VISUALSONICS

White Paper:

High-Resolution Micro-Ultrasound for Small Animal **Cancer Imaging**

Ver 1.2

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Abstract

Micro-ultrasound imaging is a flexible high-resolution real-time *in vivo* imaging modality based on the detection of soft tissue and blood flow via the non-invasive transmission and receiving of ultrasound waves. Because of its real-time temporal resolution, its spatial resolution of 30µm, and the non-invasive nature of ultrasound, micro-ultrasound is used extensively in preclinical research to monitor phenotypical, functional and dynamic changes in small animal models in a variety of disease processes. Micro-ultrasound imaging is a beneficial modality for small animal cancer imaging as it allows for non-invasive, longitudinal monitoring of cancer biology and therapeutic effectiveness including orthotopic tumor sizing and growth, tumor perfusion, flow architecture and neoangiogenesis, and targeting of molecular biomarkers *in vivo*, all in the same animal serially during disease progression. The following overview of micro-ultrasound illustrates how micro-ultrasound is a key modality and describes its utility to detect, monitor and quantify preclinical models of cancer.

The current paper will discuss the following topics:

- An introduction to Micro-Ultrasound: Real-Time Anatomical, Functional and Molecular *In Vivo* Imaging of Small Animals
- Overview Of Equipment Configuration And Set-Up
- What Can You Visualize With High-Resolution Micro-Ultrasound?
- Microbubbles Can Increase Applications And Quantification Capabilities
- Data Quantification
- Expanding Into Gene Delivery

Introduction

In the USA there were 294 120 men and 271 530 woman who were diagnosed with cancer in 2008 and of these, over 500 000 will die from their diagnosis, accounting for 23% of all deaths (American Cancer Society, 2009). While the fight against cancer is gradually improving (Espey et al. 2007), there continues to be a need for a better understanding of the pathogenesis governing cancer, coupled with the desire to develop and assess the effectiveness of new anti-cancer and anti-angiogenic drugs.

Murine models are a crucial tool in this research priority and are the most commonly used basic science and preclinical animal systems in academic and pharmaceutical research. This is due to the significant similarities and the plethora of commonly shared inherited diseases, both Mendelian and polygenic between humans and mice. Some of these diseases include atherosclerosis, cancer, heart disease, hypertension, obesity, bleeding disorders, asthma and neurological disorders. Notwithstanding, there are other mammals which also share this similarity. However, one of the major advantages of the mouse model is the wealth of resources available on its genetics, molecular and cellular pathways. Furthermore, mice are ideal for research environments as they are small and easy to maintain, cost-effective, have short gestation periods (~19–20 days) and finally, genetically uniform inbred strains are readily obtained.

Over the past 10 years mouse models have been used in cancer research to aid in the investigation of the basic biological principles of cancer, as in preclinical development of anticancer drugs, and as a tool for discovering new clinical agents and assays. As time has progressed genetically engineered mouse models are now being used successfully to detect phenotypical changes and genetic lesions observed in human tumors (Olive and Tuveson 2006). Early tumor detection and accurate monitoring of tumor size and disease progression is essential for studying cancer biology, preclinical drug development and for accurate diagnosis and treatment of cancer preclinically and in patients in a clinic setting.

Currently there are a variety of preclinical imaging modalities available for small animal cancer research. These include magnetic resonance imaging (MRI), X-ray and computed tomography (SPECT/CT), positron emission tomography (PET), fluorescent and bioluminescent imaging and micro-ultrasound. As in clinical imaging, no single imaging modality suits all biological applications. In fact, each imaging modality has its own strengths and weaknesses with respect to its temporal and spatial resolution, accessibility, sensitivity, ease of use, cost, the specific regions of the body and biological processes that can be imaged, the availability of compatible intravascular contrast agents, and contrast agent toxicity (Massoud et al. 2003).

As such, it is recommended that the appropriate imaging modality be selected based on its ability to best answer the specific biological question that is being asked. Often these modalities are used in a multi-modality format to allow researchers a more complete understanding of cancer disease processes and/or the efficacy of a preclinical therapeutic.

For the purpose of this review we will focus on micro-ultrasound as a key imaging modality in preclinical cancer research. Clinically, ultrasound is one of the most pervasive imaging modalities used in most hospital departments for real-time imaging and quantification of soft tissue ailments, cardiac function, fetal development and diagnosis, emergency medicine, screening and assessment of tumors, and as a tool for guidance of invasive procedures. In recent years, preclinical ultrasound systems ("micro-ultrasound") have been developed that provide the functionality available clinically yet allow for ultra-high spatial resolution (down to 30 microns) in order to view and quantify the detailed structures, flow dynamics and molecular biomarkers inherent in murine micro-imaging. In addition these systems have been developed to minimize perturbation of the mouse, rat or other small animal model while maximizing reproducibility of both the procedure and of the data derived.

In the past, preclinical cancer research and cancer drug development efforts often relied on end point autopsy as a means of evaluating tumor morphology as well as validation of treatment efficacy. However, this ex vivo method requires large groups of mice to generate statistically meaningful data, is time consuming, does not allow understanding of flow dynamics in and around tumors and is costly. By using micro-ultrasound, researchers are armed with

an relatively cost effective *in vivo* real-time imaging modality which allows for a significant reduction in the number of cohort animals needed to accurately examine tumors at multiple times points in response to pre- and post-treatments *in vivo* and the ability to quantify these observations within the same animal in a high-throughput manner.

Overview of Micro-Ultrasound Equipment Configuration, Imaging Set-Up And Data Quantification Capabilities

There is only one manufacturer of a dedicated preclinical high-resolution micro-ultrasound system (VisualSonics' Vevo® 770 / Vevo® 2100 Micro-Ultrasound). The Vevo operates at frequencies of 30 – 70 MHz (compared to frequencies of 3 – 15 MHz for clinical systems) allowing 30µm spatial resolution required for detailed imaging of rats, mice, chick embryos, zebra fish, and other small animals. It is a portable imaging system that requires no dedicated facilities, no iodizing radiation, and is configured and designed specifically for non-invasive small animal *in vivo* imaging, quantification and longitudinal study data analysis (Figure 1).

Each system is connected to a series of application-specific scanheads that transmit and receive non-invasive ultrasound signals that generate the real-time images. As can be seen in Figure 2, the scanhead is set-up in a Imaging Station to allow for hands-free positioning and capture of the images. The animal is anesthetized and maintained on a heated platform to ensure the comfort and maintain body temperature of the animal during imaging. Key physiological parameters are captured from the platform including heart rate, temperature, respiration and ECG. These signals are gathered through paw and temperature probes and integrated with the real-time micro-ultrasound images. An additional accessory to this Imaging Station is an injection mount that allows for the guidance of precise injections or biopsies *in vivo* through the real-time imaging of the micro-ultrasound system.

FIGURE 1: The Vevo 2100 Micro-Ultrasound System.

The complete Vevo 2100 high-resolution micro-imaging solution for small animal research includes the Vevo micro-ultrasound rolling cart, the interchangeable MicroScan Transducers.

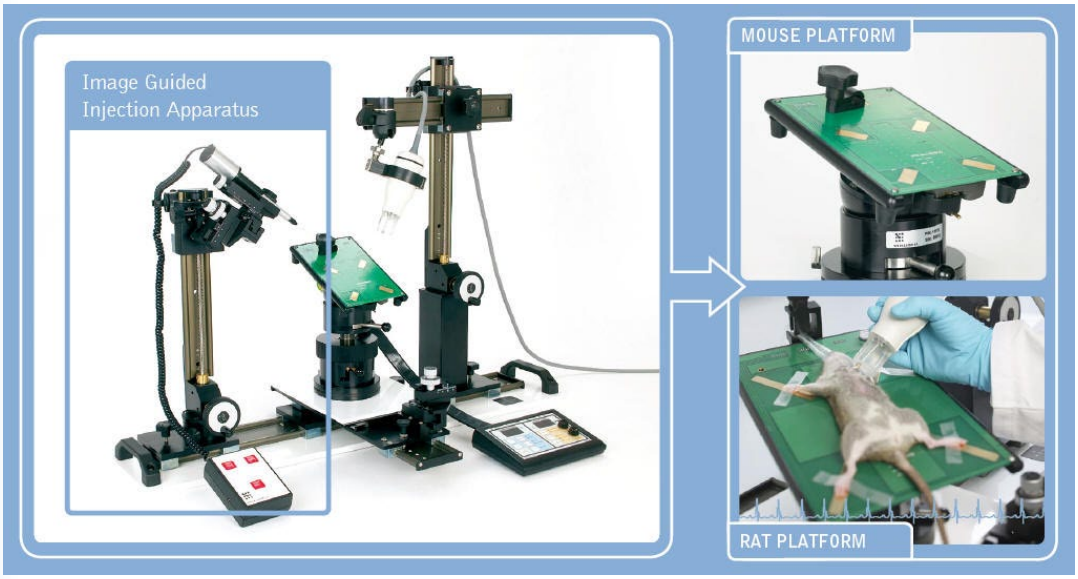

FIGURE 2: Vevo Integrated Imaging Station for Hands-Free Imaging

Complete Vevo Integrated Imaging Station including Image-Guided Injection apparatus and physiological monitoring controller. Heated platforms help to maintain animal temperature during anesthesia.

The analytic software is designed to allow for study-based aggregation of animal data for longitudinal experiments with multiple animals, multiple time-points, and multiple therapeutic interventions. Over 500 disease-specific measurements are implemented within the software to allow for quantification and analysis of the images.

Two-dimensional images are gathered and viewable in real-time without any reconstruction or delay, while 3D image are acquired rapidly and reconstruction into a 3D image in seconds by the software.

Animal physiological data can be stored with the imaging data for cardiotoxicity studies, for example. All images and measurements can easily be exported to databases for further analysis or archiving. The software is also easily networked for high-throughput studies or for use in core imaging facilities where multiple researchers use the same instrument and can quickly image their animals and then review their data and perform measurements over the network.

What Can You Visualize With High-Resolution Micro-Ultrasound?

For cancer research applications, micro-ultrasound is an imaging modality that, because of its high spatial resolution and real-time temporal resolution, can be used to visualize, characterize and quantify pre-palpable orthotopic and subcutaneous tumors in a multitude of small animal models. Tumors can be monitored and quantified from tumorgenesis through their growth stages and through to metastases to surrounding organs, lymph nodes and tissue. The software allows real-time visualization and measurement of the tumors in 2- and 3-dimensions. Blood flow, blood architecture and assessment of feeder vessels can be quantified using Power Doppler (for vessels larger than 30μm) and contrast agents for tumor perfusion and micro-circulator flow *in vivo*. As described by Xuan et al. 2007, neoangiogeneic development and tumor blood flow in prostate tumors - including visualization of each of the early stages of such development – can be clearly visualized and quantified in longitudinal studies.

Tumor Sizing and Quantification in Two- and Three-Dimensions

One of the most important measurements in oncology is quantifying the change in tumor size during disease progression and changes related to tumor response to anticancer therapy (Wirtzfeld et al. 2005; Wang et al. 2007; Feldman et al. 2008). Because micro-ultrasound is non-invasive, tumor growth and changes can be monitored repeatedly and longitudinally in the same animal, which can serve as its own control, thereby increase accuracy of the experiment and reducing the number of cohort animals required. Micro-ultrasound is routinely used in serial 2D and 3D volumetric quantification of tumor sizing *in vivo* in a variety of murine and non-rodent cancer models (Wirtzfeld et al. 2005, Graham et al. 2005, Goessling et al. 2007, Huizen et al. 2005, Wu et al. 2005 and Kiguchi et al. 2005, DeRosier et al. 2007 and Lyshchik et al. 2007). For example, Wirtzfeld et al. 2005 were the first to publish on the use of micro-ultrasound to track 3D tumor volumes in progression in a transgenic prostate cancer mouse model. 3D micro-ultrasound images correlated closely to serial histology (a correlation coefficient of 0.998 ($p<0.001$)). Furthermore, 3D micro-ultrasound measurements accurately confirmed the size and shape of these tumor masses *in vivo* (Wirtzfeld et al. 2005). The technique is highly reproducible as tumor detection sensitivity and specificity were both >90% when diagnoses were based on repeated micro-ultrasound examinations performed on separate days.

Further studies went on to show the utility of 3D micro-ultrasound to not only non-invasively track the growth of liver metastases (tumor diameter, volume and growth curve) but to also evaluate potential chemotherapeutics on these parameters in a longitudinal murine metastases model (Graham et al. 2005). In this particular study the authors showed high accuracy in detecting tumor mass progression using four different cell lines; B16F1 (murine melanoma), PAP2 (murine H-ras–transformed fibroblast), HT-29 (human colon carcinoma), and MDA-MB-435/HAL (human breast carcinoma) in a liver metastases model. Furthermore,

the longitudinal imaging of B16F1 liver metastases revealed distinct textural changes which had anechoic regions found to be areas of liquefactive necrosis. The tracking of tumor volume and textural changes can be a useful tool in tracking subtle responses to different antiangiogenic therapeutic treatments. More importantly, this study illustrated that longitudinal micro-ultrasound imaging is a powerful tool in preclinical trials and drug development since liver metastases treated with Doxorubicin a cytotoxic chemotherapeutic agent were found to have significant decreases in tumor volume at day 12 post cell injection. The authors concluded that micro-ultrasound is a vital tool for evaluating therapeutic efficacy on sequential stages of tumor development (Graham et al. 2005).

Evaluation and Quantification of Blood Flow

A key component of studying carcinogenesis in animal models is understanding how specific drugs affect angiogenesis, tumor growth and metastases. As such, micro-imaging modalities which provide insight into how a drug influences tumor vasculature and molecular expression of specific biomarkers, such as those involved in angiogenesis, have become important life science research tools. Micro-ultrasound provides such utility, allowing for the mapping and visualization of tumor vasculature using the Power Doppler which can detect blood flow ranging from 2mm/s up to 4m/s in tumors at multiple time points (Goertz et al. 2002, Xuan et al. 2007, Jugold et al. 2007). As depicted in Figure 3, Power Doppler can be used to detect subtle changes in tumor perfusion and blood vessel architecture in a non-invasive longitudinal manner (unpublished observation Xuan et al. 2006 Robarts Research Insitute, London, Canada).

Studies by Goertz et al. 2002 reported the first use of *high-frequency* micro-ultrasound 2D Power Doppler in studying the effects of an antivascular drug on blood flow in superficial human melanoma MeWo tumors. The authors reported a significant reduction in blood flow 4 hours after injection of the tumor vascular targeting agent ZD6126 followed by a recovery of flow by 24 hours after injection. They concluded that *high-frequency* Power Doppler was a highly effective non-invasive quantitative tool for longitudinally following the effects of antivascular therapy on blood flow in superficial tumors (Goertz et al. 2002).

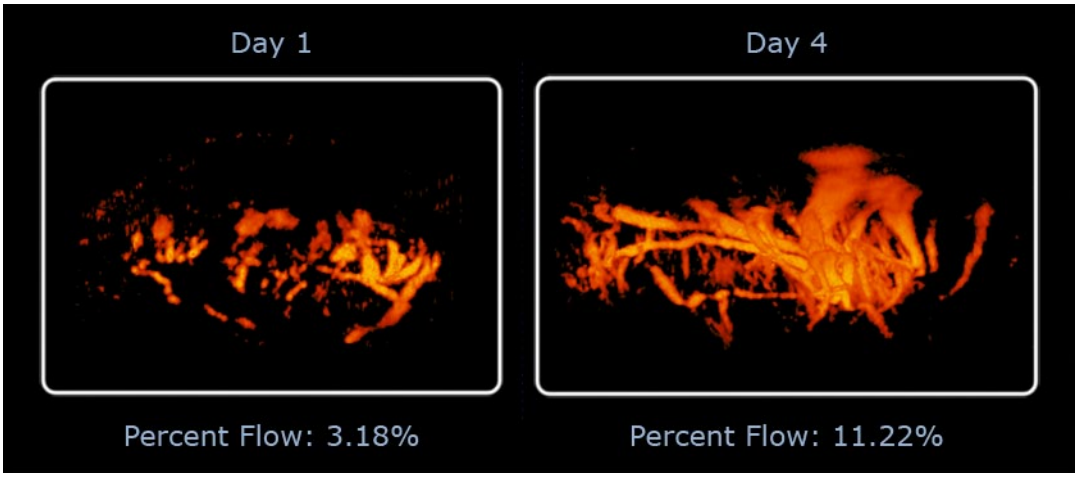

FIGURE 3: Example of 3D Power Doppler Imaging in a Murine Prostate Tumor.

3D Power Doppler was used to detect changes in tumor perfusion and flow architecture non-invasively in an adult mouse prostate tumor at days 1 (top) and 4 (bottom). Tumor growth and progression can be quantified by changes in total tumor volume and by the intensity of the Power Doppler signal which represents the vasculature and blood flow within that tumor. Figure sequence courtesy of Drs. J. Xuan, J. Lacefield and A. Fenster, Robarts Research, London, Canada

Similarly Xuan et al. 2007 recently reported the first application of high-frequency 3D Power Doppler micro-ultrasound imaging in a genetically engineered mouse prostate cancer model. They showed that 3D Power Doppler could sensitively, specifically and reproducibly depict functional neoangiogenic blood flow in prostate tumors when compared to normal prostate tissue which had little or no flow. These observations were confirmed using micro-CT and by correlation with microvessel distributions measured by immunohistochemistry and enhanced vascularity visualized by confocal microscopy. A separate study from Jugold and colleagues investigated the effects of blocking VEGF mediated pathways using a VEGFR2-blocking antibody treatment (Jugold et al. 2007). This study showed using Power Doppler imaging, that after 6 days of treatment in subcutaneous tumor (spontaneously immortalized human skin keratinocytes) in nude mice, tumor vascularity significantly decreased. These findings were additionally confirmed with immunohistochemistry staining for vascular markers, CD31 and smooth muscle actin (Jugold et al. 2007).

Finally, the high-resolution imaging capabilities of micro-ultrasound allows cancer researchers to study not only the effect of a novel drug on angiogenesis and tumor growth but also the influence of that drug on the surrounding tissues. This advantage is highly beneficial when monitoring side effects and determining the toxicity of therapeutics during drug development.

Targeting Injections and Biopsies

Because of its real-time nature, a unique utility to micro-ultrasound is its ability to guide targeted injections or biopsies without having to do surgical intervention (Figure 4). As such, intricate procedures such as the placement of probes or invasive monitors in animals, the targeted injection of stem cells into specific tumor or anatomical targets, or even the specific placement of probes *in vivo* can be visualized non-invasively using micro-ultrasound. As a result of this non-invasive guidance facilitated by micro-ultrasound, surgery and stress on the animal is minimized or eliminated, accuracy of the procedures and of the injections is increased significantly, and results are more quantitative and reproducible. This was illustrated by Goessling et al.; they demonstrate imaged-guided tumor biopsy in adult zebrafish with hepatic tumor. A 23G needle was guided into the tumor and a sample was taken and the sample was subsequently implanted in a separate recipient zebrafish (Goessling et al 2007). The implanted tumor can still be detected *in vivo* by micro-ultrasound weeks later.

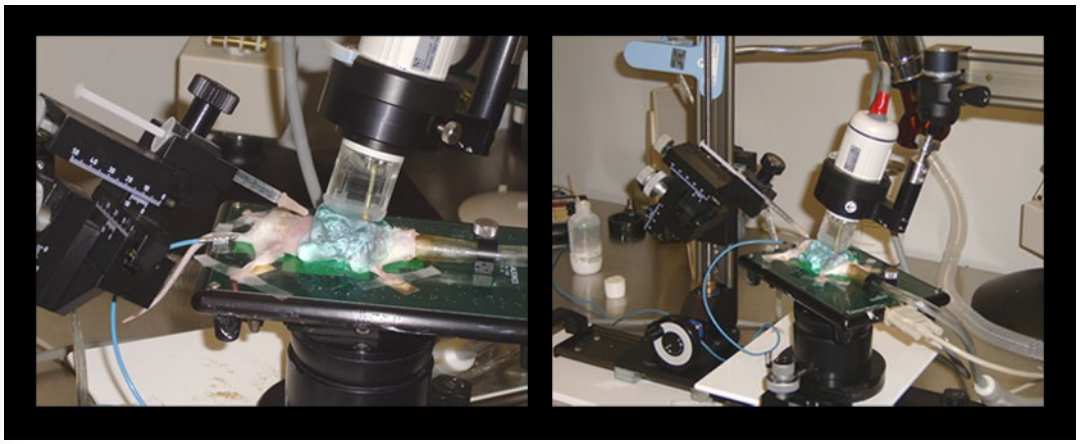

FIGURE 4: Image-Guided Injection with real-time imaging probe for visualization of targeted injections and biopsies.

This apparatus allows for real-time image guidance of precise injection and procedures in vivo such as performing in utero injections into mouse embryos, tissue biopsies and closed chest cardiac injections in adult mice as depicted in the image.

Contrast Agents for Performing Real-Time Tumor Perfusion and Targeted Molecular Imaging And Quantification of Biomarkers

Ultrasound contrast agents (UCAs) have traditionally been used to improve imaging by introducing a material into the blood stream with different acoustic properties from that of tissues (Blomley et al. 2001). The most common agents are air- or gas-filled microbubbles which increase the echogenicity of the surrounding tissue and increase the Doppler signal from blood vessels. These agents are normally injected intravenously, in the case of animal models via the tail vein or jugular vein.

Microbubbles function by providing a strongly reflective blood/gas interface making them several orders of magnitude more reflective than normal blood (Liang & Blomley 2003). As such, microbubbles enhance both normal grey-scale and flow-mediated Doppler signals. There are currently three types of microbubbles available consisting of either an albumin (eg. Albunex), lipid (e.g VisualSonics' MicroMarker™, Optison & Definity) or polyer membrane containing a gas such as nitrogen or perflurocarbon. The three key features of microbubble contrast agents are:
(1) they are true intravascular agents unlike the diffusible agents commonly used in magnetic resonance imaging or computed tomography;
(2) microbubbles have a strong nonlinear response to sound, creating a unique signature that permiting echoes from the true microcirculation to be differentiated from those of surrounding tissues; and
(3) microbubbles can be disrupted or "popped" with a controlled ultrasound pulse creating a quantitative means of probing perfusion of selected tissue regions or releasing a therapeutic payload (Liang & Blomley, 2003).

A rapidly growing area in ultrasound micro-imaging is the use of microbubble contrast agents for perfusion studies and targeted molecular imaging in cardiology, oncology and other application areas involving flow in soft tissue organs. Currently, there is a great demand for evaluating microvascular perfusion in tumors and determining which drug regimes are able to restrict angiogenesis and blood flow in carcinogenesis. The use of microbubbles coupled with micro-ultrasound is ideal as the micro-ultrasound imaging modality provides high-resolution of tumor structure while the microbubbles behave in the microcirculation like red blood cells and are therefore restricted specifically within the vasculature. As such, they can be used to determine and quantify how well vascularized a tumor is and the level of blood vessel heterogeneity within that tumor (Provenzale et al. 2007). Most importantly, the information gathered can then be used to determine whether a specific therapy, treatment regime or dosage is having the required effect on decreasing microvascular blood flow.

A recent study explored the reproducibility of contrast-enhanced ultrasound imaging. The author compared tumor image intensities between 2 subsequent contrast bolus injections (Loveless et al. 2008). Linear regression analysis yield a correlation coefficient of 0.99, suggesting a very tight correlation between repeated injections. These results show that contrast-enhanced ultrasound

imaging is a highly reproducible and reliable modality to assess tumor perfusion. Another study investigated the role of mitogen activated protein kinase (MAPK) kinase (MKK) signaling in growth and vascularization of soft tissue sarcomas (Ding et al. 2008). Nude mice with subcutaneous tumors (human firbrosarcoma cell line) were treated with anthrax lethal toxin (LeTx), which acts as an inhibitor to most MKKs. Contrast agent was infused through the tail vein to assess tumor perfusion dynamics immediately before and 24 hours after treatment. Following LeTx treatment, tumor flow significantly decreased. Kidney perfusion was also assessed and showed no difference in flow following LeTx treatment. These results show MKK signal is essential in tumor growth and vascularization (Ding et al. 2008). Tumor perfusion has also been assessed in zebrafish. Goessling et al. injected contrast agent to assess hepatic tumor flow dynamics in adult zebrafish (Goessling et al. 2007).

Another advantage of using microbubbles with micro-ultrasound is the ability to destroy or burst microbubbles using low-frequency ultrasound pulses. This approach is useful as one can introduce a bolus of microbubbles, allow them to reach a steady state within the tumor and then apply a destroy burst. The spontaneous acquisition of the images during the destruction sequence will now show an equilibrium of microbubbles re-entering or leaving the tumors microcirculation within the ultrasound beam profile. The acoustic intensity from microbubbles, or the amount of contrast enhancement at that time point, can then be used to quantify the relative microvascular blood volume in that tumor.

This technique is not limited to studying blood flow within tumor but can be applied to perfusion studies in other soft tissues and organs. As reported by Wei et al. 1998, the rate of replenishment of microbubbles and the measured rate of increase in microbubble acoustic signal within the field of view can be used quantitatively to reflect red blood cell velocity in murine myocardium. Perfusion at the microvascular level can subsequently be determined by the product of microvascular RBC velocity and volume (Wei et al. 1998). Preclinical contrast agents are also commercially available (VisualSonics, MicroMarker) that can, through a streptavidin linker on its outer shell, be targeted at site-specific biomarkers (endothelial cell markers). The methodology allows high throughput screening or the detailed evaluation of expression profiling of target ligands. In angiogenic studies, for example, the target-ready contrast agent can be quickly conjugated with an antibody against VEGFR2. The agent is then introduced into the animal through venous injection, and the animals screened for relative expression of the tagged contrast agent. The animals can then be injected with an anti-angiogenic drug and the effect of the therapeutic on the expression of the VEGFR2 receptor can be quantified. This whole procedure can occur *in vivo* in the same animals and at multiple time points over a longitudinal time course. This procedure was first described by Rychak (2007) and subsequently by Lyshchik et al. 2007, concluding that targeted contrast-enhanced high-frequency micro-ultrasound allows *in vivo* molecular imaging of VEGFR2 expression on the tumor vascular endothelium and may be used for non-invasive longitudinal evaluation of tumor angiogenesis in preclinical studies. More recently, Willmann et al. 2008 showed high VEGFR2 expression in subcutaneous tumor (mouse angiosarcoma SVR cells) grown in nude mice using targeted VEGFR2 contrast agent. VEGFR2 expression was confirmed by immunohistochemistry.

Furthermore, this contrast agent was mixed in in vitro cell culture to examine the binding affinity of the targeted contrast agent to angiosarcoma SVR cells. These results showed an average of 2 targeted VEGFR2 microbubbles binding to each angiosarcoma cell, suggesting very high binding affinity exists between targeted VEGFR2 microbubbles and angioscarcoma cell (Willmann et al. 2008).

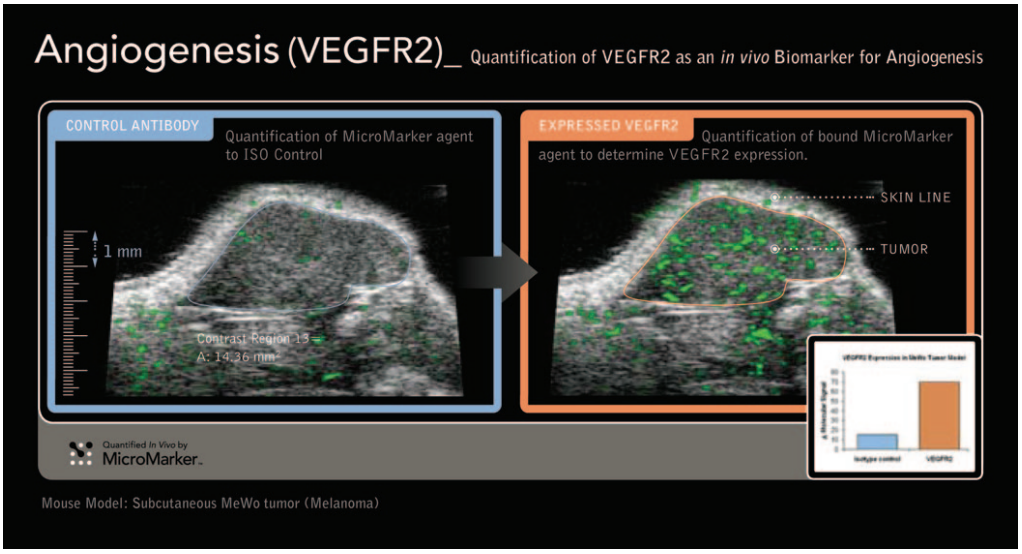

FIGURE 5: Real-Time Micro-Ultrasound Imaging of Lady Tramp Prostate Mouse Model at 48 Weeks

A 2D image of a Lady Tramp Prostate Mouse Model at 48 Weeks (left) is matched with a 3D volumetric image of the same tumor (right). Quantification of tumor volume is easily determined.

Detection and Quantification of Cardiocytotoxicity in Response to Anti-Cancer Therapy

Advancements in cancer therapy have lead to oncology patients receiving complicated therapeutic management regimes combining drugs, radiation or chemotherapy and surgery, which often result in adverse cardiovascular side effects and cardiotoxicity. Cardiotoxicity, a condition involving damage to the heart muscle resulting in decreased cardiac function, can lead to cardiomyopathy (Galderisi et al. 2007) or death if left untreated or if the response is severe. Cardiotoxicity has commonly been seen in oncology patients receiving Anthracyclines however other anti-cancer drugs such as but not limited to Alkylators, antimetabolites, antimicrotubal, cytokines and monoclonal antibodies can also result in adverse cardiac and cardiovascular effects (Myers 1998, Braverman et al. 1991, Frickhofen et al. 2002, Sevelda et al. 1994, Kammula et al. 1998 and Cersosimo et al. 2003).

Most common side effects associated with cardiotoxicity are an alteration of cardiac rhythm, changes in blood pressure and ischemia, and can also alter the ability of the heart to contract and/or relax. Detection of these symptoms can help to prevent further cardiac disease and allow clinicians to optimize or choose less toxic therapeutic regimes (Jones and Ewer 2006).

Common non-invasive methods for monitoring cardiotoxicity in patients are Pulsed-wave, tissue, and Color Doppler imaging, and advanced myocardial strain analysis. Pulse-wave and tissue Doppler measure cardiac flow and myocardial velocity profiles respectively, which are altered in abnormal cardiac function. Color Doppler imaging displays cardiac flow velocities represented by a color map. This can rapidly and efficiently identify abnormal flow disturbances. Advanced strain analysis, based on speckle tracking of the myocardial wall, can assess strain and strain rate of the myocardium. Collectively, these ultrasound imaging modes allows for identification of cardiovascular pathologies such as left ventricular (systolic and diastolic) dysfunction, valve heart disease, pericarditis and pericardial effusion, carotid artery lesions.

Since the occurrence of cardiotoxicity in patients receiving anti-cancer therapy is high, there is a great need for researchers in drug discovery and development to monitor and detect these observations at the preclinical level in animal models. This can be achieved by using the same micro-ultrasound platform to not only monitor individual tumor growth and response to anti-cancer therapy but to also use this imaging modality to study the effects of the same drugs on cardiac function. The same cardiovascular indicators and measurements are made preclinically on the micro-ultrasound as they are mode clinically on humans using a clinical ultrasound. As such, the modality and insights are completely translational from bench to bedside.

For example, Gabrielson and colleagues reported, using *high-frequency* micro-ultrasound, that doxorubicin chemotheraphy treatment induced cardiocytoxicity. They showed that 15mg of doxorubicin given to 10 week old Sprague-Dawley rats over a 6 week span induced cardiac dysfunction, indicated by decreased fractional shortening. In contrast, rats that received a lower dose of 7.5mg over the same 6 week span did not develop cardiac dysfunction (Gabrielson et al. 2007).

In a separate study, Lien and colleagues investigated the effects of the chemotherapeutic agent Adriamycin on cardiac function in wild type C57B/6 mice and double knock out (DKO) TNF receptor deficient and P55 deficient mice. The results obtained showed that three days of Adriamycin treatment resulted in cardiac dysfunction in both wild-type mice and DKO mice as indicated by the decreases of heart rate, left ventricular ejection fraction, fractional shortening, stroke volume, and cardiac output (Lien et al. 2006).

By using the same micro-utrasound imaging modality, these researchers were able to compare the cytotoxicity effect of adriamycin to phenylbutyrate, a histone deacetylase inhibitor which has shown promise in the treatment

of cancer due to its anti-oxidative effects in normal tissues. They found that phenylburyrate was cardioprotective as it was able to completely rescue the mice from the Adriamycin-induced reduction of cardiac functions exemplified by ejection fraction and fraction shortening, and increased cardiac manganese superoxide dismutase (MnSOD) protein and activity (Daosukho et al. 2007).

These studies demonstrate the great potential of using micro-ultrasound in not only monitoring the cardiotoxicity of anti-cancer therapies at the preclinical level but also in testing out new therapeutic agents and determining optimal dosage regimes.

Expanding into Gene Delivery

Another interesting application of micro-ultrasound is its potential to be used in the area of gene delivery. Low frequency ultrasound can induce cells to take up large molecules through the mechanism of acoustic cavitation. The broad ultrasound wave interacts with cavitation bubble (i.e. microbubbles described above), which concentrate acoustic energy and produce mechanical (i.e., nonthermal) effects on nearby cells. The predominant effect is cell lysis, but sublethal membrane damage can occur, in which the membrane is transiently permeable to large molecules. This phenomenon, called sonoporation, encompasses even DNA and has recently been evaluated for ultrasonically-enhanced gene transfection (Takeshita et al. 1996; Wasan et al. 1996; Manome et al. 2000; Sakakima et al. 2005). These in vitro studies have demonstrated that DNA plasmids (relatively small circular DNA segments that code for reporter proteins) entered cells during ultrasound exposure, and that some of these cells survived to express the reporter gene product in culture. The strategy of *in vivo* anticancer therapy by gene transfer into tumor cells has made tremendous progress in the last decade. Numerous clinical trials, which typically involve use of viral vectors to produce immunotherapy, are underway to evaluate safety and efficacy (Mangel et al. 2002). The desired outcome of the immunotherapy method is to stimulate immune defenses, not only to attack the treated tumor mass, but also to destroy remaining survivors and metastases throughout the body (Tuting et al. 1997). Due to the disadvantages of viral vectors and because of the need to specifically target tumors (Hwu 1997) the combination of micro-ultrasound with microbubbles shows promise in gene therapy (Taniyama 2002 & Hashiya et al. 2004).

Conclusion

Micro-ultrasound offers tremendous potential as a central modality for preclinical diagnostic and therapeutic *in vivo* imaging in the area of cancer biology. Micro-ultrasound monitoring and quantification of tumor growth and responsiveness has been proven to be effective in many subcutaneous and orthotopic tumor models and targets. Real-time quantification of tumor vascularity, flow dynamics and flow architecture allows for the study of tumor growth, vascular pathway development and for anti-angiogenic therapeutic efficacy. With the use of microbubble technology, this same imaging platform can now perform tumor perfusion imaging

and molecular imaging and quantification of targeted biomarkers *in vivo*. Cell death can also be evaluated and quantified using the digital radio frequency signal derived from the micro-ultrasound for the study of apoptosis and efficacy of chemo- and radiotherapy treatments. Cardiovascular assessments can also be performed for serial cordiotoxixity assessments, especially important in preclinical therapeutic studies. This unique modality now also offers the promise of entering the area of gene delivery using sonoporation. As one of the most pervasive clinical modalities, micro-ultrasound is quickly becoming a central *in vivo* modality for performing anatomical, functional and molecular imaging in small animal cancer research and drug development.

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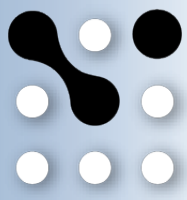

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