% NaCl solution before the experiments. The mice were given an intravenous (i.v.) injection of ONC at 2.5 to 5 mg/kg at a volume of 0.1 ml/20 g of body weight. A brief summary of treatment groups including number of mice and treatment is described in the legend of the figures.

**Histological studies preparation procedure.** For histological preparation of A549 tumors, animals with established tumors of 8-9 mm mean diameter were i.v. injected with ONC at 5 mg/kg. Animals from a saline-treated control group (N=5) and ONC-treated group (N=5) were randomly chosen. At 24-48 h intervals after ONC or saline treatment, the animals were sacrificed by an injection of a mixture of ketamine/xylazine (90/9 mg, i.p.), followed by cervical dislocation. The tumor and kidney were excised from animals immediately euthanized by a cervical dislocation. Tumor and kidney samples were placed in 10% neutral buffered formalin overnight for fixation, and then sent to the Pathology Core at the Children’s Hospital of Philadelphia (Abramson Research Center & CHOP, Philadelphia, PA, USA) for the histological studies. Once the tissues had been fixed, they were processed into paraffin blocks and made into thin microscopic sections using a microtome, then placed on slides. Routine hematoxylin and eosin (H&E) staining was performed on formalin-fixed paraffin-embedded tissues. Histological and morphological changes induced by ONC on tumor tissues and kidney were compared to saline-treated controls. Separately, slides were prepared and processed for caspase-3 antibody labeling and TUNEL staining. These slides were evaluated by microscopy for apoptosis.

X-irradiation procedure. For in vitro studies, a Mark I Cs-Irradiator (J.L. Shepherd & Associates, San Fernando, CA, USA) was used at dose rate of 1 Gy exposure (time setting = 0.79 min). Prior to X-irradiation, A549 tumor cells were incubated at 37°C for 5 h for cell attachment and incubated with ONC at 0-10 μg/ml for 24 h. Cells were then exposed to X-irradiation by placing them inside the Cs irradiator. For in vivo X-irradiation studies, a customized irradiation jig was made. Four copper tubes (of 8 mm-thick copper with an inner diameter of 29 mm and an outer diameter of 45 mm X-ray transmission ~ 3.9%) were cut and positioned on an acrylic base. Animals bearing tumors were placed on the irradiation jigs. The mice were appropriately placed so that the tumors would be at the center of the beam. Four toes of each mouse were tied with a string which were then taped and fixed in place. Photon irradiation was applied by a Phillips RT250 orthovoltage X-ray machine (Phillips Medical System, CT, USA) at 225 kV, 17 mA with 0.2 mm copper filtration at a distance of 30 cm (dose rate=3.4 Gy per min).

Biochemical analysis using non-invasive in vivo MR spectroscopy. Spin echo magnetic resonance imaging of A549 tumors was performed prior to $^1$H-MRS studies. Animal body temperature was maintained at 37°C by blowing warm air through the magnet bore. Lactate levels in tumor tissue were measured 0-2 h after ONC treatment, using a 9.4 T vertical bore spectrometer (Varian INOVA, Palo Alto, CA, USA) equipped with 55 mm, 55 G/cm gradients, and a slotted tube resonator. Lactate levels were monitored by non-localized $^1$H in vivo spectra using a SelMQC pulse sequence before and after ONC treatment (16). The MRS parameters were as follows: sfrq: 400 MHz; d1 (repetition time):
4 s; nt (# of repetition): 128; np (# of point): 1024; sw (band width): 4001 Hz; tpwr (power of the pulse): 22 dB; and pw (duration of pulse): 8190 ms.

Animal preparation and data acquisition by MRI. All MR images were acquired with a 4.7-T UNITY INOVA spectrometer (Varian Medical Systems, Palo Alto, CA, USA). For in vivo studies, mice were placed inside a H linear polarized birdcage RF coil with an inner diameter of 5 cm. Prior to the MR imaging protocol, a catheter was inserted into the tail vein of the mouse for injection of the contrast agent (Gd-DPTA). The second catheter was i.p. injected for injection of ONC. The animal was sedated with isoflurane through a nose cone. Its core temperature was monitored by a rectal thermistor and maintained at 37°C by a flow of warm air. The electrocardiogram (ECG) was also monitored by attaching a pair of subdermal needle electrodes. A bolus injection of Gd-DPTA was followed by 10 pre-acquisitions. The protocol for the T1 mapping included a multi-slice various flip angle gradient echo pulse sequence with echo time (TE)/repetition time (TR) of 2.1/200 ms, flip angles of 0°–180°, a matrix size of 128x64 (frequency direction x phase direction), field of view (FOV) of 4 cm x 2 cm (frequency direction x phase direction), a slice thickness of 2 mm, and total acquisition time of 6 min 37 s. Dynamic T1-weighted spin echo pulse sequence was recorded for thickness of 2 mm, and total acquisition time of 6 min 37 s. Dynamic T1-weighted spin echo pulse sequence was recorded for diffusion contrast-enhanced MRI perfusion with the following parameter settings: TE/TR: 8.5/200 ms; matrix size: 128x64; FOV: 4x2 cm; slice thickness: 2 mm; total slice number: 12 (kidney slice: 5, tumor slice: 7); pixel size: 0.3125x0.3125 mm2; time resolution: 12.8 s; total number of dynamic images: 300 per slice; total imaging time: 64 min; single acquisition, same field of view as the T1 map protocol and same matrix as the T1 map protocol. The changes in blood flow of the whole tumor were shown by color-grading (changes in Ktrans were color-graded) using a Toft model (17, 18).

Blood flow and blood oxygen saturation (SO2) studies using near-infrared spectroscopy (NIRS) (i.e. diffuse correlation spectroscopy (DCS) and diffuse reflection spectroscopy (DRS)). Blood flow in tumor and skeletal muscle was monitored by diffuse correlation spectroscopy (DCS) (19). In DCS, the electric field temporal autocorrelation function is explicitly related to the motion of the red blood cells. Diffusion photons can sometimes scatter from moving blood cells which cause the intensity of the diffusing light to fluctuate in time. The fluctuations are more rapid for faster moving blood cells. Therefore, one can derive information about tissue blood flow far below the tissue surface from measurements of temporal fluctuations impressed upon light diffusing through tissue. In this study, relative blood flow (rBF) was defined as the percentage change in blood flow compared to pre-treatment values. Blood oxygenation was assessed non-invasively using frequency-domain diffuse reflection spectroscopy (DRS) (19). Multi-wavelength fitting algorithms were applied to directly extract CIB (concentration of deoxy-hemoglobin) and CIBO2 (concentration of oxygenated hemoglobin) by assuming absorption was only coming from oxy- and deoxy-hemoglobin, \( \mu_a = \Sigma_j \varepsilon_j C_j (t = C_{Hb}, C_{HbO2}) \). Here \( \varepsilon \) was the extinction coefficient of a given chromophore at a given wavelength. After extracting hemoglobin concentrations, blood oxygen saturation was obtained as \( SO_2 = C_{HbO2} / (C_{HbO2} + C_{Hb}) \times 100 \).

Statistical evaluation. All measured values were presented as the mean ± standard error (SE) of each group. Significant differences within a group before and after ONC treatment were evaluated using a paired t-test. Differences between treatment groups were evaluated with an unpaired t-test. For statistical evaluation of Ktrans values before and after the ONC treatment, Ktrans values were obtained from 7 slices per tumor using an MRI method. The median (or midpoint) of Ktrans values was selected from each tumor. The mean of all median Ktrans values was selected for the statistical analysis, and the groups were compared using a t-test. Significance was set at 95% (p=0.05) for all analyses.

Results

We observed that ONC at 5-10 μg/ml sensitized the radiation response of A549 tumor cells in vitro using a clonogenic assay. This was determined by calculating the sensitization enhancement ratio (SER: defined as survival fraction (SF) of radiation alone divided by SF of radiation + ONC). SER was ~1.4 (p=0.02) for ONC at 5 μg/ml and ~1.6 (p=0.01) for ONC at 10 μg/ml at a survival fraction of 0.1 (SF0.1), as shown in Figure 1A. However, ONC at 1-2.5 μg/ml did not show similar results.

Based on our in vitro radiation sensitization by ONC, we tested the effectiveness of ONC on radiation responses in A549 tumors of nude mice. Figure 1B shows the effect of a single exposure of 5 Gy on the growth of A549 tumors. Tumor growth delay is defined as the difference in time for a treatment group to reach a four-fold volume increase, subtracting the time for the control to reach a four-fold volume increase. Untreated tumors took 11 days for a four-fold volume increase, establishing the baseline measure. After X-irradiation with 5 Gy, the tumor volume increased by a magnitude of four in 24.5 days, resulting in a growth delay of 13.5 days. When the animals were i.v. injected with ONC at 2.5 mg/kg, a four-fold increase in tumor volume took 12.4 days, demonstrating a growth delay of 1.4 days. When the animals were treated in combination with ONC at 2.5 mg/kg plus 5 Gy, the tumor volume increased by a magnitude of four was in 37 days, a growth delay of 26 days. Thus, an i.v. injection of ONC at 2.5 mg/kg alone did not effectively retard the tumor growth of A549 tumors. When ONC was i.v. injected 2 h prior to X-irradiation, ONC significantly enhanced the radiation-induced growth delay of A549 tumors in nude mice (i.e. 14.4 days delay at 5 Gy, p=0.04). It was therefore greater than the additive effects of ONC and X-irradiation on the inhibition of tumor growth of A549 in vivo.

Based on our in vivo cytotoxic studies of ONC for A549 tumors in nude mice (13), an i.v. injection of ONC at 5-10 mg/kg was effectively tumoricidal in A549 tumors. This resulted in a significant growth delay. Thus, in our present studies, we examined whether ONC induced apoptosis as one of the mechanisms for reducing the tumor volume. ONC-induced apoptosis was found in A549 tumors in vivo using three immunohistological methods, H&E staining, caspase-3
antibody labeling and TUNEL labeling (Figure 2). We observed few occurrences of apoptosis in untreated A549 tumor cell lines in vivo. However, the occurrences of apoptosis became noticeable at Day 3 after an i.v. administration of ONC at 5 mg/kg (second row in Figure 2). At Day 5, large numbers of apoptotic cells can be seen by H&E staining as well as in immunohistochemical sections of caspase-3 antibody labeling and TUNEL staining (third row in Figure 2).

Tumors exhibit enhanced rates of glucose uptake and glycolysis, which lead to an increase in lactate production in tumors (20). Thus, we examined whether ONC could alter glucose and lactate levels in A549 cell culture. Using YSI lactate/glucose analyzers (2300 Stat plus analyzer, YSI incorporated, Yellow Springs, OH, USA), we found no change in cellular levels of lactate or glucose after treatment with ONC (data not shown). However, lactate levels in the tumors were measured non-invasively after treatment with ONC using non-localized 1H in vivo spectra. An ~20% decrease (n=3 mice, p=0.03) in lactate was observed using non-localized MRS, as shown in Figure 3.

From the plasma and tissue concentration curves, Ktrans and Ve map, MRI data processing was performed sequentially. The important and critical factor was obtaining the changes in the concentration of Gd-DPTA in the vena cava of an animal over the duration of the study of each animal. The plasma concentration curve from each dynamic scan was analyzed, which provides minimum error compared to the ideal plasma concentration curve. By DCE MRI using a diffusible MRI contrast agent of Gd-DPTA, we monitored the effect of ONC on tumor perfusion. At the rim of tumors the signal enhancement after injection of Gd-DPTA rapidly increased, then rapidly decreased. However, in the core of tumors, the signal enhancement slowly increased then slowly decreased after injection of Gd-DPTA. The follow-up MRI images after treatment with ONC are shown in Figure 4. Ktrans and Ve maps for the pre- and post-treatment of ONC (post 1.5 h and post 24 h) were calculated and imaged for the entire tumor regions. The mean of the Ktrans median values from 4 tumors for the pre-ONC was 0.0812±0.014 and that of the post-ONC was 0.1237±0.0279, resulting in a 49.3±7.5% increase (N=4 mice, p=0.029). The increases in the Ktrans value of tumor regions are shown in red, mainly due to significant increases at the rim of the tumor regions at 1.5 h post-injection (7 slices; first row in Figure 4).

To confirm the above MRI blood flow data, blood flow and blood oxygen levels (SO₂) were monitored in A549 tumors and skeletal muscles before and after treatment with ONC or saline. The rBF in A549 tumors was not altered by saline alone, compared to ONC. In the ONC-treated group, the rBF in A549 tumors at 1.5 h increased by ~70% (n=8 mice, p=0.003) but remained unchanged in skeletal muscles (Figure 5A). The SO₂ in A549 tumors significantly increased from 14±4% to 32±3% (n=6 mice, p=0.02), but not in skeletal muscles in Figure 5B.

Discussion

We previously reported that ONC induced apoptosis in A549 and NCI-H1975 NSCLC cell lines using a TUNEL assay, in addition to the effect of ONC in vivo (13). In the
In continuation of in vivo ONC cytotoxic studies, we have provided the main mechanism for in vivo effectiveness of ONC by showing significant induction of apoptosis in vivo in A549 NSCLC after ONC treatment (Figure 2). Our histopathology reports using caspase-3 antibody labeling and TUNEL labeling assay in vivo showed remarkable increases in apoptosis after an injection of ONC (Figure 2). ONC may significantly increase the tumoricidal efficacy in the two (A549 and NCI-H1975) NSCLC cell lines tested, due to increased apoptosis both in vivo and in vitro (13).

This occurs regardless of epidermal growth factor receptor (EGFR) status. Thus ONC may be an adjunct therapy for molecular therapeutic gefitinib therapy (21-23).

One of our goals in this study was to determine the therapeutic effect of ONC in combination with X-irradiation. Based on our present radiation sensitization studies, ONC was an effective radiation enhancer in vitro (Figure 1A) and increased the radiation-induced tumor growth delay of A549 tumors in nude mice (Figure 1B). The growth delay was 13 days with 5 Gy alone and 26 days with 5 Gy plus ONC. Thus, it was greater than the additive effects of ONC and X-irradiation on the inhibition of tumor growth of A549 in vivo.

Our in vivo radiation studies for ONC were focused on the low dosage (almost non-toxic with ONC alone) of ONC instead of the maximal tolerance dosage, since high dosage of ONC alone significantly inhibited tumor growth in our previous report (13). Although a large (maximal tolerated dose or slightly less) dosage of ONC gave the best possible response (data not shown), complications (i.e. reduction in body weight) may occur at these high dosages. However, we believe our approach with a small dosage of ONC and fractionated X-irradiation could be translated to the clinical application.
trials for ONC as a radiation sensitizer. Furthermore, we also observed that tumor physiological parameters were modified by the small dosage of ONC in this study. This is supported by a selective increase in O2 availability in tumors, shown by the tumor blood flow and SO2 in A549 tumors (Figure 5). We are currently investigating the study of the sequence of ONC and fractionated X-irradiation in A549 and NCI-H1975 NSCLC in vivo.

We also observed that ONC did not increase radiation-induced skin damage (data not shown). This is in agreement with our results in Figure 5; the relative blood flow and SO2 in skeletal muscle was slightly modified after the ONC treatment. This suggests that the hypoxic cells in the skin of mice were not reoxygenated by ONC, which increases the therapeutic potential of ONC as a radiation enhancer. Previously Lee and his colleagues reported that ONC significantly inhibited the cellular oxygen consumption rate in various tumor cell lines including A549 tumors (14). In addition, due to the inhibition of cell proliferation in endothelial cells by ONC, the QO2 was significantly reduced, making O2 more available to the peripheral tumor regions.

The imaging construction of the kidney was also repeated on days 1-8. A subsequent T1 map of the kidney showed that the T1 values decreased after treatment with ONC (data not shown). The lack of histological changes in the kidney of ONC-treated animals measured by the H&E staining method showed that ONC caused temporary dehydration in the kidney for up to 2 days post-treatment, which then fully recovered around 4 days after ONC treatment. Thus, our MRI method may be sensitive in the detection of minimal and recoverable changes in the kidneys after ONC treatment.

This study aimed to elucidate the physiological mechanism of ONC and test its effectiveness as a potential radiation enhancer. High tumor interstitial fluid pressure (TIFP) of A549 tumors and other tumor models was significantly reduced by ONC (6, 25). The reduced TIFP improved the penetration of ONC into the tumor regions. We observed a ~50% increase in perfusion at the rim of A549 tumors using a DCE-MRI method at 1.5 h post-treatment with ONC (Figure 4). This is in agreement with our previous observations using laser Doppler flowmetry (6, 24). Since increases in viscous and geometric resistance to blood flow led to the elevated TIFP in solid tumors (1-5), the acute effect of ONC may be due to a reduction in viscous resistance, and the chronic effect of ONC on tumor vasculature may be due to a reduction in both viscous and geometric resistance.

In our present in vitro studies, ONC did not alter the cellular lactic acid levels or glucose uptake in A549 tumor cell lines, as measured by an YSI 2300 STAT Plus-glucose and lactate analyzer. However, glycolysis is tightly controlled and is adapted to meet cellular demands for ATP. We anticipate that low levels of ATP will stimulate glycolysis, which will...
lead to increased lactate levels in tumors, and high levels of ATP will inhibit glycolysis. However, in our previous report, we found that ATP levels were reduced by ONC (13) when ATP levels in A549 tumors in vivo were monitored non-invasively by non-localized $^{31}$P MR spectroscopy. The lactate levels in A549 tumors in vivo were significantly reduced by ONC, as non-invasively measured by a non-localized NMR spectroscopy method (Figure 3). This is likely due to removal of acidic metabolites by ONC-induced increases in TBF. If we are able to show a correlation between lactic acid levels
and the efficacy of ONC, we will be able to use this as an early indicator of the effectiveness of ONC on an individual patient. All of our measurements, including lactic acid levels, are performed non-invasively, making it much easier on our patients in terms of patient comfort and repeatability.

Conclusion

ONC significantly increased the radiation-induced tumor growth delay of A549 tumors in vivo and improved several physiological parameters (reduced O2, increased blood flow, increased SO2, reduced lactic acid and reduced ATP levels) with minimal side-effects. This investigation suggests the potential clinical uses of ONC with molecular therapeutic gefitinib therapy in the presence and absence of radiation therapy for the treatment of patients with NSCLC.

Acknowledgements

This work was, in part, supported by Alfacell Corporation through their sponsorship research agreement with the University of Pennsylvania (Sponsor Grant #60933-542431, P.I.: I. Lee). Dr. Sergey Magnitskys contributions were performed after his paid work hours. We sincerely appreciate Daniel Martinez at the Pathology Core, Children’s Hospital of Philadelphia (CHOP), for his histological services for processing paraffin blocks and slides for the H&E staining, caspase-3 antibody labeling, and TUNEL staining studies.

References


Accepted May 10, 2007
Revised June 18, 2007
Accepted June 27, 2007