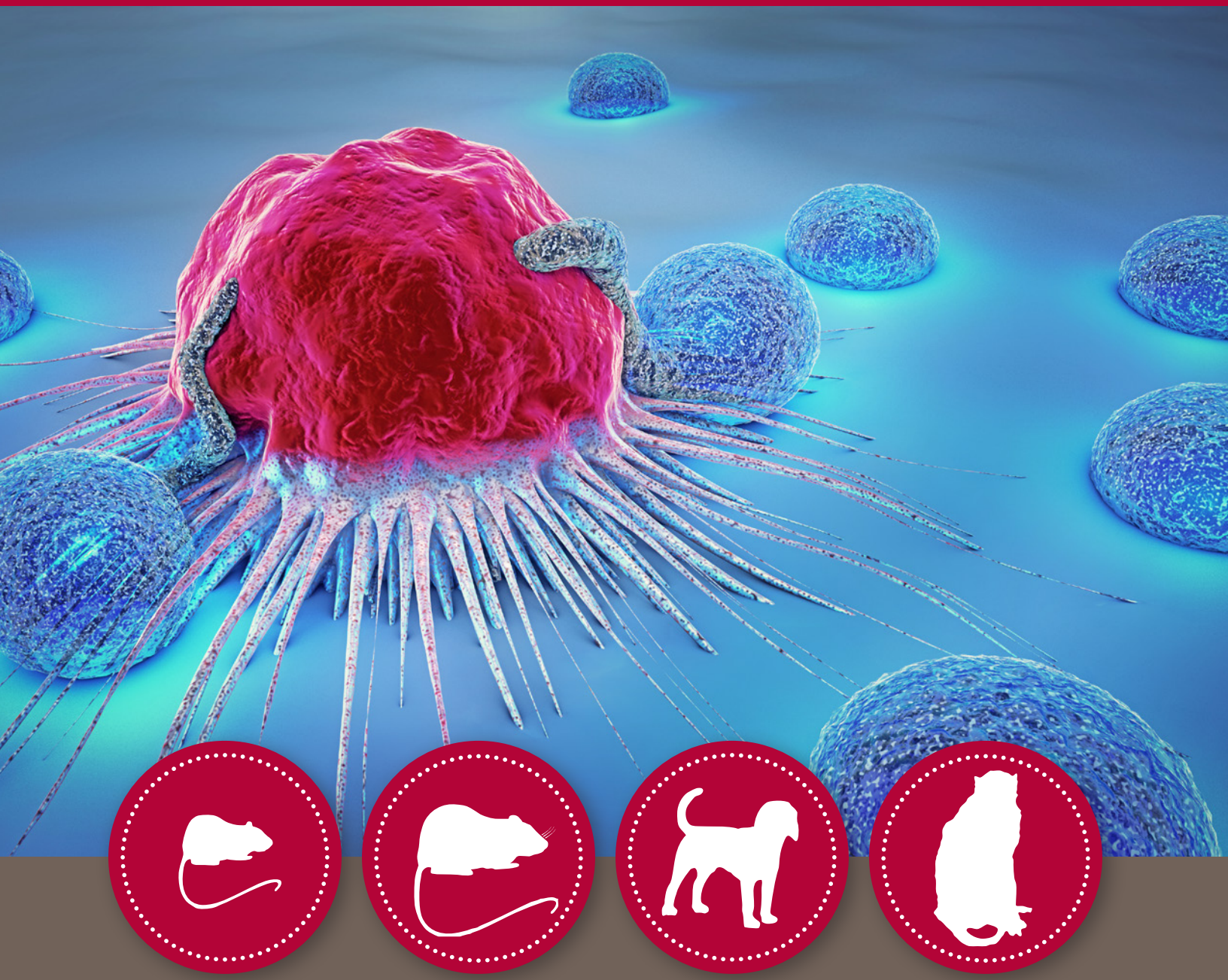
Advancing Research

Novel Approaches to Studying Cancer Physiology in Preclinical Research Models



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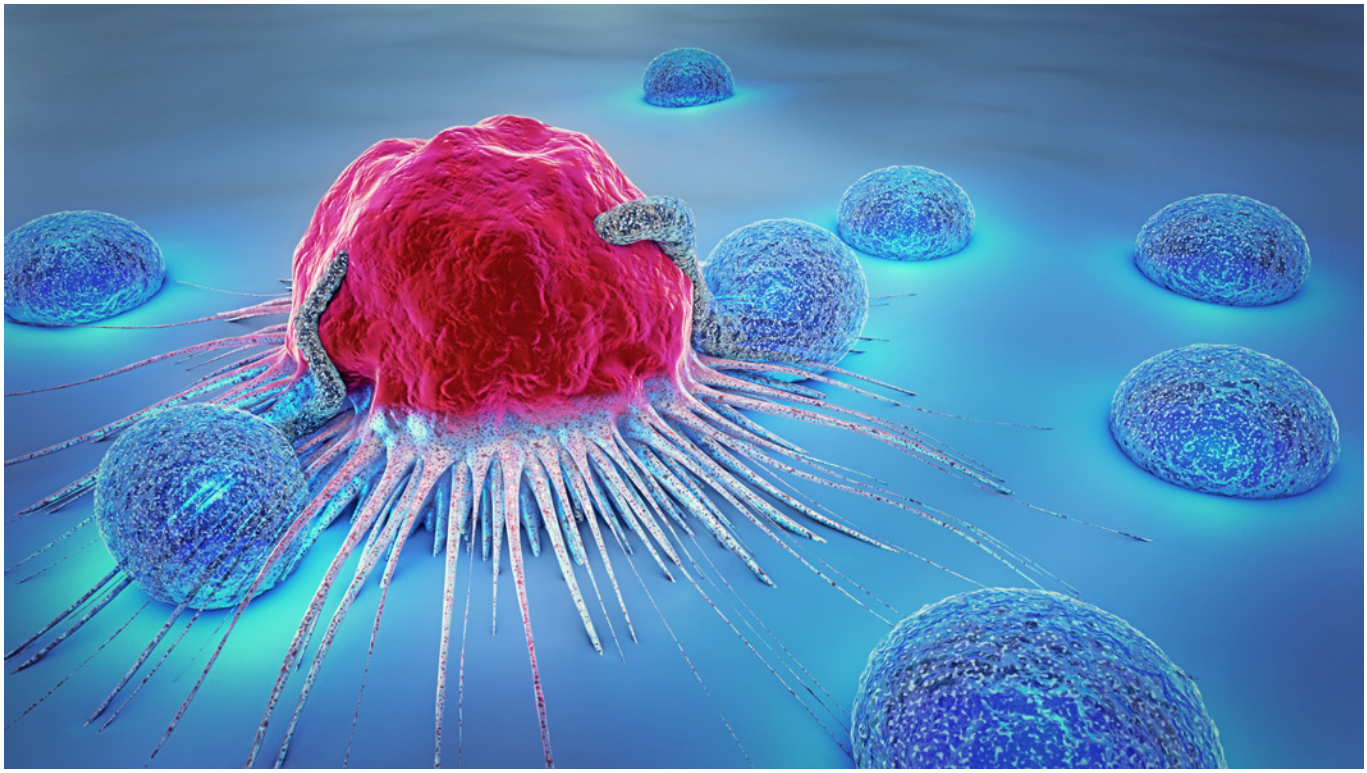
Introduction

Cancer has become one of the leading causes of death and is on track to take the lead over heart disease possibly as early as 2020. According to the World Health Organization, in 2015 cancer accounted for 8.8 million deaths worldwide and the number of new cancer cases is expected to rise by as much as 70% over the next two decades.[1]

Throughout the world, researchers from academic institutions to government initiatives are working together to find a way to beat cancer. Animal studies continue to be at the forefront of cancer breakthroughs and achievements. Treatments and diagnostics that are saving hundreds of thousands of cancer patients a year exist thanks to preclinical assessments in animal models. While cancer research has seen significant progress over the past 20 years, there are still challenges due to the complex tumor microenvironment, tumor heterogeneity, and dynamic immune and metabolic responses. Today, only 8.3% of cancer drugs receive FDA approval, up from 3.4% in 2015.[2] Biomarkers and physiological endpoint data have influenced the increased rate of approvals, and their use in preclinical models to better predict translatability and safety into the clinic will only continue to grow.

This paper provides researchers with the following information: a) novel endpoints in cancer research, b) current techniques and models used to obtain novel endpoints, c) references of relevant webinars and peer-reviewed journal articles, and d) products used to collect these endpoints.



Interstitial Fluid Pressure

Increased solid tumor pressure has been recognized as a barrier to effective cancer treatment that leads to poor prognosis in both humans and animals. Solid malignant tumors arise out of tissue or bone and make up a majority of cancer diagnoses. The pathophysiologic factors shown to cause increased tumor pressure include the tumor microenvironment, vascular abnormalities, and the proliferation of cancer cells within the tumor. Interstitial fluid pressure (IFP) of solid malignant tumors has become a novel biomarker to monitor therapeutic uptake and cancer aggressiveness.

Animals being used in research to monitor tumor IFP include small, medium and large models, with transgenic mice being the most popular due to their wide spectrum of mutations mimicking human cancers. Both invasive and noninvasive approaches to measure tumor IFP are used, including imaging techniques and pressure sensing devices. Researchers monitoring tumor pressure in animal models are able to observe a correlation between tumor growth, increased IFP and a decrease in treatment response. This data is giving researchers earlier indications as to whether a compound or treatment is having an effect on solid tumor progression and the state of the tumor microenvironment.

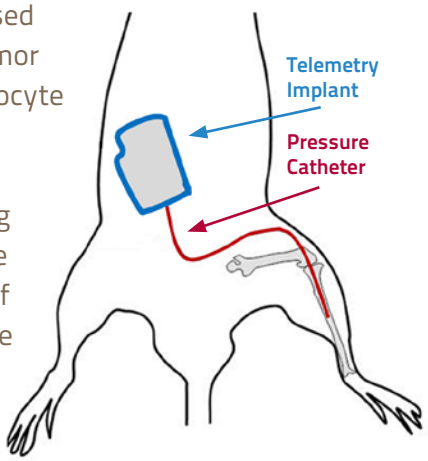
Suggested Tumor IFP Research References::

Sabelstrom, H., Iikhanizadeh, S., Miroshnikova, Y. A., Frantz, A., Zhu, W., Idilli, A., . . . Persson, A. I. (2017). Exth-23. Antisecretory Factor-Mediated Lowering Of Interstitial Fluid Pressure Produces Anti-Tumor Activity In Glioblastoma. *Neuro-Oncology,19* (Suppl_6), Vi77-Vi77. doi:10.1093/neuonc/nox168.317.

Bone Pressure

Bone tissue is one of the most common sites of metastasis and occurs when cancer cells from a primary tumor, typically breast, lung or prostate, migrate to the bone through the blood stream or lymphatic system. Secondary bone cancer, caused by metastasis, is more common than primary. Currently, there is no cure for bone metastasis, only treatment that reduces its progression.

Animal models used to measure bone pressure range from small to large animals. The most common technique used to induce tumor associated bone pressure is to inject tumor cells directly into the intramedullary cavity. Researchers are also studying naturally occurring bone metastasis by injecting cancer cells into the mammary fat pad for breast cancer or directly into the prostate. Left ventricular intracardiac injection is also a popular choice and can be used to determine distal metastasis as well as tumor cell circulation. By measuring tumor pressure within the bone, researchers are finding that the increased pressure caused by tumor growth promotes osteocyte secretion, leading to increased metastasis. Researchers are placing pressure sensors in the intramedullary cavity of the bone to obtain bone pressure and monitor tumor growth.



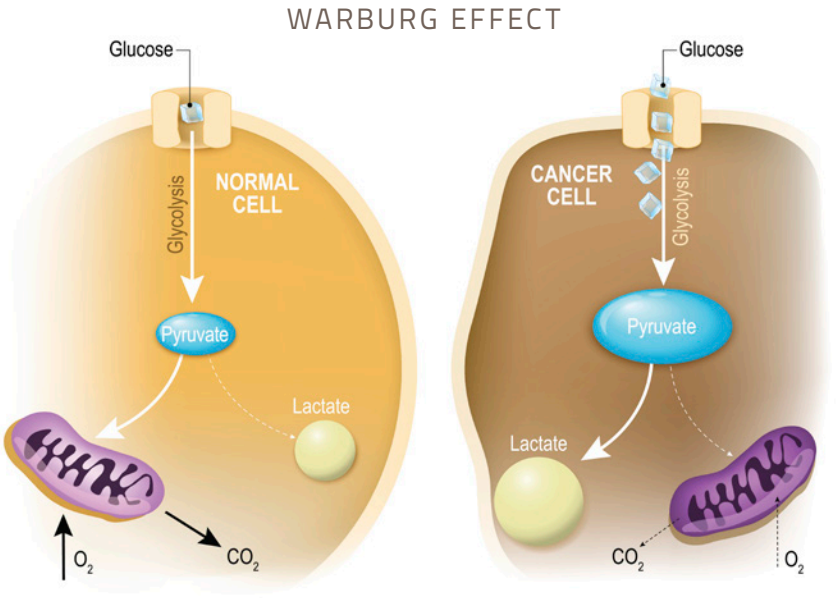
Suggested Bone Pressure Research References::

Kwon, R. Y., Meays, D. R., Tang, W. J., & Frangos, J. A. (2010). Microfluidic Enhancement of Intramedullary Pressure Increases Interstitial Fluid Flow and Inhibits Bone Loss in Hindlimb Suspended Mice. Journal of Bone and Mineral Research, 25(8), 1798–1807. http://doi.org/10.1002/jbmr.74

Sottnik, J. L., Dai, J., Zhang, H., Campbell, B., & Keller, E. T. (2015). Tumor-Induced Pressure in the Bone Microenvironment Causes Osteocytes to Promote the Growth of Prostate Cancer Bone Metastases. Cancer Research,75(11), 2151-2158. doi:10.1158/0008-5472.can-14-2493

Glucose Metabolism

Cancer cells are known for their ability to proliferate through unregulated cellular metabolism, invasion and metastasis. The driver is their ability to alter their energy production from oxidative phosphorylation to a glucose-dependent glycolytic pathway, regardless of oxygen availability, known as the Warburg effect.[3] The correlation between high blood glucose and tumor malignancy is leading researchers to look at what role diet and type 2 diabetes (T2D) may play in activating or promoting tumor cell growth. In addition, cancer treatments have been shown to alter the way humans metabolize glucose, causing induced hyperglycemia and T2D. Treatment related hyperglycemia and T2D have been shown to occur in several anticancer therapies such as anti-PD-1, TKIs, glucocorticoid dexamethasone, and L-asparaginase.[4]



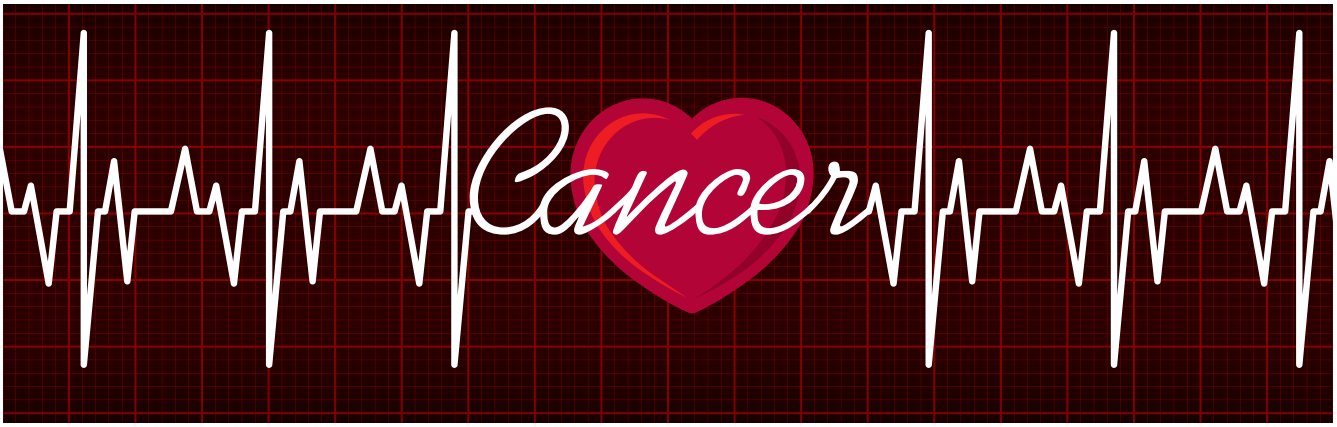
The most commonly used animal model in glucose research are mice, who are able to be genetically modified to have conditions such as obesity, insulin resistance, impaired glucose tolerance, and/or satiety hormone disorders. Genetically altered rats are also being used on a smaller scale and researchers are finding that larger animal models, like minipigs and non-human primates, are beneficial to studying translational research. Monitoring blood glucose remains the gold standard biomarker of glucose metabolism in cancer research and provides increased translatability to the clinical setting.

Suggested Glucose Research References::

Dechend, MD, R., & Schnell, C. (2015, November 18). Continuous Glucose Monitoring for Diabetes, Obesity and Metabolism Research in Rodents - InsideScientific [Video file]. Retrieved from https://insidescientific.com/webinar/continuous-glucose-monitoring-diabetes-obesity-metabolic-research-rodents-data-sciences/

Schnell, C. R., Ferrat, T., Fritsch, C., & Jensen, M. R. (2015). Abstract B108: Assessment of the hyperglycemia profile of NVP-BYL719, a selective inhibitor of the class Ia PI3K isoform alpha using real-time continuous glucose monitoring via radio-telemetry in rats. Molecular Cancer Therapeutics, 14(12 Supplement 2). doi:10.1158/1535-7163.targ-15-b108

Simonds, Ph. D., S., & Fine, DVM, M. (2018, July 12). WEBINAR: From Mouse to Monkey: Revolutionizing Research via Preclinical Continuous Glucose Telemetry [Video file]. Retrieved from https://insidescientific.com/webinar/mouse-to-monkey-preclinical-continuous-glucose-telemetry-DSI



Cardio-Oncology

While modern cancer treatments have led to improved survival rates among the most common types of cancers, various cancer treatments ranging from traditional cytotoxic agents and radiology, to newly developed immunotherapies and anti-angiogenics have been linked to adverse cardiac events. For example, anti-angiogenic cancer drugs, like VEGF receptor (VEGFR) inhibitors cause hypertension in 15 – 60% of occurrences.[5] Researchers are using cardiovascular safety assessments in animal models help to validate or rule out compounds earlier in preclinical studies.

Researchers studying cardiovascular responses and drug safety of potential cancer treatments use a variety of animal models, including rodents, rabbits, dogs and non-human primates. Long-term studies with continuous cardiovascular data can improve understanding of cardiac function post treatment, facilitating earlier interventions and improved quality of life. Primary cardiovascular endpoints include systolic and diastolic arterial pressure, left ventricular pressure, heart rate, and electrocardiogram.

Suggested Cardiovascular Research References::

Charych, D., Hoch, U., Langowski, J., Lee, S., Addepalli, M., Kirk, P., . . . Doberstein, S. (2016). NKTR-214, an Engineered Cytokine with Biased IL2 Receptor Binding, Increased Tumor Exposure, and Marked Efficacy in Mouse Tumor Models. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research, 22*(3), 680-90.

Collins, T., Gray, K., Bista, M., Skinner, M., Hardy, C., Wang, H., . . . Harmer, A. R. (2018). Quantifying the relationship between inhibition of VEGF receptor 2, drug-induced blood pressure elevation and hypertension. *British Journal of Pharmacology, 175*(4), 618-630. doi:10.1111/bph.14103

Kim, T., Kim, K., Seo, J., Park, S., & Henry, S. P. (2014). Antisense oligonucleotides on neurobehavior, respiratory, and cardiovascular function, and hERG channel current studies. *Journal of Pharmacological and Toxicological Methods, 69*(1), 49-60. doi:10.1016/j.vascn.2013.10.005

Miller, Nace, Ayala-Breton C, Steele, Bailey, Peng, & Russell. (2016). Perfusion Pressure Is a Critical Determinant of the Intratumoral Extravasation of Oncolytic Viruses. Molecular Therapy, *24*(2), 306-317.

Circadian Rhythm

Circadian rhythm is an endogenous clock that is present in nearly all organisms and is governed by the 24- hour light/dark cycle. This rhythm regulates biological functions from sleep patterns to cellular activities such as metabolism and cell division. The central clock is located within the brain's suprachiasmatic nucleus (SCN) and environmental cues stimulate the SCN to communicate information to peripheral clocks, located in organs and cells. Research has shown links between sleep disturbances, such as shift work and jet lag, and the development of certain types of cancers.

In 2007, the International Agency for Research on Cancer identified shift work as a possible carcinogen.[6] Circadian rhythm also has its place in cancer treatment, where the timing of anticancer therapies given during particular times of the day have improved efficacy. In addition, researchers are looking at the long term effects of chemotherapy and additional therapies, which can cause sleep disturbances (table 1). Sleep patterns *in vivo* can be measured by physical activity correlated to resting periods. Identifying sleep duration is often used when studying circadian rhythm. With the use of biopotential leads and synchronized video, the different stages during sleep can be identified. Sleep is determined by physiological changes in EEG together with the EMG and EOG (electrooculogram - eye movement). Other variables including temperature, blood pressure and neuroendocrine function can be added to gain extra information about the circadian process.

Suggested Circadian Rhythm Research References::

Brager, Allison J., et al. "Sleep Loss and the Inflammatory Response in Mice Under Chronic Environmental Circadian Disruption." *PLoS ONE*, vol. 8, no. 5, 2013, doi:10.1371/journal.pone.0063752.

Borniger, J. C., Ii, W. H., S., Emmer, K. M., Zhang, N., Zalenski, A. A., . . . Devries, A. C. (2018). A Role for Hypocretin/Orexin in Metabolic and Sleep Abnormalities in a Mouse Model of Non-metastatic Breast Cancer. *Cell Metabolism, 28*(1). doi:10.1016/j.cmet.2018.04.021

Borniger, J. C., Gaudier-Diaz, M. M., Zhang, N., Nelson, R. J., & Devries, A. C. (2015). Cytotoxic chemotherapy increases sleep and sleep fragmentation in non-tumor-bearing mice. *Brain, Behavior, and Immunity, 47*, 218-227. doi:10.1016/j.bbi.2014.11.001

Table 1: Cancer Treatment as a Cause of Insomnia

Cancer Treatment	Mechanism of Sleep Disturbance
Chemotherapy	Cancer-related fatigue, daytime sleepiness, mood disorders, restless leg syndrome
Radiation Therapy	Daytime sleepiness, radiation-induced emesis causing difficulty sleeping, fatigue
Hormonal Therapy	Hot flashes, night sweats, menopause-like symptoms, anxiety
Biological Therapy	Daytime fatigue, malaise
Corticosteroids	Increase in serum cortisol, hyperglycemia
Bone Marrow Transplantation	Severe anemia causing daytime fatigue and restless leg syndrome
Surgery	Pain, recovery from surgery, surgery with aesthetic or functional impairments

Cleveland Clinic Journal of Medicine. (2017, September 25). Sleep disturbances in cancer patients: Underrecognized and undertreated. Retrieved October 05, 2018, from https://www.mdedge.com/ccjm/article/95907/sleep-medicine/sleep-disturbances-cancer-patients-underrecognized-and/page/0/1

Temperature

Temperature is a valuable biomarker in understanding physiological responses in acute and chronic studies. Researchers monitor temperature as an early indicator of disease and response in animals and humans. Temperature is considered highly translatable to the clinical setting and cancer researchers use data collected to assess tumor burden, therapeutic response, immunity status, and stress.[7]

Temperature medicine has also gained ground in the past 10 years, as hyperthermia is being used as an adjunct cancer treatment in the clinical setting to promote tumor-treatment response. Temperatures of between 38.5 and 42 degrees Celsius have been shown to decrease hypoxia within the tumor microenvironment, improve efficacy of immunotherapies, and increase production of pro-inflammatory cytokines.[8]

Monitoring thermoregulation is done in a variety of species, from mouse to non-human primate. With temperature being one of the first signs of disease, it is also being used in a variety of different disease models.

To understand which temperature has the greatest impact on tumor response, researchers are monitoring core body temperature, subcutaneous temperature, and local temperature. Heart rate and physical motor activity are additional endpoints that are observed to assess for overall health of the animal. Methods of collecting temperature data include rectal thermometer, infrared imaging, tail-skin temperature, and implantable telemetry. Monitoring temperature in preclinical cancer studies leads to better, more translatable research.

Suggested Temperature Research References::

Barra, Nicole G., Palanivel, Rengasamy, Denou, Emmanuel, Chew, Marianne V., Gillgrass, Amy, Walker, Tina D., . . . Ashkar, Ali A. (2014). Interleukin-15 Modulates Adipose Tissue by Altering Mitochondrial Mass and Activity. *PLoS ONE, 9*(12), E114799.

Meyer, C., Ootsuka, Y., & Romanovsky, A. (2017). Body Temperature Measurements for Metabolic Phenotyping in Mice. *Frontiers in Physiology, 8*, 520.

Ruud, Nilsson, Engström Ruud, Wang, Nilsberth, Iresjö, . . . Blomqvist. (2013). Cancer-induced anorexia in tumor-bearing mice is dependent on cyclooxygenase-1. *Brain Behavior and Immunity, 29*(C), 124-135.

REFERENCES:

1 Cancer. (2018, February 1). Retrieved from http://www.who.int/news-room/fact-sheets/detail/cancer

2 Chi Heem Wong, Kien Wei Siah, Andrew W Lo; Estimation of clinical trial success rates and related parameters, *Biostatistics*, , kxx069, https://doi.org/10.1093/biostatistics/kxx069

3 Tekade, R. and Sun, X. (2017). The Warburg effect and glucose-derived cancer theranostics. *Drug Discovery Today*, 22(11), pp.1637-1653.

4 Goldman, J. W., Mendenhall, M. A., & Rettinger, S. R. (2016). Hyperglycemia Associated With Targeted Oncologic Treatment: Mechanisms and Management. *The Oncologist, 21*(11), 1326–1336. http://doi.org/10.1634/theoncologist.2015-0519

5 Researchers at AstraZeneca Describe Findings in Hypertension (Quantifying the relationship between inhibition of VEGF receptor 2, drug-induced blood pressure elevation and hypertension). (2018). Health & Medicine Week, 237.

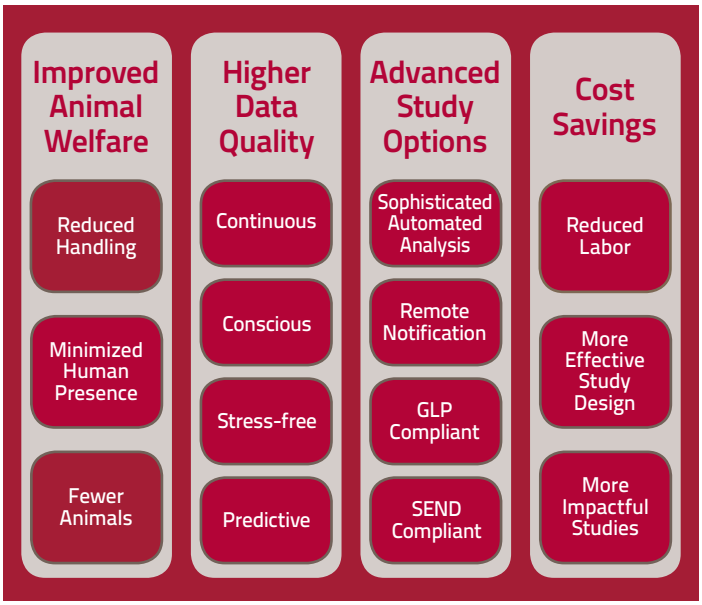
6 (n.d.). IARC - INTERNATIONAL AGENCY FOR RESEARCH ON CANCER. *Press Release N° 180*. Retrieved July 23, 2018, from http://www.iarc.fr/en/media-centre/pr/2007/pr180.html

7 J E Hunter, J Butterworth, N D Perkins, M Bateson, & C A Richardson. (2014). Using body temperature, food and water consumption as biomarkers of disease progression in mice with Eμ-myc lymphoma. *British Journal of Cancer, 110*(4), 928-34.

8 Repasky, E. A., Evans, S. S., & Dewhirst, M. W. (2013). Temperature Matters! And Why it Should Matter to Tumor Immunologists. *Cancer Immunology Research, 1*(4), 210–216. http://doi.org/10.1158/2326-6066.CIR-13-0118

The Value of Continuous Telemetry

Collecting physiological data from animals is often a challenge for any researcher. The value of the data collected must be justified by the amount of time, money and resources that go into every study. More robust data collection, under natural conditions using implantable telemetry are giving researchers new insights into animal physiology. Implantable telemetry systems provide some ease of mind for researchers by acquiring, analyzing and reporting *in vivo* physiological data of freely moving animals. Using telemetry reduces animal handling and decreases stress-related artifact, giving researchers cleaner, more reliable data. Implants are available in a variety of models, shapes and sizes to optimize data collection, maximize lab space and simplify studies. The implant transmits information wirelessly, providing the most reliable and efficient method of measuring blood pressure, tissue pressure, glucose, electrocardiogram (ECG), electroencephalogram (EEG), electromyogram (EMG), core and subcutaneous temperature, and activity.

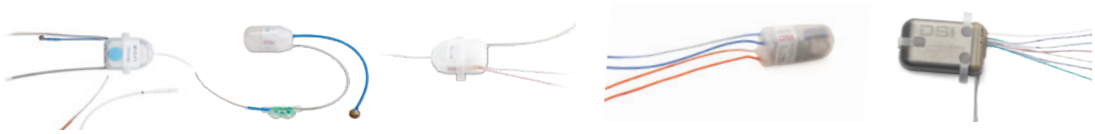


DSI: First Choice Among Researchers

Data Sciences International (DSI) has been committed to life science research for over 30 years and is the proven leader in preclinical physiological monitoring. DSI offers complete systems that sense, transmit, acquire and report physiological data. Each system is engineered with quality and performance you can trust.

- Systems available for an extensive variety of pre-clinical applications
- High-quality implants and hardwired systems that sense and transmit physiologic signals
- Accurate receivers, signal conditioners and amplifiers collect and transfer signals
- Robust software transforms signals into usable, clean, accurate data
- Knowledgeable and reliable support and services throughout your entire study to ensure the highest quality data

DSI Telemetry used in Oncology Research



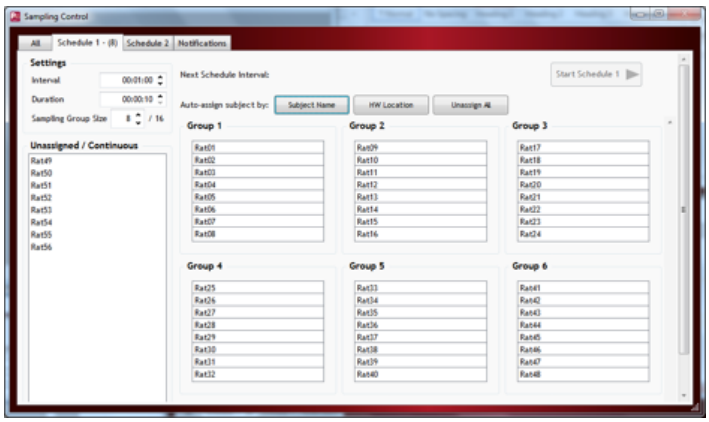
MOUSE MODEL	Pressure	Glucose	Temperature	EEG For Sleep Scoring	ECG
HD-XG		X	X		
HD-X10	X		X		
HD-X11	X		X		X
PA-C10	X				
TA-F10			X		
HD-X02			X	X	

SMALL ANIMAL MODEL	Pressure	Glucose	Temperature	EEG For Sleep Scoring	ECG
HD-XG		X	X		
HD-S10	X		X		
HD-S11	X		X		X
HD-S20	X2		X		
HD-S21	X2		X		X
TA-F40			X		
F40-TT			X2		
4ET			X	X	X
HD-S02			X	X	

LARGE ANIMAL MODEL	Pressure	Glucose	Temperature	EEG For Sleep Scoring	ECG
M0G		X	X		
M1G	X	X	X		
M10	X		X		
M11	X		X		X
L11	X		X		X
L21	X2		X		X
M00			X		
L03			X	X	X
L04			X	X	X

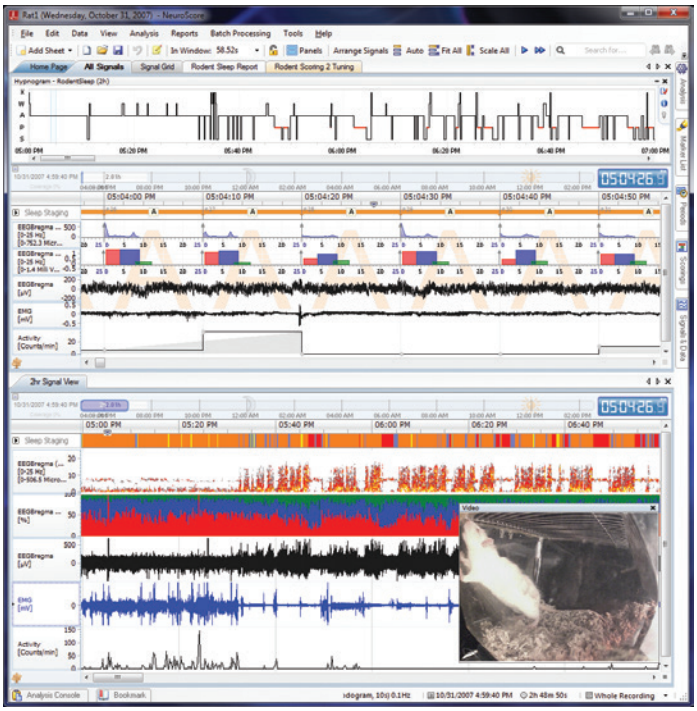
DSI Software used in Oncology Research

Ponemah is a complete physiological data acquisition and analysis system ranging from an acquisition interface unit and software to a complete PC-based scientific workstation. The multi-application platform uses state-of-the-art digital technology to automate the data analysis routinely performed in physiology, pharmacology and toxicology laboratories. The Ponemah system controls the flow of data from the collection of the incoming signal to data summary and final report generation. Ponemah integrates validated algorithms to reliably obtain accurate, consistent results and quickly deliver these results to you.



Power to Process More Data in Less Time.

NeuroScore™ is DSI's Central Nervous System (CNS) analysis software used to analyze neurophysiology data collected in Ponemah, EDF/EDF+ and other file formats. Researchers studying circadian rhythm will benefit from NeuroScore's batch processing capabilities, automated sleep scoring modules and video synchronization.



For more information on how to get the most reliable biomarker and endpoint data out of your oncology studies visit www.datasci.com

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