São Paulo Advanced School of Comparative Oncology: Abstracts

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INTRODUCTIONS

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Comparative Oncology, the theme of this School, is a new area of research and has undergone a special development over the past decades. The focus in this area has increasingly attracted the attention of researchers who are interested in etiopathogenesis, tumor biology, morphogenesis, epidemiology, genomics and cancer therapeutics. Comparative Oncology integrates the study of naturally occurring cancers in animals into studies of human cancer. These studies have enormous potential to discover novel treatments for human beings by treating pet animals, specially dogs and cats with naturally occurring cancer and that share similarities with human cancer genes.

The goal of the São Paulo Advanced School of Comparative Oncology was to promote the consolidation and expansion of basic and applied cancer research in the State of São Paulo. The international exchange of technological and scientific innovation aims to establish a globally competitive hub for talented researchers in this new area.

The ESPCA was attended by 32 speakers from different countries including Brazil, Canada, France, USA, Japan, Netherlands, Germany, Finland, and UK. The School provided an invaluable opportunity for networking and fruitful contacts with cancer researchers working with human and animals.

During the event it was created a Facebook page, on which photos and news concerning the lectures and interviews were daily updated. A journalist was hired to interview the lecturers and participants. Videos were also uploaded to an Youtube account, and the link was shared on the Facebook page. The official website of the School is http://www.comparativeoncologyspca.org/

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Institutional support was also provided by UNESP (http://www.unesp.br - PROFG, PROPe) and Brazilian Scientific Societies (ABPV – Brazilian Association of Veterinary Pathology; http://www.abpvvet.br; ABOVET – Brazilian Association of Veterinary Oncology; http://www.abrovet.org.br, and Brazilian Society of Genetics – SBG, Ribeirão Preto - SP, http://www.sbg.org.br).

A2
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FAPESP (http://www.fapesp.br/en) is a public foundation, funded by the taxpayer in the State of São Paulo, with the mission to support research projects in higher education and research institutions, in all fields of knowledge. São Paulo has a population of forty million and generates 35% of Brazil’s GNP. The constitution of the State establishes that 1% of all state taxes belong to the foundation and the government transfers these funds monthly. The stability of the funding and the autonomy of the foundation allow for an efficient management of the resources that has had a sizable impact: while São Paulo has 22% of the Brazilian population and 30% of the scientists with a doctorate in the country, the state responds for 52% of the country’s scientific articles published in international journals.

The foundation works in close contact with the scientific community: all proposals are peer reviewed with the help of area panels composed of active researchers. Besides funding research in all fields, the foundation supports large research programs in Biodiversity, Bioenergy, Global Climate Change, and in Neurosciences. FAPESP invested R$ 939 million (approximately US$ 660 million) in research projects in 2011. One third of this value goes into fellowships for graduate and undergraduate students. About 55% goes into exploratory academic research, mostly fundamental in nature. The remaining 10% is invested into application oriented research, in many cases performed in Small Businesses or in joint research performed by academia and industry. The percentage invested in applied research has been growing in recent years, consistently with the foundation’s mandate to foster the scientific and technological development in the State of São Paulo.

FAPESP maintains cooperation agreements with national and international research funding agencies, higher educational and research institutions and business enterprises. The international cooperation covers a broad range of countries and agencies (http://www.fapesp.br/en/6812) including...
the UK Research Councils, the Agence Nationale de Recherche (ANR) in France, the Deutsche Forschungsgemeinschaft (DFG) in Germany, and NSF in the U.S.

FAPESP offers many programs to support foreign scientists willing to work in research institutions in the state of São Paulo, Brazil. These include: post-doctoral fellowships (http://www.fapesp.br/en/5427), young investigator awards (http://www.fapesp.br/en/4479) and visiting researcher grants (http://www.fapesp.br/147).

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Private non-profit making institution created in 1953, Hospital A.C.Camargo is pioneer and one of the world’s biggest cancer treatment, teaching and research center. In an integrated and multidisciplinary manner, it operates in the prevention, diagnosis and ambulatory and surgical treatment of over 800 types of cancer identified by Medicine, divided into more than 40 specialties.

Every year its team treats about 15 thousand new patients from different corners of the country and abroad, totaling over one million procedures (consultations, lab and imaging tests, hospitalizations, surgeries, chemotherapy and radiotherapy, among others).

The A.C.Camargo Hospital Complex has over 62 thousand m² of built area, having one of the largest structures in the world of hospitalization in Oncology, with a total of 440 beds. It also has 62 chemotherapy rooms, 22 surgical rooms and the most complete technological assets in oncology in Latin America.

Its clinical body consists of a closed team of over 500 specialists, majority with masters and doctorate degrees. The dedication and interaction of these professionals in the interdisciplinary activities results in a treatment with better success rates, only comparable to those of the largest oncology centers in the world.

In 2012, it achieved the International Certification by the Canadian Council for Health Services Accreditation (CCHSA), certifying the efficiency of its processes in relation to patient safety.

In the teaching area, A.C.Camargo created the first Residency in Oncology in the country, in 1953, graduating its one thousandth resident in 2010. It is also responsible for the training of one in every three active oncologists in Brazil. The graduation created in 1997 is the only one in a private hospital that is accredited by the Ministry of Education and that obtained the highest score by CAPES during all this decade, thus becoming the best Oncology training center in the country among public and private schools and one of the best in Medicine in the country.

In the researching area, there is the biggest research structure in Latin America, the CIPE – International Research Center. With 4 thousand square meters, the structure of CIPE A.C.Camargo consists of research groups that work in labs with state of the art equipments and that, integrated to the entire clinical body of the hospital, conduct leading scientific projects. In 2000 it centralized the Cancer Genome in Brazil funded by FAPESP and Ludwig Institute.

It has the largest Tumor Bank and tumor tissue collection in Latin America, with 30,000 samples and the biggest scientific production of the area, with over a thousand papers published in the past decade in the major high-impact international magazines. In 2010 it was responsible for 87% of the scientific oncology production in the country.

KEYNOTE LECTURE PRESENTATIONS

K1 Modelling acute leukemias in mice: clonal evolution and the emergence of leukemic stem cells
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The concept of cancer stem cells (CSCs) is based on a hierarchical model of cancer whereby cells within a tumour exhibit distinct biological characteristics and only CSCs are able to grow indefinitely and to maintain the neoplastic process. The molecular and cellular characteristics of CSCs are thought to be due to genetic and epigenetic states reminiscent of those normal stem cells. Precisely, cancer arise from the neoplastic transformation of stem cells or committed-progenitor cells [1] through two types of events. First, normal stem cells can acquire genetic alterations that alter its growth control, increase its resistance to apoptosis and interfere with cell differentiation. Second, non-stem cells can be altered by the oncogenic process to reacquire the self-renewal properties of normal stem cells. In both cases, these characteristics confer a particular resistance to drugs, implying that CSCs are involved in the persistence of tumour cells during treatment and consequently, are responsible of relapses.

The concept of CSCs has come from pioneering studies on acute myeloid leukemia (AML) which have defined a distinct subpopulation of tumour cells, the leukemic initiating cells (LICs), characterized by their capacity to initiate the disease when transplanted into imuno-deficient mice [2,3]. A confounding issue in the field has been the equation of CSCs with the cell of origin of acute leukemias. Indeed, these original studies and subsequent work suggested that AML derived from the malignant transformation of hematopoietic stem cells (HSCs) [4,5]. However, growing evidence based on cellular and molecular studies led to the recognition that the cell of origin of AML is a committed progenitor that normally lack any potential for self-renewal [6,7]. This controversy may be reconciled by assuming that AMLs may represent in some cases a stem cell disorder, while in other cases, the reacquisition of stem cell characteristics by a committed progenitor [8].

The situation is different in paediatric acute lymphoblastic leukemia (ALL), where there is evidence that leukemia initiating activity is observed not only in the immature cell population but also in populations corresponding to a range of normal precursor cells [9,10]. Precisely, the analysis of leukemic and pre-leukemic stem cell populations in a pair of identical twins indicate that the putative stem cell responsible for initiating and maintaining B-ALL are not a fixed cell identity but evolve both in genotype and phenotype [11]. Comforting this observation, a process of clonal evolution at the level of LIC populations was provided both in B-ALL and T-ALL. Indeed, the molecular investigation of individual LIC helped to establish a complex clonal architecture of individual leukemia, showing that LIC are genetically heterogeneous due to the process of clonal evolution [12-14].

T-acute lymphoblastic leukemia (T-ALL) represents about 15% of paediatric leukemias. Several studies in these last years have, in part, elucidated the molecular mechanism of T-ALL transformation. Indeed, T-leukemogenesis is a multi-step process characterized by the acquisition of several oncogenic events. Especially, genes encoding the SCL transcription factor and its nuclear partners LM01 and LM02 are frequently deregulated in T-ALL. Furthermore, activating mutations of NOTCH1 are found in more than 50% of T-ALL cases, and are frequently associated with chromosomal abnormalities in the SCL and/or LM01/2 locus [15,16], implying that these mutational events frequently collaborate during neoplastic transformation of thymocytes. It was originally proposed that the phenotype of the tumor reflects the cell of origin of T-ALL [17]. However, recent studies indicate that fully transformed T-leukemic cells are functionally heterogeneous and may originate from the leukemic transformation of an early T-cell progenitor [18,19]. Furthermore, it has been recently shown that the overexpression of the LM02 oncogene in the thymus induce the emergence of a pre-leukemic stem cell (pre-LSC) population [20] but the identification of the cell of origin of T-ALL and the mechanisms by which these oncogenes reprogram normal thymocytes to become T-LIC remain unclear. We took advantage of a transgenic mouse model that closely reproduces paediatric T-ALL to define oncogenic events during the pre-leukemic phase.

We show that SCL-LM01 inhibits thymocyte differentiation at the double negative to double positive transition, via inhibition of two transcription factors that are essential in the thymus, HEB and E2A [21,22]. Moreover, SCL-LM01 reprograms thymocyte progenitors to confer abnormal
self-renewal capacity. The acquisition of stem cell-like properties establishes a pre-leukemic state in thymocytes by causing an expansion of the CD4+CD8- double negative (DN) population of progenitors that actively proliferate under the influence of the pre-TCR and are therefore at risk of acquiring mutations. Strikingly, our data indicate that the pre-TCR favors the acquisition of Notch1 mutations in SCL-LMO1 pre-leukemic stem cells [23]. Finally, SCL, LMO1 and NOTCH1 together induce a polyclonal disease in transgenic mice, which is comparable to that induced by transplantation of a single leukemic stem cell. We therefore conclude that these three oncogenes are sufficient to transform DN thymocytes.

In summary, we identified in T-ALL, the target cell of transformation by the SCL-LMO1 oncogenes are double negative thymocytes that acquire aberrant self-renewal activities but remain non-leukemogenic. Acquisition of activating Notch1 mutations then transforms these thymocyte progenitors into leukemic stem cells [23].

Competing interests: There are no competing interests in this presentation.

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K2 Hereditary colorectal cancer and Lynch syndrome

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The term Hereditary Nonpolyposis Colorectal Cancer, or HNPCC, has been less used for naming the classical autosomal dominantly inherited susceptibility to cancer [1]. As this susceptibility applies to tumors from different primary sites other than but including colorectal cancer (CRC), the term Lynch Syndrome (LS) is a less restrictive name. Lynch Syndrome is characterized by an autosomal dominantly inherited susceptibility to cancer, caused by inherited germline mutations in the mismatch repair (MMR) genes. It is characterized by early age of onset, predilection to the proximal colon, multiple primary CRCs, and extracolonic tumors, particularly endometrium carcinoma (EC) [2, 3]. To establish a profile of the disease, a better definition of the spectrum of related tumors has been a constant concern [4, 5]. It is therefore expected to find heterogeneity among the families regarding the susceptibility to develop tumors in different specific sites. The risk of cancer in fact varies among families with LS, although the variation does not necessarily result from genetic heterogeneity. The standards of environmental exposure must contribute to the differential gene expression, justifying at least in part this heterogeneity [6].

LS accounts for 2%–5% of all CRC cases [7]. In fact, it is believed that 20% to 30% of patients with CRC present some type of genetic susceptibility, but without meeting criteria for known typical syndromes. However, new cancer cases in the patient’s family or supplemental information on previously unknown cases can lead to a reclassification that may characterize a typical syndrome. In other situations, despite the lack of clinical criteria for determining an inherited character, molecular inquiry can define the diagnosis of inherited syndrome. For these reasons, even in the absence of typical clinical characterization, criteria must be used to direct the inquiry of an inherited condition.

The spectrum of extracolonic tumors in LS began to be the subject of several publications in which the most common cancers found were...
those affecting the endometrium, the stomach and the urinary tract [8-14]. Watson and Lynch [6] calculated the frequency of cancer in other specific sites in 1,300 high-risk individuals from 23 families having LS and demonstrated a significant increase of the risk of developing cancer in the stomach (RR:4.1), small bowel (RR:25), kidneys (RR:3.2), ureter (RR:22), and ovary (RR:3.5). The proposed extracolonic cancers associated with LS are endometrium, stomach, ovary, small bowel, ureter, renal pelvis, brain, and hepatobiliary tract. Among these tumors, endometrium, ureter, renal pelvis, and small bowel cancers present the highest relative risk, and are therefore the most specific for LS.

The Amsterdam criteria for clinical diagnosis of LS are: (1) at least three relatives must have histologically verified CRC, endometrium, ureter, renal pelvis, or small bowel cancer; (2) one must be a first-degree relative of the other two; (3) at least two successive generations must be affected; (4) at least one of the relatives with cancer must have received the diagnosis before age 50; and (5) familial adenomatous polyposis must have been excluded. Because there are families with an MMR mutation present exclusively in patients with endometrial cancer without CRC, the requirement of at least one case of CRC was suppressed [15,16].

Patients with LS may also have sebaceous adenomas, sebaceous carcinomas, and multiple keratoacanthomas, findings consonant with Torre’s syndrome variant [17,18]. The definition of LS includes a familial clustering of colorectal and/or endometrial cancer and as associated cancers stomach, ovary, ureter, renal pelvis, brain, small bowel, hepatobiliary tract, and skin (sebaceous tumors) tumors [15,19].

As discussed by Vasen [20], since it is known that LS is caused by an mismatch repair gene defect and that the hallmark of the syndrome is microsatellite instability (MSI), more attention should be given to the so-called Bethesda guidelines, which describe almost all clinical conditions in which there is suspicion of LS and in which a search for MSI is indicated, mainly early onset of colorectal adenomas and cancer. Competing interests: There are no competing interests in this presentation. References

K3

Modeling squamous cell carcinoma development and malignant progression in mice
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Squamous cell carcinoma (SCCs) of the skin and head and neck arise through the accumulation of genetic and epigenetic alterations that contribute to different stages of tumor progression and the metastatic potential of these tumors. Advanced SCCs are refractory to current therapies and exhibit high risk for relapse, and despite significant advances in prevention and in understanding the biology of SCCs, there are currently no effective treatments for patients with metastatic SCCs. Understanding the role of the molecular alterations found in SCCs may facilitate the design of novel therapies to treat these patients. The most frequently mutated gene in SCC of the skin and head and neck is the p53 gene, which is found mutated in up to 50-70% of the SCCs analyzed. The majority of the p53 mutations found in SCCs are missense mutations that result in the expression of altered forms of p53, some of which may promote tumorigenesis, indicating that p53 is not only a major tumor suppressor gene, but it may also be a primary oncogene in SCC. Genetically engineered mouse models are powerful tools to determine the role of genetic alterations found in human cancers. We generated a mouse model for sporadic head and neck cancer based on the focal activation of endogenous K-ras and p53 mutations in the oral epithelium. Using this model we demonstrated that the p53 gain-of-function mutation p53<sup>172H</sup>, but not deletion of p53, contributes to oral tumor initiation, accelerates tumor growth and promotes malignant progression.

Molecular analysis of tumors and cell lines derived from them determined that mutant p53 promotes the expression of mitotic genes and induces accelerated entry in mitosis, a mechanism that depends on oncogenic K-ras. These findings provide in vivo evidence for the oncogenic potential of mutant p53<sup>172H</sup> during SCC development and support clinical observations indicating that certain p53 mutations are associated with poor prognosis in SCC of the head and neck. I will discuss the potential implications of these findings in tumor progression, resistance to therapy and genomic instability associated with p53 mutations. As the oncogenic function of mutant p53 in this mouse model was dependent on K-ras mutations, we speculate that p53 mutants are activated in response to oncogenes. To address this question, I will further discuss the consequences of activating p53 gain- and loss-of-function mutations in the absence of additional genetic alterations in mouse models in which only p53 mutations are induced in stratified epithelia of the skin and oral mucosa. In addition, novel mouse models generated in our laboratory are shedding new light into the function of genes that cooperate with p53 mutations in the early stages of SCC formation and during malignant progression and metastasis.

Competing interests: There are no competing interests in this presentation.
Development and progression of any cancer disease are the result of various alterations at the cellular and molecular level. This comprises changes in expression of genes, display of surface molecules, composition of extracellular matrix, and homing of circulating cells to the tumor site. Most of these changes occur long before morphological changes can be detected by conventional methods.

Molecular imaging is aimed at the non-invasive in vivo characterization and measurement of these processes to assess therapy effects more promptly than classic morphological and functional imaging can provide. Additionally, visualization of these processes would provide more precise information about the disease expansion. Beyond that, novel therapy regimens may be immunotherapeutic requiring methods for tracking the therapeutic cells. Different imaging modalities are used for these purposes, originally established in cell biology labs like fluorescence imaging (FLI), bioluminescence imaging (BLI), and photoacoustic imaging (PAI) as well as in clinical routine like magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), single photon emission CT (SPECT), and ultrasound (US).

Variations in endogenous tissue contrast can be utilized for certain applications, e.g. alterations in oxygen saturation lead to signal changes in PAI and dedicated MRI sequences like BOLD sequences. However, specific contrast agents need to be designed in most assignments. These molecules usually consist of a targeting moiety that binds to molecules of interest, indicates certain functional states of the tissue, or is modified by specific enzymes on the one hand. On the other hand, another moiety is needed that changes tissue contrast in order to be detected by the chosen modality, like fluorophores for FLI, microbubbles for US, e.g. gadolinium for MRI, e.g. 18F for PET and e.g. 99mTc for SPECT. These contrast agents can either be injected into the animals whereby pharmacokinetics of the molecule itself determines the imaging protocol. Alternatively, they can be used for cell labelling in order to track these cells after injection into the animals. Most of these techniques in general provide the opportunity to be translated into clinical routine.

This toolbox of endogenous and exogenous contrast agents is completed by reporter gene imaging that allows detecting changes in gene expression. This technique is in most cases limited to preclinical imaging. To visualize changes of gene expression, genetic sequences are used that code for different fluorescent proteins for FLI, different luciferases for BLI, herpes simplex virus tyrosine kinase (hsv-tk) for PET or e.g. iron-binding or iron-storage proteins for MRI. These gene sequences are brought under the control of the promoter of interest. The vectors constructed this way are then either transfected into tumor cells for preclinical transplantaion models of cancer or used for the generation of transgenic animals.

Pancreatic ductal adenocarcinoma (PDAC) represents one of the malignancies with the poorest prognosis where incidence equals mortality. Despite considerable advances in the understanding of the molecular mechanisms involved in the carcinogenesis of PDAC during the last decade the survival of the disease was not significantly improved over the last 40 years. Therefore, novel therapeutic approaches are urgently needed. Preclinical animal imaging can provide accurate measures of tumor progression non-invasively. Molecular imaging can in addition help, a) to detect cellular and molecular processes in vivo avoiding artefacts caused by tissue collection and preservation, b) to visualize specific therapy effects on certain signalling pathways over time, and c) to reduce animal numbers due to its non-invasiveness.

PDAC is characterized by early spreading of metastatic cells and a high rate of local and distant recurrent disease even after complete surgical removal of the primary lesion as defined by histology. One key factor for this aggressiveness seems to be due to the high susceptibility towards inflammatory signals, part of which are acting in an autocrine manner since PDAC cells have been found to frequently express simultaneously ligands and corresponding receptors. From a surgical point of view local recurrent disease as well as distant metastases (mostly in the liver) limit the success of this curative therapeutic attempt, for which less then 20% of patients are eligible. There is strong evidence that inflammation drives these sometimes rapid and fulminant recurrences. Adjuvant chemotherapy has been shown to improve the outcome yet no long-term survival is achieved. Numerous different contrast agents for all modalities are available to detect surrogate markers of inflammation, such as endothelial adhesion molecules like E-selectin or VCAM, as well as enzymatic activity e.g. of matrix metalloproteases, caspases or cathepsins. Additionally, invasion of immune cells can be monitored by reporter gene imaging. A plethora of molecular alterations are thought to be responsible for the profound chemoresistance. Besides classical hallmarks of cancer such as mutations in the K-ras oncogene and the p53 tumor suppressor, the constitutive or inducible activity of transcription factor pathways is characteristic for PDAC. The nuclear factor-kB (NF-kB) has been shown besides others to be crucial for tumor development and apoptosis resistance mechanisms in this context. We have shown that NF-kB contributes to non-apoptotic signalling of death receptors, "normally" inducing apoptosis upon death-ligand-driven activation. Thus, targeting this pathway by established recombinant inhibitors or new drugs including natural compounds with anti-inflammatory potential might have great therapeutic potential.

In summary, in vivo animal models are a key element of understanding the processes of tumor development and progression as well as of validating novel strategies for tumor therapies. Clinically adapted animal models allow distinguishing between adjuvant, palliative and neo-adjuvant concepts. Preclinical animal imaging and especially molecular imaging can help to visualize molecular and cellular processes during the course of the disease non-invasively in vivo, and will allow a more valid validation of any novel therapeutic regime.

**Competing interests:** There are no competing interests in this presentation.

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**K5**

**Comparative oncology – the North American experience**

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Comparative oncology can be used to describe a discipline that integrates the study of naturally occurring cancers in animals into studies of human cancer biology and therapy. The term is most often used when referring to the study of cancers seen in companion (pet) animals. Cancers in companion and comparative oncology drug development leading the effort. There have also been a growing number of consortia and cooperative groups that have functioned successfully by uniting multinstitutional efforts, advocating for veterinary clinical trials, and emphasizing the synergy between basic science and clinical progress. With support from clients who are motivated to seek advanced care for their pets and to enroll them in investigational trials that offer new therapies, clinical research in veterinary oncology is growing in scope and importance.

Oncology clinical trials attempt to answer questions and find better ways to prevent, diagnose, or treat cancer. Their model is different from trials involving infectious or even chronic diseases because the risks involved have greater morbidity and mortality. Traditional drug development follows a strict, step-wise paradigm that begins with a phase I dose-finding trial, followed by a phase II efficacy/activity trial, and concludes with a phase III "pivotal" trial that pits a novel agent against or with the current standard of care (Table 1). Although due to its nomenclature oncology trials sometimes combine these concepts, their individual descriptions serve as the framework for new drug development. Clinical designs, pertinent endpoints and analyses, the process for drug approval, and clinical trial ethics will be explored in the following sections.

**Consortia:** One of the most exciting achievements in veterinary oncology over the last decade has been the development of successful and
collaborative consortia groups that are purposed to perform multicenter clinical trials and prospective tumor biospecimen repository collections. Consortia infrastructure teams allow larger scale clinical trials and provide the voice for collective advocacy in veterinary and comparative oncology. Their success is an example of the growing importance of the study of tumor and clinical biology. Some of these efforts are profiled here. 

**Comparative Oncology Trials Consortia: The Comparative Oncology Trials Consortium (COTC) is an active network of 20 centers (https://ccrd.cancer.gov/confluence/display/CCRCOPWeb/Comparative+Oncology+Trials+Consortium), centrally managed by the National Institutes of Health-National Cancer Institute’s Comparative Oncology Program (NCI-COP), that functions to design and execute clinical trials in dogs with cancer to assess novel therapies. The goal of this effort is to answer biologic questions geared to inform the development path of these agents for use in human cancer patients. As of 2012, the COTC has completed or is currently conducting 18 clinical trials and has been successful in promoting the utility of comparative oncology modeling within the drug development community.**

**Animal Clinical Investigation: Animal Clinical Investigation (ACI; http://www.animalci.com) is a privately organized and run specialty network of veterinary hospitals that designs, conducts, and reports clinical studies for the animal health industry. ACI trials emphasize oncology drug development but have recently expanded to include other medical conditions, including inflammatory and metabolic disease, cardiovascular disease, and arthritis. ACI provides multi-site, pivotal, or nonpivotal studies and commercialization support to help define effective novel veterinary therapeutics. It is the first example of a commercial clinical research organization (CRO) in veterinary medicine. Over 30 clinical trials have been conducted through this network over the last 10 years.**

**Canine Comparative Oncology and Genomics Consortium: The Canine Comparative Oncology and Genomics Consortium (CCOGC; http://www.cccgc.net) is an informal collaboration of veterinary and medical oncologists, pathologists, surgeons, geneticists, and molecular and cellular biologists with a common interest in the comparative study of canine and human genomics and cancer. The goals of the CCOGC are to facilitate partnerships and collaborations focused on the problem of cancer in dogs. Early priorities included advocacy for the field of comparative oncology, the development of a mechanism to share reagents and resources in the community, and the development of a biospecimen repository. The repository houses tumor tissue; normal tissues; serum, plasma, peripheral blood mononuclear cell preparations, genomic DNA, RNA, and urine samples. As of 2011 the CCOGC has collected 1600 of 3000 anticipated samples. The repository will serve as a seminal resource for the research community to acquire tissues for target biology studies, as well as its mechanism for prospective collections of unique samples. Future efforts will focus on dissemination of these tissues.**

**The traditional drug development flow:** Traditionally, *first-in-species trials* start with a *Phase I* dose-finding trial, followed by a *Phase II* efficacy/activity trial and concluding with a *Phase III* "comparative" trial that pits the novel agent against or with the current standard of care (Table I).

**Conclusions:** Clinical trials are an important research discipline to improve care and outcome for cancer patients in both human and veterinary oncology. Steps should be made to ensure study aims are achievable within a crafted study design and protocol. Rules governing design are prospective and involve questions of dose and schedule, selection, toxicity, activity, and comparison to known effective therapies. Statistical expertise is also necessary to ensure appropriate clinical trial design. Regulatory oversight of veterinary oncology trials is increasing, and new approval of veterinary oncology agents will emphasize these processes over the next decade. Comparative oncology trials also are key to the inclusion of pet animals in the evaluation of novel anticancer therapeutics, imaging strategies, and medical devices. Consortia groups will continue to advocate and advance the use and utility of veterinary oncology clinical trials. The field of veterinary oncology will continue to grow through the proper use and design of both traditional and novel clinical trials.

**Table 1 (abstract K5) Goals of Phase I–III Clinical Trials**

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>PHASE I (DOSE FINDING)</th>
<th>PHASE II (ACTIVITY/EFFICACY)</th>
<th>PHASE III (PIVOTAL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary goals</td>
<td>• Determine MTD</td>
<td>• Determine activity/efficacy in defined populations</td>
<td>• Compare a new drug or combination to therapy currently regarded as standard of care</td>
</tr>
<tr>
<td></td>
<td>• Define DLT</td>
<td>• Inform the decision to move to a Phase III trial</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Characterize type and severity of adverse events</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary goals</td>
<td>• PK/PD issues</td>
<td>• Estimate therapeutic index</td>
<td>• Quality of life comparisons</td>
</tr>
<tr>
<td></td>
<td>• Scheduling issues</td>
<td>• Expand adverse event data</td>
<td>• Comparative costs</td>
</tr>
<tr>
<td></td>
<td>• Target-modulation effects</td>
<td>• Evaluate additional dosing groups</td>
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<tr>
<td></td>
<td>• Preliminary efficacy data</td>
<td>• Expand target modulation and biomarker data</td>
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<tr>
<td></td>
<td>• Investigate surrogate biomarkers of response</td>
<td>• Quality of life measures</td>
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</tbody>
</table>

MTD, Maximum tolerated dose; DLT, dose-limiting toxicity; PK/PD, pharmacokinetic/pharmacodynamic.
the Her-2 expression to be positively associated with nuclear pleomorphism, histological grade and mitotic count. Positive association was found between nuclear pleomorphism and MIB-1 index. These results imply that some tumor biological and morphological characteristics are associated with canine mammary gland tumors as it has been seen in human breast cancer. Studying benign non-neoplastic lesions was verified that they are pathologically and immunophenotypically similar to those in the human breast. In our diagnostic routine, mixed tumors are the most common tumor types in the female canine mammary gland. These tumours exhibit a complex histological pattern due to the presence of epithelial and mesenchymal elements and have the capacity to undergo malignant transformation, resulting in carcinomas. The origin of the several mixed tumour components is a subject of a long-standing controversy and is not yet fully understood. A suggested hypothesis states that these components originate from stem cells with a high divergence capability. This assumption is grounded on immunohistochemical studies and on the observation that the epithelial and mesenchymal components of mixed tumours are monoclonal. Carcinomas in benign mixed tumors (CBMT) are the most common malignant tumor in female dogs and may serve as a model for studies on tumor progression. Versican expression in in situ and invasive carcinomatous areas of CBMT was evaluated, verifying possible associations with other classic prognostic factors and overall survival. Results suggest that versican loss in CBMT gains mesenchymal characteristics, as a consequence of p63 and α-SMA molecule expression and increase in versican expression occurs. In addition, the direct relation between versican and invasion suggests the role of this molecule in tumor progression. In CBMT were also investigated morphological aspects and their immunophenotypical profiles, through an immunohistochemical panel based on five molecular markers (ER, PR, HER2, CKs and EGFR). It was concluded that CBMT are predominately characterized as low-grade malignancy neoplasms. The various immunophenotypic profiles suggest the origin of these lesions in more than one cell type (luminal and myoepithelial). Invasive micropapillary carcinoma (IMPCa) of the mammary gland was first described by our group and should be highlighted. Despite its rare occurrence in humans and dogs, it has a distinct aggressive behaviour and poor prognosis. Were evaluated clinicopathological and immunophenotypical characteristics as well as the overall survival of canine IMPCa. Findings demonstrate that canine IMPCa are similar to human IMPCas, presenting aggressive behaviour with high rates of metastasis to regional lymph nodes and short overall survival and should be considered important lesions of the mammary gland in dogs. The distribution and intensity of lymphocytic responses in canine mammary tumors, in particular the relative abundance of the lymphocyte subpopulations which define whether the inflammatory process will act as a promoter or inhibitor of tumor development and metastasis were analyzed. Morphologic and immunohistochemical studies should be related with clinical aspects and serve as prognostics and predictive markers. Then patients suffering from mammary tumors with advanced clinical staging were included, reviewing the overall survival only with surgery compared to those complementarily treated with carboplatin and Cox-2 inhibitors, the benefit of these patients from complementary therapy was verified. Carboplatin as an only drug was indicated for treating mammary tumors of female dogs in advanced clinical staging, with minimal side effects and easy administration. Limited therapeutic resources in mammary neoplasms of the female dog, the confirmed benefits of tamoxifen in human mammary tumors and insufficient data available on the canine specie justified a study that addressed additional effects of this drug in veterinary medicine. Side effects of oral administration of tamoxifen in spayed and intact female dogs were studied and were verified similar side effects to those observed in women with breast cancer treated with this medication. It was investigated the potential prognostic value of serum markers and increased CA15.3 and LDH serum levels were directly related to the presence of regional lymph node and distant metastases. CEA showed no significant change among the groups (healthy, non-metastatic and metastatic mammary cancer groups). Thus, we suggest the possibility of using the criteria and the more extensive experience of human studies for the future, treating canine mammary gland tumors. On the other hand, these results reinforce the theory that spontaneous canine mammary tumors may be used as models for studies evaluating the mechanisms involved in mammary gland carcinogenesis, development of novel cancer therapeutics and may be relevant for human breast cancer studies.

K7

Integrative cancer informatics for the identification of prognostic and predictive biomarkers

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BMC Proceedings 2013, 7(Suppl 2) K7

Cancer development is a multi-step process that leads to uncontrolled tumor cell growth. Multiple pathways are involved; typically some signalling and regulatory pathways are activated, while others are suppressed. Systematically exploring these networks of proteins will lead to better understanding of disease initiation and progression. Integrating these data with microRNA regulatory networks may identify control mechanism that these master regulators use to affect oncogenesis. Including data on drug targets, modes of actions predicted from drug profiles and compound similarity will in turn lead to more effective patient treatment. To address these challenges, we developed a system for an integrative analysis, prediction and characterization of molecular signatures and relevant protein-protein interactions, microRNA: gene interactions, and resources for rationally identifying drug combinations for cancer treatment.

Competing interests: There are no competing interests in this presentation.

K8

Evolution of energy metabolism, stem cells and cancer stem cells: how the Warburg and Barker hypothesis might be linked

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BMC Proceedings 2013, 7(Suppl 2) K8

Two explanations for the origin of cancers exist: the “Stem Cell Theory” [1-4] or the “De-Differentiation” or “Reprogramming Theory” [5]. Concepts related to the genesis of cancers include: (a) The Multi-Stage, Multi-Mechanism concept of carcinogenesis [6]; (b) the evolution of earth’s physical environment ultimately allowed the appearance of anaerobic microbiological life forms that metabolized via glycolysis [7]; (b) the evolution of photosynthetic algae led to the oxygenation of the environment and to proto-eukaryotes after the symbiotic marriage of bacteria that could produce energy via oxidative phosphorylation; (d) the Warburg metabolism of cancers [8]; (e) the concept of “cancer stem cells” and “cancer non-stem cells”[9]; and (f) the Barker hypothesis (diseases later in life might be the result of in utero embryonic/ fetal exposures to a variety of factors [10]. To prevent and treat cancer, one must understand the complex mechanism of the multi-stage, multi-mechanism process of human carcinogenesis [11]. Starting with the initiation step of transforming a normal cell to one that is unable to terminally differentiate, and the “promotion” phase which comes about by the clonal expansion of this single initiated cell. Promotion is brought about the reversible inhibition of gap junctional intercellular communication, caused by growth factors, inflammatory stimulation, compensatory hyperplasia, due to chronic irritation or cell death, and by the ability to resist apoptotic death. A single cell can, then, accrue enough genotypic and epigenetic alterations to acquire all the “hallmarks”[12] of an invasive and metastatic cancer cell (the “progression” phase). Two important questions arise from this concept of the initiation/promotion/progression process of carcinogenesis, namely: “What is the cell that is the target cell for ‘initiation’?”, and “What are the underlying molecular mechanisms for each phase of carcinogenesis?” With the discovery of cancer- initiating cells within a tumor, the concept of “cancer stem cells” has been generated. Implicit in this concept is the idea that each tumor is a heterogeneous mixture of “cancer non-stem cells” and “cancer stem cells”. The current paradigm is based on the assumption that a somatic mortal, differentiated cell can be de-differentiated or re-programmed to become immortal, allowing it to survive long enough to accrue additional mutations and epigenetic changes to become neoplastically-transformed. This paradigm is supported by observations in the stem cell field, where “induced pluripotent stem cells” (“iPS”) can be isolated from primary in vitro cultures with various cocktails.
of embryonic "stemness" genes [13]. However, there is an alternative interpretation of the origin of these "IPS" cells, namely, they were selected adult stem cells in those primary cultures [9]. In addition, the isolation of "MUSE" cells from normal human skin has demonstrated that these rare cells in the skin are the "target cells" for the so-called "IPS" cells [14]. Normal human adult stem cells are naturally "immortal" until they are induced to terminally differentiate. Adult stem cells can be inhibited from "mortalsizing" or to remain "immortal" [15]. Dramatic demonstration has shown that only normal human breast stem cells could be efficiently blocked from "mortalsization" and then neoplastically-transformed. This observation strongly supports the stem cell hypothesis [16,17]. Individual genetic, gender, dietary, environmental, life style, medical, lifespan and cultural factors can affect each of these three phases of carcinogenesis [18-21]. Genetic predispositions, such as xeroderma pigmentosum, leading to UV-induced skin cancer and the experimental carcinogenesis studies and epidemiological findings that clearly show how diets, environmental chemicals (asbestos), drugs (DES: estrogen), life style factors (alcohol; cigarette smoking) can enhance the risk to varius cancers. What is sometimes ignored are cultural factors, such as postponement of childbearing (early child bearing is a cancer risk reducer). Other cultural factors, e.g., reduced exercise and dramatically altered diets and nutrition, have been associated with an increased caloric intake and non-nutritional diets or processed foods. A collision of biological evolutionary with cultural evolution is occurring. This allows for increased caloric intake, change in eating habits, types of foods, and even the relationship of the biological evolutionary symbiotic role of our gut microbiota to our gut biology [22]. This collision has been more pronounced with caloric over-abundance, dramatically less physical exercise, the eating of processed foods and less of the early human diet-related foods, more grilled red meat, eating at all hours of the day, the changing of our gut microbiota and dieting with supplements. Clearly, biological evolution does not work fast enough to keep up with cultural evolutionary changes that affect our diet and other life style changes (postponing marriage; living longer). While all those factors can influence any of the phases of the carcinogenic process, initiation can never be reduced to a zero risk. Every time any cell replicates, there is always a finite chance of a mutation/initiation event to occur. The longer we live, the more initiated cells we accumulate. Except for teratomas, and early childhood cancers, most adult cancers take decades for the promotion process to expand the numbers of initiated cells for more mutational and epigenetic events to occur. Therefore, the promotion phase is the most efficacious period to intervene to prevent many cancers associated with environmental, dietary, life style, exercise and other cultural factors. One of the newer concepts that might also influence our understanding of risk factors to cancers is the "Barker hypothesis". Indirect experimental studies, as well as epidemiological studies, suggest that modulation (increased or decreased) of organ-specific adult stem cells could increase or decrease the risks to organ-specific cancers later in life (e.g., DES-exposed female fetuses led to vaginal cancers in young women; soy- and caloric-restricted female fetuses of Japanese women have been associated with low breast cancer frequencies of women later in life) [22]. Two factors could reduce the risks to various cancers, namely, modulating stem cell numbers in utero by careful exposures to environmental/life style and nutritional factors (decrease the target size of 'initiation' step) and post-natally, by interfering with the "promotion" of initiated cells by those same factors.

Competing interests: There are no competing interests in this presentation.

References

K9

Comparative breast cancer research, lessons from companion animals
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BMC Proceedings 2013, 7(Suppl 2)K9

Breast cancer is the most frequent occurring tumor in humans. Current knowledge describes breast cancer as a heterogeneous disease classified in four major categories, i.e. basal-like, ErbB2 enriched, luminal subtype A or luminal subtype B. The majority of human breast cancers are hormone-receptor positive and may respond to endocrine therapy. Our understanding of mammary gland development and breast cancer progression and metastasis comes mainly from cell lines and (transgenic) mouse models. However, these models have limitations. Differences exist between human and mouse tumorigenesis and metastasis. Spontaneous mouse models in general also do not present hormone-sensitive mammary cancers. The available chemically- or virally-induced mouse models or genetically manipulated mouse models are also more...
homogeneous and represent only certain phenotypes of the human disease. Spontaneous mammary carcinomas in companion animals may present as relevant to be studied both for veterinary treatment and increase in knowledge and treatment modalities of human breast cancer. Companion animals are exposed in a similar way to humans to environmental mutagens or carcinogens in food. The annual incidence of mammary carcinomas in the dog is 2 to 3-fold higher than in humans whereas a 4-fold lower incidence of mammary cancer is found in the cat. The high incidence of spontaneous mammary cancer in dogs and the higher homology of the canine genome to the human genome than that of rodents make them relevant for human breast cancer research. Comparison of gene expression profiles of mammary cancer in humans and dogs showed that they share similar pathways involved in tumorigenesis such as integrin-signalling, Wnt-signalling, chemokine and cytokine signalling and angiogenesis. Also perturbations in cancer-related pathways such as PI3K/akt, PTEN and MAPK are found. Immunohistochemical analysis of canine mammary tumors identifies the luminal A and B and basal-like tumors as found in humans. Part of the canine mammary tumors is hormone receptor positive although the presence of progesterone- and estrogen-receptors decreases with increasing malignancy. Studies at the start of this century in women receiving hormone-replacement therapy (HRT) showed an increased incidence of breast cancer in the mammalian gland in combination with other hormones and estrogen-only use in the estrogen-only group resulting in a paradigm shift in the thinking about the role of progesterone in breast cancer development. In dogs and cats progesterone plays a dominant role in mammary tumorigenesis. We will therefore focus on the role of progesterone.

The signal transduction cascades involved in progesterone signaling start with binding of progesterone to specific receptors. The two well-known nuclear progesterone receptors (PR) are transcribed from a single gene but - through use of different promotors - two different PR isoforms are synthesized. The shorter form, PRA, contains the hormone binding domain, a hinge region and a DNA binding domain but lacks an amino-terminal sequence which is unique for the longer PRB receptor. This B-upstream region segment (BUS) contains an activation domain, AF3, which results overall in a much higher transactivation potential of the PRB in relation to PRA. PRA is mainly found in nuclei whereas PRB has both nuclear and cytoplasmatic localizations. Progesterone plays a central role in the regulation of stem and progenitor cells within the mammary gland. Cells expressing the PR act as sensors and upon progesterone binding they stimulate the stem cell compartment to proliferate and differentiate.

In the cat, endogenous progesterone and synthetic progestins induce fibroblast-like hyperplasia (FAH) of the mammary gland. In such FAH tissues we demonstrated both PR isoforms PRA (80-86 kDa) and PRB (116-120 kDa) using Western blots. In FAH examined by immunohistochemistry we found predominant staining for PR in ductal epithelium with both nuclear and cytoplasmic localization. Compared to feline mammary carcinomas, PR expression was more pronounced. As for any experimental with laboratory animals, extrapolating the results to the human situation is not a simple task, and may compromise human risk assessment. To inform risk assessment, a framework was proposed for systematic and detailed evaluation of the MoA in laboratory animals and for evaluating the human relevance of experimental results [3-5].

The standard procedure for identifying chemical carcinogens is the long-term bioassay with rodents. Although presenting a 40-year long history of usefulness, this 2-year assay bears important limitations, such as high cost and operational complexity, and also because it was not designed to provide information about the toxicological mode of action (MoA) of chemicals. As for any experiment with laboratory animals, extrapolating the results to the human situation is not a simple task, and may compromise human risk assessment. To inform risk assessment, a framework was proposed for systematic and detailed evaluation of the MoA in laboratory animals and for evaluating the human relevance of experimental results [3-5].

In conclusion, progesterone plays an important role in mammary hyperplasia and carcinogenesis in companion animals inducing among others Wnt- and cytokine signaling thereby stimulating recruitment of mammary stem cells. The spontaneous nature and high incidences make them attractive for further studies on the hormonal regulation of breast cancer development and treatment.

**Competing interests:** There are no competing interests in this presentation.

### K10

**Chemical carcinogenesis – mode of action to inform quantitative human risk**

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BMC Proceedings 2013, 7(Suppl 2):K10

The progressive public and governmental awareness that we live in a chemical world, where both natural and man-made substances impose specific risks to living organisms, raised the need for regulatory and interventional measures to protect human health. Naturally, regulatory measures depend on identification of the hazard and the risk imposed by suspected chemicals. In the absence of human data, laboratory models have been used to identify target organs, adverse effects, and critical toxicological doses, all of which contribute to subsidize specific regulations. A case study is presented to illustrate how basic research can contribute to regulatory measures aiming to control human cancer risk due to environmental contaminants.

Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) is a substituted urea herbicide acting through inhibition of plant photosynthesis. In a 2-yr long experiment, Wistar rats fed diuron at 2,500 ppm (about 157 mg/kg/day for both sexes) developed urothelial carcinomas in the urinary bladder. The non-observed effect level (NOEL) for carcinogenicity was 25 ppm (1.35 mg/kg/day), based on urothelial hyperplasia and tumors [1]. Accordingly, the U.S. Environmental Protection Agency evaluated diuron as a “known/likely carcinogen to humans” [2]. Despite this classification, the quantitative risk associated to diuron was considered of no concern [2] and the herbicide is worldwide applied on main agricultural crops such as soy, cotton and sugar cane, among other uses. The impact of chronic low-dose exposure to diuron on human health has not been determined.

The standard procedure for identifying chemical carcinogens is the long-term bioassay with rodents. Although presenting a 40-year long history of usefulness, this 2-year assay bears important limitations, such as high cost and operational complexity, and also because it was not designed to provide information about the toxicological mode of action (MoA) of chemicals. As for any experiment with laboratory animals, extrapolating the results to the human situation is not a simple task, and may compromise human risk assessment. To inform risk assessment, a framework was proposed for systematic and detailed evaluation of the MoA in laboratory animals and for evaluating the human relevance of experimental results [3-5].

The genotoxic/mutagenic potentials of diuron are accepted to be negative [1,6,7]. Therefore, the carcinogenic MoA of diuron in the rat urinary bladder should be predominantly non-genotoxic. In an early study, rats fed diuron at 2,500 ppm during 20 weeks developed urothelial necrosis – observed ultrastructurally, regenerative cell proliferation and urothelial hyperplasia [6]. To test the hypothesis that urinary crystals and amorphous precipitates could act as microabrasives to the mucosa causing cell death and regenerative hyperplasia that lead to urothelial carcinogenesis, rats were fed 2,500 ppm of diuron either with or without NH4Cl 10,000 to acidify urine during 15 to 30 weeks [7]. Groups fed diuron+NH4Cl showed reduced amount of urinary solids in the urine; nevertheless, urothelial necrosis and simple hyperplasia (SH), a stochastic preneoplastic urothelial lesion, were observed both in diuron- and diuron+NH4Cl treated animals. Therefore, chemically induced cytotoxicity and not abrasion by urinary solids seems to be the initial key event for diuron-induced rat urothelial carcinogenesis. These urothelial lesions start at the very 1st day of exposure to 2,500 ppm and encompass cell swelling followed by necrosis, exfoliation and piling up of cells indicative of hyperplasia [8]. A recent study indicated that 500 ppm can also induce urothelial cicototoxicity [9].
Regarding dose-response and the molecular pathways involved, rats were fed diuron at different concentrations from 60 ppm up to 2,500 ppm for 7 days and 20 weeks. After 7 days no significant urothelial lesions were observed by light or electron microscopy but gene expression profile (Affymetrix microarrays) indicated altered pathways related to cell-to-cell interactions in a dose-response manner, suggesting that the early influence of diuron and/or its metabolites on the urothelium may result in loss of cell-to-cell adhesion and local tissue disorganization [10]. By the week 20th, rats fed 1,250 or 2,500 ppm, but not those fed 125 ppm or lower, developed SH [11]. Accordingly, gene expression data suggested that low levels of diuron induced pathways involved in the maintenance of cellular homeostasis while higher levels were associated to increased cell metabolism, oxidative stress and cell death followed by sustained hyperplasia. Although based on preneoplastic alterations, these data allow indicating diuron at 1,250 ppm as potentially carcinogenic as 2,500 ppm and assuming 125 ppm as a no effect level (NOEL) for urothelial alterations [11].

This case study illustrates how translational non-clinical research can help to understand and eventually control environmentally-related human cancer hazards. Optical and ultrastructural microcopies, genotoxicity assays, cell proliferation assays, and genome-wide transcriptional profiling disclosed key events of diuron-induced rat urothelial cytotoxicity and potencies. They also indicated that a threshold dose may exist below which no carcinogenic process is launched. Data still missing to support sound quantitative human risk assessment refers to the evaluation of human relevance of the key events verified in rodents and the identification of the exposure levels to which people are exposed.

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References

K11
Viral carcinogenesis: virus implicated in cancer
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The first consistent observations that viruses could be associated with certain types of cancer where made almost a century ago. A great deal of effort was involved in unraveling the molecular mechanism underlying carcinogenesis implicated to animal and human viruses. As a result of these studies, a strong link between some viral agents and several human cancers has been established. Some viruses as the Epstein-Barr virus (EBV), hepatitis B virus (HBV), hepatitis C virus (HCV), human T-cell lymphotropic virus type I (HTLV-I), immunodeficiency virus type I (HIV-1) and several human papillomavirus types (including types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66) have been classified as group 1 carcinogens by the International Agency for Research in Cancer (IARC). Infection by these viruses constitutes a heavy burden for human populations as it accounts for almost 15% of all human malignancies. Furthermore, many other viral agents have been classified as probable (group 2A carcinogens) or possibly (group 2B carcinogens) carcinogenic to humans and others have been occasionally found in human tumors suggesting that this figure is just an underestimation of viral involvement in human cancer etiology. Nevertheless, viral infection appears as one of the main cancer risk factors that could be prevented. Prevention and control of infection by these agents could dramatically reduce the incidence of some prevalent cancers and, consequently, have a great impact on public health.

Competing interests: There are no competing interests in this presentation.

K12
Head and neck cancer
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More than 600,000 head and neck cancers (HNC) are diagnosed every year and the incidence and mortality rates vary according to the economical status of the country. Most are oral, pharyngeal or laryngeal squamous cell carcinomas. The most significant risk factors include lifestyle habits (tobacco, alcohol drinking and diet). More recently, exposure to some biological agents (HPV and EBV) and genetic susceptibility were also identified. Tobacco smoking increases acetaldehyde burden following alcohol consumption, and alcohol consumption enhances the activation of pro-carcinogens by induction of cytochrome P450-2E1 system. HPV and EBV are associated with the oropharyngeal and nasopharyngeal cancers, respectively. Genetic susceptibility has a significant role in non-smoking young patients and it is based on differences in the efficiencies of metabolizing carcinogens, DNA repair and cell cycle control. Familial aggregation of HNSCC has been recently reported and the responsible genes are under investigation. Possibly, the identification of a genetic risk profile may improve prevention, early diagnosis and treatment. Most HNC are characterized by local tumor invasion, frequent regional lymph nodes metastasis, and multiple primary cancers in the upper aerodigestive tract, esophagus and lung. Most patients had advanced-stage disease at diagnosis and current treatment options frequently incur significant morbidity. The significant prognostic factors in HNC patients are TNM stage, tumor site and histologic variables. However, tumors of the same site with similar histology and stage may behave differently due to their differing biological characteristics. There is a major interest in the identification of biomarkers ("biological fingerprints") that could be used in the clinical practice in order to better prognosticate and risk-stratify patients and predict treatment response. The results of these investigations can completely change clinical practice and result in effective “personalized medicine”.

Only HPV positivity and EGFR overexpression are currently considered reliable predictive markers for therapeutic response in HNC patients.
Some recently published studies have also shown that TP53 genetic polymorphisms and mutations influence tumor progression and response to therapy. Genotyping has been also utilized to probe other known gene mutations and can offer potential for identification and targeting of key pathways implicated in tumor progression to guide therapeutic strategies. Recently we have investigated the expression profile of Epithelial-Mesenchymal-Transition genes in patients with oral SCC using a cDNA microarray platform coupled to qRT-PCR and immunohistochemical analysis. We identified TWIST1 transcription factor to be overexpressed and to predict metastases and prognosis. Most prognostic information on HNC are patient and tumor-related factors and occasionally pathologic findings. These variables influence outcomes prediction at diagnosis, but do not consider several others such as response to initial therapy, complications, risk of recurrences and second primary tumors. Furthermore, the risks of recurrence, second primary cancers and non-cancer related deaths change during follow-up when a patient develops a recurrence or second primary tumor. More sophisticated prognostic evaluations are ongoing.

**Competing interests:** There are no competing interests in this presentation.

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**K13**

**Domestic animals as sentinels for environmental carcinogenic agents**

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The idea of using animals as sentinels of environmental contaminations is not new, but until now is relatively little explored. The aim of this talk is to present evidences that lymphomas in dogs of the city of São Paulo may be associated to exposure to air pollution caused by vehicle emissions and heavy traffic, and that the spatial distribution of canine lymphomas in the city of São Paulo coincides with the spatial distribution of human lymphomas in the same city.

Lymphomas are highly prevalent malignant neoplasms that affect canines, humans and other species. Several reports state that, besides a genetic breed susceptibility, environmental factors may be associated to the development of these neoplasms. One first study aimed to investigate the possible factors associated with the development of canine lymphomas. The owners of 83 dogs with lymphomas and 84 controls were interviewed through an epidemiological questionnaire. Cases and control animals lived in the same geographical areas and were admitted to the same veterinary hospitals (five locations in total), in the city of São Paulo. The results showed that age, gender, breed, diet, reproductive status, mineral or tap waters, exposure to cigarette smoke, rocks or soil where dogs walk, building materials, products for home maintenance and other factors were unrelated to the risk of malignant lymphoma. Interestingly, our study detected that dogs which were permanently kept outdoors and around 100 meters to busy streets or avenues (more than 50 vehicles per minute) had a higher risk for development of the disease. The city of São Paulo, Brazil, is the largest city in South America and the fourth largest city in the world housing a fleet of about five million cars and a million motorcycles. These results suggest that environmental factors, such as the air pollution caused by the heavy traffic, may be associated to the development of canine lymphomas.

A second study has been performed in order to verify and compare the spatial distribution of canine and human lymphomas in the city of São Paulo. Non-Hodgkin's lymphomas (NHLs) are complex and heterogeneous neoplasms characterized by malignant proliferation of lymphoid cells. Human and canine NHLs share several features. Because dogs share most environmental and living conditions with humans, we reasoned that spatial distributions of NHL cases between the two species would provide some evidence for the role of certain environmental factors, such as pollutants, in the pathogenesis of NHLs. In this study, we retrospectively analyzed the spatial distributions of 630 human NHL cases randomly selected from a database of more than 8,000 cases at the Cancer Registry of São Paulo and 579 canine NHL cases diagnosed in five referral veterinary hospitals in the city. All human and canine cases were recorded between 1996 and 2006. Here we show that human and canine cases of NHL have similar spatial distributions in the city of São Paulo, with a high incidence in the central region, which is the most polluted area of the city, due to the presence of avenues with a heavy traffic of vehicles. These results suggest that environmental pollutants may play a role in the pathogenesis of human and canine NHLs. In conclusion, the results of these two studies have suggested that canine lymphomas, and possibly human lymphomas, may be associated with the exposure to air pollution. Other studies are being conducted to better establish the relationship between air pollution and development of human and canine lymphomas. Fortunately, the government of the state of São Paulo is aware of the deleterious effects of air pollution on human health and is taking measures to better control the levels of air pollution in the city.

**Competing interests:** There are no competing interests in this presentation.
Several hereditary breast cancer syndromes have been identified to date and mutations in many different genes have been described with these forms of the disease including those in BRCA1, BRCA2, TP53, PTEN, ATM, among others. The magnitude of risk associated with mutation carriage is significant, i.e. carriers of mutations in the BRCA1 and BRCA2 genes have an estimated lifetime risk of 70-85%, compared to the lifetime risk of women in the general population which is close to 10%. In addition, women with these mutations are at increased risk for contralateral breast tumors (40-60%) compared to those without genetic forms of the disease (5%). Taking the same example of hereditary breast and ovarian cancer syndrome due to BRCA mutations, several strategies for preventive interventions have been developed over the last two decades, including risk reducing surgeries, intensified and early-onset cancer screening and development of targeted therapies for tumors mainly caused by hereditary mutations. On the other hand, several challenges are still present in the diagnosis and management of hereditary breast cancer. These include our still limited knowledge of the functional impact of certain sequence alterations in known cancer predisposition genes, the absence of specific cancer predisposing mutations in certain cases (even with an obvious hereditary phenotype), the occurrence of significant phenotypic overlap between the different syndromes rendering molecular diagnosis a difficult task in certain situations and the low sensitivity and specificity of criteria currently used to define syndromes by the patient’s family history. Finally, there is a lack of understanding of the impact of genetic modifiers and environment on the phenotype in hereditary cancer families, as well as limited knowledge on population-specific or geographically localized mutations. As example we can cite a population-specific situation related to a founder germline mutation in the tumor suppressor gene TP53.

The exact contribution of TP53 germline mutations to the overall burden of cancer is still only partially known [5]. Most of the current evidence comes from studies on few families that carry highly predisposing, hotspot TP53 mutations (TP53 mutation database). Studies in Brazil have shown that a particular mutant, p.R337H, has incomplete penetrance and may be present in a significant number of subjects due to a founder effect (estimated frequency at the populational level of 1:300 individuals in certain regions), which may not easily recognized as risk patients due to weak family history of the disease and/or later onset of tumors [6,7]. This observation raises two points: (a) the occurrence of cancer-predisposing TP53 germline mutations may be much more common than recognized so far. This is likely the case in Southern and Southeastern Brazil for p.R337H, but may also occur in other Brazilian regions, or other parts of the world, due to similar founder mutations of incomplete penetrance; and (b) the cohort of p.R337H subjects and patients from Brazil provides a basis for both mechanistic and clinical studies. In a recent study designed to assess the prevalence of p.R337H in breast cancer-affected women the mutation was present in breast cancer affected women with positive familial history and/or later onset of tumors in the literature, the steps involved in the determination of a crystal structure will be described, including how to obtain good quality crystals, how to perform the X-ray diffraction experiment itself and how to solve the phase problem and refine the final structure. Emphasis will be placed on how the end-user should be aware of the limitations of the techniques involved and the potential errors present in proteins structure deposited in the Protein Data Base. Fragment-based ligand discovery and theoretical docking offer alternative approaches for focusing the structural biologist’s weaponry on drug design problems. Of the many applications described in the literature inhibitors of HSP90 and Protein Kinase B will be used as examples of the ways in which improving specific protein-ligand interactions can be used to improve binding and pharmacokinetic properties. On the other hand, a complex structure involving a misfolding analogue will exemplify the risks of over-interpretation of crystallographic data.

Competing interests: There are no competing interests in this presentation.

K15

Structural biology and cancer

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The techniques of structural biology currently play an important role in the development of novel therapeutic agents including cancer chemotherapies. X-ray crystallography has proved to be a particularly powerful tool for both the discovery of novel compounds and their subsequent development. In this review of the literature, the steps involved in the determination of a crystal structure will be described, including how to obtain good quality crystals, how to perform the X-ray diffraction experiment itself and how to solve the phase problem and refine the final structure. Emphasis will be placed on how the end-user should be aware of the limitations of the techniques involved and the potential errors present in proteins structure deposited in the Protein Data Base. Fragment-based ligand discovery and theoretical docking offer alternative approaches for focusing the structural biologist’s weaponry on drug design problems. Of the many applications described in the literature inhibitors of HSP90 and Protein Kinase B will be used as examples of the ways in which improving specific protein-ligand interactions can be used to improve binding and pharmacokinetic properties. On the other hand, a complex structure involving a misfolding analogue will exemplify the risks of over-interpretation of crystallographic data.

Competing interests: There are no competing interests in this presentation.

K16

Tumors as complex organs: are cancers manageable through the modification of their microenvironment?

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The techniques of structural biology currently play an important role in the development of novel therapeutic agents including cancer chemotherapies. X-ray crystallography has proved to be a particularly powerful tool for both the discovery of novel compounds and their subsequent development. In this review of the literature, the steps involved in the determination of a crystal structure will be described, including how to obtain good quality crystals, how to perform the X-ray diffraction experiment itself and how to solve the phase problem and refine the final structure. Emphasis will be placed on how the end-user should be aware of the limitations of the techniques involved and the potential errors present in proteins structure deposited in the Protein Data Base. Fragment-based ligand discovery and theoretical docking offer alternative approaches for focusing the structural biologist’s weaponry on drug design problems. Of the many applications described in the literature inhibitors of HSP90 and Protein Kinase B will be used as examples of the ways in which improving specific protein-ligand interactions can be used to improve binding and pharmacokinetic properties. On the other hand, a complex structure involving a misfolding analogue will exemplify the risks of over-interpretation of crystallographic data.

Competing interests: There are no competing interests in this presentation.
Cancers behave as organoids, composed not only by genetically altered tumor cells, but also by a variety of non-tumoral cells, such as fibroblasts, macrophages and endothelial cells among others. Cellular interactions within this organoid dictate the rate of tumor progression, and therefore the accumulation of heterogeneous genotypes which characterize the tumor mass at the moment of diagnosis. Evidence for both homotypic and heterotypic cooperation between cells within the tumor microenvironment exist. We will show evidence for fluctuations in the expression of a specific tumor marker, namely galectin-3.

Galectin-3 is an animal lectin that is found in diverse cellular compartments, depending on the cellular context and functional state of cells. Normal cells tend to accumulate galectin-3 in the nucleus. Upon malignant transformation, galectin-3 may concentrate in the cytoplasm and can be secreted to the extracellular matrix, where it acts as a matricellular protein, interfering with integrin function among other cell surface glycoproteins. We had shown a focal accumulation of galectin-3 in glioblastomas. Galectin-3 expression was associated with its translocation to mitochondria, suggesting its involvement in mitochondrial homeostasis. Inhibition of galectin-3 expression led to increased cell death, indicating that galectin-3 acts as a pro-survival factor in very specific tumor microenvironments associated with glioblastoma progression. The protumoral role of galectin-3 was also observed in murine melanomas, engrafted in both wild type and galectin-3-deficient mice. Absence of galectin-3 from both tumoral and stromal compartments was associated with attenuated tumor growth, as compared to an experimental condition where both tumor and stromal cells expressed galectin-3. Intriguingly, de novo expression of galectin-3 was frequently observed in tumors derived from cells that did not express galectin-3 when these cells were engrafted in galectin-3 deficient mice. This observation prompted us to investigate whether galectin-3 expressing cells would favor tumor growth when admixed with galectin-3 negative cells. Indeed, when cells are admixed, tumors grew much more efficiently than those engraftments of either cell alone, indicating cooperation between galectin-3 expressing cells and galectin-3 negative cells. Further experiments indicated that galectin-3 expression is related with the induction of a proangiogenic tumor environment. Thus, targeting galectin-3 expression in selected tumors may prove to be an effective strategy to control tumor growth. Angiogenesis and vasculature function are critically altered in cancers, which are usually ill-perfused. Evidence for activation of both angiotensin and bradykinin-dependent pathways within the tumor microenvironment of melanomas will be discussed. Blockage of vasoactive peptide-dependent pathways may control tumor angiogenesis and therefore may control tumor growth. Indeed, epidemiological studies are now indicating that individuals receiving specific blockers of angiotensin receptors may be relatively protected from the development of tumors. In a clinical setting, however, antiangiogenic drugs would be preferably used in combination with chemotherapeutic drugs. Vasculature function, however, is also critical for the proper delivery of chemotherapeutic drugs to tumors, specific therapeutic windows for the application of antiangiogenic drugs need to be identified. This notion will be further discussed. Altogether, the examples above call for a necessary understanding of tumor physiology, which seems to be under appreciated in the design of experimental/clinical trials.

**Competing interests:** There are no competing interests in this presentation.

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### K17

#### 3D nuclear organization and genomic instability in cancer

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To understand the genetic changes that occur at tumor initiation and during tumor progression, we focus on changes in nuclear architecture that promote the onset of genomic instability [1,2]. To determine changes in nuclear organization, we measure the 3D nuclear organization of telomeres, the ends of chromosomes. The measurements of telomeres allow one to trace the positions of chromosomes in interphase nuclei and, by using a fluorescent telomere-specific probe, all telomeres can be visualized in a single image. During the past years, we have defined the organization of telomeres in nuclei of normal, immortalized and tumor cells. We have developed quantitative software that enables us to measure the three-dimensional (3D) organization of telomeres [3,4]. The parameters we measure include: number of telomeres, sizes of telomeres, nuclear distribution of telomeres, and the presence of telomeric aggregates. The latter are clusters of telomeres that are absent from normal cells. More recently, we started to automate the 3D image acquisition and analysis and we are able to scan 15 000 cells per hour [5]. We observed changes in the nuclear organization of telomeres as a result of conditional c-Myc deregulation dependent on a functional myc box II [6,7]. We found Epstein-Barr Virus-induced changes in 3D telomere organization and resulting genomic instability [8].

Encouraged by these findings, we focused on tumor initiation and progression in patient samples and observed cancer-associated 3D nuclear telomere changes in lymphoid and solid tumors [9-12]. Moreover, the use of 3D nuclear telomere profiling permitted, for the first time, the identification of patient (and tumor) subpopulations that were not detectable up to that point. For example, we blindly defined three distinct subpopulations that correlated with short-term, intermediate and long-term survival in glioblastoma [11]. Using the same 3D imaging approach, we defined subpopulations in myelodysplastic syndromes and acute myeloid leukemias [12]. Additional studies are currently ongoing.

We provided evidence that genomic instability is a result of these nuclear changes. Dynamic nuclear alterations directly result from 3D telomere aberrations [1,2,6,13-15]. These genomic changes include aneuploidy, Robertsonian fusions, breakage-bridge fusion (BBF) cycles with resulting terminal deletions and unbalanced translocations and continued rounds of BBF cycles. While these changes can be followed during cancer progression in patients, their true origin can only be examined in conditional expression studies or longitudinally in mouse models. Using conditional c-Myc deregulation, we demonstrated that changes in 3D telomere profiles precede the onset and propagation of genomic instability [6,13].

In Hodgkin’s lymphoma, in collaboration with Dr. Hans Knecht, we showed that mono-nucleated H cells become multinucleated Reed Sternberg (RS) cells through telomere dysfunction as measured by 3D nuclear profiles [10,16]. These changes coincide with localized shelterin dysfunction, aberrant centromere duplication as well as spindle formation and significantly elevated levels of DNA damage foci [10,16]. Spectral karyotyping (SKY) and super resolution 3D imaging confirmed the dynamics and complexity of these genetic changes in which RS cells are the end-stage cells generated through multiple defects traced back to the H cells and propagated from there on [15]. Most recently, we have reported that Hodgkin’s patients with recurrent/non-recurrent disease display distinct 3D telomeric profiles [17].

Ongoing studies focus on a variety of cancers where clinicians currently lack the ability to define the risk of an individual patient to progress, the ability of early detection or of monitoring of disease progression. In conclusion, 3D telomere profiling provides a platform technology able to determine normal and aberrant nuclear organization that is a measure of genomic instability and cancer.

**Competing interests:** There are no competing interests in this presentation.

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K18
Biomarker analysis in human neoplasias: superior next-generation sequencing on frozen bone marrow cells and on formalin-fixed, paraffin-embedded tumor tissues
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Background: Biomarker-based personalized treatment has improved the prognosis of many tumors. EGFR, KRAS, and BRAF mutations and EGFR antibody treatment (colorectal carcinoma) and EGFR inhibitor treatment (lung carcinoma) serve as good examples. The rapid improvement of biomarker-based treatments requires that not merely one biomarker but many biomarkers such as mutations, gene fusion, and gene copy number alterations, are to be tested simultaneously for selection of the ideal combination of molecularly targeted and individual treatment. Next-generation sequencing (NGS) makes it possible to study mutations, gene fusions, and copy numbers genome wide in one single analysis. This presentation based on our three papers [1-3] in press illustrates the feasibility, even superiority of NGS for biomarker analysis in human neoplasias.

Aims: 1) As EGFR, KRAS, and BRAF mutations are clinically relevant, mainly predictive biomarkers in colorectal and lung carcinomas, we aimed to evaluate by NGS and routinely used real-time PCR (RT-PCR) methods the feasibility of NGS for analysis of these mutations. Furthermore, we aimed, by NGS, to reveal novel mutations.

2) Anaplastic lymphoma kinase (ALK) gene fusions occur in a small proportion of non-small cell lung carcinomas. Identification of the fusions is crucial for targeted treatment decisions. We aimed to screen ALK gene fusions in lung carcinomas, and to compare the results that were studied by NGS with those detected by routine methods, including standard fluorescence in situ hybridization (FISH), immunohistochemistry (IHC), and RT-PCR. We conducted the ALK fusion analyses on 95 formalin-fixed paraffin-embedded (FFPE) tumor tissues from 87 patients with non-small cell lung carcinoma.

3) As many human neoplasias such as acute lymphoblastic leukemia, chromosomal deletions at 9p are common. To study the feasibility of NGS for detection of copy number alterations at chromosome 9p, we conducted NGS and array-comparative genomic hybridization (aCGH) analyses on 35 patients with acute lymphoblastic leukemia.

Methods: Methods for NGS, aCGH, FISH, RT-PCR, and IHC appear in the original articles (1-3). Our NGS method was based on targeted resequencing. For EGFR, KRAS, BRAF, and ALK, all the exons (for ALK also some intronic areas) were sequenced. For 9p, target regions were sequenced with a total length of 1 Mb from a 32 Mb region. For capture of target regions, baits were custom-designed to capture all exons.

Results and discussion: Comparison of NGS and PCR methods for detection of EGFR, KRAS, and BRAF mutations in lung carcinoma [1]: We observed a significant concordance (from 96.3 to 100%) of the EGFR, KRAS, and BRAF mutation detection results between targeted NGS and RT-PCR. Moreover, targeted NGS revealed seven non-synonymous single-nucleotide variations and one insertion-deletion variation in EGFR undetectable by the real-time PCR methods. The potential clinical significance of these variants requires elucidation in future studies. Our results support the use of targeted NGS in the screening of EGFR, KRAS, and BRAF mutations in FFPE tissue material.

Detection of ALK fusions in lung carcinoma by NGS, FISH, RT-PCR, and ICH [2]: All methods were successful on FFPE tumor tissue material. Of the 87 patients examined, we detected ALK fusion in 5 (5.7%). Results from resequencing correlated significantly with those from FISH, RT-PCR, and IHC. Targeted NGS provided a promising method for ALK gene fusion detection in non-small-cell lung carcinomas. Means to reduce the material and turnaround time required for analysis are, however, necessary.

NGS as a method for detection of copy number alterations [3]: In detection of copy number alterations at 9p, high concordance occurred between NGS and aCGH. Both methods revealed deletions at the CDKN2A/B locus, whereas NGS revealed additional small deletions outside the resolution of aCGH. Furthermore, NGS showed mutations and gene fusions impossible to see by aCGH. To conclude, novel copy number alterations, mutations, and structural alterations were revealed.

Conclusions: For two reasons, NGS was superior for biomarker analysis: the possibility to see a large range of gene mutations in one analysis, and the possibility to detect mutations, gene fusions, and copy number alterations simultaneously in a single test. Our studies illustrated the feasibility of NGS for clinical specimens of FFPE tumors. We also showed that in addition to mutations, gene fusions and copy number alterations can also be reliably detected by NGS. As the costs and required material for NGS analyses has decreased to near that of PCR or FISH, NGS may very soon become a method to replace all other methods for biomarker analyses of human malignancies.

Competing interests: There are no competing interests in this presentation.

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K19
INCT in oncogenetics focusing on hereditary breast-colorectal carcinoma syndrome
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The National Institute of Science and Technology in Oncogenetics (INCT) established a scientific program that links 46 researchers and supports their efforts to translate research findings into clinical practice, including clinical trials. Our initiative focused in the management of resources of three areas: research networks; chair programs and infrastructure support; transference of new knowledge, which can reduce the burden of cancer, quickly and efficiently to the Brazilian healthcare system. The National Institute of Science and Technology in Oncogenetics was created by the Brazilian Ministry of Science and Technology in search of excellence in scientific activities at an international level, by means of programs and instruments made operationally by CNPq (National Council for Scientific and Technological Development) and FAPESP (São Paulo Research Foundation).

The major aim of this study is recruit cancer patients from families with a history of family aggregation or hereditary cancer and relatives, affected or not, in order to obtain molecular and epidemiological data. We are investigating genomic alterations in the probands and in their relatives, including at least one who is a cancer carrier (not necessarily the same tumor type). Genomes vary from one another in several ways and the totality of this genetic variation is the basis of human traits heritability. Genome re-sequencing studies have shown that the bases that vary among genomes reside in CNVs (Copy Number Variations) ranging in size from kilobases (kb) to megabases (Mb), which are not identifiable by conventional chromosomal banding. Deletions, duplications, amplifications, insertions, and translocations can result in CNVs. In addition, balanced genomic inversions leading to DNA structural variations that do not cause CNV can nevertheless contribute significantly to genome instability. Despite extensive studies, the total number, position, size, gene content, and population distribution of CNVs remain elusive. Although it is estimated that CNVs may account in a large proportion of the human genome, the association between CNVs and human cancer is limited. It is anticipated that the application of array CGH techniques and next-generation sequencing will reveal a significantly larger scale of structural variation among different individuals and populations, as the majority of CNVs appear smaller in scale and are beyond the resolving capability of current arrays [1,2]. CNVs can be inherited or sporadic; large de novo CNVs are thought more likely to be disease causative. However, the phenotypic effects of CNVs are sometimes unclear and depend mainly on whether dosage-sensitive genes or regulatory sequences are affected by the genomic rearrangement. Sporadic disease can also result from a combination of CNVs that is at a single locus or theoretically from two or more CNVs at different loci from two normal parents [3].

Despite the fact that cancer is an acquired disease caused by various factors, there is clear evidence that inherited factors play a significant role. Some of these inherited factors represent loss-of-function mutations in tumor suppressor genes, resulting in a high relative cancer risk among carriers. In particular, rare constitutional CNVs may affect important cancer-associated genes or pathways, providing an explanation for high-risk cancer families. The first approach of this study is to investigate clinically and genetically the most common cancers associated with hereditary predisposition (breast, ovary, colorectal, head and neck carcinomas) in a Brazilian Network of Cancer involving Reference Health Care Cancer Centers.

It is accepted that 5 to 10% of all cancers are hereditary or familial [4]. The majority of hereditary neoplasias related to breast cancer are associated with germline mutations in BRCA1 and BRCA2. However, inherited mutations related to other genes and/or related to certain syndromes also influence the increased risk of developing cancer. Li-Fraumeni syndrome results in a mutation on gene TP53 and is related to increased risk of developing tumors at a young age. A deletion in gene CHEK2 is associated with a two-fold greater risk of the patient presenting breast cancer. Hereditary nonpolyposis colorectal cancer (HNPCC) associated with mutation in the DNA damage repair genes, such as MLH1 and MSH2, constitutes a risk factor for the development of extracolonic tumors, including breast tumors [5].

The possibility of identifying a Brazilian profile of the syndrome has permitted our group to propose new tracing strategies aimed at contributing to early detection of disease carriers among Brazilians. The Department of Oncogenetics of the AC Camargo Hospital was created in 2000 and since then, more than 4,500 patients and family members have received Genetic Counseling. The Oncotree software is used to monitor family history data. In the AC Camargo Hospital peripheral blood DNA samples of patients with hereditary cancer or family history or familial aggregation of cancer have been recruited over the years. Some of these families were selected because they presented all but one of the international criteria adopted in the categorization of a Familial Cancer Syndrome. In this project, affected family members (with screening of pathogenic mutations in the major candidate genes) are under evaluation by array-CGH and next generation sequencing. The objective is to publicize rare genomic alterations that could contain new hereditary predisposition genes, aimed at defining or identifying new markers of risk of susceptibility to cancer. Here, we discuss the new findings in probands without mutations in genes frequently described as associated with the breast and ovary syndrome, Lynch syndrome, breast and colorectal syndrome as well as future perspectives of application of these results.

Competing interests: There are no competing interests in this presentation.

References
The tumor microenvironment is a complex milieu of tumor and host cells. Host cells can include tumor-reactive T cells capable of killing tumor cells. However, more frequently tumor and host components interact to generate a highly immune suppressive environment that frustrates T cell cytotoxicity and promotes tumor progression through a variety of immune and non-immune mechanisms. Myeloid-derived suppressor cells (MDSC) are a major host component contributing to the immune suppressive environment. MDSC accumulate in most patients and experimental mice with cancer. They inhibit both adaptive and innate immunity through a diverse array of suppressive mechanisms and therefore are a significant obstacle for natural immunity and for active cancer immunotherapies. Their accumulation and suppressive potency are driven by pro-inflammatory mediators. In addition to their inherent immune suppressive function, MDSC appear to inhibit anti-tumor immunity and promote tumor progression, and the role of inflammation in promoting cross-talk between MDSC and other cells in the tumor microenvironment.

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K21

Medical applications of RNA interference (RNAi)
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During its long and rich history, medical sciences have undergone several changes of paradigms. In this quest to develop more potent drugs, less invasive surgical techniques and more sophisticated diagnostic approaches (among other goals) medicine has contributed decisively to our well being and longevity. Some of these major accomplishments, which altered the course of humanity are: the development of antibiotics, organ transplantation and radiography. Medicine has recently reached a new hallmark: RNA interference (RNAi), a Nobel prize winning technology which promises to promote medical care at the molecular level, by regulating genes in our favor.

RNAi was first successfully used in C. elegans, a laboratory model species, by microinjecting dsRNAs in the worm’s gonad. However, the works that laid the foundations for the development of the technique were initially done in plants and fungi. RNAi was accidentally discovered, when white transgenic petunias were obtained instead of deep purple ones [1]; a similar paradoxal result was obtained when white transgenic colonies of Neurospora sp were observed in place of orange ones [2]. These findings revealed the existence of an unknown potent mechanism of gene regulation, which could be tamed for diverse uses, from basic science, agriculture, animal health and medicine.

From these seminal observations, during a period of nearly 10 years, researchers aimed at characterizing, at the genetic and biochemical levels, the foundations of the development of the technique were initially done in plants and fungi. RNAi was accidentally discovered, when white transgenic petunias were obtained instead of deep purple ones [1]; a similar paradoxal result was obtained when white transgenic colonies of Neurospora sp were observed in place of orange ones [2]. These findings revealed the existence of an unknown potent mechanism of gene regulation, which could be tamed for diverse uses, from basic science, agriculture, animal health and medicine.

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Epigenetic field for cancerization: its cause and clinical implications

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Epigenetic alterations are present not only in cancer cells but also in non-cancerous tissues. Accumulated evidence of aberrant DNA methylation in non-cancerous tissues can correlate with risk of cancer development, especially in chronic inflammation-associated cancers [1-3]. The close correlation in non-cancerous tissues was prominent for epigenetic alterations, compared with genetic alterations, and formed a concept of “epigenetic field for cancerization (the epigenetic field defect)”. In gastric cancers, close correlation between methylation levels and cancer risk has been demonstrated [4]. As mechanisms for methylation induction in the stomach, infection by Helicobacter pylori (H. pylori), the major cause of gastric cancers, was implicated in humans [5], and was demonstrated in Mongolian gerbils [6]. Especially, a critical role of inflammation triggered by H. pylori infection, not by high concentrations of ethanol or salt, was demonstrated, suggesting the importance of specific chronic inflammation [7]. Gene expression analysis showed that expression levels of Il1b, Nos, and Tnf were well correlated with methylation levels induced. To dissect molecular mechanisms for induction of epigenetic alterations, a mouse colitis model induced by dextran sulfate sodium (DSS) was used. First, we isolated genes methylated in colon tumors induced by DSS and azoxymethane, and showed that these genes were methylated in non-cancerous colonic mucosa, forming an epigenetic field. Averant methylation was induced even in SCID mice, which lack functional T- and B-lymphocytes, and it was shown that lymphocytes are not essential in lymphocyte-dependent induction of aberrant DNA methylation [8]. By chromatin-immunoprecipitation-on-Chip analysis of H3K27me3, absent H3K27me3 was shown to be induced by colitis, and can be carried into cancer tissues and function as a premark for induction of aberrant DNA methylation [9].

One of the major translations of the epigenetic field for cancerization is its use as a cancer risk marker. By searching for CpG islands differentially methylated in gastric mucosa of gastric cancer patients and healthy volunteers, both of which had past infection by H. pylori, we were able to isolate and validate seven differentially methylated CpG islands. The new markers had large areas under the receiver-operating characteristic curves (0.78-0.84) and high odds ratios (12.7-36.0) even among individuals with past H. pylori infection, compared with two currently available markers (0.60-0.65, 5.0-5.7) [10]. We are currently conducting a prospective study to predict patients who suffer from metastatic gastric cancers among gastric cancer patients treated by endoscopic submucosal dissection.

Another translation is prevention of cancers. Several studies involving viral onco genes and chemical carcinogens showed that epigenetic cancer prevention is possible, but there have been no studies for the usefulness of epigenetic cancer prevention in chronic inflammation-associated cancers. We administered 5-aza-2-deoxycytidine (5-aza-dC) to Mongolian gerbils infected with H. pylori after administration of N-methyl-N-nitrosourea. It was shown that the incidence of gastric cancers was suppressed almost to half of that in gerbils without 5-aza-dC [Niwa, submitted]. These findings vividly show that the epigenetic field defect has its unique characteristics, such as ease of measurement and reversibility, and harbors a rich chance of clinical translations.

Competing interests: There are no competing interests in this presentation.

References

K23
Prion protein signaling as therapeutic target in human tumors

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Prions are proteinaceous infectious agents associated to invariable fatal neurodegenerative disorders named transmissible spongiform encephalopathies or prion diseases. The mechanism associated with disease propagation and definitions of the cellular prion protein (PrP\(^\text{Pr}\)), which acquires altered conformation, form aggregates and is resistant to proteolysis. PrP\(^\text{Pr}\) is a cell surface glycosylphosphatidylinositol (GPI)-anchored protein of 208-209 aminoacids which is codified by a single exon and whose expression is developmentally regulated. In adult animals PrP\(^\text{Pr}\) is highly expressed in the central nervous system but peripheral nervous system or other organs or tissues also express this protein.
Initial experiments conducted with PrP<sup>C</sup>-null mice established that this protein is absolutely necessary to propagate prion infection. Remarkably, these animals had no gross anatomical abnormalities or behavior alterations although higher neuronal sensitivity to stress conditions such as seizures and hypoxic-ischemic injury are present. These data pointed that PrP<sup>C</sup> would be unnecessary for embryonic development or some compensatory mechanism might be present in these animals. In fact, the later hypothesis was supported by findings that demonstrated that deletion of specific PrP<sup>C</sup> domains cause severe neurodegeneration in mice.

The use of cellular models led to the characterization of PrP<sup>C</sup> ligands and related signaling pathways allowing the definition of PrP<sup>C</sup> cellular functions. We identified three major ligands for PrP<sup>C</sup>, two proteins of the extracellular matrix, laminin and vitronectin, and a secreted co-chaperone the stress inducible protein 1 (ST1I1) or Hop (its human homologue). Comparative experiments using neurons derived from wild type and PrP<sup>-</sup>-null mice demonstrated that PrP<sup>C</sup> binding to vitronectin promoted axonal growth in dorsal root ganglion neurons (DRG). In addition, PrP<sup>C</sup> interaction with laminin activated metabotropic glutamate receptors, Ca<sup>2+</sup><sup>+</sup> mobilization and PKC activation promoting neuronal plasticity and memory formation in rats. ST1I1 was the most explored PrP<sup>C</sup> ligand. The PrP<sup>C</sup>–STI1 engagement induces PKA an ERK1/2 activation, which increase neuronal survival and differentiation. The signaling pathways modulated by PrP<sup>C</sup>–STI1 also include Ca<sup>2+</sup><sup>+</sup> influx by activation of a-7 nicotinic acetylcholine receptor, induction of PI3K and mTOR. These last two pathways were associated to increased protein synthesis. An increment on the self-renewed of neuronal progenitors is also modulated by PrP<sup>C</sup>–STI1 engagement.

The co-chaperone STI1 is known to interact with PrP<sup>C</sup> at the neuronal membrane and recently we demonstrated that this protein is abundantly secreted by astrocytes. Secreted STI1 plays an autocrine activity upon astrocytes modulating their proliferation and differentiation. The mechanism associated with STI1 secretion is under evaluation but preliminary results indicated that this protein is secreted by microvesicles derived from multitissues.

The involvement of PrP<sup>C</sup> and STI1/Hop in cellular survival and differentiation raised questions regarding their involvement in tumoral processes. In fact, the literature has been pointed that PrP<sup>C</sup> expression contributes to cancer progression and resistance to various cancer therapies. STI1/Hop expression was also associated with the proliferation of tumor cells. Our data pointed that STI1 secreted by glioblastoma cells has an autocrine function mediating proliferation of these cells by binding to PrP<sup>C</sup>. We are presently evaluating how to interfere with the PrP<sup>C</sup>–STI1 binding in order to modulate tumor growth.

Together our results point that PrP<sup>C</sup>–STI1 engagement is an interesting therapeutic target for both neurodegeneration and cancer and investments should be done to address these issues.

Competing interests: There are no competing interests in this presentation.

Acknowledgements: Supported by: FAPESP and National Institute for Translational Neuroscience.

**ORAL PRESENTATIONS**

**O1** Differentially expressed genes responsible for insensitivity of CD34+ to kinase inhibitors in patients with chronic myeloid leukemia
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**Background:** Chronic Myeloid Leukemia (CML) is a clonal myeloproliferative disorder characterized by formation of BCR-ABL fusion that encodes the p210 oncoprotein, which has a tyrosine kinase activity that confers an adaptive advantage to leukemic cells. Imatinib mesylate (IM) acts specifically on p210. Imatinib is able to reduce the differentiated cells (CD66b<sup>+</sup>) effect but it may not affect the progenitor cells (CD34+). CD34+ can be kept alive during treatment. Our aim was to identify expressed genes in CD34+ and CD66b+ cells as candidates for kinase inhibitors transport.

**Materials and methods:** CD34+ and CD66b+ cells were isolated from bone marrow (BM) and peripheral blood (PB) of five patients with CML in optimal response, and 1 control. The samples were sequenced on SOLID<sup>TM</sup> platform for whole transcriptome analysis. We analyzed the Gene Ontology annotation, and the software Cufflinks were used to identify the differential expression of genes in patients (BM x PB) and controls (BM x PB).

**Results:** In pooled patient samples, we identified the expression of SLCO2A1 influx gene in both, BM and PB samples, without any significant change (p ≤ 0.05), and expression of SLCO1A2 influx gene only in PB sample. Thus its presence could not be identified in any of the control samples. The overexpression of ABG efflux gene family (ABC1B; ABCG2; ABCC1), were found only in BM cells of patients. The presence of other two genes responsible for the drug efflux was also found exclusively in BM pool sample of patients, SLC47A1 and SLC47A2.

**Conclusions:** Over-representation of drug influx and absence of drug efflux channels in mature cells, and the reverse in stem cells of patients with CML may explain the insensitivity of CD34+ cells to IM treatment and consequent failure to eliminate minimal residual disease.

**Financial support:** Novartis Oncology of Brazil.

**O2** Characterizing the role of the alternative NF-κB pathway in diffuse large B-cell lymphoma
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**Background:** Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma in humans and dogs and has similar biology and clinical behavior in both species. Therefore, comparative approaches in understanding the disease may be beneficial to both species. The deregulation of nuclear factor kappa B (NF-κB) pathway, composed of classical and alternative pathways, is important in the pathogenesis of DLBCL. However, studies have largely focused on the classical pathway and the role of the alternative pathway is incompletely understood. In this study, we characterize the alternative NF-κB pathway in DLBCL to test its potential as a therapeutic target.

**Materials and methods:** The activation of NF-κB pathways was analyzed by the expression, nuclear translocation, and binding to the NF-κB oligonucleotide probe, of classical and alternative NF-κB proteins in primary dog DLBCL cells using western blotting and electrophoretic mobility shift assay.

**Results:** We demonstrated for the first time that the alternative NF-κB pathway, as well as the classical NF-κB pathway, is recurrently activated in primary dog DLBCL cells. The pattern of NF-κB protein expression was similar to that observed in human DLBCL cells.

**Conclusions:** We propose the alternative NF-κB pathway as a novel target for lymphoma therapies. We are currently analyzing the effect of small interfering RNAs targeting the alternative NF-κB pathway for cell proliferation/viability and changes in genome-wide gene expression using a RNA-sequencing technology. The results will provide new insights on the roles of the alternative NF-κB pathway to develop novel treatment strategies for human and dog DLBCL using comparative oncology approaches.

**Financial support:** MAF First Award Grant D12CA-302 (DI) and the University of Minnesota Animal Cancer Care and Research Program Fund.

**O3** A high-resolution system for metabolic imaging of cancer
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**Background:** Chronic Myeloid Leukemia (CML) is a clonal myeloproliferative disorder characterized by formation of BCR-ABL fusion that encodes the p210 oncoprotein, which has a tyrosine kinase activity that confers an adaptive advantage to leukemic cells. Imatinib mesylate (IM) acts specifically on p210. Imatinib is able to reduce the differentiated cells (CD66b<sup>+</sup>) effect but it may not affect the progenitor cells (CD34+). CD34+ can be kept alive during treatment. Our aim was to identify expressed genes in CD34+ and CD66b+ cells as candidates for kinase inhibitors transport.
Alterations in metabolic signaling pathways allow tumors to achieve balance between oxygen supply and consumption via activation of several genes involved in adaptation to low oxygen conditions (hypoxia). Hypoxia stimulates glucose uptake, glycolysis and changes in mitochondrial respiration in tumors as a direct result of increased HIF-1 transcriptional activity. HIF-1α stabilization induces the expression of genes involved in cell survival, proliferation, angiogenesis and glucose transport (GLUTs) and the stimulation of genes involved in glycolytic energy production. Elevated anaerobic metabolism results in increased production of lactate as an alternate cellular energy source. Recent studies suggested that increased levels of lactate in tumors are associated with tumor progression, and are useful of pretreatment, radioresistance and HIF-1 abnormal status in the tumor microenvironment. Therefore, the ability to monitor products of altered metabolism in tumors would provide valuable information to improve cancer treatment. We have developed a novel high-resolution imaging system to assess the distribution of metabolites in tumors such as lactate and ATP. Initial tests demonstrate the capacity of this technique in providing quantitative measurements of metabolites with high spatial resolution. This technology offers several important advantages over other techniques including ease of translation to the clinic, microscopic spatial localization and quantification of metabolite concentration. Another advantage on the use of this technique is the possibility of spatial co-registration of metabolites with other tumors biomarkers. The main focus of this approach is the development of novel technologies for imaging tumor metabolism. This will lead to a better understanding of the role of tumor metabolism in treatment outcome, leading to more rational therapeutic protocols.

**Financial support:** The Terry Fox Foundation, Canada.

### 04 Evaluation of interleukins 8 and 12, CA 15-3 and free circulating DNA as prognostic markers in dogs with mammary tumors


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**Background:** Mammary tumors of dogs are an excellent model to investigate the clinical and pathological diagnosis and prognosis of cancer. Interleukins play a key role in cancer, particularly interleukin-8, which has tumorigenic and proangiogenic properties and interleukin-12, with anti-metastatic and angiogenic properties. The carbohydrate antigen tumor marker (CA 15-3) has important clinical significance in the monitoring of patients with breast cancer and, in addition, free circulating DNA has been considered a candidate biomarker for cancer. The aim of this study was to measure serum levels of interleukin-8, CA 15-3 and to estimate the number of copies of CAN SINE sequences to correlate with clinicopathological parameters and survival.

**Materials and methods:** We used enzyme-linked immunosorbent assay and qPCR to evaluate 33 bitches with mammary cancer and 50 control bitches.

**Results:** High levels of interleukin-8 were found in bitches with mammary cancer and were correlated with disease progression, lymph node involvement, recurrence and death. Low levels of interleukin-12 were observed in bitches older than 10 years, with longer tumor time course, and high levels correlated with high survival rate. High levels of CA 15-3 were correlated with lymph node involvement and death and, in estimating the number of copies of CANSINEs, a significant difference was found regarding the parameters that indicate poor prognosis and low survival.

**Conclusions:** Our results show that these proteins and the effect of SINEs can be used as noninvasive prognostic markers in breast cancer research and are useful in predicting progression and tumor recurrence in bitches with mammary cancer.

**Financial support:** FAPESP.

### 05 Genomic alteration in hereditary colorectal patients without mutations in mismatch repair genes

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**BMC Proceedings** 2013, 7(Suppl 2):O5

**Background:** Lynch Syndrome (LS) is the most common hereditary syndrome of colorectal cancer (CRC), caused by mutations in mismatch repair (MMR) genes. It is estimated that 50% of families classified according Amsterdam criteria not show germline mutations in MMR genes. These findings suggest that other genetic or epigenetic factors are associated with predisposition to CRC.

**Materials and methods:** It was evaluated germline copy number variations (CNVs) in 57 patients with LS (Amsterdam Criteria), but without pathogenic mutations in MMR genes, by array CGH using the 4x180K platform (Agilent Technologies). Genomic data were extracted with Feature Extraction software and analyzed using Genomic Workbench software, statistical algorithm ADM-2 and threshold of 6.7.

**Results:** It was found 252 CNVs (4.4 ± 3.6 CNVs/individual), including 104 genomic gains and 148 losses. After comparison with a reference group, composed of 100 healthy Brazilian women (Krepschi et al., 2012) and the Database of Genomic Variants (DGV-hg18), 106 rare CNVs were identified in 41 cases and 10 new rare CNVs in six cases. Four rare CNVs, of the same size, were detected in at least three cases: 1q21.1, 7p22.3, 11q13.2 and 15q11.2. Four patients had new rare CNVs mapped at 7p22.3. In 7p22.3 and 15q11.2.

**Conclusions:** Putative candidate genes mapped on 7p22 are suggestive to be associated with hereditary predisposition to CRC. The relatives of these probands are being evaluated to confirm the segregation of the most important alterations and their association with CRC predisposition.

**Financial support:** FAPESP and CAPES.

### 06 Hematopoietic stem cells have an intrinsic expansion limit

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**BMC Proceedings** 2013, 7(Suppl 2):O6

**Background:** Hematopoietic stem cell (HSC) transplantation is the only treatment providing long-term cure in acute myeloblastic leukemia. At the apex of the hematopoietic system, quiescent HSCs escape to chemotherapy treatments and therefore can regenerate the entire blood system after drug exposure. Nevertheless, the consequence of repeated chemotherapy regimen on HSC function remains to be clarified. In this study, we investigate how massive expansion in vivo influence HSC functions.

**Methods and results:** We optimized a protocol based on 5-fluorouracil (5FU), an antimetabolite used to treat different types of cancers. We show that after one 5FU treatment, HSCs exit quiescence and enter the cell cycle. To deplete cycling HSCs, we injected a second dose of 5FU and show that the stem cell pool is disseminated. Nonetheless, the remaining HSCs proliferate extensively to re-establish the HSC pool. At this point, most HSCs have exited the cell cycle and are back to quiescence. Despite a near normal stem cell pool size and a quiescent status, HSCs from these 5FU treated mice cannot compete against untreated cells to reconstitute the host in transplantation assays. Furthermore, we show that this extensive proliferation in vivo severely impairs the clonal expansion of individual HSC as measured by the mean activity of stem cell (MAS).

**Conclusion:** Our results demonstrate that HSCs lose their competitive potential after two 5FU treatments, suggesting that HSCs have an intrinsic expansion limit beyond which their regenerative potential is impaired. We propose that chemotherapy regimens based on repeated administration of antimetabolites are likely to impair long-term stem cell functions.

**Financial support:** FRSQ, Cole Foundation.
O7
Acute, but not constitutive, loss of endothelial β3-integrin inhibits tumour growth and angiogenesis
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BMC Proceedings 2013, 7(Suppl 2):O7

Background: Angiogenesis, the formation of new vessels from pre-existing ones, is essential for tumour growth and metastasis. Endothelial cells play a central role in this process: they drive blood vessel formation in response to signals from the local environment, by a mechanism that is integrin-dependent. We are particularly interested in understanding what role αvβ3-integrin plays in governing tumour angiogenesis. αvβ3-integrin seemingly poses an ideal anti-angiogenic target. Its expression is vastly up-regulated in neo-angiogenic vessels, while its expression in quiescent vasculature is minimal. However, anti-angiogenic therapy targeting αvβ3-integrin has proven somewhat disappointing. In part this likely relates to the fact that αvβ3-integrin is not expressed solely by endothelial cells, but across a wide range of cell types that each contribute to angiogenesis. The aim of the research we present here is to elucidate the role of αvβ3-integrin in tumour growth and angiogenesis as it is expressed specifically by endothelial cells.

Methods: We have crossed β3-integrin floxed animals to two Cre transgenic models to delete β3-integrin specifically in endothelial cells. In Pdgfb.Creert2 transgenic mice, β3-integrin is deleted in a tamoxifen-inducible fashion in neo-angiogenic endothelial cells, while in Tie1.Cre transgenic mice β3-integrin is constitutively deleted in endothelial cells. In these animals, we have studied angiogenesis via the ex vivo aortic ring model, and in subcutaneous grown B16F0 and CMT19T allograft tumours.

Results: In β3-floxed/Pdgfb.Creert2 positive mice treated with tamoxifen, allograft tumours grow significantly smaller when compared to their growth in Cre negative littermate controls. This correlates with decreased microvascular density observed in Cre-positive compared to Cre-negative tumour sections. In contrast, constitutive deletion of endothelial β3-integrin via Tie1.Cre leads to enhanced tumour angiogenesis. These findings are re-capitulated in the aortic ring model. VEGF-induced microvessel sprouting is inhibited in β3-floxed/Pdgfb.Creert2 positive mice compared to Cre-negative littersmates. In marked contrast, sprouting is enhanced in β3-floxed/Tie1.Cre positive mice compared to β3-floxed/Tie1.Cre negative mice.

Conclusions: These findings strengthen the argument that endothelially-expressed β3-integrin remains a valid target of anti-angiogenic tumour therapy. However, taken together, these data suggest that the timing and length of exposure to β3-integrin endothelial genetic inhibition impacts on the angiogenic response. These data highlight the need to enhance our understanding of the molecular basis of angiogenesis in order to develop improved therapeutic treatments.

Financial support: University of East Anglia and the BigC Norfolk Cancer Charity.

POSTER PRESENTATIONS

P1
Survival study of 86 bitches with mammary tumors treated at the Veterinary Hospital of the University Anhembi Morumbi, São Paulo, Brazil
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BMC Proceedings 2013, 7(Suppl 2):P1

Background: Mammary tumors are the most common cancers in dogs. The aim of this study is to analyze the association between the survival rates and the variables: size of tumors, presence of metastases, type of treatment and stage of malignancy.

Patients and methods: In this retrospective study, clinical records of 86 animals with 154 mammary formations, treated in 2010-2011 at the Veterinary Hospital of the University Anhembi Morumbi, were analyzed for breed, age, histopathological type, size, presence or absence of metastases, survival and staging of tumor. Kaplan-Meier log rank analysis was used to evaluate survival.

Results: Among the 154 tumors analyzed, 149 (96.7%) were neoplasms (benign and malignant) and 5 (3.3%) were non-neoplastic lesions. For statistical analysis, mongrel (30.2%) and Poodles (26.7%) were a lot more affected breeds, age between 9 and 12 years (62.8%) was the most frequent of all, 60,4% (90/149) of neoplastic formations were benign and 39,6% (59/149) were malignant. 24,4% (12/49) of all malignant tumors patients had metastasis. Dogs whose tumors were less than 4 cm had no significantly increased duration of survival with a median of 15,3 months versus 17,4 months for dogs with tumors greater than 4 centimeters (p=0.327). Dogs with metastasis lived a median of 10,7 months versus 18,3 months for dogs without them (p=0.01). Patients treated with surgery had 17,3 months of median survival rate versus surgery plus chemotherapy submitted dogs had 13,8 months (p=0.319). Dogs with malignant tumors in stage I, II, III, IV and V had, respectively, 21, 18,4 , 14,9, 17,2 and 8,8 months of survival (p<0.01).

Conclusions: Survival analysis of patients with malignant mammary tumors indicated that size did not influence survival time, the animals live less if had metastasis, the type of treatment (only surgery or surgery plus chemotherapy) did not change survival rate and there was a tendency of decrease survival rate for animals in more advanced clinical staging.

P2
MicroRNAs from peripheral blood mononuclear cells as biomarkers for detection of preclinical fibrosarcoma
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BMC Proceedings 2013, 7(Suppl 2):P2

Background: Blood immune cells cooperate to prevent the progression of tumors through cancer immunosurveillance. Since activated peripheral immune cell clones trigger a sensitive transcriptional response upon recognition of tumors, which can be identified by transcriptional profiling, we hypothesised that peripheral blood mononuclear cells (PBMCs) could be used as reporters for cancer detection.

Materials and methods: We used a model system in which groups of immunocompetent BALB-c mice were subcutaneously injected with different numbers of tumorigenic B61 fibrosarcoma cells. The groups of study were: (i) tumoral group with serial injections of 10^6 to 10^7 cells; (ii) negative control group represented by sterile nonpyrogenic saline, (iii) inflammation group by Zymoza (Sigma) and (iv) bacterial infection group by injection of 10^6 colony forming units [cfu] pool from mice feces. Mouse peripheral blood was collected three days after injection; blood samples (N=10) were pooled according to experimental conditions. Mononuclear cells were separated by centrifugation on a Ficol-Hypaque cushion (GE Healthcare) and RNA was extracted using Trizol Reagent (Invitrogen). Samples were hybridized on miRNA microarrays (Agilent).

Results: We identified four microRNAs, miR-451, miR-144, miR-486 and miR-494, which were differentially expressed when compared to control groups, including inflammation and bacterial infection.

Conclusions: Our results showed that PBMC microRNA expression profiling can serve as a sensitive method for detection of preclinical cancer.

Financial support: FAPESP.

P3
Genotype and phenotype of balb/c mouse strain expressing h-2k2-tssa58-sv40 immortalizing oncogene
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BMC Proceedings 2013, 7(Suppl 2):P3
**P4**

Ezrin is required for adhesion and migration in invasive breast cancer

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**BMC Proceedings** 2013, 7(Suppl 2):P4

**Background:** Deregulation of focal adhesion (FA) dynamics has been found to contribute to tumour progression by promoting cancer cell migration and invasion. Both focal adhesion kinase (FAK) and Src are involved in FA formation, and both interact with the scaffolding protein ezrin, which our group has shown to be required for breast cancer metastasis. Our aim was to assess the role of the membrane-cytoskeletal linker protein ezrin in Src-induced adhesion and migration in the human invasive breast cancer cell line MDA-MB-231.

**Materials and methods:** shRNA-mediated knockdown (KD) of ezrin function was conducted in MDA-MB-231 cells, as well as in cells expressing constitutively active Src (MDA-Src). Adhesion and migration were assessed using collagen-I adhesion assays and wound healing assays, respectively. Expression and localization of FA proteins was assessed via immunoblotting and immunofluorescence (IF).

**Results:** Ezrin KD impaired migration of both MDA-MB-231 and MDA-Src cells. Interestingly, Ezrin KD resulted in increased cell adhesion to collagen-I, an extracellular matrix protein found in stromal tissue. IF imaging of the FA-associated proteins β1 integrin, FAK, paxillin, and vinculin revealed an increase in localization of these proteins to FA sites at the cell periphery in ezrin KD cells compared to control cells. Furthermore, immunoblotting analysis showed increases in protein expression of β1 integrin, FAK and paxillin but not vinculin, suggesting that FA formation may be increased when ezrin expression is abolished.

**Conclusions:** Ezrin is required for adhesion and migration of MDA-MB-231 cells, and may play an important regulatory role in FA dynamics.

**Financial support:** Canadian Institutes of Health Research (BEE), Ontario Graduate Scholarship (AS), Terry Fox Foundation Training Studentship in Transdisciplinary Cancer Research (AS) and Canadian Breast Cancer Foundation Fellowship (NH).

**P5**

DNA microarray profile of genes regulated by the epigenetic modifier sodium butyrate combined with etoposide in Burkitt's lymphoma cells

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**BMC Proceedings** 2013, 7(Suppl 2):P5

**Background:** The mechanisms underlying Burkitt’s Lymphoma (BL) chemoresistance and how it can be circumvented remain undetermined. The histone deacetylase inhibitors (HDACI) represent a novel class of agents which have demonstrated potent antitumor activity in preclinical models and promising clinical efficacy in cancer patients. The aim of this study was to evaluate the cell death enhancement effect of Sodium Butyrate (NaB), a HDACI combined with suboptimal concentration of etoposide (VP-16) and identify genes differentially regulated by this combination to provide rationale for novel drug combination patterns.

**Materials and methods:** Raji BL cell line was treated with NaB isolated or combined with VP-16. Growth inhibition and cell cycle were analyzed in response to treatment using trypan blue exclusion assay and flow cytometry. Gene expression profiles were determined using One-Color Microarray-Based Gene Expression (Agilent Technologies) and analyzed on Feature Extraction v 9.5.1 software.

**Results:** NaB/VP-16 combined treatment decreased cell proliferation and survival, and blocked cell cycle progression at G2/M with a concurrent decrease in S phase in Raji cells at 24h. Microarray profile showed upregulation of genes related to apoptosis, cell cycle arrest and response to DNA breaks. We also observed downregulation of genes related to cell cycle progression and angiogenesis.

**Conclusions:** Alterations in critical genes involved in cell survival, angiogenesis, cell cycle and DNA damage response were identified. Pathways identified may represent potential targets for combined therapy protocols which have been emerging to improve treatment strategies to circumvent responseless BL patients.

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**P6**

Assessment of normal tissue radiosensitivity in cervical tumors

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**Background:** In Brazil, cervical cancer is the second most common malignant tumor among women. Radiation therapy is part of its interdisciplinary management. The major challenge of modern medicine in radiotherapy is to develop predictive methods that can determine the level of radiosensitivity of the patient and the healthy surrounding tissue in order to individualize the prescribed radiation dose, and to prevent severe side effects, promoting better local tumor control. This study evaluated the acute and chronic adverse effects on the skin, lower gastrointestinal tract and urinary tract of radiotherapy in 47 patients with cervical cancer.

**Materials and methods:** After signing the informed consent agreement, a sample of peripheral blood of 47 patients was collected then the DNA was extracted. TP53 and ATM sequences were amplified to be sequenced.

**Results:** Univariate analysis showed that age was strongly associated with a risk of acute skin toxicity (p=0.023). Patients who received a high dose of external beam radiation and patients who have undergone brachytherapy, showed a significantly higher incidence of chronic urinary tract toxicity (p=0.031) and (p=0.019), respectively. The exchange G>A in the position 5557 of the ATM gene was significantly associated with the risk of acute lower gastrointestinal tract toxicity (p=0.008).

**Conclusions:** Our data corroborate the importance of investigating genetic profiles in order to predict adverse side effects in patients with...
cervical cancer undergoing radiotherapy, TP53 and ATM, known to play an important role in DNA repair pathways, are probably capable of modifying responses of normal tissues to radiotherapy.

Financial support: FINEP (Rede GENOPROT) and CNEN.

P7

Genetic variants involved in specialized DNA replication and their relation with breast cancer risk, disease progression and patient prognosis

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Background: The molecular mechanisms involved in genetic instability, which is a driving force of cancer cells from earlier stages of pathogenesis, are not fully understood. Current evidence shows that overexpression of Pol α, a “DNA repair” polymerase specialized in the replication of damaged DNA, which is altered in breast tumors, is not a passive agent in tumor development and is able to predict patient outcome. Ablating POLQ expression may be related to genetic instability, and also resistance to “replicative stress”, leading to changes in replicating parameters and consequent tumor development. The objective of this project is to analyze genetic variants related to POLQ as new population biomarkers of risk, progression and prognosis in hereditary (HBC) and sporadic (SBC) breast cancer in Brazil.

Materials and methods: Single Nucleotide Polymorphisms (SNPs) were systematically identified through the NCBI database. SNPs identified result in amino acid exchange in the protein and are located within exons or promoter regions. Most SNPs have not been tested in population-based studies. We recruited 211 breast cancer patients (94 SBC and 114 HBC) and 206 women without cancer. In this case-control study, we first genotyped seven SNPs (rs67157736, rs55748151, rs14545723, rs1381057, rs587553, rs13065220, rs3806614) using Taqman Real Time PCR. Data were analyzed using SPSS 18.0.

Results: Interestingly, the rs581553SNP located in a promoter region was associated with HBC (g.121265913T>C; HBC TT=16, Control TT=8; OR=2.01, CI95%=1.32-3.32; p<0.001). Although the Chi-Square analysis did not show any statistical difference between groups for the other SNPs, the HBC group showed more polymorphic genotypes than SBC and Control groups regarding the rs1381057 SNP (C.75387T>C; SBC TT=7; HBC TT=13, Control TT=8).

Conclusions: These results suggest that POLQ germline variation may be related to cancer progression in this patient group. Additional SNPs are being analyzed and the correlation between genotype and relevant clinical variables for breast cancer prognosis will be evaluated.

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P9

Differential expression profile of microRNAs associated with human breast cancer progression

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Background: MicroRNAs (miRNAs) negatively regulate gene expression and their deregulation is involved in cancer progression. Our aim was to verify which miRNAs may play a role in breast cancer progression, especially metastasis.

Materials and methods: MicroRNA expression profiles were generated by microarray analysis (Agilent) of 70 samples from primary breast tumors with different clinical staging. Data were analyzed using bioinformatics software (R, Cluster and Tree View), which allowed sample discrimination (metastatic vs. non-metastatic tumors) by hierarchical clustering of breast cancer expression signatures. We performed a second analysis using the ROC curve method that allowed us to identify miRNAs with greater predictive potential of malignancy. Furthermore, by Venn diagram analysis, we were able to identify 8 miRNAs that were differentially expressed in all metastatic tumors of different clinical staging, compared to the primary non-metastatic tumors. SPSS 19 software was used to generate a Kaplan-Meier curve (p<0.05) to estimate the disease-specific survival (considering the specific event such as death by cancer), based on miRNA expression patterns.

Results: We found four miRNAs previously described as potentially oncogenic (hsa-let-7a, hsa-let-7b, hsa-let-7c and hsa-miR-21) for breast cancer, being hsa-let7a, hsa-let7b and hsa-miR-21 under-expressed in the metastatic vs non-metastatic tumors, and four new miRNAs candidates for markers of metastasis (has-miR-1308, has-miR-923, has-miR-328, has-miR-494), the first two were over-expressed while the latest ones were under-expressed in metastatic vs non-metastatic tumors.

Conclusions: We identified two groups of miRNAs whose expression level were associated with worse prognosis. These findings are important for better understanding of the role of miRNAs in breast cancer progression.

Financial support: FAPESP.

P8

Tetra-O-methyl nordihydroguaiaretic acid, an inhibitor of Sp1-mediated survivin transcription, induces apoptosis and acts synergistically with chemo-radiotherapy in glioblastoma cells

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Background: Glioblastoma (GBM), one of the most malignant human neoplasms, responds poorly to current treatment modalities, being temozolomide (TMZ) the mostly used drug for treatment. Tetra-O-methyl Nordihydroguaiaretic Acid (M4N) is a global transcriptional repressor of genes dependent of Sp1 transcription factors, such as Survivin and CDK1. We evaluated expression of Survivin and CDK1 in glioblastoma and analyzed the effects of M4N combined or not with temozolomide and radiation on cell lines and primary cultures of GBM.

Materials and methods: RT-PCR assays were performed to determinate survivin-spliced variants and CDK1 mRNA expression in glioblastoma tumor samples and cell lines. Cell proliferation was measured by KTT assay, and cell cycle and apoptosis were determined by flow cytometry. Drug combination analyzes using different schedules of administration (simultaneous and sequential) were made based in Chou-Talalay method on GBM cell lines and primary cultures. Gamma radiation for clonogenic survival used the doses of 2, 4, and 6 GY.

Results: All survivin-spliced variants and CDK1 gene were expressed in GBM samples (n=16) and cell lines (n=6), except the survivin-2B variant that was expressed exclusively in GBM cell lines. M4N decreased cell proliferation separately and synergistically with TMZ, besides enhancement of radiation effects, mainly when associated with TMZ. M4N also induced apoptotic cell death, decreased mitotic index and arrested the cell cycle in G2/M phase. M4N treatment down-regulated CDK1 gene and survivin and survivin-ΔEx3 was regulated, while the survivin-2B variant was up-regulated.

Conclusions: Our results suggest a potential clinical application of M4N in combination with TMZ in GB treatment.

Financial support: CAPES and FAPESP (process number 2009/50118-2).
P10 Regulation of TRAIL expression by PRAME and EZH2 as potential therapeutic target against solid tumors
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BMC Proceedings 2013, 7(Suppl 2):P10
Background: TRAIL, a member of the TNF ligand family, was shown to selectively kill cancer cells and, therefore, to participate in the cell-mediated immunity against tumors. However, TRAIL is down-regulated in a variety of cancers. Furthermore, PRAME (preferentially expressed antigen of melanoma) is frequently over expressed in a wide variety of malignant diseases. It was shown that PRAME, in a complex with a member of the polycloumb group, EZH2, can function as a transcriptional repressor of retinoic acid receptor. Interestingly, TRAIL expression can be positively regulated by retinoic acid. Previous studies performed by us revealed that TRAIL is down-regulated and PRAME is up-regulated during development of chronic myeloid leukemia (CML) and that their normal levels are restored after complete cytogenetic remission (CCR). There was a significant, negative correlation between the expression of PRAME and TRAIL in CML patients. Over expression of BCR-ABL in the acute promyelocytic leukemia cell line HL-60 increased the levels of PRAME and decreased the levels of TRAIL. Knocking-down of either PRAME or EZH2 in K562 CML cell line resulted in TRAIL up-regulation.

Materials and methods: We are continuing this study in solid tumors and sarcomas, through qRT-PCR and tissue microarray (TMA) immunohistochemistry, using samples from human lung cancer and patient samples.

Results: Using the publicly available Oncomine Research platform, we found that PRAME was up- and TRAIL was down-regulated in several cancers. Literature data were validated by TMA immunohistochemistry, in tumor samples from patients with lung, prostate, breast and kidney cancers, melanoma and sarcoma. We are performing qRT-PCR assays to validate deregulated mRNA expression in several tumor cell lines and primary patient samples.

Conclusions: These initial data, showing PRAME overexpressed in tumors, accompanied by a decreased expression of TRAIL, corroborate our hypothesis that the presence of a complex consisting of PRAME and EZH2 is responsible for the negative transcriptional regulation of TRAIL in cancer.

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P11 Evaluation of the anti-angiogenic action of melatonin in breast cancer
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BMC Proceedings 2013, 7(Suppl 2):P11
Background: Once a tumor lesion exceeds a few millimeters in diameter, hypoxia triggers a cascade of events to allow angiogenesis and tumor progression. As angiogenesis is essential for tumor growth and metastasis, controlling tumor-associated angiogenesis is a promising tactic in limiting cancer progression. Melatonin has been suggested to inhibit angiogenesis in cancers, although this effect has not been described in breast cancer. We evaluated the effects of melatonin treatment on angiogenesis in breast cancer.

Materials and methods: MDA-MB-231 breast cancer cell line was cultured in DMEM high glucose at 37°C in 5% CO2. Cells received CoCl2 to mimic hypoxia and were then treated with melatonin (1mM). Cell viability was measured by MITT assay, and protein and gene expression were assessed by immunocytochemistry and real time PCR, respectively. We performed an in vivo study where cells were implanted in the mammary gland of athymic nude mice. Mice were treated with 1mg of melatonin or vehicle daily, administered intraperitoneally 1 hour before room lighting was switched off. Tumors were measured weekly with a digital caliper and angiogenic proteins were evaluated in mammary tumor tissues.

Results: Melatonin in vitro treatment was able to significantly decrease cell viability and protein expression of the hypoxia inducible factor 1 alpha (HIF1α), under hypoxic conditions. Furthermore, the anti-angiogenic action of melatonin was tested with breast cancer xenografts in nude mice.

Conclusion: This is the first study to show that melatonin effectively acts against angiogenesis in breast tumors, suggesting that melatonin may have potential therapeutic applications in this disease.

Financial support: FAPESP.

P12 Arylamo-no-β-lapachone derivative–induced apoptosis in human prostate cancer cells: involvement of NAD(P)H:quinone oxidoreductase (NQO1)
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BMC Proceedings 2013, 7(Suppl 2):P12
Background: β-lapachone, a DNA repair inhibitor, has been recognized as an important prototype with activity against cancer cells devoided of cytotoxicity in non-tumor cells. NQO1 is a reductive enzyme that is important for the activation of many bioreductive quinones. Thus, differential levels of NQO1 in tissues, including tumors, can provide a target for an enzyme-directed approach to cancer therapy. Herein, we aimed to evaluate the role of NQO1 on the cytotoxicity of 3-arylamo-no-β-lapachone derivative using the prostate DU-145 (NQO1-overexpressing) and LNCap (NQO1-deficient) cells.

Materials and methods: Compound cytotoxicity was evaluated by the MTT assay, and apoptosis and free radicals were observed by flow cytometry. Also, comet assay was performed to evaluate the DNA strand breaks induced by quinoid compound. For all experiments, cells were treated in the presence or absence of dicumarol (NQO1 inhibitor).

Results: The 3-arylamo-no-β-lapachone derivative showed cytotoxic activity (IC50 2.98 μM) after 24 h exposure. In order to determine the mechanisms involved in cytotoxicity, cells were treated with increasing concentrations (1, 2 and 4 μM) of compound during 4 h. After exposure, apoptosis signals, DNA damage and free radicals production were observed. Coadministration of dicumarol (50 μM) abrogated 3-arylamo-no-β-lapachone derivative-mediated cytotoxicity and downstream apoptotic end points.

Conclusions: In summary, NQO1 may be a pharmacologically exploitable target for therapy against certain tumors using lapachone compounds.

Financial Support: CAPES, CNPq, BiotechCell, FUNCAP.

P13 Evaluation of myeloid metaplasia in canine mixed mammary tumors
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BMC Proceedings 2013, 7(Suppl 2):P13
Background: Mixed tumors are among the most frequent mammary neoplasms in female dogs. Some of these tumors present bone marrow associated with the newly formed osseous tissue, characteristic of myeloid metaplasia. Our aims were to evaluate the occurrence of these lesions in a
series of mixed tumors, and to determine tumor histomorphological characteristics.

Materials and methods: 384 mammary mixed tumors from 289 animals have been reviewed. Lesions were classified according to the presence of osseous metaplasia associated with myeloid metaplasia or extramedullary hematopoiesis. Myeloid metaplasia characterization was determined from the morphological characteristics and organization of cells and adjacent tissues.

Results: The 384 cases included 206 benign and 178 carcinomas in mixed tumors. Osseous metaplasia was present in 16.1% and calcified areas exclusively in 3.1% of lesions. Among all osseous metaplasia, 33.9% presented some type of extramedullary hematopoiesis, of which 71.4% were classified as myeloid metaplasia and 28.6% as extramedullary hematopoiesis. Myeloid metaplasia cases consisted of 67% benign mixed tumors and 33% carcinomas in mixed tumors. Myeloid metaplasia was observed in 74% of mixed tumors containing osseous metaplasia and in 4% of all mixed tumors analyzed.

Conclusions: Despite these results and considering the low frequency of this lesion, additional studies are needed to understand the implications of myeloid metaplasia in canine mammary mixed tumors.

Financial support: FAPESP, CAPES and CNPq.

P14

Cancer stem cells in head and neck squamous cell carcinoma
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Background: Head and Neck Squamous Cell Carcinomas (HNSCCs) are tumors that display a high degree of heterogeneity. HNSCCs contain tumor-initiating cells (TICs) or cancer stem cells (CSCs) that are enriched within the CD44+ fraction, and are capable of initiating and maintaining tumor growth. HNSCCs also contain a stromal component, consisting of cancer associated fibroblasts (CAF)s, endothelial cells, and immune cells, which also play an important role in tumor progression. This heterogeneity can obscure results and thus obtaining pure populations of TICs, non-TICs and CAFs is essential for more stringent analysis.

Materials and methods: HNSCC specimens were dissociated into single cell suspensions, stained for cell surface markers and examined by fluorescence-activated cell sorting. A high-throughput screen for 340 cell surface markers was used to identify candidate biomarkers that could further enrich for TICs that were then functionally tested by performing in vivo limiting dilution assays. Total RNA was extracted from isolated TIC, non-TIC and CAF populations and real time PCR was performed.

Results: These candidate biomarkers were identified and one marker, CD271, resides within the CD44+ fraction and further enriches for TIC activity. In well-differentiated tumors CD271 consistently localizes to the basal layer. Moreover, TIC, non-TIC and CAF populations contain distinct expression profiles.

Conclusions: We have identified CD271 as a biomarker that enriches for TIC activity. The distinguishing expression profiles for TICs, non-TICs, and CAFs are being further analyzed to determine essential signaling cascades that exist among these populations that could potentially be targeted for HNSCC treatment.

P15

Canine osteosarcoma cells exhibit resistance to aurora kinase inhibitors
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Background: The assessment of copy number changes in the SLN is a first step to explore development of Aurora kinase inhibitors (AKIs) as novel therapeutic agents for canine osteosarcoma.

Materials and methods: We evaluated the effects of two AKIs, AZD1152 and VX680, on four canine osteosarcoma cell lines. Viability was assessed using the MTS assay, apoptosis using caspase activation and flow cytometry for Annexin V binding and 7-AAD uptake, and target modulation using Western blotting. We also evaluated gene and protein expression of Aurora kinase B, ABCB1 and ABG2.

Results: Expression of Aurora kinase B protein and mRNA was seen in all cell lines. Although Aurora kinase inhibitors induced cytotoxicity, half-maximal inhibitory concentrations were significantly higher than seen in other cancer types and induction of apoptosis was minimal at concentrations close to IC50s. AZD1152 reduced Aurora kinase B phosphorylation, indicating effective target modulation. ABCB1 and ABCG2 transporter-mediated efflux is one known mechanism of resistance against these drugs. Verapamil modestly enhanced AZD1152-mediated apoptosis and ABCG2 transporters were expressed by a small percentage of cells, suggesting that other mechanisms may contribute to resistance.

Conclusions: Canine osteosarcoma cells are resistant to AKIs and we suggest that these compounds are unlikely to be useful as monotherapy for this disease. Further investigation of resistance mechanisms and the potential utility of AKIs in combination therapy is warranted.

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P17 The diagnostic importance of invasive micropapillary carcinoma in the canine mammary gland: clinicopathological, immunohistochemical and survival analysis

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Background: Invasive micropapillary carcinoma (IMPC) of the mammary gland, despite its rare occurrence in humans and dogs, is an important neoplasm due to its aggressive behaviour. Our aim was to evaluate clinicopathological and immunophenotypical characteristics of IMPC and to determine overall survival of dogs with this tumour.

Materials and methods: Twenty-two IMPC cases were selected for survival and clinicopathological analysis. Immunohistochemistry was performed for Epidermal Growth Factor Receptor (HER-2), Epidermal Growth factor Receptor (EGFR), Oestrogen Receptor (ER), Progesterone Receptor (PR), CD-31, Cytokeratin (CK) AE1AE3, p63 and Epithelial Membrane Antigen (EMA).

Results: Of the 22 studied cases, the majority had > 3 cm neoplasms (15/19, 78.95%) and lymph node metastases (16/16, 100%), but only two cases (2/22, 9.09%) had distant metastases. The IMPCs were classified as either pure (15/22, 68.18%) or mixed (7/22, 31.82%) types. A predominance of moderate histological grade (16 grade II) tumours was observed and the average overall survival was 120 days. Positive immunohistochemical staining for EMA and negative staining for CD-31, p63 and CK AE1AE3 in cystic formations confirmed the micropapillary nature of these neoplasms. The majority of cases were positive for RE (19/20, 95%) and RP (19/20, 95%), but lacked HER-2 (16/22, 72.72%) and EGFR (15/22, 68.18%) over-expression.

Conclusions: These findings demonstrate that, similar to the situation pertaining with human IMPCs, canine IMPCs behave aggressively with high rates of metastasis to regional lymph nodes and short overall survival times.

Financial support: FAPEMIG, CAPES and CNPq.

P18 Thalidomide inhibits inflammation and angiogenesis in tumor 4T1 growth in mice

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Background: Thalidomide has proven to exert anti-inflammatory, anti-proliferative and anti-angiogenic activities in both neoplastic and non-neoplastic conditions. We investigated the effects of this compound on blood vessel formation, inflammatory cell recruitment/activation and cytokine production of 4T1 mammary tumor in mice.

Materials and methods: 4T1 cells were injected subcutaneously into Balb/c mice. After tumor engraftment (5 days), thalidomide (150 mg/kg) was administered to the treated group for 7 days. Tumors of control and treated groups were sized regularly every 12 days and inoculated after for biochemical parameters to assess neovascularization and inflammation.

Results: Daily oral dose of thalidomide was able to reduce in 46% the tumor volume. Assessment of tumor vasculization revealed a significant decrease in blood vessels formation by thalidomide. The levels of two cytokines, VEGF and TNF-α were decreased in tumor samples of thalidomide-treated group compared with the control group. Accumulation of neutrophils or macrophages in the 4T1 tumor measured by the activities of inflammatory enzymes, MPO for neutrophils and NAG for macrophages, was inhibited by the treatment.

Conclusions: By targeting key components of 4T1 tumor simultaneously, thalidomide was effective in attenuating tumor growth. This approach, suppression of inflammation and angiogenesis may provide further insights for both prevention and treatment of cancer.

Financial support: FAPEMIG, CNPq and CAPES.

P19 Targeting urorophyrinogen decarboxylase for head and neck cancer treatment

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Background: Head and neck cancer (HNC) is the 8th most common malignancy worldwide. Despite advances in therapeutic options over the last few decades, treatment toxicities and overall clinical outcomes have remained disappointing, underscoring a need to develop novel therapeutic approaches, particularly those that enhance tumor cell death, while minimizing damage to the surrounding normal tissues.

Materials and methods: An RNA interference (RNAi)-based high-throughput screen (HTS) was performed for the large-scale identification of radiosensitizing targets. An RNAi-based radiosensitizer HTS has revealed UROD as a potent target, demonstrating both proliferative and anti-angiogenic activities in both neoplastic and non-neoplastic conditions. We investigated the effects of thalidomide on 4T1 mammary tumor in mice. We investigated the effects of this compound on blood vessel formation, inflammatory cell recruitment/activation and cytokine production of 4T1 mammary tumor in mice.
P20

Immunohistochemical expression of TGFβ, E-cadherin and vimentin in benign and malignant neoplasias of canine mammary gland

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Background: Epithelial-mesenchymal transition (ETM) is a fundamental biologic process whereby epithelial cells detach from the surrounding tissue and acquire characteristics of mesenchymal cells, a unique motile, spindle-shaped cell with end-to-end polarity. EMT can be induced or regulated by various growth and differentiation factors; among these, TGFβ has received much attention as a major inducer of EMT during embryogenesis, cancer progression and fibrosis. Our aim was to correlate the immunohistochemical expression of TGFβ, E-cadherin and vimentin in canine mammary tumors.

Materials and methods: A total of 52 canine mammary tumors, among adenomas (G1, n = 12), non-metastatic carcinomas (G2, n = 24) and metastatic carcinomas (G3, n = 16), were used to evaluate the immunohistochemical expression of TGFβ1, E-cadherin and vimentin. Fisher’s Exact Test was used for statistical analysis.

Results: E-cadherin was not differentially expressed in the three tumor groups. Vimentin expression was significantly higher in malignant neoplasias (G1 vs. G2, p = 0.019) and (G1 vs. G3 p = 0.008), with no difference in cases with and without metastasis. The expression of TGFβ1 was significantly higher in adenomas compared to metastatic carcinomas (p = 0.01). There was no difference between adenomas and non-metastatic carcinomas.

Conclusion: The pathogenesis and the progression of numerous cancers have been attributed, at least in part, to disruption of normal TGFβ signaling. Here, we found that decreased expression of TGFβ1 in metastatic carcinomas was accompanied by the acquisition of a mesenchymal phenotype, raising the possibility that this cytokine may be involved in EMT in canine mammary neoplasias.

Financial support: FAPESP.

P21

The role of Met, Src and Stat3 in basal-like breast cancer invasion

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Background: The basal-like subtype of human breast cancers has no clinically defined marker profile. Recent studies have shown that Met as well as certain Src family kinases are over-expressed in basal-like breast cancers and correlate with disease progression and poor prognosis. Our group has also demonstrated a potential regulatory role of Stat3 on Met activation or turnover, adding to the previously identified roles of Stat3.

Materials and methods: We have elected to test the efficacy of inhibitors of Met (PF-02341066), Src family kinases (Dasatinib), and Stat3 (CPA7) in blocking the invasive potential of breast cancer cells, using the human basal-like breast cancer cell line MDA-MB-231 transfected with a constitutively active Src mutant (MDA-Src cells). The efficacy of inhibitors in disrupting the invasive capacity of cells was assessed using transwell invasion assays and colony growth in a 3D Matrigel culture model mimicking basement membrane matrices.

Results: Treatment of MDA-Src cells with Stat3 or Src inhibitors markedly reduced branching extensions, and induced growth arrest and cell death of colonies in a dose-dependent manner. Interestingly, treatment with the Met inhibitor also decreased branching extensions but failed to inhibit cell colony forming ability or proliferation. Furthermore, we have found that induction of morphological responses in 3-D culture occurs at ~15-fold lower concentration of the inhibitors than is necessary to observe decreased expression of drug targets in 2D culture. Transwell assays also showed a decrease in invasion of CPA7 and Dasatinib treated cells. Interestingly, PF2341066 treatment of cells did not affect transwell invasive potential, suggesting that other effectors than Met may be involved.

Conclusions: Our findings further implicate a rate-limiting role of Src, Stat3 and Met in the process of invasion in human basal-like cells, and may lead to improved predictive biomarkers for targeting of aggressive breast cancer subtypes which exploit this pathway.

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P22

Cyr61, a marker of progesterone activity in normal and hyperplasic endometrium of female dogs

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Background: Cysteine-rich protein 61 (Cyr61) is a growth factor-inducible gene, involved in the regulation of multiple cellular processes, such as cell adhesion, migration, survival, growth factor-induced mitogenesis, endothelial tubule formation, apoptosis and angiogenesis. In normal women endometrium, Cyr61 expression is elevated during the proliferative phase of the menstrual cycle, and reduced during the midsecretory phase. However, Cyr61 expression is deregulated in endometrium with estrogen dependent diseases, such as endometriosis, polycystic ovarian syndrome, endometrial hyperplasia and adenocarcinoma.

Materials and methods: Cyr61 expression in endometrium of 20 prepubertal female dogs, 20 female dogs at each phase of the estrous cycle and 24 dogs affected by cystic endometrial hyperplasia associated with pyometra, was evaluated by immunohistochemistry, analyzed by Leica QWin Plus V.3.5.0 automated system and statistically analyzed and correlated with estrogen and progesterone plasmatic levels.

Results: Cyr61 protein was detected in glandular and luminal epithelial cells of endometrium. Prepubertal female dogs had significantly the lowest expression of Cyr61, followed by proestrus, estrus and anestrus phases. Patients affected by pyometra revealed significantly the highest expression of Cyr61, followed by anestrus and estrus phases. Therefore, Cyr61 in normal endometrium was highest during the secretory phase and lowest during proliferative phase of the estrus cycle, resulting in significant positive linear correlation of cytoplasmatic staining area with progesterone levels. There was no significant correlation with estrogen levels.

Conclusions: In female dogs, Cyr61 is overexpressed in hyperplasic endometrium affected by pyometra, a process mediated by progesterone and only aggravated by estrogens. Cyr61 low expression in prepubertal endometrium and proestrus phase, suggest that Cyr61 is not regulated by estrogen, but by progesterone responsiveness, which can be caused by specific characteristics of long luteal phase, characteristic of this species.

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P23

Analysis of protein profile expressed on the membrane surface of plasma cells, stromal cells and mononuclear cells from bone marrow microenvironment of multiple myeloma patients by mass spectrometry

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For this purpose we performed gain-of-function analyses, and we assessed the genomic profile of CNAs in 16 primary cultures and evaluated the effect of the over-expression of specific CNAs in cancer cell lines. We were able to detect down-regulation of miR-10b target, a validated target, and of the miR-196a target in oral cancer cell line. Different results were observed for keratinocytes, in which we confirmed down-regulation of miR-7 targets miR-7 and miR-196a 196a, a miR-196a target, and of the miR-10b target HOXD10. Over-expression of miR-7, miR-10b and miR-196a clearly interfered with cell cycle progression, but this effect was significantly stronger in keratinocytes.

**Conclusions:** The expression levels of the target genes studied here could collaborate to the effect on cell proliferation, since they have all been previously related to this process. The role of microRNAs on gene regulation clearly depends on the cell type.

**Financial support:** FAESP and CNPq.
Background: Only a fraction of patients with metastatic colorectal cancer (mCRC) receive clinical benefit from therapy with anti-epidermal growth factor receptor (EGFR) antibodies. Gene mutations along the Ras pathway (KRAS, NRAS, BRAF, PIK3CA) correlate with poor response to anti-EGFR antibodies.

Patients and methods: By implantation and serial propagation in NOD/SCID mice, we produced large xenograft cohorts from 85 patient-derived mCRC samples ("xenopatients").

Results: Xenopatients retained the histological and genomic features of the original counterparts, responded to the anti-EGFR antibody cetuximab similarly to clinical observations, and could be prospectively stratified as responders or nonresponders based on predictive biomarkers. Genotype-response correlations indicated HER2 amplification specifically in a subset of cetuximab-resistant, KRAS/NRAS/BRAF/PIK3CA wild-type cases. In this subset, combined HER2/EGFR inhibition induced long-lasting tumor regression. We also assessed the effects of MEK and PI3K/mTOR inhibitors (AZD6244 and BEZ235 respectively) in 40 specimens harboring KRAS/NRAS/BRAF/PIK3CA mutations. Cotreatment of xenografts with AZD6244+BEZ235 induced disease stabilization in the majority of cases (70%) but did not lead to tumor regression. Monotherapy was less effective, with BEZ235 displaying higher activity than AZD6244. Triple therapy with cetuximab provided further advantage. The extent of disease control depended on prolonged treatment.

Conclusions: Our preclinical platform prospectively recapitulated biomarker-based case stratification and was instrumental in identifying HER2 as a predictor of resistance to cetuximab and of response to combination therapies against HER2 and EGFR. The prevalent growth-suppressive effects produced by MEK and PI3K/mTOR inhibition suggest that this strategy may retard disease progression in KRAS-mutant mCRC patients.

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P28
Detection of cancer stem cells by immunohistochemistry of canine mammary neoplasias

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Background: Canine mammary neoplasias are very common and, in Brazil, 70% are considered malignant. Cancer stem cells (CSCs) are able to self-renew and have abilities to form metastases. In canine mammary tumors these CSCs were isolated by flow cytometry by means of the surface markers CD44+/CD24-.

Patients and methods: A total of 130 breast cancer samples were selected from the Unesp - Department of Pathology and classified according to [1]. These samples were composed of adenomas; metastasis, solid carcinoma grades II and III; tubular, papillar and mixed tumors; carcinoma grades I, II, and III. Immunohistochemical was performed with antibodies CD44 and CD24.

Results: A regression line by Pearson correlation test was performed. The value which CD44 is positive and CD24 becomes zero is 0.4675. From 130 samples, 40 showed the phenotype CD44+/CD24-Thirty seven was metastasis, grades II and III. The immunostainings for CD44 and CD24 antibodies were respectively 62.2% and 0%. In the literature, grade III are more correlated with CD44 than CD24. In studies by flow cytometry authors found percentages of CSC similar to those found in this work.

Conclusion: The immunohistochemistry showed to be a reliable technique for detection CSCs in canine mammary neoplasias and correlates with grades II and III (poor prognosis).

Financial support: CNPq.

Reference:

P29
Investigation of ATM, TP53 and MDM2 polymorphisms and their association with outcome of radiotherapy for prostate cancer

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Background: The purpose of this study was to evaluate the association of polymorphisms of ATM and TP53 genes in prostate cancer patients with morbidity after radiotherapy. These two genes encode important proteins of the DNA repair pathways. It is believed that their polymorphisms are likely to modify the response of normal tissues to radiation.

Patients and methods: After signing the informed consent agreement, a sample of peripheral blood of 50 patients from Araújo Jorge Hospital was collected to verify the presence of a ATM (rs1801516), TP53 (rs1042522, rs1800371, rs17878362, rs17883323 and rs35117667) and MDM2 (rs2279744)
polymorphisms. The side effects were classified according to the Radiation Therapy Oncology Group (RTOG) score.

Results: On univariate analysis, hypertension was strongly associated with a decreased risk of late urinary toxicities (OR=0.048, 95% CI 0.004 - 0.620; p=0.022). Patients receiving hormone therapy had a significantly higher incidence of acute skin toxicity (OR=27.667 95% CI:1.203-636.11; p=0.029). The exchange C→T in the position 11132 (intron 3) of the TP53 gene (rs35117667) was significantly associated with the risk of acute skin toxicity (OR=0.012 95% CI. 0.0004-0.317; p=0.006). No significant associations were found for the remaining polymorphisms (p>0.05).

Conclusion(s): We conclude that hypertension seems to be protective for late urinary effects of radiotherapy. Hormonal therapy and the intronic TP53 polymorphism, rs35117667, were associated to increased acute skin radiosensitivity. This observation corroborates the importance of investigating the genetic profile to predict adverse side effects in patients undergoing radiotherapy.

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P30
Methylation profile in penile carcinoma reveals unique signature relative to surround tissue and HPV status
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Background: Penile carcinoma (PeCa) is an important public health problem in poor and developing countries. Despite the unpredictable behavior and aggressive treatment, there are few data on molecular and epigenetic mechanisms reported in PeCa. The aim of this study was to identify epigenetic profile in tumors and surrounding tissue as well as to identify molecular markers in PeCa, according to the Papillomavirus (HPV) infection.

Patients and methods: Paired PeCa and surrounding tissue samples were collected from 16 patients. Twenty tumors were also included. Methylation (Methylminer - InVirotm) and unmethylated (digested with restriction enzyme MroBC) enriched sequences were hybridized in a 244K Human DNA Methylation Microarray platform (Agilent Technologies). This assay interrogates 27,627 expanded CpG islands and 5081 shore CpG island regions. Genomic Workbench Standard (v 5.0.14) and BRB softwares were used to analyze the data.

Results: It was considered only probes with p value<0.001, FDR <0.05 and located inside or in the gene promoter. HPV positivity was detected in 43% of cases (Linear Array HPV Test Genotyping - Roche), mainly for 16 and 18 subtypes. Penile carcinoma displayed unique signature relative to surround tissue, showing 171 probes methylated and 349 unmethylated in tumor samples. Several probes related to genes involved in NOTCH and WNT pathways were altered in HPV positive cases.

Conclusions: It was found a differential methylation profile according to HPV status (positive or negative), indicating at least two disrupted pathways, one related to viral infection and the other associated with transcriptional regulation of stem cells.

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P31
Activated Stat5 levels do not increase with cell density in contrast to activated Stat3 levels in breast epithelial cell culture
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Background: The Signal Transducer and Activator of Transcription-3 (Stat3) is activated by cytokine receptors such as the IL6 receptor (IL6R) family, as well as Src and Abl. In cancer, Stat3 can become constitutively active, regulate genes involved in survival, proliferation and invasion, and has been associated with poor survival in breast cancer patients. Our group recently discovered a novel pathway of Stat3 activation triggered by engagement of cadherins, cell to cell adhesion molecules, which induce high levels of Rac and transcriptional activation of IL6. Another Stat family member, Stat5, is known to promote breast differentiation and lactation in response to lactogenic hormones, and its activation correlates with a more differentiated breast tumour phenotype.

Materials and method: To assess whether Stat5 activation also increases with cell density, HC11 breast epithelial cells were plated at different densities, and pY694Stat5, pY705Stat5 and corresponding target genes (cyclin D1 and p21) were assayed via Western blotting. Cyclin D1 and p21 were also stained and scored on a human breast cancer tissue microarray (n=61).

Results: pYStat3 expression was dramatically increased in cells at higher densities, however pYStat5 levels remained constant throughout all cell densities. This indicates that Stat3 and Stat5, although activated by similar cytokines, are differentially regulated in breast development.

Conclusions: Activated Stat5 levels do not increase with cell density in contrast to activated Stat3 levels in breast epithelial cell culture. Additionally, expression of the Stat3 target gene, cyclin D1, is correlated inversely with triple negative breast cancers, supporting a distinct role of this pathway in breast cancer subtypes. Together, our results suggest differential regulatory roles of Stat3 vs Stat5 in breast cancer.

Financial support: CBCF, CIHR, Terry Fox Transdisciplinary Studentship in Partnership with CIHR.

P32
Undernutrition in utero increases susceptibility to prostate neoplasias in adult rat after steroid exposure
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BMC Proceedings 2013, 7(Suppl 2):P32

Background: Maternal protein restriction during pregnancy promotes several alterations in the progeny. Previous studies showed that androgen/estrogen imbalances during perinatal period can program prostate cells to develop prostate lesions in adult life after a second insult. This study aimed to investigate prostate diseases susceptibility in adult rat offspring which underwent in utero low protein diet and were chronically exposed to low doses of estrogen and testosterone in adult life.

Material and methods: 16-weeks-old Wistar rats (n=48) that received in utero normal protein diet (NP group, 17% protein) or low protein diet (LP group, 6% protein) were subjected to 17-beta estradiol+testosterone administration (subcutaneous implant, NPH and LPH groups) for 16 weeks. The animals were killed at age of 35 week and the ventral (VP) and dorsolateral prostate (DLP) was excised. The animals were killed at age of 35 week and the ventral (VP) and dorsolateral prostate (DLP) was excised. The animals were killed at age of 35 week and the ventral (VP) and dorsolateral prostate (DLP) was excised. The animals were killed at age of 35 week and the ventral (VP) and dorsolateral prostate (DLP) was excised. The animals were killed at age of 35 week and the ventral (VP) and dorsolateral prostate (DLP) was excised. The animals were killed at age of 35 week and the ventral (VP) and dorsolateral prostate (DLP) was excised. The animals were killed at age of 35 week and the ventral (VP) and dorsolateral prostate (DLP) was excised. The animals were killed at age of 35 week and the ventral (VP) and dorsolateral prostate (DLP) was excised. The animals were killed at age of 35 week and the ventral (VP) and dorsolateral prostate (DLP) was excised. The animals were killed at age of 35 week and the ventral (VP) and dorsolateral prostate (DLP) was excised. The animals were killed at age of 35 week and the ventral (VP) and dorsolateral prostate (DLP) was excised. The animals were killed at age of 35 week and the ventral (VP) and dorsolateral prostate (DLP) was excised. The animals were killed at age of 35 week and the ventral (VP) and dorsolateral prostate (DLP) was excised. The animals were killed at age of 35 week and the ventral (VP) and dorsolateral prostate (DLP) was excised. The animals were killed at age of 35 week and the ventral (VP) and dorsolateral prostate (DLP) was excised. The animals were killed at age of 35 week and the ventral (VP) and dorsolateral prostate (DLP) was excised. The animals were killed at age of 35 week and the ventral (VP) and dorsolateral prostate (DLP) was excised.

Results: Both VP and DLP weight from NPH group were higher than LPH group. Serological data showed that estradiol levels were similar in both groups, but testosterone levels were lower in the LPH male offspring. Morphometric analysis verified a decrease in the height of prostate epithelium, apoptotic index and an increase of proliferation index in LPH group compared to NPH group. The incidence of prostatitis and prostatic intraepithelial neoplasia was higher in VP and DLP of LPH group. However prostate cancer was not observed.

Conclusion: Maternal protein restriction alters adult prostate response to androgen/estrogen handling and also interferes in adult prostate susceptibility to diseases.

Background: Gestational trophoblastic disease is a group of benign and malignant entities, where the hydatidiform mole is the most common, and can be divided into complete hydatidiform mole and partial hydatidiform mole. However, the histological criteria are still questionable and also, histologic evaluation alone is prone to great interobserver variability. In this way, immunohistochemical analysis for p57kip2 may be useful to aid in the diagnosis and classification of hydatidiform moles, with management and prognostic implications. p57 is the protein product of the paternally imprinted but maternally expressed gene CDKN1C. Because complete hydatidiform mole lacks a maternal genomic component, an absence of p57 staining is specific for this pathology. This study looking for demonstrates the p57kip2 immunohistochemistry as a technique useful in the differentiation between partial and complete hydatidiform mole.

Patients and methods: From 2007 to 2011, our institute handled a total of 104 patients with gestational trophoblastic disease type complete and partial mole. This study did not consider patients with other forms of trophoblastic disease different to hydatidiform mole. The residues obtained after 104 curettages were analyzed by histological studies and 86 of them were subjected to determination of p57.

Results: Of that total, 63.95% (55 patients) had a partial mole and the rest (31 patients, 36.04%) had a complete mole. There was divergence in the pre and post diagnostic immunohistochemistry in 12.79% of cases (11 patients).

Conclusions: The p57kip2 immunohistochemical technique should become a thorough job in diagnosing hydatidiform mole.

Financial support: FAPESP and CAPES.

P35

Constitutive expression and roles of interleukin-8 in canine hemangiosarcoma

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Background: Interleukin-8 (IL-8) is a pleotropic cytokine that promotes tumor cell proliferation and survival, inflammation, and angiogenesis; however, its role in the pathogenesis of canine hemangiosarcoma (HSA) is unknown.

Materials and methods: Six canine hemangiosarcoma (HSA) cell lines and 24 primary and metastatic HSA tumor tissues were used to investigate the biological functions of IL-8. Roles of IL-8 were examined by analyzing microarray data, qRT-PCR, ELISA, MTS cell proliferation assay, and sphere-forming assay.

Results: IL-8 mRNA expression was variable among the tissue samples and both IL-8 mRNA and protein were variable among the cell lines. In contrast, IL-8 receptor mRNA and protein showed minimal variance. IL-8 high” and “IL-8 low” groups were defined from the HSA tumor samples based on gene expression profiles. The “IL-8 high” group was associated with a “reactive microenvironment,” showing enrichment of coagulation, inflammation, and fibrosis networks. However, IL-8 added exogenously and IL-8 blockade using neutralizing antibodies had no effect on HSA cell proliferation, despite apparent response to these signals at the level of gene expression. Similarly, neither addition nor blockade of IL-8 protected cells from apoptosis. IL-8 mRNA was elevated in HSA cancer stem cells, but exogenous IL-8 attenuated self-renewal of these cells.

Conclusion: The results of this study suggest that IL-8 is a driver of tumor heterogeneity, steering cells away from self-renewal and towards partial differentiation. It also could act to recruit (or produce from the tumor) inflammatory and pro-angiogenic cells to the microenvironment. We are testing this hypothesis in a robust xenograft model. These experiments will establish if IL-8 plays a role in progression and metastasis of canine HSA, and allow us to define the therapeutic potential of IL-8 blockade.

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P36

HMGA2 overexpression is associated with differential expression of miRNAs in uterine leiomyomas

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Background: HMGA2 overexpression is associated with differential expression of miRNAs in uterine leiomyomas.
Background: Uterine leiomyomas (UL) are mesenchymal benign tumors extremely common that represent a significant public health problem. The deregulation of growth factors and microRNAs (miRNAs), shortening of telomeres, excessive production of disorganized extracellular matrix, loss of heterozygosity and recurrent chromosomal aberrations (including 7q22 deletion and chromosomal rearrangements in 12q15) have been suggested to contribute to the growth of fibroids. HMGA2, mapped to 12q15, is a major regulator of benign tumorigenesis from mesenchyme-derived tissues and stem-cell self-renewal. In UL, HMGA2 overexpression is frequently reported. Recently it has been shown that repression of HMGA2 by microRNA let-7s is a critical molecular regulatory mechanism associated with tumor growth. In this study, it was evaluated three miRNAs mapped to 7q22 (miR-25, miR-93, and miR-106b) and miR-let-7a (previously reported as HMGA2 regulator). These findings were compared with gene expression microarray data.

Methods and results: Seventy-eight fresh frozen UL and 20 adjacent normal myometrium (MM) were collected from 54 patients who had undergone a hysterectomy procedure. Paired analysis were performed in 20 cases. Quantitative real time RT-PCR was applied to evaluate the expression of miR-let7a, miR-25, miR-93, and miR-106b using RNU44, RNU48 and U47 as endogenous control. Array CGH and expression analysis was carried out using Agilent 4 x 44 k arrays in the same set of cases. Results: Losses of 7q22 were significantly detected in UL by array CGH. In 7q22 is mapped the miR-93 and miR-106b which were found as down-regulated (p<0.001; p=0.001, respectively), miR-let-7a was also down-regulated (p=0.001). Oligorray expression analysis confirmed HMGA2 overexpression.

Conclusions: Losses of 7q22 were associated with miR-93 and miR-106b downexpression leading to deregulation of target genes involved in ULs pathogenesis. In addition to miR-let7a, miR-93 is also a candidate for HMGA2 regulation in these tumors.

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P37 Action of melatonin in primary culture of canine mammary tumours
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Background: Breast neoplasms are the most common tumors in female dogs, representing about 50% of all cancers in this animal population. The identification of therapeutic agents that can be used as an alternative treatment for this tumor type has proven to be useful. The administration of melatonin, a hormone secreted by the pineal gland, can exercise oncostatic effects in several types of cancer. Regarding breast cancer its importance is significant. Due to the many similarities shared by humans and dogs, canine mammary tumors are an excellent experimental model. Thus, the aim of this study was to evaluate the effects of melatonin treatment in canine mammary tumors.

Materials and methods: Ten tumor fragments of female dogs were collected and stored in transport medium for performing the cell cultivation and cell treatment with melatonin. The samples was cultured in medium DMEM (E) and incubated at 37°C in 5% CO₂. It was established two groups: Control group (untreated) and Group treated with different concentrations of melatonin (0.5mM, 1mM, 2mM, 5mM and 10mM). Cell viability was verified by MTT assay. The results were analyzed evaluating the mean and standard error.

Results: Melatonin was able to reduce cell viability at all concentrations tested. 60% of samples showed a greater reduction in cell viability when cells were treated with 10mM melatonin.

Conclusions: Our results suggest that melatonin decreases the viability of the canine mammary neoplastic cells, where, the treatment with 10mM was more effective treating a promising way to the use as a therapeutic agent in cancer treatment.

Financial support: FAPESP and CNPq.

P38 TGF-β polymorphism and its expression correlated with CXCR4 expression in human breast cancer
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Background: It is known that the transforming growth factor beta (TGF-β) can act as both a tumor suppressor and as a significant stimulator of tumor progression, invasion, and metastasis. It has been suggested a link between TGF-β and CXCR4 expression in human breast cancer cells, which may be one of the mechanisms of TGF-β mediated enhancement of metastatic potential in breast cancer cells. Therefore, the objective of the present study was to investigate the TGF-β T869C polymorphism and its expression correlated with CXCR4 expression in breast cancer patients.

Patients and methods: Genomic DNA was obtained from 21 samples of peripheral blood or from normal tissue previously fixed in formalin and embedded in paraffin for TGF-β T869C polymorphism analyses. Total cellular RNA was extracted from the same 21 patients, but from fresh tissue (tumor and adjacent healthy from the same breast) to expression analysis by Real Time PCR.

Results: No significant differences were observed in genotype distribution according to clinic pathological characteristics. TGF-β mRNA expression was assessed according to T869C polymorphism and CC patients presented a higher TGF-β expression but not significant when compared to other genotypes (p=0.064). A positive correlation was observed in relative mRNA expressions of CXCR4 and TGF-β (p=0.020). It is known that overexpression of TGF-β by both tumor and stromal tissue can facilitate the development of metastases, mainly by TGF-β stimulated angiogenesis and increased tumor cell motility.

Conclusion: Our findings suggested a role of these genes as progression markers for breast carcinoma.

Financial support: CNPq, CAPES, Fundação Araucária and PROPPG-UEL.
Results: Invasion was considered when suspicious Haematoxylin-Eosin stained areas revealed a total loss of immunoreactivity for α-SMA and p63. Versican immunoreactivity was less intense adjacent to in situ carcinomatous regions when compared to invasive regions in which staining was found as more extensive areas with strong expression.

Conclusions: Obtained data reveal that, in carcinomas in benign mixed tumors, versican expression differs significantly within invasive and in situ areas, suggesting the role of this molecule in tumor progression.

Financial support: FAPEMIG, CNPq and CAPES.

P40
Evaluation of the expression of angiogenic factors in breast cancer after curcumin treatment
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Background: Angiogenesis plays an important role in the pathogenesis of several malignancies, including breast cancer. Tumor growth requires the formation of new blood vessels that are stimulated by angiogenic factors, which initiates the sprouting and proliferation of endothelial cells. Curcumin is used as food and in traditional medicine, however evidences indicate that rhizome has anticancer effects against different types of cancers. We evaluated the effects of curcumin treatment on angiogenesis in breast cancer.

Materials and methods: Breast cancer cell line MDA-MB-231 was cultured in vitro in DMEM high glucose at 37°C in 5% CO2. Cell viability was measured by MTT assay with three concentrations of curcumin (10μg, 20μg and 40μg), in 4 hours and 24 hours. In vivo, cells were injected subcutaneously in mammary gland in athymic nude mice, and each animal received daily 7.5mg of curcumin administrated intraperitoneal. Tumor size was measured weekly and angiogenic factors were evaluated in breast tumors.

Results: There was a significantly decrease in cells viability after treatment with curcumin (all concentrations). In addition, results showed that 40μg of curcumin was able to reduced 88% of cell viability after 24 hours. Furthermore, the action of curcumin as anti-angiogenic agent was tested in breast cancer xenografts established in nude mice.

Conclusions: The highest dose of curcumin was considered the optimal concentration for in vitro treatment. This study was an innovative way to evaluate the potential effectiveness of curcumin in the control of angiogenesis in breast cancer.

Financial support: FAPESP.

P41
Evaluation of different methods to obtain primary mammary epithelial cell cultures from canine spontaneous mammary gland tumors
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Background: Mammary gland neoplasms are the most prevalent tumors in dogs, and their treatment is still challenging. A crucial problem in the handling of this type of neoplasm is to obtain primary mammary epithelial cell cultures from the original tumors. The aim of this study was to determine the best conditions to culture primary mammary epithelial cells from several histological types of canine breast tumors.

Materials and methods: Several culturing conditions have been tested including enzymes such as collagenases types III and IV, trypsin and hyaluronidase, differential centrifugation and trypsinization. Four tumor samples were processed to obtain organoids, stromal and epithelial cells. The histological tumor types studied were mixed carcinoma and simple adenoma. The cells were phenotypically characterized according to an immunocytochemical panel, including cytokeratins, alpha smooth muscle actin and vimentin.

Results: Results have shown that the best method of obtaining primary epithelial cell lines comprises the use of collagenase type I, hyaluronidase and trypsin followed by serial differential trypsinization.

Conclusions: Standardization of such methodological tools in the canine model for the study of cancer will allow a more detailed analysis of the action of new antineoplastic agents, which could be applied to animals and, eventually, to humans.

Financial support: FAPESP and CNPq.

P42
Interface of post-translational glycosylation and endoplasmic reticulum stress in melanoma: target to cancer cell sensitization to chemotherapeutic agents?
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Background: Melanoma is the most lethal skin cancer, despite being less prevalent. Due to its lethality and resistance to a variety of known chemotherapeutic drugs, studies on melanoma are paramount. Tumor cells in general, and melanoma cells particularly, commonly present a disturbed metabolic rate, e.g. altered metabolism of reactive oxygen species and increased rates of protein synthesis. Altogether these perturbations would often trigger the Unfolded Protein Response (UPR); however, data from our laboratory showed that UPR markers are not constitutively expressed in tumorigenic cells. Our previous results suggest that melanoma cells have developed adaptive mechanisms to maintain an unstable equilibrium. Besides, glycosylation of tumor cells are commonly altered, due to differentiated expression rates of N-glycosylation enzymes, like N-acetylglucosaminyltransferase S (MGATS). Our hypothesis is that the expression of MGATS (and/or MGATSB) is part of an adaptive response to proteotoxic stresses, and on doing so they would serve as targets for sensitization of melanoma cells to UPR.

Materials and methods: We treated non-tumorigenic melan-a cells and tumorigenic melanoma cells Tm1 and Tm5 in vitro with tunicamycin, a drug that prevents N-glycosylation, and swainsonine, a drug that prevents Golgi apparatus glycosylation.

Results: Results showed that tumorigenic cells were more sensitive to both drugs and that they accumulated larger amounts of endoplasmic reticulum (ER) stress markers after treatments.

Conclusions: These results show that tumorigenic cells were more sensitive to both drugs and that they accumulated larger amounts of endoplasmic reticulum (ER) stress markers after treatments.

Financial support: FAPESP and CNPq.

P43
RO1 is a pseudokinase that is crucial for MET-driven tumorigenesis
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Background: Aberrant deregulation of some Receptor Tyrosine Kinases (RTKs) signalling underlies diverse facets of tumor pathobiology providing an attractive target for cancer therapy. Searching for novel cancer-associated RTKs is an important issue to discover new therapeutic opportunities. For this reason, we investigated the contribution of Receptor tyrosine kinase-like Orphan Receptor 1 (ROR1) to human cancer.

Patients and methods: The level of ROR1 protein expression, phosphorylation and cellular growth response to RNA-mediated ROR1 knockdown was evaluated by an integrated screening in a panel of 43 cancer cell lines. ROR1 auto-kinase activity and transphosphorylation were determined by biochemical assays. Functional consequences of ROR1 silencing were evaluated by several in vitro and in vivo biological assays.

Results: We demonstrated that although ROR1 is expressed in approximately 75% of the screened cancer cell lines, only gastric carcinoma cells (HS746T) and non-small cell lung carcinoma cells (NCI-H1993) exhibit high levels of ROR1 tyrosine phosphorylation and experience growth inhibition upon ROR1 suppression. Biochemical assays revealed that ROR1 is a pseudokinase lacking autophosphorylation activity. Intriguingly, the two phospho-ROR1 positive cell lines both exhibited amplification and constitutive activation of the MET oncogene. ROR1 phosphorylation was abrogated by MET inhibition, indicating MET dependent transphosphorylation of ROR1. Silencing of ROR1 in HS746T and NCI-H1993 cells impaired cellular proliferation, growth and migration in vitro and induced a dramatic inhibition of tumorigenesis in vivo.

Conclusions: Our data show that ROR1 is a pseudokinase functionally transphosphorylated by MET RTK, suggesting a critical role for ROR1 in malignant phenotypes sustained by the MET oncogene.

Financial support: AIRC special program Molecular Clinical Oncology “5x1000” and AIRC grants.

**P44**

mir-205 is involved in metastatic potential of 21T series, a breast cancer progression model

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Background: microRNA (miRNA) is a class of non-coding RNAs, which regulate gene expression at the post-transcriptional level. Many miRNAs have been implicated in several human cancers, as these regulatory molecules play important roles in key cellular processes, including cell proliferation, differentiation and response to DNA damage.

Methods: To gain insights into the mechanisms involved in breast cancer initiation and progression we conducted a miRNA global expression on 21T series. These cell lines represent an in vitro model of breast cancer progression comprising immortalized cell lines derived from the same patient diagnosed with Her-2 overexpressing ductal carcinoma. The set include a normal epithelial (16N), primary in situ ductal carcinoma (21PT and 21NT) and cells derived from pleural effusion of lung metastasis (21MT-1 and 21MT-2). To confirm microarray results, the expression of the most significantly altered miRNAs were checked by qPCR. Matrigel invasion assay was done to evaluate the migration capacity of 21Tcells and Her-2, ZEB-1 and e-cadherin protein levels were achieved by western blot.

Results: Analysis of 21T series revealed a significant downregulation of miR-205 together with an enrichment of its predicted target, the pro-metastatic factor ZEB-1 and the consequent reduction of e-cadherin levels in the invasive 21MT cells.

Conclusions: These molecular alterations, in special the downregulation of miR-205 in cancer cells, can participate on modulation of epithelial to mesenchymal transition and increase the metastatic potential on breast cancer.

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Breast and gastric cancer were more frequent at higher doses and lung cancer in lower ones.

**Conclusion:** It's an effective and flexible carcinogenesis model that can be useful for studies for multiple cancers, especially in breast, gastric, lymphoid and lungs.

**Financial support:** FAPESP.

**Acknowledgements:** The first author is presenting the abstract on behalf of the study group.

### P47

**Analysis of the DNA methylation of the H19 gene in human bladder cancer**

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**BMC Proceedings 2013, 7(Suppl 2):P47**

**Background:** H19 is a paternally imprinted gene located at 11p15.5, which encodes a non-coding transcript. Although the role of genomic imprinting in bladder cancer is not well understood, previous studies have described H19 over-expression in these tumors. It is well established that a Differentially Methylated Region (DMR) regulates its maternal monoallelic expression by acting as an insulator that precludes the binding of the transcriptional factor CTCF in the paternal allele.

**Materials and methods:** DNA methylation status of two distinct regions of the H19 gene was evaluated: the sixth CTCF-binding site located in the DMR (qMSP, Quantitative Real Time Methylation Specific Polymerase Chain Reaction) and the promoter-associated CpG island (MS-HRM, Methylation-Sensitive High Resolution Melting analysis) in a total of 48 tumoral samples (37 of them matched with normal adjacent tissue).

**Results:** Using a pool of blood samples obtained from healthy young adults as reference to the normal imprinting, higher methylation levels of the CTCF-binding site were detected in bladder tumors compared to the normal adjacent tissue (p=0.0031). Gains of methylation were more frequently detected in non-invasive (p=0.0425) and non-recurrent (p=0.0399) papillary bladder tumors. While DNA methylation levels of H19 promoter region varied from 10 to 50% in normal adjacent bladder tissues, tumoral samples showed greater variation (30 to 100% of methylation). Heterogeneous patterns of CpG methylation were also detected in nine tumoral samples.

**Conclusion(s):** Our data suggest that aberrant DNA methylation is an epigenetic change potentially associated with loss of imprinting of the H19 gene in bladder cancer.

**Financial Support:** FAPESP, CAPES.

### P48

**Açaí (Euterpe oleaceae Martius) intake and its protective effect against 1,2-DMH-induced rat colon carcinogenesis**

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**BMC Proceedings 2013, 7(Suppl 2):P48**

**Background:** Açaí (Euterpe oleaceae Martius) is an important crop consumed in Brazil that gained attention due to its potential antioxidant and anti-inflammatory properties. Various studies have shown that this exotic fruit may reduce the risk of many diseases, including cancer. The present study investigated the beneficial potential of açaí extract intake on the 1,2-dimethylhydrazine (DMH)-induced rat colon carcinogenesis.

**Materials and methods:** Five groups were studied: G1 to G4 groups received four s.c. injections of DMH (40 mg/kg) twice a week during two weeks and G5 group received similar injections of EDTA (DMH vehicle). After two weeks of colon initiation, groups were fed basal diet (G1), basal diet containing 2.5% açaí extract (G2), basal diet containing 5.0% açaí extract (G3 and G5) or basal diet containing 0.2% of N-acetylcyesteine (G4, positive control). Ten weeks after the beginning of the treatment, G1 to G4 groups were sacrificed for aberrant crypt foci (ACF) assay. Also, G1 and G3 groups were maintained on basal diet or basal diet containing 5.0% açaí extract, respectively, until week 20. Colonos were analyzed for ACF development (week 10) or tumor incidence, multiplicity and histological analyses (week 20).

**Results:** The number of total aberrant crypts (AC) was significantly reduced in G3 and G4 groups when compared to G1 group (p=0.036). Data from ACF (1-3 AC) was significantly lower in G3 and G4 groups (p=0.011 and p=0.037, respectively). In the colon tumor assay, G1 group developed invasive (75%) and noninvasive (25%) tumors whereas G3 group developed only noninvasive tumors (100%).

**Conclusions:** These results suggest that açaí extract intake is potentially beneficial for early reduction of the number of AC and may affect tumor development and late malignancy in male Wistar rats initiated for colon carcinogenesis.

**Financial support:** CAPES.

### P49

**Serum and molecular assessment of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1α (HIF-1α) in canine mammary tumors**

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**BMC Proceedings 2013, 7(Suppl 2):P49**

**Background:** Due to the many similarities shared by humans and dogs, canine mammary tumors are an excellent model to comprehend various aspects of mammary neoplasias. The tumor cells have the ability to promote changes in their functionality in order to survive. In situations of hypoxia, tumor cells produce and release pro and antiangiogenic factors that regulate the process of angiogenesis. The hypoxia-inducible factor 1 (HIF-1) is a central regulator of pathophysiologic response of mammalian cells to low oxygen levels, able to activate transcription of the gene that promotes the induction of vascular endothelial growth factor (VEGF), which in turn, promotes angiogenesis through its ability to stimulate growth, migration and invasion of endothelial cells, leading to the formation of new blood vessels and subsequent tumor growth. In this context, the aim of this study was to measure serum levels of VEGF and HIF-1 and to relate with clinicopathological parameters and survival.

**Patients and methods:** Through enzyme-linked immunosorbent assay and qPCR were evaluated and statistically related 30 female dogs with mammary tumor and 47 controls.

**Results:** High levels of VEGF were correlated with abundant irrigation (p=0.02, metastase (p=0.003), death (p=0.001) and low survival (p<0.0001)); however HIF-1 levels was not related with clinicopathological features investigated. The VEGF was superexpressed in tumors with abundant irrigation and also in female dogs with metastase, recidive and death while the HIF-1α was underexpressed.

**Conclusions:** Our results show that these proteins play an important role in angiogenesis and are useful in predicting tumor progression in canine mammary tumors.

**Financial support:** FAPESP.

### P50

**Use of immunohistochemistry in the differential diagnosis of cutaneous round cell tumours in dogs**

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Background: Cutaneous round cell tumors may have a similar morphological appearance, and a diagnosis based on routine histopathology only is often challenging. Immunohistochemistry has been proven to be one of the most important ancillary techniques in the characterization of neoplastic diseases in veterinary medicine.

Materials and methods: This work describes an antibody panel (CD117, CD3, CD79a, CD45, cytokeratin, vimentin and E-cadherin) for immunohistochemistry analyses of formalin-fixed, paraffin-embedded sections of canine cutaneous round cell tumors. Neoplastic tumors were diagnosed by history and histochemical staining and included 89 mast cell tumors, 31 cutaneous histiocytomas, 21 cutaneous lymphomas, three plasma cell tumors, and seven unclassified round cell tumors.

Results: The histologic diagnosis was modified in 43.5% of the total 147 neoplasms. The staining for CD45 and E-cadherin were variable, and therefore, the final diagnoses of cutaneous histiocytoma was made based on histology in association with negative results for CD3, CD79a, CD117 and cytokeratin. The cellular origin of unclassified round cell tumors was defined in all cases. Cutaneous B-cell lymphoma and plasma cell tumors were CD79a-positive and could be distinguished from each other by the morphological characteristics. Mast cell tumors and T cell lymphoma were CD117 and CD3 positive, respectively. The positive staining for vimentin and the negative staining for CD3, CD79a, CD117 and cytokeratin favored the diagnosis of transmissible venereal tumours.

Conclusions: The final diagnosis of cutaneous round cell tumors should be based on the interpretation of immunohistochemical results together with the cellular morphology observed by histology. Therefore, more studies to optimize the specific markers in formalin-fixed, paraffin-embedded tissues (especially for histiocytes) are required for definitive diagnosis of round cell tumors in dogs.

Financial support: PRPq-UFMG, CNpq, FAPEMIG and CAPES.

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**PS1**

Transcription profiling in papillary thyroid carcinoma reveals potential diagnostic markers and drug targets

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**Background:** Papillary thyroid carcinoma (PTC) is the most frequent malignant endocrine neoplasia with an increasing prevalence in the last decades. We aim to identify transcripts and pathways associated with PTC tumorigenesis.

**Materials and methods:** RNA from tumor and adjacent normal samples was evaluated using Sure Print G3 8x60K slides (Agilent Technologies). Sixty-five tumor (T) and four normal (N) tissues were labeled with Cy5. A pool composed by nine normal samples (without the corresponding tumor assayed) was labeled with Cy3. A co-hybridization. Statistical analysis was performed using two approaches, a paired (4N vs 4T) and an independent analysis (9N vs. 6T).

**Results:** Overlapping paired (paired Significance Analysis of Microarrays (SAM) with 0.01) and independent analysis (mean log ratios < -1 or >1 with 99% Confidence Interval) resulted in a list of 546 deregulated genes. Networks and functional analysis were generated through IPA software (Ingenuity® Systems). The major biological network identified was related to endocrine system development and function and down regulation of tyrosine metabolism was the main canonical pathway. A preliminary validation was carried out on RT-qPCR for HMGA2. A higher expression was confirmed (P<0.001) in an independent sample set (11N vs. 4T). HMGA2 expression had also diagnostic ability, correctly classifying 117/121 samples according to tumor status (sensitivity=99%, specificity=94% and area under the ROC curve=0.989).

**Conclusion:** This study unveils transcription modulations during PTC genesis and HMGA2 may be a potential diagnostic marker. Functional studies are required to confirm HMGA2 as an oncogenic driver in PTC and with a possible role as a drug target.

**Financial support:** FAPESP and CAPES.

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**PS2**

Distinct gene expression signatures in Lynch syndrome and familial colorectal cancer type X

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**Background:** Heredity is estimated to cause at least 20% of colorectal cancer. The hereditary nonpolyposis colorectal cancer (HNPPC) subset is divided into Lynch syndrome and familial colorectal cancer type X (FCCTX) based on presence of mismatch repair gene defects. We addressed the gene expression signatures in colorectal cancer linked to Lynch syndrome and FCCTX with the aim to identify diagnostic discriminators and to map signaling pathways relevant to hereditary colorectal carcinogenesis.

**Patients and methods:** RNA extracted from 123 colorectal cancers, including 39 Lynch syndrome tumors, 37 FCCTX tumors and 47 sporadic tumors was analyzed using the whole-genome cDNA-mediated annealing, selection, extension, and ligation (WG-DASL) assay containing 18k genes, where after key targets were validated by real time quantitative RT-PCR (qRT-PCR).

**Results:** Colorectal cancers linked to Lynch syndrome and FCCTX showed distinct gene expression profiles, which by significance analysis of microarrays (SAM) differed by 2188 genes. Functional pathways involved were related to G-protein coupled receptor signaling, oxidative phosphorylation, cell cycle function and mitosis and genes herein proved deregulated using qRT-PCR.

**Conclusions:** Distinct genetic profiles and deregulation of different canonical pathways apply to Lynch syndrome and FCCTX and key targets herein may be relevant to pursue in relation to refined diagnostic and therapeutic strategies for hereditary colorectal cancer.

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**PS3**

Clinical impact of the metabolic phenotype of prostate cancer: role of monocarboxylate transporters (MCTs)

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**Background:** Monocarboxylate transporters (MCTs) are transmembrane proteins that facilitate the transport of important monocarboxylates such as lactate across the cell membrane. Thus, these transporters play a central role in tumour metabolism and, as a result, are attractive targets in cancer therapy, which are now starting to be explored in the clinical context.

**Materials and methods:** Expression of key metabolic markers was assessed by immunohistochemistry in 480 prostate samples and prostate cell lines. The levels of glycolytic metabolism were assessed using commercial colorimetric assays. The effect of the MCT inhibitor and thioridazine was evaluated on cell viability by using the Sulforhodamine B assay. Finally, ultra-structural studies were performed by classical electron microscopy transmission techniques.
Results: Firstly, we observed an overexpression of proteins involved in oxidative phosphorylation and lipid β-oxidation in localized prostate cancer, which expression was already evident in precursor lesions. Importantly, only proteins involved in glycolytic metabolism were associated with poor prognosis and the same proteins, despite expressed at low levels in localized tumour, were highly expressed in the metastatic samples. Secondly, prostate cancer cell lines showed important differences at metabolic and ultrastructural levels. The less aggressive LNCaP cells exhibited a more oxidative phenotype whereas the highly aggressive and metastatic cell lines PC3 and DU145 were more glycolytic. Finally, the distant metastatic prostate cancer cell lines were more sensitive to CHC (KIT inhibitor) than LNCaP cells.

Conclusions: These studies demonstrate differences in the metabolism of prostate cancer cells, which could be relevant on the development of new diagnostic, prognostic and therapeutic strategies involving metabolic targets.

Financial support: Fundação para a Ciência e Tecnologia (FCT).

P54
Gene expression profile of stromal cells from potential metastatic sites in breast cancer patients
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Background: In breast cancer, there is increasing evidence that stromal cells may influence tumor development in the primary site, as well as in regional and distant metastatic sites. The aim of this study was to compare the expression profile of stromal cells from the primary tumor (PT), lymph nodes metastases (N+), and bone marrow (BM) from breast cancer patients.

Methods: Fibroblasts primary culture was established from 11 breast cancer patients. Expression analysis was evaluated in PT (n=4), N+ (n=3) and BM (n=4) through a customized CDNA microarray platform (4,800 ORESTES) analyzed by SAM (TMEV, FDR 0%) and functional analysis was performed using DAVID. Technical validation was performed in 6 samples and biological validation was performed in fibroblasts obtained from others 25 patients as evaluated by RT-qPCR.

Results: We observed 267 differentially expressed genes among fibroblasts obtained from the three different sites, which appropriately clustered them in accordance with their origin. Although differences between PT vs. N+ were represented by 20 genes, differences between PT vs. BM and N+ vs. BM were more significant (235 and 245 differentially expressed genes respectively). DAVID analysis revealed that these cells differed in many functions including those related to development. Among differentially expressed genes were NOTCH2 was confirmed less expressed in PT vs. N+ and USP16 confirmed less expressed in PT vs. BM.

Conclusion: In breast cancer patients, stromal cells obtained from different origins present a differential gene expression profile.

Financial Support: FAPESP (process number 2009/10088-7) and CAPES.

P55
Identification of genes located at breakpoints of uncharacterized chromosomal translocations by the use of chromosomal microdissection and next generation DNA sequencing
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BMC Proceedings 2013, 7(Suppl 2):P55

Background: Cancer results from accumulation of pathogenic genetic changes in a somatic cell. Chromosomal translocations (CT) are among the most common molecular changes associated with the development of cancer. The mechanism by which CT causes cancer is through the juxtaposition of two genes, generating a hybrid protein with oncogenic function or through the placing of a proto-oncogene under the control of a promoter region active in the cell of origin. While most genes located at the breakpoints of common CT have been identified, several, less frequent, CT remain uncharacterized.

Materials and methods: We propose to study a cohort of 17 already identified patients with hematologic malignancies that have uncharacterized CT in the neoplastic cells. To accomplish that, we will perform CT microdissection followed by amplification of chromosomal DNA, and DNA sequencing using next generation DNA sequencing.

Results: As a pilot study, we have performed microdissection and amplification of a normal chromosome 7, and tested the efficacy of the entire process by doing a PCR for the BRAF gene, which is located on chromosome 7. PCR amplification yielded a specific band with the right molecular weight, attesting the success of our method. In addition, we have successfully microdissected uncharacterized CT of 8 patients, and successfully amplified the DNA of two of these cases.

Conclusion: We are developing a platform for rapid and robust robust mutation detection of uncharacterized CTs, and will identify genes involved in CT of 17 patients with hematological malignancies.

Financial support: Hospital Albert Einstein.

P56
Mutations in C-KIT exon 11 in canine cutaneous mast cell tumors
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Background: The c-KIT proto-oncogene encodes the receptor tyrosine kinase KIT, which has been shown to play important roles in the cellular maturation, survival, proliferation, and migration of several cell types including mast cells. Mast cell tumors (MCTs) are the most common cutaneous tumor in the dog. MCTs exhibit wide variation in biological behavior. KIT mutations and aberrant KIT expression have been identified in canine MCTs. Unlike human mastocytosis patients, in which point mutations primarily occur in the kinase domain of KIT, internal tandem duplications (ITD) have been identified in the juxtamembrane domain of KIT in canine MCTs. Among several KIT mutations identified, an ITD in exon 11 has been analyzed most consistently and is significantly associated with malignant behaviour of affected tumors. The goal of this pilot study was to describe ITD KIT mutation in six high grade MTCs and four low grade MCTs.

Materials and methods: PCR amplification mutational analysis of KIT was performed using a primer pair for C-KIT exon 11, forward primer 5’ CC ATGTGATGAATCAGTGGAAG-3’ and reverse primer 5’-GTTCCCTAA AGCTTGTGTAAC-3’. PCR products were analyzed by 1.5% agarose gel electrophoresis and visualized with SYBR® Safe DNA Gel Stain. Two of six high grade MCTs (2/6) had ITD KIT mutations. All of these tumors had aberrant diffuse cytoplasmic c-KIT (CD117) localization by immunohistochemistry.

Results and conclusions: None of low grade tumors 4/4 had ITD KIT mutations, and of these tumors only one (1/4) had aberrant c-KIT immunostaining. Internal tandem duplication of KIT exon 11 mutation was associated with high grade tumors and aberrant C-KIT localization by immunohistochemistry in canine MCTs.

Financial support: FAPESP.

P57
The role of genetic polymorphisms at the chromosomes 5p15, 6p12, 6p21 and 15q25 in non-small-cell lung cancer prognosis: a Portuguese prospective study
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Background: The following candidate loci were analyzed: 5p15 (LKB1), 6p12 (TCF7L2), 6p21 (CDKN2A/CDKN2B) and 15q25 (EGR2), which are likely to be involved in lung cancer development. This study aimed to evaluate the potential role of these loci in predicting the survival of Portuguese lung cancer patients.
Introduction: Genome Wide Association Study (GWAS) variants on chromosome 15q25 and 5p15 and genetic polymorphisms on the vascular endothelial growth factor (VEGF) gene showed that they may contribute to lung carcinogenesis. Therefore, this study was performed in order to assess the role of GWAS, and VEGF variants in non-small-cell lung cancer (NSCLC) prognosis.

Materials and methods: Prospective study from February 2010 to April 2011. Median follow up was 12 months. NSCLC patient’s genotyping was performed using the Sequenom MassARRAY platform. Kaplan-Meier curve was used to assess overall survival (OS) and progression-free-survival (PFS).

Statistical significance was considered for p < 0.05.

Results: 144 NSCLC patients were consecutively genotyped in order to assess 19 single nucleotide polymorphisms (SNPs), Males were 75.8%.

Median age was 61.5 (32 – 89) years-old. Non-squamous cell histology was 77.1% and 91.4% were stages IIIb and IV. The following SNPs showed influence in OS: rs2010963 (VEGF + 405 G/C), p = 0.042, rs3025010 (VEGF intron 5 C/T), p = 0.047, rs401681 C/T on Sp15, p = 0.046, rs13489 C/A on Sp15, p = 0.029; and these SNPs showed influence in PFS: rs9297540 G/A on 6p21, p = 0.074, rs401681 C/T on Sp15, p = 0.021.

Conclusions: This was the first large study in Portugal involving NSCLC patients and assessment of 19 SNPs on chromosome 5p15.33, 6p12, 6p21, 5p21.3, and 15q25. Our study suggests that variants on chromosome 5p15 and 6p21 are prognostic biomarkers in advanced NSCLC. In the future, genome-identified patients may improve NSCLC screening strategies and therapeutic management.

Financial support: University of Minho, FAPESP and CAPES.

Characterization of primary mammary epithelial cells with loss of BRCA1 at a single cell level

PB9

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Background: Loss of BRCA1 has been linked to increased cell proliferation in human mammary epithelial cells. To determine if this phenotype is mirrored in the normal-appearing mammary epithelial cells from mouse models of BRCA1 deficiency, time-lapse imaging was performed on primary mammary epithelial cell (PMEC) cultures. Three distinct genetic models were tested to evaluate the role of p53 haploinsufficiency and p53 haploinsufficiency in the background of Erα over-expression to altered cell proliferation.

Methods: PMEC cultures were generated from 10-12 month old wild-type, Brca1f11 f11/MMTV-Cre, Brca1f11 f11/MMTV-Cre p53−/− and Brca1f11 f11/MMTV-Cre p53−/−; Tet-op-ER/MMTV-rTA mice. Live cell phase contrast time-lapse imaging performed for 5-7 days immediately after plating. Timm’s Tracking Tool software (http://www.helmholtz-muenchen.de/scd/service/scientific-services/software-downloads/index.html) was used to measure individual cell lifetimes.

Results: Mean cell lifetimes in generations 1-4 were significantly shorter in PMEC cultures from Brca1f11 f11/MMTV-Cre and Brca1f11 f11/MMTV-Cre p53−/− mice as compared to Brca1f11 f11/MMTV-Cre p53−/−; Tet-op-ER/MMTV-rTA and wild-type mice. A higher percentage of dividing cells were found in Brca1f11 f11/MMTV-Cre, Brca1f11 f11/MMTV-Cre p53−/− and Brca1f11 f11/MMTV-Cre p53−/−; Tet-op-ER/MMTV-rTA mice as compared to wild-type mice. Brca1f11 f11/MMTV-Cre p53−/−; Tet-op-ER/MMTV-rTA mice showed the highest level of colony formation and lowest numbers of apoptotic cells. Brca1f11 f11/MMTV-Cre p53−/− mice showed the lowest level of colony formation and highest number of apoptotic cells.

Conclusions: Loss of Brca1 by itself was sufficient to decrease cell lifetime however this was modificable by exposure to Erα overexpression. An inverse relationship between colony formation and numbers of apoptotic cells was found. In summary, genotype-specific differences in primary mammary epithelial cell behavior were revealed by single cell tracking.

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Exploiting monocarboxylate transporters as new molecular targets for colorectal carcinoma therapy

PB6

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**Background:** Tumour cells relies mostly on glycolysis to meet their energetic demands thus leading to an overload of lactic acid, which must be exported to the extracellular milieu, through the monocarboxylate transporters (MCTs). Due to their upregulation in cancer, MCTs are currently seen as promising therapeutic targets in cancer. Colorectal carcinoma (CRC) is the second most common type of cancer worldwide, being mainly a disease of industrialized countries. Among the chemotherapeutic agents used in the treatment of CRC, S-fluorouracil (S-FU) is one of the most efficient, although resistance to S-FU treatment has been reported. We aimed at understanding the role of MCTs in the glycolytic metabolism of CRC and exploring these transporters as putative therapeutic targets.

**Patients and methods:** We performed a detailed characterization of MCT expression in a CRC clinical series and correlated the expression with key metabolic proteins. We also studied the effects of MCT activity inhibition in normal and CRC derived cell lines and evaluated the effect of MCT inhibitors in combination with S-FU.

**Results:** Our results showed that MCT1 and MCT4, their putative chaperones (CD147 and CD44) and the glucose transporter GLUT1 are overexpressed in CRC samples. Furthermore, we observed that MCT expression is associated with the remaining proteins. In vitro assays, we demonstrated that MCT inhibition disrupted tumour cell aggressiveness and potentiated the cytotoxic effect of S-FU.

**Conclusions:** Our results provide novel evidence for the pivotal role of MCTs in CRC maintenance, supporting the exploitation of MCTs as therapeutic targets in CRC.

**Financial support:** Portuguese Foundation for Science and Technology (FCT). The NCM460 cell line was received by a licensing agreement with INCCELL Corporation, San Antonio, TX. The cells were routinely propagated under standard conditions in M3(10)-medium (INCELL).

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**P61**

**Gene expression profile of long non-coding RNA EVF-2 in medulloblastoma cell lines and tissue samples**

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**Background:** Long non-coding RNAs (lncRNAs) are molecules that have been recently described to participate in tumor progression, however, they remain poorly characterized and their role in brain cancers, such as medulloblastoma (MBD) has not been studied in detail as yet. This study aimed to assess the gene expression profile of an lncRNA named EVF-2 in MBD tissue samples and cell lines.

**Materials and methods:** We used two human MBD tissue samples, three human MBD cell lines (UW473, UW472 and DAOY); three human cerebellum tissue samples and two human cerebellum primary culture. The cell lines were characterized morphologically by light microscopy and immunophenotypically by flow cytometry. MBD cell lines and tissue samples, as well as, control cerebellum primary culture and tissue were evaluated for gene expression profile of the EVF-2 lncRNA gene by Quantitative Real Time PCR.

**Results:** MDL cell lines are morphologically presented as polygonal and fibroblastoid cells. Immunophenotypically, MDL cell lines showed a high percentage (70-99%) for CD44, CD73, CD105, CD166 and CD29 and a low or absence (0-4%) for CD144, CD31, CD34 and CD45. MDL cell lines were different for CD271 (3,26-26,9%), CD24 (20-64%), CD146 (34-90%), CD90 (3,7-99%), CD98 (0,3-6%) and were positive for the cancer stem cell marker CD133 (2,9-5,3%). The EVF-2 lncRNA showed 81 ± 13 times more expressed in MBD tissue samples when compared to cerebellum tissue and 119 ± 48, 476 ± 74, 227 ± 33 times more expressed in MDL cell lines UW402, UW473 and DAOY respectively, when compared to cerebellum primary culture.

**Conclusion:** These data demonstrate that MDL cell lines present an immunophenotype somewhat different for some markers and that EVF-2 lncRNA is more expressed in both human MBD cell lines and tumor tissue when compared to cerebellum primary culture and tissue, respectively.

**Financial support:** FAPESP and Hemeocentro de Ribeirão Preto-HCMR/USP.

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**P62**

**MicrovesSEL density is as a PROGNOSTIC factor in canine cutaneous masteR cell tumors**

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**Background:** Mast cell tumors represent almost 25% of canine skin neoplasms. These tumors are classified in three grades of differentiation, based on histological features. However, this grading system is a method based on subjective parameters, which generate intra- and interobserver variations. The microcirculation is an important feature to the primary tumor expansion, dissemination and metastasis, and there are essential evidences that increasing microvesSEL density is associated with short survival disease-free intervals. The purpose of the present study was to verify the prognostic value of the intratumoral microvesSEL density (IMVD) in a set of canine cutaneous mast cell tumors.

**Materials and methods:** Twenty-nine canine cutaneous mast cell tumors were subjected to immunohistochemical analysis using a rabbit polyclonal antibody anti-human von Willebrand Factor. Subsequently, the IMVD was determined by the average number of vessels in 5 low-power fields. The average IMVD was 9.1 vessels/field for grade I mast cell tumor cases, 14.1 for grade II and 17.2 for grade III. There was no statistically significant differences between histopathological grades with regard to IMVD (p=0.0881). Nevertheless, IMVD was significantly higher (p=0.0362) in dogs which died due to the mast cell tumor. After the identification of a cutoff point by ROC curve analysis (12.6 vessels/field), cases were divided into two groups. Survival analysis showed that mast cell tumors with higher IMVDs had a worse prognosis (p=0.0064), with median survival of 751 days.

**Conclusions:** The IMVD is a trustworthy prognostic factor, indicating the post-surgical survival in cases of canine cutaneous mast cell tumors.

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**P63**

**Evaluation of c-KIT protein expression in canine mammary tumors**

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**Results:** c-KIT protein expression on invasive mammary carcinoma were evaluated using an IHC assay. A representative area of the primary tumor was selected for analysis. The c-KIT expression was analyzed by image analysis software and expressed as percentage of positive cells. The percentage of c-KIT positive cells was 80, 55, 75 and 30% for canine mammary tumors grade I, II, III and IV respectively. The expression of c-KIT was significantly higher (p<0.05) in grade II and III compared to grade I and IV.

**Conclusion:** The evaluation of c-KIT protein expression in canine mammary tumors may be a useful tool for the prognosis and treatment planning.

**Financial support:** FAPESP and Unicamp.

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http://www.biomedcentral.com/bmcpdiscprocelements/7/S2
Background: c-KIT is a proto-oncogene that synthesizes a tyrosine kinase protein responsible for cell growth. Higher expression of c-KIT protein in women normal breasts correlates with differentiation status of normal breast epithelium, while loss of c-KIT expression is observed during progression or malignant transformation of mammary epithelium. In bitches the role of this tyrosine kinase receptor has not been established. The aim of this study was to identify c-KIT protein expression in canine breast tissue and compared results with c-KIT expression status in human breast tissue.

Materials and methods: c-KIT protein expression was assessed by immunohistochemistry in 26 canine mammary tumors samples and compared with normal mammary tissue, non-metastatic and metastatic mammary carcinomas. c-KIT expression was evaluated according to its pattern, as established in canine mast cell tumors (KIT I: perimembranous, KIT II: focal cytoplasmic and KIT III: diffuse cytoplasmic). Fisher exact test was used to determine the association between the categorical variables.

Results: Metastatic carcinoma were correlated to pattern KIT II, non-metastatic carcinoma to pattern KIT I and normal breast to pattern KIT III (p<0.05).

Conclusion: Our results differ from c-KIT protein profiles and relation to prognosis reported in canine mast cell tumors, since normal canine breast tissue had a KIT III pattern, related to worst prognosis in mast cell tumors. However, c-KIT pattern is not described in human breast cancer and correlated to prognosis.

Financial support: FAPESP.

P65 Characterization of the mechanisms underlying the crosstalk between galectins and notch in gastric cancer

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Background: Gastric cancer is the fourth most common cancer and the second leading cause of cancer-related deaths worldwide. Galectins form a family of β-galactosides binding proteins that recognize a variety of glycan-containing proteins at the cell surface and are overexpressed in various tumors, including gastric cancer. Galectins overexpression as well as changes in their subcellular distribution has been associated with gastric cancer progression and poor prognosis. It is not well understood, however, how the interaction between galectins and glycosylated receptors modulates tumor development and growth. Since Notch receptors and ligands contain glycan structures known to bind galectins, we aim to demonstrate that galectins expression in the tumor microenvironment may interfere with Notch signaling activation during tumor development and progression.

Materials and methods: Immunoprecipitation procedures with gastric cancer cell line AGS (ATCC CRL-1739) and MKN45 (ACC 409) were used to test for association between galectin-1/-3 and Notch-1 receptor. Furthermore, we transfected AGS cell line with siRNA against galectin-1/-3 or scramble using standard protocols (IDT DNA technologies), stimulate them with immobilized human recombinant delta-4 or Jagged-1 and assessed Notch-1 receptor activation.

Results: Galectin-1 and -3 interact with Notch-1 receptor and differentially modulate Notch signaling pathway upon activation by Delta/ Jagged ligands. Galectin-1 knockdown alters Notch-1 activation induced by Delta-4 whereas galectin-3 knockdown alters jagged-1-mediated Notch-1 activation. Furthermore, we found that exogenously added galectin-3 can enhance Notch-1 activation by Jagged-1.

Conclusion: Our results suggest that galectin-1 and -3 interact with Notch-1 receptor and differentially modulate Notch signaling activation induced by Jagged-1 and Delta-4.

Financial support: FAPESP and CAPES.
expression included both proliferation and apoptotic assays. Future work utilizing large-scale proteome screens as well as preclinical cell and animal models will identify calpain targets and global cellular signaling changes when calpain expression and activity are altered.

**Results:** In human breast cancer cell lines, overexpression of calpain, but not an inactive mutant leads to increased cell proliferation and renders breast cancer cells expressing the HER2 oncogene resistant to a HER2 specific drug, Trastuzumab. In addition, loss of calpain expression leads to reduced cell proliferation and decreased ability to grow tumors in mice.

**Conclusions:** Results from this study provide evidence that calpain functions as an oncogene and plays a role in regulating cell survival in response to Trastuzumab. This study can lead to the discovery of drug combinations for the improvement of breast cancer treatment.

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**P67 miRNA expression in human lung cancer and fetal lung: a comparative study**

**Study**

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**Background:** Many molecular systems involved in controlling development are also implicated in malignant transformation and are associated with poorer survival outcomes in cancer patients. In fact, lung tumours with similar gene expression profiles to fetal lung cells are associated with aggressive phenotypes of the disease. Targeting fetal developmental pathways selectively reactivated in cancer cells would be considered ideal therapeutic targets for cancer treatment. Therefore, a better understanding of the interplay between fetal lung development and lung cancer on the level of individual miRNAs will provide new insights for translational research. In this study we applied sequencing technology to identify miRNAs with similar expression patterns between fetal lung and lung tumours.

**Patients and methods:** Eighty-nine pairs of tumour and adjacent malignant lung and five fetal lung tissue samples were profiled by miRNA-sequencing. Mann–Whitney U tests were performed with Benjamini–Hochberg multiple testing correction to identify common expression patterns.

**Results:** We describe, for the first time, a comprehensive miRNA expression profile of human fetal lung. We have not only detected miRNAs that exhibit similar expression levels between fetal lung and non-malignant lung tissue which may play roles in lung tissue maintenance, but also identified multiple miRNAs with similar expression patterns between fetal lung and lung tumours. Some of these miRNAs have been previously implicated in lung cancer, while others have not yet been described to play a role in this disease. Pathways known to be targeted by miRNAs commonly expressed between fetal lung and lung tumours are involved in cell proliferation and angiogenesis. Further studies will elucidate the specific roles of these miRNAs in lung tumourigenesis.

**Conclusions:** We identified several miRNAs with similar expression profiles between fetal lung and lung tumours which potentially play critical roles in lung cancer and might lead to the identification of promising therapeutic targets.

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**P68 Evaluation of ATM protein expression in canine mammary tumors**

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**Background:** Ataxia telangiectasia mutated (ATM) synthesizes a protein kinase known as a major regulator of DNA damage response. ATM mutations in women have been associated with moderate risk to develop familial Breast Cancer. ATM transcript and protein down-regulation have been reported in sporadic breast carcinomas and the absence of ATM protein expression was also significantly associated with distant metastasis in women. Canine mammary tumors have an incidence three times higher than women and their biological behavior is similar in both species. The aim of this study was to identify the ATM protein expression in canine breast and compared the results with what occurs in women.

**Patients and methods:** In this study, we evaluated ATM protein expression by immunohistochemistry of 48 canine breasts samples, and compared ATM expression among normal breasts, benign mammary tumors (hyperplasia or adenoma), non-metastatic and metastatic mammary carcinomas. Evaluation of ATM protein expression was performed by the distribution of the positive cells (score 1: <25% cells positive, 2: 26% to 50%, 3: 51% to 75% and 4+: >75%).

**Results:** Kruskal-Wallis test and Wilcoxon test were used (P < 0.05). Lower ATM levels were significantly associated with non-metastatic and metastatic mammary carcinoma when compared to normal breast tissue and benign mammary tumors.

**Conclusions:** A similar ATM expression was found between non-metastatic and metastatic mammary carcinoma samples and this fact can be explained by the possibility that these patients could present distant metastasis in the future, once they have been monitored for just one year. These data suggests that ATM have a similar behavior in bitches and women.

**Financial support:** FAPESP.

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**P69 Genetic profile analysis of tumor stem cells in locally advanced breast cancer**

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**Background:** Breast cancer is a highly prevalent and incident disease. About half the observed cases are diagnosed in later and/or disseminated disease stages. Treatment success in advanced disease stage occurs in about 20% of cases. The identification of patients who would most benefit from neoadjuvant therapy can reduce treatment costs and avoid adverse effects in patients with low probability of response. However, is not yet possible to make such a prediction. The cancer stem cells (CSCs) paradigm relates a cell population resistant to radiotherapy, chemotherapy and cells capable of tumor initiation and recurrence. The identification and characterization of CSCs in the primary tumor can be an effective method of predicting response to neoadjuvant chemotherapy in locally advanced breast cancer.

**Materials and methods:** We aim to include 40 patients diagnosed with invasive ductal carcinoma, who will undergo neoadjuvant chemotherapy before surgery. We are in process of collecting tumor tissue biopsies and quantifying, by flow cytometry, and separating, by FACS, the CSCs. We will define CSCs genetic profiles and correlate them with pathological response
to the treatment. Biopsies have been collected from five patients to date; from these samples, CSCs were sorted and RNA was extracted and stored. We are following patients for their clinical progress.

**Results:** To date, we did not observe statistical differences in the percentage of CSCs in these five samples. We expect to find transcriptional differences between CSCs in tumors from patients who respond to neoadjuvant chemotherapy and from patients who do not respond to treatment regimens.

**Conclusions:** Although our preliminary data did not show differences, we expect a slightly different percentage of CSCs in tumor samples from responders vs. non-responders, in a larger sample set. However, CSCs transcriptome differences between the two groups of patients may yield a better understanding of neoadjuvant chemotherapy resistance, preventing unnecessary and costly treatment for many patients.

**Financial support:** FAPESP.

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**P70**

*Lafoensia pacari* extract induces apoptosis mediated by caspase-3 and inhibition of growth in human lung cancer cells

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**Background:** *Lafoensia pacari* is a native species from South America which has been used in traditional medicine as anti-ulcerogenic and anti-inflammatory for several diseases, but the antineoplastic potential still have not been elucidated so far, though its ethnopharmacological indication.

The aim of this study was to evaluate the anti-neoplastic effect of *L. pacari* ethanolic extract in three human lung neoplastic cell lines.

**Materials and methods:** For the assessment of cytotoxicity, cell lines were grown in vitro, being two originally from non-small cell lung carcinoma (A549 and H2023) and one of giant cell lung carcinoma obtained from pleural effusion (H460). Cells were grown in 96 well plates under regular cell culture condition and treated with different concentrations of *L. pacari* ethanolic extract, then analyzed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl bromide tetrazolium, widely used to determine the viability of cultured cells. The IC50 value and the regression curve were calculated with 5.0 Prism (GraphPad Software, USA). Also, the Caspase-3 Assay Kit for Live Cells (Biotium, USA) was used to determine the mode of action of *L. pacari* at IC50 concentration.

**Results:** After the treatment for 72h with the ethanolic extract of *L. pacari*, we determined a dose-dependent effect of *L. pacari* extract in all cell lineages, being the H460 cell line the most sensitive by means of lowest IC50. Thus, we also showed that this effect was due to induced caspase-3 dependent apoptosis.

**Conclusions:** The extract of *L. pacari* demonstrated an antineoplastic effect in all cell lines. The Caspase-3 activation in tumor cells after treatment with *L. pacari* suggests that the cytotoxic effect is related to activation of the intrinsic apoptotic pathway, leading ultimately to cell death. Further studies are under investigation to determine the specific substances responsible for these effects.

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